The Effect of Galactosamine on Rat Liver Cytochrome P-450 Activities

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Abstract—The effect of galactosamine on hepatic drug metabolizing activities was examined in rats. In the microsomal fraction, the contents of cytochrome P-450 (P-450) and cytochrome b5 (b5) and the activity of NADPH-cytochrome c reductase (reductase) were examined for 7 days after galactosamine administration. In addition, substrate metabolizing activities in damaged microsomes were examined using four substrates: aminopyrine, aniline, benzo(a)pyrene (B(a)P) and 7-ethoxycoumarine (7-EC). The contents of P-450 and b5 and the activity of reductase showed a minimal value after 3 days of galactosamine administration and then gradually increased, reaching to the control level after 7 days. All four substrate metabolizing activities showed a similar response as the content of P-450, but the decrement among the four activities was not uniform. The activities of B(a)P hydroxylation and 7-EC deethylation were more impaired than those of aminopyrine demethylation and aniline hydroxylation. This nonuniformity was clear on the activity based on P-450. This result suggested that galactosamine disturbed the population of multiple P-450 subtypes, and each P-450 subtype was damaged to the various extent by galactosamine administration.

The liver is primarily involved in the metabolization of hydrophobic drugs. In this organ, various hydrophobic drugs undergo oxygenization or hydroxylation by the mixed function oxidase system in their microsome (1).

The mixed function oxidase system consists of cytochrome P-450 (P-450), cytochrome b5 (b5) and NADPH-cytochrome c reductase (reductase); especially, P-450 is a key enzyme in drug metabolization. Accumulated evidences indicate that P-450s exist in multiple form (2), and each subtype of P-450 has loose substrate specificity; one P-450 subtype can metabolize several foreign substances. Some of the foreign substances can induce a specific type of P-450; for example, P-448 is induced by 3-methylcholanthrene. Accordingly, in the induced state by a drug, the population of P-450 subtypes is much different from that of the noninduced state (3). On the other hand, the question remains as to whether liver damage causes a change in the P-450 subtype population. Willson and Hart (4) and Noguchi et al. (5) reported that the content or activity of P-450 was changed in drug induced liver injury, but they did not investigate the change in the population of P-450 subtypes. For this purpose, we chose four substrates for measuring P-450 activity: aminopyrine, aniline, benzo(a)pyrene (B(a)P), 7-ethoxycoumarine (7-EC), which are widely used substrates for P-450 (6–9). Although each subtype has several substrates, some substrate specificity exists, so that P-450 can be called an “enzyme”. If liver damage causes a change in the population of P-450 subtypes, those four activity should change in response to liver damage.

In the past, no one has reported the time course of drug metabolizing activity after injury by galactosamine, so in our study, we have also stressed the importance of this.
The contents of P-450 and \( b_6 \), the activity of NADPH-cytochrome c reductase, and the activities of P-450 with respect to aminopyrine demethylation, aniline hydroxylation, B(a)P hydroxylation and 7-EC deethylation were examined.

Materials and Methods

Treatment of rats: Male Sprague-Dawley rats weighing 150 g were used in all experiments. They were fed a standard laboratory chow diet (Oriental Yeast Mfg., Ltd., Japan) and drinking water ad libitum. Galactosamine (0.9 g/kg body weight) was administered by a single intraperitoneal injection, as described by Sawamura et al. (10). The animals were fasted overnight before sacrifice and were killed by cervical dislocation at 9 A.M. Blood samples were taken from the carotid arteries.

Assays of serum transaminases: The activities of serum glutamic-pyruvic transaminase (serum GPT) and glutamic-oxaloacetic transaminase (serum GOT) were determined by the procedure of Reitman and Frankel (11), expressed in Karmen units.

Preparation of microsomes: Rat liver microsomes were prepared (12) as follows: Excised livers were thoroughly perfused with cold 0.15 M KCl and homogenized in 4 volumes of 0.15 M KCl solution containing 10 mM EDTA using a Potter-type Teflon glass homogenizer. The homogenate was centrifuged at 10,000 \( \times g \) for 15 min in a Hitachi 20PR refrigerated centrifuge. The supernatant was then centrifuged at 105,000 \( \times g \) for 60 min in a Hitachi 65P preparative ultracentrifuge. The pellet of microsomes was suspended in the homogenizing solution in the homogenizer and centrifuged again as described above. The resulting pellet was suspended in 20 mM potassium phosphate buffer, pH 7.4, containing 15% glycerol, and this sample was used for several assays described next. These operations were done at 0–4°C.

Assays of drug metabolizing activity: Drug metabolizing activity, aminopyrine demethylation, aniline hydroxylation, B(a)P hydroxylation and 7-EC deethylation were assayed as described by Nash (6), Imai et al. (7), Nebert and Gelboin (8) and Ullrich and Weber (9), respectively.

Assays of the contents of P-450 and cytochrome \( b_6 \) and the activity of NADPH-cytochrome c reductase: The content of P-450 was assayed by the method of Omura and Sato (13), and the content of cytochrome \( b_6 \) and the activity of NADPH-cytochrome c reductase were assayed by the method of Omura and Takesue (14). Microsomal protein was measured by the method of Lowry et al. (15).

All operations and assays were done within 24 hr after preparation of the microsomes.

Gel electrophoresis: Sodium dodecyl sulfate polyacrylamide slab gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (16). The apparent molecular weights of the microsomal polypeptides were estimated by comparison with pure protein standards known molecular weights: phosphorylase \( b \) (94000), bovine serum albumin (64000), ovalbumin (43000) and carbonic anhydrase (30000).

Galactosamine treated microsomes were prepared 72 hr after administration. Treatments with phenobarbital and 3-methylcholanthrene were at dosages of 100 mg/kg/day \( \times 5 \) and 25 mg/kg/day \( \times 3 \), respectively, and microsomes were prepared 24 hr after the last treatment.

Electron microscopical study: Liver specimens were fixed with a mixture of glutaraldehyde and osmium tetroxide (17, 18), dehydrated, embedded in Epon and sectioned as described previously (19). After 3 days of galactosamine administration, the sample of rat liver was prepared.

Results

The effect of galactosamine administration on liver weight, serum transaminase activity and serum total protein: Figure 1 shows the time course of liver weight/body weight, serum transaminase activity and the content of serum total protein between the time of administration of galactosamine and 7 days later.

The liver weight and the content of serum total protein decreased to a minimum value 3 days after administration, 70.5% and 62.3% of the control value, respectively, and increased again to the control value after
The activities of serum GOT and GPT showed a 50-fold increase as compared to the control value 1 day after administration (Table 1).

Table 1. Maximum value of the activities of serum GOT and GPT, minimal value of liver weight and serum total protein in galactosamine-treated rats

|                      | Liver weight (g/100 g body wt.) | Total protein (g/100 ml) | GOT (K.U./ml) | GPT (K.U./ml) |
|----------------------|---------------------------------|--------------------------|---------------|---------------|
| Nontreated (n=22)    | 6.25±0.73                       | 7.70±0.81                | 60.0±5.3      | 44.0±3.6      |
| Galactosamine treated (n=8) | 4.41±0.67*                     | 4.80±0.62*               | 3316±418*     | 1880±226*     |
| Point of max. or min.| 72 hr                           | 72 hr                    | 24 hr         | 24 hr         |

(mean±S.D.). *Significantly different from the nontreated group at P<0.001. K.U., Karmen unit.

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Electron microscopic study: A mixture of amorphous cytoplasmic debris and subcellular organelles in varying stages of degeneration were observed 3 days after galactosamine administration (Fig. 2). These findings clearly demonstrate that the livers of galactosamine-administered rats were severely damaged.

The effect of galactosamine administration on the mixed function oxidase system: Figure 3 shows the changes in the content of microsomal protein, P-450, cytochrome b₅ and NADPH-cytochrome c reductase activity following the administration of galactosamine. The content of P-450 decreased to 34.5% of the control value 3 days after treatment and gradually increased toward the control value after 7 days. The content of microsomal protein, cytochrome b₅ and the activity of NADPH-cytochrome c reductase also showed a similar response, decreasing to 70.2%, 35.5% and 39.2% of the control values, respectively, and gradually returned to the control levels (Table 2).

The effect of galactosamine on the activity of aminopyrine demethylation, aniline hydroxylation, B(a)P hydroxylation and 7-EC deethylation: The activities of the mixed function oxidase system was examined.
using four substrates, aminopyrine, aniline, B(a)P and 7-EC, between the day of galactosamine administration and 7 days later.

As shown in Fig. 4 and Table 3, the four metabolizing activities/microsomal protein showed similar response as the content of P-450 (Fig. 3). They decreased to a minimal value 3 days after galactosamine administration, aminopyrine demethylation to 30.3%, aniline hydroxylation to 21.8%, B(a)P hydroxylation to 13.8%, 7-EC deethylation to 16.7% of the control value, respectively, and then gradually increased.

The minimal activities based on nanomoles of P-450, which appeared 3 days after galactosamine administration, were aminopyrine demethylation, 65.8%; aniline hydroxy-


Table 2. Minimal value of microsomal protein, the contents of P-450 and cytochrome b₅, and the activity of NADPH-cytochrome c reductase 3 days after galactosamine administration

|                       | Microsomal protein (mg/g liver) | P-450 (nmol/mg MS prot.) | Cytochrome b₅ (nmol/mg MS prot.) | NADPH-cytochrome c reductase (nmol/mg MS prot./min) |
|-----------------------|---------------------------------|--------------------------|----------------------------------|-----------------------------------------------|
| Nontreated (n=22)     | 10.26±1.06                      | 1.25±0.16                | 0.547±0.064                     | 92.64±10.03                                   |
| Galactosamine treated (n=8) | 7.21±0.84*                      | 0.43±0.15*               | 0.194±0.035*                    | 36.32±6.83*                                   |

(mean±S.D.). *Significantly different from the nontreated group at P<0.001. MS, microsomal.

Electrophoresis of microsomes: SDS-PAGE patterns of nontreated, galactosamine-treated, PB-induced and MC-induced rat liver microsomes are shown in Fig. 5.

In PB-induced (well E) and MC-induced (well D) rats, 50K and 53K bands are deeply stained, respectively, compared with the nontreated rat (well B).

It appears that in nontreated rat liver microsomes (well B), P-450 probably exists...
in 50K, 53K and nearly region. In galactosamine treated rats, the bands of these part were generally fainter than those in nontreated rat.

Discussion

It is a general concept that drug-metabolizing activity of liver microsomes decreases when the liver is injured by virus, malnutrition, poison and so on. The main cause of the decrease of this activity is from the damage of the mixed function oxidase system in which P-450 plays the most important part.

It is generally recognized that P-450 does not exist uniformly, has multiple subtypes and each subtype has loose substrate specificity (2). Accordingly, one substrate metabolizing activity does not show the activity of one P-450 subtype. If we attempt to design the perfect experiment about the change of P-450 subtypes in damaged liver, we should prepare all antibodies of all subtypes and observe the quantitative change of each subtype. However, there is no exact answer for the question how many P-450 subtypes exist in noninduced rat liver, and no one has reported the purification and characterization of all P-450 subtypes at present.

Although each P-450 subtype has several substrates, some substrate specificity of each subtype exists so that P-450 can be called an "enzyme". Consequently, to study the general change of the P-450 population in damaged liver, measuring of several substrate metabolizing activities is a primitive but good method, and the change of the general balance of P-450 subtypes will be detected by this method.

In this experiment, we are also interested in the relation between drug metabolizing activity and the time course of liver damage, which accompanies the change of the P-450 population.

The results illustrated in Fig. 1 and Table 1 are consistent with those of Richter et al. (20) and Koff et al. (21), and they reveal that serum transaminase activity significantly increases within 24 hr after the administration of galactosamine. Moreover, the electron microscopic findings demonstrated that the liver of these rats had been severely damaged by galactosamine.

As shown in Fig. 3 and Table 2, the contents of cytochrome b5 and P-450 and the activity of NADPH-cytochrome c reductase showed a decrease after 24 hr; all data showed minimal values after 72 hr, then gradually increased, reaching to the control level after 7 days.

Figure 4 and Table 3 show metabolizing activity changes of aminopyrine, aniline, B(a)P and 7-EC in rats treated with galactosamine.

Table 3. Minimal value of the activities of aminopyrine demethylation, aniline hydroxylation, B(a)P hydroxylation and 7-EC deethylation 3 days after galactosamine administration

|                | Aminopyrine (nmol/mg MS prot./min) | Aniline (nmol/mg MS prot./min) | B(a)P (pmol/mg MS prot./min) | 7EC (pmol/mg MS prot./min) |
|----------------|----------------------------------|--------------------------------|----------------------------|----------------------------|
| Nontreated (A) (n=22) | 19.94±5.42               | 1.64±0.24                     | 118.86±13.06                | 113.42±19.40               |
| Galactosamine treated (B) (n=8) | 6.04±2.69**               | 0.36±0.17**                   | 16.45±6.79**                | 18.96±7.45**               |
| (B)/(A)×100(%)     | 30.3                          | 21.8                          | 13.8                        | 16.7                       |

(mean±S.D.). *P<0.01, **P<0.001: Significantly different from the nontreated group. #P<0.01: Significantly lower than both data of aminopyrine and aniline.

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Figure 4 and Table 3 show metabolizing
activities for the four substrates. Though these activities changed in the same fashion as the content of P-450, differences in degree of decrement among the four catalytic activities were found. Residual activities based on microsomal protein (control, 100%) were aminopyrine demethylation, 30.0%; aniline hydroxylation, 21.8%; B(a)P hydroxylation, 13.8%; and 7-EC deethylation, 16.8%; and activities based on P-450 (control, 100%) were 65.8%, 59.4%, 36.3%, 39.8%, respectively. Statistically significant differences were seen between the high percentage group (aminopyrine demethylation and aniline hydroxylation) and the low one (B(a)P hydroxylation and 7-EC deethylation) in the data based on P-450, but not between the four residual activities based on microsomal protein. This non-uniformity of various enzyme activities in a damaged liver has been reported using acetaminophen (4) and CCl₄ (5) treated rats, but both reports only showed the effect of hepatotoxin and did not refer to the cause of this phenomenon. From our results, the four substrate metabolizing activities were not uniformly damaged; especially, the non-uniformity showed clearly in the data on P-450. We suggest that the P-450 subtypes each have different susceptibilities to galactosamine, and the population of P-450 subtypes in the galactosamine damaged state is very different from that of the nontreated state.

Galactosamine, which is a popular and strong hepatotoxin, causes severe damage from impaired protein synthesis due to uridine deficiency. Therefore, in galactosamine-treated liver, microsomal enzymes should be damaged uniformly from the standpoint of protein synthesis arrest, but in this study, damage of metabolizing activity of the four substrates was not uniform. According to the reports by Lewin et al. (22) and Sadano and Omura (23), some P-450 subtypes have different turnover rates. One of the reasons for this phenomenon may be the difference of the turnover rate of P-450 subtypes.

We also have another point of view about this phenomenon. Kato (24) reported that P-450 activities in male rats is dependent on androgen to some degree. Because our experimental conditions are different from his, our results did not always agree with his, but the effect of galactosamine on androgen activity should be considered as the cause of our results.

Therefore, we can propose some explanations for our results. In either case, it is reasonable to speculate that the P-450 subtypes in noninduced rats may be damaged differently from each other, and these populations may be changed by galactosamine administration.

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