Involvement of mast cells in acute hepatic injury induced by galactosamine and lipopolysaccharide in mice

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(Communicated by Masanori OTSUKA, M. J. A., June 10, 2003)

Abstract: We investigated the possible involvement of mast cells in acute hepatic injury induced by galactosamine (GalN, 20 mg/mouse) and lipopolysaccharide (LPS, 15 ng/mouse) (GalN/LPS) in mice. Histidine decarboxylase (HDC) mRNA expression in the liver as well as serum ALT level was increased with time after GalN/LPS treatment. Steroid anti-inflammatory agent (dexamethasone), histamine H₁ receptor antagonists (diphenhydramine and pyrilamine), histamine H₂ receptor antagonists (cimetidine and ranitidine) and 5-HT₂ receptor antagonist (ketanserin) were effective in reducing the ALT level at 8 h after the treatment with GalN/LPS. Furthermore, dexamethasone, diphenhydramine and cimetidine inhibited the increase in hepatic HDC mRNA level. The increase in ALT level by GalN/LPS was much lower in mast cell-deficient mice than in the congenic normal mice. Diphenhydramine further inhibited ALT level in response to GalN/LPS in mast cell-deficient mice. Our data suggest that mast cells play an important role in the development of hepatic injury induced by GalN/LPS, and suggest that histamine and 5-HT released from mast cells and other cells are involved in this model.

Key words: Galactosamine; lipopolysaccharide; histamine; mast cells; histidine decarboxylase mRNA; mouse liver injury.

Introduction. Mast cells are known to be located in connective tissue and in the mucosal membrane of almost all organs.¹⁻³⁾ Their number varies depending on pathological conditions and the increase in mast cells have been observed in acute as well as delayed hypersensitivity and inflammatory diseases.⁴⁾ Mast cells produce and release various biologically active substances, such as histamine, serotonin, arachidonic acid derivatives, cytokines and proteases. It is known that mast cells are present in normal liver and accumulated in liver of patients with chronic or acute hepatitis,^{5,6)} and participate in the inflammatory response of acute hepatitis.⁵⁾ As the density of mast cells is increased in liver of rats treated with carbon tetrachloride (CCl₄), the density of mast cells has been suggested to be a useful marker of acute liver inflammation.⁷⁾ Histamine and tryptase, chemical mediators derived from mast cells, are known to be mitogen for cultured fibroblasts and to enhance the collagen synthesis by fibroblasts.^{8,9)} Thus, it has been considered

that mast cells may contribute to inflammatory response and fibrosis in human liver diseases.

Combination of galactosamine (GalN) and a small dose of lipopolysaccharide (LPS) causes specifically hepatic failure in rodents.^{10,11)} This model has widely been used to examine hepatic protection of compounds. Histamine has shown to be increased in the liver of GalN/LPS- or CCl₄- treated mice.^{12,13)} Synthesis of histamine from histidine is mediated by histidine decarboxylase (HDC). HDC activity is increased in the liver of mice treated with LPS, CCl₄ or LPS plus GalN.^{12,13)} These reports suggest that histamine production is up-regulated in liver after treatment with various stimuli. There is a clinical report showing that a HDC inhibitor relieves the symptoms of patients with hepatitis.¹⁴⁾ However, direct evidence for the participation of mast cells and its mediators such as histamine and serotonin in the development of liver injury in human and experimental animals is lacking. In this study, we examined the possible involvement of mast cells in acute liver injury induced by GalN/LPS in mice.

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Materials and methods. *Animals.* Six-week-old male BALB/c mice (Japan SLC, Hamamatsu, Japan) or seven-week-old mast cell-deficient male Slc: WBB6F1-W/W^v (WB-W/+×C57BL/6-W^v/+) mice and the congenic normal male Slc: WBB6F1-+/+ mice (Japan SLC, Hamamatsu, Japan) were used for the experiments. The animals were kept in an environmentally controlled room (24 ± 1 °C, 55 ± 10% humidity) and allowed free access to food and water. This study was performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

Materials. Lipopolysaccharide (*Escherichia coli*, O26:B6), galactosamine, diphenhydramine, pyrilamine, cyproheptadine, cimetidine, ketanserin and dexamethasone were purchased from Sigma (St. Louis, MO, U. S. A.). Ranitidine and atropine were obtained from Wako Chemicals (Osaka, Japan). All drugs were dissolved in pyrogen-free saline.

Galactosamine/lipopolysaccharide-induced liver injury. The BALB/c mice were intravenously injected with 15 ng/mouse of LPS and 20 mg/mouse of GalN. Mast cell-deficient mice and the congenic normal mice were intravenously administered with LPS at 10 ng/mouse and GalN at 20 mg/mouse, as they were more sensitive to LPS and died when the same dose of LPS as BALB/c mice was given. At 1 h to 8 h after GalN/LPS treatment, mice were anesthetized with diethyl ether to collect blood by heart puncture. Hepatocellular damage was evaluated by measuring serum alanine aminotransferase (ALT) using a SPOT CHEM SP-4420 analyzer (ARKRAY, Kyoto, Japan). Oral and intraperitoneal administration of test compounds were performed 30 min (but dexamethasone was 1 h) and intravenous administration was done 15 min before GalN/LPS treatment, respectively. Control mice received saline. Effects of test compounds on liver damage were examined at 8 h after GalN/LPS treatment.

RNA isolation. Livers were removed and frozen immediately in a dry ice/ethanol. Total RNA was extracted from frozen livers (about 10 mg) by the acid-guanidine-phenol-chloroform method using ISOGEN (Nippon Gene, Toyama, Japan).

Reverse transcription polymerase chain reaction (RT-PCR) analysis. About 1 µg of total RNA in the liver of GalN/LPS-treated mice was hybridized to oligo dT-adaptor primer, and the RT reaction was carried out using AMV reverse transcriptase XL (Life Sciences, St. Petersburg, FL) for 10 min at 30 °C and 1 h at 42 °C. The reaction was terminated by incubation of the mixture at

Fig. 1. Changes of serum ALT levels (A) and HDC mRNA levels (B) in the liver after GalN/LPS treatment. Values represent the mean ± SEM of 4-6 mice. *p < 0.05 and **p < 0.01 compared with 0 h. The relative quantities of HDC mRNA are presented as ratio between the intensities of HDC band relative to the intensity of the GAPDH band (C). M: marker.

99 °C for 5 min. The resulting cDNA was used as a template for PCR amplification. The PCR reaction of HDC was performed using *Ex Taq* (Takara Biomedical, Shiga, Japan). PCR primers for murine HDC were according to Shiraishi *et al.*¹⁵⁾ The synthetic forward primer (5'-GGATCCAAGATCAGATTCTACCTGTGGAC-3') and reverse primer (5'-GTCGACGACATGTGCTTGAA-GATTCTTCAC-3') were designed to amplify a 516-bp fragment. The PCR profile was as follows: denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, extension at 72 °C for 30 s, total 30 cycles or 40 cycles. The PCR reaction of murine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was similarly carried out using the forward primer (5'-ACCACAGTCCATGCCATCAC-3') and the reverse primer (5'-CCACCACCCTGTTGCTGTAG-3'). This pair of primers amplified PCR product of 451-bp. PCR products were electrophoresed on 2% agarose gel

Table I. Effects of histamine and 5-HT receptor antagonists on acute hepatic injury by GalN/LPS

Class	Drug	Dose (mg/kg)	Route	Inhibition(%)	
H ₁ receptor antagonist	Diphenhydramine	10	i.p.	48 ± 12	*
		25		83 ± 7	**
		50		85 ± 6	**
	Pyrilamine	25	i.p.	32 ± 21	
		50		70 ± 10	*
H ₂ receptor antagonist	Cimetidine	50	p.o.	51 ± 12	*
		100		78 ± 6	**
	Ranitidine	50	p.o.	63 ± 5	**
		100		73 ± 3	**
Histamine and 5-HT receptor antagonist	Cyproheptadine	10	i.p.	35 ± 20	
		25		83 ± 2	*
		50		87 ± 4	*
5-HT ₂ receptor antagonist	Ketanserin	0.5	i.v.	74 ± 6	**
		1		82 ± 3	**
		2		94 ± 2	**
Steroid	Dexamethasone	0.1	i.p.	89 ± 0.8	**
Muscarinic acetylcholine receptors antagonist	Atropine	16	i.p.	26 ± 31	

Test compounds were administered 30 min but ketanserin was 15 min and dexamethasone was 1 h before GalN/LPS treatment. Serum ALT level were measured 8 h after GalN/LPS treatment. Values represent the mean ± SEM for 4-6 mice. *p < 0.05 and **p < 0.01 compared with control (Turkey-Kramer test).

and stained with SYBR Gold (Molecular Probes, OR, U. S. A.). These oligonucleotides were synthesized by Hokkaido System Science (Hokkaido, Japan). No PCR product was amplified without reverse-transcription reaction.

Statistical analysis. Data are shown as the mean ± standard error of mean (SEM). Statistical significance of difference between the GalN/LPS-treated group and test groups was determined with Turkey-Kramer multiple comparisons test after one-way analysis of variance.

Results. *Effects of histamine and 5-HT receptor antagonists on liver injury induced by GalN/LPS.* Serum ALT levels were significantly ($p < 0.05$ or $p < 0.01$) increased at 6 h and 8 h after intravenous treatment with GalN/LPS (Fig. 1A). However, GalN or LPS alone had no effect on ALT level (data not shown). We examined the effects of histamine and 5-HT receptor antagonists on GalN/LPS-induced hepatic injury (Table I). Serum ALT level at 8 h after GalN/LPS treatment was 1306 ± 201 IU/L (11 experiments, $n = 5-7$ mice), whereas that of saline group was 67 ± 13 IU/L (9 experiments, $n = 3-5$ mice). Diphenhydramine, a histamine H₁ receptor antagonist, at a dose of 10-50 mg/kg which blocks the

LPS-induced dye leakage,¹⁶⁾ inhibited GalN/LPS-induced elevation of ALT level in a dose dependent manner. Pyrilamine, as well as diphenhydramine, reduced ALT level in response to GalN/LPS. The histamine H₂ receptor antagonists such as cimetidine¹⁷⁾ and ranitidine¹⁷⁾ were also effective in inhibiting the increase in ALT level. Cyproheptadine,¹⁸⁾ an antagonist of both histamine and 5-HT receptors, exhibited a similar to those of histamine H₁ receptor antagonists. Pretreatment with ketanserin,¹⁹⁾ a 5-HT₂ receptor antagonist, dramatically prevented the increase in ALT level by GalN/LPS. Dexamethasone, a steroid agent, strongly inhibited GalN/LPS-increased ALT level. However, pretreatment with atropine²⁰⁾ failed to protect against GalN/LPS hepatotoxicity.

Time-course of expression of HDC mRNA induced by GalN/LPS in liver. It has been reported that HDC activity is enhanced in the liver of mice treated with GalN/LPS.¹²⁾ Thus, we examined expression of HDC mRNA in the liver of mice treated with GalN/LPS. Levels of HDC mRNA were increased with time after GalN/LPS treatment (Fig. 1B). HDC mRNA level at 8 h

Fig. 2. Effects of diphenhydramine (DH), cimetidine (CIM) and dexamethasone (DEX) on HDC mRNA level in liver induced by GalN/LPS (G/L). DH (25 mg/kg, i.p.) and CIM (100 mg/kg, p.o.) were administered 30 min but DEX (0.1 mg/kg, i.p.) was 1 h before GalN/LPS treatment (A). The relative quantities of HDC mRNA are presented as ratio between the intensities of HDC band relative to the intensity of the GAPDH band (B). S: saline treatment; M: marker.

was 10-fold higher than that at 0 h (Fig. 1C). The amplified products were confirmed to be HDC gene by sequence analysis (data not shown).

Effects of diphenhydramine, cimetidine and dexamethasone on HDC mRNA expression induced by GalN/LPS in liver. We examined effects of drugs on expression of HDC mRNA in mouse liver at 8 h after treatment with GalN/LPS (Fig. 2A). Dexamethasone strongly inhibited expression of HDC mRNA in response to GalN/LPS (Fig. 2B). Diphenhydramine also reduced the up-regulation of HDC mRNA, and cimetidine slightly inhibited the GalN/LPS-induced elevation of HDC mRNA level.

ALT level in response to GalN/LPS in mast cell-deficient mice. In order to further clarify the involvement of mast cells in the GalN/LPS-induced mouse liver injury, we examined ALT level in response to GalN/LPS in mast cell-deficient mice (W/W^v) (Fig. 3A). There was a significant ($p < 0.01$) difference between mast cell-deficient mice and the congenic normal mice (+/+) at 8 h after GalN/LPS treatment. The response of mast cell-deficient mice was much lower than that of the congenic normal mice. Diphenhydramine at a dose of 25 mg/kg (i.p.) significantly ($p < 0.01$) reduced serum ALT level

Fig. 3. Serum ALT level (A) at 8 h after GalN/LPS treatment and expression of HDC mRNA (B) in mast cell-deficient mice. Values represent the mean $\pm$ SEM of 4-6 mice (A). ** $p < 0.01$ vs saline, ## $p < 0.01$ vs congenic mice. Saline ($\square$), GalN/LPS treatment ($\blacksquare$). Fig. 3B shows expression of HDC mRNA and GAPDH mRNA in the liver of mast cell-deficient mice and the congenic mice without GalN/LPS treatment. M: marker; +/+ : congenic mice; W/W^v : mast cell-deficient mice.

from 440 ± 105 IU/L ($n = 6$) to 171 ± 40 IU/L ($n = 6$) in mast cell-deficient mice treated with GalN/LPS. Expression of HDC mRNA was observed in the liver of mast cell-deficient mice without GalN/LPS treatment but its level was low compared with the congenic normal mice (Fig. 3B). Increase in HDC mRNA level by GalN/LPS was observed in the liver of the congenic normal mice but not in mast cell-deficient mice (data not shown).

Discussion. Histamine is involved in allergy and inflammatory response through the activation of histamine H₁ and H₂ receptors on various cells.^{21,22} This vasoactive amine modulates various immune responses, including the production of cytokines.²³⁻²⁶ It is known that histamine is increased in plasma of patients with acute hepatitis.²⁷ In animal experiments, increase in histamine content has been observed in liver and plasma of mice treated with GalN/LPS and CCl₄.^{12,13} 5-HT has also been implicated as an endogenous mediator contributing to liver dysfunction.^{28,29} LPS-induced accumulation of 5-

HT as well as histamine has been observed in the mouse liver.³⁰⁾ We found that histamine H₁ and H₂ antagonists are effective in inhibiting mouse liver injury in response to GalN/LPS. In addition, ketanserin, a 5-HT₂ receptor antagonist, and cyproheptadine, a histamine and 5-HT receptor antagonist, also inhibited the increase in ALT level. These results suggest that histamine and 5-HT are involved in the liver injury induced by GalN/LPS. Others have reported that cimetidine protected the hepatic injury induced by CCl₄³¹⁾ and acetaminophen³²⁾ *in vivo*. An inhibition by cimetidine may be due to protection against hepatic damage induced by acetaminophen metabolized via cytochrome P-450 system. Furthermore, cimetidine and ranitidine reduce the rat liver injury in response to galactosamine through inhibition of galactosamine phosphorylation by galactokinase.³³⁾ Recent report has shown that ranitidine reduces ischemia/reperfusion-induced rat liver injury by inhibiting neutrophil activation.³⁴⁾ Thus, it is conceivable that in addition to block of histamine H₂ receptor, other mechanisms of cimetidine and ranitidine may be involved in the inhibition of GalN/LPS-induced liver injury. On the other hand, Nakamura *et al.*³⁵⁾ have reported that dimaprit, a histamine H₂ receptor agonist, suppresses ALT level in response to GalN/LPS in mice. The mechanism of dimaprit may be due to down-regulation of TNF- α production via activation of histamine H₂ receptor.³⁵⁾ In fact, TNF- α is well known to be a central mediator in GalN/LPS-induced mouse liver injury.³⁶⁾ There is evidence for the inhibition of cytokine productions such as IL-1 and TNF- α by histamine H₂ receptor activation *in vitro*.^{18),19)} In present study, however, it is unclear whether histamine H₂ receptor plays a role in the development of GalN/LPS-induced mouse liver injury.

HDC activity has been observed in various types of cells, such as mast cells, basophils and macrophages.^{37),38)} Previous reports have shown that HDC activity is enhanced in the liver of mice treated with GalN/LPS¹²⁾ and CCl₄.¹³⁾ In this study, levels of HDC mRNA in liver were increased with time after GalN/LPS treatment, indicating that histamine production is up-regulated by GalN/LPS in the liver. Furthermore, dexamethasone inhibited expression of HDC mRNA as well as ALT level in response to GalN/LPS. Interestingly, diphenhydramine also down-regulated the level of HDC mRNA. These results suggest that a mechanism by which these compounds inhibited the increase in ALT level may be due partly to modulation of histamine production in the liver, and further support that histamine production is involved in the development of GalN/LPS-induced

mouse liver injury.

We found that ALT level in response to GalN/LPS in mast cell-deficient mice is much lower than that in the congenic normal mice. This is strong evidence that mast cells play an important role in the GalN/LPS-induced mouse liver injury. Moreover, diphenhydramine reduced ALT level in mast cell-deficient mice treated with GalN/LPS, suggesting the participation of histamine released from macrophages¹³⁾ and basophils¹⁵⁾ in this model. In fact, HDC mRNA was expressed in the liver of mast cell-deficient mice. It has been reported that histamine level and HDC activity in the liver of mast cell-deficient mice were increased by CCl₄ treatment.¹³⁾ It is, therefore, likely that histamine released from both mast cells and other cells contributes to the development of liver injury.

In conclusion, we demonstrated that mast cells play an important role in the GalN/LPS-induced liver injury. It seems that histamine and serotonin released from mast cells and other cells are involved in the process of hepatic injury.

Acknowledgements. The authors acknowledge Prof. Emeritus K. Wake (Tokyo Medical and Dental University) and Prof. T. Sato (Tsurumi University) for their invaluable advice.

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