

丹皮酚对大鼠心肌缺血再灌注损伤的线粒体膜脂的保护作用

湖北医科大学药理教研室(武汉 430071) 张卫国* 张志善

摘要 丹皮酚每天腹腔注射60mg/kg,连续15d,每天2次,然后结扎大鼠左降枝冠状动脉45min,解除结扎恢复血流灌注60min,复制大鼠心肌缺血再灌注损伤模型。观察丹皮酚对心肌细胞线粒体膜胆固醇/磷脂的比值、膜Ca⁺⁺-ATP酶活性、膜脂流动性及心肌细胞游离脂肪酸的影响。结果丹皮酚能降低线粒体膜胆固醇/磷脂的比值,减少心肌细胞游离脂肪酸含量,改善Ca⁺⁺-ATP酶活性,改善和调节线粒体膜脂的流动性。表明丹皮酚能明显改善缺血再灌注损伤心肌线粒体膜的结构和功能,与保护心肌细胞膜脂结构有关。

关键词 丹皮酚 心肌缺血再灌 线粒体 膜脂流动性。

丹皮酚是牡丹皮的有效成分之一。具有明显的抗炎、镇痛作用,以及抗动脉粥样硬化,抗血小板聚集和抗心律失常、降压作用^[1]。本室在心肌缺血再灌注损伤实验中证实该药有抗膜脂质过氧化作用。本文报道该药在线粒体膜脂的变化,研究线粒体膜脂的保护作用。

1 材料和方法

1.1 药品:丹皮酚为上海第一制药厂产品;DPH(1,6-diphenyl-1,3,5-hexatriene,1,6-二苯基-1,3,5-己三烯)为Sigma产品,其余未注明药品均为国产分析纯。

1.2 动物:健康雄性SD大鼠,体重220±25g,随机分3组。丹皮酚组按每天腹腔注射,60mg/kg,连续15d,2次/d,常规饮食喂养;假手术组和缺血再灌组腹腔注射相应体积的生理盐水。15d后分别进行心脏缺血再灌注实验。

1.3 缺血再灌注损伤模型:大鼠用20%乌拉坦腹腔注射麻醉,行气管插管,接人工呼吸机。于心尖搏动明显处上方剪断肋骨,分离心包,暴露心脏,在距主动脉根部2mm处结扎左前降支,以心电图监测结扎情况和连续记录标II导联的心电变化。结扎45min,解除结扎60min。实验末取出心脏,从结扎线以下剪取心肌组织作生化指标测定。

1.4 线粒体制备和膜流动性测定:按常规分离线粒体方法制备线粒体,每0.5g心肌组织加0.5ml,分离液制备线粒体悬浮液。膜流动性测定采用荧光偏振技术,将线粒体悬浮液加少许2μmol/LDPH,25℃水浴30min后,摇匀,以激发波长362nm,发射波长432nm,光栅狭缝10nm,测定荧光偏振度(P),膜脂微粘度(η),分析膜的流动性。

1.5 生化分析:采用Folin-Lowry法测定心肌蛋白及线粒体蛋白含量。依Falholt法测定心肌游离脂肪酸(Free Fatty Acid, FAA)。化学法测定心肌总磷脂。采用邻苯二甲酰直接显色法测定心肌胆固醇含量及与磷脂的比值。按文献^[2]测定Ca⁺⁺-ATP酶活性。

2 结果

2.1 对大鼠心肌线粒体膜脂流动性的影响:表1所示,心肌缺血再灌后,P、η值明显升高,表明心肌缺血再灌注时,线粒体膜脂流动性降低。而丹皮酚组,使升高的P、η值明显降低,趋于正常,说明丹皮酚能维持和改善缺血再灌损伤心肌线粒体膜的流动性。

*Address: Zhang Weiguo, Department of Pharmacology, Hubei Medical University, Wuhan

2.2 对线粒体膜胆固醇、磷脂及胆固醇/磷脂比值和心肌游离脂肪酸含量的影响: 表2所示, 心肌缺血再灌后, 与正常组相比, 线粒体总磷脂含量显著降低, 总胆固醇含量及胆固醇/磷脂比值均显著增加, 并使心肌游离脂肪酸明显上升。丹皮酚组线粒体膜总磷脂含量明显增加, 总胆固醇及胆固醇/磷脂比值均有显著降低。并能明显降低心肌游离脂肪酸。

2.3 对线粒体膜Ca⁺⁺-ATP酶活性的影响, 表3所示, 心肌缺血再灌后, 线粒体膜的Ca⁺⁺-ATP酶活性明显降低。丹皮酚能明显抑制缺血再灌注对膜酶活性降低作用, 恢复膜酶的活性。

表1 丹皮酚对大鼠心肌线粒体膜脂流动性的影响 ($\bar{x} \pm SD$)

组别	P	η
假手术组	0.19 ± 0.008	1.4 ± 0.1
再灌组	0.25 ± 0.01	2.4 ± 0.2
丹皮酚组	0.2 ± 0.007*	1.6 ± 0.1**

n = 8~10与再灌组比(下同) *P < 0.05 **P < 0.01

表2 丹皮酚对大鼠心肌线粒体膜胆固醇、磷脂及胆固醇磷脂比值和心肌游离脂肪酸含量的影响 ($\bar{x} \pm SD$)

组别	胆固醇(Ch) (nmol/mg.Pto)	磷脂(PI) (nmol lipid pi/mg.pro)	Ch/PI	FFA (nmol/mg.Pro)
假手术组	33.3 ± 6.3	15.1 ± 1.3	2.2 ± 0.4	0.83 ± 0.16
再灌组	46 ± 7.2	6.7 ± 1	6.9 ± 1.6	1.2 ± 0.3
丹皮酚组	35.4 ± 6.1**	13.5 ± 1.5**	2.4 ± 0.4	0.8 ± 0.1**

3 讨论

生物膜是由脂质(磷脂、胆固醇等)组成的超分子体系, 具有维持细胞完整性和正常功能的作用。有资料表明^[3], 心肌缺血再灌过程中, 生物膜易发生严重损伤, 可包括以下几方面: 膜磷脂的降解, 膜胆固醇含量

及胆固醇/磷脂比值增加, 引起膜脂流动性降低, 尤以线粒体膜脂破坏明显, 并引起膜结合的Ca⁺⁺-ATP酶活性降低^[4], 同时伴有磷脂分解产物游离脂肪酸的聚集。本文结果证实, 丹皮酚能明显改善线粒体膜脂的流动性, 明显降低心肌匀浆中的游离脂肪酸, 明显降低线粒体膜总胆固醇含量及胆固醇/磷脂比值, 改善和恢复线粒体膜Ca⁺⁺-ATP酶活性。对缺血再灌注心肌的线粒体膜脂的结构和功能有明显的保护作用。最近研究表明, 在细胞水平上膜结构和功能的破坏是再灌注损伤的发病关键环节。本文研究也初步证实丹皮酚有抗氧自由基和抑制脂质过氧化作用。说明丹皮酚对缺血再灌损伤的心肌线粒体膜脂保护作用与抗脂质过氧化有关。

表3 丹皮酚对线粒体膜Ca⁺⁺-ATP酶活性的影响 ($\bar{x} \pm SD$)

组别	Ca ⁺⁺ -ATP酶 ($\mu\text{mol pi/h.mg.pro}$)
假手术组	19.4 ± 1.3
再灌组	11.6 ± 0.8
丹皮酚组	15.8 ± 1.8**

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the content of baicalin in this preparation. As a result of stability test, the shelf life at 25°C is estimated to be 1.97 years.

(Original article on page 182)

Studies on Effective Compositions of Pinecone Ⅱ. Determination of Polysaccharides in Cone of Chinese Pine (*Pinus tabulaeformis*)

Li Haozhi, Lu Yongjun, Bai Gang, et al

Quantitative determination of polysaccharides in pine cones by phenol-sulfuric acid method was studied, and the effects of concentration of phenol-sulfuric acid and reaction temperature on color formation were investigated. It was found that the absorbance is linearly correlated to polysaccharide concentrations between 10~78µg/ml ($r=0.9999$). The analytical recovery was 99.8%, CV% was 1.2% and the minimal detectable concentration was 5µg/ml.

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Artificial Neural Network Method for Quality Estimation of Traditional Chinese Medicine

Cai Yudong, Gong Jiawen, Cheng Zhaonian, et al

An artificial neural network method for quality estimation of traditional Chinese medicine was suggested, and quality of Hou-Po was estimated by the proposed method in comparison with the analytical results of gas-liquid chromatography. The successful rate reached 100%. The results showed that the neural network method is reliable, and therefore may be referred to as an effective technique for the quality estimation of traditional Chinese medicine.

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Protective Effect of Paeonol Against Ischemia Reperfusion Damage in Cardiac Mitochondria Membrane of Rats

Zhang Weiguo and Zhang Zhishan

60mg/kg·d ip, of paeonol were given to rats for 15 days. The myocardial ischemia reperfusion injury model was produced by occluding the left coronary artery and releasing the occlusion in rats. This significantly decreased myocardial Ca^{++} -ATPase activities and CH/PL radical, FFA content, improved mitochondrial membrane fluidity and kept them away from oxygen free radical damage. These results indicated that paeonol has membrane protective effect on myocardial ischemia reperfusion injury probably by inhibiting the oxygen free radicals and subsequent lipid peroxidation.

(Original article on page 193)

An Inquiry into Preparing Diarrhea Model of Mice and Application of Diarrhea Index

Zhou Gannan, Hu Zhihua, Wang Yaxian, et al

In Order to screen antidiarrhea drugs, the standard operation of mice diarrhea model replicated with leave of *Cassia angustifolia vahl* was introduced. In experiments, diarrhea index expressed with loose stool incidence rate multiplied by loose stool grade was used as main index. It thoroughly mirrors indi.