

补髓生血冲剂对慢性再生障碍性贫血患者血清可溶性 Fas、肿瘤坏死因子、白细胞介素 2 表达作用的影响

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摘要 为探讨补肾中药补髓生血冲剂抑制慢性再生障碍性贫血(CAA)细胞凋亡的疗效机理,分析了 30 例 CAA 患者血清可溶性 Fas(sFas)、肿瘤坏死因子(TNF- α)、白细胞介素 2(IL-2)水平在用药前后的变化规律,并用再障生血片进行对照研究。结果,患者疗前血清 sFas、TNF- α 、IL-2 表达均明显高于正常组($P < 0.05$ 或 $P < 0.01$),疗后 sFas、TNF- α 水平接近正常组($P > 0.05$),而 IL-2 水平仍高于正常组($P < 0.05$)。表明:(1)CAA 患者血清存在着细胞凋亡相关因子的表达异常;(2)补髓生血冲剂是通过调节 TNF- α 水平来调控 CAA 患者细胞表面 Fas 分子的表达,并影响血清 sFas 的表达,而并非通过调节 IL-2 表达水平来影响 CAA 细胞凋亡。

主题词 贫血,再生障碍性/中药疗法 @ 补髓生血冲剂

再生障碍性贫血(Aplastic Anemia, AA)是一种与全血细胞减少有关的血液系统疾病,其发病机制十分复杂。近期的研究表明,造血干/祖细胞过早或过度凋亡在 AA 发病机制中占有重要的地位。我们课题组的前期工作提示,骨髓造血干细胞存在着细胞凋亡现象,证实了 AA 时骨髓造血细胞凋亡明显增加,这与有关报道一致^[1]。本文用免疫学方法检测慢性再生障碍性贫血(Chronic Aplastic Anemia, CAA)患者血清 sFas、肿瘤坏死因子(TNF- α)、白细胞介素 2(IL-2)表达水平,并观察用药前后的变化规律,以进一步探讨 CAA 发病中的凋亡机制及补肾中药补髓生血冲剂治疗 CAA 的疗效机理。

1 临床资料

所有 CAA 患者的诊断均符合 1987 年中华血液学会第四届全国再生障碍性贫血学术会议制定的 AA 及 CAA 诊断标准^[2]。采用单盲法将 48 例 CAA 患者随机分为补髓生血冲剂实验组(以下简称实验组)30 例,再障生血片对照组(以下简称对照组)18 例。实验组男 17 例,女 13 例,年龄 9~62 岁,中位年龄 35.5 岁;对照组男 6 例,女 12 例,年龄 6~65 岁,中位年龄

37 岁。其病程、病情、年龄等与实验组大致相似。另设正常对照 30 例,男 16 例,女 14 例,平均年龄 38 岁,皆为健康献血员。

2 方法

2.1 治疗方法:实验组口服补髓生血冲剂(熟地黄 5 份、山茱萸 3 份、枸杞子 4 份、淫羊藿 5 份、巴戟天 5 份、鹿茸 5 份、黄芪 10 份、人参 3 份等 12 味中药),每次 1 袋,每日 3 次。对照组用再障生血片,主要成分:黄芪、阿胶、人参、鹿茸等,吉林省辽源市中药厂生产,吉卫药准字(84)730017。每次 5 片,每日 3 次口服。如感染严重可加用抗生素;出血严重者可适当使用止血药;贫血严重者可输血缓解病情。实验组与对照组均以 3 个月为 1 个疗程,两个疗程后按照 1987 年中华血液学会第四届全国再生障碍性贫血学术会议制定的 AA 及 CAA 疗效判定标准判定疗效^[2]。

2.2 观察指标及方法

2.2.1 标本处理:取 CAA 患者和正常献血员的静脉血 2ml,注入加有抗凝剂 EDTA 的无热原和内毒素的试管内,4℃,3500r/min 离心 20 分钟,取上清,按一次用量分装,冻存于-20℃待测。注意去除溶血或高血脂标本。

2.2.2 检测方法:sFas、TNF- α 的测定采用酶联免疫法(ELISA),试剂盒分别由深圳晶美生物工程有限公司和北京邦定生物有限公司提供,操作按说明书进行。IL-2 测定采用放射免疫法(RIA),试剂盒由北京邦定生物有限公司提供,操作按说明书进行。

2.3 统计学方法:采用 t 检验及相关分析。

3 结果

3.1 补髓生血冲剂与再障生血片疗效比较:从表 1 可见,补髓生血冲剂治疗 CAA 30 例,结果为基本治愈 1 例,缓解 12 例,明显进步 9 例,无效 8 例,总有效率为 73.33%。对照组 18 例,基本治愈 0 例,缓解 5 例,明显进步 6 例,无效 7 例,总有效率为 61.11%。两组疗效

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表 1 两组临床疗效比较[例(%)]

组别	例数	基本治愈	缓解	明显进步	无效	总有效
实验组	30	1(3.33)	12(40.00)	9(30.00)	8(26.67)	22(73.33)
对照组	18	0	5(27.78)	6(33.33)	7(38.89)	11(61.11)

表 2 两组治疗前后血清 sFas、TNF- α 和 IL-2 水平变化比较 ($\bar{x} \pm s$)

组别	例数		sFas(ng/ml)	TNF- α (OD 值)	IL-2(ng/ml)
正常组	30		5.05 $\pm$ 0.15	85.55 $\pm$ 13.73	5.13 $\pm$ 0.15
实验组	30	疗前	6.39 $\pm$ 0.05*	216.85 $\pm$ 30.17**	6.15 $\pm$ 0.17*
		疗后	5.10 $\pm$ 0.06 $\Delta\Delta$	88.29 $\pm$ 8.39 $\Delta\Delta$	6.08 $\pm$ 0.17*
对照组	18	疗前	6.38 $\pm$ 0.04*	216.67 $\pm$ 30.43**	6.19 $\pm$ 0.17*
		疗后	5.14 $\pm$ 0.06* Δ	147.82 $\pm$ 13.56* Δ	6.10 $\pm$ 0.12*

与正常组比较, * $P < 0.05$, ** $P < 0.01$; 与本组疗前比较, $\Delta P < 0.05$, $\Delta\Delta P < 0.01$; 与对照组比较, $\Delta P < 0.05$, $\Delta\Delta P < 0.01$

差异显著 ($P < 0.05$)。

3.2 实验组和对照组疗前、疗后血清 sFas、TNF- α 和 IL-2 水平与正常组比较:由表 2 可见,两组疗前血清 sFas、TNF- α 及 IL-2 水平均高于正常,差异有显著性 ($P < 0.05$ 或 $P < 0.01$);疗后两组 sFas、TNF- α 水平均有所降低,与疗前比差异显著 ($P < 0.05$, $P < 0.01$),实验组与正常组比差异无显著性 ($P > 0.05$),而对照组与正常组及实验组相比差异均显著 ($P < 0.05$, $P < 0.01$);IL-2 水平虽有所降低,但与疗前比差异无显著性 ($P > 0.05$),与正常组比差异仍显著 ($P < 0.05$),两组间比较差异无显著性 ($P > 0.05$)。

4 讨论

慢性再生障碍性贫血是临床难治的血液系统疾病,其致病原因多样,发病机制复杂。近期研究表明,再生障碍性贫血与细胞凋亡有关,但其凋亡机制不十分清楚。Maciejewski 等^[3]研究了 Fas 抗原表达在 AA 病理生理过程中的意义,发现 AA CD₃₄⁺细胞 Fas 抗原表达高达 43.0%,并抵抗 Fas 抗原 McAb 介导的造血抑制效应敏感性增加,说明 Fas 系统与 AA 的细胞凋亡密切相关。Fas 系统是细胞凋亡过程中重要的信号传递系统。由蛋白水解酶裂解膜结合 Fas 或 Fas 变异体 mRNA 翻译而成的 Fas 不含跨膜区,以可溶性形式存在于血液中。血清中可溶性 Fas 通过与细胞 Fas 分子竞争结合 Fas 配体 (FasL) 而阻断 Fas 介导的细胞凋亡^[4]。TNF 作为凋亡诱导因子之一,其诱导细胞凋亡的机制和途径是通过增加细胞内 Ca²⁺ 浓度和 DNA 的断裂实现的,且主要通过上调 Fas 水平而促进凋亡的发生。Philpott 的研究^[5]发现,AA CD₃₄⁺细胞体外受 TNF- α 刺激后,表达高水平的 Fas 抗原。我们的研究结果显示,CAA 患者血清 sFas、TNF- α 均显著高于正常水平,经补髓生血冲剂治疗后,两者水平都接近正常。我们推测可能由于促凋亡因子 TNF- α 水平的上升,导致了细胞表面 Fas 分子表达的增加,细胞 Fas 分子竞争结合 FasL,致使血清中 sFas 水平升高。我们研制的补肾中药补髓生血冲剂可下调 TNF- α 水平,从而降低细胞表面 Fas 分子的表达。细胞表面 Fas 分子的表达水平降低后,血清中 sFas 竞争结合 FasL,其在

血清中的水平则下降。

IL-2 是一种主要由辅助性 T 细胞分泌的淋巴因子,与细胞凋亡之间的相互关系很复杂。IL-2 可以通过不同的机制,对不同细胞的凋亡起抑制或促进作用。IL-2 主要通过活化蛋白激酶 C 和酪氨酸蛋白激酶等细胞内传递系统来影响自身基因 Fas、Bcl-2 等的表达,进而调控细胞凋亡。目前有关 IL-2 与 Fas 在 AA 细胞凋亡中的协同作用的报道很少。而本实验初步观察到,CAA 的 IL-2 水平较正常人明显升高,使用补肾中药治疗后 IL-2 未能降低到正常水平,这说明补肾中药对 CAA 患者 IL-2 的异常升高没有影响。

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ABSTRACTS FROM ORIGINAL ARTICLES

Observation on Therapeutic Effects of Different Needling Methods of Acupuncture at Cervical Jiaji Points on Cervical Spondylopathy

Du Yuzheng, Li Dajun

104 cases of cervical spondylopathy were treated with acupuncture of Jiaji (EX-BL) points with different needling methods. In 53 cases of conduction group, the needle was inserted at the level of affected vertebral body into a depth of 1.5–2.0 cun based on indication of X-film, with reducing method of lifting and thrusting, making the upper limb of the affected side vibrating 1–2 times, and twirling and rotating method was used in the health side. Routine needling method was used in the control group. Results indicated that the total effective rate, the transient analgesic effect and long-term analgesic effect in the conduction group were significantly superior to those of the control group (all $P < 0.01$).

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Key words: Cervical spondylopathy/acup — moxibustion therapy Acupoint, Jiaji

(Original article on page 534)

Clinical Observation on 36 cases of Hashimoto's Thyroiditis Treated with Chinese Drugs Plus Small Dose of Jia Zhuang Xian Tablet

Pang Jie, Ye Liya

In order to observe clinical therapeutic effect of combined treatment of Chinese medicine and western medicine on Hashimoto's thyroiditis and its effect on immunological function, and preliminarily approach to mechanisms of the therapeutic effect, 68 cases Hashimoto's thyroiditis (HT) were randomly divided into treatment group (36 cases) treated with drugs for warming and tonifying spleen and kidney plus small dose of Jia Zhuang Xian Tablet, and control group (32 cases) treated with Western medicine Jia Zhuang Xian Tablet. Serum anti-thyroid auto-antibody (TGAb, TMAB) and T helper lymphocyte (TH) subgroup were detected before and after treatment. Results indicated that the cured rate, markedly effective rate, effective rate and the total effective rate were 8.33%, 13.89%, 69.45%, 91.67% in the treatment group and 0, 6.25%, 65.63%, 71.88% in the control group, with significant difference between the two groups ($P < 0.05$). Additionally, TGAb and TMAB levels before treatment in the patient of HT were higher than normal and number and ratio of TH were abnormal; after treatment the TGAb and TMAB levels decreased in the treatment group with significant difference as compared with the control group ($P < 0.05$) and ratio of TH subgroups tended to balance state.

Authors' address: China — Japan Friendship Hospital, Beijing 100029

Key words: Thyroiditis, Auto-immunity/ combined treatment of traditional Chinese medicine and Western medicine

(Original article on page 543)

Effects of Bu Sui Sheng Xue Instant Granules on Expression of Serum Soluble Fas, TNF- α and IL-2 in the Patient of Chronic Aplastic Anemia

Sun Weizheng, Luo Haihong, Li Haixia, et al.

In order to approach the mechanisms of Chinese drug Bu Sui Sheng Xue Instant Granules for tonifying kidney inhibiting apoptosis in the patient of chronic aplastic anemia (CAA), levels of soluble Fas (sFas), tumor necrosis factor (TNF- α), interleukin-2 (IL-2) before and after treatment were determined in 30 cases of CAA and Zai Zang Sheng Xue Tablet was used as control. Results indicated that expression of serum sFas, TNF- α and IL-2 before treatment was significantly higher than those of the control group ($P < 0.05$, $P < 0.01$), and after treatment the levels of sFas and TNF- α approached to the normal ($P > 0.05$), and the IL-2 level still was higher than the normal ($P < 0.05$). It is suggested that there is abnormal expression of correlative factors of apoptosis in serum of the patient of CAA; Bu Sui Sheng Xue controls expression of Fas on the surface of cells and influences expression of serum sFas in the patient of CAA through regulating TNF- α level, and apoptosis of CAA is influenced not through regulating expression of IL-2.

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Key words: Anemia, Aplastic/Chinese drug therapy @Bu Sui Sheng Xue Instant Granules

(Original article on page 546)

Study on Application of Damp — heat Syndrome Model of Traditional Chinese Medicine

Wu Shijiu, Yang Yungao, Tong Li, et al.

Basical study of application of TCM damp — heat model includes development of damp — heat animal model and mechanism study of removing heat and dampness. Results indicated that under the guidance of classical theory of TCM febrile diseases, on the basis of comparison of five factors and six methods of developing animal model, and the damp — heat models developed by means of combined factors (diet + climatic surroundings + pathogenic biological factors) approached to damp — heat syndrome of TCM in conditions of attack, affected Zang — and Fu — organs, and main symptoms and signs, with characteristics of good replace and simple manipulation.

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Key words: Damp — heat/TCM pathogenesis

(Original article on page 553)