Species and Sex Differences in the Inhibitory Action of the Corticosteroid Alclometasone Dipropionate on the Hepatic Drug-Metabolizing System

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Abstract—The effect of successive administration of the corticosteroid alclometasone dipropionate (ACM) on the hepatic drug-metabolizing system was examined using male and female rats. Although some pharmacological changes such as increases in plasma enzyme activity, lipid level and protein concentration appeared similarly in ACM-treated male and female rats, the activities of 7-alkoxy-coumarin O-dealkylase, especially the O-depropylation activity, decreased dose-dependently by ACM administration only in male rats. ACM did not affect the hepatic drug-metabolizing activity in female rats and mice of both sexes. Also, ACM did not inhibit androgen-independent aniline hydroxylase activity even in male rats. The time course of changes of the drug-metabolizing system in male rats showed a rapid decrease in cytochrome P-450 content and O-depropylation activity following successive treatments with ACM, but there was a slow onset in the decreases of the O-demethylation and O-deethylation activities of 7-alkoxy-coumarin. When ACM was withdrawn, the O-demethylation and O-deethylation activities rapidly returned to their control levels, while recovery of the O-depropylation activity was slow. These results suggested that ACM inhibits the hepatic drug-metabolizing enzyme activity associated with a specific form(s) of androgen-dependent cytochrome P-450 in male rats.

The corticosteroid alclometasone dipropionate (ACM: 7α-chloro-11β,17,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione 17,21-dipropionate) is a local anti-inflammatory drug (1) with the chemical structure shown in Fig. 1. This compound was characterized as possessing enhanced topical anti-inflammatory activity with a potential for lesser side effects (1, 2). However, sub-chronic toxicity studies of ACM revealed that the liver drug-metabolizing enzyme activity in male rats was depressed by its successive administrations (3, 4).

Cytochrome P-450 is a terminal enzyme participating in the oxidative biotransformation of xenobiotics and endogenous compounds (5). Various drugs or chemicals are known to induce or inhibit the cytochrome P-450-dependent monooxygenase in liver microsomes (6, 7), followed by modifications in the pharmacological or toxicological activities of the drugs (8–10). Therefore, the

Fig. 1. Chemical structure of alclometasone dipropionate (ACM).
present study was conducted to examine whether ACM inhibits the cytochrome P-450-dependent monoxygenase activity in various animals.

Materials and Methods

Animals: Slc-Wistar strain rats, 8 weeks old, were used. In some experiments, 8-week-old DS strain mice were also used. The animals were kept in an air-conditioned room (23±2°C, 55±10% relative humidity) lighted 12 hr a day (8:00-20:00) and maintained on commercial rat chow (CA-1, Clea Japan, Inc., Tokyo) and water ad libitum.

Administration of test compound: ACM was suspended in 1% (w/v) arabic gum and administered subcutaneously at the volume of 1 ml/kg (rat) or 5 ml/kg (mouse). Control animals were given 1% arabic gum alone.

Determination of biochemical parameters in plasma: Rats were placed under pentobarbital anesthesia, and heparinized blood samples were withdrawn from the vena cava. Biochemical parameter such as GOT, GPT and LDH activities and concentrations of total protein, total cholesterol and triglyceride in heparinized plasma samples were determined using a Technicon auto-analyzer SMAC-III system (Technicon Instrument Co., New York, NY, U.S.A.).

Determination of hepatic drug-metabolizing activity: Liver samples obtained were homogenized in 0.25 M sucrose containing 50 mM Tris-HCl buffer (pH 7.4), and the resulting homogenates were used as enzyme sources. 7-Alkoxycoumarin O-dealkylase and aniline hydroxylase activities at the homogenate level were measured as described previously (11, 12). UDP-glucuronyltransferase activity was determined in the presence of 0.03% Triton X-100 using 7-hydroxycoumarin as a substrate (13). Cytochrome P-450 content and UDP-glucuronyltransferase activity also showed dose-dependent decreases. However, the drug had little or no effect in female rats. Interestingly, aniline hydroxylase activity in both male and female rats was not affected by ACM administration.

Results

Effect of alclometasone dipropionate on liver drug metabolizing activity: Alterations hepatic drug-metabolizing enzyme activities in rats were examined following the subcutaneous administration of various doses of ACM for two weeks. As shown in Fig. 2, 7-alkoxycoumarin O-dealkylase activities in male rats decreased gradually with increasing doses of ACM, and a more marked depression was detected in the O-depropylation activity than that of the O-demethylation or O-deethylation activity. Cytochrome P-450 content and UDP-glucuronyltransferase activity also showed dose-dependent decreases. However, the drug had little or no effect in female rats. Interestingly, aniline hydroxylase activity in both male and female rats was not affected by ACM administration.

To compare the effects of ACM on male and female mice, various doses of ACM were given subcutaneously for 2 weeks, and their enzyme activities were measured. As shown in Fig. 3, the O-depropylation and aniline hydroxylation activities were little modified in both male and female mice, although slight decreases in aniline hydroxylase activity were detected in male mice treated with 0.5 or 1.0 mg/kg dose of ACM. Normal values were also found for the O-demethylation and O-deethylation activities of 7-alkoxycoumarin in the treated mice (data not shown).
Changes in hepatic 7-alkoxycoumarin O-dealkylase activity in rats. Male and female rats were administered subcutaneously various doses of alclometasone dipropionate (ACM) for 2 weeks, and liver samples were obtained one day after the last administration. Activities of 7-alkoxycoumarin O-demethylase (A), O-deethylase (B) and O-depropylase (C), aniline hydroxylase (D) and UDP-glucuronyltransferase (E) were assayed using liver homogenates as enzyme sources. Cytochrome P-450 contents in the liver homogenates are also shown in the figure (F). The values in the figure represent the mean and standard error of 5 rats. *, **: Statistically significant (P<0.05 and P<0.01, respectively) against the control.

Changes in body and liver weights and plasma biochemical parameters: The toxicological effects of ACM were examined to evaluate whether the ACM-induced decrease of hepatic drug-metabolizing enzyme activities in male rats had developed secondarily following the hepatotoxic action of the drug. Rats were given subcutaneously various doses of ACM for 2 weeks, and body and liver weights were checked 24 hr after the last administration. As shown in Table 1, body weight gain during the administration period was suppressed in both male and female rats by increasing doses of ACM, while there was no marked difference in the absolute liver weight between the control and ACM-treated groups of male rats. Thus, the relative liver weight (ratio of liver to body weights) in male rats showed a tendency to increase with increasing doses of ACM. On the other hand, slight decrease in liver weight was detected in ACM-treated female rats, and thus the relative liver weight in females was not changed much by ACM treatment. Dose-dependent increases in plasma enzyme levels and lipid concentrations were found in ACM-treated rats of both sexes (Table 2). The total protein concentration in plasma seemed to increase in the treated animals. Other components of plasma (plasma levels of glucose, albumin, creatinine, urea-N, Na+, K+, Cl−, and so on) in male and female rats were only
Table 1. Body and liver weights of rats after successive subcutaneous administrations of alclometasone dipropionate for 14 days

| Sex  | Dose (mg/kg) | Body weight (g) | Liver weight |      |
|------|--------------|----------------|--------------|------|
|      |              | Initial | Final | Absolute (g) | Relative (% B.W.) |
| Male | Control      | —      | —     | —            | —                |
|      | ACM 0.1      | 210±2  | 234±4*| 9.7±0.3      | 4.13±0.05       |
|      | ACM 0.5      | 210±2  | 212±3*| 10.0±0.2     | 4.74±0.06**     |
|      | ACM 1.0      | 209±3  | 198±4*| 9.9±0.4      | 4.97±0.09**     |
| Female | Control   | —      | —     | —            | —                |
|       | ACM 0.1     | 154±2  | 157±2 | 5.5±0.1**    | 3.52±0.03       |
|       | ACM 0.5     | 156±2  | 146±2**| 5.8±0.1     | 3.95±0.07       |
|       | ACM 1.0     | 154±3  | 141±3**| 5.5±0.1**   | 3.92±0.03       |

Male and female rats (8 weeks old at the start) were treated with various doses of ACM for 14 days. Body and liver weights were measured 24 hr after the last administration. The values represent the mean±S.E. of 6 animals. *: Statistically different (P<0.01) from the control.

Table 2. Biochemical findings in rat plasma

| Sex   | Dose (mg/kg) | GOT (U/I) | GPT (U/I) | LDH (U/I) | T. Protein (g/dl) | T. Cholesterol (mg/dl) | Triglyceride (mg/dl) |
|-------|--------------|-----------|-----------|-----------|-------------------|------------------------|----------------------|
| Male  | Control      | 63±2      | 44±2      | 95±10     | 5.7±0.1           | 57±2                   | 104±15               |
|       | ACM 0.1      | 73±5      | 58±1**    | 124±22    | 5.9±0.1           | 77±3**                 | 189±16**             |
|       | ACM 0.5      | 112±8**   | 131±19**  | 137±10*   | 6.2±0.1**         | 113±9**                | 219±17**             |
|       | ACM 1.0      | 112±14*   | 126±19**  | 164±29    | 6.2±0.1**         | 131±6**                | 214±14**             |
| Female | Control     | 54±1      | 38±3      | 69±7      | 5.4±0.1           | 70±3                   | 40±4                 |
|       | ACM 0.1      | 68±1**    | 47±4      | 92±7*     | 5.4±0.1           | 84±4*                  | 41±3                 |
|       | ACM 0.5      | 102±14*   | 102±16*   | 131±19*   | 5.9±0.0**         | 132±7**                | 93±6**               |
|       | ACM 1.0      | 110±6**   | 127±10**  | 146±9**   | 5.9±0.0**         | 153±2**                | 87±16*               |

Heparinized blood samples of rats were obtained after 14-day ACM treatment. Activities of plasma enzymes and levels of total protein (T. Protein), total cholesterol (T. Cholesterol) and triglyceride were determined as described in Methods. The values in the table represent the mean±S.E. of 6 animals. *: Statistically significant (P<0.05 and P<0.01, respectively) against the control.
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slightly changed by the administration of ACM (data not shown).

Fig. 3. Hepatic 7-propoxycoumarin O-depropylase and aniline hydroxylase activities in mice. Male and female mice were given subcutaneously various amounts of ACM for 2 weeks. The values represent the mean and standard error of 5 animals. **: Statistically significant (P<0.01) against the control.

Morphological examination of ACM-treated rat liver: Male and female rats were given ACM for 2 weeks at the dose of 1.0 mg/kg, and the livers were histologically examined 24 hr after the last administration. Compared with the histological profile of the control animals, a significant change was observed in the ACM-treated rat livers (Fig. 4). Liver sections stained with hematoxylin and eosin showed vacuoles inside the liver cells of the treated rats, while such vacuoles were not detected in the control animals. The PAS-stained sections indicated a marked increase of PAS-positive substances (glycogen) concomitant with the appearance of intracellular vacuoles (data not shown). The liver cells in ACM-treated rats were then examined electron microscopically. As shown in Fig. 5, ACM treatment caused a marked increase in the glycogen area inside the hepatocytes, and the cellular organelles such as mitochondria and endoplasmic reticulum were observed mostly in the peripheral regions of the cells. This observation agreed with the findings shown in Fig. 4. Such a morphological change of liver cells is characteristic of physiological responses resulting from an increase of glycogen.

Histological observations of ACM-treated female rats were also carried out to compare the changes between male and female rats. Similar changes were detected in both groups of rats, although the morphological changes were less in female rats compared with those in the male (data not shown). The results

Fig. 4. Histological observations of rat liver cells. Tissue samples obtained from (A) control and (B) ACM-treated male rats were fixed and then stained with hematoxylin and eosin. The treatment of ACM was 1 mg/kg/day for 14 days. ×230.
shown in Tables 1 and 2 and Figs. 4 and 5 indicate that the ACM-induced pharmacological changes appeared similarly in both male and female rats, suggesting that the ACM-induced decreases of drug-metabolizing activities in male rats were not a secondary effect due to the hepatotoxic effect of ACM.

**Time course in the effect of ACM on hepatic drug-metabolizing system:** Male rats were given subcutaneously ACM at 0.5 mg/kg, once daily for 1–14 days, and the hepatic 7-alkoxycoumarin O-dealkylase activity and cytochrome P-450 content were determined 24 hr after the last administration of each treatment. The O-depropylation activity in the liver was depressed gradually by repeated administrations of ACM, with the lowest activity being detected following the treatment for 3–7 days. Cytochrome P-450 also showed a rapid decrease following the ACM administration, while the O-demethylation and O-deethylation activities showed a slow onset in the decrease (Fig. 6).

The decreased cytochrome P-450 content and the drug-metabolizing enzyme activities returned gradually to the control levels with time after the withdrawal of the drug (Fig. 7). Interestingly, a rapid recovery was observed in the O-demethylation and O-deethylation activities, while the O-depropylation activity returned slowly to the normal level. These results indicated that the inhibitory effect of ACM on the hepatic drug-metabolizing system in male rats is reversible.

**Discussion**

Sex-related differences in hepatic drug-metabolizing activity have been well-established specifically for rats (17). Various
Fig. 7. Time course in the recovery of the ACM-induced alterations of hepatic drug-metabolizing system in male rats. Animals were given ACM at 0.5 mg/kg for 7 days, and their livers were removed 1–21 days after the last administration. 7-Alkoxycoumarin O-dealkylase activity and cytochrome P-450 content were determined using liver homogenate, and their relative values against the control are plotted in the figure as a function of withdrawal period. Each value represents the mean and standard error of 5 animals. *: Statistically significant (P<0.05) against the control. **: Statistically significant (P<0.01) against the control.

cytochrome P-450-dependent reactions are classified as being androgen-dependent, while some, such as the aniline hydroxylation reaction, are independent (18). Using 7-alkoxycoumarin O-dealkylation activities as indicators, sex-related differences in the O-dealkylation activities, especially the O-depropylation activity, were demonstrated by Kamataki et al. (19). Studies on the postnatal changes of hepatic 7-alkoxycoumarin O-dealkylase revealed that the sex-related differences in the O-dealkylase appeared in rats after the development of sex-specific phenomena at the age of 5–6 weeks (20). Recent studies demonstrated the existence of male- and female-specific forms of cytochrome P-450 in rat livers (21). Furthermore, testosterone 6β-hydroxylation, specifically detected in male rats but not in female, was demonstrated to be catalyzed by cytochrome P-450 6β (22). Androgen-independent aniline hydroxylase is catalyzed by cytochrome P-450 1 (23). As described in this paper, administration of the corticosteroid ACM caused decreases in hepatic cytochrome P-450 content and 7-alkoxycoumarin O-dealkylase activity only in male rats. No inhibitory effect of ACM was detected in female rats and both sexes of mice (Figs. 2 and 3). Furthermore, hepatic drug-metabolizing enzyme activities of male and female dogs were not affected by ACM administration (24). These results suggested that ACM inhibits the drug-metabolizing activity catalyzed by specific form(s) of cytochrome P-450 peculiar to male rats. In fact, specific decreases in cytochrome P-450 6β content and testosterone 6β-hydroxylation activity were detected in ACM-treated male rats (T. Matsubara, unpublished result). Horie et al. reported the inhibition of the liver microsomal monooxygenase system by the antibiotic aztreonam in male but not female rats (25). LeBlanc and Waxman reported the feminization of rat liver cytochrome P-450 by cisplatin treatment (26). These findings suggest that the inhibitory mechanism of ACM is similar to that of aztreonam or cisplatin. ACM treatment also depressed the UDP-glucuronyl-transferase activity in male rats (Fig. 2), but its mechanism is not yet clear.

The corticosteroids have numerous and diversified physiological functions and pharmacological effects (27): suppression of body weight gain (Table 1) and increase in liver glycogen (Figs. 5 and 6) are characteristic
responses observed in corticosteroid-treated animals. Alteration in plasma enzyme level and lipid content detected in ACM-treated rats (Table 2) have also been reported in animals administered corticosteroid, prednisolone or betametasone (28, 29). Interestingly, these pharmacological effects of ACM were observed in both male and female rats, although the effects were slightly weaker in females. They suggested that the inhibitory mechanism of ACM against the androgen-dependent drug-metabolizing system was different from the pharmacological or physiological action mechanism of corticosteroids. The mechanism by which ACM affects the drug-metabolizing system in male rats remains to be clarified.

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