中西医结合杂志 1985 年第 5 卷第11期

丹参酮对人白细胞趋化性影响的观察

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内容提要　本文应用琼脂糖玻片法进一步证实了丹参酮的抗炎效应，并探索了应用体外法白细胞趋化性实验进行抗炎药物筛选的可行性。研究表明，当健康人白细胞(5×10^7/ml)与 50ng/ml 丹参酮共同孵育 1 小时时，可使白细胞趋化性发生明显抑制；而对白细胞的随机运动无影响。若孵育时间持续 19 小时，则 5 ng/ml 丹参酮足以使白细胞趋化性及随机运动均发生有意义的抑制。

中药丹参(*Salvia miltiorrhiza* Bunge)的乙醚提取物丹参酮，具有明显的抗炎作用，已为一系列临床及实验性研究所证实[1~3]。作者根据国外在进行抗炎药物筛选中，也常规地检测药物对离体白细胞趋化性的影响[4,5]，以及根据国内制订的《抗炎药筛选规程(试用稿)》[6]的要求，对丹参酮对白细胞趋化性的影响，进行了体外法的实验性研究。目的在于进一步阐明丹参酮的抗炎效应并探索应用体外法白细胞趋化性实验筛选抗炎药物的可行性。

材料和方法

丹参酮(中国医学科学院药物研究所提供)、二甲基砜（DMSO、金山化工厂)、琼脂糖（上海东海制药厂)、趋化因子 AB 血型人血清（健康献血者)、RPMI 1640(日水制药株式会社)。

白细胞趋化性测定方法，选用 Nelson 琼脂糖玻片法(1975)[7]。在建立方法过程中，本研究室对 Nelson 原法略做改进[8]。

一、琼脂糖玻片制备：将20%琼脂糖液（48°C 水浴中保温)与 48°C 预热的 2 ×RPMI 1640-HEPES 液(加有 10%灭活的小牛血清)等量混合后，平铺于灭菌的玻片(26×76mm)上。凝固后放 4 °C 冰箱中 30 分钟。用模板和打孔器打孔，孔径 3 mm；孔距 2 mm，每片打 3 排× 6 孔。

二、白细胞悬浮液制备：用右旋糖酐法分离健康献血者白细胞，用 RPMI 1640 将白细胞调制成 1×10^7/ml 白细胞悬浮液。

三、丹参酮液配制：以 DMSO为助溶剂，配制成一定浓度的储备液做为原液备用。

四、实验步骤：

1.以向每 ml 白细胞悬浮液中添加 1 μl 的 DMSO 为对照组(即 0 组)；以向每 ml 白细胞悬浮液中添加

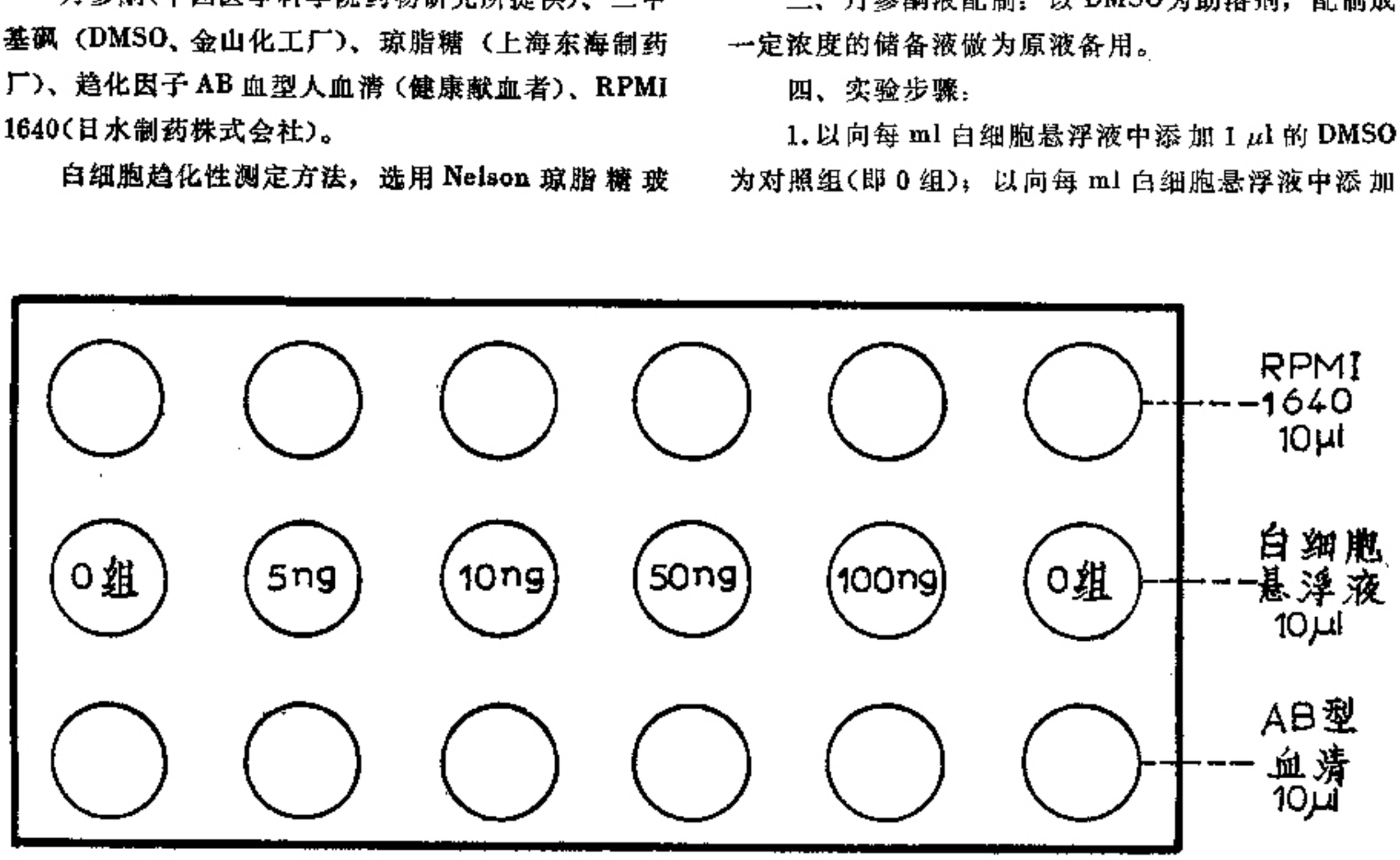

图1　琼脂糖玻片各孔配列图

5 ng、10ng、50ng、100ng 丹参酮为实验组，内含 DMSO 量亦均为 1 μl。

2. 添加药物后的白细胞悬浮液混匀后，置 37°C 孵箱培养 1 小时。弃上清取白细胞，又进而分为两组：（1）清洗组：将培养后白细胞用 RPMI 1640 冲洗两次后，调制成 5×10^7/ml 白细胞悬浮液，即丹参酮仅作用 1 小时；（2）不清洗组：将培养后白细胞用RPMI 1640 调制成 5×10^7/ml 白细胞悬浮液，即丹参酮可持续地发挥作用。

3. 依图1分别向琼脂糖板各孔中添加RPMI 1640、白细胞悬浮液和趋化因子各 10μl。置 37°C 培育18 小时。

五、结果判定：将培养后的琼脂糖板乙醇固定30分钟，剥去琼脂，染色后光镜下用测微尺准确测定白细胞随机运动及趋化性的距离，并依下式计算二者的抑制百分率[9]。

$$\frac{\text{实验组距离} - \text{对照组距离}}{\text{对照组距离}} \times 100$$

结　果

一、当丹参酮作用 1 小时时，5～50ng/ml 的丹参酮对白细胞的随机运动无有意义的影响，只有当浓度增至 100ng/ml 时，随机运动才见消失。但丹参酮在 50ng/ml 浓度时，却可使白细胞趋化性发生明显抑制，抑制率达 37.7%（P＜0.025）。至 100ng/ml 时，趋化性亦见完全消失（参见图 2）。

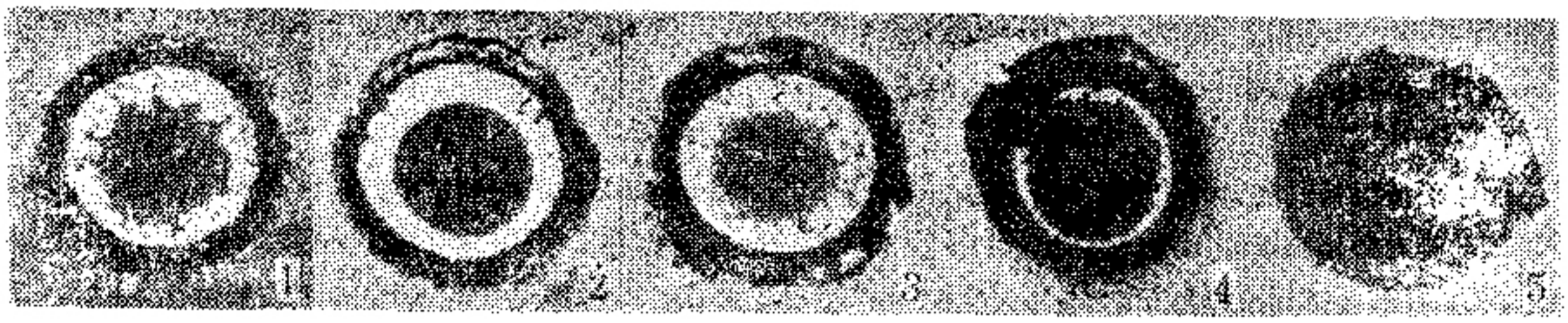

图2　不同浓度丹参酮对白细胞趋化性（琼脂糖下）的影响（光镜20倍）

① 0 组　② 5 ng/ml组　③ 10ng/ml组　④ 50ng/ml组　⑤ 100ng/ml组

二、当丹参酮持续作用 19 小时时，50ng/ml 浓度的丹参酮，就足以使白细胞随机运动、趋化性均发生有意义的抑制，抑制率分别为 79%（P＜0.001）、81.3%（P＜0.001）。

讨　论

随机运动（random motility）是白细胞所固有的不受趋化因子影响的无方向的运动。而趋化性（chemotaxia）则是白细胞向趋化因子方向移动的有方向的运动。在炎症过程中，炎区所出现的大量白细胞聚集，正是由于白细胞趋化性所决定的。高玉桂等[2]应用体内法观察到丹参酮可明显地抑制白细胞向炎区的游走，而本研究则证实，游走的减少系由于丹参酮抑制了白细胞趋化性的结果。因此，本文对揭示丹参酮的抗炎原理，进一步提供了实验依据。

目前，国内在抗炎药物筛选研究中，主要采用体内法观察药物对白细胞向炎区游走的抑制，但由于从炎区所搜集的白细胞数误差较大，因此，基本属于半定量方法。近年来，国外在筛选抗炎药物时，除采用体内法外，还必进行体外法观察药物对白细胞趋化性的影响。本研究首次报道了应用琼脂糖玻片法进行体外观察药物对白细胞趋化性的影响，证明本法作为抗炎药物筛选的指标，是可行的，值得广为应用。

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The Effect of Sodium Ferulate on Complement-Activated Hemolysis

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Sodium Ferulate (SF) is one of the ingredients derived from Radix Angelicae Sinensis and Rhizoma Ligustici Chuanxing. Red blood cells from normal persons or paroxysmal nocturnal hemoglobinuria (PNH) patients were incubated with SF and the effect of SF on Cobra Venom Factor (CoF) hemolysis test, activation of complement (C_3), the binding of C_{3b} with red blood cell membrane and red cell deformability was observed. The result revealed that SF could definitely reduce the hemolysis induced by CoF and the binding of C_{3b} to red cell membrane was decreased, but no influence on the activation of complement through alternate pathway or effect on red cell deformability was observed. The mechanism of action was briefly discussed.
(Original article on page 681)

Observation on Inhibitory Actions of Tanshinone on Leukocyte Chemotaxis

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As has been demonstrated by a series of clinical practices and experimental researches, Tanshinone (ether extract from the root of *Salvia miltiorrhiza* Bunge) possesses antibiotic and antiinflammatory actions. However it has not been reported whether Tanshinone has inhibitory action on leukocyte chemotaxis in vitro. This paper reports the observation of actions of Tanshinone on leukocyte random locomotion and chemotaxis in vitro by agarose plate assay for elucidation of Tanshinone's action. The results shows though human leukocytes chemotaxis was inhibited when Tanshinone (50 ng/ml) and leukocytes suspensions (10^7/ml) preincubated for 1 hour at 37°C, random locomotion showed no changes. When action of Tanshinone on leukocytes lasted 19 hours, both chemotaxis and random locomotion were inhibited by Tanshinone with the same concentration. The results demonstrate and confirm the antiinflammatory action and mechanism of Tanshinone.
(Original article on page 684)

Effect of Disodium Cantharidate and Injectio Herbae Sar Candrae on Energy and Cyclic Nucleotide Metabolism in Hepatoma 22 Cells and Liver Tissues of Tumor-Bearing Mice

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Effect of Disodium Cantharidate (DSC) and injectio herbae Sarcandrae on oxygen consumption, respiratory control ratio, oxidative phosphorylation efficiency, succinate dehydrogenase activity, catalase activity, cAMP and cGMP levels and phosphodiesterase activities in Hepatoma 22 cells and liver tissues of tumor-bearing mice were studied in vivo and in vitro.

The present data suggest that these two drugs are respiration inhibitors and can improve the energy metabolism of tumor cells and tumor-bearing mice. They also increase the catalase activity, showing a decrease in toxohormone level of these mice. The improvement of energy metabolism may be one of their ways to control or moderate carcinogenesis.

The cAMP/cGMP ratio was found much lower in Hepatoma cells than in livers of normal mice. It was also found that DSC not only elevated the intracellular cAMP level and cAMP/cGMP ratio, but also inhibited cAMP phosphodiesterase activity (low Km) in Hepatoma cells. These results suggest that the antitumor mechanism of DSC may be partly due to modulating cAMP level by inhibiting the activity of cAMP phosphodiesterase.
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