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LIVER INJURY SUPPRESSION

All-trans retinoic acid suppresses liver injury induced by Propionibacterium acnes and lipopolysaccharide in rats

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Abstract
All-trans retinoic acid (ATRA) has been reported to exert major effects on the immune system, including monocytes/macrophages. The present study was designed to determine whether ATRA would modulate macrophage-associated liver injury induced by Propionibacterium acnes and lipopolysaccharide (LPS) in rats. All-trans retinoic acid administration alleviated the liver injury and reduced the incidence of death following hepatic failure. Serum alanine aminotransferase (ALT) levels 5 h after, and survival rates within 12 h after the administration of LPS were significantly lower in the ATRA-treated group (134±119 IU/L and 72.7%) compared with the control group (713±411 IU/L and 18.2%; P<0.05). Histological findings supported these results. These effects may be due to suppression of tumour necrosis factor-α (TNF-α) and superoxide anions produced by activated macrophages. Serum levels of TNF-α 1 h after LPS administration were significantly lower in the ATRA-treated group (60.5±7.0 ng/mL) as compared with the control group (105.2±39.3 ng/mL; P<0.05). Formazan deposition that was generated by the perfusion of the liver with nitroblue tetrazolium, also suggested suppression of the release of superoxide anions from hepatic macrophages. These results suggest that ATRA acts as an immunomodulator in liver injury by suppressing the activation of liver macrophages.

Key words: all-trans retinoic acid, immunomodulator, Kupffer cell, liver injury, LPS, Propionibacterium acnes, superoxide, TNF-α.

INTRODUCTION

Retinoids, natural and synthetic derivatives of vitamin A, exert marked effects on cellular proliferation and differentiation, and also on the immune system. The anti-inflammatory effects of retinoids have been demonstrated in dermatological diseases in humans1 and in animal models of inflammatory diseases, such as adjuvant arthritis2 and ultraviolet-induced erythema.3 Most of the effects of vitamin A (retinol) are linked to the oxidized form, all-trans retinoic acid (ATRA), via the ligand-dependent activation of two families of nuclear hormone receptors, retinoid acid receptors (RAR)6 and retinoid X receptors (RXR).7 We previously studied the effects of ATRA, retinal and retinol on the function of isolated rat Kupffer cells, which are resident macrophages in the liver. Of the three retinoids, ATRA is most potent in suppressing the production of tumour necrosis factor-α (TNF-α) and nitric oxide (NO) by lipopolysaccharide (LPS)-stimulated Kupffer cells.8 This suggests that ATRA may inhibit inflammation in some types of liver injury by suppressing the production of inflammatory cytokines and free radicals from Kupffer cells or hepatic macrophages. The present study was designed to determine whether ATRA would exert any effect on experimental liver injury in the rat. Propionibacterium acnes (P. acnes, Corynebacterium parvum) and LPS were used to induce liver injury in this model. Severe liver injury and circulatory shock can be induced by the injection of a low dose of LPS to mice or rats pretreated with heat-killed P. acnes.9,10 Hepatic macrophages are considered to be mainly involved in the development of the liver injury in this model.9,11

METHODS

Animals
Male Wistar rats (170–200 g, Laboratory of Animal Experiments, Kyushu University, Japan) were maintained on a basal pelleted diet and water ad libitum in a room under normal laboratory lighting conditions. Protocols
Procedure for liver perfusion with nitroblue tetrazolium

The procedure for liver perfusion was carried out as previously described with some modifications. Under anaesthesia with diethyl ether, the liver was perfused successively with Ca\(^{2+}\)-free Hank’s balanced salt solution (HBSS) for 5 min, Dulbecco’s minimal essential medium (Nipro, Tokyo, Japan) with 0.05% NBT (Wako) for 10 min or with NBT and 60 U/mL of SOD (from bovine erythrocytes, Sigma) as previously described.

The perfusion was performed at a flow rate of 20 mL/min at 37°C with a continuous supply of O\(_2\). The perfusate contained 30 mmol/L HEPES, pH 7.4. The excised liver was then fixed in formalin and embedded in paraffin, and stained with nuclear fast red.

Histological evaluation

The histological extent of liver injury was classified according to the area of coagulative necrosis in hepatic lobules as follows: (1) no coagulative necrosis, (2) mild or rare (Fig. 1b); only two of six rats showed grade 1; (3) moderate (Fig. 1a); five of six rats showed the necrosis of grade 2; (4) severe coagulative necrosis: two of six rats showed grade 3.

The degree of liver injury was classified according to the area of coagulative necrosis in hepatic lobules as follows: grade 0, no coagulative necrosis; grade 1, mild or rare coagulative necrosis; grade 2, moderate coagulative necrosis; grade 3, severe coagulative necrosis.

The granuloma size is the mean of largest diameter of 30 granuloma randomly selected on the printed picture magnified x40. The number of granulomas is the total number counted from five fields randomly selected under magnification x100.
The administration of LPS increased the serum concentration of TNF-α in the control group. This increase was significantly attenuated by treatment with ATRA (P < 0.05; Table 1).

**DISCUSSION**

Endotoxins are potent immunomodulators that are toxic to a number of tissues. Because the liver is the major organ responsible for the clearance of soluble and particulate materials (including bacterial products such as LPS) from the circulation, this organ is highly susceptible to the deleterious effects of endotoxins. As a consequence of the liver’s functions, a wide array of bioactive substances are released. Among them are reactive oxygen intermediates which are involved in the pathophysiological responses that accompany endotoxemia. Tumour necrosis factor is believed to be the principal mediator of the deleterious effect of LPS. Neutralization of TNF-α activity with TNF-α antibodies abolishes many of the adverse effects of LPS and prevents death from severe endotoxemia. Macrophages play important roles in the development of liver injury by producing proinflammatory cytokines and free radicals. Inhibition of these inflammatory mediators is an important target in the treatment of liver diseases.

In the control group, the marked deposition of formazan was observed not only surrounding granulomas but also in perisinusoidal areas throughout the liver (Fig. 3a). Poorer deposits of formazan were observed in the ATRA-treated group (Fig. 3b). These deposits of formazan nearly disappeared in the rats that were simultaneously perfused with NBT and SOD (Fig. 3c). The administration of LPS increased the serum concentration of TNF-α in the control group. This increase was significantly attenuated by treatment with ATRA (P < 0.05; Table 1).

**REFERENCES**


