Effect of Gadolinium Chloride Treatment on Concanavalin A-Induced Cytokine mRNA Expression in Mouse Liver

Toshihiro Okamoto¹, Osamu Maeda¹, Naoki Tsuzuki² and Kaoru Hara¹

¹Research Laboratories, Nippon Chemipharm Co., Ltd., 1–22 Hikokawato, Mutsu, Saitama 341–0005, Japan
²Zeria Pharmaceutical Co., Ltd., 2512–1 Konan-machi, Ohsato-gun, Saitama 360–0111, Japan

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ABSTRACT—The effect of Kupffer cell depression on concanavalin A (Con A)-induced cytokine mRNA expression in the liver was studied. Gadolinium chloride (GdCl₃) is a commonly used Kupffer cell inhibitor. GdCl₃ (40 mg/kg, i.p.) was injected into each mouse, and 24 hr later, Con A (0.2 mg/mouse) was administered. Plasma was obtained at 24 hr after Con A treatment for alanine aminotransferase (ALT) measurement. GdCl₃ treatment inhibited Con A-induced elevation of ALT. However, it did not inhibit Con A-induced interleukin-2 or tumor necrosis factor-α mRNA expression. The present results suggest that Kupffer cells are not responsible for Con A-induced cytokine expression in the liver.

Keywords: Concanavalin A, Gadolinium chloride, Cytokine

A mouse T-cell dependent liver injury model involving concanavalin A (Con A) has been developed (1). Injection of Con A caused the release of cytokine protein into the plasma of mice. The Con A-induced cytokine release is regarded to be a result of T-cell activation (2). However, the Con A-induced cytokine protein level in the plasma does not accurately reflect the Con A-induced T-cell activation in the liver. Recently, we reported Con A-induced cytokine mRNA expression in mouse liver, which might be a result of T-cell activation in the liver (3). In addition to T-cells (4, 5), Kupffer cells are also a source of cytokines in the liver (6). Thus, it is possible that Kupffer cells may be a source of Con A-induced cytokines. Treatment a mouse with silica particles depressed Kupffer cells, and this Kupffer cell-depressed mouse was resistant to Con A treatment (1), indicating a contribution of Kupffer cells to the development of Con A-induced hepatitis. Gadolinium chloride (GdCl₃) is a commonly used Kupffer cell inhibitor (7). In the present study, we determined the effect of Kupffer cell depression with GdCl₃ on Con A-induced cytokine mRNA expression in the liver. We measured Con A-induced cytokine mRNA expression by the polymerase chain reaction (PCR).

Female BALB/c mice obtained from Charles River Japan, Inc. (Atsugi) were used at 7–10 weeks of age. The animals were kept in an air-conditioned room and given chow and water ad libitum. Con A was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Con A dissolved in pyrogen-free saline was administered to the mice (0.2 mg/mouse) via a tail vein (in a volume of 100 μl). GdCl₃ was obtained from Wako Pure Chemical Industries (Osaka). GdCl₃ dissolved in acidic 0.9% NaCl was intraperitoneally administered (in a volume of 200 μl). To measure plasma transaminase activity, mice were anesthetized with ether and then blood was collected from an abdominal vein in a syringe with a sodium heparin film. Plasma transaminase activity, i.e., that of alanine aminotransferase (ALT), was measured by the standard photometric method with an automatic analyzer (3). RNA isolation (8), PCR analysis (9) and the PCR primers (9) were described previously. cDNA was amplified by 30 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 45 sec, and extension at 72°C for 1.5 min. For relative quantification of the PCR products, the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an internal control. Messenger RNA expression was examined using a CanoScan 600 (Canon, Tokyo) and quantified with NIH image 1.56 analysis software.

The results were analyzed by means of the F test, followed by the t-test with Welch's correction.

GdCl₃ is a commonly used Kupffer cell inhibitor (7). The effects of three different doses of GdCl₃ (20, 30 and 40 mg/kg, i.p., n = 4) on Con A-induced hepatitis were examined; 40 mg/kg of GdCl₃ was found to significantly inhibit Con A-induced hepatitis (data not shown). Thus, in the following study, GdCl₃ was used at the dose of 40
mg/kg. GdCl₃ (40 mg/kg) was injected into each mouse, and 24 hr later Con A was administered. Plasma was obtained at 24 hr after Con A treatment. Treatment with GdCl₃ (40 mg/kg) inhibited Con A-induced hepatitis (Fig. 1). Thus, Kupffer cells may be involved in the development of Con A-induced hepatitis. Then we examined the effect of GdCl₃ (40 mg/kg) treatment on Con A-induced cytokine mRNA expression in the liver. GdCl₃ was injected into each mouse, and 24 hr later, Con A was administered. The liver was removed for RNA analysis at 1 hr after Con A treatment. GdCl₃ treatment inhibited neither interleukin-2 (IL-2) nor tumor necrosis factor-α (TNF-α) mRNA expression (Fig. 2: A and B). Thus, Kupffer cells may not be the source of these Con A-induced cytokine mRNAs in the liver. This supports the notion that Con A-induced cytokine mRNA expression in the liver may be a result of T-cell activation (4, 5), but it does not involve Kupffer cells.

Other investigators proposed that Con A may be a substitute for the signals provided by Kupffer cells and that T-cells are activated on recognition of the substitute signals from Con A (1). For T-cell activation, they stressed the importance of the coupling of Con A with Kupffer cells and T-cells. However, in the present study, treatment with Con A induced cytokine mRNA expression even if the Kupffer cells were depressed with GdCl₃. Thus, in contrast to the proposals of other investigators, Con A may activate T-cells independently of Kupffer cell function.

TNF-α is an important mediator in the development of hepatitis in animal liver injury models (2, 10, 11).

![Fig. 1. Effect of GdCl₃ on Con A-induced elevation of the plasma ALT level. Each mouse was pretreated with GdCl₃ (40 mg/kg, i.p.), and 24 hr later, Con A was injected. Plasma was obtained at 24 hr after Con A treatment (n=5). *P<0.01 vs result with Con A alone.](image)

![Fig. 2. RT-PCR analysis of the effect of GdCl₃ treatment on Con A-induced cytokine gene expression. GdCl₃ (40 mg/kg, i.p.) was administered to each mouse, and at 24 hr later, Con A was injected. A liver sample for RNA analysis was obtained at 1 hr after Con A treatment. A: Messenger RNAs from liver samples from 4 Con A control mice and 3 GdCl₃-treated mice were amplified for 30 cycles with IL-2, TNF-α and GAPDH gene-specific primers. B: The relative amounts of the PCR products in panel A were determined with a computerized laser densitometer. The strongest band was expressed as 100. The density of each amplified cDNA band for each cytokine was normalized with respect to the density of each corresponding band for GAPDH.](image)

However, TNF-α administration alone does not induce any hepatitis without sensitization of the animals to TNF-α by transcriptional arrest both in vivo and in vitro (12). In the present study, in Kupffer cell-depressed mice, although Con A administration induced TNF-α mRNA in the liver, hepatitis was not induced. This suggests that Kupffer cells may contribute to make mice sensitive to TNF-α, possibly through transcriptional arrest.

Although Kupffer cells are a source of cytokines in the liver, it may not be involved in the Con A-induced cytokine mRNA expression. This model may be useful for investigating the function of Kupffer cells to induce liver injury by mechanisms other than cytokine production.
REFERENCES


