JIANG SU YAO LI TONG XUN

JIANGSU RMACOLOGICAL BULLITIN

第九期

江苏药理通讯

9

2011

江苏省药理学会主办

主办单位：江苏省药理学会

内部资料　　免费交流

本期协办单位：
徐州医学院药学院

目　录

江苏省药理学会2010年度工作总结

2010年江苏省药理学会在江苏省科协、江苏省民政厅、卫生厅、药监局等各级的帮助和支持下，认真贯彻学习胡锦涛总书记在纪念中国科协成立50周年大会和中华人民共和国成立60周年上的重要讲话，认真总结药理学会工作取得的成绩和经验，为进一步推动我省药理工作更好更快的发展，团结全省广大药理工作者，推动我省药理学科技创新发展而努力工作。回顾2010年，学会工作取得的成绩主要有以下几方面:

（一）加强学会建设

1．江苏省药理学会坚持把服务于国家医药卫生事业发展作为药理学会的根本宗旨，坚持把团结和动员广大药理科技工作者、推进药理学科技进步为学会工作的中心任务，坚持把完善组织建设作为学会工作的首要任务。在2010年学会在组织建设、学术交流、编辑出版、科学普及、继续教育和科技发展等方面取得较好的成绩，为学会工作的持续发展打下重要的基础。

2．2010年1月17日在中国药科大学会议室召开了江苏省药理学会第二届理事会第四次会议，会上认真学习了胡锦涛总书记在全国科协成立50周年大会上的讲话和徐耀新主席在省科协第七届五次全委扩大会议的工作报告，坚持“三服务一加强”的工作定位和“搭建平台，资源共享”的工作思路，通过学习全体理事为解放思想，开拓创新坚定了信心。秘书长朱萱萱教授传达了江苏省科协学会工作总结要求，汇报了2009年江苏省药理学会工作。以及贯彻落实中央和省委关于新社会组织深入学习实践科学发展观活动的有关精神。

会上布置了2010年学会工作计划，讨论江苏省药理学会第四届学术会议安排，定为徐州医学院，时间为2010年4月23日至25日，以大会报告的形式，报告人员安排由南京大学徐强教授；南京医科大学胡钢教授；李胜男教授；中国药科大学王广基教授；南京中医药大学方泰惠教授；扬州大学张洪泉教授；苏州

大学秦正红教授；徐州医学院印晓星教授；江南大学金坚教授等作报告。江苏省药理学会第四届学术会议主席台人员名单由徐州医学院会议会场定。学术论文编辑人员由江苏省药理学会朱萱萱教授和新药临床前药理专业委员会刘毅教授负责。江苏省药理学会第四届学术会议召开同时成立新药临床前药理专业委员会、江苏省药理学会中药药理分会第三届学术会、临床药理分会第八届学术会和教学分会第一届学术会。江苏省药理学会理事会议和各专业的工作会议也一并召开。讨论 2010 年江苏省药理学会科普活动的开展，根据江苏省科学技术协会的要求，及时收集资料和佐证材料，2010 年增加科普活动次数，扩大影响力。原有科普活动的要继续开展。会议表彰了 2009 年度的先进个人，根据 2009 年度对学会工作贡献突出的个人进行表彰，会议一致通过张洪泉教授和朱萱萱教授获 2009 年度江苏省药理学会学会工作贡献突出奖。与会人员一致认为本届理事会在理事长王广基教授领导下，管理规范、卓有成效的做了大量的工作。但也指出了一些不足之处，如发展会员力度不够，工作资料留存不全等。

3. 2010 年 3 月 24 日江苏省药理学会第二届中药药理专业委员会第一次会议在长澳药业一楼会议室召开，出席会议的还有长澳药业总经理李战、长澳药业副总经理曹阳、栖霞区王平主任。长澳药业总经理李战首先介绍长澳药业的基本情况，长澳药业 2005 年在新加坡上市，57 个品种，两个研究所。同位素标志的药代动力学研究开发中心。长澳有一半，产业化的研发企业。随后栖霞区王平主任介绍栖霞区政府生命科技园规划，介绍园区简介和成立背景（成立于 2009 年 1 月）栖霞区政府将生命科技园作为医药研发的区域　定位：生态化生命科技高编产业特色园区　一个基地，两个中心。吸引中国药科大学，中医药大学和南京大学三所学校到工业园区共同发展，提供公共分析平台。园区优势：配套设施，交通方便，大学有 12 所学生 15 万人。生活配套，商业配套。随后学会副理事长方泰惠宣布第二届中药药理专业委员会名单并颁发了聘书，秘书长朱萱萱教授传达了江苏省科协学会工作总结要求，汇报了 2009 年江苏省药理学会工作总结，讨论 2010 年中药药理专业委员会工作计划，全体委员都表示愿为江苏医药研发积极作贡献。

4. 江苏省药理学会第二届中药药理专业委员会第二次委员会议于 2010 年 4 月 24 日在徐州举行，会议由南京中医药大学方泰惠教授主持，会议现场气氛热烈，委员们踊跃发言。会议讨论了中药药理专业第四届学术会议的相关事宜，明确了第四届中药药理学专业会议于今年 5 月份由扬州大学承办，会议拟定的主题是“中药药理与安全性评价”，大部分委员认为讲座的内容不应仅局限于中药药理，还应注重学科的交叉融合，比如请化学、生物学等相关学科的专家进行讲座，同时开展科研学术沙龙，对感兴趣的学术问题各抒己见，进行积极地交流与讨论；会议的标牌与江苏省药理学会一致；各委员一致提议中药药理专业会议增设“优秀论文奖”（需由导师推荐学生，一、二、三等奖各位 1 名、2 名、3 名），以对参会研究生进行鼓励，给予研究生展示自我的平台，同时为表彰对江苏省药理学做出杰出贡献的学者增设“江苏省药理学会杰出贡献奖”；会议拟定于今年 10 月份进行科普活动。

委员们对如何办好中药药理学会、提高科研水平、促进江苏省医药事业发展等问题也进行了认真的分析与讨论。顾振纶教授认为，我们的科学研究要积极为企业服务，让这种服务由“被动”变为“主动”，这样才能做到“产学研”结合，更好为江苏省医药事业、为地方经济做出贡献；当前，中药现代化遇到了发展瓶颈，如何取得突破，是每一个中药药理工作者需要认真思考的问题，顾教授指出我们要以中医的理论基础来指导中药的发展，同时也要建立规范、可制的研究新方法。

杨娴处长就我省新药临床前研究中存在的问题进行了简要分析，她认为江苏省是教育、科技大省，具备良好的新药研发条件，应注重自身宣传，吸引企业在本省做新药临床前研究，建议企业与高校做一些有特色的项目，比如医院独有的特色药以及中药注射剂再评价，加强新药申报的规范化、制度化。

5. 2010 年 4 月 24 日在徐州召开江苏省药理学会教学专业委员会第二次会议，季晖教授总结教学专业委员会成立以来的工作，提出今后开展工作思路，提请大家讨论。委员们交流讨论各高校药理学教学情况，如师资队伍、课程设置、精品课程、规划教材、远程教学、实验教学等。并提出一些问题如由于政策原因导致

对教学工作重视不够，投入不足等。苏州卫生职业技术学院的韦翠萍副教授提出学会支持申报教学研究课题的有关事项。讨论教学专业委员会开展科普活动的安排，提出开展科普活动的形式如合理用药咨询、报告、展板、传单或科普宣传小册子等。并落实各地开展科普工作的责任人（徐州：谷淑玲教授；盐城：秦红兵副教授；淮阴：刘斌副教授；南通：张伟副教授；扬州：葛晓群教授；苏州：梁中琴教授；镇江：李永金副教授；南京：洪浩副教授）。讨论如何进一步发展会员，决定由秦红兵副教授负责发展高职学院教师入会工作，梁中琴教授负责发展苏大新会员。会议提出《江苏药理学通讯》是否能让每位会员每期人手一册，以便大家即时了解学会动态。讨论学术活动安排，会议决定教学委员会学术活动安排在省药理学会两年一度的学术交流会期间，延长半天进行学术交流，并建议学会增设教学论文奖（1次/2年）。

6. 2010年4月23日在徐州召开江苏省药理学会第二届理事会第二次理事大会，主要日程包括江苏省药理学会2009年的工作总结；传达江苏省科协对学会工作的要求和2010年学会工作计划；讨论2010年江苏省药理学会科普活动；讨论江苏省药理学会专业分会标牌；讨论江苏省药理学会设立年度表彰；调研您认为江苏省药理学会今后应如何发展；作为药理学会的理事，您今后对学会的工作、会员发展、学术交流和科普宣传方面打算如何做。（附表填写）

7. 深入学习实践科学发展观开创药理学会建设美好未来，江苏省药理学会在全党开展的深入学习实践科学发展观活动中成绩突出被评为省科协学习实践科学发展观先进奖。

8. 2009年学会被评为省科协学术先进奖，学会2009年会员郝海平获省科协江苏省青年科技奖，张陆勇获省科协江苏省先进科技工作者奖。

（二）学会学术工作

当今世界，科技发展突飞猛进，创新创造日新月异，是一个自主创新源泉充分涌流，创造活力竞相迸发的时代，是一个全面发展科学素质全民提高的时代，是一个科技社团蓬勃发展、科技创新人才辈出的时代，江苏省药理学会学会学术工作在2010年也取得较好的成绩。

1. 学会的江苏省药理通讯编委会，于 2010 年 7 月出版了《江苏药理通讯》第八期 250 册，分别发送学会理事和会员，通讯主要介绍了江苏省药理学会 2009 年学会工作总结内容和 2010 年在徐州医学院召开的江苏省药理学会第四届学术会议暨新药临床前药理专业委员会成立大会，期刊还介绍了我省临床药理工作者近期科技成果和科研内容 36 篇，其中包括实验研究、药物临床研究、病例报告和临床管理方面的内容。

2. 2010 年 4 月 24 日在徐州召开江苏省药理学会第四届学术会议，来自全省各地近 140 名药理学工作者汇聚在历史文化名城徐州，徐州医学院吴院长出席了开幕式并对本次学术会议的如期举办表示了热烈的祝贺。会议由徐州医学院印晓星院长主持，江苏省药理学会理事长中国药科大学副校长王广基教授代表本届理事会致开幕词，并代表江苏省药理学会，向这次大会的召开表示最热烈的祝贺，向来自江苏各医药院校、科研院所及医药企业的代表们表示诚挚的欢迎。同时对江苏省药理学会近年来的工作做了汇报，江苏省是医药大省，省药理学会作为全省药理学界最重要的学术团体之一，团结和凝聚了一大批药学和药理学科技工作者。在这里有为药学和药理学发展做过杰出贡献的老一辈学者，有科研、教学及产业的中坚力量和新秀。按照“为经济社会发展服务，为全民科学素质服务，为科技工作者服务，加强自身建设”的学会工作定位，积极努力工作，对全省药学和药理学教学科研水平的提高、学科的发展和人才的培养发挥了积极的作用！本次会议共收到 68 篇学术论文，与会的代表们围绕神经药理、免疫药理、药代动力学、中药药理、临床药理等领域进行了广泛和深入的交流。南京医科大学李胜男教授作了《CRF 家族肽生物作用多样性的研究李胜男》的学术报告，就 CRF 家族肽在体内作用复杂进行全面阐述，在不同情况下对机体发挥完全不同的作用，提示 CRF 家族肽-受体系统的平衡状态对机体的重要性，许多疾病可能由于该系统失衡有密切关系，左右其功能可能成为治疗疾病的重要药物治疗手段。南京大学生命科学学院副院长、长江学者徐强教授作了《化学生物学—药理学工作者的新领域》的学术报告，报告中阐述了药理学研究的是药物与机体的相互作用原理，其中主要是小分子药物与机体作用的生物学过程，小分子与蛋白质等大

分子的相互作用已成为药理学研究的重要内容。化学生物学则使用小分子作为工具研究生物学问题，具体内容之一就是借助小分子干扰/调节蛋白质等大分子物质从而了解其生物学功能。在这个意义上，化学生物学和药理学之间存在某种接点，比如使用小分子调节目标蛋白质与制药公司发展新药类似，表明化学生物学的研究将有助于新药的发现。中国药科大学副校长王广基教授作了《中药复方药代动力学研究》的学术报告，并从中药药代动力学研究意义研究思路；关键技术的研究；确有疗效中药制剂药代动力学研究等方面作了详细的叙述，并指出在体内外物质组研究的基础上，阐明药效物质组，确定中药的 PK/PD 标记物，进行多组分药代动力学研究与模型整合，开展临床 PPK/PPD 结合研究，探讨中药临床给药方案优化措施，开展确有疗效中药制剂的纵贯式深入研究。南京中医药大学方泰惠教授作了《中药注射剂再评价的关键问题》的学术报告，就近年来，中药注射剂不良反应报告逐年增加。尤其是鱼腥草、刺五加、双黄连、清开灵等注射剂引起的严重过敏反应，导致多名患者死亡，使人们对中药注射剂的安全性日益关注。从已有的报导来看，中药注射剂引起的不良反应占中药不良反应的 50%以上，成为中药引起不良反应的主要因素作了详细的报告。

南京市鼓楼医院肖大伟主任药师作了《lims 系统在实验室管理的应用》的报告，报告中阐述了实验室信息管理系统在国际和国内上已取得很快的发展和巨大的成就。特别在药物研究领域中，数据的采集和管理趋向于完全的集成化和统一化的 LIMS 管理系统；现代药物研究遵从法规主要致力于患者安全，产品质量和数据的完整性。随着法规要求更严格的控制和更佳的可溯源性，没有计算机为基础的实验室信息管理系统，实验室流程管理变得更加困难。LIMS 已经逐渐成为一个成熟和高水平实验室的标志性因素。徐州医学院印晓星院长作了《以人为本药学服务，积极推进临床药学专业教育》的报告，报告中总结了临床药学的核心是以人为本，药学服务，从而使病人获取最佳的治疗结果。当前我国医院药师正由传统的药品提供者转变成药学服务者，但我国药学教育与此要求相比尚有距离，临床药学专业教育迫切需要完善和提高。临床药学专业应紧扣当今医药改革之要求明确人才培养目标，以医药并重的原则，合理调整课程体系和教学内容，

强化医学相关课程的比重，使学生具有合理的医学和药学知识背景，为今后的临床药师及新药临床研究工作打下坚实的基础。苏州大学秦正红教授作了《p53 signals apoptosis and autophagy in mediating excitotoxicity》的报告，报告内容丰富、学术气氛活跃，大家踊跃参会，认真听讲，深入讨论，为今后江苏省药理学的发展奠定了良好的基础。最后大会评出了青年优秀论文一、二、三等奖并颁发了证书。

3. 江苏省药理学会教学专业委员会职业教育分会2010年会于2010年12月3日在盐城卫生职业技术学院召开。江苏省药理学会副理事长、江苏省药理学会教学专业委员会主任季晖教授，江苏省药理学会秘书长朱萱萱教授，江苏省药理学会教学专业委员会秘书长洪浩副教授参加本次大会，并分别代表省药理学会和教学专业委员会讲话；盐城卫生职业技术学院李玉华副院长出席会议，盐城卫生职业技术学院常唐喜副院长代表学院领导看望了出席会议的专家和代表。全省共有14 所医药卫生类职业院校的代表出席了本次会议。会议决定江苏省药理学会教学专业委员会职业教育分会拟每两年举办一次年会，旨在为省内医药卫生职业院校药理学教师提供一个学习、交流的平台，促进医药卫生职业院校药理学教学改革，提升药理学教师的整体教学水平。会议进行了说课比赛、教案及课件制作三项竞赛。参赛的有9个说课、17份教案和16个课件，分别从药理教学教研的不同角度，展现出先进的职教理念、扎实的专业功底，上乘的教学技能，充分显示我省医药卫生类职业院校药理学教师的风采。季晖、朱萱萱、洪浩等专家对说课进行了精彩的点评，并评选出说课、教案和课件制作一、二等奖。

会后全体代表还参观了盐城卫生职业技术学院制药技术实训中心、新四军纪念馆和海盐馆，使代表受到一次爱国主义教育和了解了盐的历史。通过代表们的共同努力，会议圆满完成了预定的目标。

4. 2010年12月10日由江苏省药理学会主办的“创新药物成药性评价高层学术论坛”暨中国药学会应用药理专业委员会第四届学术年会和中国药理学会制药工业专业委员会第十四届学术年会在南京先声药业会议中心隆重召开，来自全国130多名药学专家、药理、药代科技工作者会聚紫荆山畔，参加了本次会议。中

国工程院院士刘昌孝、中国药科大学校长吴晓明、中国药理学会理事长杜冠华、中国药科大学副校长王广基教授、原江苏省政协主席沙人麟等药学专家、领导出席本次会议开幕式并为本次论坛致词，对本次会议隆重召开表示热烈祝贺。

会议全面落实《国家中长期科学和技术发展纲要（2006-2020）》和国务院批准的“重大新药创制”科技重大专项的实施方案，贯彻执行党中央、国务院关于发挥科技重大专项在战略性新兴产业培育、促进经济发展方式转变、深化医药卫生体制改革中支撑作用的指示精神，以“创新药物成药性评价高层学术论坛”形式展开，其主旨紧扣“十一五”重大创新药专项主体，对我国创新药物体系的开发有着积极的推动作用。本次会议通过广泛征求意见、民主协商，本着“公平、公正”的原则，对中国药学会应用药理学专业委员会进行了换届选举，优化了委员会的专业结构，为中国应用药理学更快发展奠定了坚实基础。

与会专家为本次大会做了精彩、深入的学术报告，报告以创新药物成药性评价为主题，内容涉及创新药物成药性评价、药理新理论、中药复方药代动力学研究、转化医学、成药性及其风险等方面，与会代表踊跃提问深入探讨，会议学术交流气氛浓厚。中国工程院院士、药学专家刘昌孝院士作了《转换研究与新药的成药性》的精彩学术报告，对转换研究在药物创新中的应用做了详细深入的介绍，并深入地剖析了转换研究在中药研究中的应用，为我国创新药物体系的发展指明了方向。中国医学科学院药物研究所杜冠华教授以抗帕金森药物研究为例，为与会代表做了《新药临床前成药性研究的关键问题探讨》的学术报告，详细列举了新药临床前成药性研究的关键问题及对策。中国药科大学副校长王广基教授作了《中药复方药代动力学研究探讨》的学术报告，介绍了中药复方药代动力学研究的关键技术，为中药复方药代动力学研究提供了新的方法及思路。SFDA药品审评中心审评五部审评九室彭健教授以创新化学药药理毒理评价及成药性评价为中心，消息探讨了创新药药理毒理研究的策略及我国审批管理的进展，并展望了我国药理毒理评价质量和风险控制工作的发展方向。北京大学基础医学院药理系张永鹤教授以成药性研究中的机遇和风险为切入点，列举了历史上有名的成功案例，深入浅出地为与会代表剖析了成药性研究中的方法及思路。此外，药品审评

中心笪红远主任药师、先声药物研究院CSO王鹏博士也为大会作了精彩的学术报告。

此次大会，以“创新药物成药性评价高层学术论坛”形式展开，来自全国各地的130多名药学科技工作者参与，规模大，层次高，为我国创新药物的发展提供了一个良好的交流平台。与会专家的精彩报告，代表们积极参与探讨的精神，充分折射出此次高层学术论坛浓厚的学术交流气氛，活跃了国内创新药物成药性评价研究的思想。刘昌孝院士为此次高层学术论坛做了总结性发言，至此本次大会获得了圆满的成功，并得到了业内人士的一致赞扬。

5.接待国际学术团体 6 次，其中包括接待 2010 年 3 月 25 日，接待悉尼资深教授 Zheng.hui 访问，美国衣阿华大学药学院 John P.N. Rosazza 教授； 2010 年 4 月 15 日接待英国斯特莱斯克莱德大学成员并于 2010 年 8 月 31 日签署协议；2010 年 7 月 29 日负责接待美国新泽西州州立大学 Tony Kong 教授；2010 年 11 月 8 日负责接待日本岐阜药科大学永濑久光教授等多个国际学术团体。

6.积极开展科普活动， 2010 年由江苏省药理学会主办的科普活动分别在南京、扬州等地开展。2010 年 7 月 26 日江苏省药理学会与扬州干休二所联合举办联欢活动共庆八一建军节活动，中国人民解放军是一支具有光荣革命传统和辉煌战斗业绩的人民军队。83 年来，人民军队始终与中华民族命运共系，与中国人民血肉相连，在中国共产党的领导下，经历了血与火的洗礼，为人民解放、民族独立、国家富强，进行了英勇顽强、艰苦卓绝的斗争，建立了卓越功勋。中国人民解放军不愧为人民民主专政的坚强柱石，不愧为捍卫国家主权和领土完整的钢铁长城，不愧为社会主义建设的重要力量，不愧为全心全意为人民服务的子弟兵。值此中国人民解放军建军 83 周年的光辉节日即将到来之际，江苏省药理学会、扬州干休二所为庆祝八一建军节，联合举办联欢活动。江苏省药理学会副理事长方泰惠教授、朱萱萱秘书长携会员，扬州干休二所离退休老干部及全体工作人员，扬州大学女教授合唱团等参加了本次庆八一联欢活动。并邀请维杨区公安分局徐兆华政委为本次庆八一联欢活动特别嘉宾。活动于上午 9 时 30 分在一阵阵热烈的掌声中拉开序幕。扬州市干休二所陶涛所长主持了本次庆八一联欢活动，并为

本次联欢活动贺词，其热情洋溢的语言充分表露了于会的各位人员的喜悦心情。

借此机会，江苏省药理学会全体会员对多年来为祖国繁荣富强、社会稳定发展作出重大贡献的离退休老干部表达了崇高敬意，并组织省内知名专家教授做重要的科普知识讲座。与会的各位离退休干部对科普知识讲座的内容表示非常满意，并作了充分肯定。讲座分为四部分进行，内容涉及老年人疾病治疗及合理用药、老年人健康保健饮食及调理等。江苏省中医院王淑云主任对心血管疾病药物治疗为广大离退休干部做了深入浅出的讲解，为老年人心血管疾病的合理治疗作出了有益的指导。南京中医药大学方泰惠教授就老人健康保健知识为与会的各位离退休干部做了详细的讲解。江苏省药理学会秘书长、江苏省中医院朱萱萱教授做了老年人日常最佳饮食调理的知识讲座，为老年人的健康保障提出了新的理念。扬州大学孙云教授对老年人合理用药做了详细的剖析。江苏省药理学会组织本次科普知识讲座丰富了此次联欢活动的内容，为离退休干部的健康护理、日常保健及合理用药提出了有益的帮助。

为颂扬中华民族的繁荣富强，为歌颂并继续发扬艰苦奋斗的精神，活动以别开生面的合唱联欢结束本次庆祝八一建军节联欢活动，江苏省药理学会副理事长方太惠教授致答谢词，扬州大学女教授合唱团与扬州干休二所老干部表演了《思念》、《洪湖水浪打浪》、《十送红军》、《我是一个兵》《没有共产党就没有新中国》等颂扬中华民族革命历史及辉煌历程的节目。

7. 2010 年 10 月 30 日江苏省药理学会“关爱生命，合理用药”广场大型科普及咨询活动在汉中门广场举办，本次活动由江苏省药理学会主办，江苏省药理学会教学专业委员会和中国药科大学学生会承办，参加咨询活动的专家有方泰惠（南京中医药大学）、王淑云（江苏省中医院）、许惠琴（南京中医药大学）、李庆平（南京医科大学）、李新宇（中国医学科学院皮肤病研究所）、朱萱萱（江苏省中医院）、季晖（中国药科大学）、肖大伟（南京鼓楼医院）、周永刚（解放军八一医院）、洪浩（中国药科大学）、袁红宇（江苏省人民医院）。以及中国药科大学药学院 30 余名学生。本次活动内容以宣传合理用药知识和方法，发放合理用药知识宣传材料，解答市民们提出的药物应用中的问题，指导合理用药；回答市民

们提出的饮食、运动、心理和疾病等健康相关的问题，解答市民们提出的药物应用中的问题，给市民们量血压、腰围，测体重、身高，并计算体重指数。活动内容还包括发放健康小礼品和健康知识问卷调查。

通过本次活动帮助广大市民们掌握正确合理用药的知识和方法，了解饮食、运动、心理与健康和疾病的关系，不断提高我市市民的健康素质起到了积极推动的作用。参与活动的广大市民对本次合理用药知识宣传的内容表示非常满意，数百人健康知识问卷调查结果对此次活动作了充分肯定。并希望今后多开展此类关爱生命，合理用药的活动。

（三）学会财务工作

江苏省药理学会是江苏省药理学工作者进行广泛交流科研成果的场所，本学会以会员为本，2009 年施行缴纳会费制度，该会费用于学术活动交流的支出。2009 年已收取 183 人的会费，本年度新增加会员 34 名，已交纳会费的单位主要是：中国药科大学（21 人）、南京医科大学（18 人）、江苏省中医院（17 人）、南京中医药大学（17 人）、徐州医学院（20 人）、江南大学（16 人）、扬州大学（12 人）、江苏大学（10 人）、江苏省药品检验所（9 人）、省中医药研究院（8 人）、南通大学（5 人）、医科院皮肤病研究所（6 人）、无锡高等卫生职业学校药学系（6 人）江苏职工医科大学（2 人）、盐城卫生职业技术学院（1 人）、淮阴卫生高等职业学校（10 人）泰州职业技术学院医学技术学院（2 人）、江苏康缘药业（2 人）、南京军区总医院（2 人）、苏州大学（17 人）、镇江卫校（1 人）、苏州卫生职业技术学院（3 人）江苏省人民医院（2 人）江苏正大天晴（6 人）、江苏省食品药品监督管理局（1 人）、中国药科大学高等职业技术学院（2 人）南京大学（1 人）、江苏省苏北人民医院（1 人）、南京市中医院（6 人）、南京市第一医院（8 人）、南京市食品药品监督管理局（1 人）、南京市市级机关医院（1 人）、南京理工大学医院（1 人）、江苏省南通市肿瘤医院（11 人）、东南大学药学院（1 人）、盐城卫生职业技术学院（1 人）、扬州环境资源学院（1 人）、南京鼓楼医院（5 人）、南京海辰药业（1 人）、苏州市冯氏（1 人）。

（四）2011 年学会工作计划

2011 年继续全面贯彻胡锦涛总书记在全国科协成立 50 周年大会上的讲话，坚持“三服务一加强”的工作定位和坚持“搭建平台，资源共享”的工作思路，贯彻落实中央和省委关于新社会组织深入学习实践科学发展观活动的有关精神，团结带领全省广大药理工作者，解放思想，开拓创新，坚定信念，扎实工作，为江苏省药理学科率先科学发展、和谐发展作出积极贡献。

1、坚持以会员为本

会员是学会的立会之本，联系会员、服务会员、发展会员是学会的基础性工作，把会员是否满意作为衡量学会工作的主要标准，努力建设充满生机和活力的现代科技社团，把江苏省药理学会建设成为江苏药理学工作者的“和谐大家庭”。积极做好会员发展工作，继续做好会员和团体会员的收费制度。

2、坚持搭建平台

学会是国家创新体系的重要组成部分，学会作为科技工作者自愿组成的社会团体，是科学共同体的重要组织形式，负有推进自主创新、传播科学文化，规范学术行为、提供服务和反映诉求的重要职责，在国家创新体系建设中发挥着重要作用。充分发挥学会的学科优势、人才自愿优势和组织网络优势，积极搭建不同层次、不同形式的服务平台，为开展学术交流、促进创新人才成长服务，为开展科普活动、提高全民科学素质服务，为开展决策咨询和建言献策，增强自主创新能力服务，实现自愿共建共享。2011 年 4 月拟在扬州举办江苏省药理学会新药临床前药理专业委员会第二届、中药药理专业第四届和教学药理专业第二届学术研讨会。

3、坚持创新发展

围绕党和国家工作的大局，不断深化学会改革，创新工作机制和方式，建立健全学会规章制度，民主办会，规范学会工作，提高学会自主活动、自主发展、自我约束的能力，自觉制止学术上的不正之风和不端行为，营造民主讨论、平等代人、严肃批评的学术氛围，加强科学道德建设，促进科学技术健康发展。

4、坚持落实各项规章制度

强化责任意识和服务意识，严格按照所制定的职责分工和各项规章制度，

认真履行各项职责和义务。增强全体会员参与学会工作的责任感和积极性，积极为学会工作建言献策，并对理事会的工作进行监督，不断提高学会工作的水平和规范程度。增加科普活动和技术咨询。

5、规范学会财务管理制度

不断完善财务管理制度，促进财务管理制度化，规范化，不断提高财务管理水平，确保学会资金公开透明使用安全有效。

在新的一年内，继续贯彻落实中央和省委关于新社会组织深入学习实践科学发展观活动的有关精神，以科学发展观为指导，扬长避短，凝心聚力，做学会的有心人，多动脑筋，广纳各地区、各行业及各层次的药理学工作者，积极开展科普及社会服务工作，团结带领全省广大药理工作者，解放思想，开拓创新，坚定信念，扎实工作，为江苏省药理学科率先科学发展、和谐发展作出积极贡献。力争 2011 年学会工作再上新台阶！

总之，在省科协、民政厅、卫生厅、药监局等部门的领导下，在江苏省药理学会理事会及全省药理工作者的共同努力下，我省药理学会的工作将会取得更好更大的成绩！

江苏省药理学会

2010-12-20

1. 南京中医药大学

题目：宫血饮对不完全流产大鼠子宫出血量、子宫组织形态学性激素水平及受体表达的影响

作者：崔　姣　　指导老师：许惠琴

Influence of GONG XUE YIN on Stanching Effect, Gonadal Hormone, Estrogen Receptor and Progesterone Receptor of Endometrium on Incomplete-abortion Induced by Medicine in Early Pregnancy Rats

南京中医药大学 Nanjing University of Chinese Medicine

Postgraduate : Cui Jiao
Tutor : Prof. Xu Huiqin

1 Background

Dysfunctional uterine bleeding (DUB) is defined as abnormal uterine bleeding without a demonstrable organic cause. It is a commonly encountered gynecologic disease. DUB occurs most commonly at the extremes of reproductive age (20% of cases occur in adolescence and 40% in patients over age 40). DUB can be divided into ovulatory and anovulatory. In adolescent and perimenopausal period groups, 90% of DUB is anovulatory.

In TCM theory the most important mechanism for the generation of menstruation is the co- ordinative effect of Kidney-Qi, Tiangui, thoroughfare, conception vessels (Chong
Channel and Ren meridian) and the uterus.

The therapeutic principles used to treat metrorrhagia and metrostasis are: to stop bleeding, determine the cause and deal with the principal aspect and most importantly, restore normal menstruation.
Chinese herbal medicinal compound prescriptions compounds can ameliorate the morphosis of the adenohypophysis and adjust the endocrine function of the adenohypophysis, promote ovarian follicle growth, corpus luteum formation and ovulation .

2 Methods

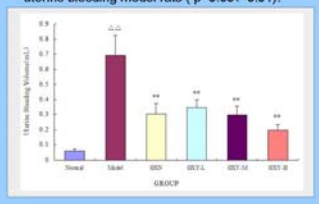
The incomplete-abortion rat model was induced by misoprostol and mifepristone on seven-days pregnant rats.

measurement index

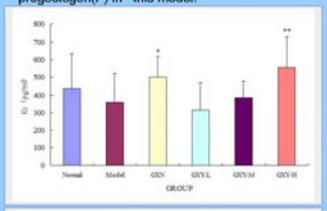
- Uterine Bleeding Volume:
  Using cotton balls to collect uterine bleeding and immerse with NaOH ,then determinate the OD value of the wavelength of 546 nm
- E_2 and P :
  The radio-immunity method was used to examine the serum estradiol (E_2), progestogen (P) .
- ER mRNA and PR mRNA levels
  Polymerase chain reaction was (PCR)used to detect the level of estrogen receptor and progesterone receptor of endometrium.
- pathological examination
  Observed the number of endometrial in lamina propria, the change of stromal vascular and fibrous connective tissue.

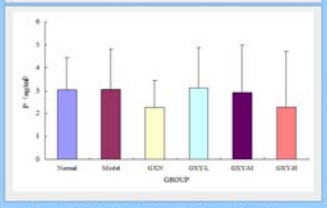
3 Results

1.GONG XUE YIN(11、22、44 g/kg) could markedly reduced uterine bleeding quantity in uterine bleeding model rats (p<0.05、0.01).

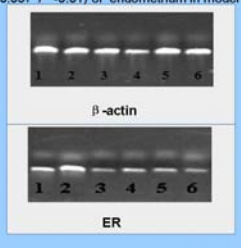


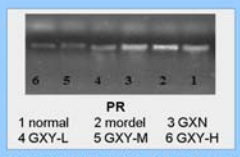
2. Gong XUE YIN (22g/kg) could significantly increase the level of serum estradiol (E_2) in model rats. But it can not regulate the level of progestogen(P) in this model.



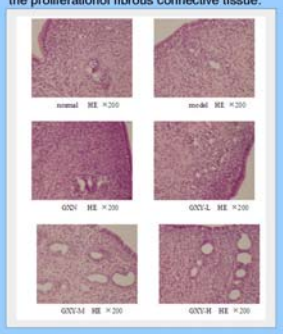


3. Gong XUE YIN (5.5g/kg、11g/kg、22g/kg) could significantly decrease the level of estrogen receptor (ER) and progesterone receptor (PR) (P <0.05、P <0.01) of endometrium in model rats.





4 GONG XUE YIN YIN(22、44 g/kg) could increase the number of endometrial in lamina propria, reduce dilatation and congestion of stromal vascular and inhibit the proliferationof fibrous connective tissue.



4 Conclusion

- It has been shown that GONG XUE YIN could significantly reduce the amount of uterine bleeding. The mechanism may related to contraction of uterine blood vessels, reducing clotting time, promotion the function of platelet aggregation and adhesion.
- It has been shown that GONG XUE YIN could increase the levels of serum estrogen, decreasing the expression of ER and PR, while it has little effect on progesterone. It suggested that the model may be in high level of P and the medicine can not regulate.
- It has been shown that GONG XUE YIN could increase the number of endometrial in lamina propria, reducing dilatation, congestion of stromal vascular and inhibiting the proliferation of fibrous connective tissue.
- **In conclusion, the present study demonstrated that Gong XUE YIN has the effect on reducing uterine bleeding quantity, and regulating both gonadal hormone and estrogen receptor of endometrium in model rats.**

5 References

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Funding Source: key project of medical technology development of nanjing

2. 南京中药大学

题目：隐丹参酮对黑素瘤细胞系 B16F10 的抗癌作用及其机理

作者：黄臣虎　　指导老师：陆　茵

The Anti-cancer Effect and Mechanism of Cryptotanshinone on Melanoma Cell Lines B16F10

Huang Chenhu

College of Pharmacy, Nanjing University of Chinese Medicine

Abstract

Purpose

Cryptotanshinone is a major active component of Salvia miltiorrhiza, which is often used as Chinese herbal medicine in cancer therapy. Here, we systematically assessed the anti-tumor effect of Cryptotanshinone on melanoma cell lines B16F10 in vivo. and in vitro.

Materials and methods

Animals

Female C57BL/6 mice (6–8 weeks old) were purchased from the Slac Animal Inc (Shanghai, China). Throughout the experiments, mice were maintained in plastic cages at 21 ±2° C, on a 12-h light/dark cycle and with free access to food and water.

Cell lines and culture condition

These studies utilized C57BL/6 mice-derived melanoma cell lines, B16F10 (high-lung-metastatic potential), which were kindly provided by Nanjing University. The cells were cultured as a monolayer in DMEM (Gibco, Grand Island, NY, USA), containing 10% v/v fetal bovine serum (Hyclone, Canada).All cells were grown in a humidified atmosphere, containing 5% CO2 at 37° C.

Experimental metastasis model

The suspension of B16F10 cells was injected through the tail vein of a 6- to 8-week-old female C57BL/6 J mice.All mice were killed 23 days after the injection of the tumor cells. The lungs were then removed, weighed and fixed. The metastastic foci on the surfaces of the lung were photographed and counted and the survival of mouse must be recorded.

Spontaneous metastasis model

The suspension of B16F10 cells was injected into the foot pad of a 6- to 8-week-old female C57BL/6 J mice and allowed to growth. The tumor volum of mouse must be measured from 13-23 days after the injection of the tumor cells and the survival of mouse must be recorded.

MTT assay

In this study, 100 mM stock solution of Cryptotanshinone (Xi'an Helin Biological Engineering Co., Ltd. Xi'an, China, purity >95%) was prepared in ethanol, then filtered by 0.2μm membrane and diluted as indicated. Solvent control was also prepared for the treatment of cultures. The growth inhibition effect of Cryptotanshinone on melanoma cells was carried out using the MTT assay.

LDH assay

Cytotoxicity was determined by measuring cell membrane damage through the release of lactate dehydrogenase (LDH). For this experiment, All methods was performed according to the manufacturer's instructions. The LDH kit was purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

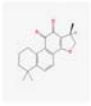
Annexin V assay

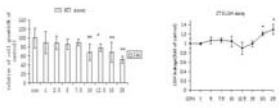
Cells were then treated with Cryptotanshinone (0, 1, 15 and 40 μM) for 24 h.Cell apoptosis was assessed by Annexin V-FITC stainingusing a flow cytometric apoptosis detection kit (Cat.No.556420, BD Biosciences Pharmingen, San Jose, CA).

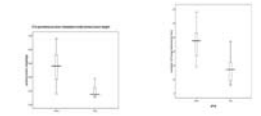
Western blotting assay

To detect the effects on metastatic-associated protein expression in B16F10 cells. Cells in culture were then treated with different concentrations of Cryptotanshinone or media only (control) for 24 h. After that, protein was extracted by RIPA. Protein samples were resolved by SDS–PAGE and transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA). p38,p53, FAK,bcl-2,bax,mmp-2,mmp-9 (1:200, Bioworld Technology, Bioworld, USA), or GAPDH (1:5000, Santa Cruz Biotechnology, Santa Cruz, CA), then followed by incubation with peroxidase-coupled secondary antibodies. A Supersignal kit (Pierce, Rockford, IL) was used to visualize the bands according to the manufacturer's instructions.

Results







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Contact:
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3. 南京中医药大学

题目：活血化瘀中药有效成份抗肿瘤血管生成研究

作者：陶　丽　　指导老师：陆　茵

A Pilot study on compounds with anti-angiogenic activity from Chinese medicine for activating blood circulation and dissolving stasis

Li Tao, Yin Lu *, Shi zhong Zheng, Ai yun Wang, Wen xing Chen
epartment of Clinical Pharmacy, Nanjing University of Chinese Medicir

Introduction

The role of angiogenesis in tumor growth and metastasis is well established. Identification of a small molecule that blocks tumor angiogenesis and is safe and affordable has been a challenge in drug development. Accumulative evidences have confirmed the perspective application of Chinese medicine as nature candidates targeting tumour-associated angiogenesis. By biomedical literature mining and preliminary experimental study, we found a full source active compounds is derived from those activating blood circulation and dissolving Stasis. We further analyzed the feasibility in other special issues of these hemorrheologic agents as tumor angiogenesis inhibitors.

Methods

1. search strategy: Searching the biomedical literature for the past 10 years on anti-angiogenic Chinese medicine.

(1) Topic terms:

#1: "Medicine, Chinese Traditional traditional"[Mesh] OR Chinese medicine OR Chinese herbal drugs OR medicinal plants OR medicinal herbs

#2: "neoplasm"[Mesh] OR cancer OR tumor OR malignancy

#3: "neovascularization, Pathlogic"[Mesh] OR angiogenesis OR vascularization

#4: #1 AND #2 AND #3

(2) Database：MEDLINE, EMBASE and web of science

2. Retrieving informations:

(1) Loading retrieved literatures into EndNote (X4) software, selected components with clear targets or signaling pathways and building list table.

(2) 2D or 3D structure of compounds obtained from Pubchem compound database (URL: http://www.ncbi.nlm.nih.gov/pccompound).

(3) CID number obtained for each compound and clustering by structural similarity.

3. Statistical analysis: Sorting hit compounds for screening traditional Chinese medicine ingredients. Building biological regulatory network of tumor angiogenesis for each preventative hemorrheologic ingredient.

Results

Structure feature of anti-angiogenic Chinese medicine and more than half of them came from hemorheologic agents:

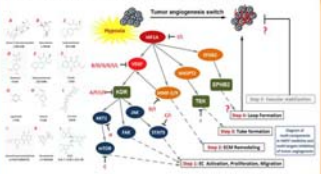
Type	Frequency	Hemorheologic agents
Flavonoids	11/40	Quercetin; Hydroxysafflor yellow A; Baicalein; Luteolin; Genistein.
Phenols	8/40	Danshensu; Gallic acid; EGCG; Paeonol; Curcumin; Tanxic acid.
Terpenes	7/40	Beta-elemene; Acetyl-11-keto-beta-boswellic acid; Norcantharidin; 20(R)- ginsenoside Rg3.
Alkaloids	6/40	Protoberberine; Ligustrazine.
Quinones	5/40	Cryptotanshinone; beta-hydroxysovalerylshikonin; Dihydrotanshinone I; Emodin.
Polysaccharides	2/40	Acidic mucopolysaccharide
Minerals	1/40	---
Total	40	22
percentage		55%

Reports for the past 10 years on anti-angiogenic Chinese medicine:

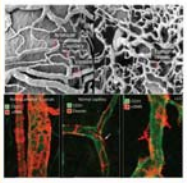
Compounds	Type	Mechanism	First Author	YEAR
Indirubin	Alkaloids	Inhibiting JAK/STAT3 signaling pathway.	Zhang X et al.	2011
Isoxanthohumol	Flavonoids	Inhibiting TGF-beta and JAK/STAT signaling pathway ; Inhibiting IFN-gamma, IL-4 and IL-6 expression.	Serova A et al.	2011
Danshensu	Phenols	Inhibiting VEGF and MMP-2/9 expression.	Zhang LL, Lu Y* et al.	2010
Daurinone	Alkaloids	Inhibiting NF-kappaB signaling pathway;	Yang Z et al.	2010
Isoliquiritigenin	Flavonoids	Inhibiting mTOR and ERK signaling pathway; Activating JNK signaling pathway.	Cabecadas J et al.	2010
Cryptotanshinone	Quinones	Inhibiting AKT/mTOR signaling pathway.	Chen W'etal	2010
WS25	Polysaccharides	Inhibiting BMP/Smad/Id1 signaling pathway.	Qiu H et al.	2010
Celastrol	Terpenes	Inhibiting AKT/mTOR signaling pathway.	Pang X et al.	2010
Scopoletin	Alkaloids	Inhibiting VEGFR2 phosphorylation;	Pan R et al.	2010
Quercetin	Flavonoids	Inhibiting HIF-1alpha, AP-1, VEGF and Pin1 expression.	Oh SJ et al.	2010
Gallic acid	Phenols	Inhibiting PI3K/Akt and Ras/MAPK signaling pathway; Inhibiting ADAM17 expression.	Lu Y et al.	2010
Cucurbitacin E	Terpenes	Inhibiting VEGFR2 mediated JAK2/STAT3 signaling pathway.	Dong Y et al.	2010
EGCG	Phenols	Inhibiting HIF-1 alpha and VEGF expression.	Domingo DS et al.	2010
Beta-elemene	Terpenes	Inhibiting VEGF expression.	Chen W et al.	2010
Acidic mucopolysaccharide	Polysaccharides	Inhibiting VEGF and MMP-2/9 expression.	Zhang WL Lu Y* et al.	2009
Hydroxysafflor yellow A	Flavonoids	Inhibiting VEGF and bFGF expression.	Xi S et al.	2009
Acetyl-11-keto-beta-boswellic acid	Terpenes	Inhibiting VEGFR2 signaling pathway.	Pang X et al.	2009
Morelloflavone	Flavonoids	Inhibiting Rho-GTPases; Inhibiting ERK signaling pathway.	Pang X et al.	2009
Scopolin	Alkaloids	Inhibiting IL-6, VEGF and FGF-2 expression.	Cabecadas J et al.	2009
beta-hydroxysovalerylshikonin	Quinones	Inhibiting VEGFR2 and Tie2 phosphorylation.	Komi Y et al.	2009
Paeonol	Phenols	Inhibiting Akt signaling pathway; Inhibiting MMP-2/9 expression.	Kim SA et al.	2009
Protoberberine	Alkaloids	Inhibiting VEGFA activated ERK1/2 signaling pathway; Inhibiting MMP2 expression.	Gao JL et al.	2009
Norcantharidin	Terpenes	Inhibiting integrin beta1 and vimentin expression; Inhibiting JNK and ERK signaling pathway.	Chen YI et al.	2009
Ligustrazine	Alkaloids	Inhibiting VEGF and MMP-2/9 expression.	Chen L, Lu Y* et al.	2009
Gambogic acid	Flavonoids	Inhibiting VEGFR2 signaling pathway.	Yi T et al.	2008
Baicalein	Flavonoids	Inhibiting HIF-1 alpha and PHD2 expression.	Cho H et al.	2008
Dihydrotanshinone I	Quinones	Inhibiting VEGF expression.	Bian W et al.	2008
Curcumin	Phenols	Inhibiting NF-kappaB and STAT3 signaling pathway.	Lin YG et al.	2007
		Inhibiting HIF-1 alpha expression.	Bae MK et al.	2006
20(R)-ginsenoside Rg3	Terpenes	Inhibiting MMP-2/9 expression.	Yue PY et al.	2006
arsenic trioxide	Minerals	Inhibiting VEGF expression.	Xiao YF et al.	2006
Emodin	Quinones	Inhibiting VEGFR2 signaling pathway.	Kwak HI et al.	2006
ON-III	Flavonoids	Inhibiting VEGFR2 signaling pathway.	Zhu XF et al.	2005
Genistein	Flavonoids	Inhibiting VEGF, PDGF, TF, uPA and MMP-2/9 expression and upregulating PAI-1, endostatin, angiostatin and thrombospondin-1 expression.	Su SJ et al.	2005
Resveratrol	Terpenes	Inhibiting MMP-2 activity.	Cao Y et al.	2005
Shikonin	Quinones	Inhibiting VEGFR2 signaling pathway.	Yong Y et al.	2004
Pseudolaric acid B	Terpenes	Inhibiting HIF-1 alpha expression	Li MH et al.	2004
ursolic	Flavonoids	Promoting ECs' F-actin and beta-tubulin depolymerizing.	Gong YQ et al.	2004
Luteolin	Flavonoids	Inhibiting PI3K/p70 S6K signaling pathway.	Bagli E et al.	2004
Tanxic acid	Phenols	Inhibiting CXCL12/CXCR4 signaling pathway.	Chen X et al.	2003
Honokiol	Phenols	Inhibiting TNF-alpha expression.	Bai X et al.	2003

SCI papers published by our lab on anti-angiogenesis activity of HXHY TCM:

Multi-components in HXHY TCM and multi-targets inhibition tumor angiogenesis:



Schematic diagram of the angiogenic response induced by Ad-VEGF-Δ^{PH} (Modified from Pettersson et al (2000)



Legends: The structure and function of the tumor's vascular network is considered abnormal. The normal vasculature is arranged in a hierarchy of evenly spaced, well-differentiated arteries, arterioles, capillaries, venules and veins; the tumour vasculature is uneven; distributed and chaotic. Tumour vessels often exhibit a serpentine course, branch irregularly and form arterio-venous shunts (Warren, 1979). Blood flow through tumours does not follow a constant, unidirectional path. Not all open vessels are perfused continuously, and, over a few minutes, blood flow may follow different paths and even proceed in alternating directions through the same vessel (Chaplin et al, 1987). Tumour blood vessels are more abundant at the tumour-host interface than in central regions. Also, vascular density tends to decrease as tumours grow, leading to zones of ischaemia and ultimately necrosis as tumours 'outgrow their blood supply' (Peterson, 1991). Finally, tumour blood vessels are structurally

Discussions

Angiogenesis, the growth of new blood vessels from pre-existing vasculature, is a key process in several pathological conditions, and excessive regulation underlies tumor growth and metastasis. Targeting the VEGF pathway with various inhibitors has led to FDA approval of multiple drugs for the treatment of advanced cancer. Such treatments conferred clinical benefits, including increased overall survival. Researchers found that the vasculature of solid tumours is morphologically aberrant and characterized by dilated and fragile vessels, intensive vessel sprouting and loss of hierarchical architecture.

However, there are still many aspects of physiological angiogenesis that are not fully understood, such as the exact mechanisms behind lumen formation and pericyte investment and the maintenance of vascular quiescence, whereas this step can not be completed in tumor context. It will be important to establish whether perturbing lumen formation and keep a stable vascular is an additional anti-angiogenic strategy. Further understanding of these issues should allow us to ask new questions regarding their potential role of Chinese medicine for activating blood circulation

Reference

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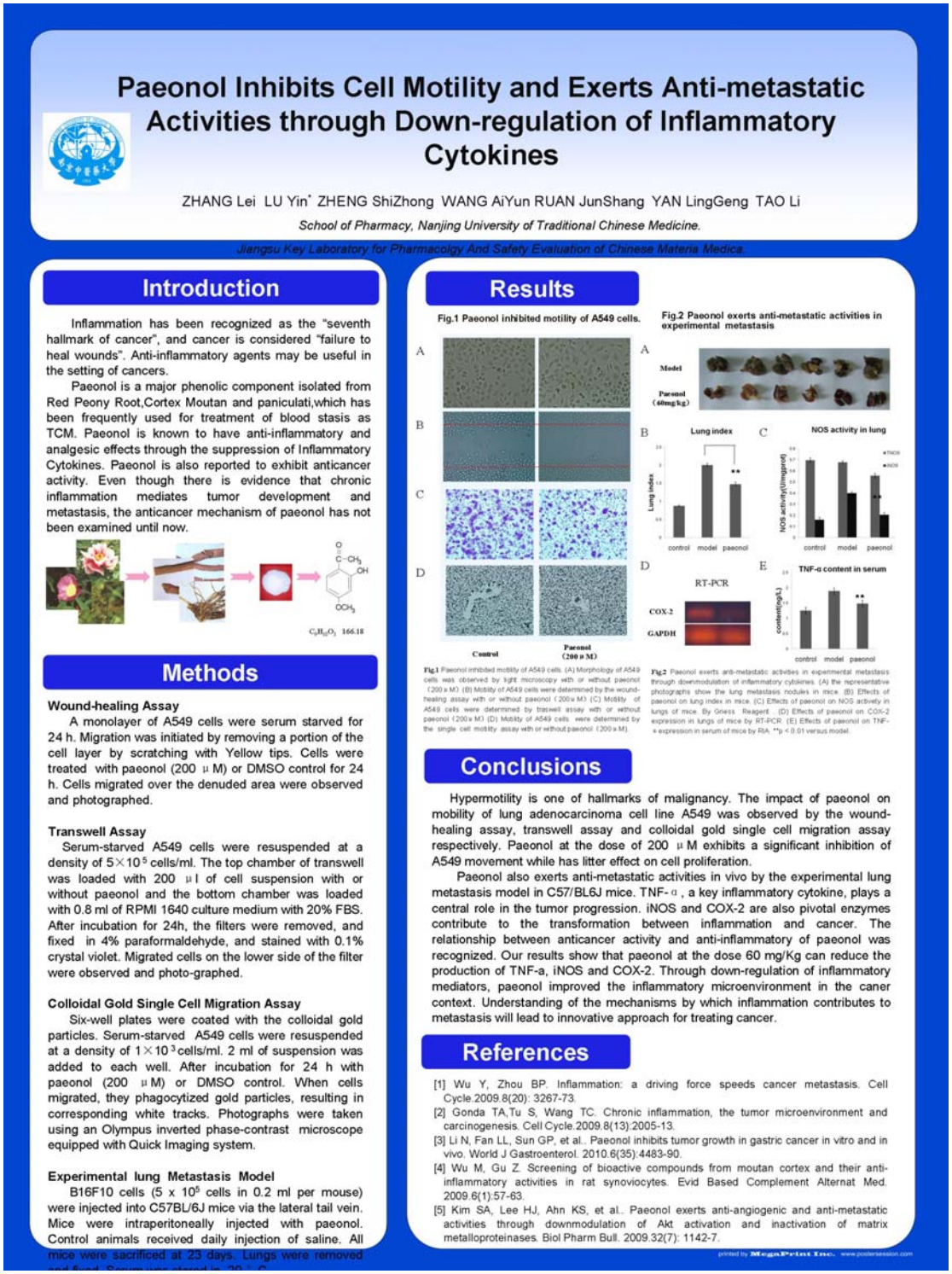
[2] Dai Fukumura, Rakesh K. Jain. Tumor microvasculature and microenvironment: Targets for anti-angiogenesis and normalization [J]. ***Microvascular Research***, 2007, 3 (5): 211-268.

[3] Pries AR, Höpfner M, le Noble F, Dewhirst MW, Secomb TW. The shunt problem: control of functional shunting in normal and tumour vasculature [J]. ***Nat Rev Cancer***, 2010, 10 (8): 587-593.

4. 南京中医药大学

题目：丹皮酚下调肿瘤炎性微环境抑制肿瘤细胞活动性和转移

作者：张　蕾　　指导老师：陆　茵



Paeonol Inhibits Cell Motility and Exerts Anti-metastatic Activities through Down-regulation of Inflammatory Cytokines

ZHANG Lei LU Yin* ZHENG ShiZhong WANG AiYun RUAN JunShang YAN LingGeng TAO Li

School of Pharmacy, Nanjing University of Traditional Chinese Medicine.

Jiangsu Key Laboratory for Pharmacology And Safety Evaluation of Chinese Materia Medica.

Introduction

Inflammation has been recognized as the "seventh hallmark of cancer", and cancer is considered "failure to heal wounds". Anti-inflammatory agents may be useful in the setting of cancers.

Paeonol is a major phenolic component isolated from Red Peony Root,Cortex Moutan and paniculati,which has been frequently used for treatment of blood stasis as TCM. Paeonol is known to have anti-inflammatory and analgesic effects through the suppression of Inflammatory Cytokines. Paeonol is also reported to exhibit anticancer activity. Even though there is evidence that chronic inflammation mediates tumor development and metastasis, the anticancer mechanism of paeonol has not been examined until now.

$C_9H_{10}O_3$ 166.18

Methods

Wound-healing Assay

A monolayer of A549 cells were serum starved for 24 h. Migration was initiated by removing a portion of the cell layer by scratching with Yellow tips. Cells were treated with paeonol (200 μM) or DMSO control for 24 h. Cells migrated over the denuded area were observed and photographed.

Transwell Assay

Serum-starved A549 cells were resuspended at a density of 5×10^5 cells/ml. The top chamber of transwell was loaded with 200 μl of cell suspension with or without paeonol and the bottom chamber was loaded with 0.8 ml of RPMI 1640 culture medium with 20% FBS. After incubation for 24h, the filters were removed, and fixed in 4% paraformaldehyde, and stained with 0.1% crystal violet. Migrated cells on the lower side of the filter were observed and photo-graphed.

Colloidal Gold Single Cell Migration Assay

Six-well plates were coated with the colloidal gold particles. Serum-starved A549 cells were resuspended at a density of 1×10^3 cells/ml. 2 ml of suspension was added to each well. After incubation for 24 h with paeonol (200 μM) or DMSO control. When cells migrated, they phagocytized gold particles, resulting in corresponding white tracks. Photographs were taken using an Olympus inverted phase-contrast microscope equipped with Quick Imaging system.

Experimental lung Metastasis Model

B16F10 cells (5 x 10^5 cells in 0.2 ml per mouse) were injected into C57BL/6J mice via the lateral tail vein. Mice were intraperitoneally injected with paeonol. Control animals received daily injection of saline. All mice were sacrificed at 23 days. Lungs were removed

Results

Fig.1 Paeonol inhibited motility of A549 cells.

Fig.2 Paeonol exerts anti-metastatic activities in experimental metastasis

Fig.1 Paeonol inhibited motility of A549 cells. (A) Morphology of A549 cells was observed by light microscopy with or without paeonol (200 μM). (B) Motility of A549 cells were determined by the wound-healing assay with or without paeonol (200 μM). (C) Motility of A549 cells were determined by transwell assay with or without paeonol (200 μM). (D) Motility of A549 cells were determined by the single cell motility assay with or without paeonol (200 μM).

Fig.2 Paeonol exerts anti-metastatic activities in experimental metastasis through downmodulation of inflammatory cytokines. (A) the representative photographs show the lung metastasis nodules in mice. (B) Effects of paeonol on lung index in mice. (C) Effects of paeonol on NOS activity in lungs of mice. By Griess Reagent. (D) Effects of paeonol on COX-2 expression in lungs of mice by RT-PCR. (E) Effects of paeonol on TNF-α expression in serum of mice by RIA. **p < 0.01 versus model.

Conclusions

Hypermotility is one of hallmarks of malignancy. The impact of paeonol on mobility of lung adenocarcinoma cell line A549 was observed by the wound-healing assay, transwell assay and colloidal gold single cell migration assay respectively. Paeonol at the dose of 200 μM exhibits a significant inhibition of A549 movement while has litter effect on cell proliferation.

Paeonol also exerts anti-metastatic activities in vivo by the experimental lung metastasis model in C57/BL6J mice. TNF-α, a key inflammatory cytokine, plays a central role in the tumor progression. iNOS and COX-2 are also pivotal enzymes contribute to the transformation between inflammation and cancer. The relationship between anticancer activity and anti-inflammatory of paeonol was recognized. Our results show that paeonol at the dose 60 mg/Kg can reduce the production of TNF-a, iNOS and COX-2. Through down-regulation of inflammatory mediators, paeonol improved the inflammatory microenvironment in the caner context. Understanding of the mechanisms by which inflammation contributes to metastasis will lead to innovative approach for treating cancer.

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5. 南京中医药大学

题目：海参粘多糖通过体内外抑制血管生成和肿瘤细胞的迁移发挥抗肿瘤作用

作者：张伟伟　　指导老师：陆　茵

Acidic mucopolysaccharide from holothuria leucospilota has antitumor effect by inhibiting angiogenesis and tumor cell invasion in vivo and in vitro

Weiwei Zhang,Yin Lu, Bo Xu, Jiaming Wu, Lijuan Zhang,Ming Gao, Shizhong Zheng, Aiyun Wang,Changbin Zhang, Lei Chen and Na Lei

INTRODUCTION

The metastatic process involves a series of distinct steps that are required for the metastatic cancer cells to be established in a new environment. Metastatic cancer cells must first be able to survive in the bloodstream, come to lodge in blood vessels in the potential target organ, and finally extravasate through the endothelium into the distant organ. In order to form a metastasis, metastatic cancer cells need to generate new blood vessels from existing blood supply for continued cell growth.

Wide arrays of dietary plants and animals have been reported to possess significant anti-metastasis and anti-angiogenic activities. Holothurian is natural marine resource which has been used as a traditional medicine for a long time in China. It contains a lot of nutrients and is employed as a tonic. HS is a new type of acidic mucopolysaccharide isolated from Holothuria leucospilota (Brandt), a species of Holothurian. HS has been widely used as an anti-throm-botic drug. Its average relative molecular weight was estimated to be 50,000 by high performance liquid chromatography (HPLC).

In this report, we evaluated the anti-metastasis activity of HS. We hypothesize that HS inhibits tumor growth and metastasis by inhibiting tumor angiogenesis or tumor cells invasion. We found that HS inhibited tumor metastasis in both spontaneous and experimental tumor metastasis models. We also examined the effect of HS on angiogenesis, and found that HS reduced VEGF-induced HUVEC proliferation, invasion, migration, tube formation, vessel sprouting in vitro, and vessel formation in a matrigel plug and chick chorioallantoic membrane in vivo. Moreover, HS inhibited the invasive ability of B16F10 cells through the Matrigel-embedded Boyden chamber. HS treatment significantly reduced the expression of MMP-2,-9 and VEGF in B16F10 cells and tumor model. All these results suggest that HS has anti-metastasic effect possibly via its anti-angiogenesis induced by downregulation of VEGF and suppression of invasive ability of cancer cells mediated by downregulation of MMP-2, -9 and their activities.

RESULTS

1. HS inhibits VEGF-induced proliferation of HUVECs and B16F10 cells growth.

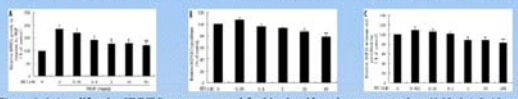

Figure 1. (A) proliferating HUVECs were pretreated for 30 min with various concentrations (0.08, 0.4, 2, 10 or 50 μM) of HS before exposure to VEGF (10 ng/ml). After 30 h, cell growth and viability were measured by MTS assay. Media-treated controls were normalized to 100%. (B and C) HUVECs were incubated for 30 h with various concentrations of HS (0.08, 0.4, 2, 10 or 50 μM) without VEGF treatment. B16F10 cells were seeded in 96-well plates and after an overnight incubation, B16F10 cells were treated with various concentrations (0, 0.001, 0.01, 0.1, 1, 10 or 100 μM) of HS for 24 h.

2.HS inhibits VEGF-induced endothelial cell migration and B16F10 cell invasion.

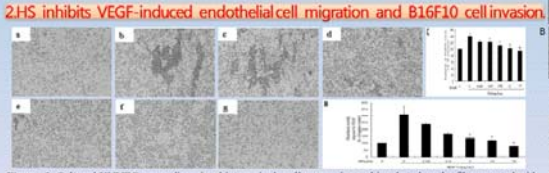

Figure 2. Cultured HUVECs were dissociated into a single cell suspension and incubated on the filters coated with gelatin on the lower surfaces in the absence or present of HS (0.08, 0.4, 2, 10 or 50 μM). The migration was evaluated in response to 10 ng/ml VEGF. B16F10 cells starved overnight in serum-free DMEM containing 0.1% BSA prior to initiation of the assay were dissociated into a single cell suspension and incubated on the filters coated with Matrigel on the upper surfaces in the absence or present of HS for 6h. The invasion was evaluated in response to10% bovine serum. (A) the photographs show the lower chamber of different groups. (a) negative control; (b) VEGF stimulated group; (c) 0.08 μM HS treated with VEGF; (d) 0.4 μM HS treated with VEGF; (e) 2 μM HS treated with VEGF; (f) 10 μM HS treated with VEGF; (g) 50 μM HS treated with VEGF. (B and C) the migrated or invaded cells were counted by an inverted light microscope at 400 magnification. Five random fields were chosen for each membrane. Negative controls were normalized to 100%.

3.HS inhibits endothelial cell morphogenesis.

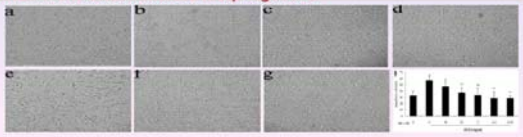

Figure 3. HS inhibits the VEGF-induced differentiation of endothelial cells. The inhibitory effect of HS on capillary tube formation was assessed by exposure to HS of HUVECs on fibrin gel in present of 10 ng/ml VEGF. After 24 h, changes of cell morphology were captured with an inverted microscope at a x100 magnification. (A) representative endothelial tubes were shown. (a) negative control; (b) VEGF stimulated group; (c) 0.08 μM HS treated with VEGF; (d) 0.4 μM HS treated with VEGF; (e) 2 μM HS treated with VEGF; (f) 10 μM HS treated with VEGF; (g) 50 μM HS treated with VEGF. (B) tubes forming intact networks were counted under a microscope at a 100 magnification. Five random fields were chosen for each well.

4.HS inhibits angiogenesis in vivo.

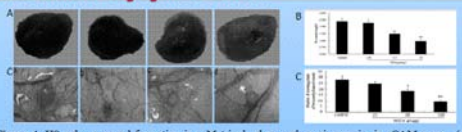

Figure 4. HS reduces vessel formation in a Matrigel plug and angiogenesis in CAM assay. (A and B) C57BL/6 mice injected with Matrigel plugs containing b-FGF were treated intraperitoneally with HS dissolved in saline at doses of 1.04, 5.2 or 26 mg/kg according to the weight of the mice, the control group received saline. After 7 days, mice were sacrificed, and neovascularization and hemoglobin content of Matrigel pellets were evaluated. (A) the photographs show macroscopic analysis of Matrigel. (B) hemoglobin content of Matrigel plugs. Eight mice were used as a group.(C) HS or saline were loaded on gelatin sponges which were applied to the chick chorioallantoic membranes of chick embryos 10-day-old. The zones of neo-vascularization under and around the loaded sponge were photographed with a Cannon digital camera after 3 days, and the number of crossing points of two formed vessels was counted. The representative photographs show the angiogenesis in CAM of different groups. (a) saline control, (b) CAM treated with HS 25 μg/egg, (c) CAM treated with HS 50 μg/egg, (d) CAM treated with HS 100 μg/egg.

5. HS inhibits spontaneous and experimental melanoma lung metastasis but does not inhibits primary tumor growth in mice bearing B16F10 cells.

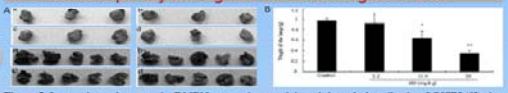

Figure 5. In experimental metastasis, B16F10 suspension was injected through the tail vein of C57BL/6J mice. HS, dissolved in physiological saline, were given by intraperitoneal injection at dose of 5.2,11.6 or 26 mg/kg and the control group received saline only. After 23 days, the weigh of lung were measured to show effects of HS on tumor spontaneous metastasis. (A) the representative photographs show the lung metastasis nodules in experimental metastasis model. (a) saline control, (b) HS 5.2 mg/kg, (c) HS 11.6 mg/kg,(d) HS 26 mg/kg. (B) effects of HS on lung weigh in experimental metastasis model.

6.HS inhibits protein expression ofVEGF and CD34 in local tumor anddownregulates VEGF and MMP-2, -9 expression in B16F10 cells.

Figure 6.HS inhibits expression of VEGF and CD^{34}in local tumor and neovascularization and downregulates MMP-2, 9 and VEGF expression in B16F10 cells. (A and B) the footpads bearing the local tumors were embedded in paraffin and stained immunohistochemically using the labeled(strept)avidin-biotin method. Representative pictures of VEGF and CD^{34}-positive immunohistochemical staining of tumor cross-sections in the control and HS-treated mice were taken by a light microscope. (A) immunohistochemical analysis of VEGF protein expression in tumor tissues; (B)immunohistochemical analysis of CD^{34} protein expression in tumor tissues. (a) saline control, (b) HS 5.2 mg/kg, (c) HS 11.6 mg/kg, (d) HS 26 mg/kg. (C) Western blots shown the expression of VEGF, MMP-2 and MMP-9 in exponentially growing B16F10 cells.

DISCUSSION

This is the first study to demonstrate that the nature acidic mucopolysaccharide isolated from Holothuria leucospilota (Brandt), HS. HS has anti-metastasic properties possibly via its anti-angiogenesis induced by downregulation of VEGF and suppression of invasive ability of cancer cells mediated by downregulation of MMP-2, 9. Taken together, our findings suggest that HS would be beneficial in inhibiting tumor metastasis and angiogenesis. However, the other mechanisms underlying its antitumor activities need further detailed study.Furthermore, HS has a rela- tively low toxicity, which means that HS may have a potential to develop pharmaceutical medicine for treatment of cancer.

6. 南京中医药大学

题目：海参粘多糖在凝血系统中对肿瘤细胞恶性生物学行为的作用

作者：赵　杨　　指导老师：陆　茵

The effects of Holothurian glycosaminoglycan on tumor malignant biological behavior mediated by coagulation system

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Abstract

Blood coagulation disorders often associate with cancer, even in its early stages. Tissue factor, the initiator of the extrinsic coagulation pathway ,is expressed on the surface of many kinds of tumor cells that participates in many tumour-related processes that contribute to malignant tumor progression. In this study, holothurian glycosaminoglycan(hGAG) can decrease the expression of TF that control the tumor induced thrombosis and interference with blood coagulation of hGAG exerts effects on inhibiting tumor metastasis.

Introduction

It has been well accepted that coagulation and tumor progression form a vicious circle, in which hypercoagulability facilitates the aggressive biology of cancer and vice versa. TF)is known to be the initiator of the extrinsic coagulation cascade that belongs to the class II cytokine receptor superfamily. TF expressing tumor cells initiate the coagulation pathways within the tumor microenvironment and shedding of procoagulant activity is a key factor for the disseminated coagulation activation frequently detected in advanced cancer. In this study, We found that holothurian glycosaminoglycan(hGAG) could decrease the tumor induced thrombosis which was due to affect the expression of TF, furthermore, hGAG had the inhibitory effects on tumor metastasis through reversing the abnormal coagulation state.

Method

1 Cell culture: B16F10 Cells were grown in DMEM supplemented with 10% fetal calf serum and antibiotics.

2 Real time PCR: Total RNA was extracted from cells using TRIzol Reagent. Real time PCR was performed using the SYBR Green JumpStart kit.

3 western blot: Cell extracts and tissues were prepared using a lysis buffer. All blots were normalized to GAPDH expression.

4 Elisa: TF、TNF-a、uPA and PAI were measured with Elisa kit.

5 Experimental metastasis model

6 HE staining and immunohistochemistry

7 plasmid construction and transfection

Results

1. hGAG inhibits TF mRNA expression

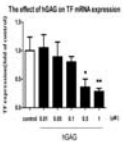


Fig.1 hGAG inhibits TF mRNA expression, Real time PCR demonstrates that hGAG decreases TF mRNA expression in the dose-dependent manner. Values are normalized to β-actin expression. (*P<0.05 and **P<0.01)

2. hGAG inhibits TF protein expression

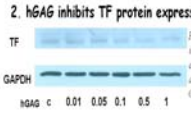


Fig.2 hGAG inhibits TF protein expression. All blots are normalized to GAPDH expression.

3. Immunofluorescence for TF

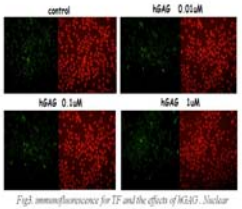


Fig3. immunofluorescence for TF and the effects of hGAG . Nuclear was stained with Topro3 and TF was labeled with 10H10.

4. TF signaling pathway and the effects of hGAG

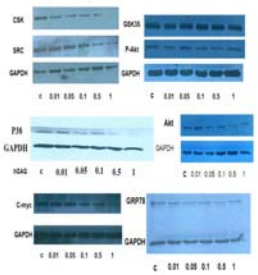


Fig4 The western blot for the downstream factor of the TF. hGAG can decrease the protein of P38 MARK, which may be the signaling pathway that affects the expression of TF. This result requires more evidence.

5. Experimental metastasis model

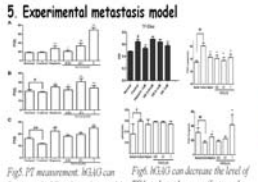


Fig5. PT measurement. hGAG can Decrease the PT in three phases of the Experimental metastasis model.

Fig6. hGAG can decrease the level of TF but almost have no effects on the uPA、PAI、TNF-a in the plasma

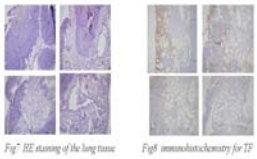


Fig7 HE staining of the lung tissue

Fig8 immunohistochemistry for TF

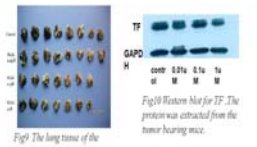


Fig9 The lung tissue of the experimental metastasis model. Lung nodules are counted

Fig10 Western blot for TF .The protein was extracted from the tumor bearing mice.

6. TF promoter assay

Fig11 TF promoter plasmid has been constructed and Luciferase Will be performed the next step.

Conclusion

1 hGAG can decrease the TF mRNA and protein expression in the dose dependent manner.

2 hGAG exerts effects of reducing TF expression may be attributed to the P38-MARK signaling pathway.

3 hGAG can reduce the PT significantly in the experimental model and decrease the secretion of TF from tumor cell but has no effects on the secretion of TNF-aand uPA.

4 hGAG can dramatically reduce the metastasis rate of experimental metastasis model ,which related to the TF expression.

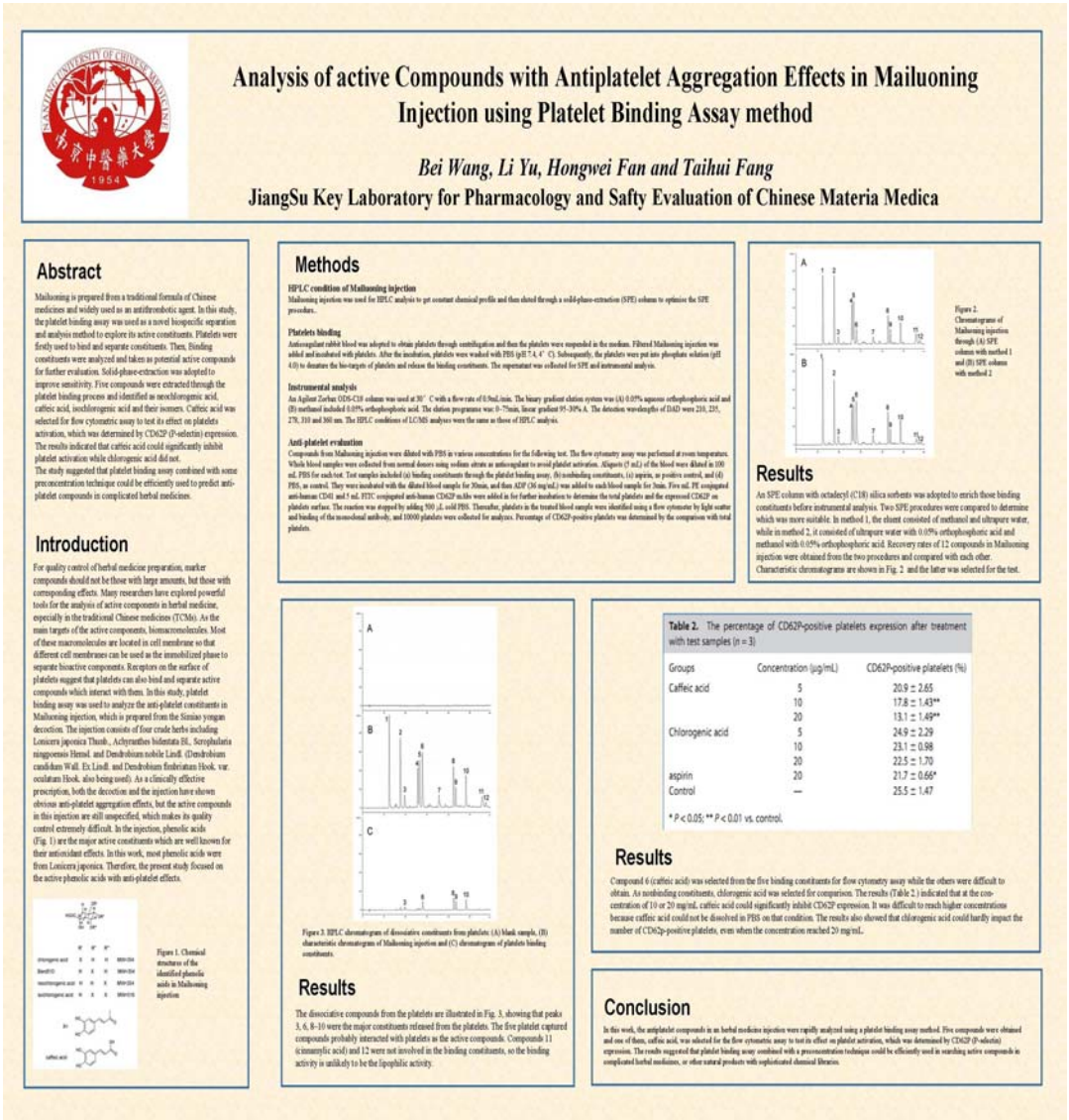
Acknowledgements

Financial support from National Nature Science Foundation of China (Project NO30371727) and Nature Science Foundation of Jiangsu Province (Project No BK2003113).

7. 南京中医药大学

题目：血小板结合实验方法用于分析脉络宁注射液抗血小板聚集作用的有效成分

作者：王　蓓　　指导老师：方泰惠



Analysis of active Compounds with Antiplatelet Aggregation Effects in Mailuoning Injection using Platelet Binding Assay method

Bei Wang, Li Yu, Hongwei Fan and Taihui Fang

JiangSu Key Laboratory for Pharmacology and Safty Evaluation of Chinese Materia Medica

Abstract

Mailuoning is prepared from a traditional formula of Chinese medicines and widely used as an antithrombotic agent. In this study, the platelet binding assay was used as a novel biospecific separation and analysis method to explore its active constituents. Platelets were firstly used to bind and separate constituents. Then, Binding constituents were analyzed and taken as potential active compounds for further evaluation. Solid-phase-extraction was adopted to improve sensitivity. Five compounds were extracted through the platelet binding process and identified as neochlorogenic acid, caffeic acid, isochlorogenic acid and their isomers. Caffeic acid was selected for flow cytometric assay to test its effect on platelets activation, which was determined by CD62P (P-selectin) expression. The results indicated that caffeic acid could significantly inhibit platelet activation while chlorogenic acid did not.

The study suggested that platelet binding assay combined with some preconcentration technique could be efficiently used to predict anti-platelet compounds in complicated herbal medicines.

Introduction

For quality control of herbal medicine preparation, marker compounds should not be those with large amounts, but those with corresponding effects. Many researchers have explored powerful tools for the analysis of active components in herbal medicine, especially in the traditional Chinese medicines (TCMs). As the main targets of the active components, biomacromolecules. Most of these macromolecules are located in cell membrane so that different cell membranes can be used as the immobilized phase to separate bioactive components. Receptors on the surface of platelets suggest that platelets can also bind and separate active compounds which interact with them. In this study, platelet binding assay was used to analyze the anti-platelet constituents in Mailuoning injection, which is prepared from the Similao yongan decoction. The injection consists of four crude herbs including Lonicera japonica Thunb., Achyranthes bidentata Bl., Scrophularia ningpoensis Hemsl. and Dendrobium nobile Lindl. (Dendrobium candidum Wall. Ex Lindl. and Dendrobium fimbriatum Hook. var. oculatum Hook. also being used). As a clinically effective prescription, both the decoction and the injection have shown obvious anti-platelet aggregation effects, but the active compounds in this injection are still unspecified, which makes its quality control extremely difficult. In the injection, phenolic acids (Fig. 1) are the major active constituents which are well known for their antioxidant effects. In this work, most phenolic acids were from Lonicera japonica. Therefore, the present study focused on the active phenolic acids with anti-platelet effects.

Figure 1. Chemical structures of the identified phenolic acids in Mailuoning injection

Methods

HPLC condition of Mailuoning injection

Mailuoning injection was used for HPLC analysis to get constant chemical profile and then eluted through a solid-phase-extraction (SPE) column to optimize the SPE procedure..

Platelets binding

Anticoagulant rabbit blood was adopted to obtain platelets through centrifugation and then the platelets were resuspended in the medium. Filtered Mailuoning injection was added and incubated with platelets. After the incubation, platelets were washed with PBS (pH 7.4, 4° C). Subsequently, the platelets were put into phosphate solution (pH 4.0) to denature the bio-targets of platelets and release the binding constituents. The supernatant was collected for SPE and instrumental analysis.

Instrumental analysis

An Agilent Zorbax ODS-C18 column was used at 30° C with a flow rate of 0.8mL/min. The binary gradient elution system was (A) 0.05% aqueous orthophosphoric acid and (B) methanol included 0.05% orthophosphoric acid. The elution programme was: 0-75min, linear gradient 95-30% A. The detection wavelengths of DAD were 210, 235, 278, 310 and 360 nm. The HPLC conditions of LC/MS analyses were the same as those of HPLC analysis.

Anti-platelet evaluation

Compounds from Mailuoning injection were diluted with PBS in various concentrations for the following test. The flow cytometry assay was performed at room temperature. Whole blood samples were collected from normal donors using sodium citrate as anticoagulant to avoid platelet activation. Aliquots (5 mL) of the blood were diluted in 100 mL PBS for each test. Test samples included (a) binding constituents through the platelet binding assay, (b) nonbinding constituents, (c) aspirin, as positive control, and (d) PBS, as control. They were incubated with the diluted blood sample for 30min, and then ADP (30 mg/mL) was added to each blood sample for 2min. Five mL PE conjugated anti-human CD41 and 5 mL FITC conjugated anti-human CD62P-nAbs were added in for further incubation to determine the total platelets and the expressed CD62P on platelets surface. The reaction was stopped by adding 500 μL cold PBS. Thereafter, platelets in the treated blood sample were identified using a flow cytometer by light scatter and binding of the monoclonal antibody, and 10000 platelets were collected for analyses. Percentage of CD62P-positive platelets was determined by the comparison with total platelets.

Figure 2. Chromatograms of Mailuoning injection through (A) SPE column with method 1 and (B) SPE column with method 2

Results

An SPE column with octadecyl (C18) silica sorbents was adopted to enrich those binding constituents before instrumental analysis. Two SPE procedures were compared to determine which was more suitable. In method 1, the eluent consisted of methanol and ultrapure water, while in method 2, it consisted of ultrapure water with 0.05% orthophosphoric acid and methanol with 0.05% orthophosphoric acid. Recovery rates of 12 compounds in Mailuoning injection were obtained from the two procedures and compared with each other. Characteristic chromatograms are shown in Fig. 2 and the latter was selected for the test.

Figure 3. HPLC chromatogram of dissociative constituents from platelets: (A) blank sample, (B) characteristic chromatogram of Mailuoning injection and (C) chromatogram of platelets binding constituents.

Results

The dissociative compounds from the platelets are illustrated in Fig. 3, showing that peaks 3, 6, 8–10 were the major constituents released from the platelets. The five platelet captured compounds probably interacted with platelets as the active compounds. Compounds 11 (cinnamylic acid) and 12 were not involved in the binding constituents, so the binding activity is unlikely to be the lipophilic activity.

Table 2. The percentage of CD62P-positive platelets expression after treatment with test samples (n = 3)

Groups	Concentration (μg/mL)	CD62P-positive platelets (%)
Caffeic acid	5	20.9 ± 2.65
	10	17.8 ± 1.43**
	20	13.1 ± 1.49**
Chlorogenic acid	5	24.9 ± 2.29
	10	23.1 ± 0.98
	20	22.5 ± 1.70
aspirin	20	21.7 ± 0.66*
Control	—	25.5 ± 1.47

* $P < 0.05$; ** $P < 0.01$ vs. control.

Results

Compound 6 (caffeic acid) was selected from the five binding constituents for flow cytometry assay while the others were difficult to obtain. As nonbinding constituents, chlorogenic acid was selected for comparison. The results (Table 2.) indicated that at the concentration of 10 or 20 mg/mL, caffeic acid could significantly inhibit CD62P expression. It was difficult to reach higher concentrations because caffeic acid could not be dissolved in PBS on that condition. The results also showed that chlorogenic acid could hardly impact the number of CD62p-positive platelets, even when the concentration reached 20 mg/mL.

Conclusion

In this work, the antiplatelet compounds in an herbal medicine injection were rapidly analyzed using a platelet binding assay method. Five compounds were obtained and one of them, caffeic acid, was selected for the flow cytometric assay to test its effect on platelet activation, which was determined by CD62P (P-selectin) expression. The results suggested that platelet binding assay combined with a preconcentration technique could be efficiently used in searching active compounds in complicated herbal medicines, or other natural products with sophisticated chemical libraries.

8. 南京中医药大学

题目：白芷熏前与熏后药效与毒性比较

作者：陈　晨　　指导老师：方泰惠

The Comparision of Efficacy and Toxicity of Sulfur-Fumigated and Unfumigatd of Radix Angelicae Formosanae

ChenChen　Bohua Xu　LiYu　Taihui Fang

NanJing University of Chinese Medicine
JiangSu Key Laboratory for Pharmacology and Safty Evaluation of Chinese Materia Medica

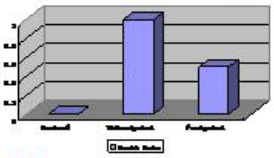
Objective

To compare the efficacy and toxicity of sulfur-fumigated and unfumigatd of radix angelicae formosanae to promote its application

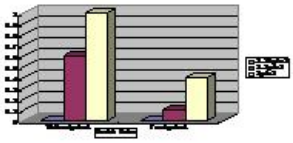
Methods

- **Acute Toxicity Experiment**
- **Efficacy Experiment**
- **Long-Term Toxicity Tests**

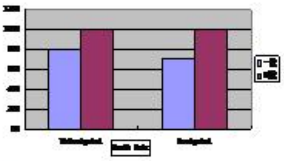
A Observation of the toxicity by the same dosage



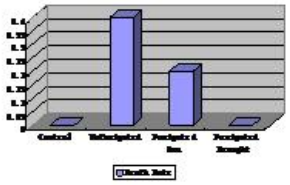
B Observation of the toxicity by the different dosages



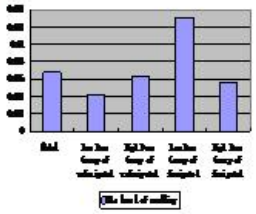
C Observation of the toxicity by the different times



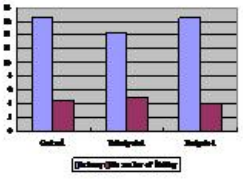
D The toxicity of radix angelicae formosanae from various ways



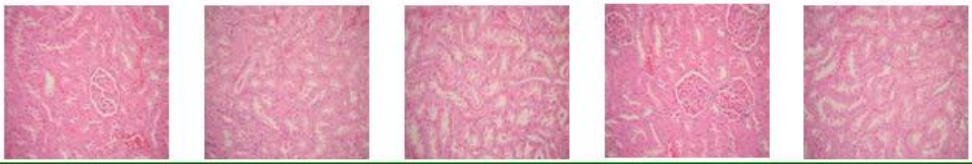
E Anti-inflammatory test



F Analgesia test



G Long-term toxicity tests for three months (kindey)



Results

The efficacy and toxicity of sulfur-fumigated Radix Angelicae Formosanae are declined. The toxicity of sulfur-fumigated herb to kindey is irreversible, while unfumigated is reversible.The cause might be the reduce of all chemical composition in the herb.

9. 南京中医药大学

题目：隐丹参酮通过激活 p38/JNK 并抑制 Erk1/2 诱导肿瘤细胞凋亡

作者：陈文星　　指导老师：陆　茵

LSU Health Sciences Center

Cryptotanshinone activates p38/JNK and inhibits Erk1/2 leading to apoptosis in cancer cells

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[1]Department of Biochemistry and Molecular Biology, [2]Feist-Weiller Cancer Center, Louisiana State University Health Sciences Center, Shreveport, LA 71130-3932

ABSTRACT

Cryptotanshinone (CPT), a natural compound isolated from the plant Salvia miltiorrhiza Bunge, is a potential anticancer agent. Our recent studies indicate that CPT induces apoptosis of cancer cells. This study was set to identify the underlying mechanism. We found that CPT induced apoptosis of human cancer cells (Rh30, DU145, and MCF-7). Concurrently, CPT increased phosphorylation of p38 MAPK and JNK, but inhibited phosphorylation of Erk1/2. Inhibition of p38 MAPK with SB202190 or JNK with SP600125 partially prevented CPT-induced apoptosis. Similarly, downregulation of p38 or c-Jun also in part attenuated CPT-induced cell death. In contrast, overexpression of constitutively active MEK1 conferred resistance to CPT inhibition of Erk1/2 phosphorylation and induction of cell death. Furthermore, we found that all of these were associated with CPT induction of reactive oxygen species (ROS). This is supported by the following findings: (i) CPT induced ROS in a concentration dependent manner. (ii) CPT induction of ROS was inhibited by N-acetyl-L-cysteine (NAC), a ROS scavenger. (iii) NAC blocked CPT activation of p38/JNK, inhibition of Erk1/2, and induction of apoptosis. The results suggest that CPT induced apoptosis of cancer cells through induction of ROS, resulting in activation of p38/JNK pathways and inhibition of Erk1/2 pathway.

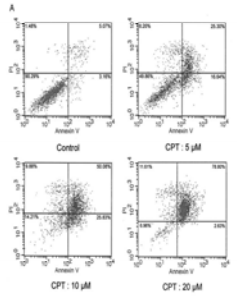


Fig.1 CPT induces cell apoptosis in cancer cells. DU145 (A) and Rh30 (data not shown) cells dispersed in complete medium were seeded in 6-well plates (1×10⁵ cells/well) and treated with different concentration of cryptotanshinone (0-40 μmol/L) for 24 hrs, followed by cell apoptosis analysis, cells were stained by Annexin V-FITC and Propidium Iodide and then analysized by Flowcytometer.

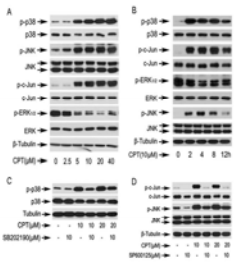


Fig.2 CPT increases phosphorylation of JNK/p38 , but inhibits phosphorylation of ERK1/2. DU145 cells were seeded in 6-well plates and treated with cryptotanshinone(0-40 μM) for 8 hrs (A), or with 10 uM for an indicated time (B), then the cellular lysates were subjected to western blot analysis. (C) DU145 cells were treated with cryptotanshinone (10 or 20 μM) followed by pretreatment with or without SP600125 (10 μM), then collect cell lysates for western blot analysis for c-Jun, JNK; (D) DU145 cells pretreated with or without SB202190 (10 μM), were treated with cryptotanshinone (10 or 20 μM) following western blot analysis for p38.

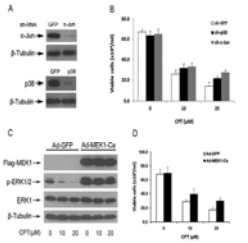
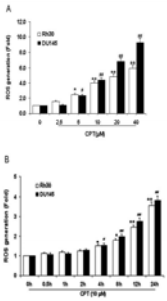


Fig.3 Silence the p38 and c-Jun and overexpress the MEK1 in cancer cells attenuate the inhibition of CPT. Lentivirus to downregulate the express of p38 and c-Jun and adenovirus to overexpress the MEK1 were constructed and transfected into Rh30 cells. (A) Rh30 cells express p38 less after transfection with sh-RNA-p38 from the analysis of western blot. Results of cell counting show that compared with sh-GFP control, sh-RNA-p38 Rh30 cells show more resistant to cryptotanshinone. (B) The western blot analysis shows that expression of c-Jun has been downregulated significantly. Cell viability analysis illustrates that downregulating c-Jun could prevent cell from death in some contents. (C) Flag-tagged MEK1 results in overexpression of phosphorylation of ERK1/2 in Rh30 cells and inhibition of phosphorylation of ERK1/2 and cell viability induced by cryptotanshinone is blocked. Results are presented as mean ± SE, n = 3. *P < 0.05, **P < 0.01, difference vs. control (0 μM CPT).



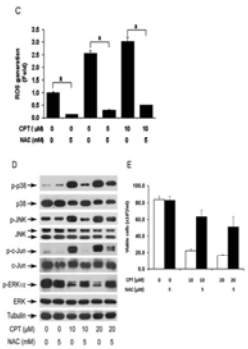


Fig.4 CPT induces ROS generation by a dose- and time-dependent way, and NAC can reverses CPT-induced phosphorylation of p38 and JNK and dephosphorylation of ERK1/2. DU145 and Rh30 cells in 96-well plates treated with CPT in a dose-dependent manner (A) for 24 hrs or with 10 μM CPT in a time-dependent manner (B) were detected for ROS generation using CM-H2DCFDA reagent kit. (C) DU145 cells seeded in 96-well plates, were pretreated with or without NAC (5 μM) for 30 mins and then treated with CPT (10μM and 20μM) for 24 hrs, finally followed by detecting ROS by the same way as above. DU145 cells seeded in 6-well plates were pretreated with or without NAC (5 μM) for 30 mins and treated with CPT (10μM and 20μM) for 24hrs, cell lysates were subjected to western blot analysis against indicated antibodies (D), or sequential cell counting (E) using Beckman Coulter Z1 particle counter by Olympus inverted phase-contrast microscope equipped with Quick Imaging system were carried out. Results are presented as mean ± SE, n = 6. *, # P < 0.05, **, ## P < 0.01, difference vs. control (0 μM CPT).

Conclusion

1. CPT induces cancer cell apoptosis.
2. CPT induces ROS generation.
3. CPT activates JNK/p38 MAPK pathway, but inhibits ERK1/2 pathway.
4. CPT induces cancer cell apoptosis through activating ROS-mediated JNK/p38 MAPK pathway, but inhibiting ROS-mediated ERK1/2 pathway..

10. 南京中医药大学

题目：大蒜二烯丙基三硫化物抑制血小板激活诱导的人乳腺癌细胞的粘附转移侵袭

作者：王颖钰　　指导老师：陆　茵

The Role of Diallyl Trisulfide as an Metastasis Suppressor in actived-platelets induced Human Breast Cancer Cells adhesion, migration and invasion

Ying-yu Wang, Yin Lu* , Shi-zhong Zheng, Wen-hui Qian
(College of Pharmacy, Nanjing University of Chinese Medicine)

南京中医药大学 Nanjing University of Chinese Medicine

INTRODUCTION

Breast cancer is the most prevalent cancer in the world and metastasis is the major cause of mortality in Breast cancer patients. Therefore, the search for new therapeutic targets and the development of inhibitors of tumour cell resettlement and metastatic growth is an ongoing challenge.

The formation of distant hematogeneous metastases requires that the blood-borne tumor cells implant within the microcirculation, usually by adhesion to endothelial cells in a blood vessel and to the underlying basal lamina matrix. Subsequently, the tumor cells must penetrate the cell and matrix layers of the blood vessel to reach the extra vascular tissues where they can survive and proliferate to form metastatic colonies. so inhibition the activation of platelets have an indirectly Significance to suppress tumor metastasis.

The identification of new drugs from plants has a long and successful history, and chinese medicines for promoting blood circulation have been widely used in inhibit tumor metastasis for thousands of years. Diallyl Trisulfide(DATS) is the major fat-soluble component of traditional blood circulation drugs garlic. DATS is a natural substance, which has a dual activity of anti-clotting and anti-metastasis. This brings us a question whether the anti-tumor metastasis activity of DATS is based on its inhibitory effect on platelet activation?

In this report, we investigated the in vitro effects of DATS on active-platelets induced tumor metastasis. Our data indicated that DATS can suppresses breast cancer cells adhesion, migration and invasion via inhibiting the activation of platelets.

RESULTS

1.Inhibitory effect of DATS on MDA-MB-231 cell growth.

Cell prolife

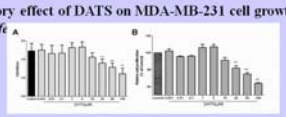


Fig. 1 Effects of DATS on tumor cell proliferation.
Human breast tumor MDA-MB-231cells were treated with various concentrations(0.001~100μM) of DATS for 24 h. cell growth and viability were measured by MTT assay incubated with DATS at various concentration range from 0.001~100μM for 24h.The proliferation in a 24-hour period is shown. The results were measured by the MTT assay. The reading of control was normalized to 100%, and readings from DATS-treated cells were expressed as %of control. Error bars represent the SEM. **, $P < 0.01$, compared with the control. (A) OD_{490nm} of MTT assay of MDA-MB-231 cells treated with various concentration of DATS;(B)The realitive cell proliferation of DATS treated cell of control(%).

2.Inhibitory effect of DATS on platelets aggregation and activation.

Platelet ag

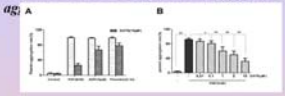


Fig. 2.1 Effect of DATS on platelet aggregation.
Washed platelets were incubated for 10 min withDATS at 10μM, platelet aggregation was induced byPAF(5nM),ADP(10μM)andThrombin(0.1U/ml) measured with an aggregometer device. (A)Platelets incubated with 10μM DATS can neutralization the platelet aggregate effect of PAF(5nM)strongly. (B)We used PAF to investigate the inhibiton effects of DATS at various concentration(0.01~10μM) on platelet aggregation. Displayed is the mean of aggregation for independently performed experiments, each done in triplicate. *, $P < 0.05$and **, $P < 0.01$, compared with the control.

RIA-radioimmunoassay.

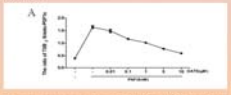


Fig. 2.2 Effect of various concentration of DATS on RIA production of TXB_2 /6-keto-PGF1α of PAF-actived platelets.
Washed platelets were incubated for 30 min with different concentrations of DATS. platelet aggregation was induced by PAF(5nM) ; nse Radioimmunoassay kit to test TXB_2 and 6-keto-PGF1α productions (A)The ratio of TXB2/6-keto-PGF1α productions. Displayed is the mean of aggregation for independently performed experiments, each done in triplicate. *, $P < 0.05$and **, $P < 0.01$, compared with the control.

3. Inhibitory effect of DATS on actived-platelets induced MDA-MB-231 cell mobility.

Wound-healing mobility assay.

Fig. 3. Effect of DATS on MDA-MB-231 cell motility in vitro. Monolayers of MDA-MB-231cells were incubated with PAF-actived platelets for 1h at 37℃ and scraped the cells and treated with DATS (0~10μM),the cells in the denuded zone was photographed after indicated times (0~18 h). Black lines indicate the wound edge.

4. Inhibitory effect of DATS on actived-platelets induced MDA-MB-231 cell migration and invasion.

Boyden chamber migration assay.

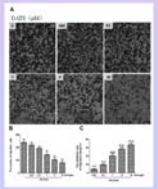


Fig. 4.1 Effect of DATS on PAF-actived platelets induced MDA-MB-231 cell migration in vitro.
2×10^5 MDA-MB-231 cells cultured in the upper well. DATS of indicated concentrations were put in the upper wells and add 10%FBS medium in lower wells containg 3×10^7 PAF-actived platelets.Observa the inhibit impact of DATS on cell migration by blocking platelet aggregation. migration of the cells were determined by measuring the ability to pass through the filters. (A)Migrated cells under the membrane after 6 hours on invert microscope(x200);(B)The number of migration cells;(C)The inhibition rate of migration assay each done in triplicate. *, $P < 0.05$and **, $P < 0.01$, compared with the control.

Collagen invasion assay.

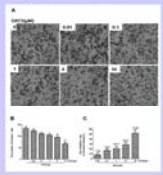


Fig. 4.2 Effect of DATS on PAF-actived platelets induced MDA-MB-231 cell invasion in vitro.
2×10^5 MDA-MB-231 cells cultured in the upper well. DATS of indicated concentrations were put in the upper wells and add 10%FBS medium in lower wells containg 3×10^7 PAF-actived platelets.Observa the inhibit impact of DATS on cell invasion by blocking platelet aggregation. invasion of the cells were determined by measuring the ability to pass through the filters. (A)Invasion cells under the membrane after 24 hours on invert microscope(x200);(B)The number of invasion cells;(C)The inhibition rate of invasion assay each done in triplicate. *, $P < 0.05$and **, $P < 0.01$, compared with the control.

5. DATS regulates the protein expression levels involved with actived-platelets induced metastasis in MDA-MB-231 cells

Western blotting assay.

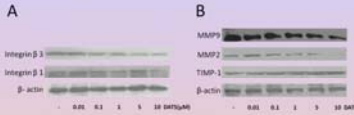


Fig. 5 DATS down-regulates protein expression of Integrinβ1, Integrinβ3,MMP-2 ,MMP-9 and up-regulate Expression of TIMP-1 in MDA-MB-231 breast cancer cell. Western blot showed the expression of MMP-2 and MMP-9 in exponentially growing MDA-MB-231cells. Specific bands are indicated by the arrows to the left and the other bands are nonspecific staining. DATS dose-dependently inhibited MMP-2 ,MMP-9 expression and promoted TIMP-1 expression of MDA-MB-231cells.

6. DATS regulates the mRNA expression levels involved with actived-platelets induced metastasis in MDA-MB-231 cells

RT-PCR and Quantitative real-time PCR assay.

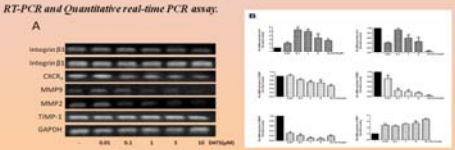


Fig. 6 DATS down-regulates protein expression of human CXCR4,MMP-2 ,MMP-9 and up-regulate Expression of TIMP-1 in MDA-MB-231 breast cancer cell. Expression of Integrinβ3 were also down-regulated by DATS, but DATS had no significant effect on the expression of Integrin β1.

11. 南京中医药大学

题目：川芎嗪以 Bcl-2 介导的 caspase 依赖性机制体外诱导大鼠肝星状细胞凋亡研究

作者：张　峰　　指导老师：郑仕中

Ligustrazine-induced apoptosis is involved in its regulation of expression of Bcl-2 and Bax by caspase-dependent mechanism in rat hepatic stellate cells

Feng Zhang, Na Lei, Shizhong Zheng *, Yin Lu, Jin Ma

Department of Clinical Pharmacy, Nanjing University of Chinese Medicine

南京中醫藥大學

Background

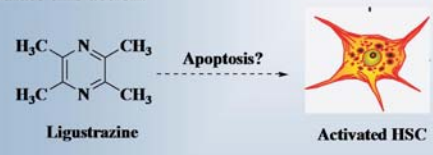
Hepatic fibrosis (HF) is a pathogenesis of excessive deposition of extracellular matrix (ECM) as a result of wound-healing responses to chronic liver injury. Abnormal activation of hepatic stellate cells (HSCs) is the key event in fibrosis progression, leading to ECM accumulation in pathological contexts of fibrosis.

Fibrosis is demonstrated as a malignant process, but there has been evidence that fibrosis can undergo regression via HSC apoptosis. Thus driving HSCs to undergo apoptosis may be an effective strategy for fibrosis prevention, and this concept may hopefully renovate current antifibrotic therapy.

Hypothesis and purpose

Ligustrazine, an alkaloid isolated form *Rhizoma Chanxiong*, has shown significant activity against fibrogenesis through stimulating ECM degradation, demonstrating a potential implication in the therapy against liver fibrosis.

We hypothesized that ligustrazine could induce apoptosis of hepatic stellate cells resulting in ECM degradation and thereby fibrosis regression, and we further elucidated the molecular mechanisms that mediate this action.



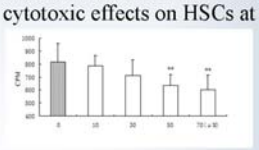
Materials and methods

- Rat hepatic stellate cell line T6 was cultured
- ^{3}H-TdR incorporation test for analysis of proliferation inhibition
- Cytotoxicity test by analysis of LDH release
- Apoptotic rate assay using flow cytometry
- Western blotting for analysis of expression of Bcl-2, Bax and caspase-3
- RNA isolation and real-time PCR

Results

Ligustrazine inhibits HSC proliferation without prominent cytotoxicity dose-dependently

Ligustrazine inhibited uptake of ^{3}H-TdR during HSC proliferation dose-dependently. Decreased CPM values paralleled increased doses of ligustrazine. LDH release assay demonstrated that ligustrazine had no significant cytotoxic effects on HSCs at doses of 10-70 μ M.

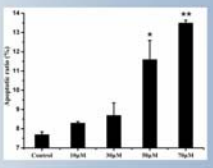


^{3}H-TdR incorporation assay

LDH release assay

Ligustrazine induces apoptosis in HSCs significantly

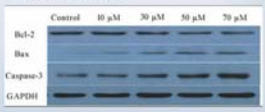
Increasing HSC apoptosis existed in drug-treated groups and prominent HSC apoptosis emerged with ligustrazine dose increasing to 70 μ M.



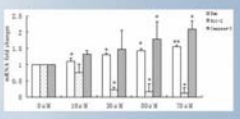
HSC apoptosis assay

Ligustrazin-induced HSC apoptosis is involved in its regulation of expression of Bcl-2, Bax and caspase-3

Ligustrazine led to decreased expression of Bcl-2 with concomitantly increased expression of Bax and caspase-3 in a dose-dependent manner at protein and mRNA levels.



Western blot analysis



Real-time PCR analysis

Summary

Our investigations have identified that ligustrazine-induced apoptosis of rat hepatic stellate cells *in vitro* is involved in its regulation of expression of Bcl-2 and Bax through a caspase-dependent mechanism. These findings strongly suggest that ligustrazine may be exploited as a potential option for treating and reversing hepatic fibrosis.

12. 南京中医药大学

题目：川穹嗪衍生物 H168 对肝星状细胞凋亡地影响及其分子机制研究

作者：倪春燕　　指导老师：郑仕中

Effects of ligustrazine derivative H168 on hepatic stellate cell apoptosis and the molecular mechanisms

Chunyan Ni, Shi zhong Zheng *, Yin Lu, Jin Ma

Department of Clinical Pharmacy, Nanjing University of Chinese Medicine

Introduction

Hepatic fibrosis (HF) represents a pathogenesis of excessive deposition of extracellular matrix (ECM) as a result of wound-healing responses to chronic liver injury[1]. Abnormal activation and recruitment of hepatic stellate cells (HSCs) is the dominant event in fibrosis progression, leading to ECM accumulation in pathological contexts of fibrosis[2]. Fibrosis is demonstrated as a progressive and malignant process, but there has been clear evidence that fibrosis can undergo regression via HSC apoptosis[3]. Thus driving HSCs to undergo apoptosis may be an effective strategy for fibrosis prevention, and this concept may hopefully renovate current antifibrotic therapy.

It is urgent to search for potent antifibrotic agents. Our previous experiments showed that ligustrazine, an alkaloid isolated form Rhizoma Chanxiong, has shown significant activity against fibrogenesis via stimulating ECM degradation, demonstrating a potential therapeutic implication in the therapy against liver fibrosis[4]. But we found that H168,ligustrazine derivative properties has an stronger activity against fibrogenesis. Now,we studied H168 against hepatic fibrosis from the mechanism of apoptosis.

Materials and methods

Cell culture

Rat hepatic stellate cell line T6 were cultured. Cells aged at passages 4-8 were used for experiments. H168 was dissolved in phosphate buffered solution with standard antibiotics.

HE staining

HSCs were seeded and treated with H168 at indicated concentrations. They were fixed by 4% poly-MeOH, then HE stained.

Cytotoxicity test

Analysis of lactate dehydrogenase (LDH) release was used to evaluate the cytotoxic effect of H168. HSCs were seeded and treated with H168 at indicated concentrations. LDH release assay Kit was used to detect LDH release.

Apoptosis assay using flow cytometry

HSCs were seeded and treated with H168 at indicated concentrations. Then they underwent apoptosis assay using Annexin V-FITC Apoptosis Assay Kit.

Western blotting analysis

Whole cell protein extracts were prepared from H168-treated HSCs. The concentration was determined using BCA assay kit. After transblotting, the separated proteins were detected using primary antibodies Bcl-2, Bax and caspase-3, and horseradish peroxidase conjugated goat anti-rabbit IgG as the secondary antibody. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an invariant control.

Statistical analysis

Student's t-test was used for determination of statistical difference. Values of $P<0.05$ were considered to be statistically significant. The data represent means ± SD from three independent experiments at least.

Results

Firstly, H168 inhibits HSC proliferation without prominent cytotoxicity dose-dependently.

Secondly, H168 significantly induces apoptosis in HSCs .

Thirdly, H168-induced HSC apoptosis is involved in its regulation of expression of Bcl-2 and Bax .

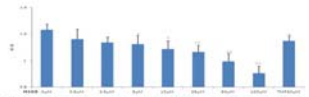


Fig1: Our data showed H168 suppressed HSC proliferation in a dose-dependent manner.

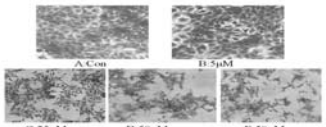


Fig 2: HSCs were seeded and treated with H168 at indicated concentrations. They were fixed by 4% poly-MeOH, then HE stained.

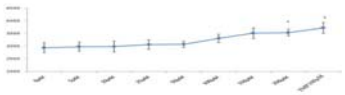


Fig3: LDH release assay demonstrated that H168 had no significant cytotoxic effects on HSCs at doses of 5-150μM

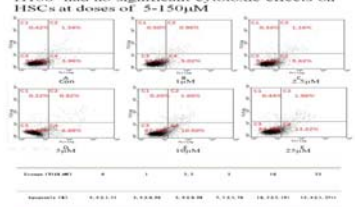


Fig4:Apoptosis analysis using flow cytometry showed that increased HSC apoptosis existed in H168-treated groups with doses lower than 10μM and especially, prominent HSC apoptosis emerged with ligustrazine dose increasing to 25μM.

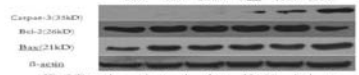


Fig5:Results showed that H168 led to decrease of Bcl-2 with a concomitant increase of Bax dose-dependently, indicating that H168 exerted its apoptotic effect upon HSCs through downregulation of the ratio of Bcl-2 to Bax

Conclusions

Our investigations have identified that H168-induced apoptosis of rat hepatic stellate cells *in vitro* is involved in its regulation of expression of Bcl-2 and Bax by a caspase-dependent mechanism. These findings strongly suggest that H168 may be exploited as a potential option for treating and reversing hepatic fibrosis.

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13. 南京中医药大学

题目：木犀草素通过抑制整合素 β1 抑制缺氧诱导的人源 A549 上皮间质转化

作者：阮君山　　指导老师：陆　茵

Luteolin Inhibits Hypoxia-induced Epithelial Mesenchymal Transition through Suppression Integrin ß1 in Human Lung Cancer Cell A549

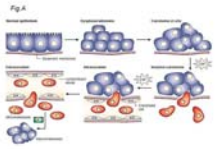
Junshan-Ruan Yin-Lu* Shizhong-Zheng Aiyun-Wang Linggen-Yan Lei-Zhang Li-Tao

School of Pharmacy, Nanjing University of Traditional Chinese Medicine

Jiangsu Key Laboratory for Pharmacolgy and Safety Evaluation of Chinese Materia Medica

Introduction

Currently, hypoxia-induced epithelial mesenchymal transition is thought to be a key step for cancer metastasis(Fig.A).The development of metastasis requires the movement and invasion of cancer cells from the primary tumor into the surrounding tissue. To acquire such invasive abilities, epithelial cancer cells must undergo several phenotypic changes. , by decreased epithelial markers such as E-cadherin and increased mesenchymal markers such as fibronectin. Many EMT inducers have been identified, and the molecular mechanisms related to the highly invasive characteristics of cancer cells have been intensively investigated (Fig.B). Luteolin（Fig.C), a flavonoid, widely distributed in Chinese Herbs, is considered as the major antioxidant, anti-inflammation, antifibrotic, and anticancer constituent. This is the first study to demonstrate that luteolin alleviates hypoxia induced Epithelial-Mesenchymal Transition in human lung adenocarcinoma cells line A549.

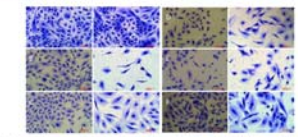


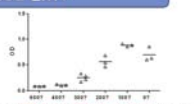
Abstract

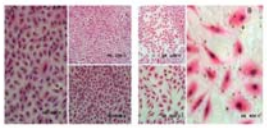
The TCM of Activating Blood and Resolving Stasis, has been known as an anti-metastasis for thousands of years. Luteolin, an active flavonoid compound, isolated from the herbs, has a spectrum of biological activities. Whether luteolin has a direct inhibitory effect on hypoxia induced Epithelial-Mesenchymal Transition has not been established. In this study, we examined the effects of luteolin on hypoxia induced Epithelial-Mesenchymal Transition in vitro. We found in A549 cells line, Luteolin could Inhibit Hypoxia-induced Epithelial Mesenchymal Transition. Then we explored the possible mechanism of this action.

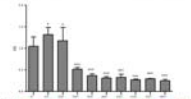
Results

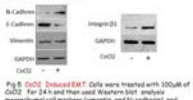
Model of Hypoxia Induced EMT



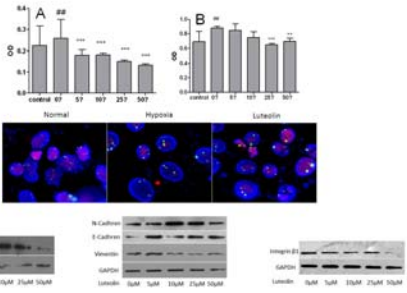








Effect Of Luteolin On Hypoxia Induced （EMT）



Summary

- Our in vitro studies reveal that luteolin suppressed Hypoxia–induced EMT by increase of epithelial marker E-cadherin expression and decrease of myofibroblast markers, N-cadherin, Ezrin, Vimentin expression in human A549 cells. Luteolin reduced epidermal growth factor gene mutations and suppression integrin ß1 expression.
- In hypoxia tumor microenvironment epidermal growth factor and Integrin is closely related to EMT, the above finding suggest that inhibition of EGFR and Integrin is the possible mechanism for Luteolin Inhibits Hypoxia-induced Epithelial Mesenchymal Transition.

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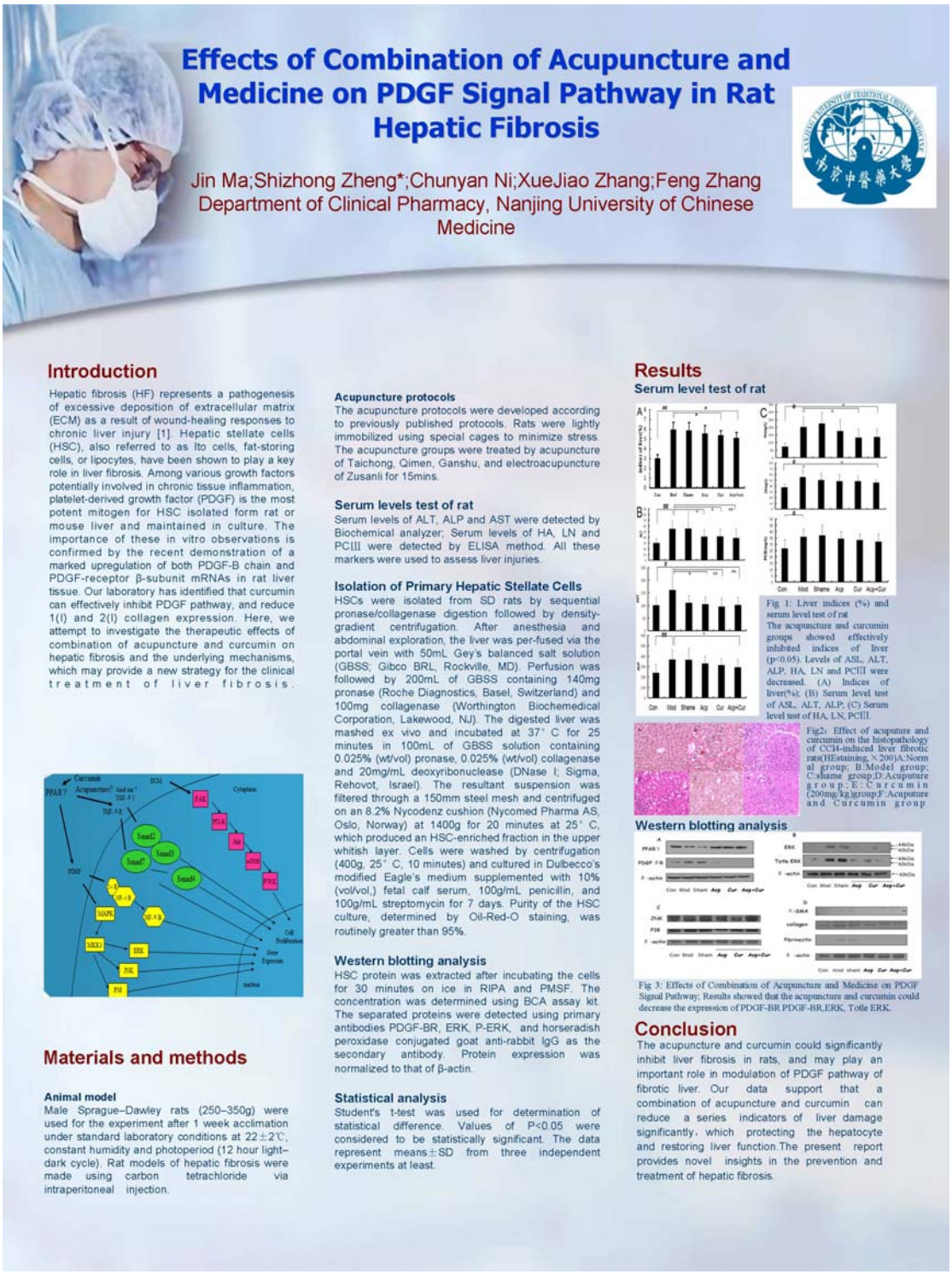
14. 南京中医药大学

题目：针药结合对肝纤维化大鼠 PDGF 信号通路的作用研究

作者：马　进　　指导老师：郑仕中



Effects of Combination of Acupuncture and Medicine on PDGF Signal Pathway in Rat Hepatic Fibrosis

Jin Ma;Shizhong Zheng*;Chunyan Ni;XueJiao Zhang;Feng Zhang
Department of Clinical Pharmacy, Nanjing University of Chinese Medicine

Introduction

Hepatic fibrosis (HF) represents a pathogenesis of excessive deposition of extracellular matrix (ECM) as a result of wound-healing responses to chronic liver injury [1]. Hepatic stellate cells (HSC), also referred to as Ito cells, fat-storing cells, or lipocytes, have been shown to play a key role in liver fibrosis. Among various growth factors potentially involved in chronic tissue inflammation, platelet-derived growth factor (PDGF) is the most potent mitogen for HSC isolated form rat or mouse liver and maintained in culture. The importance of these in vitro observations is confirmed by the recent demonstration of a marked upregulation of both PDGF-B chain and PDGF-receptor β-subunit mRNAs in rat liver tissue. Our laboratory has identified that curcumin can effectively inhibit PDGF pathway, and reduce 1(I) and 2(I) collagen expression. Here, we attempt to investigate the therapeutic effects of combination of acupuncture and curcumin on hepatic fibrosis and the underlying mechanisms, which may provide a new strategy for the clinical treatment of liver fibrosis.

Materials and methods

Animal model

Male Sprague–Dawley rats (250–350g) were used for the experiment after 1 week acclimation under standard laboratory conditions at 22±2℃, constant humidity and photoperiod (12 hour light–dark cycle). Rat models of hepatic fibrosis were made using carbon tetrachloride via intraperitoneal injection.

Acupuncture protocols

The acupuncture protocols were developed according to previously published protocols. Rats were lightly immobilized using special cages to minimize stress. The acupuncture groups were treated by acupuncture of Taichong, Qimen, Ganshu, and electroacupuncture of Zusanli for 15mins.

Serum levels test of rat

Serum levels of ALT, ALP and AST were detected by Biochemical analyzer; Serum levels of HA, LN and PCⅢ were detected by ELISA method. All these markers were used to assess liver injuries.

Isolation of Primary Hepatic Stellate Cells

HSCs were isolated from SD rats by sequential pronase/collagenase digestion followed by density-gradient centrifugation. After anesthesia and abdominal exploration, the liver was per-fused via the portal vein with 50mL Gey's balanced salt solution (GBSS; Gibco BRL, Rockville, MD). Perfusion was followed by 200mL of GBSS containing 140mg pronase (Roche Diagnostics, Basel, Switzerland) and 100mg collagenase (Worthington Biochemedical Corporation, Lakewood, NJ). The digested liver was mashed ex vivo and incubated at 37° C for 25 minutes in 100mL of GBSS solution containing 0.025% (wt/vol) pronase, 0.025% (wt/vol) collagenase and 20mg/mL deoxyribonuclease (DNase I; Sigma, Rehovot, Israel). The resultant suspension was filtered through a 150mm steel mesh and centrifuged on an 8.2% Nycodenz cushion (Nycomed Pharma AS, Oslo, Norway) at 1400g for 20 minutes at 25° C, which produced an HSC-enriched fraction in the upper whitish layer. Cells were washed by centrifugation (400g, 25° C, 10 minutes) and cultured in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol,) fetal calf serum, 100g/mL penicillin, and 100g/mL streptomycin for 7 days. Purity of the HSC culture, determined by Oil-Red-O staining, was routinely greater than 95%.

Western blotting analysis

HSC protein was extracted after incubating the cells for 30 minutes on ice in RIPA and PMSF. The concentration was determined using BCA assay kit. The separated proteins were detected using primary antibodies PDGF-BR, ERK, P-ERK, and horseradish peroxidase conjugated goat anti-rabbit IgG as the secondary antibody. Protein expression was normalized to that of β-actin.

Statistical analysis

Student's t-test was used for determination of statistical difference. Values of $P<0.05$ were considered to be statistically significant. The data represent means±SD from three independent experiments at least.

Results

Serum level test of rat

Fig 1: Liver indices (%) and serum level test of rat
The acupuncture and curcumin groups showed effectively inhibited indices of liver ($p<0.05$). Levels of ASL, ALT, ALP, HA, LN and PCⅢ were decreased. (A) Indices of liver(%); (B) Serum level test of ASL, ALT, ALP; (C) Serum level test of HA, LN, PCⅢ.

Fig2: Effect of acuputure and curcumin on the histopathology of CCl4-induced liver fibrotic rats(HEstaining, ×200)A:Normal group; B:Model group; C:sham group;D:Acuputure group; E: Curcumin (200mg/kg)group;F:Acuputure and Curcumin group

Western blotting analysis

Fig 3: Effects of Combination of Acupuncture and Medicine on PDGF Signal Pathway; Results showed that the acupuncture and curcumin could decrease the expression of PDGF-BR,PDGF-BR,ERK, Totle ERK.

Conclusion

The acupuncture and curcumin could significantly inhibit liver fibrosis in rats, and may play an important role in modulation of PDGF pathway of fibrotic liver. Our data support that a combination of acupuncture and curcumin can reduce a series indicators of liver damage significantly, which protecting the hepatocyte and restoring liver function.The present report provides novel insights in the prevention and treatment of hepatic fibrosis.

15. 南京中医药大学

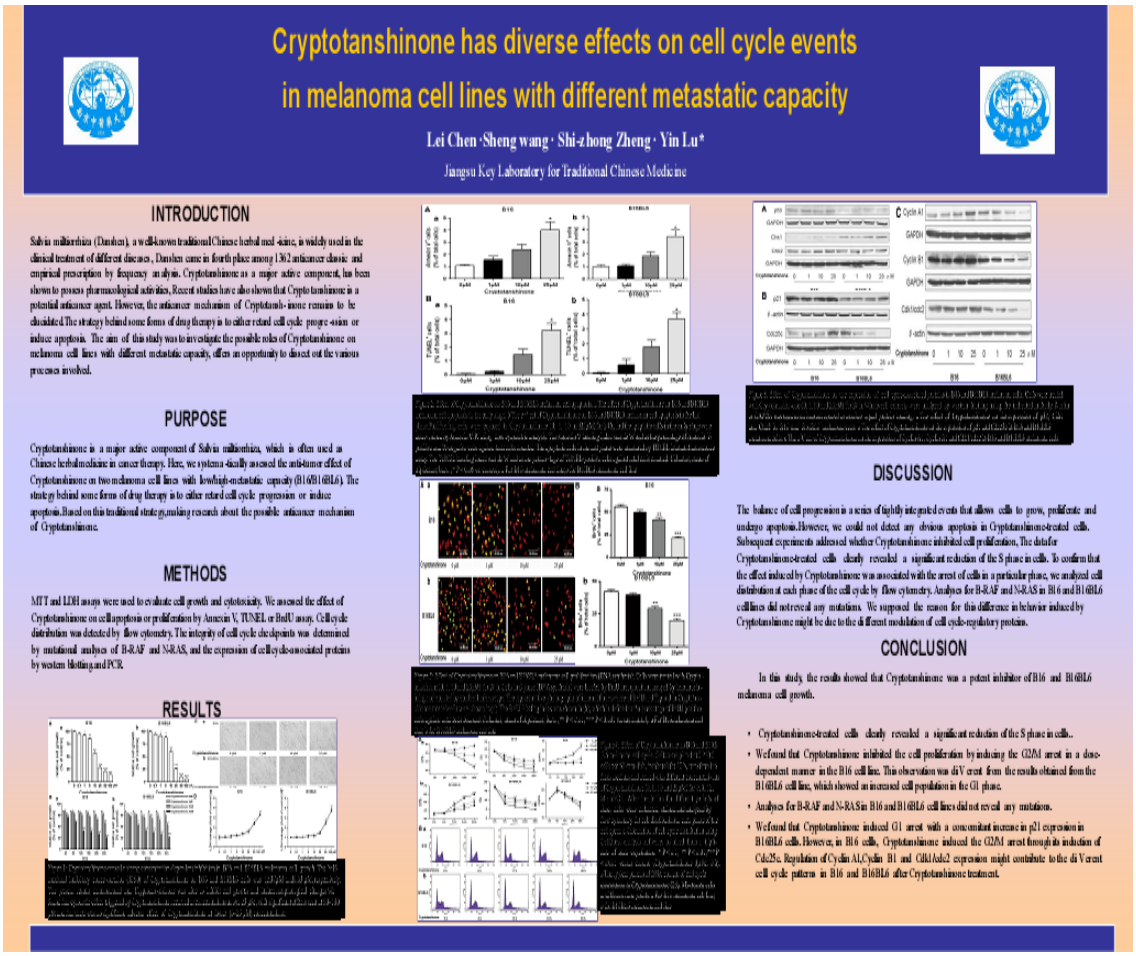
题目：地黄山茱萸对糖尿病大鼠降糖作用机制的研究

作者：吴　诚　　指导老师：许惠琴

16. 南京中医药大学

题目：隐丹参酮对黑色素瘤细胞株周期的不同作用来调控其侵袭能力

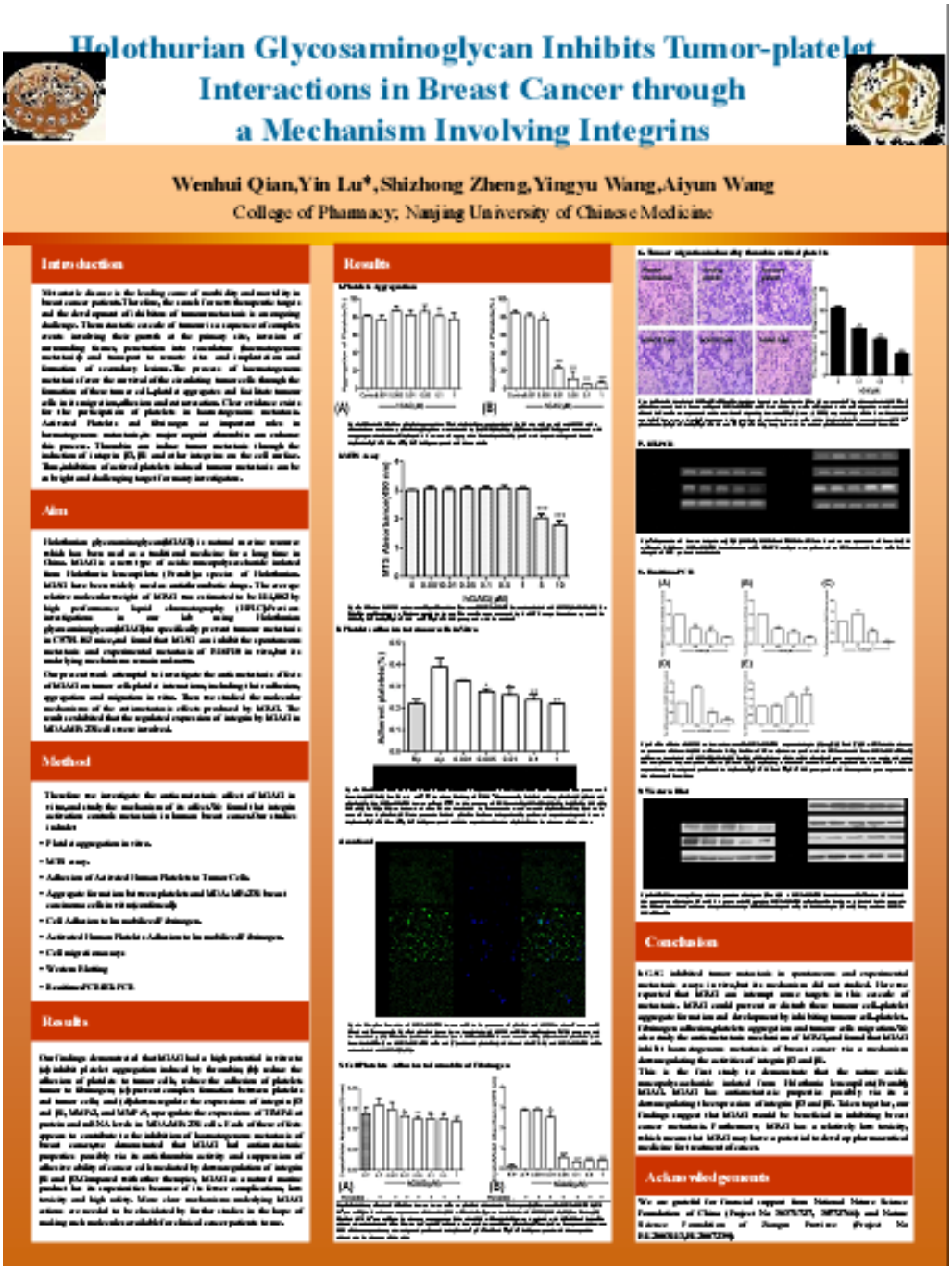
作者：王　生　　指导老师：陆　茵



17. 南京中医药大学

题目：海参糖胺聚糖通过对整合素 β3 及 β1 的调控来抑制血小板-乳腺癌细胞间的相互作用

作者：钱文慧　　指导老师：陆　茵



18. 南京中医药大学

题目：川穹嗪衍生物 H168 对肝星状细胞增殖地影响及其机制研究

作者：张雪娇　　指导老师：郑仕中

The effect of ligustrazine derivative H168 on hepatic stellate cells proliferation and its possible mechanism

Zhang Xue-jiao, Zheng Sheng-zhong*, Lu Yin, Ma Jin
(Nanjing University of Chinese Medicine, college of pharmacy，Department of Clinical pharmacy，Nanjing, 210029,China)

Introduction:

HSCs located in Disse space adhere to hepatocytes. In normal liver, HSCs accounting for 5-11% of cell population contain large amount of vitamin A, and play a role in vitamin A metabolism and ECM synthesis. In pathological context, HSCs serve as the primary source of ECM deposition, and their activation is considered as the centre event in hepatic fibrogenisis. Moreover, HSC activation is strikingly characterized by its proliferation. Thus inhibition of HSC proliferation can significantly attenuate HSC activation, and provide novel insights into the molecular mechanisms and therapeutic strategy of live fibrosis.

Ligustrazine, known as tetramethylpyrazineis, is a naturally occurring alkaloid product isolated from Chinese medicine Rhizoma Chanxiong. Pharmacological studies demonstrated that ligustrazine has effects of microcirculation improvement, anti-oxidant, immunoloregulation and liver protection. Structural modifications of ligustrazine led to its derivative H168 with higher water solubility. We herein investigated the in vitro anti-proliferative effects of H168 on HSC-T6, and the underlying molecular mechanisms

Materials and methods:

Cell culture

Rat hepatic stellate cell line T6 were cultured. Cells aged at passages 4-8 were used for experiments. Ligustrazine hydrochloride was dissolved in phosphate buffered solution with standard antibiotics.

Cell Viability Assay

For the quantification of cell viability, we performed the MTT assay using the 3-(4,5-dim-ethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide reagent。

Cell cycle assay using flow cytometry

HSCs were seeded and treated with ligustrazine derivative H168 at indicated concentrations. Then they underwent cell cycle assay assay using PI dual staining Assay Kit.

Western blotting analysis

Whole cell protein extracts were prepared from H168-treated HSCs. The concentration was determined using BCA assay kit. After transblotting, the separated proteins were detected using primary antibodies P21 and P27, and horseradish peroxidase conjugated goat anti-rabbit IgG as the secondary antibody. β –actin was used as an invariant control.

RNA isolation and real-time PCR

Total RNA was isolated and real-time PCR was then performed. β –actin was used as an invariant control. Fold changes in the mRNA levels of target genes related to the invariant control GAPDH were calculated.

Statistical analysis

Student's t-test was used for determination of statistical difference. Values of P<0.05 were considered to be statistically significant. The data represent means ± SD from three independent experiments at least.

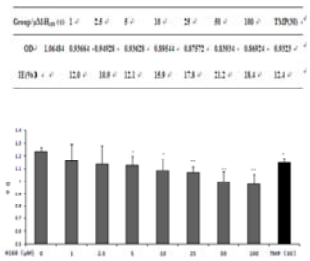


Fig.1 Cell viability assay

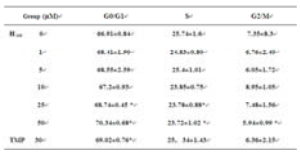


Fig.2 Cell cycle assay using flow cytometry

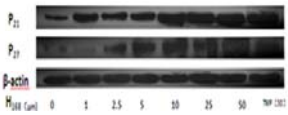


Fig.3 Western blotting analysis. GAPDH was internal control for equal loading.

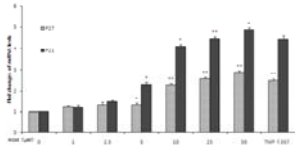


Fig.4 Real-time PCR analysis. GAPDH was invariant control for calculating fold changes in mRNA levels.

Results：

ligustrazine derivative H168 inhibits HSC proliferation with prominent dose-dependently

Our data showed that ligustrazine derivative H168 can inhibit the proliferation of HSC-T6 obviously ,with the increased of drug concentration ,the potential efficacy on inhibition of HSC-T6 proliferation shows an dose-dependent manner(Fig. 1).

ligustrazine derivative H168 significantly induces cell cycle in HSCs

Cell cycle analysis using flow cytometry showed that the effect of HSC existed in H168 -treated groups with doses of 25μM . Our data showed that the cells accumulated in G0/G1, S phase and reduced G2 / M phase cells and inhibit cell proliferation. (Fig. 2).

ligustrazine derivative H168 inhibit cell proliferation is involved in its regulation of expression of p21 and p27

We performed Western blotting analysis targeting p21 and p27. Western blotting results showed that ligustrazine derivative H168 can inhibit HSC-T6 proliferation by promoting P21/P27 protein expression (Fig. 3);Further use of Real time PCR found that ligustrazine derivative H168 promotes expression of P21/P27 mRNA (Fig. 4).

Conclusions:

Our investigations have identified that H168 inhibited of hepatic stellate cells proliferation in vitro is involved in its regulation of expression of P21 and P27. These findings strongly suggest that H168 may be exploited as a potential option for treating and reversing hepatic fibrosis.

Acknowledgements:

ligustrazine derivative H168 was generously supplied by Pfizer Li.

19. 中国药科大学

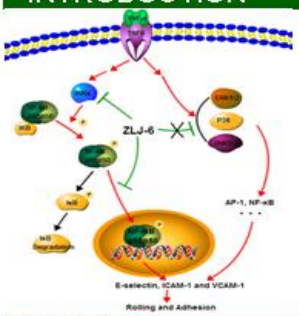
题目：新的环氧合酶/5-脂氧合酶抑制剂 ZLJ-6 通过环氧合酶/5-脂氧合酶依赖性途径抑制 TNF-α 诱导的内皮选择蛋白、细胞粘附因子-1、血管细胞粘附因子-1 表达及单核细胞-内皮间的相互作用

作者：陈　莉　　指导老师：季　晖

ZLJ-6, a novel COX/5-LOX inhibitor, attenuates TNF- α -induced endothelial E-selectin, ICAM-1 and VCAM-1 expression and monocyte-endothelial interactions via a COX/5-LOX-independent mechanism

Li Chen, Qian Zhao, Xu-Liang Wang, Ran You, Yi-Hua Zhang, Hui Ji*, Yi-Sheng Lai*
Department of Pharmacology, China Pharmaceutical University
* Corresponding author

INTRODUCTION



◆ Adhesion and migration of circulation monocytes to the blood vessel wall, based on the expression of adhesion molecular, are important characterizes of inflammation reactions.

◆ NSAIDs are previous found to posses additional prostaglandins and leukotrienes-independent anti-inflammatory mechanisms. However, the chronic use of NSAIDs is limited by their severe side effects.

◆ As a consequence, COX/5-LOX dual inhibitors, interfering both with the production of prostaglandins and the biosynthesis of leukotrienes (LTs), have emerged as a possibility to avoid side effects related to selective COX inhibition.

◆ The aim of this study was to investigate the effect of ZLJ-6, an imidazolone COX/5-LOX dual inhibitor, on TNF-α-induced adhesion molecular expression on HUVECs, monocyte-endothelial interactions and the possible mechanism.

METHOD

- Static and dynamic endothelial-monocyte interaction.
- Surface expression of adhesion molecules.
- NF-κB and MAPK signaling pathway analysis.
- Electrophoretic mobility shift assay (EMSA).
- IκB kinase assay.α

RESULTS

Fig. 1

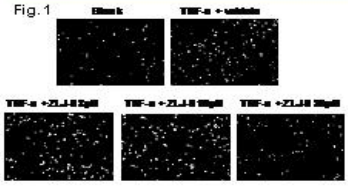


Fig. 2

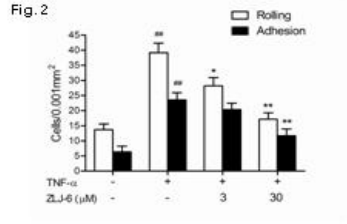
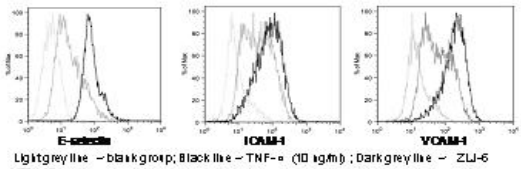


Fig. 3



Light grey line – blank group; Black line – TNF-α (10 ng/ml); Dark grey line – ZLJ-6 (30 μ M)

Fig. 4

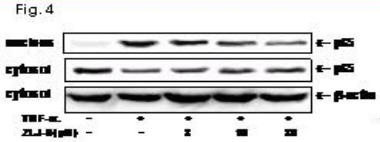


Fig. 5

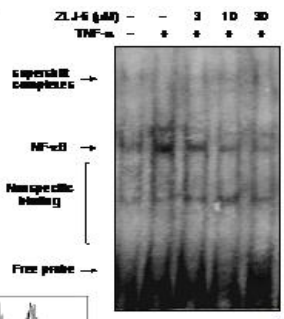


Fig. 6

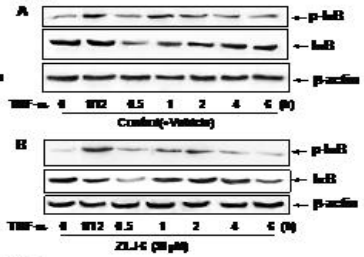
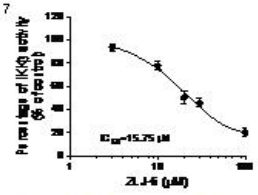


Fig. 7



A. ZLJ-6 potently inhibited both the static and dynamic monocyte-endothelial interactions (Fig.1 and Fig.2). However, COX-2 inhibitor celecoxib and 5-LOX inhibitor zileuton failed to affect the interactions (data not shown).
B. ZLJ-6 decreased E-selectin, ICAM-1 and VCAM-1 expression on HUVECs (Fig.3).
C. ZLJ-6 suppressed the NF-κB translocation and binding activities in HUVECs (Fig.4 and Fig.5).
D. The inhibition of ZLJ-6 on NF-κB activation was regulated by preventing IκB phosphorylation and IKKβ activation (Fig.6 and Fig.7).

CONCLUSION

These findings suggest that :

➢ZLJ-6 potently inhibited TNF-α-induced monocyte-endothelial interaction and expression of adhedion molecular expression (E-selectin, ICAM-1 and VCAM-1).

➢The inhibitory effects of ZLJ-6 were regulated by NF-κB signaling pathway rather than its primary pharmacological target COX-2 or 5-LOX.

Funded by: "Scientific and Technological Major Special Project-Significant Creation of New Drugs (No. 2009ZX09103) in the Eleventh Five-Year" and Specialized Research Fund for the Doctoral Program of Higher Education of China (No. 20090094110001).

20. 中国药科大学

题目：银杏内酯 B 对大鼠短暂局灶性缺血模型内皮和基底节的治疗作用

作者：方伟蓉　　指导老师：李运曼

Therapeutic Effects of Ginkgolide B on Cortex and Basal Ganglia in a Rat Model of Transient Focal Ischemia

Peng Lv†, Weirong Fang†, Xiaohan Geng, Qichuan Yang, Yunman Li, Lan Sha.

† These two authors contributed equally to this work.

Department of Physiology, China Pharmaceutical University.

Introduction: Ischemic stroke is the third leading cause of death and the main reason for severe disabilities in the world. In clinical settings, many patients suffering from stroke often seek medical assistance and diagnosis with considerable delay, and result in irreversible ischemic damage. Therefore, pharmacological strategies limiting the delayed phase of the brain damage are probably more important in stroke therapy, and drugs with the potential for clinical stroke treatment need to have a rather large therapeutic window of at least several hours. Cerebral cortex and basal ganglia are two brain regions with main vascular supply from middle cerebral artery and are vulnerable to ischemic damage from middle cerebral artery occlusion (MCAO). Considering that there are no previous reports in this study, we evaluated the therapeutic effects of ginkgolide B on cortex and basal ganglia in a clinically relevant model of stroke, MCAO.

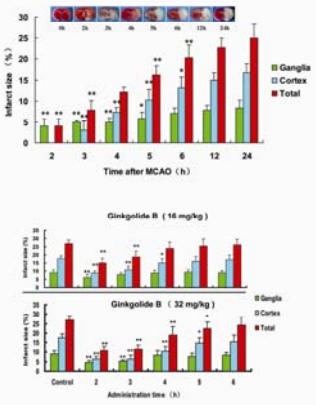


Fig.4 Post-ischemic treatment with ginkgolide B on cerebral infarction in MCAO rats. ginkgolide B (32 mg/kg i.v.) treatment significantly decreased the total infarct rate and the cortical infarct rate at 2, 3, 4, 5h after MCAO and the basal ganglia infarct rate at 2, 3h compared to vehicle-treated group. Ginkgolide B (16 mg/kg i.v.) treatment significantly decreased total infarct rate at 2, 3h, cortical infarct rate at 2, 3, 4h, and basal ganglia infarct rate at 2h compared to vehicle-treated group.

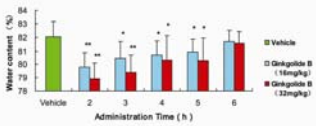


Fig.5 Post-ischemic treatment with ginkgolide B on cerebral edema in MCAO rats. Brain water contents in vehicle-treated group were significantly higher than other groups, indicating a significant edema formation at post-stroke (82.1±1.1%). Post-treatment with ginkgolide B (32 and 16mg/kg i.v.) reduced edema formation by decreasing water contents at 2, 3, 4 and 5 h after MCAO.

Fig.1 Infarct rate in cerebral cortex and basal ganglia following MCAO.Compared to 24h after MCAO, there was significant difference in total and basal ganglia infarction at 2, 3, 4, 5h, and in cortical infarction at 2, 3, 4, 5, 6h.

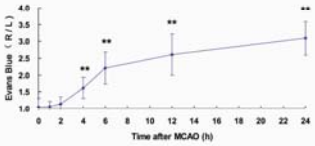


Fig.2 Tissue EB leakage rate at 1, 2, 4, 6, 12, and 24h following MCAO. As shown in Fig. 2, the tissue EB leakage rate at 1, 2, 4, 6, 12, and 24h was 1.0±0.3, 1.1±0.2, 1.1±0.2, 1.6±0.3, 2.2±0.5, 2.6±0.6, 3.1±0.5. In the right hemisphere, EB content is greater than that of the left hemisphere at all time points except for 0h group.

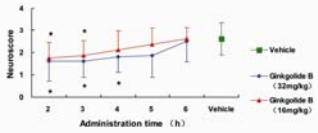


Fig.3 Post-ischemic treatment with ginkgolide B on neurological deficits in MCAO rats. Ginkgolide B (32 mg/kg) treatment at 2, 3, and 4 h after MCAO produced significant improvement in neurological score compared to vehicle-treated group. Ginkgolide B (16 mg/kg) treatment at 2 and 3h after MCAO also produced significant improvement in neurological score compared to vehicle-treated group.

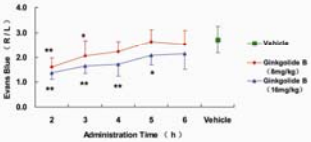


Fig.6 Effect of post-treatment of ginkgolide B on the extravasation of Evans Blue. Extravagation of Evans Blue in vehicle-treated group was 2.71±0.5. Ginkgolide B (32 mg/kg i.v.) reduced extravagation of Evans Blue by decreasing the tissue EB leakage rate at 2, 3, 4, and 5 h after MCAO ($P < 0.05$, $P < 0.01$) Ginkgolide B (16 mg/kg i.v.) reduced extravagation of Evans Blue by decreasing the tissue EB leakage rate at 2, 3 and 4 h after MCAO ($P < 0.05$, $P < 0.01$).

21. 江苏省中医院

题目：乌鳖颗粒治疗卵巢早衰的机制研究

作者：王海丹　　指导老师：朱萱萱

MECHANISMS OF WU BIE GRANULES ON PREMATURE OVARIAN FAILURE

Wang Haidan, Zhu Xuanxuan

Nanjing University of Chinese Medicine

INTRODUCTION

◆POF is biochemically characterized by low levels of gonadal hormones (estrogens and inhibins) and high levels of gonadotropins (LH and FSH) .

◆Heterogeneity of POF is also reflected by the variety of possible causes, including autoimmunity, toxics, drugs, as well as genetic defects.

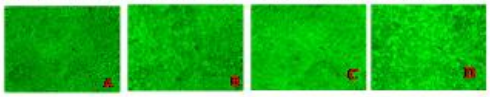
◆ Objectives: To Explore the Mechanisms of Wu Bie granules on premature ovarian failure .

METHODS

Using GWT , CDDP , D-galactose and ZP3 set up different POF model. To determine the oestrous cycle by observing vaginal cells every timing. Testing the level of E_2 ,FSH and LH.Calculation ovaries and uterine index. Ovarian for organization morphological observation, counted the number of various follicular and corpus luteum . Detected T lymphocyte cell subsets of the spleen and Fas, FasL protein expression of ovarian by immunohistochemistry.

RESULTS

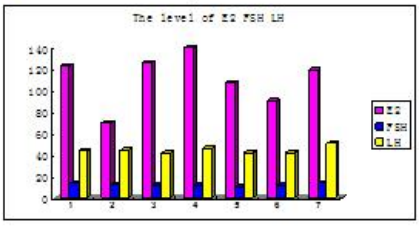
◆ **vaginal cells of different oestrous cycle**



A:proestrus B:oestrus C:metoestrus D:dioestrus

Oestrous cycle of model control normal group mice were dysfunction and anoestrum were prolongation.

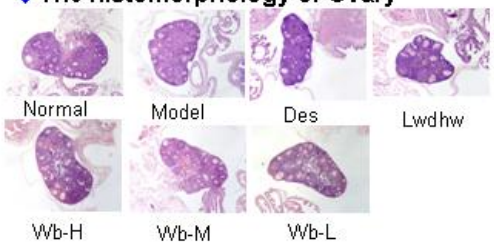
◆**The level of E2 ,FSH and LH**



1:Normal
2: Model
3:Des
4:Lwdhw
5:Wb-H
6:Wb-M
7:wb-L

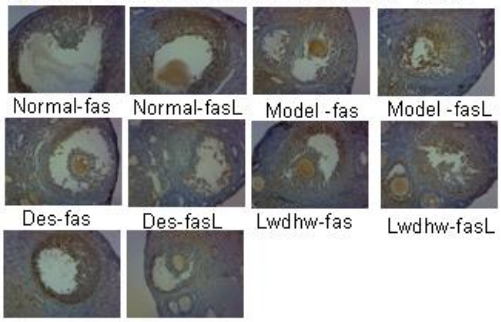
Wb could significantly increase the level of E2 and decrease the level of FSH and LH in model rats.

◆**The histomorphology of Ovary**



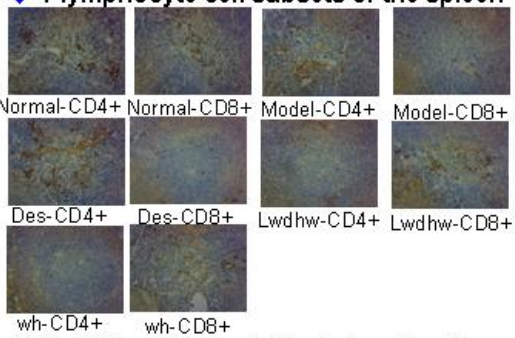
Wb could significantly promote the growth of follicle compared with the model group.

◆**Fas, FasL protein expression of ovary**



WB could significantly increase the fas protein expression and decrease the Fas-L protein expression compared with the model.

◆ **T lymphocyte cell subsets of the spleen**



The CD4+/CD8+ of WB was significantly lower than the model group, compared with the model group with significant difference.

CONCLUSION

◆ Wu Bie granules can promote the development of uterus and ovary , improve The morphology of ovarian tissue,adjust ovarian hormones.

◆Wu Bie granules can regulate CD4 + / CD8 + and the Fas / FasL system.

22. 江苏省中医院

题目：润喉开音颗粒对体外培养人声带小结成纤维细胞的影响

作者：朱吾元　　指导老师：朱萱萱

The effects of runhoukaiyinkeli on Vocal nodules fibroblast cultured in vitro

Wu-yuan Zhu [1],Xuan-xuan[2] zhu,F.Sheng[2]
1. Nan Jing University of traditional Chinese medicine,2. Jiang Su province hospital of TCM,210029

INTRODUCTION

Vocal nodule is among the most common laryngopathies that cause vocal functional disorders. Often the first step to the disease is phonotrauma,it leads to the injury of the vessels, hemorrhage, leakage of fibrin, thrombosis, proliferation of capillaries,and then fibrosis. Extensive studies indicate that fibrosis account for approximately 93% of all vocal nodels.So it may be play an inportant role in the treating of vocal nodules to restain the proliferation of fibroblast and promote its apoptosis.

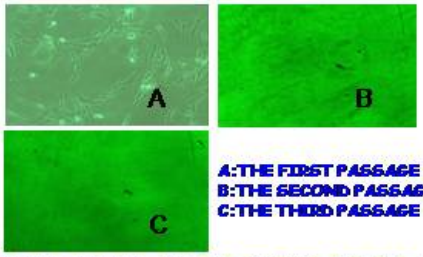
METHOD

1.Human vocal nodules tissue obtained from patients was rinsed by PBS-antibiotic solution,cuted into 1-mm3 pieces,and incubated in 5% carbon dioxide at 37℃.A confluent monolayer is established within 14 days.For this experiment ,fibroblast cells from three passage was used.The cultured cells of experimental group were treated with runhoukaiyinkeli at varing concentrations of30mg/ml,21mg/ml,14.7mg/ml.

2.The proliferation level of fibroblast cultured in vitro was measured by MTT.The apoptosis level was detected by Hoechst33258.Cell cycle was analyzed by flow cytometry.

RESULTS

Pictures of cell cultured in vitro



Pictures of apoptosis level detected by Hoechst33258.

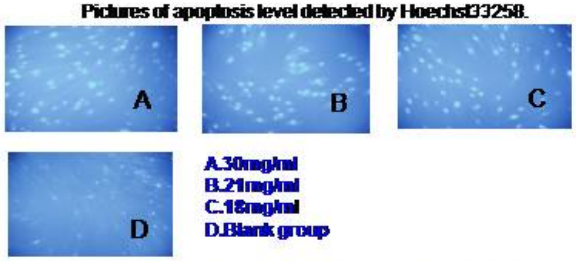


Chart one:the effect of runhoukaiyinkeli on proliferation of fibroblast

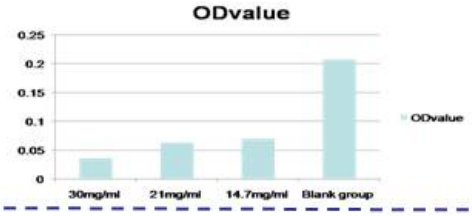
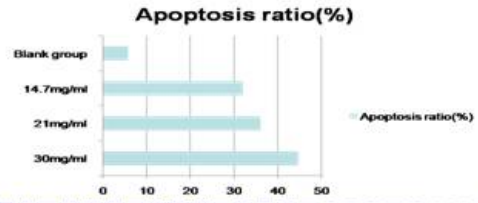
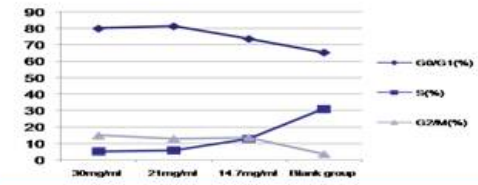
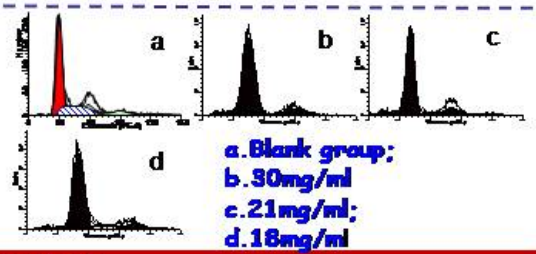


Chart two:the effect of runhoukaiyinkeli on apoptosis of fibroblast



The effect of runhoukaiyinkeli on cell cycle





CONCLUTION

Runhoukaiyinkeli can inhibit the proliferation and DNA synthesis of fibroblast cultured in vitro, which may be accomplished by blocking the cell cycle.

23. 江苏省中医院

题目：冠心平对过氧化氢引起血管内皮细胞损伤的影响

作者：万　盟　　指导老师：朱萱萱

The effects of Guanxinping on vascular endothelial cell injured by H_2O_2

Wan M[1], Zhu XX[2], Yan SH[2], Li QY[2],Zhu WY[1],Wang HD[1],Fu R[2],Shi LF[1]
1. NanJing University of traditional Chinese medicine,2.JiangSu province hospital of TCM,210029

INTRODUCTION

◆The pathological basis of coronary heart disease (CHD) is atherosclerosis (AS), while the endothelial dysfunction (EDF) is the initial factor of AS.

◆Vascular endothelial cells (VEC) is simple squamous or polygonal cells overlay on vascular intima surface.It is both induction cells and effector cells, that not only perceive inflammatory signal, hormone levels, shear stress and pressure information , but secrete a variety of vascular active substances .

◆Oxygen free radicals could damage endothelial cells , such as H_2O_2 could increase inflammatory factors secreted by VEC , induce more substance which could damage cells , even inducing apoptosis.

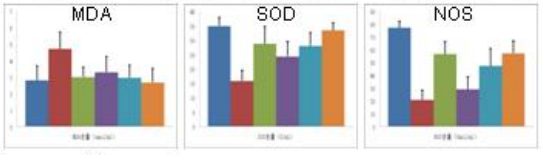
◆Clinical research and vitro studies suggest Guanxinping have good effect on EDF, it also could adjust the oxidative stress, relieve inflammatory reaction and so on.

METHOD

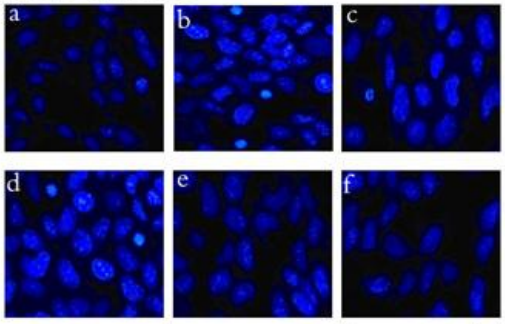
■By adopting serum pharmacology method and preparing Guanxinping medicated serum, we cultivate endothelial cells with medicated serum , observing the the protective effect of Guanxinping on VEC injured by H_2O_2.The indicators include oxidation and antioxidant balance, inflammatory factors secreted by cells and the influence of cell apoptosis. In order to explore the mechanism of Guanxinping on protecting endothelial cells..

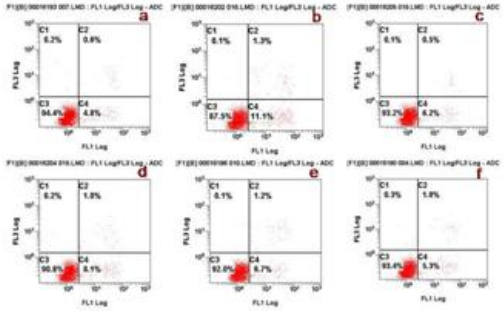
RESULTS

■(a) 正常组 ■(b) 模型组 ■(c) 维拉帕米组 ■(d) 小剂量组 ■(e) 中剂量组 ■(f) 大剂量组

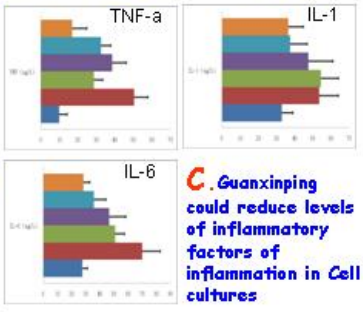


A. Guanxinping may have antioxidant action to improve the NOS level, increase the level of NO and decrease the synthesis of ET





B. Guanxinping could decrease the rate of apoptosis , protect cells state stability. Meanwhile, after pretreatment of Guanxinping , NF - κ B protein expression drops significantly, and to a certain extent a dose dependencies.



C. Guanxinping could reduce levels of inflammatory factors of inflammation in Cell cultures

CONCLUSION

These findings suggest that Guanxinping could through the following aspects in playing the role of protecting vascular endothelial cells:

- protect the VEC from oxidative damage with a certain effect of antioxidation
- medicated serum could decrease rate of apoptosis
- reduce the secretion of inflammatory factors by injured cell, play the role of protecting VEC.

24. 扬州大学

题目：柏木醇抑制大鼠关节炎的实验研究

作者：邱　夏　　指导老师：孙　云

Experimental esearch of Kashiwagi alcohol inhibited response on arthritis model in rat

XIA QIU,TING HU,YUN SUN*
College Medicine, Yangzhou University

INTRODUCTION

◆Kashiwagi alcohol is a sesquiterpene alcohol present in the cypress and fir oils.It is insoluble in water, slightly soluble in glycerol, mineral oil,and soluble in ethanol, methyl benzoate. Kashiwagi alcohol has anti-inflammatory granuloma in rats Effect.It can reduce the weight of granulation tissue in rats and can better control the early inflammatory response in rats.

◆Rheumatoid arthritis is an arthritis in response to persistent joint damage leading to progressive joint dysfunction and disability of chronic inflammatory disease characterized.

◆The present study, we ilustrate the mechanism of inflammatory response, improvation of early pathological changes of morphological structure and its anti-inflammatory damage in early RA.

METHOD

※Rats were grouped into control group (NS), Kashiwagi alcohol high, medium, low (40,20,10mg/kg)dose groups, dexamethasone group (0.525mg/kg), the weight of granuloma were measured at last.

※Rats were randomly grouped into: kashiwagi alcohol high,medium, low dose groups, indomethacin group (10mg /kg),gingerol group(500mg/kg) and control group, After the last administration. carrageenan was injected into rat paw to build the rat model of adjuvant induced joint swelling.After the last administr-ation, the paw volume and pain threshold were measured by instrument.

RESULTS

A.Kashiwagi alcohol can significantly inhibite the rat granuloma.Compared with the saline control group, there was significant differ- ence (P <0.05).Compared with the hydroco- rtisone group was no significant difference (P> 0.05) B.Swelling of the indome- thacin group dec- reased significantly compared with the con- trol group (p<0.01).Swelling of Kashiwagi alcohol group decreased significantly compared with the control group (p<0.01),and positively correlated with the dose.Swelling of gingerol group decreased relative to the control group (p <0.05).

table2.Impact of Kashiwagi alcohol on carrageenan induced rat paw edema

		Swelling (ml)			Inhibition(%)		
group	Induced granuloma ory	1h	3h	5h	1h	3h	5h
Blank	1.68±0.15	0.44±0.14	0.93±0.16	0.80±0.13			
Ind.	1.66±0.17	0.27±0.06	0.36±0.05	0.34±0.07	0.389	0.616	0.575
H	1.62±0.13	0.30±0.10**	0.69±0.13**	0.65±0.09**	0.306	0.251	0.187
M	1.69±0.16	0.32±0.08**	0.72±0.15**	0.67±0.11**	0.265	0.224	0.167
L	1.64±0.11	0.34±0.05**	0.82±0.09**	0.76±0.07**	0.211	0.121	0.052
Gingerol	1.54±0.18	0.39±0.07**	0.89±0.11**	0.79±0.12**	0.101	0.039	0.018

Compared with the control group: #p<0.05, # # p<0.01

Summary

table1.Impact of Kashiwagi alcohol on carrageenan induced rat paw edema

	dose mg/kg	Weight (g)	
		paper	Tampon
blank	0	0.5000±0.009	0.0655±0.002
positive	0.525	0.0265±0.009--	0.0202±0.002--
high	40.0	0.0251±0.006--	0.0544±0.007--
medium	20.0	0.0255±0.004--	0.0625±0.007-
low	10.0	0.0512±0.002--	0.0665±0.007

Compared with the control group: #p<0.05, # # p<0.01

table3.Rat pawtenderness values (g)

	0h	2h	4h	6h
blank	296.333	259.367	256.6	237.45
Indomethacin	282.92T	382.26T	316.933	354.15
high	264.133	316.6	301.2	308.55
medium	250.85	310.225	291.233	283.317
low	250.25	279.933	269.55	260.075
Gingerol	241.525	256.275	296.475	299.225

CONCLUSION

➢These findings suggest that :

➢Kashiwagi alcohol has anti-inflammatory effects in rat granuloma model.It can reduce the weight of granulation tissue in rats and can better control the early inflammatory response in rats.

➢Kashiwagi alcohol reduce the symptoms in mice 24h after the model carrageenan-induced edema,at the same time,decrease the tenderness value, indicating that there is resistance to acute inflammation in a dose-dependent.

25. 扬州大学

题目：百日咳杆菌效应 CpG-ODNs 调控变应性哮喘 TOLL 信号分子的实验研究

作者：张宝袁　　指导老师：孙　云

The experimental research on modulating Toll-like signal path of allergic asthma with CpG-ODNs from *Bordetella pertussis*

ZHANG Bao-yuan, CHI Shen, SUN Yun*
Department of Pharmacology, Medical College of Yangzhou University, Yangzhou 225001, China

INTRODUCTION

◆Allergic asthma is one chronic airway inflammation with overexpression of IgE, infiltration of eosinophil and mast cells, airway hyperresponse and remodeling.

◆Th1/Th2 balance becomes the most important principle of preventing and therapeutic of asthma, many scientists found that Th1/Th2 balance could be regulated by some Chinese traditional drugs, vaccine of whole cell of bacteria, chemicals and CpG-ODNs.

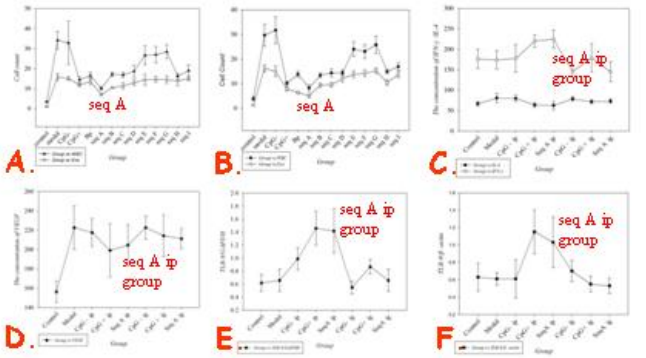
◆CpG-ODNs are one kind of PAMP, they can enter cell by inner swallow and combine with TLR-9 to be dimmers, after that TLR signal way would be activated to stimulate NF-kappa B and AP-1, then Th1/Th2 balance would be modulated to preventing and therapeutic of asthma.

◆Bordetella pertussis is one sort of microorganism which can induce cough and asthma. Vaccine of Bordetella pertussis could reduce the morbidity. CpG-ODNs extracted from Bordetella pertussis may be one potent intense Th1 inducer. So we studied the mechanism and function of the CpG-ODNs for regulating asthma.

METHOD

■ICR mice were sensitized with OVA to be acute or chronic allergic asthma model accompany administration of CpG-ODNs by ip and ig routes, and the BALF was extracted for counting inflammatory cell, measuring the concentration of Th1/Th2 cytokines and VEGF by ELISA protocol, TLR-9 mRNA of spleen and the protein of lung were extracted from the tissues and measured by RT-PCR and western blot.

RESULTS



A. The number of EOS and WBC in BALF after administration of *CpG-ODNs* from Bordetella pertussis by ig route.

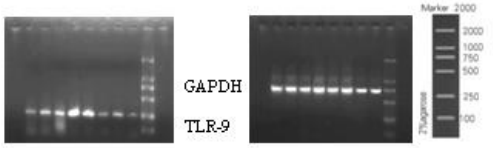
B. The number of EOS and WBC in BALF after administration of *CpG-ODNs* from Bordetella pertussis by ip route.

C. The concentration of IL-4 and IFN-γ in BALF after administration of *CpG-ODNs* from Bordetella pertussis.

D. The concentration of VEGF in BALF after administration of *CpG-ODNs* from Bordetella pertussis.

E. The expression of TLR-9 mRNA in spleen after administration of *CpG-ODNs* from Bordetella pertussis.

F. The expression of TLR-9 protein in lung after administration of *CpG-ODNs* from Bordetella pertussis.



Expression of TLR-9 mRNA in spleen of mice. Total RNA was exacted from spleen with Trizol reagent. Expression of TLR-9 mRNA was determined by reverse transcription polymerase chan reaction (RT-PCR). We use 2% gel to analyse cDNA by agarose-gel electrophoresis. Lane: 1: Control group; 2: Model group; 3: CpG- ip group; 4: CpG+ ip group; 5: seq A ip group; 6: CpG- ig group; 7: CpG+ ig group; 8: seq A ig group; 9: DNA Marker DL 2000.

Expression of TLR-9 protein in lung of mice. Protein was obtained by RIPA buffer from lung tissue and resolved on 10% SDS-polyacrylamide gels. After that, the protein bands were transferred onto polyvinylidene fluoride membranes, blocked 2h with 3% bull serum albumin(BSA) and incubated with Anti-Mouse TLR-9 antibody(1:500) all over a night. After that, the membranes were incubated with HRP-Goat Anti-Mouse IgG (1:1000). After washing, the membranes was incubated with Diaminobenzidine for colobration. Lane: 1: Control group; 2: Model group; 3: CpG- ip group; 4: CpG+ ip group; 5: seq A ip group; 6: CpG- ig group; 7: CpG+ ig group; 8: seq A ig group.

CONCLUSION

These findings suggest that *CpG-ODNs* extracted Bordetella pertussis can modulate Th1/Th2 to reaulate asthma through TLR-9 signal way:

- **Regulate inflammatory cells (WBC/EOS);**
- **Modulate Th1/Th2 cytokines (IFN-γ/IL-4) and can not modulate VEGF;**
- **Upregulate TLR-9 mRNA in spleen and TLR-9 protein in lung.**
- **Administrating *CpG-ODNs* by ip route is better than that by ig route.**

揚州大学 YANGZHOU UNIVERSITY

26. 扬州大学

题目：山绿茶总黄酮对 AS 大鼠模型的影响

作者：邱　夏　　指导老师：孙　云

Effect of of hainan holly leaf total flavonoids on AS model in rats

QIU Xia ， SUN Yun* ， College of Medicine, Yangzhou University

INTRODUCTION

◆Over the past decade, we have com e to appreciate a prominent role for inflamm-ation in atherosclerosis and its complications. It is now well-established that from early lesion to vulnerable plaque formation, numerous in flammatory factors participate in the disease process.

◆ Hainan holly leaf have the efficacy of Clearing and detoxifying, swelling and pain, Huoxuetongmai.

◆In this study, we investigate the therapeutic action and mechanism of hainan holly leaf total flavonoids to inhibit CRP、TNF-α and atherosclerosis in rat with high fat diet.

METHOD

■SD rats were randomly divided into group , drug intervention for 8 week , Using automatic biochemical analyzer measured the level of serum lipid and measuring the level of TNF-α 、CRP in the serum by Elisa , observing liver's and aortic's morphological structure.

RESULTS

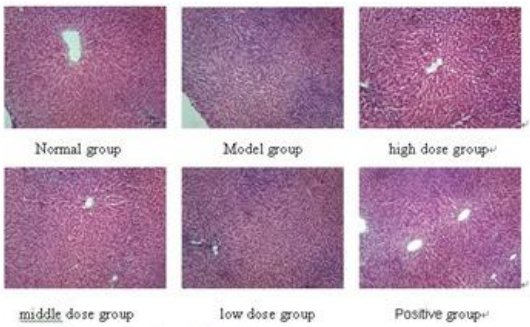


Figure 1 Hainan holly leaf total flavonoids effect on the pathology morphology of the liver (×200)

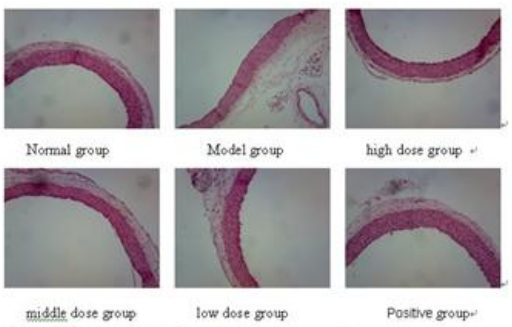


Figure 2 Hainan holly leaf total flavonoids effect on the morphology pathology of aortic (×200)

Table 1. Effect of hainan holly leaf total flavonoids on the value of serum lipid level in AS rat ($\bar{x}\pm s$)

group	TC(mmol/L)	TG(mmol/L)	LDL-C(mmol/L)	HDL-C(mmol/L)
Normal group	1.46±0.11**	0.37±0.04**	0.12±0.02**	1.75±0.06
Model group	7.15±0.86ΔΔ	0.74±0.08ΔΔ	0.51±0.08	1.22±0.08ΔΔ
Positive group	6.07±0.78**	0.53±0.06**	0.37±0.04**	1.77±0.09**
High dose	5.88±0.85	0.44±0.03**Δ	0.39±0.05**	1.79±0.03**
middle dose	6.71±0.87Δ	0.47±0.05**	0.44±0.05Δ	1.74±0.05**
low dose	7.08±0.93ΔΔ	0.49±0.07**	0.48±0.07	1.70±0.07**

Note: Compared with normal group: Δ p <0.05, ΔΔ p <0.01; Compared with model group: * p<0.05, ** p <0.01.

Table 2. Effect of hainan holly leaf total flavonoids on the value of serum level of TNF-α、CRP in AS rat ($\bar{x}\pm s$)

group	n	TNF-α (ng/L)	CRP (μg/L)
Normal group	10	50.19±5.48**	1.85±0.36**
Model group	10	84.98±3.95ΔΔ	2.76±0.25ΔΔ
Positive group	10	52.56±4.63**	1.95±0.14**
High dose	10	51.25±3.58**	1.89±0.05**
Middle dose	10	54.76±5.72**	1.97±0.34**
low dose	10	56.43±4.37**	2.03±0.21**

Note: Compared with normal group: Δ p <0.05, ΔΔ p <0.01; Compared with model group: * p<0.05, ** p <0.01.

CONCLUSION

Hainan holly leaf total flavonoids can inhibit the inflammatory reaction and reduce the generation of TNF-α and CRP , so as to reduce the formation of foam cell, and then inhibit the formation of atherosclerotic plaque , prevent or delay occurrence and development of atherosclerosis.

27. 扬州大学

题目：半枝莲总黄酮降低细胞磷脂转运蛋白表达的实验研究

作者：祝娉婷　　指导老师：卜　平

The effect of Barbata flavonoids interfere with the expression of PLTP from recombinant PLTP gene cell

ZHU Ping-Ting BO Ping* Sun Yun Li Xiang-Ming Zhen Xin-Mei

Medical School of Yangzhou University

INTRODUCTION

◆phospholipid transfer protein(PLTP) is one combine protein of which molecular weight is 81 Kda, it is mainly synthesised in lung and liver.

◆PLTP makes an inportant role in the metabolism and reconstruction of high density lipoprotein(HDL), it can affect the function of HDL and modify the processes of atherosclerosis(AS).

◆Epidemiologic Studies found the concentration of PLTP is extremely high in the serum of AS patients.

◆There is no report for therapeutic of AS through PLTP signal way in China.

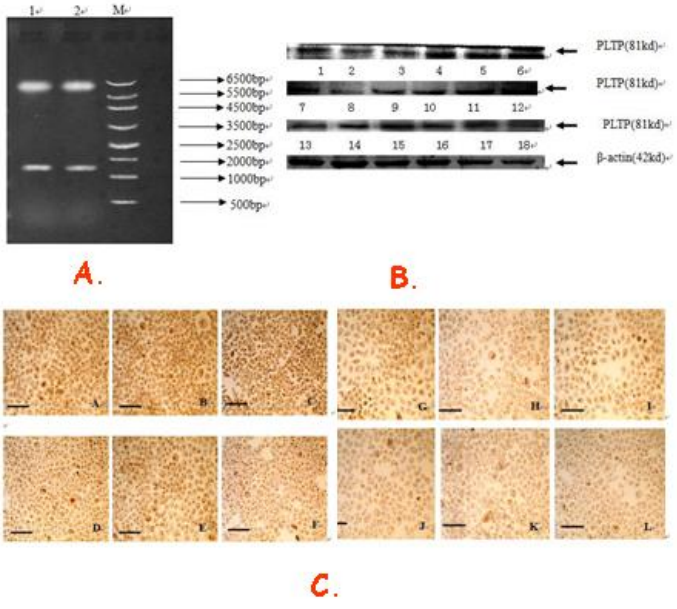
◆We construct recombinant PLTP gene cell and evaluate the effects mechanisms of Barbata flavonoids,through the test of their suppressive function of PLTP in the Bel-7402 cells.

METHOD

■PLTP cDNA flag was amplified with reverse transcription-polymerase chain reaction(RT-PCR), PLTP/p3XFLAG-CMV-14 was constructed and transfected into Bel-7402 cells. The Bel-7402 cells influenced by Barbata flavonoids were analyzed by immunohistochemical assay.

RESULTS

TLRs mRNA expression in mDCs (upper graphs) and % decrease relative to unstimulated cultures (lower graphs)



A Enzyme cut of plasmid PLTP/p3XFLAG-CMV-14

B. Western blot analysis for the expression of PLTP treated with Barbata flavonoids

C. Immunocytochemical staining of PLTP treated with Barbata flavonoids after 24h and 48h (×200)
A:model for 24h; B: model for 48h ; C: treated 24h(40 mg/L); D: treated 48h(40 mg/L); E: treated 24h(80 mg/L); F: treated 48h(80 mg/L);G:treated 24h(160 mg/L); H:treated 48h(160 mg/L); I:treated 24h(Guggulsterones 80 mg/L); J:treated 48h(Guggulsterones 80 mg/L); K:treated 24h(Ginkgo Capsule 80 mg/L); L:treated 48h(Ginkgo Capsule 80 mg/L) Bar=40um

CONCLUSION

The total flavone can inhibit the Lipid peroxidation, Free Radical Scavenging, blood lipid regulation, platelet aggregation, Increased capillary permeability, increased brittleness, the formation and development of AS.

Our research is basis on receiving PLTP cDNA from the tissue of fetal liver and constructing gene recombination cells. The expression of PLTP of constructing gene recombination cells was down regulated after the administration of Barbata flavonoids. It means the function of Barbata flavonoids may concern with the inhibition of PLTP and effecting the route of metabolism of lipid protein.

28. 扬州大学

题目：南蛇藤提取物通过血管内皮生长因子信号转导通路抑制肿瘤血管生成并从体内和体内说明其对肝癌的抗癌活性

作者：钱亚云　指导老师：刘延庆

Celastrus orbiculatus extract inhibits tumor angiogenesis by targeting VEGF signaling pathways and shows potent antitumor activity in hepatocarcinomas *in vitro* and *in vivo*

Yayun Qian[a, *], Hua Zhang[a], Ying Hou[a], Lin Yuan[a, b], Guoqing Li[a], Shiyu Guo[c], Hisamits Tadashi[c], Yanqing Liu[a, *]

INTRODUCTION

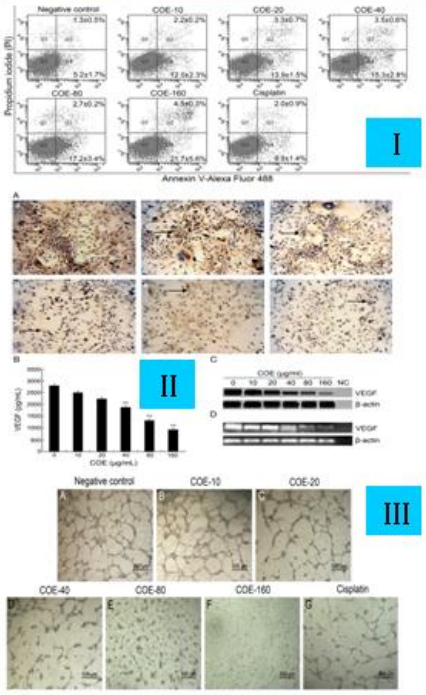
Celastrus orbiculatus Thunb has been used for thousands of years in China as a remedy against cancer and inflammatory diseases.

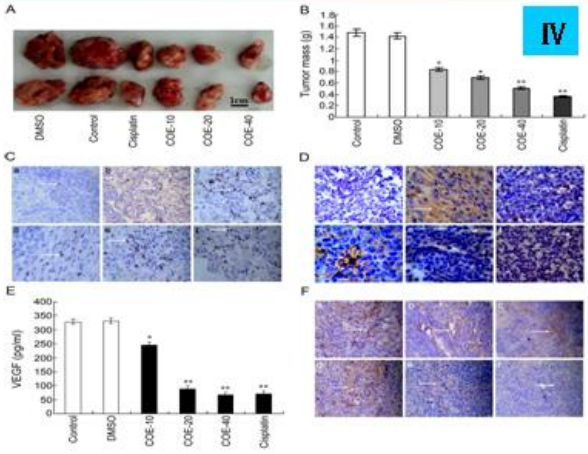
This study aims to investigate whether *C. orbiculatus extract* (COE) could inhibit angiogenesis, which is the pivotal step in tumor growth, invasiveness, and metastasis.

METHOD

In this study, the extract from the stem of Celastrus orbiculatus was used. Mouse hepatic carcinoma cells (Hepal-6) were treated with COE in different nontoxic concentrations (10, 20, 40, 80, and 160 μ g/mL). The mRNA and protein expression levels of vascular endothelial growth factor (VEGF) were detected by RT-PCR and Western Blot, respectively; the active fractions were further tested on C57BL/6 mice and human umbilical vein endothelial cells (HUVEC) for any anti-angiogenic effects.

RESULTS





Ⅰ, COE significantly inhibited proliferation and induced apoptosis in Hepal-6 cells.

Ⅱ, COE inhibited VEGF expression at both the mRNA and protein levels.

Ⅲ, COE inhibited the formation of the capillary-like structure in primary cultured HUVEC in a dose-dependent manner.

Ⅳ, *In vivo*, COE significantly reduced the volume and weight of solid tumors with low adverse effects, and it decreased tumor angiogenesis.

CONCLUSION

COE could be used to treat hepatic carcinoma. The mechanisms of the antitumor activity of COE may possibly be due to its effects against tumor angiogenesis by targeting the VEGF protein.

Funded by: Plans of Colleges and Universities in Jiangsu Province to Postgraduate Research and Innovation (No.CX09B-321Z) and State Administration (No. 04-05ZP35) of Traditional Chinese Medicine of People's Republic of China.

29. 扬州大学

题目：南蛇藤提取物对 HCCLM6 细胞增殖及细胞中 VEGF-C 表达的抑制作用

作者：张　华　　指导老师：刘延庆

Inhibition of Celastrus orbiculatus Extracts on Proliferation and VEGF-C Expressionin HCCLM6 Ce

ZHANG Hua, QIAN Ya-yun ,YUAN Lin, HOU Ying, LIU Yan-qing
Medical College of Yangzhou University

INTRODUCTION

◆Hepatocellular carcinoma(HCC)is the secondary cancer "killer" in China. Metastatic recurrence is still the main obstacle to the improvement of treatment efficacy. Therefore, researches on metastasis and recurrence are still important issues.

◆Studies have shown a correlation between tumor expression of Vascular endothelial growth factor C (VEGF-C) and lymph node metastasis. VEGF-C induces lymphangiogenesis via VEGFR-3 and stimulates metastasis.

◆*Celastrus orbiculatus* Thumb is a plant belonging to Celastraceae , which has been used traditionally in the treatment of arthritis and other inflammatory diseases. Some studies have shown that *C. orbiculatus* extracts(COE) exhibits potent antitumor activity with low adverse effects in vitro and in vivo.

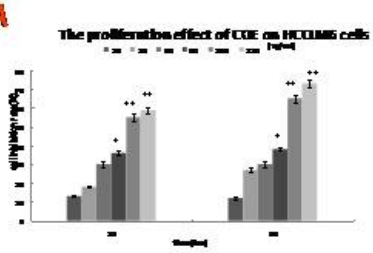
◆Here we investigated whether COE could inhibite Proliferation and VEGF-C Expression and increase the activities of p38 MAPK to some degree in HCCLM6 Cells.

METHOD

- The proliferation inhibition effect of COE on HCCLM6 cells was detected by microculture tetrazolium (MTT) assay.
- Immunohistochemistry was applied to examine the protein expression of VEGF-C.
- Phosphorylation activity of p38 MAPK was detected by Western Blotting assay.

RESULTS

A

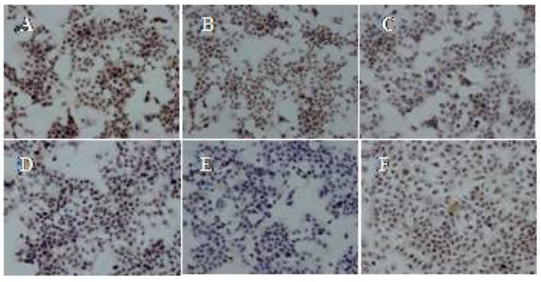


A. COE could significantly inhibit HCCLM6 cells proliferation.

B. COE could inhibit VEGF-c expression in HCCLM6 cells

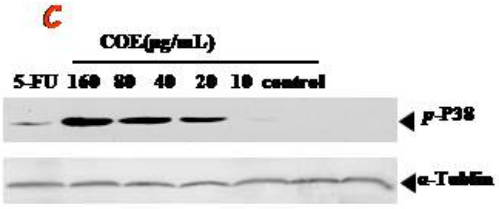
C. COE could increase the activities of p38MAPK to some degree in HCCLM6 Cells.

B



A: control B: COE20ug/ml C: COE40ug/ml
D: COE80ug/ml E: COE160ug/ml F: 5-Fu

C



CONCLUSION

These findings suggest that COE can significantly :

- inhibit HCCLM6 cells proliferation
- inhibit VEGF-C expression of HCCLM6 cells
- increase the activities of p38 in HCCLM6 cells

30. 苏州大学

题目：蛇床子素通过抑制心肌 TGF-β1 的表达，治疗异丙肾上腺素诱导的小鼠心肌纤维化

作者：陈　蓉　　指导老师：谢梅林

Osthole inhibits isoprenaline-induced myocardial fibrosis in mice by reduction of myocardial TGF- β 1 expression

Rong Chen (陈蓉) , Mei-lin Xie (谢梅林)

Department of Pharmacology, College of Pharmaceutical Science, Soochow University

INTRODUCTION

✱ Myocardial fibrosis occurs with hypertension, myocardial infarction and heart failure, and so on. Transforming growth factor (TGF)- β 1 is critical for the generation and development of myocardial fibrosis.

✱ Peroxisome proliferator-activated receptor (PPAR) α and PPAR γ ligands may inhibit the fibrotic gene expressions through interference with nuclear factor (NF)- κ B. NF- κ B is a nuclear transcription factor that initiates the gene transcription of profibrogenic mediators, such as TGF- β 1.

✱ Osthole, an active constituent isolated from the fruit of *Cnidium monnieri* (L.) Cusson, may be a dual PPAR α / γ agonist.

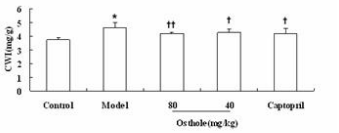
✱ In the present study, we investigated the inhibitory effect of osthole on myocardial fibrotic formation in mice and its possible mechanisms.

METHODS

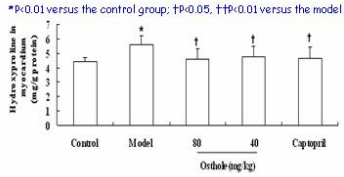
✱A mouse model with myocardial fibrosis was induced by hypodermic injection of isoprenaline (ISO) when these mice were simultaneously treated with osthole 40mg/kg, 80 mg/kg and positive drug captopril 25 mg/kg for 40 days.

✱ The cardiac weight index (CWI) and hydroxyproline content in myocardial tissue were determined, partial myocardial tissues were embedded in paraffin for H&E and Masson staining and immunohistochemistry assay, respectively. The rest of the ventricles were quickly frozen for measurement of RT-PCR.

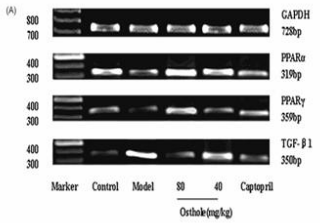
RESULTS

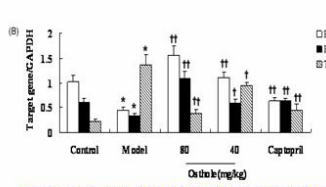


*P<0.01 versus the control group; †P<0.05, ††P<0.01 versus the model group.

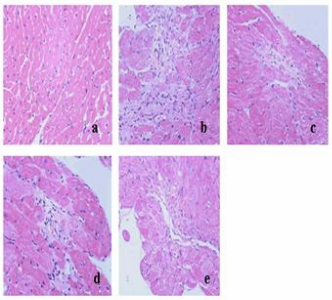


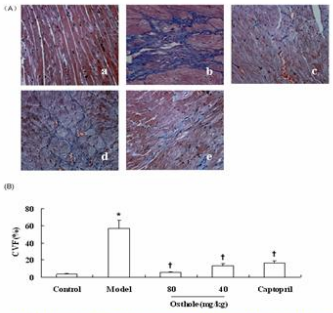
*P<0.01 versus the control group; †P<0.01versus the model group.



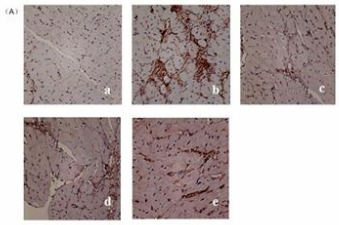


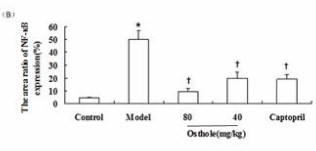
*P<0.01 versus the control group; †P<0.05, ††P<0.01 versus the model group.



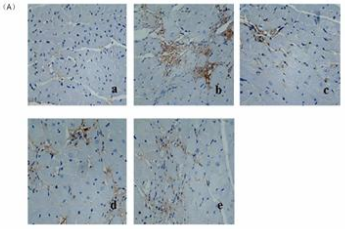


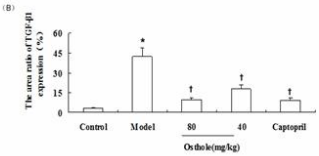
*P<0.01 versus the control group; †P<0.01versus the model group.





*P<0.01 versus the control group; †P<0.01versus the model group.





*P<0.01 versus the control group; †P<0.01versus the model group.

✱ CWI and hydroxyproline content in myocardial tissue were decreased;

✱ The degree of collagen accumulation in heart was improved significantly;

✱ The mRNA expressions of PPAR α / γ in myocardial tissue were increased, while the mRNA expression of TGF- β 1 and protein levels of NF- κ B and TGF- β 1 in myocardial tissue were decreased.

a: Control; b: Model; c: Osthole 80mg/kg; d: Osthole 40mg/kg; e: Captopril

CONCLUSION

✱ Osthole can prevent ISO-induced myocardial fibrosis in mice;

✱ Its mechanisms may be related to reduction of TGF- β 1 expression via activation of PPAR α / γ and subsequent inhibition of NF- κ B in myocardial tissue.

This work was supported by grants from the Postgraduate Innovative Foundation of Jiangsu Province (№ CX10B-056Z)

31. 苏州大学

题目：蛇床子素通过增强脂肪肝大鼠脂联素释放改善胰岛素抵抗

作者：亓志刚　　指导老师：谢梅林

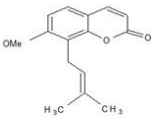
Osthole Ameliorates Insulin Resistance by Increment of Adiponectin Release in High-Fat and High-Sucrose-Induced Fatty Liver Rats

Zhi-gang Qi (亓志刚), Mei-lin Xie (谢梅林)
Department of Pharmacology, College of Pharmaceutical Science, Soochow University

INTRODUCTION

◆Insulin resistance (IR) is defined as a decreased response of the peripheral tissues to insulin action, and is an important pathophysiological mechanism in the development of fatty liver.
◆Peroxisome proliferator-activated receptors (PPARs), including three subtypes of PPARα, β/δ and γ, belong to the nuclear receptor family. They may control the glucose and lipid metabolism.
◆Osthole is an active compound isolated from the fruit of *Cnidium monnieri* (L.) Cusson, a traditional Chinese medicine.
◆ Our previous studies and literature data suggested that osthole might be a dual PPARα/γ agonist.
◆In the present study, we further investigated the effect of osthole on IR in high-fat and high-sucrose-induced rat model and its possible mechanism of treating IR.

Chemical structure of osthole.

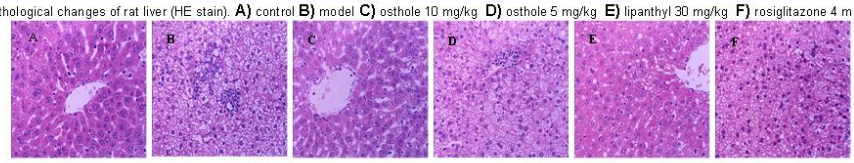


METHODS

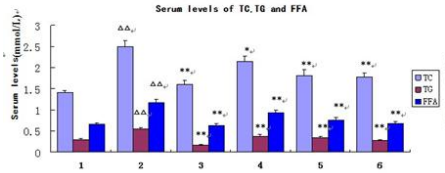
➢ The rat model was established by orally feeding high-fat and high-sucrose emulsion by gavage for 9 weeks. The experimental rats were treated with osthole 5 and 10 mg/kg, lipanthyl 30 mg/kg, and rosiglitazone 4 mg/kg after oral high-fat and high-sucrose emulsion for 6 weeks and were sacrificed 4 weeks after administration.
➢The total cholesterol (TC), triglycerides (TG) and free fatty acids (FFA) in serum and hepatic tissue, fasting blood glucose (FBG), fasting serum insulin (FINS), serum adiponectin and liver weight were measured. The homeostasis model assessment of insulin resistance (HOMA-IR) and coefficient of hepatic weight were calculated.
➢We also determine the effect of osthole on related target gene expressions such as carnitine palmitoyltransferase 1 (CPT-1), diacylglycerol acyltransferase (DGAT), and sterol regulatory element binding protein-1c (SREBP-1c) in the liver, and glucose transporter-4 (GLUT-4) in skeletal muscle.

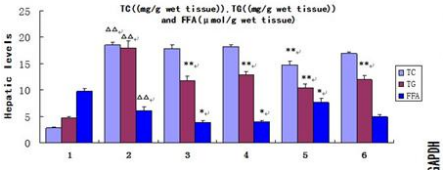
RESULTS

Histopathological changes of rat liver (HE stain). **A)** control **B)** model **C)** osthole 10 mg/kg **D)** osthole 5 mg/kg **E)** lipanthyl 30 mg/kg **F)** rosiglitazone 4 mg/kg.

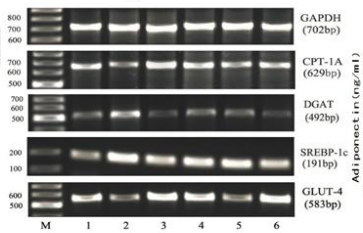


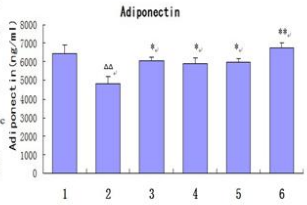
Data are presented as mean ± SEM. ΔΔ*P*<0.01 *vs.* contro1; **P*<0.05, ** *P*<0.01 *vs.* model.
1: control; **2:** model; **3:** osthole 10 mg/kg; **4:** osthole 5 mg/kg; **5:** lipanthyl 30 mg/kg; **6:** rosiglitazone 4 mg/kg

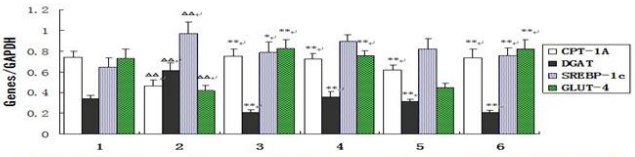




Genes measured by RT-PCR







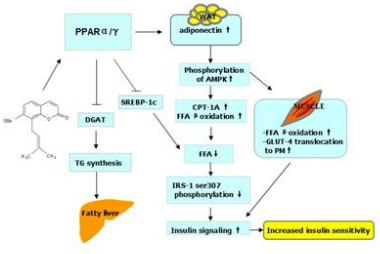
Levels of FBG, FINS and HOMA-IR

Group	FINS (ng/mL)	FBG (mmol/L)	HOMA-IR
1	338.3±14.0	6.00±0.12	90.0±4.0
2	402.5±21.0ΔΔ	7.33±0.17ΔΔ	130.6±6.6ΔΔ
3	301.8±8.9**	6.52±0.19**	87.4±2.9**
4	286.5±18.3**	7.07±0.16	90.7±6.6**
5	274.0±19.2**	8.63±0.21**	106.2±9.2**
6	306.4±15.3**	6.56±0.19**	90.2±6.6**

➢After treatment with osthole, the serum levels of TC, TG and FFA, the contents of TG and FFA in hepatic tissue and body weight gain were lowered, especially in the osthole 10 mg/kg group.
➢The histological evaluation of liver specimens demonstrated that the steatosis and inflammation in liver in osthole-treated groups were improved, especially in the 10 mg/kg group.
➢ Importantly, the levels of FBG, FINS and HOMA-IR in the osthole 10 mg/kg group were decreased, while the level of serum adiponectin in the osthole-treated groups, like PPARα agonist lipanthyl and PPARγ agonist rosiglitazone, was increased.

CONCLUSION

I. Osthole can improve the IR induced by high-fat and high-sucrose emulsion in fatty liver rats.
II. Osthole may activate PPARα or/and γ, and increase the expression and release of adiponectin in WAT. Adiponectin then stimulates the phosphorylation of AMPK and activates AMPK, and subsequently increases the rate of skeletal muscle fatty acid β-oxidation via increment of CPT-1 expression, the serine phosphorylation of IRS-1 stimulated by overmuch FFA interrupting insulin signaling, and then causing IR is also decreased. Insulin signaling cascade is improved by doing so.
III. Another key effect of AMPK activation in skeletal muscle is stimulation of glucose uptake due to increasing expression of GLUT4 and its translocation to plasma membrane.



32. 徐州医学院

题目：黄连素对链挫霉素诱导的糖尿病大鼠脑内淀粉 beta 缩氨酸水平的提高无作用

作者：刘耀武　　指导老师：刘晓东

No influence of berberine on the increased amyloid beta-peptide (1-40) levels in brain of streptozotocin-induced diabetic rats

Yao-wu Liu[a,b], Li Liu[b], Xiao-dong Liu[b,*] [a]*Department of Pharmacology, Xuzhou Medical College, Xuzhou 221002,* [b]*Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009.*

INTRODUCTION

◆Progressive accumulation of the amyloid beta-peptide (Aβ) appears in brain of diabetic rats, which maybe one of causes to the CNS complication of diabetes mellitus (DM).

◆Berberine(Ber) (benzyltetrahydroxyquinoline), an isoquinoline-type alkaloid, is a major ingredient of Huanglian (*Coptidis rhizome*), also exists in Huangbai etc.

◆Besides antidiarrheal, antimicrobial, and anti-inflammatory effects, Ber had innovative pharmacological activities, such as cholesterol-lowering drug with a mechanism of action differing from that of the statins, blood glucose-lowering drug as an effective insulin sensitizing and insulinotropic agent.

◆Ber was an AchE inhibitor similar to Galanthamine and a low-molecular-weight neurotrophic drug to neurodegeneration disorder.

◆Ber could decrease extracellular Aβ levels by modulating Aβ precursor protein processing in the cultured H4 neuroglioma (APPNL-H4) cells.

◆Here, we will examine whether Ber has a comprehensive effect on clearance of the elevated Aβ in brain of streptozotocin-induced diabetic rats.

METHOD

■Diabetic rats were induced by i.p. administration of 65 mg/kg streptozotocin (STZ). Rats with fasting blood glucose level higher than 11.1 mM on 7th day of STZ injection were considered to be diabetic rats, which were randomly divided into 2 groups, administrated i.g. Ber (100mg/kg) and 0.25% CMC-Na respectively once a day for four weeks. The body weight and FBG level were monitored once every two weeks. Temporal cortex and hippocampus were obtained for Aβ (1-40) with an ELISA kit on the 35th day after induction.

RESULTS

Table 1 Body weights and plasma glucose levels of the age-matched normal (Cont.), diabetic (DM), and Ber-treated diabetic (DM + Ber) rats after STZ or vehicle injection.

	Body weight (g)			Blood glucose (mM)		
	Cont.	DM	DM + Ber	Cont.	DM	DM + Ber
Week 1	193 ± 5	163 ± 7**	165 ± 6**	6.17 ± 0.47	18.49 ± 4.35**	17.74 ± 5.96**
Week 3	264 ± 12	168 ± 21**	167 ± 19**	6.19 ± 0.75	23.98 ± 5.60**	15.77 ± 7.67##**
Week 5	294 ± 17	157 ± 34**	165 ± 33**	6.10 ± 0.87	30.01 ± 2.45**	13.51 ± 7.37##**

Data are means ± SD (n=6). ** p<0.01, when compared with the normal. ## p<0.01, when compared with the diabetic.

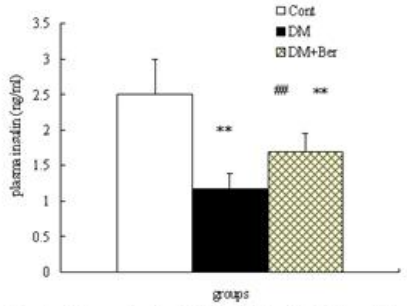


Fig.1. Levels of plasma insulin in Cont., DM and DM+Ber rats five weeks after STZ or vehicle induction. Insulin level in plasma was measured with an ELISA kit. * * p<0.01 when compared with the control rats, ## p<0.01 when compared with the diabetic rats (n=6).

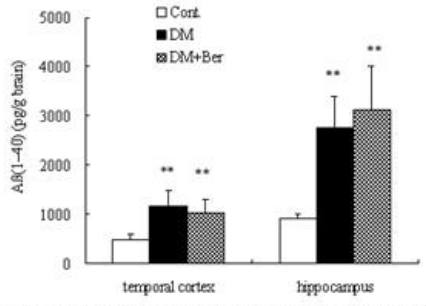


Fig.2. Levels of brain Aβ (1-40) in Cont., DM and DM+Ber rats five weeks after STZ or vehicle induction. Aβ (1-40) level in brain was measured with an ELISA kit. * *p<0.01 when compared with the control rats (n=6).

CONCLUSION

➢**Ber actually had an effect on decreasing blood glucose and increasing insulin level in plasma in STZ-induced diabetic rats.**

➢**Ber did not exert an effect on the elevated Aβ (1-40) levels in brain of STZ-induced rats by i.g. administration for its trace plasma concentration mostly caused by its both poor intestinal absorption and rapid substantial biotransformation in the liver.**

This work was supported by the National Science foundation of China (No. 30672499) and the Project of Innovation in Graduate Education, Jiangsu Province, China (No. 2006).

33. 徐州医学院

题目：通过调节ERK1/2，cPLA$_2$及Bcl-2/Bax调控缺氧/再灌注:盐酸戊乙奎醚的一种潜在神经保护效应

作者：王　允　　指导老师：谷淑玲

Regulated hypoxia/reperfusion-dependent modulation of ERK1/2, cPLA$_2$ and Bcl-2/Bax : a potential mechanism of neuroprotective effect of penehyclidine hydrochloride

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School of pharmacy, XuZhou Medical College
1 These two authors contributed to this work equally
* Corresponding Author

INTRODUCTION

◆Stroke is the leading cause of morbidity and a common cause of mortality worldwide, however, the pathophysiology involved in ischemic stroke is complex and not yet fully understood.

◆Recently, researches show that PHC (an anticholinergic agent, with only high degree of selectivity for M1 and M3 receptor subtypes) can exert protective effects on cerebral ischemia. We also showed PHC could reduce the forebrain ischemia-reperfusion injury in gerbils. However, the mechanism is not well known.

◆It seems more plausible that maissive Ach could act on M1 receptors, then activate ERK1/2 and cPLA$_2$ signal, and yield ischemic damage.

◆ We hypothesize that PHC maybe depress the activation of ERK1/2-cPLA$_2$ pathway, then regulate expression of Bcl-2 and Bax as well as Caspase-3, finally relieve ischemia damage.

徐州医学院 药学院 School of Pharmacy , Xuzhou Medical College

METHOD

■Hippocampal slices subjected to hypoxia/reoxygenation were used in this study. The structure of the hippocampal CA1 area pyramidal cells was observed by HE staining. Expression of ERK1/2, cPLA$_2$, Caspase-3, Bcl-2 and Bax was detected by immunohistochemistry and/or western blotting method.

RESULTS

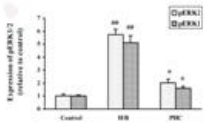


Fig 1

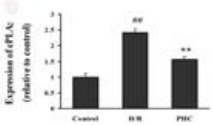


Fig 2

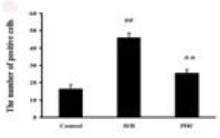


Fig 3

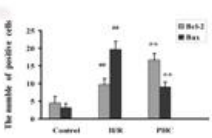


Fig 4

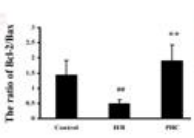


Fig 5

A. It was observed that the pattern of close arrangement of the hippocampus CA1 area pyramidal cells normal cells and their normality were significantly well presevered in PHC group.

B. PHC can reduce cell injury induced by H/R and depress expression of Caspase-3.

C. PHC can diminish expression of pERK1/2 and cPLA2 caused by H/R damage, depressed expression of Bax, highten expression of Bcl-2 and the ratio of Bcl-2/Bax.

CONCLUSION

Our study here strongly suggests that this protective effect of PHC may be exerted by regulating expression of pERK1/2, cPLA2 and Caspase-3 as well as the ratio of Bcl-2/Bax. As a anticholinergic agent, with only high degree of selectivity for M1 and M3 receptor subtypes, this study may widen the application of PHC and therapeutic agents of stroke.

Funded by: Fund of XuZhou Medical College and Open Fund of Jiangsu Province Key Laboratory of Anesthesiology

34. 徐州医学院

题目：左旋瓜氨酸对缺血再灌注引起的大鼠急性胃粘膜损伤的保护作用

作者：缑灵山　　指导老师：刘　毅

PROTECTIVE EFFECT OF L-CITRULLINE AGAINST ACUTE GASTRIC MUCOSAL LESIONS INDUCED BY ISCHEMIA-REPERFUSION IN RAT

Gou Lingshan (缑灵山), Yin Cui (尹翠), Yin Xiaoxing (印晓星), Liu Yi (刘毅)*
School of Pharmacy, Xuzhou Medical College, 84 West Huaihai Road, Xuzhou, Jiangsu, 221002, China

INTRODUCTION

◆NO plays a biphasic role in the ulcerogenic response of the gastric mucosa depending on the NOS isozyme; a protective effect by cNOS/NO, and a proulcerogenic effect by iNOS/NO.

◆It has been demonstrated that L-arginine can elicit a gastric protective effect by inhibiting the increased inducible NOS (iNOS) activity and by preserving constitutive NOS (cNOS) activity in the gastric mucosa.

◆In the kidney, vascular endothelium and other tissues, L-citrulline can be readily converted to L-arginine, thereby providing a recycling pathway for the conversion of L-citrulline to NO via L-arginine. Because of close relation to NO production in vivo, its heathcare and medicinal values are attracting increasing attention.

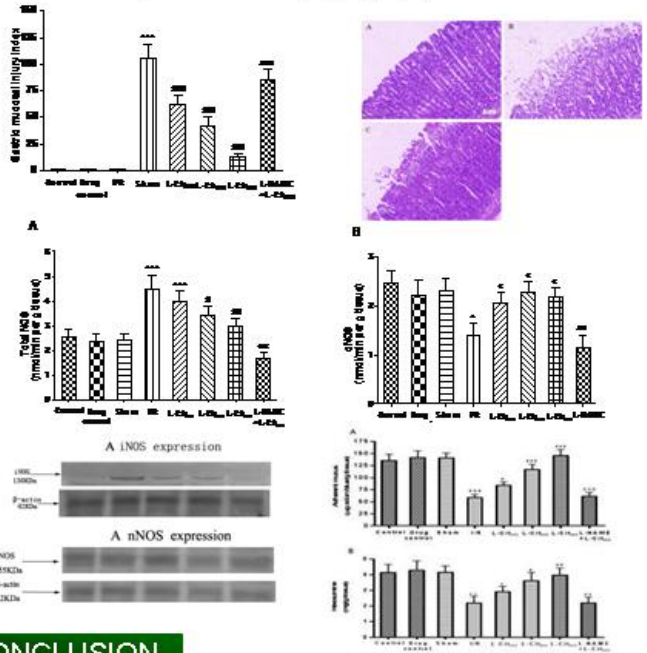
◆We hypothesized that L-citrulline might inhibit I/R-induced gastric mucosa injury by preserving NO production via cNOS in the gastric mucosal tissue, therefore we studied the gastric protective effect of L-citrulline using a gastric ischemia-reperfusion model.

METHOD

Sixty minutes before ischemia, L-citrulline at doses of 300, 600, 900 mg/kg was administered intragastrically. After the experiment, the stomachs were removed for biochemical and histological examinations. Moreover, the expression of iNOS and nNOS protein in the gastric mucosa were determined.

RESULTS

TLRs mRNA expression in mDCs (upper graphs) and % decrease relative to unstimulated cultures (lower graphs)



A L-citrulline had significant protective effect against I/R-induced gastric mucosal injury,. However, this protective effect was significantly reversed by prior administration of L-NAME

B. The MPO activities of L-citrulline groups were significantly decreased compared to the I/R group.

C. Treatment with L-citrulline significantly decreased the total NOS and iNOS activity compared to the I/R group. Moreover, pretreatment with L-citrulline induced a significantly increase of the cNOS activity up to sham group.

D. L-citrulline preadministration significantly attenuated the increase of iNOS expression induced by gastric I/R. In addition, no significant difference was found among I/R group, sham and L-citrulline-treated group.

CONCLUSION

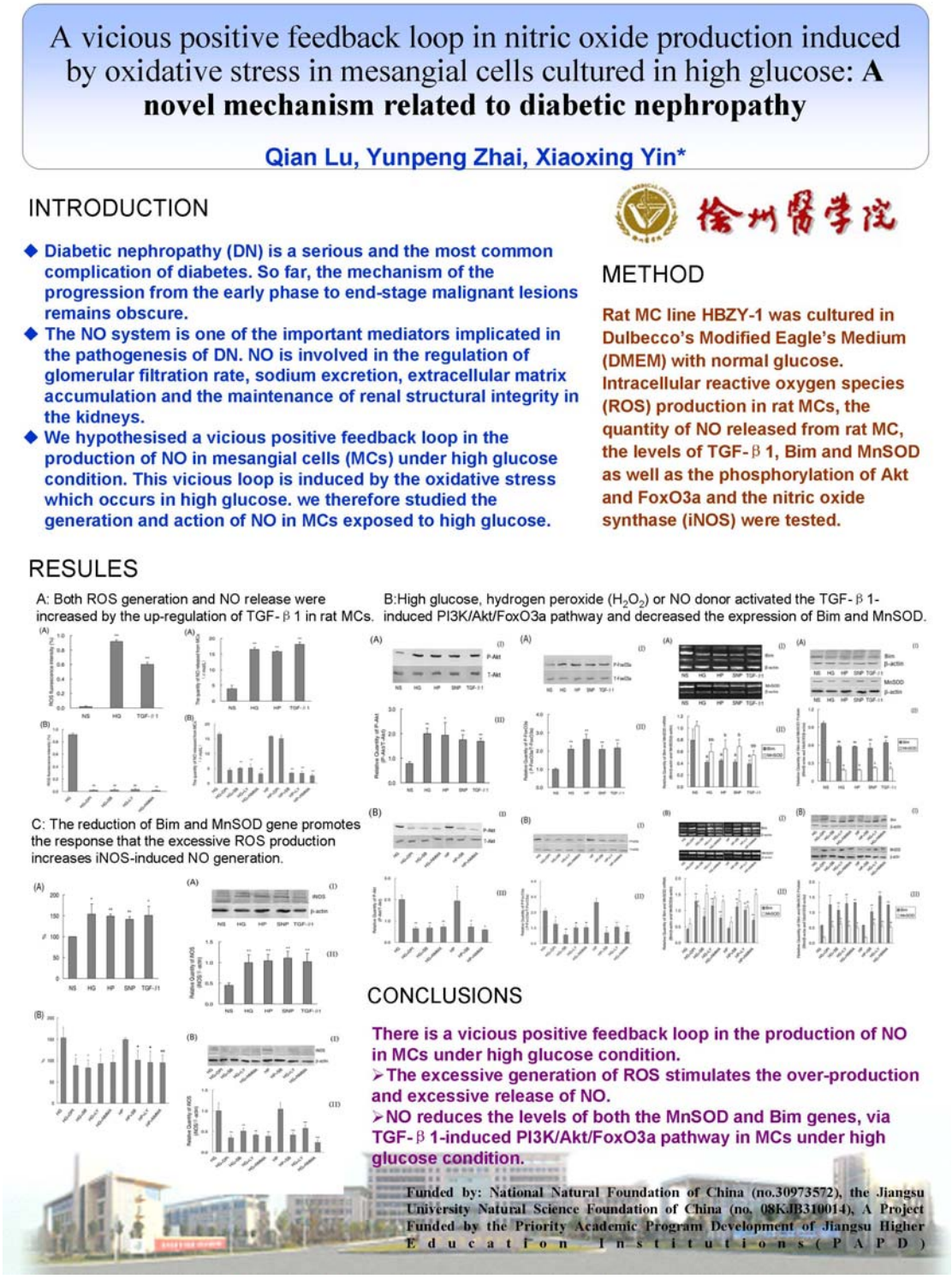
This study demonstrated that L-citrulline had protective effects on I/R-induced gastric injury in rats. This protective effect occurs, at least in part, via the preservation of mucus as well as reducing neutrophil infiltration into the gastric mucosa tissues, which are related to the maintenance of cNOS activity. Moreover, the suppression of an increase in gastric mucosal iNOS protein expression may be also involved in the gastric protective effect of L-citrulline.

Funded by: Natural Science Foundation of Jiangsu Province, Foundation of Xuzhou Medical College for Postgraduate.

35. 徐州医学院

题目：氧化应激诱导体外培养肾小球膜细胞 NO 产生的恶性正反馈通路:与糖尿病性肾病相关的新机制

作者：翟云鹏、鲁茜　　指导老师：印晓星



36. 徐州医学院

题目：甲基化寡核苷酸对体外培养人 786-0 肾癌细胞中 Ki-67 基因的影响

作者：牟　杰　　指导老师：印晓星

THE EFFECT OF METHYLATED OLIGONUCLETIDE TARGETING Ki-67 GENE IN HUMAN 786-0 RENAL CARCINOMA CELLS

Jie Mou, Yi Liu, Dao-Quan Tang, Xiao-Xing Yin*
School of Pharmacy, Xuzhou Medical College, Xuzhou 221002, China

INTRODUCTION

◆Ki-67 protein is strictly associated with cell proliferation. It is present during all active phases of the cell cycle, G1, S, G2, and M, but is absent from resting cells.

◆Ki-67 protein is not only associated with cell proliferation but also integrated into the regulatory protein network that drives the cell division cycle. Since the growth of tumor cells is frequently associated with high cell proliferation, Ki-67 may represent a potential target for cancer therapy.

◆DNA methylation occurs almost exclusively at CpG nucleotides and has an important contributing role in the regulation of gene expression and the silencing of repeat elements in the genome.

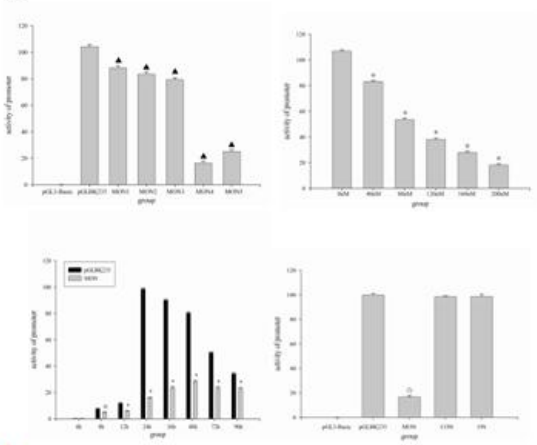
◆We hypothesised methylated oligonucleotide targeting Ki-67 gene could inhibit Ki-67 expression and consequent proliferation of human renal carcinoma cells.

METHOD

■The activity of Ki-67 promoter was detected by dual-luciferase reporter assay system. The expression of Ki-67 in 786-0 cells was detected by RT-PCR and immunohistochemistry respectively. The proliferation of 786-0 cells was determined by WST-8. The apoptosis was measured by Annexin V and Propidium Iodide .

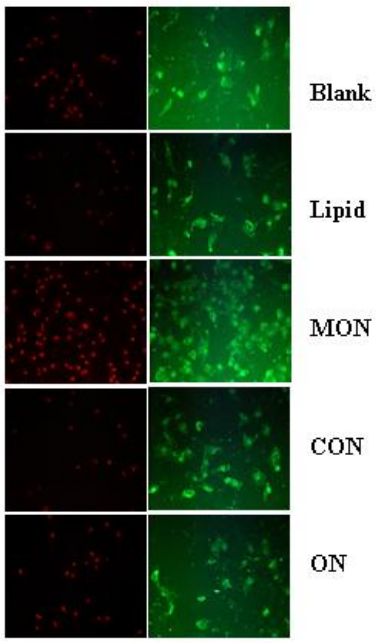
RESULTS

A



A. The activity of Ki-67 promoter in 786-0 cells. The 786-0 cells were transfected with pGL3-Basic/pGLBK235 or were cotransfected with pGLBK235 and oligonucleotides (MON1-5/CON/ON).

B



B. The cells apoptosis at earlier period and midanaphase were observed by fluorescence microscope.

CONCLUSION

These findings suggest that methylated oligonucleotide targeting Ki-67 significantly inhibited:

- the activity of Ki-67 promoter,
- the expression of Ki-67,
- the proliferation of 786-0 cells.

江苏省药理学会第六届学术研讨会
暨第三届会员代表大会第一轮会议通知

各有关单位：

当今世界，科技进步日新月异，创新浪潮风起云涌。科技作为第一生产力在当代经济社会发展中的地位和作用更加突出。为促进我省药理学科的快速发展，交流药理学学术成果和研究经验。经研究决定于 2012 年将召开“江苏省药理学会第六届学术研讨会暨第三届会员代表大会”。本次会议以青年药理工作者的科技创新，药理科技发展和创新药开发为主题，搭建多学科的学术交流平台，着力吸引和聚集青年人才，充分发挥我省药理青年工作者思维、眼界开阔、思想解放的优势，促进学术创新和学术繁荣，促进科技创新和产学研结合，加快我省药理学科发展，充分发挥我省药理人才资源优势，以青年药理科技工作者人才引领药理学科高水平发展，努力形成药理学科创新人才大量涌现的生动局面。现将有关事项通知如下：

一、参会对象：

各医药院校、制药企业、科研院所、医疗机构相关专业人员。

二、会议报告内容：

1. 基础科学与技术在药物发现中的应用；2.药物靶标发现与确认；3.药物筛选技术；4.合理化药物设计；5.小分子药物发现的前沿进展；6.以基因组为基础的药物发现与研发；7.中医与以天然产物为基础的药物发现；8.药物发现研究与发展和转化医学

三、征文要求：

参会代表需提交尚未公开发表的论文中英文摘要或全文。截稿日期 2012 年 4 月 30 日。请参会代表在论文截稿日前发致 E-mail:zhuxuanxuan@sina.com，本次会议将设立“江苏省药理学会青年优秀论文奖”，欢迎 40 周岁以下的药理工作者和研究生踊跃投稿。（评奖论文需全文）

四、授予学分：

参会代表可获得江苏省继续教育学分。

江苏省药理学会

2011 年 9 月 22 日