Comparison of Inhibitory Effects of New Quinolones on Drug Metabolizing Activity in the Liver

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Abstract—The effects of three new quinolones (enoxacin (ENX), norfloxacin (NFLX) and ofloxacin (OFLX)) on acetaminophen-induced liver injury in rats were examined and compared with their effects on the elimination half-life (T1/2) of theophylline (in vivo) and on the 7-ethoxycoumarin (7-EC) O-deethylase activity in liver microsomes (in vitro). ENX, NFLX and OFLX (75 or 300 mg/kg) were administered orally to rats 1 hr before, simultaneously with, and 1 hr after the acetaminophen injection (800 mg/kg). Biochemical liver function tests, drug metabolizing activity in liver microsomes, the total glutathione content of the liver and histological changes were examined 5 hr after the acetaminophen injection. ENX markedly reduced acetaminophen-induced liver injury and NFLX slightly but significantly did so, but no protective effect was observed with OFLX treatment. ENX markedly and NFLX slightly prolonged the T1/2 of theophylline, but OFLX did not affect it. In addition, ENX markedly and NFLX slightly inhibited the 7-EC O-deethylase activity in liver microsomes, but OFLX again had no effect. These findings indicated that ENX markedly inhibited the activity of cytochrome P-450 in liver microsomes and NFLX did so slightly, while OFLX had no such effect. Slight variations in the structures of these quinolones might explain the differences in their effects on cytochrome P-450 activity.

In recent years, several new quinolones have been developed as effective antibacterial agents and widely used to treat a variety of infections. In 1984, Wijnands et al. (1) first reported that co-administration of enoxacin (ENX), one of the new quinolones, with theophylline raised the plasma theophylline concentration, and that eight of the ten patients who received theophylline in combination with ENX developed serious nausea and vomiting. Since then, many investigators have demonstrated that ENX raises the plasma theophylline concentration and prolongs its elimination half-life (T1/2). In contrast, other new quinolones, such as norfloxacin (NFLX) and ofloxacin (OFLX), have no or only a slight effect on the pharmacokinetics of theophylline (2–8). It appears that the increase in the plasma theophylline concentration is due to a reduced metabolic clearance of theophylline in the liver induced by ENX, while NFLX and OFLX seem to have little effect on drug metabolizing activity in the liver. However, little in vitro evidence has been reported that the new quinolones affect the activity of drug metabolizing enzymes in liver microsomes.

Cimetidine, which is known to inhibit the drug metabolizing activity in liver microsomes (9), has been reported to prevent acetaminophen-induced liver injury (10, 11), but there have been no reports concerning the effects of the new quinolones on acetaminophen-induced liver injury.

In the present study, the protective effects of three new quinolones (ENX, NFLX and OFLX) on acetaminophen-induced acute liver injury in rats were investigated and compared with each other. Moreover, the effects of these new quinolones on the T1/2 of theophylline (in vivo) and on the 7-ethoxycoumarin (7-EC) O-deethylase activity in rat liver microsomes (in vitro) were also examined.
and compared with the data on acetaminophen-induced liver injury.

Materials and Methods

1. Effects of ENX, NFLX and OFLX on acetaminophen-induced liver injury

   a) Pretreatment of animals: Male Fischer rats, each weighing 200 g, were fed a standard pellet diet and drinking water ad libitum. According to the method of Murase et al. (11), the rats were injected intraperitoneally with a single dose of 3-methylcholanthrene (3-MC) (25 mg/kg) in olive oil to increase the sensitivity of the rats to acetaminophen, and the rats in this induced state were used as the control. Rats were used for the experiments 72 hr after pretreatment with 3-MC after an overnight fast. Fasting was maintained throughout the duration of each experiment, although the animals were allowed free access to tap water.

   b) Administration of drugs and sampling: Acetaminophen was dissolved in 5 ml of dimethyl sulfoxide and diluted with saline to give 20 ml of a 8% (w/v) solution, which was administered intraperitoneally to the rats at a dose of 800 mg/kg. ENX, NFLX and OFLX were suspended in 0.5% tragacanth gum and administered orally at a dose of 75 or 300 mg/kg, 1 hr before, simultaneously with, and 1 hr after the acetaminophen injection. These doses were used because treatment with ENX (300 mg/kg) was reported to prolong the T1/2 of theophylline in rats (12). Blood samples were taken from the jugular vein 5 hr after the injection of acetaminophen, and the rats were sacrificed by cervical dislocation. Part of the liver was used for the histological study, and the rest was used for the preparation of microsomes and the measurement of total glutathione content.

   c) Biochemical liver function tests: The activities of serum glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were measured by the standard UV method of the German Society for Clinical Chemistry (13) and expressed as international units. The content of serum total protein was determined by the biuret reaction (14). Then the serum protein fraction was assayed by cellulose acetate electrophoresis, and the serum albumin content was calculated.

   d) Assays of drug metabolizing activity in liver microsomes and the total glutathione content of the liver: Rat liver microsomes were prepared as reported previously (15). The microsomal protein content was measured by the method of Lowry et al. (16). The content of cytochrome P-450 (P-450) was assayed by the method of Omura and Sato (17), and aminopyrine N-demethylase activity was determined by the method of Nash (18). Total glutathione content of the liver was measured by the method of Tietze (19).

   e) Histological study: The liver sections were fixed in 10% (v/v) formalin and stained by a routine method with hematoxylin and eosin for microscopic study.

2. In vivo effects of ENX, NFLX and OFLX on the T1/2 of theophylline

   a) Animals and administration of drugs: Male Wistar rats, each weighing 200 g, were fed a standard pellet diet and drinking water ad libitum. After an overnight fast, theophylline ethylenediamine diluted with saline was injected to the rats via the jugular vein at a dose of 10 mg/kg. ENX, NFLX and OFLX were administered 1 hr before, simultaneously with, and 1 hr after the theophylline injection, as in the above experiments.

   b) Measurement of the T1/2 of theophylline: Blood samples were taken from the jugular vein at 1.5, 3.0, 4.5 and 6.0 hr after the injection of theophylline. Serum theophylline concentrations were measured by an enzyme immunoassay (EMIT®) (20), and the T1/2 of theophylline was read from the linear part of the time concentration curve on a semilog graph.

3. In vitro effects of ENX, NFLX and OFLX on 7-EC O-deethylase activity in liver microsomes

   a) Animals and preparation of microsomes: Male Wistar rats, each weighing 200 g, were used, and liver microsomes were prepared as reported previously (15) after an overnight fast.

   b) Assay of 7-EC O-deethylase activity: 7-EC O-deethylase activity was determined according to the method of Ullrich and Weber (21). 7-EC was dissolved in 1 M Tris-HCl buffer (pH 7.6) to give a 10^{-3} M solution. ENX, NFLX and OFLX were dissolved in 6 ml
of 0.1 N NaOH and diluted with distilled water to give 100 ml of a 5 mM solution. The reaction mixture consisted of microsomes, $10^{-4}$ M 7-EC and $10^{-4}$ M NADPH in 1.0 ml of $10^{-1}$ M Tris-HCl buffer (pH 7.6). ENX, NFLX and OFLX were added to the mixture to give concentrations of 0.5, 1.0, 1.5 and 2.0 mM. These concentrations were used because it was reported that cimetidine significantly inhibited 7-EC O-deethylase activity at such concentrations, but famotidine did not (22). An equivalent volume of the solvent was added in the control assay. The reaction was started by adding NADPH (10 μl, $10^{-2}$ M), and the mixture was incubated aerobically at 37°C. The increase in fluorescence intensity with time was measured at 460 nm with an excitation of 372 nm using a fluorescence spectrophotometer (Hitachi, 650-60).

4. Chemicals

The drugs used were as follows: Acetaminophen and 3-MC (Sigma Chemical Co., St. Louis, MO, U.S.A.), 7-EC (Nacalai Tesque, Kyoto), theophylline ethylenediamine (Theophylline® (Inj.), Eisai Co., Tokyo). ENX, NFLX and OFLX were generously provided by Dainippon Pharmaceutical Co. (Osaka), Kyrin Pharmaceutical Co. (Tokyo) and Daiichi Pharmaceutical Co. (Tokyo), respectively.

5. Statistical analysis

![Figure 1](image_url)

Fig. 1. Effects of enoxacin (ENX), norfloxacin (NFLX) and ofloxacin (OFLX) on serum glutamic-oxaloacetic transaminase (GOT) levels in rats treated with and without acetaminophen (AA). The drugs were administered as follows: AA, 800 mg/kg, i.p.; ENX, NFLX and OFLX, 75 or 300 mg/kg, p.o., 1 hr before, simultaneously with, and 1 hr after the AA injection. Blood samples were taken 5 hr after the administration of AA. Vertical bars represent the mean ± S.D.
The data on acetaminophen-induced liver injury were examined by analysis of variance followed by Bonferroni's multiple range test. Concerning the data on the T1/2 of theophylline, the significance of difference was determined by Student's t-test for unpaired variables.

Results

1. Effects of ENX, NFLX and OFLX on acetaminophen-induced liver injury
   a) Effects of ENX, NFLX and OFLX on biochemical liver function tests: The effects of ENX, NFLX and OFLX on the serum biochemical parameters in the rats treated with and without acetaminophen are shown in Figs. 1-3 and Table 1.
   The serum GOT, GPT and albumin levels were not significantly affected by ENX, NFLX and OFLX at 75 or 300 mg/kg.
   In the rats treated with acetaminophen alone, serum GOT and GPT rose markedly to 19,700±4,730 and 7,440±3,600, respectively; and the serum albumin level was obviously lower than in the control group. In contrast, in the groups treated with ENX (75 or 300 mg/kg) plus acetaminophen, the increase in serum GOT and GPT were considerably less. In these groups, the decrease in serum albumin induced by acetaminophen
was obviously attenuated as the dose of ENX increased; no significant difference was observed between the serum albumin level in the group treated with 300 mg/kg of ENX plus acetaminophen and that in the control group.

In the groups treated with NFLX plus acetaminophen, the increase in serum GOT was significantly suppressed as the dose of NFLX increased. The increase in serum GPT in the group treated with 300 mg/kg of NFLX plus acetaminophen was significantly less than in the group treated with acetaminophen alone. In the group treated with 300 mg/kg of NFLX plus acetaminophen, the decrease in serum albumin also tended to be attenuated, but was not significantly different from the group treated with acetaminophen alone.

In the group treated with 75 mg/kg of OFLX plus acetaminophen, the serum GOT and GPT levels increased and the serum albumin level decreased to similar levels as in the group treated with acetaminophen alone. All the rats treated with 300 mg/kg of OFLX plus acetaminophen died within 5 hr of the acetaminophen injection.

b) Effects of ENX, NFLX and OFLX on drug metabolizing activity in liver microsomes: The effects of ENX, NFLX and OFLX on the drug metabolizing activity in the rats treated with and without acetaminophen are shown in Figs. 4–6 and Table 2. The microsomal protein and P-450 contents and the aminopyrine N-demethylase ac-
Table 1. Effects of three new quinolones on serum transaminase and albumin levels in rats treated with and without acetaminophen

|                | s-GOT (IU/l) | s-GPT (IU/l) | s-Albumin (g/dl) |
|----------------|-------------|--------------|------------------|
| Control        | 293±80      | 55±18        | 3.42±0.31        |
| ENX 75 mg/kg   | 162±48      | 39±4.0       | 3.37±0.07        |
| ENX 300 mg/kg  | 308±43      | 44±5.5       | 3.64±0.20        |
| NFLX 75 mg/kg  | 169±14      | 45±2.1       | 3.59±0.13        |
| NFLX 300 mg/kg | 172±6       | 51±3.0       | 3.66±0.36        |
| OFLX 75 mg/kg  | 273±31      | 49±2.9       | 3.38±0.42        |
| OFLX 300 mg/kg | 238±29      | 47±14        | 3.24±0.24        |
| AA alone       | 19.700±4.730** | 7.440±3.600** | 2.63±0.32**     |
| AA+ENX 75 mg/kg| 1.130±1.120 | 526±797      | 3.13±0.28*       |
| AA+ENX 300 mg/kg| 827±762   | 234±296      | 3.45±0.41        |
| AA+NFLX 75 mg/kg| 15.000±4.400** | 8.930±3.200** | 2.76±0.27**     |
| AA+NFLX 300 mg/kg| 9.400±4.400** | 5.570±3.380** | 2.85±0.17**     |
| AA+OFLX 75 mg/kg| 21.800±7.200** | 10.700±5.050** | 2.71±0.27**     |
| AA+OFLX 300 mg/kg| (n=11)      | All rats died within 5 hr. | |

The drugs were administered as follows: Acetaminophen (AA), 800 mg/kg, i.p.; enoxacin (ENX), norfloxacin (NFLX) and ofloxacin (OFLX), 75 or 300 mg/kg, p.o., 1 hr before, simultaneously with, and 1 hr after the AA injection. Blood samples were taken 5 hr after the administration of AA. Each value represents the mean±S.D. * : Significantly different from the control (*P<0.05; **P<0.01).

Table 2. Effects of three new quinolones on the contents of microsomal protein and cytochrome P-450, and aminopyrine N-demethylase activity in rats treated with and without acetaminophen

|                | Microsomal protein (mg/g liver) | P-450 (nmol/mg MS prot.) | Aminopyrine N-demethylation (nmol/mg MS prot./min) |
|----------------|--------------------------------|--------------------------|-----------------------------------------------|
| Control        | 24.86±2.21                    | 2.09±0.31                | 17.02±3.12                                   |
| ENX 75 mg/kg   | 26.52±1.26                    | 1.99±0.11                | 17.19±0.85                                   |
| ENX 300 mg/kg  | 24.50±2.63                    | 1.85±0.19*               | 15.04±1.51                                   |
| NFLX 75 mg/kg  | 26.87±1.44                    | 1.88±0.16                | 16.34±1.20                                   |
| NFLX 300 mg/kg | 27.45±1.27*                   | 1.77±0.11*               | 14.46±2.54                                   |
| OFLX 75 mg/kg  | 24.69±1.37                    | 2.01±0.13                | 16.53±1.68                                   |
| OFLX 300 mg/kg | 28.05±3.83*                   | 1.73±0.22**              | 14.70±3.05                                   |
| AA alone       | 19.12±2.92**                  | 1.38±0.17**              | 12.03±1.61**                                 |
| AA+ENX 75 mg/kg| 24.85±1.24                    | 1.83±0.26*               | 14.24±3.08*                                  |
| AA+ENX 300 mg/kg| 27.08±1.93*                   | 1.95±0.11                | 13.96±1.75**                                 |
| AA+NFLX 75 mg/kg| 18.53±2.21**                  | 1.37±0.16**              | 12.09±3.01**                                 |
| AA+NFLX 300 mg/kg| 19.75±2.26**                  | 1.57±0.32**              | 14.13±1.79**                                 |
| AA+OFLX 75 mg/kg| 17.12±1.55**                  | 1.49±0.26**              | 12.39±1.92**                                 |
| AA+OFLX 300 mg/kg| (n=11)                      | All rats died within 5 hr.| |

The drugs were administered as follows: Acetaminophen (AA), 800 mg/kg, i.p.; enoxacin (ENX), norfloxacin (NFLX) and ofloxacin (OFLX), 75 or 300 mg/kg, p.o., 1 hr before, simultaneously with, and 1 hr after the AA injection. Liver microsomes were prepared for assay 5 hr after the administration of AA. Each value represents the mean±S.D. * : Significantly different from the control (*P<0.05; **P<0.01).
Activity in the groups treated with ENX, NFLX and OFLX (75 or 300 mg/kg) remained at similar levels to those in the control group. The microsomal protein and P-450 contents and the aminopyrine N-demethylase activity were obviously reduced in the rats treated with acetaminophen alone. In contrast, treatment with ENX (75 or 300 mg/kg) plus acetaminophen noticeably attenuated these changes to similar levels as in the control group. For the microsomal protein and P-450 contents, the degree of the attenuation was dependent on the dose of ENX administered.

In the group treated with 300 mg/kg of NFLX plus acetaminophen, the decrease in aminopyrine N-demethylase activity was significantly attenuated. In this group, the decrease in P-450 content also tended to be attenuated, but was not significantly different from the group treated with acetaminophen alone. The co-administration of NFLX did not affect the microsomal protein content in the rats treated with acetaminophen.

In the group treated with 75 mg/kg of OFLX plus acetaminophen, the microsomal protein and P-450 contents and the aminopyrine N-demethylase activity were reduced to similar levels as in the group treated with acetaminophen alone, and no significant difference was observed between these two groups.

c) Effects of ENX, NFLX and OFLX on the total glutathione content of the liver: The ef-

Fig. 4. Effects of enoxacin (ENX), norfloxacin (NFLX) and ofloxacin (OFLX) on the microsomal protein content in rats treated with and without acetaminophen (AA). The drugs were administered as follows: AA, 800 mg/kg, i.p.; ENX, NFLX and OFLX, 75 or 300 mg/kg, p.o., 1 hr before, simultaneously with, and 1 hr after the AA injection. Liver microsomes were prepared for assay 5 hr after the administration of AA. Vertical bars represent the mean±S.D.
The effects of ENX, NFLX and OFLX on the total glutathione content in the rats treated with and without acetaminophen are shown in Fig. 7 and Table 3. The total glutathione content of the liver in the groups treated with ENX, NFLX and OFLX (75 or 300 mg/kg) remained at a similar level to that in the control group.

The total glutathione content of the liver was markedly reduced in the rats treated with acetaminophen alone. In contrast, treatment with ENX (75 or 300 mg/kg) plus acetaminophen noticeably attenuated the decrease in total glutathione content to a similar level as in the control group.

In the groups treated with NFLX plus acetaminophen, the decrease in total glutathione content tended to be attenuated as the dose of NFLX increased, but no significant difference was observed compared with the group treated with acetaminophen alone.

In the group treated with 75 mg/kg of OFLX plus acetaminophen, the total glutathione content of the liver was reduced to a similar level as in the group treated with acetaminophen alone, and no significant difference was observed between these two groups.

d) Effects of ENX, NFLX and OFLX on histological findings in the liver: In the livers of rats treated with acetaminophen alone, severe hemorrhagic necrosis was observed throughout the hepatic lobules, predominantly in the centrilobular regions (Fig. 8A).
In contrast, in the group treated with 300 mg/kg of ENX plus acetaminophen, although slight hemorrhage was still observed, the degree of hepatic necrosis was obviously less than in the group treated with acetaminophen alone (Fig. 8B). In the group treated with 75 mg/kg of ENX plus acetaminophen, hemorrhagic necrosis of the liver was also attenuated, but to a considerably lesser extent than in the group treated with 300 mg/kg of ENX plus acetaminophen (not shown).

On the other hand, in the groups treated with 75 (not shown) or 300 mg/kg ofNFLX (Fig. 8C), and 75 mg/kg of OFLX (Fig. 8D) plus acetaminophen, severe hemorrhagic necrosis was observed, the degree of which was similar to the group treated with acetaminophen alone.

2. In vivo effects of ENX, NFLX and OFLX on the T1/2 of theophylline

The theophylline T1/2 in untreated rats was 2.61 ± 0.29 hr. In the rats treated with ENX (75 or 300 mg/kg), the theophylline T1/2 was noticeably prolonged to 5.01 ± 0.54 and 6.49 ± 0.62 hr, respectively (Fig. 9). In the rats treated with 300 mg/kg of NFLX, the theophylline T1/2 was significantly prolonged to 3.27 ± 0.57 hr, but that in the rats treated with
75 mg/kg of NFLX (2.68±0.52 hr) was not significantly different from the control group (Fig. 10). On the other hand, OFLX treatment (75 or 300 mg/kg) did not affect theophylline T1/2; the values in these groups were 2.71±0.26 and 2.89±0.49 hr, respectively (Fig. 11).

3. In vitro effects of ENX, NFLX and OFLX on 7-EC O-deethylase activity in liver microsomes

The 7-EC O-deethylase activity in untreated rats was 277±26 pmol/mg microsomal protein/min (100%). As shown in Table 4, increasing concentrations of ENX added to the incubation mixture markedly inhibited the 7-EC O-deethylase activity in liver microsomes. Increasing concentrations of NFLX also inhibited 7-EC O-deethylase activity, but to a considerably lesser extent than did ENX. The addition of OFLX to the incubation mixture did not affect the 7-EC O-deethylase activity in the liver microsomes.

Discussion

Acetaminophen is known to be metabolized in the liver and primarily excreted as a sulfate or glucuronide conjugate. A small amount (about 5%) of the dose administered is biotransformed through the mixed-function oxidase system in liver microsomes into toxic intermediates, which are preferentially conjugated with glutathione and then detoxified...
When a large dose of acetaminophen is administered or the activity of P-450, a key enzyme in the mixed-function oxidase system, is induced, the increased amount of toxic intermediates cannot all be detoxified because the hepatic glutathione content becomes depleted, and then acute liver injury occurs. Therefore, agents which inhibit the activity of P-450 can prevent the transformation of acetaminophen into toxic intermediates and protect against acetaminophen-induced liver injury. Mitchell et al. (10) first showed that cimetidine, a H₂-receptor antagonist that is known to inhibit the mixed-function oxidase system in liver microsomes (9), protected against acetaminophen-induced liver injury. Murase et al. (11) also reported that cimetidine markedly reduced acetaminophen-induced liver injury, while ranitidine and famotidine, other H₂-receptor antagonists that do not inhibit the mixed-function oxidase system (22, 24), had little or no effect.

### Table 3. Effects of three new quinolones on the total glutathione content in rats treated with and without acetaminophen

|                | Glutathione (mg/g liver) |
|----------------|-------------------------|
| Control        | (n=10)                  | 1.48±0.27               |
| ENX 75 mg/kg   | (n=4)                   | 1.32±0.12               |
| ENX 300 mg/kg  | (n=6)                   | 1.31±0.17               |
| NFLX 75 mg/kg  | (n=4)                   | 1.28±0.13               |
| NFLX 300 mg/kg | (n=4)                   | 1.39±0.37               |
| OFLX 75 mg/kg  | (n=4)                   | 1.28±0.18               |
| OFLX 300 mg/kg | (n=4)                   | 1.14±0.29*              |
| AA alone       | (n=9)                   | 0.20±0.09**             |
| AA+ENX 75 mg/kg| (n=9)                   | 1.12±0.41**             |
| AA+ENX 300 mg/kg| (n=10)                 | 1.18±0.44*              |
| AA+NFLX 75 mg/kg| (n=10)                | 0.25±0.10**             |
| AA+NFLX 300 mg/kg| (n=11)               | 0.29±0.14**             |
| AA+OFLX 75 mg/kg| (n=9)                  | 0.20±0.07**             |
| AA+OFLX 300 mg/kg| (n=11)                | All rats died within 5 hr.

The drugs were administered as follows: Acetaminophen (AA), 800 mg/kg, i.p.; enoxacin (ENX), norfloxacin (NFLX) and ofloxacin (OFLX), 75 or 300 mg/kg, p.o., 1 hr before, simultaneously with, and 1 hr after the AA injection. The total glutathione content of the liver was assayed 5 hr after the administration of AA. Each value represents the mean±S.D. *, **: Significantly different from the control (*P<0.05, **P<0.01).

### Table 4. Effects of enoxacin (ENX), norfloxacin (NFLX) and ofloxacin (OFLX) on 7-ethoxycoumarin O-deethylase activity in rat liver microsomes

| Concentration (mM) | ENX       | NFLX      | OFLX      |
|--------------------|-----------|-----------|-----------|
| 0.5 (n=4)          | 54.9±12.5 | 87.7±5.6  | 93.7±2.2  |
| 1.0 (n=4)          | 43.7±9.3  | 76.9±6.0  | 93.7±12.2 |
| 1.5 (n=4)          | 28.2±2.8  | 73.5±5.4  | 98.5±20.4 |
| 2.0 (n=4)          | 18.1±1.3  | 66.1±3.9  | 94.3±5.4  |

Control activity was 277±26 pmol/mg microsomal protein/min (100%) (n=4). Each value represents the mean±S.D.
In this study, ENX was strongly protective against acetaminophen-induced liver injury. NFLX also significantly prevented liver injury, but to a considerably lesser extent than did ENX. In contrast, OFLX had no protective effect on acetaminophen-induced liver injury. These findings suggested that ENX markedly and NFLX slightly inhibited the activity of P-450 in liver microsomes, while OFLX did not affect it.

Theophylline is known to be metabolized chiefly in the liver. Its elimination from the blood is dependent mainly on drug metabolizing activity in liver microsomes, and other factors such as hepatic blood flow or binding to plasma proteins are generally recognized to have little influence on theophylline elimination (25). The in vivo experiments in this study showed that ENX markedly and NFLX slightly prolonged the T1/2 of theophylline in rats, but OFLX did not affect it. Many investigators have reported a similar result in human subjects, that ENX markedly raised the plasma theophylline concentration and prolonged its T1/2, whereas NFLX and OFLX had little or no effect on the pharmacokinetics of theophylline (1-8). Sekine et al. (12) and Okazaki et al. (26) have shown similar results in rats concerning ENX and OFLX. Plasma protein-binding and renal clearance of theophylline are reported to remain unchanged in patients treated with ENX or OFLX (1, 2, 5), so that the
prolongation of the T1/2 of theophylline is considered to be due to reduced metabolic clearance in the liver. In rats, ENX and OFLX are also reported not to affect serum protein binding (26).

The in vitro experiments in this study also showed that ENX markedly and NFLX slightly inhibited the 7-EC O-deethylase activity in rat liver microsomes, while OFLX did not affect this activity. Okazaki et al. (26) recently demonstrated that OFLX exhibited a weak but significant inhibition of 7-EC O-deethylase activity and N-demethylase activities toward benzphetamine and aminopyrine in rat liver microsomes, while ENX inhibited these activities to greater extents. Their results differed from the observation in this study that OFLX did not affect the 7-EC O-deethylase activity in rat liver microsomes. The reason for the difference is not clear, but a difference in the P-450 species contained in the microsomes used in the respective experiments may partly explain it; that is, they used liver microsomes from rats pretreated with phenobarbital, while untreated rats were used in this study.

The effects of ENX, NFLX and OFLX on acetaminophen-induced liver injury, the changes in the T1/2 of theophylline (in vivo), and the alterations in the 7-EC O-deethylase activity in liver microsomes (in vitro) all correlated with each other. All the results obtained indicated that ENX markedly and NFLX slightly inhibited P-450 activity in the mixed-function oxidase system in liver microsomes, while OFLX did not affect it.

The mechanism by which quinolones influence the drug metabolizing activity in liver microsomes is still a matter of controversy. Two hypotheses have been proposed. Tamura et al. (7) compared the effect of NFLX on theophylline metabolism and its chemical
structure with those of ENX and suggested that the nitrogen at position 8 of the core of the quinolone molecule is the factor which affects theophylline metabolism. (The chemical structures of the quinolones discussed in this report are shown in Fig. 12.) Niki et al. (8) compared the effects of 6 quinolones on theophylline metabolism. They reported that among the 6 drugs investigated, ENX and pipemidic acid both considerably increased the serum theophylline concentration and both had nitrogen at positions 1 and 8 (naphthyridine or pyridopyrimidine ring), while the other drugs had nitrogen only at position 1 (quinoline ring). However, NFLX, which has a quinoline ring structure, significantly inhibited P-450 activity in this study. Pefloxacin (PFX) and ciprofloxacin (CPFX), which also have quinoline ring structures, have been reported to slightly but significantly decrease the metabolic clearance of theophylline, while nalidixic acid, which has a naphthyridine ring structure, has been reported not to affect it (4). Therefore, the hypothesis that the nitrogen at position 8 affects the drug metabolizing activity in liver microsomes should be ruled out.

Wijnands et al. (4) compared the effects of

Fig. 11. Effect of ofloxacin (OFLX) on the elimination half-life (T1/2) of theophylline in rats. Theophylline ethylenediamine (10 mg/kg) was injected intravenously and OFLX (75 or 300 mg/kg) was administered orally 1 hr before, simultaneously with, and 1 hr after the theophylline injection.

Fig. 12. Chemical structures of quinolones.
5 quinolones on theophylline metabolism and showed that the extent of the decrease in the metabolic clearance of theophylline correlated with the 24-hr urinary excretion of the 4-oxo metabolite of each quinolone. For example, 14.7% of ENX was recovered in the urine as the 4-oxo metabolite, and ENX markedly decreased the metabolic clearance of theophylline. PFX and CPFX, for which the urinary excretion of the 4-oxo metabolite was 6.4% and 4.3%, respectively, slightly inhibited theophylline metabolism. In contrast, no 4-oxo metabolite could be found in the urine after administration of nalidixic acid and only traces after OFLX administration, and neither of them affected theophylline pharmacokinetics. They thus suggested that the 4-oxo metabolite seemed to be responsible for the interaction with theophylline clearance. Since the 4-oxo metabolite is known to be produced from NFLX (27), which slightly inhibited P-450 activity in this study, their hypothesis might seem to be correct. However, the in vitro experiments in this study showed that ENX and NFLX inhibited the 7-EC O-deethylase activity in liver microsomes even from the beginning of the reaction; at that time, no metabolite could have been produced from the parent drugs. Okazaki et al. (26) have also reported similar results from in vitro experiments. Recently, Mulder et al. (28) investigated the effects of ENX, its metabolite oxoenoxacin and OFLX on the oxidative metabolism of theophylline in isolated rat hepatocytes. They demonstrated that ENX inhibited the formation of 1,3-dimethyluric acid by 67% at 1.0 mM, while neither oxoenoxacin nor OFLX had any inhibitory effect. Therefore, the hypothesis that the 4-oxo metabolite causes the inhibition of the drug metabolizing activity in liver microsomes also should not be accepted.

From the observations in this study, neither differences in the fundamental chemical structure of the parent drugs nor their metabolites can explain the differences found in the inhibitory effects of quinolones on drug metabolizing activity. Therefore, it appears possible that minor differences in the structures of the quinolone molecules, for example, in the residues or in the three-dimensional configurations, might affect the activity of P-450. Recently, Takagi et al. (29, 30) demonstrated that T-3262, a new synthetic quinolone that produces no 4-oxo metabolite, reduced the metabolic clearance of theophylline to a similar extent as did PFX and CPFX, while ENX reduced it to a greater extent. They proposed that the difference in the inhibitory effects of ENX and T-3262 could be attributed either to differences in the residues (ENX = alkyl residue, T-3262 = aryl residue) at position 1N of the naphthyridine ring, or to differences in the three-dimensional structure between the fluorine residue at position 6 and the residues (ENX = piperadine ring, T-3262 = aminopyrrolidine ring) at position 7. Their report supports the observations in this study.

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