EVALUATION OF ORALLY ADMINISTERED ACTIVATED CHARCOAL ON INTESTINAL DIALYSIS OF INTRAVENOUSLY ADMINISTERED M79175, AN ALDOSE REDUCTASE INHIBITOR, IN RATS

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The effect of oral administration of activated charcoal on serum elimination of $^{14}$C-M79175 injected intravenously in rats was studied. In situ single-pass perfusion studies showed that M79175 and/or its metabolites was transported into the small intestinal lumen. Total radioactivity expressed as equivalents of M79175 exsorbed in the perfusate and excreted in the bile juice was 3.2% and 2.0%, respectively, of dose. Oral administration of multiple doses of activated charcoal significantly decreased the serum concentration and serum half-life of $^{14}$C-M79175. The fecal excretion of $^{14}$C-M79175 after treatment with activated charcoal was increased when compared to that in the control. On the other hand, urinary excretion of $^{14}$C-M79175 after treatment with activated charcoal was decreased. However, there was no significant difference in the cumulative amounts of total excretion (faecal plus urinary excretion) between rats with activated charcoal treatment and rats without charcoal treatment. These results suggest that intestinal dialysis by oral activated charcoal is a reasonable method to enhance the elimination of M79175 from the serum in case of overdose of the drug.

Keywords — activated charcoal; M79175; exsorption rate; biliary excretion; fecal excretion; urinary excretion; intestinal dialysis; gastrointestinal tract; serum half-life

INTRODUCTION

A variety of diabetic ocular complications which include retinopathy, cataract and corneal epitheliopathy, have been proposed to be due to the accumulation of sorbitol. Under conditions of high plasma glucose concentrations in diabetes mellitus, excess glucose is converted to sorbitol by aldose reductase. Several drugs including sorbinil, ONO-2235 and alrestatin are aldose reductase inhibitors which decrease the accumulation of sorbitol, leading to improvement of these diabetic complications.

A new potent inhibitor of aldose reductase, M79175 [(2R, 4S) 6-fluoro-2-methyl-spiro[chroman-4,4′-imidazolidine]-2′,5′-dione], has been reported to be effective in delaying the development of cataract formation and the other diabetic complications of the cornea in diabetic rats. Because of the drug’s extended elimination half-life, its pharmacological effects are of long duration. However, an overdose of the drug can cause adverse effects due to the prolonged period it remains in the body. It is therefore essential to find a method to remove the drug to prevent drug poisoning.

It is well known that activated charcoal prevents the absorption of various drugs from the gastrointestinal (g.i.) tract and in some cases increases the rates of elimination of drugs from blood. Recently, it has been noted that the clearance of intravenously administered drugs is accelerated by orally administered charcoal. We have also demonstrated that fecal excretion of polychlorinated dibenzofuran was promoted by oral doses of activated charcoal in rats. These findings suggest that activated charcoal can remove the drugs, which have been parenterally administered or have already been absorbed into the systemic circulation, from the g.i. tract. We have previously demonstrated that intravenously administered theophylline, phenobarbital and phenytoin were transported into the small intestinal lumen (exsorption) to a significant extent and into the bile juice (biliary excretion) to a lesser extent in rats. The mechanism by which the elimination of intravenously administered drugs was accelerated by orally administered activated charcoal was the adsorption of these drugs which were secreted into the g.i. tract by activated charcoal.

The present study was designed to further investigate the characteristics of exsorption and/or
excretion of M79175 into the gastrointestinal tract, and to evaluate whether an exsorbed drug can be removed by adsorption by orally administered activated charcoal.

As for the metabolism of M79175 in rats, total M79175 and its metabolites excreted in 24 h was 82% as unchanged M79175, 11% as metabolite 1 (2-hydroxy derivative) and 5% as metabolite 2 (unidentified) in the urine and 50% as unchanged M79175, and minor amounts as metabolites 1 and 2 and the rest as polar compounds in the bile.

MATERIALS AND METHODS

Materials — Both unlabelled and 14C-labelled M79175 (specific activity 25.2 Ci/mol) (2R, 4S)-6-fluoro-2-methyl-spiro [chroman-4,4′-imidazolidine]-2′,5′-dione (whose structure is shown in Fig. 1) were supplied by Eisai Co., Tokyo. Scintillation fluids (Instagel®) and solubilizer (Soluene-350®) were products of United Technologies Packard, USA. Activated charcoal was a product of Inuhinode Seiyaku Co., Osaka and the particle size used in this study was less than 62 μm (250 mesh). All other chemicals used in this study were of analytical grade.

Exsorption Study — Intestinal exsorption experiments were conducted by the in situ single-pass perfusion technique as previously reported. Male rats of Sprague-Dawley strain, weighing 180–330 g, were fasted overnight with free access to water. Under anesthesia with ether, 14C-M79175 at a dose of 0.2 mg/kg was administered intravenously via the caudal vein. In the case of treatment with activated charcoal, the activated charcoal suspension in water (150 mg/ml) was administered orally with an initial dose of 300 mg at time zero and additional doses of 150 mg each at 1, 2, 3, 4, 6, 10 and 24 h after the intravenous (i.v.) administration of 14C-M79175. No suspending agent was employed and the suspension was administered soon after shaking. In the case of the control experiment, water corresponding in volume to the volume of activated charcoal used was administered orally at each dose time. During these periods, the animals were kept in metabolic cages. Blood samples were collected periodically from a cut at the tip of the tail. Urine and feces were collected separately at various time intervals for a period of 96 h. The urine was diluted with water to 50 ml prior to assay and fecal material was weighed after drying at room temperature and crushed in a mortar with a pestle.

Analytical Method — Aliquots of serum (10–50 μl) were solubilized in 0.75 ml of Soluene-350®/isopropanol (1/1) and decolorized with 50 μl of hydrogen peroxide (30%). After standing overnight, total serum radioactivity was measured after adding 5 ml of Instagel® scintillation fluid. Aliquots of perfusate (1 ml) and urine (0.1–1 ml) were counted directly in 15 ml and bile (10–50 μl) in 5 ml of Instagel®. Aliquots of feces (20 mg) moistened with water (0.1 ml) were solubilized in 1 ml of Soluene-350® for 10 h at 50 °C. After cooling,
0.5 ml of isopropanol and 0.4 ml of hydrogen peroxide (30%) were added for decolorization and the resultant solution was incubated for 2 h at 50 °C. The samples were counted for radioactivity after adding 15 ml of Instagel® scintillation fluid. The results were corrected for background radioactivity. Average recoveries of about 95% and 74% were obtained when known amounts of 14C-M79175 were added to blank feces and feces-charcoal (1:1) mixture, respectively.

Pharmacokinetic Analysis — The time-course of serum levels of 14C-M79175 was fitted to a two compartment open model by a least square regression analysis using the following equation, 
\[ C = Ae^{-\alpha t} + Be^{-\beta t} \]
where \( C \) is the serum drug concentration and \( A \), \( B \), \( \alpha \) and \( \beta \) are hybrid parameters. Elimination half-life was determined from the relationship \( t_{1/2\beta} = 0.693/\beta \). The area under the serum concentration-time curve (AUC) was calculated by the trapezoidal rule and was extrapolated to infinity. Total body clearance (CLtot) was determined by: 
\[ CL_{\text{tot}} = \frac{\text{Dose}}{\text{AUC}} \]
The mean residence time in the systemic circulation (MRT) was calculated by: 
\[ MRT = \frac{\text{AUMC}}{\text{AUC}} \]
where AUMC is the area under the first moment curve. The steady-state volume of distribution (Vss) was calculated by: 
\[ V_{\text{ss}} = \frac{\text{Dose} \cdot \text{MRT}}{\text{AUC}} \]
The unpaired t test was used to assess the effect of charcoal treatment of the pharmacokinetic parameters.

RESULTS
Transport of M79175 into Intestinal Lumen

Figure 2 shows the concentrations of M79175 as equivalents in the serum and the bile juice, and the exsorption rate of the drug into the perfusate isotonic phosphate buffer, pH 6.0, following i.v. administration of 14C-M79175 to rats at the dose of 0.2 mg/kg by the in situ single-pass perfusion technique. Radioactivity was detected in the perfusate which indicated that M79175 and/or its metabolites were exsorbed into the small intestinal lumen. The exsorption rate of total radioactivity into the perfusate was decreased as the concentrations of total radioactivity in the serum and the bile juice were decreased. The average amount of M79175 as equivalents exsorbed into the perfusate in 120 min was 3.2% of dose. On the other hand, total radioactivity in bile after i.v. administration of 14C-M79175 was approximately ten-fold as high as serum levels.

FIG. 2. The Concentrations in the Serum and the Bile Juice and Exsorption Rate into the Perfusate (Isotonic Phosphate Buffer, pH 6.0) of M79175 after i.v. Administration of 14C-M79175 (0.2 mg/kg) to Rats

Each point represents the mean ± SEM of 5 rats.

FIG. 3. Serum Levels of M79175 after i.v. Administration of 14C-M79175 (0.2 mg/kg) to Rats with or without Treatment with Activated Charcoal

Each point represents the mean ± SEM of 6 (no charcoal treatment) or 8 (treatment with charcoal) rats. a) \( p < 0.01 \); b) \( p < 0.05 \).

Key: ○, no charcoal treatment; ●, treatment with charcoal.
TABLE I. Pharmacokinetic Parameters of M79175 after i.v. Administration of $^{14}$C-M79175 (0.2 mg/kg) to Rats with or without Treatment with Activated Charcoal

<table>
<thead>
<tr>
<th></th>
<th>$AUC_{0-\infty}$ (h·μg/ml)</th>
<th>MRT (h)</th>
<th>$Cl$ (l/h/kg)</th>
<th>$t_{1/2\beta}$ (h)</th>
<th>$V_{dss}$ (l/kg)</th>
</tr>
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<tbody>
<tr>
<td>Control, $n=6$</td>
<td>8.83</td>
<td>40.4</td>
<td>0.027</td>
<td>30.2</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>±1.35</td>
<td>±7.12</td>
<td>±0.004</td>
<td>±2.58</td>
<td>±0.34</td>
</tr>
<tr>
<td>Treatment</td>
<td>4.73 $^b$</td>
<td>28.6</td>
<td>0.053 $^c$</td>
<td>16.1 $^a$</td>
<td>1.79</td>
</tr>
<tr>
<td>with activated</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>charcoal, $n=8$</td>
<td>±1.01</td>
<td>±5.30</td>
<td>±0.011</td>
<td>±1.97</td>
<td>±0.63</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM of 6 or 8 rats. $^a$) $p < 0.01$; $^b$) $p < 0.05$; $^c$) $p < 0.10$.

Thus, the results suggested that the drug was excreted into the intestinal lumen via the bile tract to an appreciable extent. The average amount of the drug as equivalents excreted into the bile juice in 120 min was 20% of dose. These results suggested that a considerable amount of M79175 was transported into the g.i. tract via mucosal membrane or the bile tract.

Effect of Activated Charcoal on M79175 Clearance

Figure 3 shows the time course of total radioactivity as equivalents of M79175 levels after i.v. administration of 0.2 mg/kg dose of $^{14}$C-M79175 to rats with or without oral activated charcoal treatment. The serum radioactivity declined exponentially for 96 h after i.v. administration. As shown in Fig. 3, the oral administration of multiple doses of activated charcoal reduced the M79175 levels, particularly in the $\beta$-phase as compared with the control treatment. The mean serum level at 48 h was significantly decreased from 31.2 to 12.9 ng eq/ml by multiple doses of activated charcoal.

Pharmacokinetic parameters following i.v. administration of $^{14}$C-M79175 with or without activated charcoal are shown in Table I. It was observed that oral administration of multiple doses of activated charcoal significantly decreased $t_{1/2\beta}$ and $AUC$ by 53% and 54%, respectively. Although oral administration of activated charcoal tended to decrease MRT and to increase $Cl_{tot}$, there was no significant difference ($p > 0.05$) between the treatments in these parameters because of their marked intersubject variations. The volume of distribution was not significantly different between the two treatments.

To confirm that the enhanced elimination of the drug by oral activated charcoal after i.v. administration of M79175 was due to adsorption of the drug, which was desorbed into the g.i. tract, by the charcoal, urinary and fecal excretions of total radioactivity after i.v. administration of $^{14}$C-M79175 were investigated. Figures 4 and 5 show the rate of fecal and urinary excretion of radioactivity, respectively, after i.v. administration of $^{14}$C-M79175 to rats with or without treatment with multiple doses of oral activated charcoal. It was observed that the fecal excretion rate of total radioactivity as equivalents of M79175 with oral activated charcoal

![Excretion rate of M79175 in Feces after i.v. Administration of $^{14}$C-M79175 (0.2 mg/kg) to Rats](image-url)

Each bar represents the mean ± SEM of 5 rats. $^a$) $p < 0.01$; $^b$) $p < 0.05$.

Key: □□□□□ treatment with charcoal; □ no charcoal treatment.
FIG. 5. Excretion Rate of M79175 in Urine after i.v. Administration of $^{14}$C-M79175 (0.2 mg/kg) to Rats. Each bar represents the mean ± SEM of 5 rats. a) $p < 0.05$

Key: ■ treatment with charcoal; □ no charcoal treatment.

FIG. 6. Urinary and Fecal Excretion Curves of M79175 after i.v. Administration of $^{14}$C-M79175 (0.2 mg/kg) to Rats. Each point represents the mean ± SEM of 5 rats. a) $p < 0.01$; b) $p < 0.05$.

Key: No charcoal treatment; □ feces, Δ urine; ○ feces plus urine.

Treatment with charcoal: ■ feces; ▲ urine; ● feces plus urine.

treatment was significantly larger than that in the control, particularly in the initial stages. The fecal excretion rates of the drug in 24 and 48 h with activated charcoal treatment were about twice those in the control. This result suggests that a considerable amount of M79175 or its metabolites, which was adsorbed by activated charcoal, was excreted in the feces with activated charcoal, which prevented their reabsorption.

On the other hand, as shown in Fig. 5, the urinary excretion rate of total radioactivity as equivalents of M79175 with activated charcoal treatment was significantly smaller than that in the control during the 96 h period. These results suggested that the drug in systemic circulation which is eliminated from the body by renal route is decreased by oral administration of activated charcoal.

Figure 6 shows the cumulative urinary and fecal excretion of total radioactivity as equivalents of M79175 after i.v. administration of 0.2 mg/kg dose of $^{14}$C-M79175 to rats with or without oral activated charcoal treatment. It was shown that the fecal excretion of total radioactivity with activated charcoal treatment was markedly increased as compared with that in the control. The cumulative amounts of the radioactivity excreted in feces in 96 h with or without charcoal were 36% and 19%, respectively. On the other hand, the urinary excretion of total radioactivity with activated charcoal treatment was significantly decreased as compared with that in the control. The cumulative amounts of the radioactivity excreted in urine in 96 h with and without charcoal are 28% and 41%, respectively. However, there were no significant differences in the amounts of total excretion (fecal plus urinary excretion) between both treatments. These results strongly indicated that the enhanced excretion of the drug by oral administration of activated charcoal is due to prevention of reabsorption and acceleration of exsorption of the drug by charcoal.

DISCUSSION

Our results indicated that multiple oral doses of activated charcoal decrease the serum concentration, $t_{1/2B}$ and $AUC$ and tended to increase the clearance of the i.v. administered drug. The mechanism was confirmed to be adsorption of the drug secreted into the g.i. tract to the charcoal by in situ exsorption studies. We have previously shown that the fraction of exsorption of theophylline, phenobarbital and pheny-
toin⁵ into the perfusate was about 12%, 6.5% and 1.1%, respectively and the fraction of excretion into the bile juice of these drugs was less than 1.0% of 10 mg/kg dose in 2 h, and furthermore that the clearance of phenytoin was affected only slightly by oral activated charcoal whereas the clearance of both theophylline and phenobarbital was enhanced by oral charcoal.

In general, it has been shown that the exsorption of drugs through the intestinal membrane varies enormously, depending upon the nature of the drug, such as the extent of binding to serum protein, volume of distribution, lipophilicity and molecular size of the drug. The lack of effect of activated charcoal on clearance of phenytoin may be due to the inability of the drug to be excreted into the g.i. tract. For exsorption, only the unbound or free drug can permeate through capillary walls and phenytoin is more highly (about 90%) bound to serum protein than theophylline (about 60%)⁵¹, phenobarbital (about 60%)⁵² or M79175 (about 50%).⁶ Thus, it is considered that the amount of the drug transported into the g.i. tract plays an important role in intestinal dialysis. Although the relative amount of M79175 exsorbed and/or excreted into the g.i. tract was smaller than those of theophylline and phenobarbital, our observation shows that the serum level of M79175 was significantly reduced by activated charcoal.

There are several reports which may provide likely explanation for the effect. For example, Park et al.²⁳ have reported that activated charcoal has relatively little effect in increasing digoxin elimination in normal subjects who have short digoxin half-lives but has a significant effect in increasing digoxin elimination in renal failure subjects and increasing elimination of digitoxin which exhibits a long half-life. These authors suggested that the reason for the difference in the effect by charcoal might be due to the much smaller apparent volume of distribution in digitoxin (0.5 l/kg) than that in digoxin (7 l/kg) and/or the lower endogenous clearance of digitoxin (0.2 l/h vs. 17 l/h for digoxin). Goldberg et al.²⁴ have also reported that the oral administration of multiple doses of activated charcoal had no effect on the serum half-life or systemic clearance of imipramine and that the lack of effect is due to the very large volume of distribution in imipramine.

Our data showed longer $t_{1/2\beta}$ (30 h) and smaller $V_{\text{dss}}$ (1.22 l/kg) and $Cl$ (0.027 l/h/kg) values for M79175 than for those values in digoxin and imipramine. Therefore, in view of these parameters of M79175, it is suitable to perform intestinal dialysis by oral administration of activated charcoal. That is, exsorption of the drug from blood into the g.i. tract is increased because M79175 levels in systemic circulation were considerably higher than those in the fluid of the g.i. lumen. Furthermore, since the total clearance of a drug during treatment with activated charcoal is the sum of the endogenous clearance and the clearance through the g.i. tract by charcoal, the percentage change in total body drug clearance caused by the charcoal will be increased as the endogenous drug clearance (by metabolism or renal elimination) is decreased if activated charcoal produces a constant intestinal drug clearance.

In conclusion, it was confirmed that the oral administration of activated charcoal can enhance the elimination of M79175 from blood even if the drug has already been absorbed into the systemic circulation from the g.i. tract. Thus, intestinal dialysis by oral administration of activated charcoal is a reasonable method to shorten the period of intoxication in patients with increased serum drug concentrations.

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REFERENCES


6) Unpublished data in animals, Eisai Co. Tokyo.