Effect of Simultaneous Administration of Drugs on Absorption and Excretion. VIII. Effect of Plasma-Protein Binding Displacement on the Intestinal Absorption of Sulfonamides in Rabbits

YORISIGE IMAMURA and HISASHI ICHIBAGASE

Faculty of Pharmaceutical Sciences, Kumamoto University

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Effect of plasma-protein binding displacement on the intestinal absorption of sulfonamides in rabbits was investigated by using salicylic acid and phenylbutazone as displacing drugs. Salicylic acid and phenylbutazone significantly reduced the in situ intestinal absorption of sulfadimethoxine that showed high binding to plasma proteins. These two displacing drugs also enhanced the in situ intestinal exsorption of sulfadimethoxine. However, salicylic acid and phenylbutazone showed no significant effect in the transport of sulfadimethoxine from mucosal to serosal side solution through the intestinal membrane or the uptake of sulfadimethoxine by the intestinal preparation in vitro. In addition, salicylic acid and phenylbutazone did not affect the in situ intestinal absorption and exsorption of sulfanilamide that showed little binding to plasma proteins. From these results, it is concluded that the displacement of one drug from its plasma-protein binding sites by another drug is an important determinant affecting drug absorption.

Keywords—intestinal absorption; plasma-protein binding; sulfonamides; salicylic acid; phenylbutazone; displacement; rabbit

It has been known that the intestinal absorption of drugs is influenced by factors related to the physiological conditions of experimental animals such as gastric emptying and intestinal

2) Location: 5-1 Ot-honmachi, Kumamoto, 862, Japan.
motility or by factors concerning the physiochemical properties of drugs such as lipid solubility and stability. For example, Nimmo, et al.5) have revealed that the gastric emptying modified by the concurrent administration of metoclopramide or propantheline affects the intestinal absorption of acetaminophen. Schanker6) has pointed out the importance of lipid solubility in barbiturates absorption.

The previous experiments in our laboratory9) have demonstrated that salicylic acid decreases the permeation rate of sulfadimethoxine and sulfamethoxypyridazine from internal to external solution through a cellulose membrane when the external solution contains bovine serum albumin, and that the decreasing effect of salicylic acid is due to its displacing activity. These results suggest that the displacement of one drug from its plasma-protein binding sites by another drug may become one of important factors affecting drug absorption.

In the present paper, to elucidate a role of plasma-protein binding in the intestinal absorption of drugs, the effect of typical displacing drugs such as salicylic acid and phenylbutazone1,6,7) on the absorption of sulfonamides from the rabbit small intestine was investigated by use of in situ and in vitro recirculating perfusion techniques.

Experimental

Materials—Sulfonamides, salicylic acid and phenol red were obtained from commercial sources. Phenylbutazone was kindly supplied by Fujisawa Pharmaceutical Industry Co., Ltd., Osaka, Japan. All drugs were used without further purification. Phenol red was used for the in situ intestinal absorption experiment as a volume change indicator.

Animals—Male rabbits weighing 2.5—3.3 kg were fasted for about 24 hours prior to experiments, but drinking water was allowed ad libitum.

In Situ Intestinal Absorption Experiment9)—A male rabbit was anesthetized with urethane (1.2 g/kg, i.p.), and the small intestine was exposed by a midline abdominal incision. Two glass cannulae were inserted through small slits at the upper and lower jejunal portion. The intestine was flushed with saline solution maintained at 37°C. The length of the intestine used was 40 cm. The outflow and inflow glass cannulae were connected with rubber tubes to a flask containing 150 ml of drug solution which was prepared by dissolving sulfonamides (200 µg/ml) and phenol red (5 µg/ml) in pH 7.4 isotonic phosphate buffer solution and a perfusion pump, respectively. The drug solution was recirculated at the rate of 30 ml/min through the small intestine at 37°C. Aliquots were pipetted out at periodical intervals.

In Vitro Intestinal Absorption Experiment—In vitro intestinal absorption experiment was carried out according to a recirculating perfusion technique. A Wiseman type apparatus reformed by Kojima, et al.9,10) was used for this experiment. A male rabbit was anesthetized with urethane, and the small intestine was removed carefully. The intestine was cut to give a length which required slight stretching to reach the lower projection from upper one of the apparatus. The end of intestinal segment was tied on the projection with ligature. The length of the intestine used was 20 cm. The upper chamber was filled with 50 ml of drug solution which was prepared by dissolving sulfadimethoxine (200 µg/ml) in pH 7.4 Krebs-Ringer phosphate buffer solution, and the lower chamber was filled with 220 ml of pH 7.4 Krebs-Ringer phosphate buffer solution, and then the apparatus was kept in a water-bath at 37°C. The drug solution was recirculated through the small intestine. Aliquots were pipetted out at periodical intervals.

In Situ Intestinal Exsorption Experiment—In situ intestinal exsorption experiment was carried out according to the single-pass perfusion technique of Kakemi, et al.11) The procedure of operation was the same as that for the in situ intestinal absorption experiment. The small intestine was perfused with pH 7.4 isotonic phosphate buffer solution at the rate of 7 ml/min at 37°C, and then sulfonamides were administered intravenously to the rabbit with dose of 50 mg/kg. The perfusate was collected every 5 minutes, and the exsorption rate of sulfonamides was calculated from the amount of the drugs in the perfusate.

9) G. Wiseman, J. Physiol., 120, 63 (1953).
Analytical Methods—Sulfonamides were analyzed spectrophotometrically by the method of Bratton and Marshall. Phenol red was analyzed spectrophotometrically after alkalization by adding 1 N sodium hydroxide solution.

Results and Discussion

Effect of Salicylic Acid and Phenylbutazone on the in Situ Intestinal Absorption of Sulfonamides in Rabbits

Tables I and II show the effect of salicylic acid and phenylbutazone on the absorption of sulfonamides from the rabbit small intestine. Salicylic acid and phenylbutazone administered intravenously 5 minutes before the beginning of the in situ intestinal absorption experiment. Since semilogarithmic plots of percentage of sulfonamides remaining in the rabbit small intestine versus time were linear during 150 minutes, the absorption rate constants of the drugs were calculated from the slope of the straight lines. As can be seen from Table I, salicylic acid and phenylbutazone significantly decreased the absorption rate of sulfadimethoxine that showed high binding to plasma proteins. On the other hand, as can be seen from Table II, these displacing drugs did not affect the absorption rate of sulfanilamide that showed little binding to plasma proteins. Furthermore, as shown in Fig. 1, salicylic acid and phenylbutazone were found to decrease the absorption rate of sulfadimethoxine from the rabbit small intestine when these displacing drugs were administered intravenously 60 minutes after the beginning of the in situ intestinal absorption experiment. These findings suggest that the plasma-protein binding may be an important determinant for the absorption of sulfonamides from the rabbit small intestine.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Absorption rate constants (hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>0.252</td>
</tr>
<tr>
<td>2</td>
<td>0.252</td>
</tr>
<tr>
<td>3</td>
<td>0.262</td>
</tr>
<tr>
<td>4</td>
<td>0.277</td>
</tr>
<tr>
<td>Mean</td>
<td>0.261</td>
</tr>
<tr>
<td>SD</td>
<td>0.010</td>
</tr>
</tbody>
</table>

a) Significantly different from control value, p<0.01.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Absorption rate constants (hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>0.227</td>
</tr>
<tr>
<td>2</td>
<td>0.235</td>
</tr>
<tr>
<td>3</td>
<td>0.239</td>
</tr>
<tr>
<td>4</td>
<td>0.248</td>
</tr>
<tr>
<td>Mean</td>
<td>0.237</td>
</tr>
<tr>
<td>SD</td>
<td>0.008</td>
</tr>
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</table>

a) Not significantly different from control value.

Previous paper in this series\textsuperscript{b)} has demonstrated that salicylic acid decreases the permeation rate of sulfadimethoxine from internal to external solution through a cellulose membrane when the external solution contains bovine serum albumin, and that the decreasing effect of salicylic acid is due to displacing activity. Thus the results of the in\textit{ situ} experiment may be explained in terms of the displacement of sulfadimethoxine from its plasma-protein binding sites by salicylic acid and phenylbutazone.

![Graph showing effect of salicylic acid and phenylbutazone on intestinal absorption](image)

**Fig. 1.** Effect of Salicylic Acid and Phenylbutazone on the in\textit{ Situ} Intestinal Absorption of Sulfadimethoxine in Rabbits

Salicylic acid and phenylbutazone were injected into the ear vein with dose of 100 and 50 mg/kg, respectively. Representative data were shown in this figure. Similar patterns were obtained for the other experiments.

![Graph showing semilogarithmic plots](image)

**Fig. 2.** Semilogarithmic Plots of Percentage of Sulfadimethoxine Remained in the Mucosal Side Solution \textit{versus} Time

Each value is expressed as the mean of 4 experiments.

\(\bigcirc\), sulfadimethoxine alone; 
\(\bullet\), with salicylic acid; 
\(\times\), with phenylbutazone.

**Table III.** Effect of Salicylic Acid and Phenylbutazone on the in\textit{ Vitro} Intestinal Absorption of Sulfadimethoxine in Rabbits

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Disappearance rate constants (hr\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>1</td>
<td>0.133</td>
</tr>
<tr>
<td>2</td>
<td>0.154</td>
</tr>
<tr>
<td>3</td>
<td>0.189</td>
</tr>
<tr>
<td>4</td>
<td>0.189</td>
</tr>
<tr>
<td>Mean</td>
<td>0.166</td>
</tr>
<tr>
<td>SD</td>
<td>0.024</td>
</tr>
</tbody>
</table>

\(a\) Not significantly different from control value.

**Table IV.** Effect of Salicylic Acid and Phenylbutazone on the Transport of Sulfadimethoxine through the Intestinal Membrane and the Uptake of Sulfadimethoxine by the Intestinal Preparation

<table>
<thead>
<tr>
<th></th>
<th>Transport\textsuperscript{a)} (%) of dose</th>
<th>Uptake\textsuperscript{a)} (\mu g/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.1 ± 0.8</td>
<td>294 ± 77</td>
</tr>
<tr>
<td>With salicylic acid</td>
<td>4.0 ± 1.0\textsuperscript{b)}</td>
<td>291 ± 79\textsuperscript{b)}</td>
</tr>
<tr>
<td>With phenylbutazone</td>
<td>3.5 ± 0.9\textsuperscript{b)}</td>
<td>264 ± 77\textsuperscript{b)}</td>
</tr>
</tbody>
</table>

\(a\) These values were determined 60 minutes after the beginning of the \textit{in vitro} intestinal absorption experiment.

\(b\) Not significantly different from control value.
Effect of Salicylic Acid and Phenylbutazone on the \textit{in Vitro} Intestinal Absorption of Sulfadimethoxine in Rabbits

Table III shows the effect of salicylic acid and phenylbutazone on the disappearance of sulfadimethoxine from mucosal side solution in the isolated rabbit small intestine. Salicylic acid and phenylbutazone were added into mucosal side solution. Since sulfadimethoxine disappeared from mucosal side solution according to first order process during 60 minutes as shown in Fig. 2, the disappearance rate constant of sulfadimethoxine was determined from the slope of linear phase. Evidently these displacing drugs were found not to affect the disappearance rate of sulfadimethoxine from mucosal side solution in the isolated rabbit small intestine. Moreover, the effect of salicylic acid and phenylbutazone on the transport of sulfadimethoxine from mucosal to serosal side solution through the intestinal membrane and the uptake of sulfadimethoxine by the intestinal preparation were examined under the same conditions. As can be seen from Table IV, no significant effect of these displacing drugs was observed in either the transport or the uptake of sulfadimethoxine. These findings were in conflict with those obtained by the \textit{in situ} intestinal absorption experiment.

Recently, mesenteric blood flow has been shown to influence the intestinal absorption of drugs in various animals.\textsuperscript{13-15} Since mesenteric blood flow participates in the \textit{in situ} intestinal absorption but does not participate in the \textit{in vitro} intestinal absorption of sulfadimethoxine, it is considered that the presence of mesenteric blood flow is a prerequisite for the sulfadimethoxine-displacing drug interaction. This fact also suggests that the plasma-protein binding may play an important role in the absorption of sulfadimethoxine from the rabbit small intestine.

Effect of Salicylic Acid and Phenylbutazone on the \textit{in Situ} Intestinal Exsorption of Sulfonamides in Rabbits

In order to clarify further the effect of salicylic acid and phenylbutazone on the \textit{in situ} intestinal absorption of sulfadimethoxine in rabbits, the \textit{in situ} intestinal exsorption experiment was performed according to the method of Kakemi, \textit{et al.}\textsuperscript{11} As shown in Fig. 3, the exsorption rate of sulfadimethoxine to the rabbit small intestine was obviously increased when salicylic acid and phenylbutazone were administered intravenously. If the increasing

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{Effect of Salicylic Acid and Phenylbutazone on the \textit{in Situ} Intestinal Exsorption of Sulfadimethoxine in Rabbits}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4}
\caption{Effect of Salicylic Acid and Phenylbutazone on the \textit{in Situ} Intestinal Exsorption of Sulfanilamide in Rabbits}
\end{figure}

effect of salicylic acid and phenylbutazone is due to the change in either the permeability characteristics of intestinal membrane or the rate of mesenteric blood flow, the exsorption rate of sulfanilamide to the rabbit small intestine also will be affected more or less by salicylic acid and phenylbutazone. Nevertheless, as shown in Fig. 4, the exsorption rate of sulfanilamide was not affected at all. The results of these experiments indicate that only the unbound fraction of sulfonamides in plasma is available for the exsorption of the drugs to the rabbit small intestine. Therefore, it is concluded that the absorption of sulfadimethoxine from the rabbit small intestine is reduced as a result of the marked increase of unbound drug in plasma due to the displacement of the drug from its plasma-protein binding sites by salicylic acid and phenylbutazone.

Many investigators\(^{16-18}\) have pointed out the binding of drugs to plasma proteins is one of important physicochemical factors affecting drug absorption. However, there is no evidence on the basis of the experimental results. In the present study, we confirmed experimentally the importance of plasma-protein binding for the intestinal absorption of sulfonamides in rabbits.


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**Protein Binding of Sulfonylureas. I. Interaction of Some Substituted Benzenesulfonyl Propylureas to Bovine Serum Albumin**

**FUJIO KAMETANI and YASUKO SUMI**

*Faculty of Pharmaceutical Sciences, University of Tokushima*\(^1\)

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Binding affinities of five 4-substituted benzenesulfonyl propylureas to bovine serum albumin (BSA) were investigated by equilibrium dialysis method. Those of chlorpropamide (CPU) to BSA was also investigated by dynamic dialysis method using \(^{38}S\) labelled CPU. Results of both methods agreed very well.

Binding parameters of these compounds to BSA were obtained assuming that there were two classes of binding sites. CPU and 4-iodobenzenesulfonyl propylurea were bound to BSA stronger than benzenesulfonyl, \(p\)-toluenesulfonyl and 4-aminobenzenesulfonyl propylureas with regard to the primary binding site.

**Keywords**—protein binding; chlorpropamide; benzenesulfonyl propylurea; \(p\)-toluenesulfonyl propylurea; 4-aminobenzenesulfonyl propylurea; 4-iodobenzenesulfonyl propylurea; equilibrium dialysis; dynamic dialysis

Chlorpropamide (4-chlorobenzenesulfonyl propylurea, CPU), one of the hypoglycemic agents, differs significantly from tolbutamide (\(p\)-toluenesulfonyl butyurea, TBU) in that CPU is not readily metabolized to a physiologically inactive compound as TBU is to carboxytolbutamide.\(^2\) This would partly be due to the difference of the binding affinity of these sulfonylureas to serum or cell proteins. Johnson, *et al.* determined the rate of disappearanec

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1) Location: Shomachi, Tokushima, 770, Japan.