

• 实验研究 •

补阳还五汤对血管壁抗血栓功能的影响*

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内容提要 用人脐静脉灌流方法研究了补阳还五汤对血管壁抗血栓特性的影响, 结果表明, 补阳还五汤具有抑制凝血酶刺激血管壁释放 vW 因子的作用, 并抑制凝血酶凝固纤维蛋白原的活性, 但不影响血管壁对凝血酶的吸附。实验未观察到补阳还五汤对凝血酶刺激血管壁释放前列环素和纤溶抑制活性的作用有何明显影响。

补阳还五汤是治疗脑血管病的中医名方, 对脑血栓形成等病症疗效显著^①。近年来研究证明, 脑血栓形成与血管壁受损关系十分密切。为此, 我们采用人脐静脉灌流方法检测了补阳还五汤对血管壁抗血栓功能的影响。

材料与方

一、材料

1. 补阳还五汤: 由黄芪40g、当归15g、赤芍12g、川芎15g、桃仁12g、红花12g、地龙10g组成。先将各味药分别制备成注射剂(去鞣酸与蛋白), 再将各药按上述比例混合, 调pH为7.3~7.4, 生药含量为1g/ml, 冷藏。

2. 牛抗凝血酶[■]: 按Takehiko等^②所述用肝素亲和层析柱制备。

3. 牛蛋白质C粗提物: 按Comp等^③所述简化。即用枸橼酸钡吸附, EDTA洗脱, 硫酸铵分段盐析, 透析后冷藏。

4. 含纤溶酶原的牛纤维蛋白原: 在枸橼酸钠抗凝的牛血浆中加入1/13体积的1M氯化钡, 充分混合30min后离心去沉淀, 再加入聚乙二醇2000至7%浓度, 离心后用pH为5.4的枸橼酸缓冲液溶解沉淀, 再加入聚乙二醇2000至7%浓度, 离心去上清后用pH为7.4的Tris缓冲液溶解沉淀, 透析后分装冷藏。

5. 白陶土脑磷脂悬液: 在50ml pH为7.4的Tris缓冲液中加入白陶土1g, 脑磷脂150mg, 混匀后分装冷藏。

6. 凝血酶: 中国医学科学院血液研究所生产, 用pH为7.4的磷酸盐缓冲液(PBS)配制。

7. 尿激酶: 日本シドリ株式会社生产, 用pH7.4

的PBS配制。

8. von Willebrand因子(vW因子)酶联免疫测定药盒和6-酮-前列腺素 $F_{1\alpha}$ (6-keto- $PGF_{1\alpha}$)放射免疫测定药盒: 由苏州医学院血栓与止血研究室提供。

二、方法

1. 脐静脉灌流: 新鲜脐带用Hank氏液将脐静脉冲洗干净, 剪成等长两段。每段两端插入塑料管系牢, 浸入Hank氏液中。脐静脉一端接恒流泵, 另一端与恒流泵一端一同插入含RPMI1640灌流液的试管中, 这样灌流液将在脐静脉内反复循环。整个系统除恒流泵外均置于37°C水浴中。先以3.2ml/min灌流15min平衡, 再换预热的1640灌流液灌流30min, 此为基准灌流液。再换含凝血酶0.7u/ml、蛋白质C粗提物250μg/ml和补阳还五汤生药10mg/ml的1640灌流液灌流30min。另一条脐静脉灌流与此相同, 但作为对照在最后的灌流液中不含补阳还五汤。灌流结束后立即将灌流液置入冰浴, 用3.5%碳酸氢钠调pH与未灌流1640液一致(约7.4)后待测。

2. 凝血酶活性测定: 取含凝血酶灌流液100μl于瓷板凹孔中, 加入含240mg/dl的牛纤维蛋白原100μl, 挑丝计时, 记录纤维蛋白丝出现时间。由标准曲线计算凝血酶活性。

3. 活化的蛋白质C活性测定: 按Francis等^④所述改良。即以观察对白陶土部分凝血酶时间(KPTT)的影响检测蛋白质C活性。取灌流液100μl, 加抗凝血酶[■](800μg/ml)25μl, 10u/ml肝素15μl。室温下放置30min再加入0.1%硫酸鱼精蛋白10μl混合。于瓷板凹孔内加正常兔血浆100μl、上述混合液100μl、白陶土脑磷脂悬液50μl, 计时, 3min后加入0.025M氯化钙100μl, 挑丝记录血浆凝固时间。

4. 纤溶抑制活性测定: 55孔酶标板孔内加灌流液100μl, 2.5u/ml尿激酶50μl, 37°C湿盒内保温5min

*国家自然科学基金资助课题 [△]现在湖南医科大学

后加入20u/ml凝血酶及240mg/dl含纤溶酶原的纤维蛋白原溶液各50 μ l, 37°C湿盒内保温3h后用GXM-201型酶标光度计(四川分析仪器厂产品)在390nm处测光密度。在标准曲线上求得纤溶活性后再按下式计算纤溶抑制活性: 灌流液中纤溶抑制活性(u/ml) = (2.5 - 实测纤溶活性) \div 2。抑制1u尿激酶纤溶活性所需的纤溶抑制活性为1u。

5. vW因子抗原测定: 采用双抗体夹心法酶联免疫吸附试验测定。以稀释50倍的正常人混合血浆的vW因子含量为1u。

6. 6-keto-PGF_{1 α} 测定: 采用放射免疫分析法。

7. 统计学处理: 均采用配对t检验。

结 果

一、补阳还五汤对凝血酶活性及蛋白质C活性的影响: 含与不含补阳还五汤的灌流液经脐静脉灌流后其中凝血酶活性均明显下降(均为 $P < 0.01$, 见表1); 这两种灌流液中的凝血酶活性分别下降 $29.1 \pm 25.9\%$ ($M \pm SD$, 下同)和 $34.9 \pm 22.7\%$, 差异无显著性意义($P > 0.10$)。补阳还五汤本身对凝血酶凝固纤维蛋白原的活性有直接抑制作用, 抑制率为 $22.7 \pm 11.7\%$ 。

含与不含补阳还五汤的灌流液经脐静脉循环后均使KPTT延长(均为 $P < 0.01$), 提示灌流液中的蛋白质C活化。由于补阳还五汤本身对凝血酶有抑制作用, 因此未能将两种灌流液中的活化蛋白质C活性加以比较, 但应认为补阳还五汤对蛋白质C活化并无明显影响。

表1 脐静脉灌流液中凝血酶的灭活与蛋白质C的活化 ($M \pm SD$)

		凝血酶活性 (u/ml)	KPTT (s)
不含补阳还五汤灌流液	未灌流	$0.70 \pm 0.00(8)$	$23.6 \pm 4.6(8)$
	灌 流	$0.46 \pm 0.17^*$	$31.2 \pm 33.8^*$
含补阳还五汤灌流液	未灌流	$0.54 \pm 0.08(8)$	$28.0 \pm 5.8(8)$
	灌 流	$0.39 \pm 0.17^*$	$32.2 \pm 7.0^*$

注: 同一灌流液灌流前后比较, $*P < 0.01$; () 内为样本数

二、补阳还五汤对脐静脉释放vW因子与6-keto-PGF_{1 α} 的影响: 灌流液经脐静脉循环后, 出现vW因子及6-keto-PGF_{1 α} 如表2所示, 对照组与实验组基础灌流液中vW因子水平差异无显著性意义($P > 0.10$)。在凝血酶刺激下血管壁释放于灌流液中的vW因子增加。含与不含补阳还五汤的灌流液中的vW因子水平分别升高24.5%与43.4%。含补阳还五汤灌流液中的vW因子水平明显低于不含补阳还五汤灌流液中的

vW因子水平, 差异具有显著性意义($P < 0.05$)。这两种灌流液中6-keto-PGF_{1 α} 水平亦较基础灌流液中的高, 但上升幅度十分相近。

表2 补阳还五汤对vW因子和前列环素(PGI₂)释放的影响 ($M \pm SD$)

	组 别	vW 因子 (u/cm ²)	6-keto-PGF _{1α} (ng/cm ³)
对 照	基础灌流液	$0.597 \pm 0.223(9)$	$168.3 \pm 73.3(6)$
	含凝血酶灌流液	$0.856 \pm 0.552^*$	$228.5 \pm 105.3^*$
药 物	基础灌流液	$0.511 \pm 0.337(9)$	$161.2 \pm 117.7(6)$
	含凝血酶、补阳还五汤灌流液	$0.636 \pm 0.381\Delta$	$223.0 \pm 87.0^*$

注: 与基础灌流液比 $*P < 0.05$; 与对照组比 $\Delta P < 0.05$; () 内为样本数

三、补阳还五汤对纤溶抑制活性的影响: 凝血酶也刺激血管壁释放纤溶抑制活性。含与不含补阳还五汤的灌流液中的纤溶抑制活性分别由基础灌流液的 0.06 ± 0.05 u/ml和 0.09 ± 0.02 u/ml上升到 0.14 ± 0.04 u/ml和 0.21 ± 0.02 u/ml(均为 $P < 0.05$), 但两种灌流液中的纤溶抑制活性差别无显著性意义($P > 0.10$)。

讨 论

血管内皮细胞可以结合循环中的凝血酶⁽⁶⁾, 其膜上的血栓调制蛋白大大加速凝血酶对蛋白质C的激活。血管内皮细胞还可释放vW因子和前列环素(PGI₂), 后者很快转变为稳定的6-keto-PGF_{1 α} 。本实验表明, 含凝血酶及蛋白质C的灌流液经脐静脉循环后, 灌流液中凝血酶活性即下降, 并出现活化的蛋白质C活性及vW因子抗原和6-keto-PGF_{1 α} , 这些结果与内皮细胞的上述特性及Esmon等⁽⁷⁾用狗冠脉灌流的结果是一致的, 说明脐静脉灌流是一种研究人血管壁抗血栓特性的可行方法。

对补阳还五汤的作用机理曾有不少研究^(8,9), 但在其对血管壁抗血栓功能的影响方面报道尚少。用脐静脉灌流方法显示, 补阳还五汤具有抑制凝血酶刺激血管壁释放vW因子的作用。vW因子参与血小板粘附于内皮下组织, 在止血及血栓形成中起重要作用, 在脑血栓性疾病中常升高。补阳还五汤抑制vW因子释放, 有利于防止血栓形成及其增大。本实验还表明, 补阳还五汤具有直接抑制凝血酶凝固纤维蛋白原的作用, 这也可能是其发挥疗效的机理之一。有实验证明, 具有抗凝作用的水蛭素及丝氨酸活性中心阻断剂二氯磷酸盐也可阻断凝血酶刺激血管内皮细胞释放vW因子⁽¹⁰⁾, 提示凝血酶凝固纤维蛋白原与刺激vW

因子释放的活性可能均是通过丝氨酸活性基团来实现的。补阳还五汤是否也是通过某种成分阻断同一活性基团而起作用,值得进一步研究。

补阳还五汤在抑制凝血酶活性的同时,并不影响血管壁对凝血酶的灭活。在本实验所用浓度的补阳还五汤存在的情况下,蛋白质C也仍可活化。但由于补阳还五汤本身对凝血酶的抑制作用,用KPTT作为检测蛋白质C活性的观察终点显然不能表现补阳还五汤对蛋白质C活化的影响,进一步的研究有赖于测定方法的改进。

组织型活化素(tPA)与活化素抑制物是调节机体纤溶的重要物质。从血管内皮细胞体外培养得到的实验结果表明,凝血酶可加速二者从内皮细胞的释放,但对tPA释放的促进作用更为明显^[11]。而本实验表明,在凝血酶作用下血管壁主要释放纤溶抑制活性。这是否与血管平滑肌参与纤溶抑制活性的释放^[12]有关还尚待研究。

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外用“梔黄散”治疗关节扭伤150例

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我们用自制梔黄散治疗急性单纯性关节扭伤150例,疗效较好,介绍如下。

一般资料 150例中男123例,女27例。年龄最小者11岁,最大者60岁。踝关节扭伤89例,膝关节扭伤20例,腕关节扭伤36例,肘关节扭伤5例。病程最短者1小时,最长者72小时,平均2小时。

治疗方法 取生梔子、生大黄各等份,用粉碎机粉碎后消毒备用。用法:将扭伤部位洗净后,取药粉适量,24小时内就诊者以醋调外敷,24小时后就诊者以酒精调敷。有外伤者按常规清创消毒后调敷上药。敷药范围以直径大于肿痛区2cm为度,药厚0.5cm,

用塑料薄膜及绷带包扎固定,一般24小时换药1次。若药物干燥可用酒精直接外滴,保持湿润,亦可用原药重新调敷。

结 果 本组病例全部治愈,用药12小时即可止痛。其中24小时开始消肿、48小时痊愈者130例,72小时内愈者15例,96小时内愈者5例。平均治愈时间为52小时。

体 会 关节扭伤证见红肿热痛,属瘀血内阻,蕴而生热。梔黄散用酒精或醋调敷患处,可借其发散之力渗入肤内,起到活血化瘀、清热解表、通利关节、消肿定痛之作用。此法便、廉、验,值得推广。

Studies of the Effects of Buyang Huanwu Decoction(补阳还五汤)on the Antithrombotic Functions of Vessel Wall

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The effects of Buyang Huanwu Decoction(BYHWD) on the antithrombotic functions of vessel wall were studied with human umbilical vein perfusion. It was observed that the both of Von Willebrand factor release stimulated by thrombin from the vessel walls and conversion of fibrinogen to fibrin catalyzed by thrombin were inhibited by BYHWD. There were no obvious effects of BYHWD on the thrombin adsorption to the vessel walls and the thrombin induced release of PGI₂ as well as fibrinolysis inhibiting activity from the vessel walls.

(Original article on page 545)

Experimental Studies on the Relationship Between Kidney and Reproduction

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To elucidate the mechanism of TCM in treating polycystic ovary syndrome, the authors tried to produce experimental PCO by sterilizing Kunming mice with 1 mg testosterone in various ages of day (5, 8, 11, 14, 17, 21 days). Every litter consisting of 10 young mice was divided into two groups: testosterone given group (T) and control group (C). At the age of 72~75 day, ovaries and uteri were weighed and all ovaries were investigated histologically. Mating was attempted in the two groups. 19 of the 20 ovaries in T₅ group, but none of the other groups showed polycystic features. 4 of the 15 mice in T₅ group and all of the other groups resulted in pregnancy ($P < 0.05$). It was suggested that polycystic ovary was formed in the Kunming mice at age of less than 5 days administered with testosterone.

For observation of tonifying Kidney drugs on the ovaries and uteri of the polycystic ovary model in Kunming mice, testosterone was given to Kunming mice of various age (2, 5, 8, 11, 14 days). The tonifying Kidney drugs for anovulatory menstrual disorder were separated into two parts, water soluble part(MS) and ester soluble part (ML). At the age of 56 days (8 weeks), two parts of the drug were given for 14 days to every group of the mice respectively. One day after the administration, uteri were weighed, ovaries were investigated microscopically, and mating was attempted in both groups. All uteri in mice taken water soluble drugs(MS) increased in weight ($P < 0.05$), but not in the ML groups. All ovaries in groups of 2-day age (T₂) and most ovaries in T₅ groups showed polycystic features. No significant difference of the ovulatory and pregnant rates was found between all MS groups and ML groups with their control groups (CS, CL) respectively. Interstitial glands in the ovary of the MS groups were increased in size, but not the ML groups. It was suggested that the MS of tonifying Kidney drugs had an important effect on the dysfunctional ovary in mice, and this polycystic ovary animal model may be taken as the experimental animal model in TCM study on regulating reproductive endocrinological function.

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Effect of Chang Chun Dan(长春丹)on Noradrenaline Level of

Brain and Plasma in Aged Rats

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In this paper, the rats were randomly divided into three groups: (1) Young (Y); (2) Aged (A); (3) Aged rats treated with Chang Chun Dan (AT) for 3 months. The level of noradrenaline (NA) in brain and plasma of three group rats had been examined by radioenzymatic assay. The changes of fluorescent intensity of NA in locus coeruleus had been observed by histochemical method. The levels of NA in hypothalamus of three groups were 162 ± 9.93 , 209 ± 13.2 , 167 ± 11.8 ng/g wet tissues and in plasma were 1.29 ± 0.16 , 0.84 ± 0.08 , 0.96 ± 0.08 ng/ml respectively. The results showed that the NA level of hypothalamus in AT group was lower than that of A group ($P < 0.05$), and the NA level in Y group was raised markedly comparing with A group ($P < 0.01$). The plasma NA level in A group was significantly lower than that in Y group ($P < 0.05$). The NA level in AT group was raised a little comparing with A group. But the fluorescent intensity of NA in locus coeruleus between AT and A group was no difference. The results suggested that the changes of neurotransmitter induced by Chang Chun Dan may be basis of delaying aging effect and improving symptom of Kidney deficiency induced by aging.

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