

真武汤对人胎肾小球系膜细胞外基质作用的实验研究

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摘要 采用血清药理学方法,探讨真武汤对人胎肾小球系膜细胞外基质(ECM)不同成分的作用,为真武汤防治肾小球硬化的临床应用提供实验依据。结果真武汤能够明显降低ECM成分中层粘连蛋白(LN)和Ⅳ型胶原(Col Ⅳ)的含量。表明真武汤对ECM的增殖具有明显的抑制作用。

主题词 真武汤/药理学 肾小球膜/药物作用

近年来,随着动物实验模型的深入研究和细胞分子生物学技术的开展,对慢性肾功能衰竭(CRF)渐进性发展机制有了更进一步的认识。特别是随着对肾小球硬化机制认识的不断深入,肾小球系膜(MC)、细胞外基质(ECM)及各种细胞因子在肾功能进行性减退过程中所起的重要作用倍受关注。抑制MC增殖,减少ECM的堆积,拮抗细胞因子的不良作用,已成为防治肾小球硬化,延缓CRF病情进展的重要环节。鉴此,本研究采用血清药理学的方法,观察了真武汤对ECM中纤维连结蛋白(FN)、层粘连蛋白(LN)及Ⅳ型胶原(Col Ⅳ)含量的影响。冀从细胞生物学方面探讨其抑制肾小球硬化的作用机理。

1 材料和方法

1.1 药物与试剂:真武汤由附子 8g,白术 6g,茯苓 9g,芍药 9g,生姜 9g 组成。苯那普利(Benazepril)由瑞士诺华制药公司生产,批号 012200;Ⅴ型胶原酶、HEPES 均系 Sigma 产品;RPMI1640、胰蛋白酶、胰岛素均为美国 GIBCO 产品;小鼠抗人单克隆 FN 抗体、兔抗人 FN 多抗、羊抗兔 IgG-HRP 均购于北京中山生物技术有限公司;LN、Col Ⅳ放射免疫分析试剂盒均购于上海海研医学生物技术中心。

1.2 含药血清制备:SD 大鼠 18 只(由南京医科大学动物实验中心提供),体重 200 ± 20 g。真武汤组(Ⅲ组)、苯那普利组(Ⅱ组)及空白组(Ⅰ组)共 3 组,每组各 6 只动物。每组每天灌胃两次。Ⅲ组中药水煎浓缩,每次灌胃 1ml/200g 体重(1.45g/kg 体重,同临床剂量),Ⅱ组每次灌胃 1ml/200g 体重(89.3 μ g/kg 体重,相当于临床剂量),Ⅰ组每次灌胃生理盐水 1ml/200g 体重。以上 3 组共灌胃 3 天后,处死,无菌操作动脉插管取血 2ml,离心后取出血清, -20℃冻放。

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1.3 MC 培养与鉴定:无菌取胎肾,分离提取肾皮质小球组织,按照于方力方法^[1],进行原代和继代 MC 常规培养,实验中使用传代 5 代的 MC。培养的细胞经免疫病理鉴定证实为单纯的 MC,无上皮细胞、肾小管细胞及成纤维细胞污染。

1.4 分组及 MC 上清的收集:吸取培养的第 5 代 MC 培养瓶中液体,加胰酶 1ml,置孵箱(5%CO₂, 37℃)中消化 3min,加 10%FCS1640 终止液 2ml,抽吸于离心管中,800r/min 离心 3min,弃上清,15%FCS1640 培养液于离心管中,吹打,混匀。用计数板计数后,倾入加样槽中,用 8 道加样枪,按每孔 200 μ l,密度 4000/孔铺板。将 96 孔板按列从左至右依次分为 I 组、Ⅱ组、Ⅲ组,共 3 组(列),每列 6 孔,待细胞贴壁 24h 后,每孔加无血清 1640 培养液 200 μ l 同步 24h。然后,各组分别加入 20%的相应含药血清,待刺激 48h 后,依次收集上清待测。

1.5 ECM 中 FN 的含量测定:采用双抗体 ELISA 双抗体夹心法。

1.6 ECM 中 LN、Col Ⅳ含量测定:采用放射免疫法(RIA)。

1.7 统计学处理:数据均采用数理统计应用软件进行 *t* 检验。

2 结果

真武汤对 ECM 不同成分含量的影响,见附表。附表示,在对 ECM 中 LN、Col Ⅳ含量的影响方面,Ⅲ组和Ⅱ组与Ⅰ组比较,差异均有显著性($P < 0.05$ 或 $P < 0.01$),提示真武汤和苯那普利对 LN、Col Ⅳ的含量有明显抑制作用,但对 FN 含量无明显影响。

附表 真武汤对 ECM 不同成分含量的影响($\bar{x} \pm s$)

组别	鼠数	FN(OD 值)	LN(ng/ml)	Col Ⅳ(g/ml)
Ⅰ组	6	2.000 $\pm$ 0.203	170.48 $\pm$ 36.29	66.35 $\pm$ 11.28
Ⅱ组	6	1.840 $\pm$ 0.148	89.77 $\pm$ 32.80**	41.06 $\pm$ 9.67**
Ⅲ组	6	1.709 $\pm$ 0.264	104.30 $\pm$ 36.04*	36.92 $\pm$ 10.19**

与Ⅰ组比较,* $P < 0.05$,** $P < 0.01$

3 讨论

肾小球 MC 是肾脏血管周围的固有细胞。正常情况下,MC 的数量、形态和位置保持相对稳定,且合成基质的能力也较小^[2]。在病理状态下,多种损伤因子和有害物质均可促进 MC 增殖,而 MC 过度增殖,一方

面突入肾小球毛细血管管腔,导致管腔狭窄或闭塞;另一方面又可造成 ECM 的大量产生与积聚^[3]。大量的研究表明,MC 增殖与 ECM 的增多有关,而 ECM 聚积还可导致系膜清除功能障碍,使进入系膜区的致病因子不能被及时清除,而导致肾损害进一步加重^[4]。

诸多临床研究表明,CRF 以脾肾阳气衰败|水湿内聚为其主要病机特点,真武汤在改善 CRF 的临床症状,延缓 CRF 的发展进程上确有明显的治疗效果^[5~7]。真武汤是温阳利水的代表方。方中附子辛热,通三焦散阴凝,其善补命门真火,启动下焦气化,且能入脾温土,助脾运化,固护脾肾真阳;茯苓利水渗湿,《用药心法》谓其“淡能利窍,甘以辅阳,除湿之圣药也”,与附子相配,共奏温阳利水之功;白术、生姜相伍,温中焦而健脾胃,化湿浊而通水道;芍药既可敛阴益血,制附子辛热刚燥之性,又能除血痹而利小便;诸药合用,温补脾肾之阳气,化浊利水,针对 CRF 脾肾阳气衰败,水湿内聚的主要病机甚为合拍,于慢性肾衰正虚邪实,虚实夹杂之体颇为适宜。药理研究证实,真武汤能够提高心肌收缩力、增加尿量、降低肌酐、尿素氮,调整实验大鼠的渗透压调定点,减少 ADH 的分泌;促进 Na⁺、K⁺ 的排泄,明显改善 HCA 肾阳虚大鼠的肾功能,改善肾小球滤过膜的通透性,促使代谢产物 Cr、

BUN 的排出,减少血浆白蛋白的大量丢失,对机体的特异性和非特异性免疫机能均有增强作用^[8]。

本实验结果表明,真武汤对细胞外基质中的 LN、Col IV 的含量具有明显的降低作用,其作用与苯那普利相当。提示 MC 是真武汤发挥治疗作用的重要靶细胞,减轻 ECM 的蓄积可能是真武汤延缓肾小球硬化的重要作用机制之一。

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ABSTRACTS FROM ORIGINAL ARTICLES

The Protective Action of Xin He Ke Li Ji on Endothelial Injury in Coronary Diseases—A Clinical Study

Li Xiao, Ding Shuwen, Jiang Ping

Patients with unstable angina pectoris who conform with the category of impairment of the channels and collaterals due to stagnated heat and insufficiency of ying-yin were enrolled in the study and randomly assigned to treatment and control groups. In the treatment group (N=45) Xin He Ke Li Ji, a prescription of our own, was administered with routine Western drugs; in the control group (N=42) only with routine drugs. The baseline values of P-selectin, tumor necrotizing factor (TNF) and interleukin-1 (IL-1) were higher than normal subjects (N=10). The total effective rate of angina pectoris was 86.6%, that in EKG examination, 53.3% and that in symptoms in TCM terms, 88.9% after treatment and was significantly better than in control group ($P < 0.05$ or 0.01). The blood P-selectin, TNF and IL-1 of the patients were decreased both in the treatment and control group and the decrease in the treatment group was significantly greater than that of the control group ($P < 0.01$). The results suggested that impairment of the channels and collaterals due to stagnated heat is one of the important mechanisms in occurrence of angina. It is showed that Xin He Ke Li Ji can decrease the content of P-selectin, TNF and IL-1, protect the endothelium against injury and further against vascular spasm, thrombus formation and the rupture of the unstable plaques.

Authors' add: Shandong University of TCM and Pharmacy, Jinan 250011

Key words: coronary diseases/TCM treatment, endothelium, vascular/pharmacological action @Xin He Ke Li Ji

(Original article on page 661)

Treatment of Chronic Hepatitis B with Tiao Zhi Qu Xie Liao Gan Fang in 240 Cases

Zhang Shaojian

240 cases of chronic hepatitis B were treated with Tiao Zhi Qu Xie Liao Gan Fang, a prescription of our own. The prescription has the following actions: supplementing qi and invigorating the spleen; tonifying the liver and kidney; enriching the liver and blood; promoting the flow of qi by soothing the liver; promoting the circulation of blood circulation and removing stasis; detoxifying, expelling the pathogenic dampness and heat. In the control group, 100 cases were treated with concentrated pills of Yi Gan Ling plus composite Shu She tablets. The total and markedly effective rates of the treatment group were significantly higher than the

control group ($P < 0.05$ or $P < 0.01$). The negative turnover rates of HBeAg and HBV-DNA were also significantly higher ($P < 0.01$) than the control group.

Authors' add: TCM Hospital of Zuoshui County, Shaanxi Province 711400

Key word: hepatitis B, chronic/TCM treatment

(Original article on page 664)

An Experimental Study on the Effect of Modified Xian Xiong Tao Cheng Tang Plus Shen Mai Zhen in Treating Pulmonary Injury after Abdominal Infection in Rats

Ma Chaoying, Geng Yun, Cheng Linhui, et al

To elucidate the mechanism of Modified Xian Xiong Tao Cheng Tang plus Shen Mai Zhen, a prescription of our own, in treating acute respiratory failure. We established an animal model by inducing abdominal infection after ligation of the caecum. In comparison with the model group, the contents of tumor necrotizing factor (TNF α), thromboxan (TXB₂), and prostaglandin (6-keto-PGF 1α) in plasma were significantly decreased in the treatment group ($P < 0.05$ or $P < 0.01$). The morphologic changes were also much less prominent. The result showed that this prescription is effective in treating acute respiratory failure in the model animals; its mechanism is probably contributed by inhibition of the inflammatory cells and their mediators.

Authors' add: Jiangxi TCM College, 330006

Key words: Modified Xian Xiong Tao Cheng Tang, Shen Mai Zhen, ligation of caecum, ARDS, experimental study, infection/complication, pulmonary diseases/TCM treatment, abdominal cavity/microbiology

(Original article on page 683)

The Experimental Study of Zhen Wu Tang on the Extracellular Matrix Synthesis of Fetal Glomerulomesangial Cells

Geng Jianguo, Wang Changsong

To elucidate the mechanism of Zhen Wu Tang in preventing and treating sclerosis of the glomeruli we quantitatively determined the components of the extracellular matrix of the fetal glomerulomesangial cells after giving the preparation. The significantly decreased concentrations of laminin (LN) and collagen IV (Col IV) in the serum suggested that Zhen Wu Tang is markedly inhibitory in their synthesis.

Authors' add: Nanjing University of TCM and Pharmacy, Nanjing 210029

Key words: Zhen Wu Tang/pharmacology, glomerulomesangial cells/pharmacological action

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