Nicotine metabolites and metabolic pathways in mammals have been characterized (Kyerematen and Vesell, 1991). Nicotine is absorbed through the lungs with smoking and is rapidly metabolized in humans. Although it is mainly metabolized in the liver, the effects of liver injuries on nicotine metabolism are not clear. The purpose of this study was to clarify the effects of liver injuries on nicotine metabolism. Rats were treated with D-galactosamine (GalN) or thioacetamide (TA), to induce acute hepatitis or liver cirrhosis, respectively. Serum transaminase levels were significantly elevated in model rats with both types of liver injury. Cytochrome P450 (CYP) and cytochrome P2B contents in liver microsomes were decreased significantly in TA-treated cirrhotic rats but not in GalN-treated hepatic rats. The major metabolic pathways of nicotine, i.e., cotinine formation catalyzed by CYP and nicotine-1′-N-oxide formation catalyzed by flavin-containing monooxygenase, were investigated in these rat liver microsomes. Formation of cotinine and nicotine-1′-N-oxide from nicotine was not changed in GalN-treated hepatic rats, in comparison with the controls, but was significantly decreased in TA-treated cirrhotic rats. By immunoblotting, decreases in CYP1A2, CYP2B2, CYP2G, and CYP2E1 protein were recognized in liver microsomes from TA-treated cirrhotic rats. It was also shown that the maximal velocity values for nicotine-1′-N-oxide formation in TA-treated cirrhotic rats were significantly decreased, compared with the controls. These results suggested that the reduction of nicotine metabolism in cirrhosis was due to decreases in CYP and flavin-containing monooxygenase protein expression levels.

Nicotine, a major constituent of tobacco, is a nicotinic cholinergic receptor agonist and exerts a number of physiological effects. In addition, an important property of nicotine is its addictiveness. In smoking, nicotine is rapidly absorbed through the lungs and eliminated from the blood. The rapid elimination of nicotine has been attributed to its metabolism, as well as distribution to some tissues (Kyerematen and Vesell, 1991). The liver plays a major role in the detoxification and elimination of foreign compounds. Although tobacco might be consumed by patients with liver injuries, the effects of liver injuries on nicotine metabolism are not clear. To obtain better understanding and management of dependence problems, as well as of numerous health hazards associated with tobacco consumption, it is desirable to understand nicotine metabolism with liver injuries. In recent years, considerable information on nicotine metabolism has been obtained (Benowitz and Jacob, 1987). A number of metabolites have been characterized (Kyerematen and Vesell, 1991). The primary metabolic pathways in mammals involve C-oxidation, i.e., cotinine formation, and N-oxidation, i.e., nicotine-1′-N-oxide formation (fig. 1).

In some previously reported models for liver injuries, rats were treated with carbon tetrachloride (Proctor and Chatamra, 1983), GalN2 (Visen et al., 1993), TA (Kasahara, 1977), halothane (Kenna et al., 1992), ethionine (Katoh et al., 1991), or alcohol (Nanji et al., 1993). Treatment with carbon tetrachloride is frequently used to produce cirrhosis in the liver. Acute or chronic treatments with GalN or TA induce acute hepatitis or cirrhosis, respectively (Visen et al., 1993).

Abbreviations used are: GalN, D-galactosamine; TA, thioacetamide; CYP, cytochrome P450; FMO, flavin-containing monooxygenase; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.
1993; Kasahara, 1977). Treatment with halothane is frequently used to produce hepatitis (Kenna et al., 1992). Treatment with ethionine or alcohol induces fatty liver or alcoholic liver injury, respectively (Kato et al., 1991; Nani et al., 1993). In this report, GaN and TA were chosen to provide model rats with liver injuries, because these compounds induce liver diseases that are morphologically similar to viral hepatitis and liver cirrhosis in humans, respectively (Keppler et al., 1968; Brodehl, 1961). In the present study, the effects of acute hepatitis and liver cirrhosis in humans, respectively (Keppler et al., 1993). GaN and TA were purchased from Sigma Chemical Co. (St. Louis, MO). TA and transaminase C II (testwako) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Nicotine-1'-N-oxide was synthesized as described previously (Nakajima et al., in press). NADP\(^+\), glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were from Oriental Yeast (Tokyo, Japan). Anti-rat CYP1A1, CYP2B1, CYP2C11, and CYP2E1 goat sera were purchased from Daiichi Pure Chemicals (Tokyo, Japan). Other chemicals were of the highest grade commercially available.

**Animal Treatment.** All experiments were carried out using male Sprague-Dawley rats (170–220 g) supplied by Saitama Experimental Animals Supply (Saitama, Japan). The rats were maintained under conditions of controlled temperature and light, with access to food and water *ad libitum*. For the induction of acute hepatitis, rats (*N* = 6) received a single ip injection of GaN (400 mg/4 ml/kg body weight, in sterile saline), as previously described (Visen et al., 1993). Control rats received an equivalent injection of saline. Blood samples were collected from the caudal vein 12 and 24 hr after the injection. Treated rats were sacrificed 24 hr after the injection, for preparation of liver microsomes. For the induction of liver cirrhosis, TA was administered to rats (*N* = 6) ip at a dose of 200 mg/5 ml/kg body weight (in sterile saline), three times each week for 8 weeks, as described by Kasahara (1977). Control rats received an equivalent injection of saline. Blood samples were collected at week 4 and week 8 after the first injection. Treated rats were sacrificed 24 hr after the final injection, for preparation of liver microsomes. The separated sera were stored at −20°C for subsequent analysis of enzyme activities in serum.

**Estimation of Enzyme Activities in Serum.** GOT and GPT activities in sera were determined by use of a transaminase C II (testwako).

**Preparation of Microsomes.** Rat liver microsomes were prepared as described previously (Kamataki and Kitagawa, 1974) and were stored at −80°C until use. Protein concentrations were measured according to the method of Lowry et al. (1951). CYP and cytochrome *b*\(_{5}\) content were estimated by the methods of Omura and Sato (1964a,b). NADPH-cytochrome *c* reductase activity was also determined (Phillips and Langdon, 1962). Cytochrome fractions from each rat liver were obtained from the 105,000 g centrifugation during microsome preparation, as a source of aldehyde oxidase.

**In Vitro Nicotine Metabolism.** A typical incubation mixture for cotinine formation contained 50 mM potassium phosphate buffer (pH 7.4), 100 μM nicotine, an NADPH-generating system (0.5 mM NADP\(^+\), 5 mM glucose-6-phosphate, 5 mM MgCl\(_2\), and 1 unit/ml glucose-6-phosphate dehydrogenase), 1 mg/ml microsomal protein, and 3 mg/ml cytosolic protein, in a final volume of 0.5 ml. The reaction was initiated by the addition of the NADPH-generating system, after a 2-min preincubation at 37°C. After incubation for 30 min, 0.5 ml of ice-cold acetone was added to stop the reaction. To this mixture was added caffeine (100 ng) as an internal standard. After removal of protein by centrifugation, the reaction mixtures were extracted by the same method as described above. The organic fraction was evaporated under a gentle stream of nitrogen at 40°C. The residue was redissolved in 50 μl of mobile phase, and then a 20-μl portion of the sample was subjected to HPLC. A typical incubation mixture for nicotine-1'-N-oxide formation contained 50 mM potassium phosphate buffer (pH 8.4), 100 μM nicotine, an NADPH-generating system, and 0.5 mg/ml microsomal protein, in a final volume of 0.5 ml. The reaction was initiated by the addition of nicotine, after a 2-min preincubation at 37°C. After incubation for 20 min, 0.5 ml of ice-cold acetone was added to stop the reaction. To this mixture was added caffeine (100 ng) as an internal standard. After removal of protein by centrifugation, the reaction mixtures were extracted by the same method as described above. The organic fraction was evaporated under a gentle stream of nitrogen at 40°C. The residue was redissolved in 50 μl of mobile phase, and then a 20-μl portion of the sample was subjected to HPLC.

**Materials and Methods**

**Chemicals.** (S)-Nicotine, (S)-cotinine, and GaN were purchased from Sigma Chemical Co. (St. Louis, MO). TA and transaminase C II (testwako) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Nicotine-1'-N-oxide was synthesized as described previously (Nakajima et al., in press). NADP\(^+\), glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were from Oriental Yeast (Tokyo, Japan). Anti-rat CYP1A1, CYP2B1, CYP2C11, and CYP2E1 goat sera were purchased from Daiichi Pure Chemicals (Tokyo, Japan). Other chemicals were of the highest grade commercially available.

**HPLC Conditions.** HPLC analyses were performed using a DG-980–50 degasser (Jasco, Tokyo, Japan), PU-980 intelligent pump (Jasco), AS-950 intelligent sampler (Jasco), and 807 IT integrator (Jasco) with a Capcell Pak C\(_8\) UIG120 column (4.6 × 250 mm, 5 μm; Shiseido, Tokyo, Japan). The eluent was monitored at 260 nm using a UV-970 intelligent UV/visible detector (Jasco). The mobile phase for cotinine separation was 6% acetonitrile containing 0.01% acetic acid and 1 mM sodium heptane sulfonate, and for nicotine-1'-N-oxide separation was 7.5% acetonitrile containing 0.01% acetic acid and 1 mM sodium heptane sulfonate. The flow rate was 1.0 ml/min, and the column temperature was 35°C. Quantitation of the metabolites was performed by comparing HPLC peak heights with those of authentic standards with reference to an internal standard.

**SDS-PAGE and Immunoblotting.** SDS-PAGE and immunoblotting with peroxidase/antiperoxidase staining of rat liver microsomes were carried out essentially as described by Laemmli (1970) and Guengerich et al. (1982), respectively. Diaminobenzidine was used as a substrate for peroxidase. Antibodies to purified rat CYPs (CYP1A1, CYP2B1, CYP2C11, or CYP2E1) were raised in goats as described previously (Isogai et al., 1990), and anti-rat CYP3A2 antibodies were raised in rabbits (Iimoka et al., 1990). Anti-rat CYP1A1 antibodies reacted with rat CYP1A1 and CYP1A2. Anti-rat CYP2B1 antibodies reacted with CYP2B1 and CYP2B2. These forms were separable by SDS-PAGE. Anti-rat CYP2C11 antibodies reacted with CYP2C11, CYP2C6, and CYP2C13. Therefore, the CYPs that cross-reacted with the antibodies to CYP2C11 are referred to as CYP2C. Anti-rat CYP2E1 reacted with CYP2E1 and CYP2C11, and anti-rat CYP3A2 reacted with CYP3A1 and CYP3A2. However, these forms were separable by SDS-PAGE. The intensities of immunostained bands were measured with a densitometer (Quick Scan R&D, Helena Laboratories, Beaumont, TX).

**Statistics.** The results are expressed as mean ± SE throughout the text. Statistical analysis was performed by Student’s *t* test for unpaired samples. Differences were considered significant at *p* < 0.05.

**Results**

**Enzyme Activities in Sera of GaN-Treated Hepatic Rats and TA-Treated Cirrhotic Rats.** As shown in table 1, the GOT and GPT values in sera of GaN-treated hepatic rats were higher than those of the controls at 12 hr, indicating liver injury at that time. After 24 hr, progressive liver injury was evident through progressive elevation of GOT and GPT values in GaN-treated rats. In TA-treated rats, the GOT values tended to be higher and the GPT values were significantly higher than those of the controls at 4 weeks. After 8 weeks, the GOT and GPT values were significantly higher than those of the controls.

**Effects of GaN or TA Treatment on Body Weight and Liver Weight in Rats.** The body weights of GaN- or TA-treated rats just before sacrifice were significantly reduced, compared with the control rats (table 2). The relative liver weights were significantly lower in GaN-treated hepatic rats, in comparison with the controls, and significantly higher in TA-treated cirrhotic rats, in comparison with the controls.

**Effects of GaN or TA Treatment on Drug-Metabolizing Enzymes in Rats.** Treatment with GaN did not affect the CYP or
5 was also decreased significantly, to 48% of the control level (comparison with the controls (treated cirrhotic rats, the CYP content was decreased to about 34%, in activity in rat liver microsomes (table 3). In microsomes from TA-cytochrome b contents or the NADPH-cytochrome c reductase activity in rat liver microsomes (table 3). In microsomes from TA-treated cirrhotic rats, the CYP content was decreased to about 34%, in comparison with the controls (p < 0.01). The content of cytochrome b2 was also decreased significantly, to 48% of the control level (p < 0.05). On the other hand, NADPH-cytochrome c reductase activity was not changed in microsomes from TA-treated rats, in comparison with the controls.

**In Vitro Nicotine Metabolism in GalN-Treated Hepatitic Rats and TA-Treated Cirrhotic Rats.** As shown in fig. 2, cotinine formation from nicotine in GalN-treated hepatitic rats (0.151 ± 0.017 nmol/mg/min) was not changed significantly, in comparison with the controls (0.192 ± 0.016 nmol/mg/min). In contrast, cotinine formation in TA-treated cirrhotic rats (0.045 ± 0.008 nmol/mg/min) was decreased significantly (0.220 ± 0.036 nmol/mg/min, p < 0.001). Cis- and trans-nicotine-1’-N-oxide formation from nicotine in GalN-treated hepatitic rats (0.386 ± 0.123 and 0.106 ± 0.015 nmol/min, respectively) was not changed significantly, in comparison with the controls (0.491 ± 0.067 and 0.129 ± 0.025 nmol/mg/min, respectively). Formation of both nicotine-1’-N-oxide diastereomers in TA-treated cirrhotic rats (0.096 ± 0.016 and 0.063 ± 0.004 nmol/mg/min, respectively) was decreased significantly, in comparison with the controls (0.483 ± 0.151 and 0.182 ± 0.163 nmol/mg/min, respectively, p < 0.0005).

**Effects of GalN or TA Treatment on Expression Levels of CYP Isoforms in Rat Liver Microsomes.** Anti-rat CYP1A1, CYP2B1, and CYP3A2 antibodies recognized only CYP1A2, CYP2B2, and CYP3A2, respectively, because of low levels of expression of CYP1A1, CYP2B1, and CYP3A1 in male rat liver microsomes. As shown in fig. 3, GalN treatment of rats did not affect the levels of expression of CYP1A2, CYP2B2, CYP2C, CYP2E1, and CYP3A2. On the other hand, TA treatment significantly decreased the expression levels for all CYP isoforms determined in this study, except CYP3A2. In addition, the levels of expression of CYP2B2 and CYP3A2 in the control rats for TA treatment were significantly lower than those in the control rats for GalN treatment (fig. 3, B and E).

**Kinetics of Nicotine-1’-N-oxide Formation in GalN-Treated Hepatitic Rats and TA-Treated Cirrhotic Rats.** In rat livers, cis-nicotine-1’-N-oxide formation predominated over trans-nicotine-1’-N-oxide formation from nicotine. Eadie-Hofstee plots for both cis- and trans-nicotine-1’-N-oxide formation in rat liver microsomes were linear (Nakajima et al., in press). The *Km* values for cis- and trans-nicotine-1’-N-oxide formation in the control rats were 0.228 ± 0.020

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GOT</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 hr</td>
<td>24 hr</td>
</tr>
<tr>
<td>Control</td>
<td>103.3 ± 5.6</td>
<td>140.5 ± 4.5</td>
</tr>
<tr>
<td>GalN</td>
<td>601.0 ± 167.0a</td>
<td>1015.8 ± 368.4ba</td>
</tr>
</tbody>
</table>

* a p < 0.05, compared with the control group.
* b N = 5.
* c p < 0.01, compared with the control group.

### Table 2

**Effects of GalN or TA treatment on body weight and relative liver weight in rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body Weight</th>
<th>Liver Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>g/100 g body weight</td>
</tr>
<tr>
<td>Control</td>
<td>214.8 ± 2.8</td>
<td>4.34 ± 0.14</td>
</tr>
<tr>
<td>GalN</td>
<td>191.8 ± 5.8a</td>
<td>3.87 ± 0.11a</td>
</tr>
<tr>
<td>Control</td>
<td>451.7 ± 12.4</td>
<td>2.88 ± 0.08</td>
</tr>
<tr>
<td>TA</td>
<td>326.8 ± 23.2a</td>
<td>4.58 ± 0.25a</td>
</tr>
</tbody>
</table>

* a p < 0.01, compared with the control group.
* b p < 0.05, compared with the control group.
* c p < 0.005, compared with the control group.

### Table 3

**Effects of GalN or TA treatment on CYP and cytochrome b contents and NADPH-cytochrome c reductase activity in rat liver microsomes**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CYP</th>
<th>Cytochrome b</th>
<th>NADPH-Cytochrome c Reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/mg protein</td>
<td>nmol/mg protein</td>
<td>units/mg protein</td>
</tr>
<tr>
<td>Control</td>
<td>0.81 ± 0.11</td>
<td>0.27 ± 0.02</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>GalN</td>
<td>0.78 ± 0.08</td>
<td>0.26 ± 0.03</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>Control</td>
<td>1.05 ± 0.14</td>
<td>0.40 ± 0.06</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>TA</td>
<td>0.36 ± 0.06b</td>
<td>0.19 ± 0.02b</td>
<td>0.10 ± 0.01</td>
</tr>
</tbody>
</table>

* a p < 0.01, compared with the control group.
* b p < 0.05, compared with the control group.
Drug elimination is frequently altered in patients with liver disease (Sotaniemi et al., 1980). One major reason for this is thought to be impaired activity of the hepatic microsomal drug-metabolizing enzymes. The enzymes that have been studied in liver disease are CYPs (Paintaud et al., 1996). Some CYP isoforms are decreased and other isoforms are not altered by liver injury. In addition, the effects on CYP isoforms differ among liver diseases (Guengerich and Turvy, 1991). Another major drug-metabolizing enzyme in microsomes is FMO. In contrast to CYPs, the changes in FMO activity in liver disease have not been studied so far.

Nicotine, one of the few natural liquid alkaloids, exerts a number of physiological effects involving the central and peripheral nervous systems, the cardiovascular system, and the endocrine system. In most mammalian species, nicotine is rapidly and extensively metabolized, primarily in the liver (Kyerematen and Vesell, 1991). The major metabolic pathways of nicotine in mammals are C-oxidation and N-oxidation, i.e. cotinine and nicotine-1’-N-oxide formation, respectively. In humans, 70–80% of nicotine is converted to cotinine. Cotinine is extensively metabolized, and only about 10–15% of cotinine is excreted unchanged in urine. About 4% of nicotine is converted to nicotine-1’-N-oxide, which is largely (if not entirely) excreted in urine without further metabolism (Jacob et al., 1988). In rats, nicotine is excreted as cotinine and nicotine-1’-N-oxide, both at about 10% (Kyerematen et al., 1988). It was reported that CYP catalyzes the formation of cotinine from nicotine and FMO catalyzes the formation of nicotine-1’-N-oxide in mammals (Cashman et al., 1992). Therefore, nicotine is a good probe to simultaneously estimate changes in CYP and FMO after liver injury. A variety of methods for the determination of nicotine and its metabolites in biological samples have been reported in the literature (Schepers and Walk, 1988; Vonenck et al., 1989; Rustemeier et al., 1993). Because nicotine and its metabolites are moderately polar compounds, reverse-phase C₁₈ ion-pair chromatography was used for separation of these compounds in this study.

Changes in drug metabolism may depend on histological changes in the liver (acute or chronic hepatitis or cirrhosis) but may also depend on the origin of the liver injuries (viral, toxic, or immunological). Liver diseases can be defined by the anatomical changes observed (Sherlock and Dooley, 1993). Acute hepatitis is characterized by necrosis of hepatic cells and acute inflammation. In chronic hepatitis, in addition, fibrosis may be present. Cirrhosis is defined as a diffuse process with fibrosis and nodule formation, but necrosis is also generally present. Acute hepatitis can also be described as mainly cytolytic or cholestatic. In this study, rat models for acute hepatitis and cirrhosis were selected.

A single injection of GalN to rats induces liver disease that is morphologically similar to drug-induced or viral hepatitis in humans (Keppler et al., 1968; Jonker et al., 1990). The treatment results in depletion of UTP and other uracil nucleotides. This depletion interferes with the normal synthesis of mRNA, thus impairing cellular integrity (Keppler et al., 1974) and changing the liver plasma membrane lipid composition (Petkova et al., 1987). TA is hepatotoxic because of its effects on DNA, RNA, and protein synthesis (Fizhugh and Nelson, 1948). In our study, TA was selected because it induces liver cirrhosis in rats, with histopathological findings corresponding closely to those for liver cirrhosis in humans (Brodehl, 1961).

Serum transaminases are considered to be sensitive indicators of liver injury. The hepatic damage produced by GalN or TA treatment was indicated by increases in GOT and GPT levels. These changes result from the leakage of enzymes from the hepatocytes. These increases of transaminases by GalN or TA treatment are consistent with previous reports (Visen et al., 1993; Aguiar et al., 1987). The elevation of serum transaminases was milder in TA-treated cirrhotic rats than in GalN-treated, acutely hepatitic rats. Liver damage induced by chronic treatment with TA differs from that induced by GalN, which leads to liver cell necrosis and consequently elevated levels of serum transaminase. The slight increases in transaminases in cirrhotic rats are due to increases in protein synthesis in rat liver after TA intoxication (Chakrabartty et al., 1982). Chronic intoxication with TA induces liver damage with cholangiolar cell proliferation, cholangio-fibromatosis, and damage of liver cell membranes (Brodehl, 1961).

The livers of GalN-treated hepatic rats appeared pale and of reduced density, compared with the control organs. In TA-treated cirrhotic rats, distorted architecture caused by enhanced regeneration of the liver cells and hyperplasia of the fibrous connective tissue was observed. Atrophy or hypertrophy of the liver was indicated by decreases or increases of relative liver weights in GalN-treated hepatic rats or TA-treated cirrhotic rats, respectively. These results
agreed with previous reports (Keppeler et al., 1968; Chakrabartty et al., 1982).

Cotinine formation from nicotine is a two-step reaction in mammalian liver. The first step is the conversion of nicotine to nicotine-$\Delta^{1,5}$-iminium ion by CYP. The second step, the conversion of the iminium ion to cotinine, is mediated by cytosolic aldehyde oxidase (Kyerematen and Vesell, 1991). Therefore, cytosolic protein was also incubated with nicotine and microsomal protein to determine the formation of a source of aldehyde oxidase. Cotinine formation was not changed in GalN-treated hepatic rats but was decreased significantly in TA-treated cirrhotic rats. Because CYP has been reported to be a rate-limiting enzyme responsible for cotinine formation (Brandänge and Lindblom, 1979), it was thought that the decrease in TA-treated cirrhotic rats was due to decreases in CYP activity or content. The decrease of total CYP content was demonstrated by CO difference spectra. Subsequently, the changes in levels of expression of some CYP isoforms in liver microsomes of these model rats were investigated by SDS-PAGE and immunoblotting. The expression levels of CYP1A2, CYP2B2, CYP2C, and CYP2E1 were significantly decreased in TA-treated cirrhotic rats. However, the levels of CYP3A2 were not changed. These results suggested that the effects of liver injuries on CYPs differ among isoforms. We previously reported that the human CYP isoform that is involved in cotinine formation from nicotine is CYP2A6 (Nakajima et al., 1996). In contrast, it has been reported that the CYP isoform that is involved in cotinine formation in rats is CYP2B1 or CYP2C11 (Nakayama et al., 1993; Hammond et al., 1991). Therefore, the decrease of cotinine formation was attributed to decreases in expression levels of CYP2B1 and/or CYP2C11 protein.

In this study, the control rats for TA treatment were 8 weeks older than the control rats for GalN treatment, because of chronic treatment. It has been reported that the expression levels of CYP2B and CYP3A are lower in older rats (Imaoka et al., 1991). Therefore, the significant differences in CYP2B2 and CYP3A2 levels in the control rats for GalN and TA treatments were considered to be attributable to the difference in age.

Recently, the role of cytochrome $b_5$ in CYP-catalyzed reactions has been investigated. Cytochrome $b_5$ provides the second of the two electrons needed for oxygen activation. It also exerts a sparing effect by preventing uncoupling or causing metabolic switching, thereby increasing product formation while decreasing hydrogen peroxide formation. More recently, the suggestion was made that cytochrome $b_5$ binds to some CYP isoforms and serves as an electron buffer; the two-hemoprotein complex becomes a two-electron acceptor, thereby obviating the need for two sequential interactions with the reductase (Schenkman et al., 1994). Although the role of cytochrome $b_5$ in nicotine metabolism is not clear, the decrease in cytochrome $b_5$ content in TA-treated cirrhotic rats might also affect the decrease in cotinine formation from nicotine.

Although $C$-oxidation (i.e., cotinine formation) is the major pathway of nicotine metabolism, the $N$-oxidation pathway (formation of $N$-oxide derivatives of nicotine) constitutes an important route of nicotine biotransformation (Booth and Boyland, 1970; Thompson et al., 1985). In rat liver microsomes, $cis$-nicotine-$1''$-$N$-oxide formation predominated over $trans$-nicotine-$1''$-$N$-oxide formation. Therefore, the changes in $cis$- and $trans$-nicotine-$1''$-$N$-oxide formation in rat liver microsomes after GalN or TA treatments were parallel. It was reported that the reduction of nicotine-$1''$-$N$-oxide to nicotine could occur in vivo (Duan et al., 1991). However, it was confirmed in our previous study (Nakajima et al., in press) that this reduction of $cis$- or $trans$-nicotine-$1''$-$N$-oxide formation was not observed during the incubation and extraction procedures under aerobic conditions. By kinetic analysis of nicotine-$1''$-$N$-oxide formation, it was demonstrated that the decrease in nicotine-$1''$-$N$-oxide formation from nicotine was due to a decrease in the $V_{\text{max}}$ value and not the $K_{\text{m}}$ value. This suggests that the FMO protein content was decreased in TA-treated cirrhotic rats and the affinity of the enzyme for nicotine was not changed.

In conclusion, formation of cotinine and nicotine-$1''$-$N$-oxide from nicotine in liver microsomes did not change in acute hepatitis, in comparison with the controls. This is important information on nicotine metabolism, because little work has been performed concerning drug metabolism in acute hepatitis, compared with chronic hepatitis, cirrhosis, or liver cancer. In contrast, formation of cotinine and nicotine-$1''$-$N$-oxide from nicotine was significantly decreased in cirrhosis, because of the decreased levels of CYP and FMO proteins responsible for nicotine metabolism. It was determined that FMO expression and CYP levels were decreased in cirrhosis. For humans, it is thought that the plasma nicotine concentration after smoking might be elevated in patients with liver cirrhosis because of reduced nicotine metabolism, possibly leading to greater dependence on nicotine.

References


Brandänge S and Lindblom L (1979) The enzyme "$\text{aldehyde oxidase}''$ is an iminium oxidase: reaction with nicotine $\Delta^{1,5}$ iminium ion. Biochem Biophys Res Commun 91:991–996.


