培养基中加 17-β-雌二醇和雌酮处理，琼脂糖凝胶电泳观察 DNA 片段. 结果：17-β-雌二醇 0.01－1 μmol·L$^{-1}$和雌酮 10－20 μmol·L$^{-1}$均能以剂量依赖方式诱导巨噬细胞产生凋亡的典型形态学改变和特征性 DNA 片段，staurosporine、放线菌酮和他莫西芬能取消这种作用. 结论：雌二醇和雌酮能诱导小鼠腹腔巨噬细胞凋亡，这一过程与蛋白激酶 C 的活化和合成新的蛋白质有关.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 May; 18 (3): 270－273

# Effect of tripterine on collagen-induced arthritis in rats

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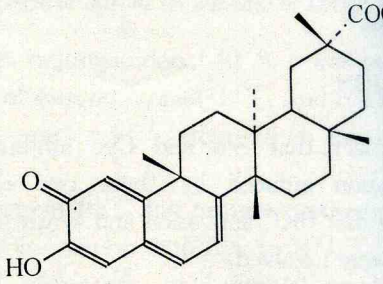
**KEY WORDS** collagen; arthritis; tripterine; antibodies; delayed hypersensitivity; interleukin-1; interleukin-2

**AIM**: To study the therapeutic effect of tripterine (Tri) on collagen-induced arthritis (CIA). **METHODS**: Collagen type II (Col) 1.5 mg was injected intradermally to induce CIA in rats. Hind paw volumes of rats were measured with a water displacement method. The serum anti-collagen antibody was measured by an enzyme-linked immunosorbent assay. Delayed hypersensitivity was reflected by skin response to Col. Interleukin-1 (IL-1) and interleukin-2 (IL-2) activities were evaluated by [$^3$H]TdR uptake. Joint was evaluated histologically. **RESULTS**: Tri 15 and 30 mg·kg$^{-1}$·d$^{-1}$ given ig to rats 3 d after the first sign of arthritis reduced inflammatory swelling, suppressed humoral and skin response to Col, inhibited IL-2 and IL-1 production, reduced pathological progression of joint. **CONCLUSION**: Tri has a therapeutic effect on CIA.

Tripterine (Tri), one of the active components first isolated from *Tripterygium wilfordii* Hook f in China, inhibited not only humoral and cellular immune responses but also some inflammatory responses[1,2]. *In vitro*, Tri inhibited IL-1 activity of murine peritoneal macrophages induced by lipopolysaccharides (LPS), IL-2 production from concanavalin A (Con A)-activated murine splenocytes, $PGE_2$ releasing from synovial cells[3]. T-cell proliferation is dependent on IL-1 and IL-2 synthesis[4], and IL-1 is one of the important proinflammatory cytokines in arthritis[5,6].

Intradermal injection of native heterologous or homologous collagen type II (Col) in Freund's incomplete adjuvant induces polyarthritis in rats named collagen-induced arthritis (CIA)[7]. It is only caused by Col from cartilage without any other bacterial components. This model of arthritis is similar to the chronic proliferative synovitis characteristics of rheumatoid arthritis (RA), and has well-defined cellular and humoral responses[8,9]. However, as any other animal models, there are still several differences between rheumatoid and collagen arthritis[10]. CIA is widely used in screening new drugs. The present work was to study the effect of Tri on CIA.




**Tripterine**

Received 1996-10-10 Accepted 1997-01-14

## MATERIALS AND METHODS

**Rats** Wistar rats (♀, $n = 18$) weighing 80 − 120 g, were provided by Shanghai Experimental Animal Center (ZK 005), Chinese Academy of Sciences.

**Mice** BALB/c mice ♀, ♂, 4 − 6 wk old and $C_{57}$BL/c ♀, ♂, 9 − 12 wk old were provided by the Department of Experimental Animals, Shanghai Medical University.

**Immunization procedures** Col isolated by our laboratory using a modified method[7] was dissolved at 4 ℃ in acetic acid 0.01 mol·L$^{-1}$ overnight at a concentration of 3 g·L$^{-1}$. Its stable emulsion was made in an equal volume of Freund's incomplete adjuvant. Each rat was immunized with 1 mL of the cold emulsion by intradermal (id) injection on back and 1 or 2 injections into the base of the tail.

**Treatment with Tri** Tri isolated by Prof PAN De-Ji (red cubic crystal, mp 199 − 201 ℃), was ground and suspended in normal saline containing 0.5 % CMC. The placebo preparation was made of the same vehicle. At 3 d after paw edema appeared, rats were grouped and given ig Tri 15 and 30 mg·kg$^{-1}$·d$^{-1}$ or placebo for 10 d.

**Assessment of arthritis** Rats were examined daily. The onset of arthritis was defined as the time when erythema and swelling of limb first appeared. The volume of both hind legs below the ankle joint was measured with a water displacement method.

**Immunoassay of antibody to Col**[11] Sera from rats were obtained by tail bleeding. Anti-collagen antibody was measured by an enzyme-linked immunosorbent assay (ELISA). Briefly, micro-ELISA plates was coated with Col dissolved in phosphate-buffered saline (PBS) 0.2 mol·L$^{-1}$ (50 mg·L$^{-1}$), 100 μL/well. Sera was diluted 1∶400, 100 μL/well. Peroxides-conjugated rabbit anti-rat IgG (provided by Prof SUN Bing in our laboratory) was added at a 1∶1600 dilution. Levels of antibody were expressed as rectified absorbance ($A_{490\ nm}$). The passive sera obtained from CIA rats 50 d after immunization were pooled and diluted 1∶400, and carried in each ELISA plate.

Rectified $A_{490\ nm} = 1.0 \times A_{490\ nm}$ of tested sera/$A_{490\ nm}$ of the passive sera

**Measurement of skin reaction to Col**[8] Col 50 μg dissolved in 0.05 mL PBS was injected intradermally in the rat left ear. The opposite ear was injected with an equal volume of PBS and served as control. Delayed hypersensitivity was expressed as the difference in thickness between the collagen- and PBS-injected ears.

**IL-1 assay**[3] Peritoneal cells of CIA were cultured with LPS (1 mg·L$^{-1}$) for 24 h to collect supernatants for IL-1 assay. Thymocyte from BALB/c mice with diluted supernatants (1∶32) (Con A 2 mg·L$^{-1}$) 100 μL/well was cultured for 72 h, at the final 6 h [$^3$H] TdR (Shanghai Institute of Nuclear Research, Chinese Academy of Sciences) 9.25 kBq/well was added to measure the [$^3$H]TdR uptake.

**IL-2 assay**[3] Spleen cells of CIA were cultured with Con A 3 mg·L$^{-1}$ for 24 h to collect supernatants for IL-2 assay. Spleen cells of $C_{57}$BL/6 mice (Con A 3 mg·L$^{-1}$) were incubated for 48 h to get activated cells. Cell viability was always more than 90 % and adjusted to $2 \times 10^9$ cells ·L$^{-1}$. The cell suspension and supernatants (dilution 1∶32) were added 100 μL/well and incubated for 24 h, at the final 6 h [$^3$H]TdR 9.25 kBq/well was added. IL-2 activity was evaluated by [$^3$H]TdR uptake.

**Histological evaluation**[9] Metotarsal-phalangeal and ankle joints were used in the study. Totally 12 joints were taken from each rat. The most severe histopathological alteration of 12 joints was blindly graded by a pathologist and assigned a score of I to IV based on the following criteria: I minimal synovitis, primary infiltration of mononuclear inflammatory cell into synovial membrane; II mild synovitis; pannus forming, cartilage degeneration; III proliferation and infiltration of a large amount of mononuclear cells; subchondral bone erosion; superficial cartilage damage; IV severe destruction of cartilage and subchrondral; complete disorganization of the joint space; fibrous thickening and severe fibrosis, bony ankylosis.

**Statistics** Results were expressed as $\bar{x} \pm s$, the significance of the differences obtained was evaluated by $t$ test.

## RESULTS

**Effect of Tri on CIA** Inflammatory polyarthritis was induced in all immunized rats (18/18). The peak incidence occurred on 9.6 ± 1.0 d after immunization. Treatment with Tri 15 and 30 mg ·kg$^{-1}$·d$^{-1}$ for 10 d diminished the rat paw volumes even when the therapy began 3 d after the disease on set (Fig 1).

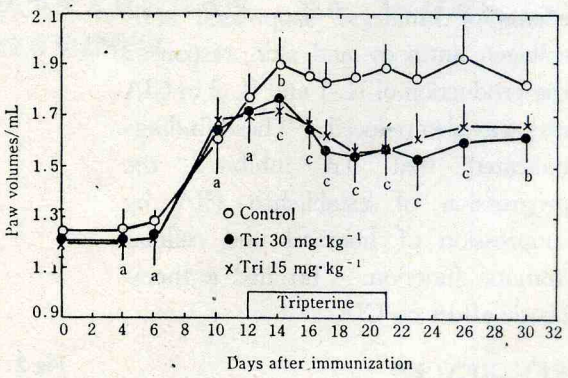



**Fig 1. Effect of Tri on paw volumes of collagen-induced arthritis.** $n = 6$ rats, $\bar{x} \pm s$. [a]$P > 0.05$, [b]$P < 0.05$, [c]$P < 0.01$ *vs* control.

Tri 15 and 30 mg·kg$^{-1}$·d$^{-1}$ suppressed sera antibody levels even 8 d after Tri administration was over. The suppression effect of Tri on skin reaction to Col lasted 17 d after the therapy stopped (Tab 1).

**IL-1 and IL-2 production** Tri decreased the IL-2 production from murine splenocytes and IL-1 production from peritoneal macrophage *in vivo*. Tri 30 mg·kg$^{-1}$·d$^{-1}$ had a better effect (Tab 1).

**Joint pathology** Histological analyses of joints indicated a reduction of the progression of ongoing arthritic disease in CIA. In Tri 15 mg·kg$^{-1}$·d$^{-1}$ group, 5 rats were graded II, 1 rat was graded I. The main changes were the panus and cartilage degeneration; In Tri 30 mg·kg$^{-1}$·d$^{-1}$ group, mononuclear inflammatory cell infiltrated into synovial membrane was graded I; In the control group, 4 rats were graded IV, 2 rats were III, all had severe bone destruction (Fig 2).

## DISCUSSION

In our previous study, the CIA rats proved to have not only high levels of anti-collagen antibody and skin reaction but also IL-2 production; Tri inhibited IL-1 and IL-2 activity *in vitro*. In this study Tri inhibited edema of hindlegs, suppressed anti-collagen antibody and skin response, the production of IL-1 and IL-2 in CIA rats was also reduced. These findings indicated that Tri inhibited the progression of established CIA by suppression of humoral and cellular immune function. Tri had a therapeutic effect on CIA.

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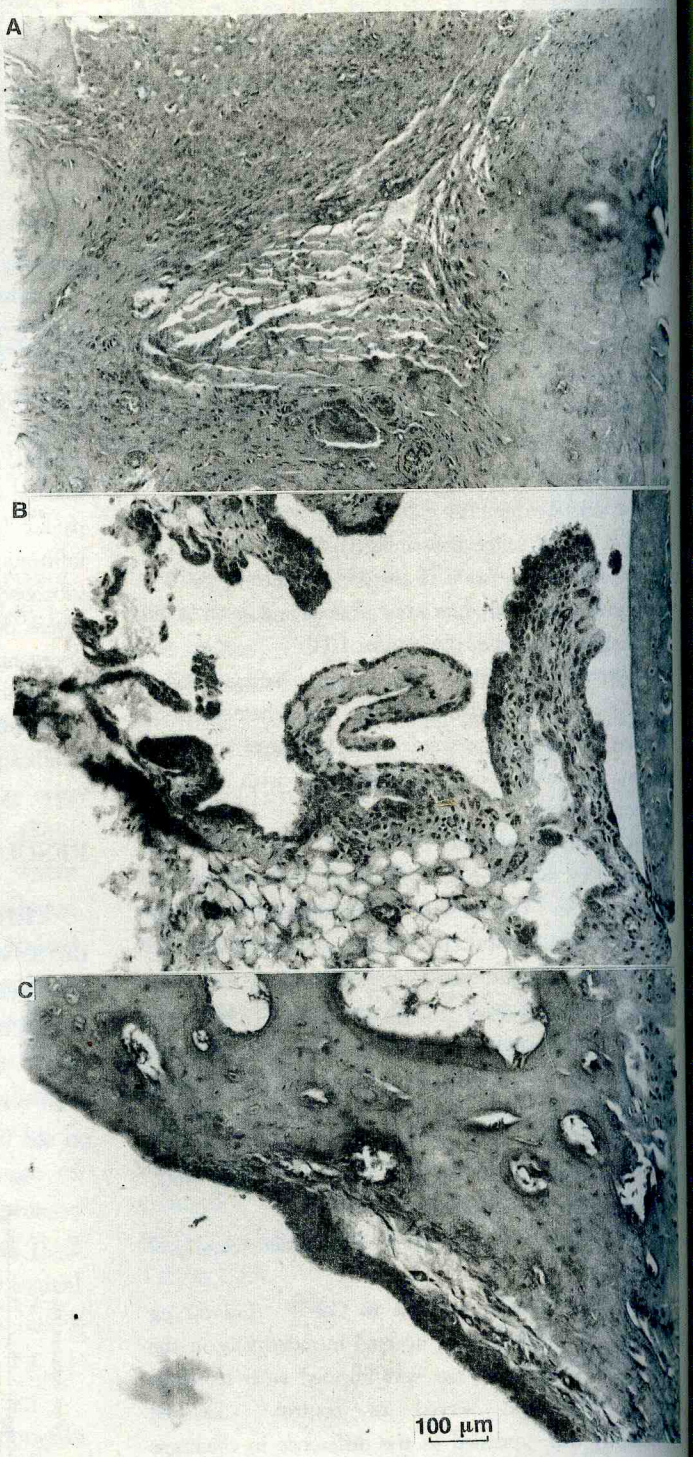


**Fig 2. Effect of Tri on pathology. A) Control: Grade IV, complete disorganization of the joint space. B) Tri 15 mg·kg$^{-1}$: Grade II, mild synovitis and pannus. C) Tri 30 mg·kg$^{-1}$: Grade I, minimal synovitis, primary infiltration of mononuclear inflammatory cell into synovial membrane. HE stain, ×100.**

**Tab 1. Effects of Tri on serum anti-collagen antibody levels ($n$ = 6 rats), cellular immunity to Col (delayed hypersensitivity, $n$ = 6 rats), IL-1 and IL-2 activity ($^3$H]TdR uptake, $n$ = 4 rats), in collagen induced arthritis. $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control; $^fP<0.01$ *vs* Tri 30 mg·kg$^{-1}$.**

| | Tripterine/mg·kg$^{-1}$·d$^{-1}$ | | |
|---|---|---|---|
| | 0 | 15 | 30 |
| Rectified absorbance at 490 nm | | | |
| d 21 | 1.26±0.08 | 1.08±0.12$^b$ | 1.04±0.13$^c$ |
| d 29 | 1.45±0.05 | 1.23±0.11$^c$ | 1.27±0.08$^c$ |
| d 42 | 1.24±0.07 | 1.21±0.07$^a$ | 1.27±0.11$^a$ |
| Ear thickness/mm | | | |
| d 21 | 0.88±0.06 | 0.67±0.14$^c$ | 0.52±0.14$^c$ |
| d 38 | 0.83±0.14 | 0.67±0.09$^b$ | 0.52±0.09$^c$ |
| IL-1 activity/Bq | 63±9 | 35±3$^{cf}$ | 23±4$^c$ |
| IL-2 activity/Bq | 299±23 | 227±39$^{cf}$ | 127±16$^c$ |


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## 雷公藤红素对胶原性关节炎的作用


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**关键词** 胶原；关节炎；雷公藤红素；抗体；迟发型变态反应；白细胞介素-1；白细胞介素-2



**目的**：研究雷公藤红素(Tri)对胶原性关节炎的作用. **方法**：足肿测量仪测定足肿变化；酶联免疫吸附法测定血清抗胶原 II 型抗体；皮肤迟发型变态反应水平测定；白细胞介素-2 和白细胞介素-1 的测定采用[$^3$H]TdR掺入法. 对后足趾－跖关节作病理切片. **结果**：Tri 15 和 30 mg·kg$^{-1}$·d$^{-1}$，发病 3 天后给药，改善足肿，抑制抗胶原 II 型抗体水平和迟发型变态反应水平；抑制白细胞介素-1 和白细胞介素-2 产生；病理检查显示 Tri 明显抑制关节炎大鼠病理改变. **结论**：Tri 对胶原性关节炎有治疗作用.