EFFECT OF DICYCLOMINE ON INTESTINAL ABSORPTION, DISPOSITION AND BILIARY EXCRETION OF DEXAMETHASONE

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The effect of dicyclicmine, a cholinergic blocking agent, on the in situ intestinal absorption, plasma clearance, biliary excretion of dexamethasone was examined in rats. The plasma concentrations and area under the plasma concentration-time curve (AUC) of dexamethasone after both a single and repeated oral coadministration with dexamethasone phosphate (4 mg/kg) and dicyclicmine (4 mg/kg) were significantly reduced compared with those after dexamethasone alone, without the alteration of elimination rate. The in situ absorption study also indicated that the absorption of dexamethasone was reduced to about a half after repeated coadministration of the two drugs. The renal plasma flow (RPF) in coadministration group was significantly enhanced compared with that of dexamethasone alone. The biliary excretion of dexamethasone was reduced, in proportion to the plasma concentrations, by dicyclicmine. Therefore, dicyclicmine should be administered taking much care in the corticosteroid treatment, because of producing the decrease in absorption.

**Keywords** — dexamethasone; in situ absorption; plasma clearance; biliary excretion; decreased absorption; dicyclicmine effect

INTRODUCTION

Dexamethasone, having a potent antiinflammatory activity because of the presence of fluorine atom, has been extensively used in the treatment of rheumatoid arthritis and allergic disorders.

This drug, like other corticosteroids, has undesirable side effects such as ulceration and Cushing's syndrome on prolonged therapy, but having relatively low sodium-retaining potency. In order to prevent the ulceration, dexamethasone is frequently coadministered with antacids, protective agents of mucous membrane and cholinergic blocking agents. However, it is shown that, when corticosteroids and antacids are simultaneously administered, the bioavailability of corticosteroids is reduced by the drug adsorption on antacid surface. On the other hand, little informations are available to the drug interactions between dexamethasone and cholinergic blocking agents, and the overall effects of the agent administration on dexamethasone pharmacokinetics has received little attention. The present study was undertaken to elucidate the effect of cholinergic blocking agent, dicyclicmine, on the plasma level, intestinal absorption and biliary excretion of dexamethasone in addition to the effect on the hepatic and renal functions in rats. The disposition of dexamethasone after repeated administration of both drugs was compared with that of a single dosing to clarify the effect following a prolonged treatment.

EXPERIMENTAL

**Materials** — Dexamethasone sodium phosphate (dexamethasone phosphate) and dicyclicmine hydrochloride (dicyclicmine) were obtained from Takeda Pharmaceutical Industries, Ltd. and Shionogi Pharmaceutical Co., Ltd., respectively. Prednisone, an internal standard for high performance liquid chromatography (HPLC), was purchased from Nakarai Chemicals, Ltd. Alkaline phosphatase (7 unit/mg) and \( \beta \)-glucuronidase-aryl sulfatase (solution) were
purchased from Sigma Chemical Co., Ltd. and Boehringer Mannheim Co., Ltd., respectively. All other chemicals used were of special grade.

**Animals and Treatment** — Male Wistar rats weighing 250–300 g were used throughout. Dexamethasone phosphate and dicyclicone were given orally as an aqueous solution in 2% acacia in a volume of 0.5 ml/100 g. In the comparative experiment, dexamethasone phosphate (2 mg/kg) was administered intravenously (i.v.) as a solution in saline in a volume of 0.1 ml/100 g. In a single oral administration, animals were treated with either dexamethasone phosphate (4 mg/kg) alone or in combination with dicyclicone (4 mg/kg). In repeated oral administration, animals were treated with every other day administration of dexamethasone phosphate (4 mg/kg) alone from day 1 to 7 or with dexamethasone as described above and for 7 d with daily administration of dicyclicone (4 mg/kg). After the final treatment, whole blood was collected at arbitrary intervals from tail vein in a heparinized syringe and the plasma was separated immediately by centrifugation at 1400 × g for 10 min. Rats were fasted for 12 h prior to the final administration.

**Determination of Dexamethasone in Plasma** — Dexamethasone in plasma was determined according to the HPLC method of Alvinerie et al. using a Shimadzu high performance liquid chromatograph LC-3A equipped with a variable-wavelength ultraviolet (UV) spectrophotometer, Shimadzu SPD-2A, at 254 nm. Separation was performed on a stainless steel column (6 × 100 mm) packed with ERC-Silica (Erma Optical Works, Ltd.) at room temperature and a flow rate of 1.5 ml/min. The solvent for elution was methylene chloride–methanol–acetic acid (96:4:0.4, by volume).

**In Situ Intestinal Absorption** — Animals were treated with repeated oral administration of either dexamethasone phosphate alone or in combination with dicyclicone, and fasted for 12 h at least prior to the experiment. The intestinal absorption of dexamethasone phosphate was estimated by the **in situ** intestinal absorption method 1 h after the final dosing. The decline of the drug in perfusate was followed at 10 min intervals for 60 min. The perfusate contained dexamethasone phosphate, 1 mg/100 ml, dissolved in Krebs-Ringer phosphate buffer (pH 7.4). The flow rate was 4 ml/min and dexamethasone phosphate in perfusate was determined following hydrolysis by alkaline phosphatase (6.65 unit, at 37°C for 1 h) according to the HPLC method described above.

**Determination of Indocyanine Green and p- Aminohippuric Acid in Plasma** — Rats were treated with repeated oral administration of dexamethasone phosphate alone or in combination with dicyclicone as described above. Sodium p-aminohippurate (30 mg/rat) or indocyanine green (5 mg/kg) separately was injected intravenously 2 h after the final treatment. p-Aminohippurate (PAH) and indocyanine green (ICG) in plasma were determined according to the method of Brun and Anger and Shimizu et al., respectively.

**Biliary Excretion Study** — The animals were treated from day 1 to 7 as described in the experimental method. After the final treatment, the animals were anesthetized with pentobarbital sodium (50 mg/kg) to allow rapid cannulations. In order to avoid the fall of body temperature during the experiments, an electric lamp was placed over the animal. After the anesthesia, the animal was placed on operation board spina
dy and followed by an abdominal incision at 30 min postdosing. The common bile duct was cannulated with a polyethylene tube (PE-10) and then bile samples were collected at 1 h intervals for 6 h. Dexamethasone and its conjugates in bile samples were determined before and after the hydrolysis with β-glucuronidase–aryl-sulfatase mixture (0.02 ml, at 37°C for 6 h) according to the HPLC method.

**Pharmacokinetic Analysis** — The graphic presentation of logarithmic dexamethasone concentration-time curve suggested that the concentration declined monoexponentially with time. Accordingly, the plasma concentration data following a single and repeated i.v. dosing...
were fitted to the one-compartment model by a nonlinear least squares fitting program (MULTI) of Yamaoka et al. The half-life \( t_{1/2} \) was calculated as \( t_{1/2} = 0.693/k_{el} \), where \( k_{el} \) is the elimination rate constant. The apparent volume of distribution \( V \) was given by the equation, \( V = \text{Dose}/C_0 \), where \( C_0 \) is the plasma concentration extrapolated to zero time. Total body plasma clearance \( (Cl_{tot}) \) was calculated by the following equation: \( Cl_{tot} = V \cdot k_{el} \). The area under the plasma concentration-time curve \( (AUC) \) was determined by the trapezoidal rule up to the last sampling point and the area beyond last observed plasma concentration \( (C_n) \) was added by integration \( (C_n/k_{el}) \). ICG clearance was estimated as the product of the \( k_{el} \) and \( V \) of ICG. Renal plasma flow \( (RPF) \) was calculated according to the following equations: \( RPF = V_{PAH} \cdot \ln 2/t_{1/2} \), where \( V_{PAH} \) is the apparent volume of distribution of PAH. The absorption rate constant \( (k_d) \) for the in situ intestinal absorption was obtained from the slope of a plot of the logarithmic drug concentration in perfusate against time (the slope $= -k_d/2.303$).

Results are expressed as the mean ± S.D. Statistical analysis was performed by the non-paired Student's \( t \)-test and a \( p \)-value of 0.05 or less was considered significant.

RESULTS

Plasma Concentrations of Dexamethasone after Single and Repeated i.v. Administrations of Dexamethasone Phosphate

The semilogarithmic plasma level data of dexamethasone following a single and repeated \( i.v. \) administrations were plotted up to 10 h after dosing. As shown in Fig. 1, the plasma decay curve of dexamethasone indicated monoeXponential kinetics, and the pharmacokinetic parameters were therefore analyzed according to the one-compartment open model. In the single \( i.v. \) dosing studies, the \( k_{eb} \), \( V \) and \( AUC \) were 0.311 ± 0.056 h\(^{-1}\), 1.19 ± 0.23 l/kg, and 4.43 ± 0.81 \( \mu \)g h/ml, respectively, whereas in the repeated \( i.v. \) dosing those were 0.256 ± 0.017 h\(^{-1}\), 1.06 ± 0.31 l/kg and 5.67 ± 1.07 \( \mu \)g h/ml, respectively. There was no significant difference in these parameters between both treatment, although slightly higher plasma levels were shown in the repeated treatment.

Plasma Concentrations of Dexamethasone after Single and Repeated Oral Administrations of Dexamethasone Phosphate Alone or in Combination with Dicyclomine

As shown in Fig. 2 (A), the plasma concentrations of dexamethasone after a single oral treatment with dexamethasone phosphate alone or in combination with dicyclomine also declined according to a single first-order function. In the presence of dicyclomine, the plasma concentrations of dexamethasone were significantly reduced compared with those after dexamethasone alone, showing no alteration in the elimination of dexamethasone. Some pharmacokinetic parameters calculated from these data are listed in Table I. It is noted that coadministration with dicyclomine produced a significant decrease in the \( AUC \) for dexamethasone. The plasma concentrations of dexamethasone following the repeated oral administrations of the drug with or without dicyclomine are depicted in Fig. 2 (B).

![Fig. 1. Semilogarithmic Plots of Plasma Dexamethasone Concentration after Single or Repeated i.v. Administration of Dexamethasone Phosphate](image-url)
Mean plasma concentrations of dexamethasone after coadministration with dicyclomine were significantly lowered at all sampling points compared with those after dexamethasone alone, without significant change in the $k_{el}$ and $t_{1/2}$ between the two groups as summarized in Table II. The decreased plasma levels after coadministration with dicyclomine agreed well with the result of the single administration (Fig. 2 (A)). The AUC for dexamethasone in the coadministration group was 54.8% of that after dexamethasone alone ($p < 0.01$) and the decrease was more extensive than that (35.4%) after a single administration.

**In Situ Intestinal Absorption of Dexamethasone Phosphate after Repeated Treatment with the Corticoid Alone or in Combination with Dicyclomine**

In order to clarify the effect of repeated treat-

![Graph showing plasma concentration over time](image)

**FIG. 2. Semilogarithmic Plots of Plasma Dexamethasone Concentration after Single and Repeated Oral Administrations of Dexamethasone Phosphate Alone or in Combination with Dicyclomine**

Dose; dexamethasone phosphate, 4 mg/kg and dicyclomine, 4 mg/kg body weight. (A) single, (B) repeated, the animals were treated for 7 d with every other day dosing of dexamethasone phosphate alone or with dexamethasone phosphate and daily dosing of dicyclomine. After the final treatment or a single dosing, blood was collected at arbitrary intervals and dexamethasone in plasma was determined. ●: dexamethasone alone, ○: dexamethasone-dicyclomine. Each point represents the mean ± S.D. of 4 rats. a) $p < 0.001$, b) $p < 0.01$ and c) $p < 0.05$ respectively compared with dexamethasone alone.

**TABLE I. Pharmacokinetic Parameters after a Single Oral Administration of Dexamethasone Phosphate Alone or in Combination with Dicyclomine**

<table>
<thead>
<tr>
<th></th>
<th>$K_{el}$ (h$^{-1}$)</th>
<th>$t_{1/2}$ (h)</th>
<th>AUC (µg·h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone alone</td>
<td>0.258 ± 0.035</td>
<td>2.732 ± 0.410</td>
<td>10.436 ± 0.351</td>
</tr>
<tr>
<td>Dexamethasone-dicyclomine</td>
<td>0.269 ± 0.072</td>
<td>2.698 ± 0.630</td>
<td>6.823 ± 0.937$^a$</td>
</tr>
</tbody>
</table>

*Each value represents the mean ± S.D. of 4 rats. a) $p < 0.001$ compared with dexamethasone alone.*
ment with dexamethasone phosphate and dicyclomine on the intestinal absorption, the in situ intestinal absorption of dexamethasone phosphate was estimated. In the group treated with both dexamethasone phosphate and dicyclomine, the $k_a$ (0.135 ± 0.018 h$^{-1}$) was significantly decreased compared with that (0.301 ± 0.096 h$^{-1}$) in dexamethasone alone ($p < 0.05$). Thus, the fraction of drug absorbed for 1 h was also significantly reduced from 25.14 ± 7.82% for the dexamethasone group to 14.77 ± 1.60% for the coadministration group ($p < 0.05$).

**ICG and PAH Clearance after Repeated Treatment with Dexamethasone Phosphate Alone or in Combination with Dicyclomine**

Hepatic and renal clearance in the rats pre-treated with dexamethasone alone or in combination with dicyclomine were approximately measured using ICG and PAH, respectively. The plasma decline curves of ICG and PAH are shown in Fig. 3. There was no statistically significant difference in hepatic clearance of ICG between the both groups (13.97 ± 1.78 ml/min/kg for dexamethasone alone and 13.43 ± 1.62 ml/min/kg for the combination). The RPF (36.07 ± 5.9 ml/min·kg) in the group coadminis-

**TABLE II. Pharmacokinetic Parameters of Dexamethasone after Repeated Oral Administration of Dexamethasone Phosphate or in Combination with Dicyclomine**

<table>
<thead>
<tr>
<th></th>
<th>$K_{el}$ (h$^{-1}$)</th>
<th>$t_{1/2}$ (h)</th>
<th>$AUC$ (µg·h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone alone</td>
<td>0.238 ± 0.019</td>
<td>2.928 ± 0.226</td>
<td>11.960 ± 1.667</td>
</tr>
<tr>
<td>Dexamethasone-dicyclomine</td>
<td>0.250 ± 0.025</td>
<td>2.781 ± 0.234</td>
<td>6.554 ± 0.845a)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 3—5 rats. a) $p < 0.01$ compared with dexamethasone alone.

**FIG. 3. Semilogarithmic Plots of Plasma Concentration after a Single i.v. Injection of p-Aminohippuric Acid or Indocyanine Green to Rats Treated Repeatedly with Dexamethasone Phosphate Alone or in Combination with Dicyclomine**

- p-Aminohippuric acid (30 mg) (A) or indocyanine green (5 mg/kg) (B) were injected 2 h after administration of drug. ●; dexamethasone alone, ○; dexamethasone-dicyclomine. Each point represents the mean ± S.D. of 3 rats.
tered with dicyclomine was significantly higher than that (26.72 ± 1.32 ml/min · kg) for dexamethasone alone (p < 0.05), as shown in Table III.

**Biliary Excretion of Dexamethasone after Repeated Treatment with its Phosphate Alone or in Combination with Dicyclomine**

The biliary excretion of dexamethasone and bile volume were estimated after repeated treatment with dexamethasone phosphate alone or in combination with dicyclomine. There was a slight but significant difference in the cumulative bile volume for 6 h between the two groups (6.05 ml for dexamethasone alone and 4.72 ml for the combination (p < 0.05)). When compared with the dexamethasone group, the coadministration with dicyclomine significantly lessened the biliary dexamethasone concentrations and consequently the amount of drug excreted in bile (Fig. 4), of which the major part (>96%) existed as the conjugate form. A marked reduction in the biliary excretion rate (dAe/dt, where A e is the cumulative amount of drug excreted) was also observed in the dicyclomine treated group compared with that in the group of dexamethasone alone as shown in Fig. 4, in which the decrease was almost the same as the decreased plasma concentrations of dexamethasone following coadministration with dicyclomine (compare with Fig. 2 (B) and Table II).

**DISCUSSION**

Dexamethasone phosphate is known to produce its pharmacological action after being converted to free dexamethasone in the body. Kitagawa et al. showed that the hydrolysis of prednisolone phosphate ester is extremely rapid in rats, rabbits and dogs, with peak plasma free steroid levels occurring almost immediately following i.v. injection of the ester. Their observation is in accord with our finding that peak plasma dexamethasone levels were obtained immediately following i.v. doses of dexamethasone phosphate ester (Fig. 1). The monoexponential decline in plasma dexamethasone levels and the close similarity in the k d values following i.v. and oral doses of dexamethasone phosphate indicate that the ester is also rapidly hydrolyzed after oral dosing. The AUC after a single i.v. administration (2 mg/kg) was approximately equal to that, normalized by the dose, after a single oral dosing (4 mg/kg) (Tables I and II), suggesting that the absorption of dexamethasone phosphate following oral dosing is complete.

The present study showed that the AUC for dexamethasone following coadministration with dicyclomine in both single and repeated oral dosing schedules was significantly decreased compared with those of dexamethasone alone (Tables I and II). This result suggests that dicyclomine coadministered substantially inhibits the intestinal absorption of dexamethasone phosphate. Thus, to further clarify the inhibition of absorption, the *in situ* intestinal absorption of dexamethasone phosphate was estimated after repeated treatment with the drugs. The absorption study confirmed well that the coadministra-

**Table III. RPF Values and Renal Function after Repeated Oral Administration of Dexamethasone or in Combination with Dicyclomine**

<table>
<thead>
<tr>
<th></th>
<th>K e (h⁻¹)</th>
<th>V d (dl · kg⁻¹)</th>
<th>RPF (ml/min · kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone alone</td>
<td>0.051 ± 0.004</td>
<td>5.295 ± 0.683</td>
<td>26.72 ± 1.32</td>
</tr>
<tr>
<td>Dexamethasone-dicyclomine</td>
<td>0.062 ± 0.007 a)</td>
<td>5.885 ± 0.985</td>
<td>36.07 ± 5.90 a)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 3—5 rats. a) p < 0.05 compared with dexamethasone alone.
tion with dicyclomine caused the evident decrease in rate and amount of dexamethasone phosphate absorbed. Considering changelessness in elimination rate of dexamethasone after coadministration with dicyclomine (Tables I and II), a dramatic decrease in the AUC following coadministration could be ascribed to the reduction in intestinal absorption of dexamethasone phosphate, probably due to a nonspecific direct relaxant action of dicyclomine on intestinal smooth muscle.\textsuperscript{11}

The absorption rate constant $k_a$, obtained

FIG. 4. Biliary Excretion of Dexamethasone after Final Administration in Repeated Oral Treatment of Dexamethasone Phosphate Alone or in Combination with Dicyclomine

The animals were treated for 7 d (A) with every other day dosing of dexamethasone phosphate (4 mg/kg) alone or (B) with dexamethasone phosphate and daily dosing of dicyclomine (4 mg/kg). At 30 min after the final treatment, bile samples were collected from the common bile duct cannulated with a polyethylene tube. (C) Semilogarithmic plots of biliary excretion rate. $Ae$: the cumulative amount of dexamethasone excreted. $\bullet$ : dexamethasone alone, $\bigcirc$ : dexamethasone-dicyclomine. Each value represents the mean $\pm$ S.D. of 4 rats. a) $p < 0.05$ and b) $p < 0.01$ respectively compared with dexamethasone alone.
from the in situ intestinal absorption experiment, in dexamethasone–dicyclomine group was about one half of that in dexamethasone group. The observation strengthens the notion that the decreased AUC in the coadministration group is mainly due to the decline of intestinal absorption of the corticoid.

Judging from the clearance of PAH, it was found that the renal plasma flow after repeated oral dose with dexamethasone phosphate and dicyclomine was significantly enhanced, from 26.72 to 36.07 ml/min·kg, compared with that of dexamethasone group. This may be due to the vasodilation based on the decrease in spasm of renal blood vessel by dicyclomine, although the action of the drug on kidney is not reported. On the other hand, the RPF value in the group administered with dexamethasone alone was approximately similar to that of normal control rat (25.0 ml/min·kg). This suggests that no alteration of renal plasma flow is caused by the repeated oral administrations of dexamethasone phosphate alone. The increased renal plasma flow after coadministration appears to little affect the plasma clearance of dexamethasone. This would be explained by that only 2.6% of the dose was excreted in urine as the unchanged drug for 24 h after dosing, and the elimination of unchanged dexamethasone therefore would be less responsible to the change of renal blood flow. In rat, corticosteroids are largely metabolized in the liver and excreted into feces in contrast to humans in which the drugs are mainly excreted into urine. In order to investigate the effect of dicyclomine on dexamethasone handling in liver, hepatic clearance of ICG and biliary excretion of dexamethasone were estimated separately after repeated oral administration of dexamethasone phosphate with or without dicyclomine. The fact that the hepatic clearance of ICG was not significantly different between dexamethasone plus dicyclomine and dexamethasone alone suggests that dicyclomine may not induce the extreme alteration of liver function. However, a marked reduction in the biliary excretion rate and cumulative amount of the drug excreted was observed after coadministration. This decreased elimination (44% reduction, Fig. 4) of dexamethasone into bile was approximately the same as the decreased amount (45% reduction of AUC, Table II) of the drug in the systemic circulation following coadministration with dicyclomine. Thus, the reduction of hepatic uptake of dexamethasone by dicyclomine is largely due to the decreased plasma concentration, and it is unlikely that dicyclomine affects the liver function such as conjugate formation based on the result obtained.

In conclusion, the present study indicated that oral administration of dicyclomine, cholinergic blocking agent, induced the decrease in the absorption rate and in the amount of dexamethasone absorbed, consequently resulting in the decreased AUC for dexamethasone. Additionally, dicyclomine produced the reduction in biliary excretion of dexamethasone in proportion to the decline of its plasma level. On the other hand, the pharmacokinetic parameters were scarcely influenced by dicyclomine, despite the fact that renal plasma flow was significantly enhanced after coadministration with dicyclomine. Therefore, dicyclomine should be administered taking much care in the treatment with corticosteroids.

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