Interaction of Drugs with Bile Components. II. Effect of Bile on the Absorption of Indomethacin and Phenylbutazone in Rats\textsuperscript{1)}

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The effect of endogenous bile on the intestinal absorption of indomethacin and phenylbutazone was investigated in control rats and in bile fistula rats. Oral administration of both drugs in suspension gave lower plasma concentrations in bile fistula rats than in control animals. On the other hand, studies in which solutions of the two drugs were administered intraduodenally to control and bile fistula rats indicated that bile had no effect on their plasma levels. These results suggest that bile plays an important role in the dissolution step in the absorption processes of indomethacin and phenylbutazone.

Keywords---indomethacin; phenylbutazone; absorption; bile effect; micellar solubilization; wetting effect; rats

In the previous paper,\textsuperscript{1)} it was confirmed that bile salts such as sodium desoxycholate and sodium cholate markedly enhance the dissolution characteristics of the non-steroidal antiinflammatory drugs indomethacin and phenylbutazone, which are very poorly soluble in water. Since the dissolution rate is often a rate-limiting step in the absorption of drugs with low solubility, it is extremely interesting that bile salts appear to affect the dissolution of these drugs in the intestine, presumably influencing their bioavailability.

Bates et al.\textsuperscript{3,4)} have shown that bile salts markedly increase the solubility and dissolution rate of poorly water-soluble drugs. They suggested that one of the steps in the intestinal absorption of relatively insoluble drugs involves preliminary solubilization of the drugs by bile salts.

Other investigators have indicated that bile plays a significant role in the intestinal absorption of lipid and lipid-soluble vitamins.\textsuperscript{5-8)} However, there are very few reports describing the effect of endogenous bile on the absorption of poorly soluble drugs.\textsuperscript{9-11)}

The present study was undertaken to examine the effect of whole bile on the absorption of suspensions of indomethacin and phenylbutazone in rats.

Experimental

Materials---Indomethacin and phenylbutazone were obtained from Sigma Chemical Co. They were used without further purification. All other materials used were of analytical grade.

Procedure for Absorption Studies---Male Wistar rats, weighing 180 to 200 g, were fasted overnight before the experiment. They were divided into two groups: control and bile fistula groups.

2) Location: 1-1 Keyakidai, Sakado, Saitama 350-02, Japan.
11) A.J. Ware and B. Combes, Gastroenterology, 64, 1150 (1973).
a) Control Rats: The animals were anesthetized with urethane (1.3 g/kg) injected intraperitoneally. These animals were treated in the same manner as the bile fistula rats, except that the bile duct was not cannulated. Indomethacin (5 mg/rat) and phenylbutazone (10 mg/rat) were administered orally via a stomach tube as a 1 ml suspension in 0.1% methylcellulose.

The method used to follow blood plasma levels after intraduodenal administration of drug solutions to rats was as follows: the upper part of the small intestine was exposed and 1 ml of a 10 mg/100 ml (indomethacin) or 2 ml of a 20 mg/100 ml (phenylbutazone) drug solution was injected by means of a syringe into the duodenum.

In both cases, blood collections were made at 30, 60, and 120 min from the jugular vein using a syringe. Each blood sample (1 ml) was diluted with 1 ml of 0.9% NaCl and the plasma was separated immediately by centrifugation.

b) Rats with Bile Fistula: The bile duct was surgically exposed by a midline abdominal incision and cannulated with polyethylene tubing. This was done to prevent accumulation of bile in the upper segment of the intestine. At this time, drug samples were administered orally or intraduodenally in the same manner as for the control rats.

Analytical Methods—a) Indomethacin: Plasma samples containing indomethacin were assayed by a modification of the fluorometric procedure described by Hucker et al., using a Jasco FP-4 fluorospectrophotometer.

b) Phenylbutazone: The concentration of phenylbutazone in plasma samples was determined by gas–liquid chromatography (GLC). The method of McGilveray et al. was used with slight modifications, in that the drug was extracted into 4 ml of heptane from 0.5 ml of the plasma.

GLC conditions were as follows: apparatus, Ohkura Denki model 701 gas chromatograph with a flame-ionization detector; column, 1 m glass tubing with 1.5% SE 30 on Gaschrom Q(100—120 mesh); injection port temperature, 300°C; column temperature, 230°C; detector temperature, 300°C; nitrogen (carrier gas) flow rate, 30 ml/min. The retention times for diphenylphthalate (internal standard) and phenylbutazone were 4.3 and 3.2 min, respectively.

Results

In order to compare the absorption of indomethacin in the presence and absence of endogenous bile, plasma concentrations of the drug were determined in rats with normal bile flow and in bile fistula rats. The results of these absorption studies are summarized in Table 1.

Figure 1 presents the mean plasma concentrations after oral administration of indomethacin as a suspension to rats; the plasma concentrations are plotted as a function of time. It is apparent that the plasma concentrations of indomethacin in bile fistula rats were significantly lower than those in control rats, and fell to about 30% of the control levels over the experimental period.

<table>
<thead>
<tr>
<th>Experimental subjects</th>
<th>Route of administration</th>
<th>No. of rats</th>
<th>Plasma concentration (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Control</td>
<td>Oral&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9</td>
<td>6.8±1.1</td>
</tr>
<tr>
<td></td>
<td>Intraduodenal&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>2.5±0.1</td>
</tr>
<tr>
<td>Bile fistula</td>
<td>Oral&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5</td>
<td>1.8±0.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Intraduodenal&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4</td>
<td>2.5±0.2</td>
</tr>
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</table>

<sup>a</sup> Oral administration of 5 mg of drug suspended in 1 ml of 0.1% methylcellulose.
<sup>b</sup> Intraduodenal administration of 1 ml of a 10 mg/100 ml drug solution (pH 7.3) in dilute NaHCO₃.
<sup>c</sup> Values are given as mean±S.E.
<sup>d</sup> Significantly different (p<0.05) from the control.
<sup>e</sup> Significantly different (p<0.005) from the control.

15) Gasukuro Kogyo Co.
16) Wako Pure Chemical Industries.
Intraduodenal administration of the drug solution to bile fistula rats resulted in plasma levels essentially identical to those of the control rats, $p > 0.5$ (Table I). This result is in accord with that reported by Moriyama et al., in their study on the plasma concentrations following intraduodenal administration of solutions of indomethacin to rabbits.

This finding rules out an effect of bile on the absorption from solution and suggests that bile may enhance the dissolution rate of the poorly soluble drug, indomethacin, and thereby promote its intestinal absorption.

Similar investigations were carried out with phenylbutazone and the results are summarized in Table II. Figure 2 shows the plasma concentrations of phenylbutazone obtained following oral administration of the drug suspension to control and bile fistula rats. Plasma levels of phenylbutazone were considerably lower in bile fistula rats than in control animals.
On the other hand, no differences in phenylbutazone plasma levels were observed in control and bile fistula rats when the drug was administered intraduodenally as a solution (Table II).

Discussion

Oral administration of indomethacin in suspension gave lower plasma concentration in bile fistula rats than in control animals. A similar result was obtained for the plasma levels of phenylbutazone after oral administration. These results indicate that indomethacin and phenylbutazone are both more efficient absorbed from the intestinal tract in the presence of bile than in its absence.

Since both drugs are extremely insoluble in water, it is reasonable to consider that the presence of bile increases the dissolution of the drugs in the intestinal lumen and thereby enhances the absorption rate. The mechanism by which bile enhanced the drug dissolution probably involves micellar solubilization as well as the wetting effect.17 This view is based on the results obtained by Nightingale et al.10 in their study on the role of bile flow in the absorption of a poorly water-soluble sulfa drug, sulfadiazine. In our previous studies, bile salts have been found to increase markedly the dissolution rates of the present drugs by micellar solubilization and/or wetting action.

Not only bile salts3,4 but also phospholipids18,19 which are normal endogenous components of bile, may enhance the dissolution rates of poorly soluble drugs. Extensive studies are required to determine the effects of phospholipids and a mixed bile salt system on the degree of micellar solubilization.

Studies in which a solution of indomethacin was administered intraduodenally to control and bile fistula rats indicated that the bile had no effect on the plasma levels of indomethacin. This was also the case for phenylbutazone.

Bile salts have some effect on drug absorption from solution by their action on membrane permeability.17 The absorptions of phenol red20,21 and sulfaguanidine,20 poorly absorbed drugs, were enhanced by bile salts. On the other hand, the absorption of sulfanilamide (a moderately well-absorbed drug) from solution was not affected by bile salts.20 Since both indomethacin13 and phenylbutazone22 are known to be readily absorbed from the gastrointestinal tract, it is reasonable to consider that bile does not affect the absorption of the two drugs from solution.

The absorption of griseofulvin has been shown to be enhanced when the drug was administered with a high fat meal.23,24 A similar effect was observed when ethynylestradiol-3-cyclopentyl ester was administered with lipid diets.25 High fat concentrations stimulate the bile flow into the small intestine, which may enhance the dissolution and absorption of these poorly soluble drugs.26 Hence, it seems of interest to examine the effect of diet on the absorption of indomethacin and phenylbutazone with respect to drug bioavailability.