中南大学学报(医学版) 2012, 37(4) 390-399 DOI: 10.3969/j.issn.1672-7347.2012.04.013 ISSN: 1672-7347 CN: 43-1427/R

论著

三羟基二苯乙烯及其对肺动脉平滑肌细胞生长抑制和致凋亡作用的研究

王欣[1], 谢立[1], 胡建国[1], 蒋新宇[2], 向大雄[3], 高洁生[4], 高戈[5,6]

1. 中南大学湘雅二医院心胸外科, 长沙 410011;
2. 中南大学化学技术学院, 长沙 410083;
3. 湘雅二医院药剂科, 长沙 410011;
4. 湘雅二医院风湿免疫科, 长沙 410011;
5. 湘雅医学院检验系, 长沙 410013;
6. 湘雅医学院生理学教研室, 长沙 410013

摘要:

目的: 以虎杖为原料提取的白藜芦醇(Res),再经甲基化反应合成的3,5,4'-三羟基二苯乙烯(3,5,4'-trimethoxystilbene,TMS),对TMS进行结构鉴定和药代动力学测定。检测TMS对肺动脉平滑肌细胞(PASMCs)的生长抑制和致凋亡作用。**方法**: 用紫外吸收光谱、红外吸收光谱、^{1}H-NMR谱、^{13}C-NMR 图谱、质谱检测等方法对TMS进行结构鉴定。测定大鼠口服TMS后的生物利用度,肠道吸收特点,体内组织分布和体内排泄。测定TMS对小鼠的急性毒性。利用大鼠肺动脉制备PASMCs。将细胞分为8组。A组(空白对照组):不加TNF-α,TMS,Res;B组(TNF-α组):100 pg/mL TNF-α;C~E组(低~高浓度TMS组):TNF-α (100 pg/mL) +TMS (5,10,20 μmol/L);F~H组(低~高浓度Res组):TNF-α (100 pg/mL)+ Res (50,100,200 μmol/L)。MTT法测定TMS对PASMCs增殖的影响。流式Annexin V/PI法测定TMS对PASMCs凋亡的影响。**结果**: TMS的紫外吸收图谱为$\lambda_{max(MeOH)}$:318, 306.2, 217.8 nm;红外光谱解析为IRv_{max}^{KBr}/cm:2999,2935,2836,1591,1511,1456/cm;^{1}H-NMR谱解析显示结构含有3个羟基; ^{13}C-NMR图谱解析为17个碳信号,并且其结构中含有对称的结构片段。质谱图谱解析为ESI-MS m/z:271$[M+H]^+$,256$[M+H-CH_3]^+$,241$[256-CH_3]^+$,相对分子质量为270,推测分子式为$C_{17}H_{18}O_3$,结构中含有甲基。样品元素分析得分子结构式可用$C_{17}H_{18}O_3$表示,因此可确定化合物为反-3,5,4'-三羟基二苯乙烯。TMS绝对生物利用度为45.4%,在小肠上段有较好吸收,通过粪便、胆汁排泄,在各组织中均有分布,最大耐受量(MTD)约5.85 g/kg。TMS干预PASMCs 24 h后经MTT法测定显示对其生长具有显著的抑制作用,其强度呈剂量依赖性改变。A~H组的生长抑制率分别为(4.07±2.12)%,(6.54±4.78)%,(9.35±4.26)%,(16.75±5.34)%,(23.74±7.07)%,(6.78±5.58)%,(8.81±5.16)%,(17.81±6.03)%。流式Annexin V/PI法检测结果显示:TMS干预PASMCs 24 h组细胞凋亡率显著高于TNF-α干预组,且其作用呈剂量依赖性。A~H组的细胞凋亡率分别为(2.63±0.74)%,(3.54±0.81)%,(5.77±4.62)%,(11.68±5.35)%,(18.79±4.15)%,(4.11±3.59)%,(6.33±4.8)%,(12.47±5.06)%。**结论**: TMS的分子式可用$C_{17}H_{18}O_3$表示,结构式为反-3,5,4'-三羟基二苯乙烯。TMS的生物利用度为45%。TMS呈剂量依赖性地抑制PASMCs的增殖并诱导其凋亡。

关键词: 反-3,5,4'-三羟基二苯乙烯 白藜芦醇 肺动脉高压 肺动脉平滑肌细胞 凋亡

Trimethoxystilbene and its effects on the proliferation and apoptosis of PASMCs

WANG Xin[1], XIE Li[1], HU Jianguo[1], JIANG Xinyu[2], XIANG Daxiong[3], GAO Jiesheng[4], GAO Ge[5,6]

1. Department of Cardiothoracic Surgery, Second Xiangya Hospital, Central South University, Changsha 410011;
2. College of Chemistry and Chemical Engineering, Central South University, Changsha 410083;
3. Institute for Clinical Pharmacy and Pharmacology, Second Xiangya Hospital, Central South University, Changsha 410011;
4. Department of Rheumatology and Immunology, Second Xiangya Hospital, Central South University, Changsha 410011;
5. Faculty of Laboratory Medicine, Xiangya School of Medicine, Central South University, Changsha 410013;
6. Department of Physiology, Xiangya School of Medicine, Central South University, Changsha 410013, China

Abstract:

Objective: To synthesize 3, 5, 4' -trimethoxystilbene (TMS) by methylation of resveratrol (Res), a natural compound extracted from polygonum cuspidatum, to identify the chemical structure of TMS, to test its pharmacokinetics, and to determine the effects of TMS on the growth inhibition and apoptosis in pulmonary artery smooth muscle cells (PASMCs). Methods: The chemical structure of TMS was analyzed by UV- and IR- absorption spectrometry, ^{1}H-NMR and ^{13}C-NMR spectroscopy and mass spectrometry. We measured the bioavailability, the characteristics of intestinal absorption, and the

distribution of TMS in body and excretions of SD rats after oral administration of TMS. The acute toxicity of TMS in mice was tested. PASMCs were prepared from pulmonary artery of SD rats. The PASMCs were divided into 8 groups. Group of A (control) was cultured without TNF-α, TMS, or Res. Group of B (TNF-α) was cultured with 100 pg/mL TNF-α. Groups of C–E (low–high concentrations of TMS) were cultured with 100 pg/mL TNF-α and 5, 10, 20 μmol/L TMS, respectively. Groups of F–H (low–high concentrations of Res) were cultured with 100 pg/mL TNF-α and 50, 100, 200 μmol/L Res, respectively. The proliferation of PASMCs after treatment was determined by MTT assay. The apoptosis of PASMCs after treatment was determined by flow cytometry. Results: The UV absorption map of TMS showed $\lambda_{max(MeOH)}$ at 318, 306.2, and 217.8 nm. Analysis of infrared spectrum of TMS showed IRv_{max}^{KBr}/cm at 2999, 2935, 2836, 1591, 1511 and 1456/cm. The ^{1}H-NMR map showed that the synthetic product contained three hydroxy groups, while ^{13}C-NMR map showed 17 carbon signals and some symmetrical structural fragments. Electospray ionization mass spectrometry of the productshowed m/z peaks corresponded to 271$[M+H]^+$, 256$[M+H-CH_3]^+$ and 241$[256-CH_3]^+$; the implied relative molecular weight is 270 and the implied molecular formula is $C_{17}H_{18}O_3$. These data confirm the product is 3,5,4' –trimethoxystilbene. The absolute bioavailability of TMS was 45.4%. TMS was well absorped in the upper small intestine; it was excreted in stool and bile and distributed into several tissues. The maximal tolerance dose (MTD) of TMS was 5.85 g/kg. MTT assay showed TMS inhibited the proliferation of PASMCs in a dose-dependent manner. The extent of growth inhibition in A–H groups were (4.07±2.12)%, (6.54±4.78)%, (9.35±4.26)%, (16.75±5.34)%, (23.74±7.07)%, (6.78±5.58) %, (8.81±5.16) %, and (17.81±6.03) %, respectively. Flow cytometry showed the extent of apoptosis in PASMCs (after being treated with TMS for 24 h) was significantly higher than that in PASMCs treated only with TNF-α. The apoptosis rates of A–H groups were (2.63±0.74)%, (3.54±0.81)%, (5.77±4.62)%, (11.68±5.35)%, (18.79±4.15)%, (4.11±3.59)%, (6.33±4.8) %, and (12.47±5.06)%, respectively. Conclusion: We have confirmed our synthetic product as 3,5,4'-trimethoxystilbene (TMS),with the molecular formula of $C_{17}H_{18}O_3$ and appropriate molecular weight and absorbption and NMR spectra. The bioavailability of TMS was to 45%. It strongly inhibits the proliferation of PASMCs in a dose-dependent manner and induces apoptosis of PASMCs.

Keywords: trimethoxystilbene resveratrol pulmonary artery hypertension pulmonary artery smooth muscle cells apotosis

收稿日期 2011-01-19 **修回日期** **网络版发布日期**

DOI: 10.3969/j.issn.1672-7347.2012.04.013

基金项目:

通讯作者: 王欣,Email: wxbox@netease.com

作者简介: 王欣,博士,主治医师,主要从事心脏损伤方面的研究。

作者Email: wxbox@netease.com

参考文献：

1. Farber HW,Loscalzo J.Pulmonary arterial hypertension ［J］.N Engl J Med,2004, 351(16):1655–1665.

2. Schuuring MJ,Bouma BJ,Cordina R,et al.Treatment of segmental pulmonary artery hypertension in adults with congenital heart disease ［J］.Int J Cardiol,2011, 14: 5–10.

3. Macchia A, Mariani J, Comignani PD, et al. Clinical trials using vasodilators in pulmonary arterial hypertension:where do we go from here? ［J］.Rev Recent Clin Trials,2011,6(3):228–234.

4. Humbert M, Sitbon O, Simonneau G. Treatment of pulmonary arterial hypertension ［J］.N Engl J Med,2004, 351(16):1425–1436.

5. Kopp P. Resvaratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the French paradox? ［J］.Eur J Endocrinol,1998,138(6):619–620.

6. 向海艳,周春山,陈龙胜,等.酶法提取虎杖中白藜芦醇新工艺研究［J］.林产化学与工业,2004,24 (4):77–79. XIANG Haiyan,ZHOU Chunshan,CHEN Longsheng,et al.Semi–bionic extraction tiger in new technology staff resveratrol ［J］.Chemical and Industrial of Forest Products, 2004, (4) :77–79.

7. 王桂英,宋翠淼,张丽男,等. 白藜芦醇对豚鼠离体心房肌收缩力和心率的影响［J］.中国中药杂志,2007,32 (13):1317–1319. WANG Guiying, SONG Cuimiao, ZHANG Llinan, et al. Roles of potassium channel in effects of resveratrol on isolated myocardial contractility and heart rate research in guinea pig ［J］. China Journal of Chinese Materia Medica,2007,32(13):1317–1319.

8. Shen M, Jia GL, Wang YM, et al. Cardioprotective effect of resvaratrol pretreatment on myocardial ischemia–reperfusion induced injury in rats ［J］.Vascul Pharmacol, 2006,45(2):122–126.

9. Dong Z. Molecular mechanism of the chemopreventive effect of resveratrol ［J］. Mutat Res,2003, (523/524):145–150.

10. Makagawa H, Kiyozukay Y, Vemura YS, et al. Resveratrol inhaibits human breast cancer cell growth and may mitigate the effect of linoleicaicid, a potent breast cancer cell stimulate ［J］. Cancer Res Clin

Oncol,2001,127(4):258–264.
11. 马宁,刘文英,李焕德,等.HPLC-MS法测定大鼠血浆中白藜芦醇衍生物(E)-3,5,4′-三甲氧基二苯乙烯的浓度［J］.药物分析杂志,2008,30(3):215–220. MA Ning, LIU Wenying, LI Huande,et al. High–performance liquid chromatographic analysis of resveratrol analog 3,5,4,–trimethoxystilbene in rat plasma［J］. Chinese Journal of Pharmaceutical Analysis,2008,30(3):215–220.
12. Hussain AR, Uddin S, Bu R,et al. Resveratrol suppresses constitutive activation of AKT via generation of ROS and induces apoptosis in diffuse large B cell lymphoma cell lines［J］. PLoS One, 2011,6(9):e24703.
13. 马宁,刘文英,李焕德,等. RP-HPLC法研究白藜芦醇衍生物(BTM-0512)在大鼠血浆与组织中的分布［J］. 药学学报, 2007,42(11):1183–1188. MA Ning, LIU Wenying, LI Huande,et al.RP–HPLC study of resveratrol derivative (BTM–0512) in rat plasma and tissue distribution ［J］. Acta Pharmaceutica Sinica,2007,42 (11):1183–1188.
14. Levenson AS, Gehm BD, Pearce ST, et al. Resveratrol acts as an estrogen receptor (ER) agonist in breast cancer cells stablytransfected with ER alpha［J］. Int J Cancer,2003,104 (5) :587–596.
15. Wouria M,Gukoskays AS,Juang Y,et al.Food derived polyphenols inhibit pancreatic cancer growth through mibochondrial cytechrome crelease and apoptosis［J］.Int J Cancer,2002,98(5):761–769.

本刊中的类似文章

1．张杰1,2， 周春山2， 刘韶3， 陈皓1， 杨超3.鬼臼毒素抗胃癌细胞株SGC 7901作用的实验研究[J]. 中南大学学报(医学版), 2008,33(08): 718-722
2．徐军美； 胡冬煦； 常业恬； 倪斌； 邹永华；.缺血预处理抑制缺血再灌注所致兔在体心肌细胞凋亡[J]. 中南大学学报(医学版), 2001,26(6): 505-
3．杨扬； 陈胜喜； 张卫星；.缺血预处理对人在体肺组织细胞凋亡及调控基因蛋白bcl-2表达的影响[J]. 中南大学学报(医学版), 2002,27(1): 43-
4．晓希； 牛晓红； 周智广； 苏恒； 蒋铁建；.完全弗氏佐剂诱导脾脏T淋巴细胞凋亡预防非肥胖性糖尿病鼠1型糖尿病[J]. 中南大学学报(医学版), 2002,27(2): 105-
5．肖涛； 李康华； 方建珍； 王万春； 李海声；.三氧化二砷诱导骨肉瘤MG-63细胞凋亡的实验研究[J]. 中南大学学报(医学版), 2002,27(2): 111-
6．陈慧玲； 廖兰； 雷闽湘； 宋惠萍；.H2O2对平滑肌细胞凋亡及p38MAPK活性的影响[J]. 中南大学学报(医学版), 2002,27(5): 402-
7．杜巍； 胡建国； 周新民； 刘锋； .30岁以上动脉导管未闭患者的手术治疗[J]. 中南大学学报(医学版), 2003,28(1): 90-
8．黄凤英； 林秋华； 方小玲； 张志胜； 王新；.Bcl-2和Bax蛋白在子宫内膜异位症的表达[J]. 中南大学学报(医学版), 2003,28(2): 102-
9．徐军美； 谭嵘； 胡冬煦； 常业恬； 曹丽君； .缺血预处理对兔缺血再灌注心肌bcl-2,bax,p53基因表达的影响[J]. 中南大学学报(医学版), 2003,28(2): 111-
10．陈子华； 冯斌；.新辅助化疗诱导大肠癌凋亡caspase-3活性的研究[J]. 中南大学学报(医学版), 2003,28 (2): 117-
11．黄健； 丁依玲； 朱付凡； 皮丕湘； 夏晓梦；.足月妊娠合并原发性肺动脉高压一例[J]. 中南大学学报(医学版), 2003,28(2): 140-
12．唐荣, 周巧玲, 舒金勇, 汤天凤, 敖翔, 彭卫生, 张义德.冬虫夏草提取液对肾小管上皮细胞Klotho 表达和凋亡的影响
[J]. 中南大学学报(医学版), 2009,34(04): 300-307
13．刘敏 周后德 何玉玲 谢辉 廖二元.核结合因子促进骨髓间质细胞MBA-1凋亡[J]. 中南大学学报(医学版), 2006,31(01): 14-18
14．杨聘 董克礼 曾望远 .益智健脑颗粒对SAMP/8快速老化小鼠行为学及神经元凋亡的影响[J]. 中南大学学报(医学版), 2006,31(01): 56-59
15．鄂顺梅 肖卫民 王慷慨 王秋鹏 刘梅冬 刘可 肖献忠.HSF1抑制热应激所致RAW264.7巨噬细胞凋亡[J]. 中南大学学报(医学版), 2006,31(02): 162-166