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Estrogens induce apoptosis in mouse peritoneal macrophages[1]

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KEY WORDS estradiol; estrone; DNA; agar gel electrophoresis; cycloheximide; tamoxifen; staurosporine; apoptosis; peritoneal macrophages

AIM: To study whether estrogen might induce apoptosis in mouse peritoneal macrophages (MPM). **METHOD:** The MPM were isolated and incubated in culture medium containing 17-β-estradiol, estrone, or equal volume of 100 % ethanol as control. DNA fragmentation was visualized by agarose gel electrophoresis. **RESULTS:** 17-β-Estradiol 0.01 - 1 $\mu mol \cdot L^{-1}$ or estrone 10 - 20 $\mu mol \cdot L^{-1}$ elicited typical morphological apoptosis and DNA fragmentation in a concentration-dependent manner in MPM. Staurosporine (Sta) 0.01 $\mu mol \cdot L^{-1}$, cycloheximide (Cyc) 1 $mg \cdot L^{-1}$, and tamoxifen (Tam) 10 $\mu mol \cdot L^{-1}$ inhibited the DNA fragmentation induced by 17-β-estradiol 1 $\mu mol \cdot L^{-1}$ or estrone 20 $\mu mol \cdot L^{-1}$. **CONCLUSION:** Estradiol and estrone induced apoptosis in MPM.

The importance of estrogens in the development of breast cancer and autoimmune diseases is commonly accepted[1-3]. However, the effects of estrogens on macrophages in association with breast cancer and autoimmune diseases are still unknown. Many pathological conditions and chemical agents, such as anticancer drugs, hyperthermia, viral infection, antigens, and hormones could induce apoptosis[4,5]. This raises the possibility that estrogen may cause macrophage death primarily by apoptosis. The purpose of this study is to examine these effects of 17-β-estradiol and estrone in mouse macrophages in relation to its receptor antagonist, protein kinase C (PKC) activation, and protein synthesis.

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MATERIALS AND METHODS

Chemicals Estrone, 17-β-estradiol, tamoxifen (Tam), staurosporine (Sta), cycloheximide (Cyc), M199 medium, and Hoechst 33258 were purchased from Sigma Chemical Co.

Cell culture Mouse peritoneal macrophages (MPM) were obtained from ♀ Kunming mice aged 6 - 8 wk and cultured in M199 medium with 15 % heat-inactivated fetal bovine serum. Estrone, estradiol, and Tam were dissolved in ethanol and the final concentration of alcohol was <0.2 %. After H-E stain, the cells were examined for apoptosis microscopically.

DNA electrophoresis At the end of each incubation period, cellular DNA was extracted by a salt-out procedure. About 4 μg DNA was electrophoretically fractionated on 1.5 % agarose gel and visualized by ethidium bromide[6].

DNA fragmentation assay MPM were lysed, and spun at 15 000 × *g* for 20 min to separate intact DNA from fragmented DNA. The pellet was sonicated for 10 s in another 1.5 mL lysis buffer. DNA in supernatant and pellet was determined using Hoechst 33258 fluorochrome[7]. The data were expressed as the % of DNA in supernatant to the total cellular DNA.

Statistics The results were expressed as $\bar{x} \pm s$, and assessed by ANOVA and *t* test.

RESULTS

Morphological changes 17-β-Estradiol- or estrone-treated MPM showed typical cell changes of apoptosis. The cell volume is reduced, indicating shrinkage of cytoplasm and the plasma membrane remained well defined, in agreement with trypan blue exclusion. The chromatin became condensed and nucleus marginated to the periphery of the cell membrane (Fig 1).

DNA fragmentation MPM were incubated with 17-β-estradiol (0.5, 1, 10, 100, and 1000 $nmol \cdot L^{-1}$). DNA electrophoresis showed a typical ladder of DNA (about 180 bp) first seen after 12 h of incubation in the presence of 10 $nmol \cdot L^{-1}$ (Fig 2).

DNA fragmentation assay showed that significant DNA fragmentation (40 %) was seen in

Fig 1. MPM after incubation with estradiol 1.0 μmol·L^{-1} (or estrone 20 μmol·L^{-1}) for 8 h. ×700.
A: normal macrophages; B: apoptotic macrophages.

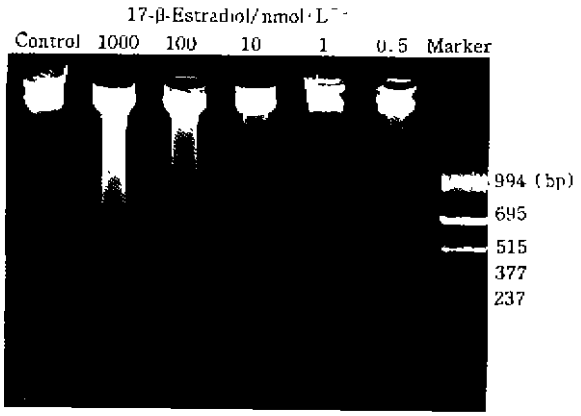

Fig 2. Effect of estradiol on a ladder of DNA.

the presence of 17-β-estradiol 10 nmol·L^{-1}, and reached 72 % with 1000 nmol·L^{-1} after 12 h of incubation (Tab 1).

Tab 1. Effects of estradiol and estrone on DNA fragmentation. $n = 5$. $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.01$ vs control.

Preincubation	DNA fragmentation/%
Estrone/nmol·L^{-1}	
Control	15.4 ± 6.9
1	23.3 ± 1.5^a
10	40.5 ± 8.4^b
1000	72.3 ± 5.6^b
Estrone/μmol·L^{-1}	
Control	13.7 ± 4.4
0.2	21.1 ± 3.0^a
2	40.0 ± 4.8^b
20	86.4 ± 5.1

When MPM were incubated with estrone (0.02, 0.2, 2, 10, 20, 200 μmol·L^{-1}), DNA electrophoresis also showed that a typical ladder of DNA seen after 24 h of incubation in the presence of estrone 10 μmol·L^{-1} (Fig 3).

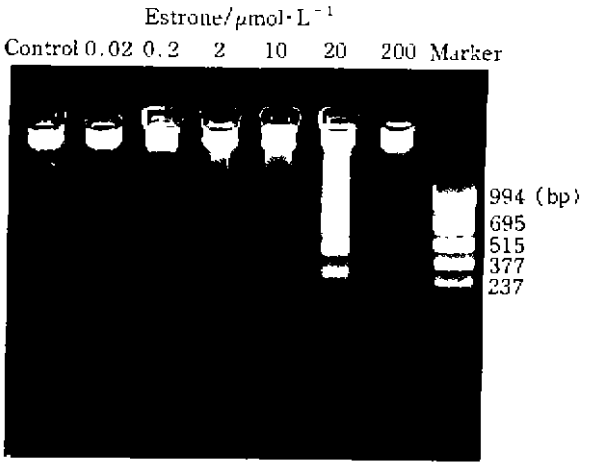

Fig 3. Effects of estrone on a ladder of DNA.

DNA fragmentation (40 %) was seen in the presence of estrone 2 μmol·L^{-1}, and reached 86 % with 20 μmol·L^{-1} after 24 h of incubation (Tab 1).

Effects of PKC and protein synthesis inhibitor

After coincubation with Cyc 1 mg·L^{-1}, a protein synthesis inhibitor[8], and Sta 10 nmol·L^{-1}, a PKC inhibitor[9] for 24 h during pretreatment of the

MPM with estrone 20 μmol·L^{-1} or for 12 h during pretreatment of the MPM with 17-β-estradiol 1 μmol·L^{-1}, DNA electrophoresis showed that Cyc and Sta inhibited DNA fragmentation induced by estrone and 17-β-estradiol (Fig 4).

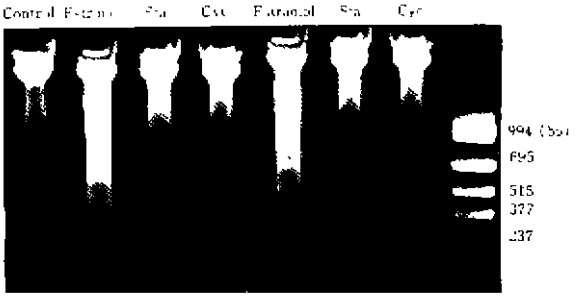

Fig 4. Staurosporine (Sta) 0.01 μmol · L^{-1} and cycloheximide (Cye) 1 mg·L^{-1} inhibited apoptosis induced by estrone 20 μmol · L^{-1} and estradiol 1 μmol · L^{-1} in macrophages.

Effects of Tam MPM after coincubation with Tam (0.1, 1, 10 μmol · L^{-1}) and estrone 20 μmol·L^{-1} for 24 h or 17-β-estradiol 1 μmol·L^{-1} for 12 h, DNA electrophoresis showed that Tam 10 μmol·L^{-1} inhibited the elicitment of a ladder of DNA bands (Fig 5).

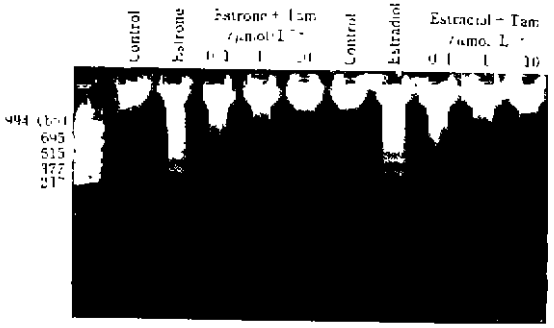

Fig 5. Tamoxifen (Tam) inhibited apoptosis induced by estrone 20 μmol · L^{-1} and estradiol 1 μmol · L^{-1} in macrophages.

DISCUSSION

The fact that Sta and Cyc inhibited DNA fragmentation induced by these two estrogens, indicating that PKC activation and synthesis of new proteins were involved.

There exist estrogen receptors in macrophages[2,10]. Tam, a specific estrogen antagonist, inhibited DNA fragmentation elicited by these two estrogens, suggesting that estrogens directly affect macrophages at its receptor sites. It is speculated that estrogens may inhibit the ability of macrophages to phagocytize breast cancer cells and apoptotic process of lymphocytes may be related to the development of breast cancer and autoimmune diseases.

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关键词 雌二醇; 雌酮; DNA; 琼脂糖凝胶电泳; 放线菌酮; 他莫西芬; staurosporine; 凋亡; 细胞凋亡 腹腔巨噬细胞

目的: 研究雌激素诱导小鼠腹腔巨噬细胞凋亡的作用. 方法: 分离、培养小鼠腹腔巨噬细胞, 在

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.

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Effect of tripterine on collagen-induced arthritis in rats

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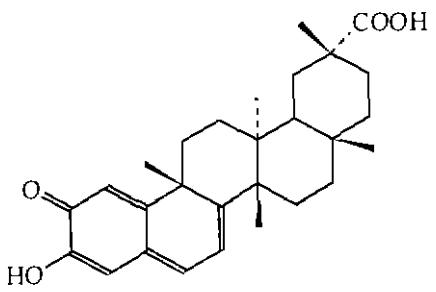
KEY WORDS collagen; arthritis; tripterine; antibodies; delayed hypersensitivity; interleukin-1; interleukin-2

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

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