ULTRASTRUCTURAL AND ULTRACYTOCHEMICAL EXAMINATION OF THE EFFECTS OF PREADMINISTRATION OF XIAO-CHAI-HU-TANG ON HEPATIC DISORDERS INDUCED BY D-GALACTOSAMINE HCL

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The effect of preadministration of Xiao-Chai-Hu-Tang on hepatic disorders, induced by D-galactosamine HCl, were examined morphologically and histocytochemically. A single intraperitoneal injection of 800 mg/kg or 1500 mg/kg D-galactosamine HCl induced remarkable histological and cytological changes in the rat liver. Light microscopically, the liver showed diffuse parenchymal damage, in which hepatic cell cords were disorganized and a marked accumulation of lipid droplets were found in the hepatocytes. Ultrastructurally, disruption of lamellar arrangement of rough endoplasmic reticulum, dissociation of intrahepatic cell space and an increase in the number of autophagic vacuoles were observed in the control groups after the administration of D-galactosamine HCl. Histo- and cytochemical detection of 5'-nucleotidase and alkaline phosphatase activities revealed disruption of the bile canaliculi system and a disturbance of plasma membrane. Activity of the glucose-6-phosphatase disclosed an uneven distribution of enzymes cell by cell.

However, no conspicuous pathological and histocytochemical changes were found in the liver preadministered with Xiao-Chai-Hu-Tang. Biochemical assay revealed that much higher enzyme activities were preserved in the groups preadministered with Xiao-Chai-Hu-Tang. From these results, it can be seen that the preadministration of Xiao-Chai-Hu-Tang is undoubtedly effective in preventing changes induced by D-galactosamine HCl.

Xiao-Chai-Hu-Tang is a blended traditional oriental medicine, which consists of seven crude extracts of Bupleuri radix, Pinelliae tuber, Scutellariae radix, Zizyphi fructus, Ginseng radix, Glycyrrhizae radix and Zingiberis rhizoma. It has been reported (1, 17, 29) that Xiao-Chai-Hu-Tang has many pharmacologic effects including anti-inflammatory action, anti-allergic action, immunoactivating action, cell membrane stability action and enhancement action of inner steroid effect.

Recently, the interest in traditional oriental medicine has been revived. Since there is no effective western medicine for chronic hepatic disorders and oriental medicine is free from harmful adverse effects, the clinical application of Xiao-Chai-Hu-Tang has become popular. In general, traditional oriental medicine has been developed on the basis of clinical experience and practice, whereas western medicine has been based on experimental pathology and pharmacology. Therefore, the
basic knowledge, including chemical, pharmacological and experimental pathological studies, concerning traditional oriental medicine has been limited.

In our present paper, in order to estimate the effects of preadministration of Xiao-Chai-Hu-Tang, hepatic disorder was experimentally induced by the injection of D-galactosamine HCl in two groups: (a) control rats and (b) rats preadministered with Xiao-Chai-Hu-Tang. Then, the effects of Xiao-Chai-Hu-Tang on experimental hepatic disorders were morphologically, histocytochemically and biochemically examined.

MATERIALS AND METHODS

Female Wistar rats weighing 130 to 150 gr were used. Hepatic disorders were produced in the following two experimental groups of rats by a single intraperitoneal injection of D-galactosamine HCl (800 or 1500 mg/kg body weight): (a) normal control rats and (b) rats preadministered with Xiao-Chai-Hu-Tang (Tsumura Juntendo Ltd., Tokyo, Japan). They had previously received a daily intraperitoneal injection of 20 mg Xiao-Chai-Hu-Tang per kg body weight for four consecutive days. Animals were sacrificed at 2, 4 and 7 days after the injection of D-galactosamine HCl.

Each experimental group was examined using the following four procedures; (1) conventional light microscopic observation with hematoxylin and eosin stain, and Sudan III stain, (2) ultrastructural observation with the conventional electron microscopic technique, (3) histo- and cytochemical detection of 5'-nucleotidase, alkaline phosphatase and glucose-6-phosphatase activity, and (4) biochemical assay of the enzyme activities of the above three phosphatases.

Tissue for light microscopy was conventionally fixed with 10% formalin, dehydrated, embedded in paraffin and stained with hematoxylin and eosin. Some materials fixed with formalin were stained with Sudan III.

For routine morphological electron microscopic observation, the liver was fixed by perfusion through the portal vein for 5 min with 2.5% glutaraldehyde in a 0.1 M phosphate buffer, pH 7.4, at room temperature. The liver was cut into small blocks and immersed in the same fixative for 1 hr. After rinsing, the blocks were postfixed for 1 hr in 1% osmium tetroxide in the 0.1 M phosphate buffer, pH 7.4, dehydrated through graded ethanol solution and propylene oxide and finally embedded in Spurr's mixture. Ultrathin sections were cut on an LKB Ulrotome III, stained with uranyl acetate and lead citrate, and examined with a JEM 100CX electron microscope.

For the histo- and cytochemical study, the livers were fixed by perfusion with 0.5% glutaraldehyde in 30 mM NaOH-PIPES, pH 7.2, containing 8% sucrose, for 5 min at 0–4°C. Since the choice of buffer, as well as fixatives, is in some cases (26, 27) critical for establishing the exact and true localization of the enzyme activity without loss of activity in the fixation for enzyme cytochemistry, NaOH-PIPES buffer was employed as a vehicle for aldehyde fixative instead of routinely used cacodylate buffer in our present study. Following the fixation, the liver was cut into slices less than 1 mm thick, which were rinsed in the same buffer as used in the aldehyde fixation. For light microscopy, frozen sections of 15 μm in thickness were cut with an electrofreezing microtome. They were incubated in a medium of
5'-nucleotidase (24) for 30 min at 37°C, alkaline phosphatase (15) for 60 min at 37°C, and glucose-6-phosphatase (25) for 30 min at room temperature. Then the sections were washed in distilled water, treated with 1% yellow ammonium sulfide, washed in distilled water, mounted on slide glasses and observed under a light microscope.

For electron microscopy, non-frozen sections of 50 μm in thickness were cut with a Microslicer and then incubated in the same mixtures as used for light microscopic observation. However, in the detection of 5'-nucleotidase activity the cerium-based method devised by Robinson and Karnovsky was applied in our present study (19). After the incubation, sections were rinsed and postfixed with 1% osmium tetroxide in 30 mM NaOH-PIPES buffer for 30 min at 0-4°C, dehydrated and embedded in Spurr's mixture. Ultrathin sections were stained with uranyl acetate and examined in electron microscopy.

These three enzyme activities were determined biochemically by the absorption of inorganic phosphorus at 720 nm after the incubation of homogenates in the above same mixture as those used for the cytochemical detection, without lead and cerium.

RESULTS

Hepatic alteration induced by D-galactosamine HCl

The single intraperitoneal injection of 800 mg and 1500 mg D-galactosamine HCl per kilogram of body weight induced remarkable histo- and cytologic changes in the liver of the control rats. At 2 days after the injection of 800 mg/kg D-galactosamine HCl, the histological features showed an acute hepatic injury, in which degenerative and regenerative foci coexisted in the lobules, accompanied by inflammatory infiltration (Fig. 1). Each hepatocyte showed variation in size, shape and staining pattern. Some hepatocytes were swollen, their cytoplasm finely granular and eosinophilic in appearance, while other cells were shrunken and deeply eosinophilic. Almost all hepatocytes showed some histologic signs of injury, which consisted of ballooned cells, acidophilic degeneration and acidophilic necrosis. The disorganization of hepatic cell cords and accumulation of lipid droplets in hepatocytes were observed as most striking alterations at this stage. The inflammatory infiltrations were also seen in peripheral regions of hepatic lobules. These hepatic alterations were more severe in the experimental groups injected with 1500 mg/kg of D-galactosamine. The areas of degeneration of parenchymal cells were more extensive, and portal inflammation more prominent than in the rats which received 800 mg/kg of D-galactosamine. In certain hepatic lobules intralobular hemorrhages were observed. The same histologic alterations were seen at 4 days after the injection.

Electron microscopically, it was frequently difficult to identify various kinds of cells coexisting within hepatic lobules and to recognize the three-dimensional organization of hepatic cell cords, since in each hepatocyte there appeared variation in size and shape depending on the severity of damage. The interhepatic space was widely dissociated, and severely injured hepatocytes were extruded from the hepatic cell cords into the sinusoidal space and were observed as isolated cells. Many lipid droplets were accumulated within the cytoplasm of hepatocytes, and consequently each cytoplasmic organella was widely separated (Fig. 2). Reduction of
microvilli of sinusoidal membrane and disappearance of the lamellar structure of rough endoplasmic reticulum were consistent alterations. An increase in both number and size of large autophagic vacuoles was frequently observed (Fig. 3).

However, at 7 days after the injection, these hepatic alterations were not light and electron microscopically conspicuous (Fig. 4). Although some degenerative foci and regenerative changes still remained in certain regions, many of the hepatic structures returned to almost normal in appearance in both the experimental groups which received 800 mg/kg and 1500 mg/kg D-galactosamine HCl.

The effect of preadministration of Xiao-Chai-Hu-Tang on hepatic alterations induced by D-galactosamine HCl

In order to estimate the effects of Xiao-Chai-Hu-Tang, the results of both experimental groups 2 and 4 days after the injection were examined. Light microscopically, conspicuous pathological alterations were not observed after the injection of both 800 mg/kg and 1500 mg/kg D-galactosamine HCl in livers of the rats which had received Xiao-Chai-Hu-Tang (Fig. 5). Some eosinophilic degeneration appeared deeply eosinophilic in the cytoplasm and condensed nucleus, mitotic figures of hepatocytes and a small number of inflammatory infiltrations were observed in certain experimental specimens. However, no lobular disarray and marked accumulation of lipid droplets in the hepatocytes existed. The morphological appearance of the hepatic lobules was quite the same as those of normal rats.

In electron microscopic observation, such conspicuous ultrastructural alterations as induced by D-galactosamine HCl in the control rats were not found in the rats preadministered with Xiao-Chai-Hu-Tang (Fig. 6). Small vacuoles were sometimes seen in certain hepatocytes. However, hepatic cell cords appeared normal in organization, and features of the plasma membrane and rough endoplasmic reticulum showed the normal structure. The preadministration of Xiao-Chai-Hu-Tang was clearly effective in preventing changes induced by D-galactosamine HCl.

Histo- and cytochemical observation

In order to examine the functional changes of both the plasma membrane, which is the first protecting barrier of cells and a marker of cell damage, and the endoplasmic reticulum, where drug-metabolizing enzymes are present, representative marker enzymes of each organelle were observed using histo- and cytochemical procedures. 5'-nucleotidase and alkaline phosphatase activities as a marker enzyme of plasma membrane, and glucose-6-phosphatase activity as one of endoplasmic reticulum were used in our present study.

Light microscopically, 5'-nucleotidase activity of the liver of normal untreated rats was demonstrated in the bile canaliculi and in the sinusoidal wall regions in the lead-based method described by Uusitalo and Karnovsky (1977) (Fig. 7). In the electron microscopic observation, reaction products of 5'-nucleotidase were present on the whole plasma membrane of hepatocytes. However, reaction products of 5'-nucleotidase were observed as coarse deposits and showed a very patchy distribution. In our present study, the cerium-based method devised by Robinson and Karnovsky (1983) was applied in the electron microscopic observation. The size of reaction products of the cerium-based method was much finer than that of the lead-based method. The cerium-based method exhibited a consistent and uniform distribution of reaction products on the plasma membrane. Reaction prod-
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Figs. 1-3.
Figs. 4-6.
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Figs. 7-10.
Figs. 11-13.
ucts of 5'-nucleotidase activity existed on the whole plasma membrane of hepatocytes of the normal untreated rats (Fig. 8). No diminishing of activities was found in the addition of 2.5 mM levamisole as a potent inhibitor of alkaline phosphatase. Reaction products were abolished in the substrate-free medium.

The administration of 1500 mg/kg D-galactosamine HCl to the control rats caused a marked decrease of 5'-nucleotidase activity of the bile canaliculi region,

Figs. 1–3. Routine morphological preparation from control rats 2 days after the injection of 800 mg/kg D-galactosamine HCl.
Fig. 1. Light micrograph of hematoxylin and eosin stain. Acute galactosamine intoxication shows diffuse parenchymal damage. Arrangement of hepatic cell cords is disrupted. Many hepatocytes are occupied by lipid droplets. ×250
Fig. 2. Electron micrograph. The cytoplasm of hepatocytes is occupied by many lipid droplets, and consequently each organella is widely separated. The reduction of microvilli sinusoidal membrane is observed. ×7,000
Fig. 3. Electron micrograph. Many autophagic vacuoles are observed in hepatocyte administered with D-galactosamine HCl. ×34,000
Fig. 4. Light micrograph for control rat 7 days after the injection of 800 mg/kg D-galactosamine HCl. Although portal inflammation still remains in this picture, hepatic structure returns to almost normal. H & E stain. ×250
Figs. 5 and 6. Routine morphological preparation from rats preadministered with Xiao-Chai-Hu-Tang 2 days after injection of 800 mg/kg D-galactosamine HCl.
Fig. 5. Light micrograph of H & E stain. No pathological signs of alterations induced by D-galactosamine HCl are observed. Hepatic cell cords are in an orderly arrangement. ×250
Fig. 6. Electron micrograph. Hepatocytes show normal ultrastructure. The arrangement of rough endoplasmic reticulum is well organized and microvilli of the sinusoidal membrane show a normal appearance. ×6,900
Figs. 7 and 8. 5'-nucleotidase activity in normal rat liver.
Fig. 7. 5'-nucleotidase activity in the light microscopy. Incubation was performed for 30 min at 37°C in the lead-based method described by Uusitalo and Karnovsky (1977). The activity is present both in the bile canaliculi and sinusoidal regions. ×400
Fig. 8. 5'-nucleotidase activity in the electron microscopy. Incubation was carried out for 30 min at 37°C using the cerium-based medium described by Robinson and Karnovsky (1983). Reaction products are uniformly distributed in all sinusoidal membranes of hepatocytes. ×10,000
Figs. 9 and 10. 5'-nucleotidase activity of control rats 2 days after the injection of 1500 mg/kg D-galactosamine HCl.
Fig. 9. 5'-nucleotidase activity. The activity of bile canaliculi markedly decreases, while that of the sinusoidal region is still demonstrated. ×400
Fig. 10. 5'-nucleotidase activity. Though the activity is present on the sinusoidal membrane, microvilli apparently decrease in number and length. ×14,000
Figs. 11 and 12. Alkaline phosphatase activity in normal rats.
Fig. 11. Alkaline phosphatase activity in the light microscopy. Incubation was performed for 60 min at 37°C in the medium described by Mayahara et al. (1967). Reaction products can be observed only in the bile canaliculi in the light microscopic level. ×250
Fig. 12. Alkaline phosphatase activity in the electron microscopy. Reaction products are demonstrated not only on the bile canaliculi, but also on the sinusoidal and lateral membranes of rat hepatocyte. ×17,000
Fig. 13. Alkaline phosphatase activity of control rat 2 days after the injection of 1500 mg/kg D-galactosamine HCl. Although activity still remains in some bile canaliculi, many bile canaliculi appear dilated, disrupted in many sites and lose normal connection through the bile canaliculi system. ×300
while that of the sinusoidal wall region was still demonstrated in the light microscopic level (Fig. 9). Ultracytochemically, most of the bile canaliculi membrane lost enzyme activity, whereas it existed on the sinusoidal and lateral membrane. Compared with the normal untreated rats, a decrease of microvilli of sinusoidal membrane in number and length was observed (Fig. 10). In the rats injected by 800 mg/kg of D-galactosamine HCl, the decrease of 5'-nucleotidase activity was found in the some groups, which was not a consistent result.

The alkaline phosphatase activity of the liver of normal untreated rats was weakly demonstrated in the bile canaliculi regions in the light microscopic observation (Fig. 11). Electron microscopically the activity could be observed not only on the bile canicular membrane but also on the sinusoidal and lateral membranes (Fig. 12): That is, the alkaline phosphatase activity was present on the whole plasma membranes of rat hepatocytes.

The administration of 1500 mg/kg D-galactosamine HCl to the control rats also induced changes in localization and intensity of alkaline phosphatase activity. Light microscopically, the beautiful polygonal-shaped pattern showing the distribution of bile canaliculi was disrupted in many parts of the bile canaliculi, became dilated and lost normal connection through the bile canaliculi system (Fig. 13). Electron microscopically, the same results were observed. The activity was observed on the plasma membrane, which showed uneven distribution. Dilatation of bile

![Fig. 14](image1.png)

Fig. 14. 5'-nucleotidase activity of rats preadministered with Xiao-Chai-Hu-Tang 2 days after injection of 1500 mg/kg D-galactosamine HCl. The activity is well preserved both in the bile canaliculi and sinusoidal regions. ×400

![Fig. 15](image2.png)

Fig. 15. Alkaline phosphatase activity of rats preadministered with Xiao-Chai-Hu-Tang 2 days after injection of 1500 mg/kg D-galactosamine HCl. Well organized bile canaliculi are observed. ×250
canaliculi and decrease of microvilli of sinusoidal membrane in number and length were also frequently observed. However, in the rats injected by 800 mg/kg D-galactosamine HCl, the results were not consistent.

However, no such conspicuous changes of staining pattern were demonstrated in the experimental groups preadministered with Xiao-Chai-Hu-Tang. A fine and well connected bile canaliculi system was demonstrated in the detection of 5'-nucleotidase and alkaline phosphatase activities (Figs. 14, 15). Electron microscopically, both enzyme activities were demonstrated on the whole plasma membrane of hepatocytes without any alterations.

The glucose-6-phosphatase activity was demonstrated as a homogeneous cytoplasmic staining on the hepatocytes in the light microscopic observation (Fig. 16). Electron microscopically, reaction products were present in the cisterna of smooth endoplasmic reticulum, rough endoplasmic reticulum and the nuclear envelope of hepatocytes. The administration of 800 mg/kg and 1500 mg/kg D-galactosamine HCl also induced remarkable changes of the staining pattern in glucose-6-phosphatase activity. Some cells still retained intense activity, while other showed weak activity, or lacked activity (Fig. 17). The same results were observed in the electron microscope (Fig. 18). A certain hepatocyte showed enzyme activity, whereas neighbouring hepatocytes lacked it. The disarrangement of the lamella structure of rough endoplasmic reticulum could be more easily observed in the cytochemical

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**Fig. 16.** Glucose-6-phosphatase activity of normal rat. Incubation was carried out for 30 min at room temperature in the medium described by Wachstein and Meisel (1965). Activity is demonstrated in the cytoplasm of all hepatocytes. \( \times 250 \)

**Fig. 17.** Glucose-6-phosphatase activity of control rat 2 days after injection of 1500 mg/kg D-galactosamine HCl. Although activity is still present in some hepatocytes, many hepatocytes exhibit weak or no activity. \( \times 250 \)
TABLE 1. Biochemical assay (The activity is expressed in micrograms of Pi released per hour per milligram tissue)

<table>
<thead>
<tr>
<th>5'-nucleotidase</th>
<th>controls 9.67 μg Pi/hr/mg tissue</th>
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<tbody>
<tr>
<td></td>
<td>800 mg/kg</td>
</tr>
<tr>
<td></td>
<td>2 days</td>
</tr>
<tr>
<td>untreated group</td>
<td>8.04</td>
</tr>
<tr>
<td>Xiao-Chai-Hu-Tang group</td>
<td>9.46</td>
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<tr>
<th>alkaline phosphatase</th>
<th>controls 3.95 μg Pi/hr/mg tissue</th>
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<tr>
<td>untreated group</td>
<td>3.12</td>
</tr>
<tr>
<td>Xiao-Chai-Hu-Tang group</td>
<td>3.33</td>
</tr>
</tbody>
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<table>
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<tr>
<th>glucose-6-phosphatase</th>
<th>controls 6.97 μg Pi/hr/mg tissue</th>
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<tr>
<td>untreated group</td>
<td>5.72</td>
</tr>
<tr>
<td>Xiao-Chai-Hu-Tang group</td>
<td>7.70</td>
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preparation. However, in the experimental groups which had previously received Xiao-Chai-Hu-Tang, no alterations of glucose-6-phosphatase activity induced by D-galactosamine HCl were observed. Glucose-6-phosphatase activity was demonstrated on all hepatocytes within hepatic lobules (Fig. 19).

Biochemical assay

These three phosphatase activities were biochemically assayed. The administration of 800 mg/kg D-galactosamine HCl induced in the control rats a decrease of glucose-6-phosphatase activity, while no notable decrease was found in the 5'-nucleotidase and alkaline phosphatase activities. In the control groups administered 1500 mg/kg D-galactosamine HCl, three phosphatase activities showed a decrease in comparison with those of normal untreated rats. 2 days after the injection of 1500 mg/kg D-galactosamine HCl, a decrease of approximately 40% in 5'-nucleotidase activity, 30% in alkaline phosphatase activity and 50% in glucose-6-phosphatase activity was found (Table 1). However, no such decrease of these three enzyme activities, as those of the control rats induced by D-galactosamine, was found in the groups preadministered with Xiao-Chai-Hu-Tang. The three phosphatase activities were preserved at the same level as those of normal untreated rats.

Fig. 18. Glucose-6-phosphatase activity of rat 2 days after injection of 1500 mg/kg D-galactosamine HCl. One hepatocyte shows enzyme activity, whereas neighboring hepatocytes are devoid of it. Arrangement of rough endoplasmic reticulum is disrupted. ×14,000

Fig. 19. Glucose-6-phosphatase activity of rats preadministered with Xiao-Chai-Hu-Tang 2 days after injection of 1500 mg/kg D-galactosamine HCl. All hepatocytes possess the enzyme activity. Rough endoplasmic reticulum is well organized. ×15,000
DISCUSSION

The present study clearly demonstrated with various procedures including conventional light and electron microscopic observation, histocytochemical techniques and biochemical assay of enzyme activity, that preadministration of Xiao-Chai-Hu-Tang is undoubtedly effective in preventing experimental hepatic disorders. For this study, it would be most ideal that a chronic hepatic disorder be experimentally induced in the animals and then the effects of the medicine concerned examined. However, up to date, there have been no reliable and well-reproducible drugs and procedures which induce in animals a chronic hepatic disorder (4). In our present study, as an experimental model, acute liver injury was induced in rats by a single intraperitoneal injection of a large amount (800 mg/kg or 1500 mg/kg) of D-galactosamine HCl.

Galactosamine hepatitis was first introduced as an experimental model of acute hepatic injury by Keppler et al. (11). Thereafter this hepatic model has been reported by many researchers (2, 5, 6, 14, 20), because of its morphologic and functional features similar to acute human viral hepatitis. Administration of D-galactosamine causes an accumulation of a high concentration of metabolites of D-galactosamine in the liver, which is followed by a selective trapping of uracil nucleotides and subsequently a decrease of hepatic UTP and UDP-galactose concentration (8, 10). This uracil nucleotide deficiency, in turn, results in a variety of metabolic disorders including the inhibition of RNA (22), glycoproteins (13) and glycolipids (7) synthesis. Moreover, the metabolites of D-galactosamine themselves inhibit certain enzymes (18). Finally, various hepatic alterations at the subcellular level are induced in the plasma membrane (2, 14), nucleus (9, 21), Golgi apparatus (3), lysosomes (23) and endoplasmic reticulum (11).

The administration of both 800 mg/kg and 1500 mg/kg D-galactosamine HCl induced remarkable histological and cytologic alterations in the liver of the control rats. Light microscopically, diffuse parenchymal damage through the hepatic lobules and disruption of hepatic cell cords was observed. Ultrastructurally, the cytoplasm of many hepatocytes was occupied by a large number of lipid droplets, and consequently prominent alterations were observed in the plasma membrane and endoplasmic reticulum. An increase of autophagic vacuoles was frequently observed. Accompanied by these morphological changes, alteration of enzyme activity existing in the plasma membrane and endoplasmic reticulum was also found by the histo-cytochemical technique and biochemical assay. As Koudstaal and Hardonk (1979) have reported (14), the preferential decrease of bile canaliculi 5'-nucleotidase was observed on the light microscopic level. Electron microscopically, decrease of 5'-nucleotidase activity was found not only on the bile canaliculi but also in the sinusoidal lateral membrane. Decrease of alkaline phosphatase activity was also demonstrated. Reduction in UTP, UDP and UDPG to less than 15% of control values inhibits biogenesis of membrane glycoproteins, which is considered to be a lethal event (2). It might be due to the difference of dose administered that no significant decrease of enzyme activities of 5'-nucleotidase and alkaline phosphatase was histocytochemically and biochemically found in the control rats administered with 800 mg/kg D-galactosamine HCl. The changes of glucose-6-phosphatase activity were found in both control groups administered with 800 mg/kg and
1500 mg/kg D-galactosamine HCl. Because D-galactosamine HCl induces diffuse parenchymal damage, the intensity and localization of glucose-6-phosphatase activity varied cell by cell, which was found to correspond to the severity of damage of each hepatocytes.

The exact mechanisms by which Xiao-Chai-Hu-Tang prevents hepatic disorders induced by D-galactosamine HCl are unknown in our present study. The onset of hepatitis consists of two pathologic processes: (1) cell damage and necrosis and (2) subsequent inflammatory reactions. It has been reported by Mizoguchi et al. (17) that the immunomodulator effect provoked by Xiao-Chai-Hu-Tang plays an important role in the treatment of chronic type hepatitis. According to Abe et al. (1), saikosaponin in Xiao-Chai-Hu-Tang has a protecting effect on hepatocytes from various acute hepatic injuries without relation to the pathogenesis including CCl4, D-galactosamine and virus. Our present study revealed that no alteration of 5'-nucleotidase and alkaline phosphatase activities which are considered to be marker enzymes of plasma membrane, nor that of glucose-6-phosphatase, a marker of endoplasmic reticulum, was histocytochemically or biochemically found in the groups preadministered Xiao-Chai-Hu-Tang after the injection of D-galactosamine HCl (28). Therefore, it is likely that Xiao-Chai-Hu-Tang directly affected the hepatocytes, possibly on the membrane system or intracellular metabolic pathways, although the exact organella still remains to be clarified.

REFERENCES

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