

· 实验研究 ·

三七总皂甙抗炎和镇痛作用及其机理探讨

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内容提要 本研究通过建立炎症动物模型,应用三七总皂甙(TSPN)、电针(EA)进行治疗,并用纳洛酮(Nx)阻断两者作用,用组织化学的方法对TSPN和EA的效应进行比较。结果可见痛阈(PT)在TSPN组、EA组和EA加TSPN组均显著提高,且硝基蓝四氮唑试验(NBT)阳性的多形核白细胞(PMN)和醋酸萘酚酯酶(ANAE)的点型亚群计数均明显增加,以上的全部效应均被纳洛酮部分阻断;TSPN组与Nx组腹腔肥大细胞脱颗粒率无明显差异。由此可见,TSPN与EA效应相似,具有明显的抗炎镇痛及免疫调整作用。这提示三七总皂甙可能是阿片样肽受体的激动剂而不具有成瘾副作用。

关键词 三七总皂甙 电针 纳洛酮 痛阈 细胞免疫

三七(*Panax notoginseng*(Burk) F. K. Chen))为五加科(Araliaceae)人参属植物,近来研究表明它的药理作用已由原来单一的对血液系统的作用扩大至对机体多系统的作用,但尚未见到有关三七在抗炎镇痛过程中对辅助性T细胞、粒细胞和肥大细胞影响的文献报道。已知阿片样肽(OLP)在针刺镇痛中起主要作用^[1],纳洛酮(Nx)是特异的OLP受体阻断剂,本实验通过Nx的阻断作用,观察针刺效应与三七药物效应的关系,从而探讨三七总皂甙(TSPN)的抗炎镇痛机理。

材料与方法

一、实验动物及处理

1. 正常雄性封闭群昆明种小鼠42只,体重20g左右(由本校实验动物中心提供),随机分成4组,首先给予每只小鼠腹腔注射无菌液体石蜡0.1ml,建立炎症动物模型,24h后测痛阈(用WQ-9E痛阈测量仪,钾离子透入法),剪尾取少许抗凝血,制血滴片,随后进行4种不同的治疗。(1)TSPN组:腹腔注射TSPN 100 mg/kg(由中国科学院昆明植物研究所杨崇仁教授提供)。(2)电针(EA)组:腹腔注射等量生理盐水20 min后,以国产CDML-2型双频针麻治疗仪施以电针双侧“足三里”穴20 min,频率1~2 Hz,空载电压1.5 V。(3)Nx加TSPN组:腹腔注射Nx(1 mg/kg)20 min后,同TSPN组治疗。(4)EA加TSPN组:同TSPN组治疗20 min

后,施以电针(同EA组),均每天治疗1次,7天后,测痛阈,剪尾取血。

2. 正常雄性封闭群昆明小鼠18只,体重20g左右(由本校实验动物中心提供),随机分成3组,摘眼球放血脱颈致死,腹腔注入1 ml MCM(mast cell medium),按摩腹部1 min,取腹腔液,离心(500 r/min, 5 min)弃上清,用MCM洗1次,离心(1000 r/min, 5 min),分别加入终浓度为2%的TSPN, 10^{-6} mol的Nx以及等量生理盐水,后者作为对照,37℃孵育15 min,然后再用MCM洗2次离心(1000 r/min, 5 min)加入终浓度为 10^{-10} mol/L的P物质37℃孵育15 min,用MCM洗2次,离心(1000 r/min, 5 min),制成细胞悬液备用。

二、标本制备及染色

1. 酸性非特异性酯酶(ANAE)组化染色 取血制成血滴片,以15%福尔马林钙固定10 min,经蒸馏水洗后,放入ANAE培育液[4%盐酸副品红0.6 ml+4%亚硝酸钠0.6 ml混匀1 min,加入20 ml/1/15 mol磷酸缓冲液(pH 7.0~7.1)+ α -醋酸萘酯10 mg溶解于0.4 ml丙酮,调pH至5.8],立即置37℃1.5 h,水洗后以1%甲绿复染,油镜观察计数点型,T辅助性细胞亚群。

2. 硝基蓝四氮唑(NBT)还原试验 取20 μ l抗凝血加40 μ l NBT应用液(等量0.2%NBT生理盐水溶液及0.15 mol/L磷酸葡萄糖缓冲液混合)滴加于凹面玻片孔内混匀,立即置37℃15 min,滴片,室温干燥后用15%福尔马林钙固定,0.01%中性红复染,油镜观察计数,每个细胞含有10个以上甲臞

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颗粒者为阳性。

3. 甲苯胺蓝(TB)组化染色 经体外孵育后的腹腔液, 用10%中性福尔马林PBS固定10 min(腹腔液: 固定液=3:1), 滴加于载玻片上, 4℃干燥后置0.5%甲苯胺蓝50%酒精染液中1~2 min, 水洗, 经0.01%伊红染色, 95%酒精分色, 100%酒精脱水, 油镜下观察计数肥大细胞, 呈现脱颗粒象的肥大细胞为阳性。

结 果

一、痛阈(PT) 用每例治疗前后痛阈差值进行不同治疗组之间的比较, EA组与TSPN组、EA组与EA加TSPN组均无显著性差异($P>0.05$); 而EA组与Nx加TSPN组之间具有显著性差异($P<0.01$); TSPN组与Nx加TSPN组之间差别也具有显著性($P<0.05$), 见附表。

二、细胞免疫功能 以ANAE所显示的点型(胞质内有1~3个局灶样棕红色颗粒)计数检测细胞免疫功能(代表辅助性T细胞亚群, TH)⁽²⁾, 用每例治疗前后ANAE一点型率差值进行不同治疗组间的比较, EA组与TSPN组之间, EA组与EA加TSPN组之间均无显著性差异($P>0.05$), 而EA组与Nx加TSPN组之间、TSPN组与Nx加TSPN组之间均差异显著($P<0.01$), 见附表。

三、粒细胞杀菌功能 以NBT显示的阳性反应(细胞质内有深蓝色点状甲臆颗粒)检测多形核白细胞的杀菌功能, 用每例治疗后NBT阳性率进行不同治疗组之间的比较, EA组与TSPN组之间、EA组与EA加TSPN组之间均无显著性差异($P>0.05$); 而EA组与Nx加TSPN组之间, TSPN组与Nx加TSPN组之间差别均显著($P<0.05$), 见附表。

附表 EA、TSPN及Nx对致炎小鼠PT、ANAE、NBT的影响 ($\bar{x} \pm S$)

组 别	PT (mA)	ANAE (%)	NBT (%)
EA	16.6±3.0	10.8±1.3	9.3±0.7
TSPN	15.0±3.4*	10.4±1.1*	10.4±1.3*
EA+TSPN	13.8±1.8*	11.5±1.5*	8.7±0.4*
Nx+TSPN	10.4±2.7**△	2.5±2.0**△△	5.8±0.7***△

注: 与EA组比较* $P>0.05$, ** $P<0.01$, *** $P<0.05$; 与TSPN组比较, △ $P<0.05$, △△ $P<0.01$ 。

四、肥大细胞脱颗粒率 以TB组化染色显示体外孵育后, 肥大细胞释放5-羟色胺、组织胺、P物质、缓激肽等活性物质的情况, 结果可见Nx组与TSPN组之间无显著性差异($P>0.05$); 而对照

组与Nx组以及TSPN组之间均有显著性差异($P<0.01$)。

讨 论

一、三七对痛阈的影响 已知OLP在镇痛效应中起主要作用, 针刺可使中枢神经系统和外周组织OLP释放增加⁽³⁾, 从而提高痛阈, Nx作为OLP受体的阻断剂可阻断电针的镇痛效应, 本实验TSPN的镇痛作用亦可被Nx阻断, 这提示TSPN的镇痛作用可能通过OLP起效应。

二、三七对细胞免疫功能及粒细胞杀菌功能的影响 我们⁽⁴⁾曾报道针刺可提高ANAE点型细胞百分率, 可提高体液免疫、粒细胞吞噬活动以及网状内皮系统功能。本实验见到Nx加TSPN组与EA组、与TSPN组在ANAE点型率及NBT阳性率之间均有显著性差异($P<0.01$, $P<0.05$), 由此提示的药效可被Nx阻断, 其作用与针刺原理相似, 除抗炎镇痛外, 尚有“扶助人体正气, 增加机体抵抗力和调整代谢”的作用。

三、三七对肥大细胞的影响 研究表明^(5,6), 针刺不但具有镇痛效应, 且可使针刺局部穴位及远隔部位和腹膜及腹腔肥大细胞脱颗粒, 本实验显示用TSPN、Nx分别体外孵育肥大细胞, 其脱颗粒率无显著性差异($P>0.05$), 我们认为这与三七的药理特性有关, 它通过抑制肥大细胞释放组织胺等活性物质, 抗渗出, 改变炎症早期的血管通透性而具抗炎作用, 这与刘杰等人⁽⁷⁾研究证明的三七皂甙能对抗缓激肽、组织胺、5-羟色胺等所致毛细血管通透性增强及炎症组织PG的释放的效应相符。

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group (31 cases) and normal control group (28 cases). Treated group used intravenously injected Ligustrazine 160 mg a day for 10 days. The plasma lipid peroxidation (LPO), superoxide dismutase (SOD) and sulfhydryl group were measured before and after treatment in the treated group, in comparing with normal control group. The results showed the levels of LPO significantly reduced ($P < 0.01$), and SOD, sulfhydryl group was obviously increased after Ligustrazine administration ($P < 0.05$). Attack of angina pectoris was reduced. Between these two group, the difference was significant ($P < 0.05$). Mechanism of Ligustrazine in reducing LPO and increasing SOD could be the inhibiting of platelet aggregation, regulating the TXA_2/PGI_2 ratio in plasma, protecting myocardial cell membrane and improving the myocardial ischemia. Ligustrazine could reduce the production of LPO due to the accelerating clearance of oxygen free radical.

Key words Ligustrazine, coronary heart disease, lipid peroxidation, superoxide dismutase

(Original article on page 26)

Study of *Andrographis Paniculata* Extracts on Antiplatelet

Aggregation and Release Reaction and its Mechanism

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63 patients of cardiac and cerebral vascular diseases were observed at 3 hours and/or one week after taking *Andrographis paniculata* (AP) extracts. Results showed that both 1 min. and 5 min. platelet aggregation induced by ADP were significantly inhibited ($P < 0.001$), 33 cases of them were observed for one week. The aggregation rate was even significantly lower than that of 3 hours. Serotonin (5-HT) release reaction from platelet was observed in 20 volunteers taking AP. Result showed 5-HT released from platelet decreased ($P < 0.01$), but plasma 5-HT level remained unchanged. The ultrastructural observation showed that AP could inhibit the releasing of dense and α agranules from platelet and dilating of canalicular system. It suggested that a raise of platelet cAMP level might be the mechanism of antiplatelet effect of AP.

Key words *Andrographis paniculata*, platelet aggregation, platelet release, serotonin

(Original article on page 28)

Relationship between Fissured Tongue with Flow and Components of Saliva

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Observation on 30 patients with fissured tongue (FT) and 42 normal subjects, through the measurement of flow and the elements of saliva, pH value, the Na and K content, SIgA, it was shown that: in FT patients, the flow of saliva and pH decreased, while the Na/K ratio and the content of SIgA increased (each $P < 0.01$, compared with the normal). It was also showed that the formation of FT has to a certain extent something to do with the electrolyte disturbance in aqueous solution, malnutrition, as well as immunity and endocrinic function of patients.

Key words oral membranous disease, fissured tongue, saliva

(Original article on page 31)

Effects and Mechanism of Total Saponins of *Panax Notoginseng* on

Anti-Inflammation and Analgesia

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In this study the effects of total saponins of *Panax notoginseng* (TSPN) and electroacupuncture (EA) were compared. Liquid paraffin was intraperitoneally injected (0.1ml/mouse) to establish the animal model with inflammation. The mice were randomly divided into 4 groups with different treatments for 7 days: EA group, TSPN group (100mg/kg intraperitoneal administration), Naloxone (Nx) plus TSPN group and EA plus TSPN group. The pain threshold was measured by a detector