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CHANGE OF HEPATIC HISTAMINE CONTENT DURING HEPATIC FIBROSIS

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Abstract—Hepatic function was studied by measuring the time courses of several variables in blood and liver using a chronic liver-injury model produced by administering CCl_4 consecutively for 12 weeks in rats. A marked increase in liver histamine content occurred after 10 weeks of treatment with CCl_4. At weeks 10 and 12, liver histamine levels in the CCl_4-treated group were 1.95 and 4.61 times higher, respectively, than in the control group. This change in liver histamine content appeared after that in other variables such as glutamic pyruvic transaminase, alkaline phosphatase, and white blood cells, but it corresponded to a change in liver hydroxyproline. Increased mast cells were seen in fibrotic foci around Glisson's sheath by microscopic morphological observation of the liver 12 weeks after treatment with CCl_4. The histamine concentration in plasma tended to decrease after CCl_4 treatment, and at week 12 the decrease was statistically significant compared with control. The liver activities of histamine-metabolizing enzymes, histamine-*N*-methyltransferase and histaminase, decreased to 1/3.4 and 1/6.0 times those of the nontreated group, respectively, 12 weeks after treatment with CCl_4, whereas blood histaminase increased about 9.2 times. The increase in histamine content in injured liver was presumedly derived from the increase in mast cells in the inflamed area of the liver; also, the deficiency of histamine-metabolizing enzymes in liver might have caused the high histamine content in the liver. On the other hand, the decrease in plasma histamine concentration might have occurred as a consequence of the enzyme leakage from hepatocytes that accompanied the breakdown of hepatocytes by CCl_4 and thus, of the histamine metabolism in blood by the leaked enzymes. The same kind of experiment was performed using a dimethylnitrosamine-induced liver injury model in rats. The increase of hydroxyproline in the liver occurred 11 days after that of histamine content in liver. These results suggest the possibility that increased histamine in the liver may participate in the biosynthesis of collagen.

Histamine has been shown to exert a regulatory influence on several aspects of inflammation, not only as a chemical mediator participating in the early stage of inflammation and type I allergy [1, 2] but also as a modulator to regulate fibrosis and delayed type inflammation [3–6]. Although the mechanisms are still uncertain, involvement of histamine in wound healing and collagen biosynthesis has long been accepted [7, 8]. Fitzpatrick and Fisher [7] reported a correlation between histamine metabolism and wound healing in rats, and Gorski and coworkers [8] revealed that compound 48/80 treatment in rats enhances the tensile strength in wounded skin compared to that of nontreated rat skin. *In vitro* histamine formation by human tissues including skin was studied by Russell *et al.* [9] and Boucek and Noble [10], and the study of stimulation of fibroblast growth by histamine in human wounds was studied by Kahlson *et al.* [11, 12].

In liver cirrhosis and fibrosis, acceleration of collagen biosynthesis as one of the processes in chronic inflammation is very important [13]. Being interested in the role of histamine in liver fibrosis, we studied the relation between the histamine content in liver and other variables in blood and liver of chronically CCl_4-treated rats. Recently, Fujiwara *et al.* [14, 15] reported that one-shot i.p. treatment with dimethylnitrosamine (DMN) in rats induced fibrosis in the liver in 3 or 4 weeks. The change in collagen and histamine in the liver was also investigated using this model.

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MATERIALS AND METHODS

CCl_4-induced chronic hepatic injury model in rats. Male Wistar rats weighing 169–190 g obtained from Nippon Laboratory Animals Inc. (Tokyo, Japan), were fed laboratory chow and tap water *ad lib.* CCl_4 (0.5 ml/kg, 25% v/v solution in olive oil) was given subcutaneously two times each week for 12 weeks. Treated (ten) and control (ten) rats were killed 2, 4, 7, 10, and 12 weeks after the first CCl_4 treatment. Blood was gathered from the dosal aorta, and plasma and serum were obtained after centrifugation. The liver was rapidly excised, weighed, homogenized, and stored at below −20°. All measured variables were expressed as a ratio relative to values for the control group.

DMN-induced hepatic injury model in rats. Male Wistar rats weighing from 149 to 176 g were treated with DMN (40 mg/kg), intraperitoneally, and housed in cages in the same conditions mentioned above. Rats were killed at 1 and 3 days and at 1, 2, 3 and 4 weeks after one-shot administration of DMN [14]. Control and DMN-treated groups consisted of five rats.

Blood variables. Glutamic pyruvic transaminase

(GPT, EC 2.6.1.2) in serum was measured using a test kit (GPT-UV Wako Pure Chemicals, Osaka, Japan). Alkaline phosphatase (ALP, EC 3.1.3.1) in serum was determined according to the method of Hansen [16]. Histamine in plasma was determined according to the method of Håkanson *et al.* [17] with some modification. To exclude the fluorescence yield of spermidine, 0.8×10^{-3} M $CdCl_2$ was added before reaction of histamine with *o*-phthalaldehyde (OPT), and the condensation reaction was done under pH 12.5, at 0° for 90 min. The samples were acidified with citric acid to pH 3.3 after condensation reaction. To assure that histamine was measured specifically by this assay, the samples prepared from plasma and liver by the usual method [18] were measured using high performance liquid chromatography (column: Lichrosorb PR-18, 5 μm; solvent: 0.2 M NaCl/MeOH (pH 3.0) 50/50; flow speed: 0.6 ml/min; detector/fluorometer: 350/450 nm). Only one peak was observed at the same position of authentic histamine-OPT fluorophore from the samples. The numbers of white blood cells (WBC) and red blood cells (RBC) in all blood were determined using an automatic blood cell counter (ELT-8, Ortho Instruments Inc., U.S.A.).

Hepatic variables. Histamine and hydroxyproline (HYPRO) were determined using the methods described by Håkanson *et al.* [17] and Bondjers and Bjorkerud [19] respectively, with some modification. Histaminase in liver and plasma was measured according to the method of McEwen [20]. Histamine-*N*-methyl transferase was measured according to the method of Snyder and Axelrod [21].

Morphological studies. Fresh tissue was fixed in formalin and embedded in paraffin. Sections were stained with toluidine blue to observe mast cells.

Chemicals. *S*-Adenosyl-L-[*methyl*-^{3}H]methionine was purchased from Amersham International Limited (Buckinghamshire, England). Other reagents used in these experiments were all analytical grade.

RESULTS

Change of several variables of hepatic function in rats treated with CCl_4. Figure 1 shows the time courses of blood variables. GPT in serum was elevated promptly after CCl_4 treatment. This elevation continued for 12 weeks (>700 mI.U./ml). ALP in serum, measured as a marker for injury of the bile duct area, began to increase at week 4 and after 7 weeks showed a 3.5 to 4.5 times higher value than normal rats. WBC in blood increased at week 4, and continued to increase gradually after that, while RBC in blood showed almost the same change as non-treatment rats except for a slight decrease at week 12. With respect to HYPRO, measured as an indicator of collagen increase in liver, an increase was recognized after 10 weeks. At week 12, a 4.5-fold increase compared with control was observed (Fig. 2). Histamine content in liver showed almost the same change as HYPRO, that is, it began to increase at week 10 and increase 4.6 times by week 12, while the concentration of serum histamine decreased according to the time change after CCl_4 treatment. The mean values of GPT, ALP, WBC, RBC and

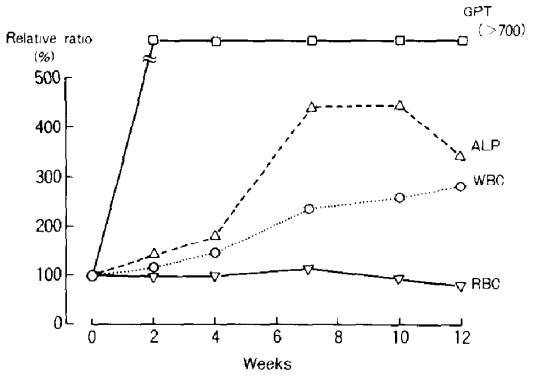

Fig. 1. Time courses of several variables with respect to hepatic function in blood after chronic administration of CCl_4. Rats were treated with CCl_4 two times a week for 12 weeks, and were killed at the indicated days to measure the variables. Each value in the treated group is expressed as a ratio relative to the value for the control group. Serum GPT in the treated group was over seven times that in control. GPT and ALP measured after CCl_4 treatment increased significantly compared with each control throughout the 12 weeks ($P < 0.01$). WBC was increased significantly from control except at week 2 ($P < 0.01$). RBC was significantly different at week 12 only ($P < 0.01$). Key: (□) GPT, (△) ALP, (○) WBC and (▽) RBC.

histamine in blood of nontreated rats (mean values of all rats from 0 to 12 weeks) were: 7.65 ± 0.63 mI.U./ml, 28.7 ± 2.4 K-A units/ml, $(6.74 \pm 0.27) \times 10^3$ cells/mm^3, $(6.98 \pm 0.10) \times 10^3$ cells/mm^3 and 42.7 ± 2.9 ng/ml.

The mean values of histamine and HYPRO in the livers of nontreated rats calculated by the same method were 1.57 ± 0.07 μg/g liver and 1.68 ± 0.09 μmoles/g liver.

By microscopic observation, the increase in collagen fiber was observed around Glisson's sheath in the liver tissue of rats after CCl_4 treatment for 10 weeks, and the progression of fibrosis was even more marked in the livers of 12-week CCl_4-treated rats.

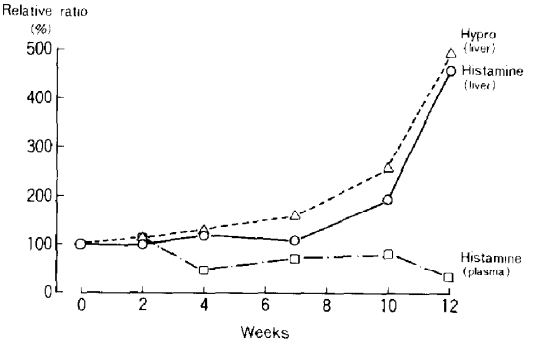

Fig. 2. Time course of histamine and HYPRO in plasma and liver. Rats were treated as described in the legend of Fig. 1. At weeks 10 and 12, the concentrations of HYPRO and histamine in liver were significantly different from control ($P < 0.01$). In plasma histamine, the difference was significant at the fourth, seventh and twelfth weeks ($P < 0.05$). Key: (□) histamine in plasma, (△) HYPRO in liver and (○) histamine in liver.

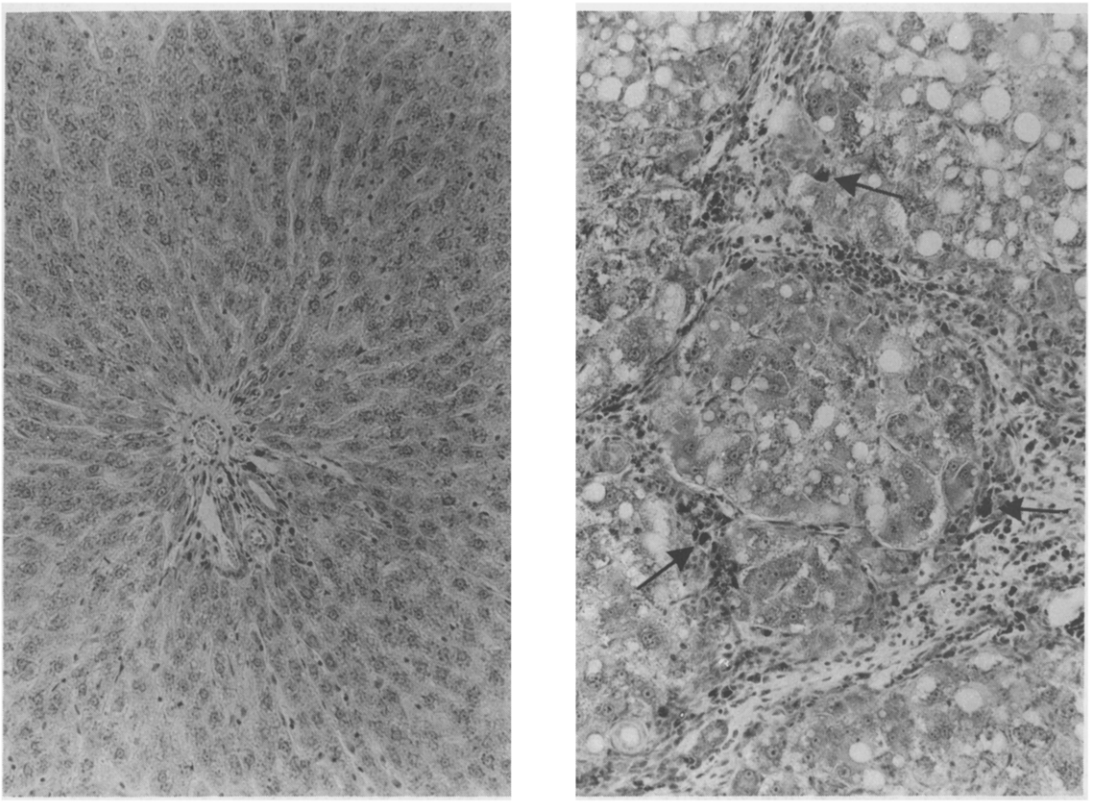

Fig. 3. Comparison of liver histology between rats treated with CCl_4 for 12 weeks (right side) and nontreated rats (left side). Fibrosis with small round cell infiltration is marked in and around the tract, and mast cells stained with toluidine blue are observed in or along with fibrosis. Arrows indicate mast cells. Magnification: (×100).

Mast cells began to appear in and around the collagen fiber, corresponding to the development of fibrosis. The difference in the number of mast cells between the liver tissue after CCl_4 treatment for 12 weeks and the nontreated control was remarkable, because in Glisson's sheath in the normal rats very few mast cells stained with toluidine blue were observed. Typical pictures are presented (Fig. 3).

Change of metabolizing enzymes of histamine. It has been reported that histamine-*N*-methyltransferase (HMT) and histamine (Hase) primarily metabolize histamine *in vivo*. We measured the

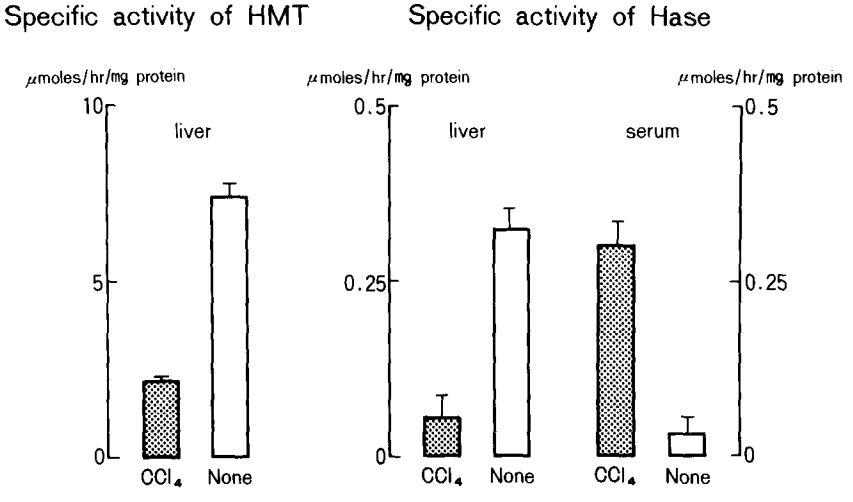

Fig. 4. Specific activities of histamine-*N*-methyl transferase (HMT) in liver of CCl_4-treated rats (N = 10) and control rats (N = 10) (left side) and of histaminase (Hase) in liver and serum of both groups (right side). Results are mean ± S.E.M.

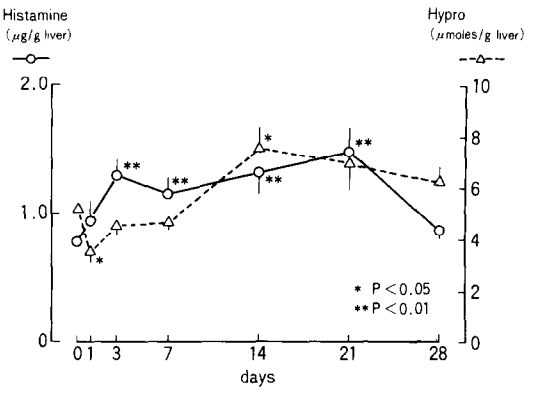

Fig. 5. Time course of histamine and HYPRO in liver after one-shot administration of DMN (40 mg/kg). Key: (○) histamine in liver, and (△) HYPRO in liver. Single and double asterisks indicate $P < 0.05$ and $P < 0.01$ vs control respectively.

activities of HMT and Hase in liver and serum of rats treated with CCl_4 for 12 weeks. As shown in Fig. 4, both enzymes in liver decreased about 1/3.4 and 1/6.0 times in specific activity, respectively; on the other hand, Hase in serum increased about 9.2 times compared with control.

Changes of variables of hepatic function in blood and liver of DMN-induced hepatic-injury model in rats. Figure 5 demonstrates the time course change of HYPRO and histamine in liver of DMN-treated rats. An increase in liver HYPRO at weeks 3–5 was observed (Fig. 5); increase in liver histamine occurred ahead of the increase in HYPRO, i.e. by day 3 high values, statistically significant from control, appeared and continued for 3 weeks.

DISCUSSION

Histamine has been focused on recently as a modulator controlling the function of several inflammatory cells, as well as a chemical mediator of type I allergy [1–8]. With respect to the modulating action of histamine on collagen biosynthesis, there are several reports. Some researchers have demonstrated that histamine enhances the biosynthesis of collagen in fibroblasts [9, 10]; on the other hand, some reports suggest a suppressive action of histamine on the biosynthesis of collagen through the H_2 receptor in granulomas induced by subcutaneous implantation of formaline-soaked filter paper disks in rats [22]. Thus, the mechanism of action of histamine on collagen biosynthesis is still obscure.

It is well known that collagen biosynthesis in rats is activated in livers chronically injured by hepatotoxins [23, 24]. Using the chronic liver-injury model in rats, we investigated the relation between the concentrations of histamine and collagen in liver as a function of time to determine the participation of histamine in liver fibrosis. A good correlation between an increase in HYPRO and the histamine content in liver after 10 weeks of treatment with CCl_4 was observed (Fig. 2). As shown in Fig. 1, changes in other hepatic variables such as GPT, ALP, and WBC in blood did not correspond to the changes in histamine and HYPRO. A good correlation between histamine and HYPRO was also observed in another liver-injury model (induced by DMN one-shot administration). In this model, the concentrations of GPT, ALP and albumin in serum changed during the early stage of liver injury after treatment with DMN but returned to normal by day 7 (data not shown). On the other hand, histamine increase in liver began at day 3 and continued for 3 weeks, whereas HYPRO began to increase 2 weeks after DMN treatment (Fig. 5). This result suggested that the increase in liver histamine was associated with the biosynthesis of collagen. When a histological study was done on the chronic liver-injury model with CCl_4, parenchymal necrosis and vacuolar degeneration were observed at weeks 2, 4 and 6 after CCl_4 treatment, but the increase in fibrosis had not appeared yet. After 10 weeks, the increase in fibrosis and pseudolobular formation occurred, and many mast cells were observed in the fibrotic foci after staining with toluidine blue. A comparison of the histological pictures of the livers in the CCl_4-treated group and the nontreated control is shown in Fig. 3. One of the reasons for the increase in liver histamine after 10 weeks of CCl_4 treatment may have been the increase in mast cells. Kurokawa [25] reported that histopathologically the increased number of mast cells is correlated with changes in the stroma, such as fibrosis of Glisson's sheath, destruction of limiting plates, and cell infiltration of portal tracts. Though he also mentioned the hypothesis that materials released from mast cells, especially histamine, accelerate the fibrosis in injured liver, the relation between the content of histamine and fibrosis was not understood quantitatively. Ishii and Tsuchiya [26] reported that, after administration to rabbits of homologous liver antigen with histamine, liver injury closely resembling chronic active hepatitis could develop and progress into liver cirrhosis with pseudolobular formation. Our results show a good correlation between the increase in HYPRO and the increase in histamine in liver, and support their hypothesis that increased histamine may control collagen biosynthesis. However, the materials released from mast cells were not restricted to histamine only. 5-Hydroxytryptamine and leucotrienes C_4 and D_4 which are recognized as having a strong contracting actions of the aorta may contribute to the disturbance of the microcirculation in liver and to the stimulation of fibroblasts in liver, inducing collagen biosynthesis.

As shown in Fig. 4, the activities of enzymes that metabolize histamine in liver were decreased. The metabolism of histamine, released from mast cells by some stimulant, may be delayed due to the decrease in histamine-metabolizing enzymes in hepatocytes; as a consequence, an increase in histamine might occur. This may be the second reason for the increase in histamine in injured liver.

We did not observe an enhancement of histamine in rat plasma as has been reported in humans and dogs elsewhere. In acute CCl_4-treated dogs and rabbits, the concentration of histamine in plasma has been reported to increase promptly after CCl_4 treatment [27, 28]; this change was attributed to a decrease in the activities of liver histamine-metab-

olizing enzymes due to liver damage by CCl_4, resulting in the increase in blood histamine level. In human chronic active hepatitis, high histamine levels in plasma were also reported by Ishii *et al.* [29, 30], and this phenomenon was explained by the deficiency of histamine-metabolizing enzymes in liver as in dogs and rabbits. However, as shown in Fig. 4, in the liver-injury model of rats with CCl_4, histamine in plasma declined in proportion to the severity of the CCl_4-induced liver injury. The activity of histaminase in plasma of CCl_4-injured rats was increased about 9.3 times compared with control. These results are ascribed to breakdown of liver parenchymal cells and leakage of histamine-metabolizing enzymes from hepatocytes into blood, which would result in increased enzyme activity in plasma and decreased histamine concentration due to metabolism by these enzymes. The discrepancy between the results from rats and from other animals including humans may be due to species differences. When the metabolizing enzymes can be measured, clearer results will be obtained.

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