

• 实验研究 •

脉络通治疗家兔原位免疫复合物型肾小球肾炎的实验研究

同济医科大学附属同济医院内科(武汉 430030) 汪琼玲 孙世澜 刘晓城

同济医科大学病理教研室 黄寿珍

内容提要 应用阳离子化牛血清白蛋白复制家兔原位免疫复合物型肾小球肾炎, 以活血化瘀中药脉络通治疗, 结果治疗组尿蛋白较对照组明显减少, $P < 0.01$; 其组织病变较对照组减轻, 肾小球毛细血管内未见微血栓, 未见红细胞、血小板聚集及白细胞嵌塞, 系膜细胞增生及中性白细胞浸润明显减少, $P < 0.01$; 肾小球无明显纤维化。提示脉络通的治疗作用可能与其抑制血小板聚集、调节血栓素 A_2 /前列环素系统平衡失调、促进免疫复合物吸收以及抑制肾小球纤维化有关。

关键词 原位免疫复合物型肾小球肾炎 脉络通 实验研究

根据Border的方法^[1], 用阳离子化牛血清白蛋白(c-BSA)静脉注入家兔, 复制原位免疫复合物型肾小球肾炎。植入在肾小球基底膜(GBM)上的抗原与抗体在原位形成免疫复合物, 结合并激活补体, 释放炎性介质损伤肾小球; 且诱发凝血和纤溶系统活动的障碍以及血栓素 A_2 和前列环素系统平衡失调, 更进一步加重了疾病。因此, 西医除用免疫抑制剂控制其免疫发病机理外, 也常采用抑制血小板聚集的药物治肾炎, 且取得了一定的疗效。我们根据中药活血化瘀的原理, 试用脉络通治疗肾炎, 以探讨其作用机理。

材料与方法

一、材料

1. 动物: 日本大耳白家兔17只, 雄性, 体重2.0~2.5kg(由本校实验动物中心提供)。

2. 抗原: 主要用碳化二亚胺(由美国Sigma公司生产)改变天然牛血清白蛋白(N-BSA, 上海生物制品研究所出品)的羧基端而使其等电点提高至9.1, 变成c-BSA, 然后将其冷冻干燥并分装贮存于 -40°C 冰柜备用。

3. 药物: 脉络通由川芎、当归、赤芍、地黄、红花、桃仁、黄芪、牛膝、枳壳、桔梗制成片剂, 每片含生药量0.5g(由同济医院制药厂提供)。

二、实验方法

17只家兔分成C、M、MC三组。C组(7只)为肾炎阳性对照组; M组(6只)为脉络通治疗组; MC组(4只)为空白对照组, 但与M组同时喂中药。第1周:

C、M两组动物, 从兔耳静脉注射c-BSA 1mg和大肠杆菌内毒素 $1\mu\text{g}$ (上海生物制品所生产)作预免疫。第2周开始C、M两组动物每只均从耳缘静脉注射c-BSA 10mg/日, 6次/周, 共5周。第7周起剂量加倍, 共6次。MC组每日每只注射生理盐水2ml, 共6周。第3周, M、MC两组动物开始喂中药, 按 $0.5\text{g}/\text{kg}\cdot\text{d}$ 给药, 直到第8周末处死动物为止。

三、观察指标

从第2周末开始导尿作尿蛋白定量, 每周1次。从第3周开始抽耳血作血尿素氮(BUN)及肌酐(Cr)检查, 每周1次。第8周末处死动物时取新鲜肾皮质作冰冻切片, 用FITC标记的羊抗兔IgG(上海生物制品所提供)及FITC标记的鼠抗兔 C_3 (中山医科大学肾脏研究所提供)分别作直接荧光染色、Leitz荧光显微镜观察。另取一份新鲜肾皮质小块, 按电镜常规固定、脱水包埋、半薄切片定位, LKB-V型超薄切片机切片, 醋酸铀及柠檬酸铅染色, Opton 10C型透射电镜观察。

结 果

一、尿蛋白的变化见附表。C、M组动物于第2周末均出现蛋白尿。C组随着时间的延长而逐渐加重。M组自第3周用脉络通后逐渐下降, 至第8周末M组尿蛋白明显减少, 与C组相比差异有显著意义($P < 0.01$)。MC组尿蛋白始终阴性。

二、血清BUN和Cr C组动物除个别略有升高外, 其余均在正常范围内。

附表 各组动物尿蛋白值的比较 (mg/dl, $\bar{x} \pm S$)

组别	动物数	2 周末	4 周末	6 周末	8 周末
C	7	582.85 $\pm$ 280.63	1141.43 $\pm$ 758.01	1018.57 $\pm$ 695.45	1075.00 $\pm$ 1026.59
M	5	538.00 $\pm$ 365.54	1012.00 $\pm$ 645.25	998.00 $\pm$ 656.02	372.00 $\pm$ 135.17*

注: 与 C 组比, * $P < 0.01$

三、免疫荧光观察: 荧光显微镜下显示 C 组动物肾小球毛细血管壁上 IgG 及 C₃ 均呈弥漫性颗粒状荧光, 或连成线条样; M 组也见同样荧光, 其强度有的比 C 组弱, 有的与 C 组差不多。两组动物在荧光标记

上差别不大(图 1)。MC 组荧光阴性。

四、光镜检查: C 组肾小球肿大, 肾小球丛毛细血管与球囊壁层粘连, 部分球囊内可见蛋白渗出液, 甚至新月体形成(4/7), 见图 2; 1 只动物肾小球节段性

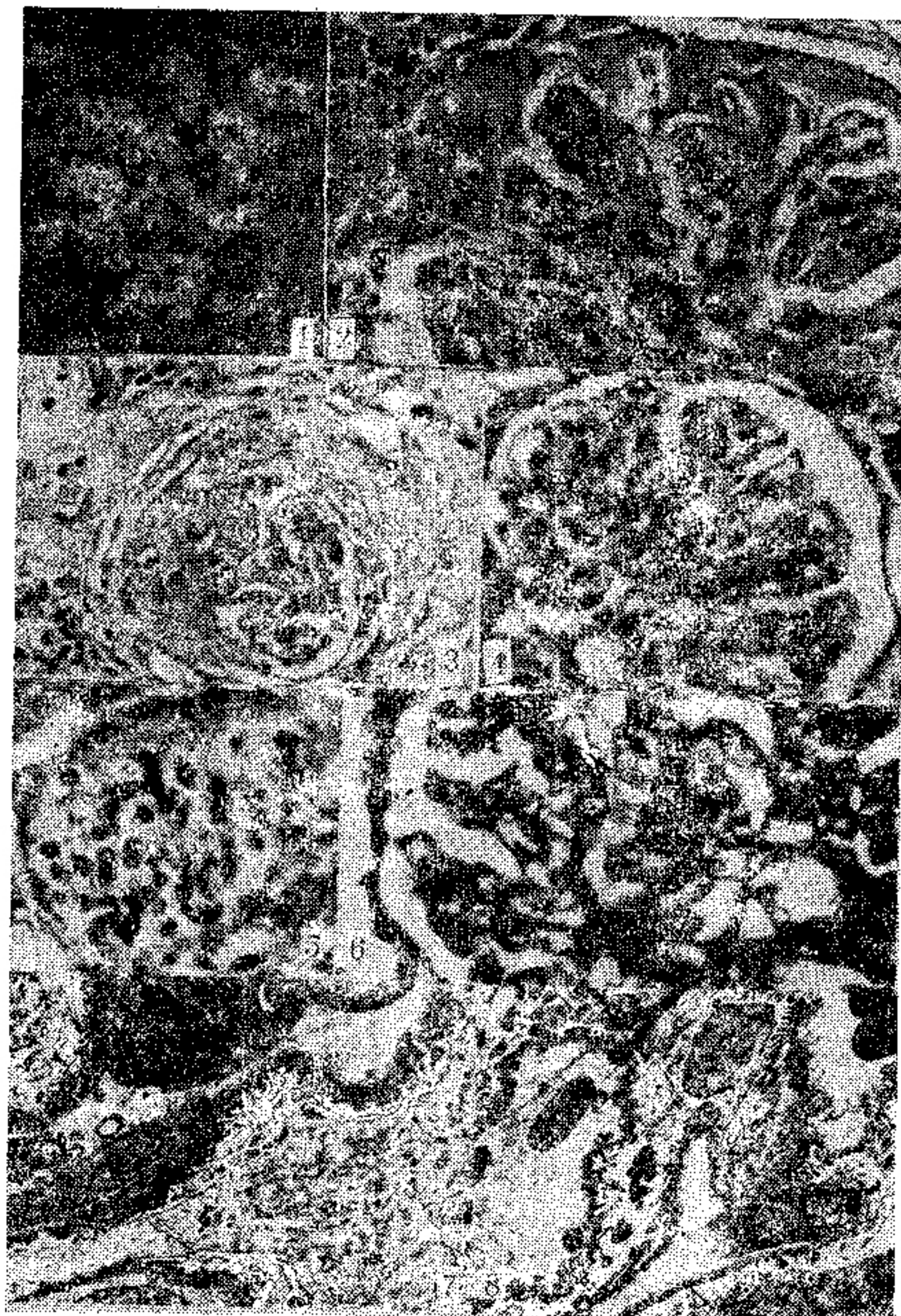


图1 肾小球毛细血管壁上显颗粒状线性荧光 FITC 标记羊抗兔 IgG $\times 150$

图2 肾小球丛毛细血管与囊壁粘连 HE $\times 600$

图3 新月体已纤维化, 周围炎细胞浸润 HE $\times 300$

图4 肾小球丛系膜细胞增生, 炎细胞浸润。肾小球细胞数明显增多 HE $\times 300$

图5 肾小球毛细血管内微血栓 (↑) PTAH $\times 300$

图6 肾小球分叶状。系膜基质增多 PASM $\times 600$

图7 肾小球毛细血管基底膜因条块状电子致密物于上皮下沉积而增厚(▲), 毛细血管腔内血小板粘附聚集(↑), 腔内微血栓形成 EM $\times 3750$

图8 治疗组基底膜上电子致密物(△), 基底膜轻度增厚, 管腔通畅 EM $\times 3750$

硬化(图3):肾小球细胞数增多,其中以系膜细胞明显增生及中性白细胞浸润为主(图4):C、M、MC三组肾小球毛细血管丛内细胞数(个, $\bar{x} \pm S$)分别为 99.40 ± 18.53 、 77.69 ± 17.89 、 66.55 ± 7.75 ,三组相比差异均有显著性($P < 0.01$)。PTAH染色,C组部分动物肾小球毛细血管内有微血栓(3/7),见图5。PASM染色C组显GBM增厚,系膜基质增多,肾小球呈分叶状,见图6。

五、电镜观察:C组GBM呈不均匀性增厚,与脏层上皮细胞间出现条块状电子致密物沉积,该处上皮细胞空泡变性,足突融合,甚至上皮细胞从GBM上脱落,使GBM裸露于尿腔;有的肾小球毛细血管腔内红细胞、血小板聚集,白细胞嵌塞,微血栓形成,内皮细胞空泡变性或脱落,见图7。M组虽见GBM不规则增厚及上皮下电子致密物沉积,但病变较C组轻,且毛细血管通畅,未见微血栓,见图8。

讨 论

一、本实验的原位免疫复合物型肾小球肾炎模型是成功的,且成功率达100%。从C组及M组的颗粒线性荧光、尿蛋白的出现,光镜和电镜的形态学改变都证实家兔发生了肾炎。其发病机理是血循环中抗c-BSA在GBM上与植入的c-BSA发生了抗原抗体反应,激活补体,释放炎性介质,而致肾小球损害。又因免疫复合物在GBM原位结合可直接损伤内皮细胞,进而启动血小板聚集、微血栓形成,使血凝和纤溶系统的活动过程障碍以及血栓素 A_2 /前列环素系统平衡失调,进一步加重了肾小球的损害。本实验的动物模型光镜下很象膜增生性肾炎,但电镜下仅见上皮下条块状电子致密物沉积,未见系膜插入内皮下及内皮下电子致密物沉积,我们认为仍属膜性肾炎模型。之所以系膜细胞增生,可能与我们所制的c-BSA的等电点高(9.1),致使GBM上沉积的免疫复合物及补体多有关。有研究表明^[2],补体介导血小板在肾小球内聚集,后者又间接引起系膜细胞增生及中性白细胞浸润,确切机理有待进一步阐明。

二、脉络通以桃红四物汤加黄芪制成,其以活血化瘀的药理作用,疏通微循环,改善血液供应,加强对免疫复合物的吸收和运转;同时抑制成纤维细胞合成胶原,促进胶原分解^[3],故抑制肾小球纤维化,从而改善肾炎的临床症状,明显减轻了蛋白尿。脉络通中川芎成分具有抑制血小板聚集及释放反应,调节血栓素 A_2 /前列环素平衡作用^[4],有人认为其抗聚集作用与阿斯匹林、潘生丁相似^[5]。抑制血小板聚集,使系膜细胞增生减少,这对防止肾小球进一步损害及硬化有重要的意义^[2]。红花除有上述作用外,还能减轻肾小球滤过膜的损害,从而减少尿蛋白量,并能加速上皮下免疫复合物的吸收和溶解^[6]。M组动物用脉络通治疗后病变减轻,肾小球毛细血管内未见微血栓,未见红细胞及血小板聚集及白细胞嵌塞,这表明该药物调整了血凝及纤溶系统、血栓素 A_2 /前列环素系统的平衡失调,阻抑了炎性细胞的渗出。虽然它不能从根本上阻断免疫复合物的形成,却能促进免疫复合物的吸收和溶解,抑制肾小球纤维化,从而保护了肾脏,为这一中药治疗肾炎提供了实验依据。详细的机制有待进一步探讨。

参 考 文 献

1. 章友康,等.用阳离子化牛血清白蛋白制作原位免疫复合物型肾炎模型.中华肾脏病杂志 1985; 1(1):7.
2. Richard J, et al. Platelet-complement interactions in mesangial proliferative nephritis in the rat. American Journal of Pathology 1991; 138(2):313.
3. 李景德,等.活血化瘀治则研究论文专辑.天津:中国医学科学院血液学研究所,1982:5.
4. 余真,等.川芎冲剂对冠心病患者血小板功能及前列腺素代谢的影响.中西医结合杂志 1987; 7(1):8.
5. 徐理纳,等.几种活血化瘀药和乙酰水杨酸对大鼠动脉壁前列腺素样物质的生成和血小板聚集性的影响.中西医结合杂志 1981; 1(1):36.
6. 许光辉,等.毛冬青甲素与苯基咪唑、藏红花对实验性原位肾炎的中长期疗效观察.中华肾脏病杂志 1989; 5(5):279.

· 消息 ·

安徽省高校科技联合培训部中医函授部面向全国招生

本部经安徽省教委批准面向全国招生。选用《全国高等中医院校函授教材》,所设12门中西医课程,与高等教育中医自学考试紧密配合。由专家教授教学和全面辅导,凡具有高中语文程度者均可报名,来函请寄至合肥市阜阳路48号安徽省高校联合培训部(邮政编码 230001),简章备索。

that PAD possessed the effect in preventing 274 persons on motion sickness. The total effective rate of PAD group was 83.9%, while that of Dramamine group was 60.8%. PAD revealed better effect than that of Dramamine. Therefore, the authors realize that PAD is a better preventive drug for motion sickness.

Key words Royal Made Ping An Dan, prevention, motion sickness

(Original article on page 19)

Clinical and Experimental Study on Yifei Jianshen Mixture(益肺健身合剂) in Preventing and Treating infantile Repetitive Respiratory Infection

Zhang Xiang -ping (张湘屏), et al

The Affiliated Hospital of Shandong College of TCM, Jinan (250011)

In recent years, the incidence of infantile repetitive respiratory infection has been increasing. In order to prevent and treat this disease, the authors suggest that pathogenesis of this disease is mainly due to insufficiency of Lung, Spleen and Kidney, which caused the Qi Deficiency and Blood Stasis Syndrome, and thus formulated Yifei Jianshen Mixture (YFJSM). The clinical and experimental study was carried out accordingly. The results showed that this mixture could increase the immune function of human body, and improve microcirculation, and has the function of warming up Yang and replenishing Qi, nourishing blood and activating blood circulation and eventually of preventing and treating diseases. 305 cases were clinically observed. the total effective rate being 95.1%. The effect of treatment is obviously better than that of control using Yupingfeng (玉屏风) powder ($P < 0.01$). The adrenocortical function, plasma nucleotide, immune function, microcirculation and others were the main technical indexes of models, which were nearly the same as clinical study. The toxicity test also showed that the YFJSM was non-toxic and had no side effect. The study proved that the mixture was highly effective and had no side effect.

Key words Yifei Jianshen Mixture, infantile repetitive respiratory infection, immune functions, microcirculation

(Original article on page 23)

Analysis of Clinical Effect and Experimental Study on 13 Herbs Anti-Cough-Dyspnea Decoction in Treating Chronic Bronchitis

Chen You-quan (陈友泉), Zhang Yan-ping(张燕萍)*

The 3rd People's Hospital, Shantou, Guangdong (515073)

**Xiyuan Hospital, Beijing (100091)*

In treating chronic bronchitis, the effect of 13 Herbs Anti-Cough-Dyspnea decoction was better than that of other traditional prescriptions such as Ephedria—almond decoction etc. The effective rate of this decoction in relieving cough, sputum, bronchial spasm and eliminating wheezing sound were 98.6%, 98.32%, 91.52% and 85.35% respectively. The total effective rate was 98%. The animal experiment revealed that the decoction was given to isolated trachea after medication for 30 min, the effective rate in easing bronchial spasm was $99.1 \pm 30.2\%$, which was two times than that of other prescriptions.

Key words chronic bronchitis, Herbs Anti-Cough-Dyspnea decoction

(Original article on page 27)

Research on Immune Complex in Situ Type Glomerulonephritis Treated with Mai-Luo-Tong (脉络通) in Rabbits

Wang Qiong-ling (汪琼玲), Huang Shou-zhen(黄寿珍)*, Sun Shi-lan(孙世澜), et al

Tongji Hospital, Tongji Medical University, Wuhan (430030)

**Dept. of Pathology, Tongji Medical University, Wuhan (430030)*

Applying c-BSA to duplicate immune complex in situ type glomerulonephritis in rabbits and treating it with Blood Circulation Promoting and Stasis-Removing Drugs Mai-Luo-Tong, the results