Effect of OKY-046 and ONO-3708 on Liver Injury in Mice

Hiroichi NAGAI, Motonori AOKI, Tsukasa SHIMAZAWA, Ikuhisa YAKUO, Akihide KODA and Masao KASAHARA

Department of Pharmacology, Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502, Japan

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Abstract—The effects of OKY-046, a selective thromboxane A\textsubscript{2} (TXA\textsubscript{2}) synthetase inhibitor, and ONO-3708, a novel TXA\textsubscript{2} receptor antagonist, on liver disease were investigated in mice. The liver injury was induced by either an injection of anti-basic liver protein (BLP) antibody into DBA/2 mice that had been previously immunized with rabbit IgG or by an injection of bacterial lipopolysaccharide (LPS) into Corynebacterium parvum (C. parvum) pretreated DDY mice. 1) In both injury models, clear elevation of glutamate transaminase (GOT and GPT) activity due to extensive liver parenchymal cell damage was observed; this was confirmed by significant histopathological changes in the liver. 2) Typical histopathological changes in the liver were submassive hepatocellular necrosis in the anti-BLP antibody-induced injury model and focal necrosis in the LPS-induced model. Inflammation and increased cell infiltration in portal connective tissue were observed in both cases. 3) Administration of OKY-046 (50 mg/kg) and ONO-3708 (0.5, 1.0 and 2.0 mg/kg) suppressed the elevation of serum GOT and GPT levels and histopathological changes in both experimental liver injury models. 4) Indomethacin inhibited the development of liver disease caused by anti-BLP antibody but not by bacterial LPS. Prostaglandin I\textsubscript{2} inhibited the elevation of serum GOT and GPT levels and histopathological changes of the liver in the mice treated with anti-BLP antibody and showed the tendency to inhibit the development of liver injury caused by bacterial LPS.

Thromboxane A\textsubscript{2} (TXA\textsubscript{2}) is a powerful platelet aggregating agent and stimulator of contractile activity of smooth muscle including blood vessel and trachea (1, 2). However, because of the lability of TXA\textsubscript{2}, its roles in the pathophysiology of certain diseases are not yet fully elucidated. In the cardiovascular system, many evidences indicate that a balance of production of TXA\textsubscript{2} and prostaglandin I\textsubscript{2} (PGI\textsubscript{2}) is important for homeostasis in the cardiovascular system and that an imbalance towards the overproduction of TXA\textsubscript{2} leads to certain diseases such as angina and myocardial infarction (3, 4). Concerning to the role of TXA\textsubscript{2} in liver disease, there are some reports suggesting that TXA\textsubscript{2} may be involved in hepatorenal syndrome and experimental liver diseases (5–8). In addition to these reports, Araki and Lefer (9) and Hamilton et al. (10) reported a protective effect of PGI\textsubscript{2} on hepatocytes in certain liver diseases. The present study, therefore, was conducted to investigate the effects of OKY-046, a selective TXA\textsubscript{2} synthetase inhibitor (11, 12); ONO-3708, a TXA\textsubscript{2} receptor antagonist (13–15); indomethacin; and PGI\textsubscript{2} on experimental liver injury including the fulminant hepatitis model in mice.

Materials and Methods

Animals: DBA/2 and DDY male mice weighing 18 to 20 g were used. Animals were housed in an air-conditioned room at 24°C and fed the usual laboratory diet and given...
water ad libitum. All animals were purchased from Japan SLC, Inc. (Hamamatsu).

Drugs: (E)-3-[(p-(1H-imidazol-ylmethyl)-phenyl]-2-propenoic acid (OKY-046) was kindly supplied from Kissei (Matsumoto) and Ono (Osaka) Pharmaceutical Companies. Dideoxa-9a,11a-dimethylmethano-11,12-methano-13,14-dihydro-13-aza-14-oxo-15-cyclo penty-16,17,18,19,20-pentanor-15-epi-thromboxane A\(_2\), (ONO-3708) was supplied from Ono Pharmaceutical Company (Osaka). PGI\(_2\), Corynebacterium parvum (C. parvum) and bacterial lipopolysaccharide (LPS) were purchased from Funa koshi Yakuhin (Tokyo). OKY-046 and ONO-3708 were administered by the method described in the legends of Figs. 3 and 4.

Experimental liver injury: Experimental liver injury was caused by the previously described (16). a) Anti-basic liver protein (BLP)-induced liver injury: BLP was prepared according to the method of Mafune (17). The antiserum containing anti-BLP antibody was obtained from rabbits which had been immunized by weekly intramuscular injection of 1.0 ml of an emulsion containing 300 \(\mu\)g BLP and complete Freund’s adjuvant (CFA) for 4 weeks. Antiserum was obtained 7 to 10 days after the last injection and absorbed with homologous erythrocytes and kidney homogenate after inactivation of complement at 56°C for 30 min. Liver injury was caused in DBA/2 mice which had been previously immunized by intraperitoneal (i.p.) injection of 0.5 mg rabbit IgG (RGG) emulsified with 0.25 ml of CFA. Five days later, 0.6 ml of antiserum containing anti-BLP antibody was injected intravenously (i.v.). In order to evaluate the severity of symptoms, blood samples were collected at 18 hr after injection of anti-BLP antibody for measurement of glutamic pyruvic transaminase (GPT) and glutamic oxalacetic transaminase (GOT) in serum. Pathological changes in the liver (formation of submassive necrosis) were evaluated in a semi-quantitative fashion after staining with hematoxylin and eosin.

b) C. parvum and bacterial LPS-induced liver injury: LPS-induced liver injury in DDY mice pretreated with C. parvum was carried out according to the method of Ferluga et al. (18). In brief, 1 mg of C. parvum suspended

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Fig. 1. The changes of serum biochemical parameters in anti-BLP antibody and bacterial LPS-induced liver injury mice. Serum was obtained 18 hr after the injection of anti-BLP antibody and 2 hr after the injection of bacterial LPS. Each column represents the mean±S.E. of 8 to 15 animals. □□□□: Normal mice. ■■■■: Treated mice. *P<0.05, **P<0.01.
TXA₂ in hepatorenal syndrome and experimental liver diseases (5–8). Since the TXA₂ level in the liver was elevated after the injection of bacterial LPS (H. Nagai et al., unpublished data) and many symptoms in the bacterial LPS-induced liver injury model are very close to those in human fulminant hepatitis, the role of TXA₂ in fulminant hepatitis should be studied. If TXA₂ plays a role in fulminant hepatitis, OKY-046 and ONO-3708 are useful for the treatment of such types of diseases. In conclusion, the present study indicated the efficacy of TXA₂ inhibitors, both a synthetase inhibitor (OKY-046) and a receptor antagonist (ONO-3708), on liver injury models in mice.

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The efficacies of OKY-046 and ONO-3708 on anti-BLP antibody and bacterial LPS-induced liver injuries in mice. Moreover, the different efficacies between indomethacin and PGI$_2$ on each liver injury model suggest the different participation of PGI$_2$ and TXA$_2$ for the development of liver injury in the above two models.
There are many reports to indicate the efficacies of OKY-046 and ONO-3708 on thrombosis and vasoconstriction in experimental animals (11–15). From the previous results, the efficacies of both agents are assumed to be the inhibition of TXA₂ synthesis and TXA₂ receptor antagonism directly besides the accumulation of PG₁₂ indirectly. In the present study, OKY-046, ONO-3708 and indomethacin clearly inhibited the anti-BLP antibody-induced liver injury. However, PG₁₂ slightly showed the tendency to inhibit the development of anti-BLP antibody-induced liver disease. These results suggest that direct action against TXA₂ is mainly involved in the inhibition of anti-BLP antibody liver injury by OKY-046 and ONO-3708. From our unpublished data, the level of TXB₂ in the liver homogenate from mice treated with anti-BLP antibody increased three times more than that normal mice. Therefore, TXA₂ may act directly to cause anti-BLP antibody-induced liver injury, and the above two agents may inhibit the direct action of TXA₂.
Contrary to anti-BLP antibody induced liver injury, bacterial LPS-induced liver injury was inhibited by PG12. In addition to the above data, the doses of OKY-046 and ONO-3708 employed in the present study are sufficient to elevate the PG12 levels in serum and lung tissues as indicated in previous reports. These data suggest that the inhibitory mechanism of OKY-046 and ONO-3708 on LPS-induced liver injury is mainly due to the increase of PG12 level. To clarify this point, more experiments including measurements of TXA2 and PG12 levels in the liver and serum after the injection of OKY-046 and ONO-3708 are necessary to determine the precise mechanism.

As for the role of TXA2 in liver disease, there are some reports indicating the participation of...
in 0.5 ml saline was injected i.p. into mice. Nine days later, LPS, at a concentration of 10 μg/0.5 ml saline, was injected i.v. Animals were killed 2 hr after the injection of LPS. Evaluation of the severity of disease was carried out by the same method as described above. Histopathological change was evaluated by counting the number of granulomatous nodules.

**Statistics:** Results were statistically evaluated Student’s t-test. In the histopathological studies, statistical significance was tested using Willcoxon’s U-test.

**Results**

**Experimental liver injury:** To characterize each of the liver injury models, changes in serum parameters were measured, and histopathological studies of livers were carried out. After an injection of anti-BLP antibody or bacterial LPS, clear elevation of serum GOT, GPT and LDH activities were observed (Fig. 1). Figure 2 shows the histopathological picture of the liver from each group. Livers from the anti-BLP antibody injected mice exhibited submassive hepatocellular necrosis and infiltration of granulocytes and lymphocytes into the portal tract and sinusoid in the necrotic lesion. In mouse liver treated with bacterial LPS, focal necrosis and inflammation and increased cell filtration in periportal connective tissue were observed.

**Effect of OKY-046 or ONO-3708 on experimental liver injury:** After intravenous administration of OKY-046 and intraperitoneal administration of ONO-3708, the elevation of serum GOT and GPT was inhibited in both models (Figs. 3 and 4). Histopathological studies indicated that both drugs inhibited the pathological changes of the liver (Table 1).

**Effect of indomethacin or PGI2 on experimental liver injury:** As indicated in Fig. 5, indomethacin inhibited the elevation of serum GOT and GPT in anti-BLP antibody-induced liver injury mice, but did not inhibit the elevation of transaminase in bacterial LPS-induced liver injury mice. Histopathological studies indicated that indomethacin inhibited the histological changes of the liver from mice treated with anti-BLP antibody, but did not inhibit those from mice treated with LPS (Table 2). On the contrary, PGI2 inhibited the change of each parameter in bacterial LPS-induced liver injury, but not in anti-BLP antibody-induced liver injury (Fig. 6 and Table 2).

**Discussion**

The results reported here indicate the