Effect of Experimental Acute Renal Failure on Barriers to Permeation of a Polar Drug in Rat Jejunum: An Electrophysiological Analysis

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The effect of experimental acute renal failure on the permeability and transport function of the intestinal membrane was analyzed in the isolated rat jejunum by using in vitro electrophysiological methods. The mucosal-to-serosal flux rate of sulfanilic acid (SA), which was used as a model of the polar drug, increased significantly in the membrane obtained from the rat with acute renal failure, indicating a reduction of the barrier function of the intestinal membrane. This effect of renal failure was neither dependent on the procedure used to induce the disease nor correlated with the extent of the disease state, shown by the level of blood urea nitrogen. Furthermore, it was clarified that the change in the permeability to SA occurred mainly in the transcellular pathway but not in the paracellular one. The electrical resistance of the jejunal membrane did not change in rats with acute renal failure. Thus, it was suggested that the structure of the tight-junctional portion of the epithelial layer remained unchanged, while the permeability of the cellular membrane was altered by the disease state. The activity of the intestinal membrane to transport Na⁺ or glucose might not be influenced by the disease state since the transmembrane potential difference and the short-circuit current of the membrane showed no significant change from their normal values.

Keywords—acute renal failure; isolated rat jejunum; membrane permeability; electrical resistance; short-circuit current; paracellular route; transcellular route; sulfanilic acid

Introduction

The high frequency of occurrence of adverse drug reactions in patients with renal failure is thought to be due to the higher plasma levels and the accumulation of administered drugs, caused by reduction of renal excretory functions and changes in hepatic metabolism or other pharmacokinetic parameters.¹⁻² Thus, to justify the dosage-schedules for patients with renal failure, kinetic studies have often been undertaken for various drugs.³⁻⁶

On the other hand, it has been reported that the intestinal absorption of nutrients, such as glucose or amino acids, is influenced during renal failure.⁷⁻⁸ Morphological or enzymatic abnormalities in the intestine have been found in patients with chronic renal failure.⁹ In our previous reports, it has been pointed out that the intestinal absorption of various drugs increases in rats with acute renal failure.¹⁰⁻¹¹ These facts raise the possibility that the alterations in drug absorption from the gastrointestinal tract also play some roles in the occurrence of adverse drug reactions. Further, we have discussed the possible mechanisms of the increased absorption of poorly-absorbable and well-absorbable drugs, individually.¹¹

In the present work, by using in vitro electrophysiological techniques, we have examined the changes in barrier functions of the intestinal membrane in order to clarify the mechanisms
of increased absorption of poorly-absorbable drugs caused by experimental acute renal failure.

Materials and Methods

Animals—Acute renal failure was induced in male Wistar rats weighing 200—220 g by an intramuscular injection of 50% glycerol (10 ml/kg) or by 5/6 nephrectomy. The experiments were performed at 24 h after glycerol injection or at 48 h after 5/6 nephrectomy. Glycerol-treated (GL) and nephrectomized (NP) rats were allowed free access to standard diet and water until the experiment.

Preparation of Jejunal Sheets—A portion of lower jejunum, about 10 cm in length, was isolated from a control, GL or NP rat and immediately mounted between two Lucite half chambers. Both sides of the membrane were filled with glucose-containing HCO₃⁻-Ringer solution (normal Ringer solution) and bubbled with 95% O₂-5% CO₂ mixed gas. Its composition in millimoles per liter was as follows: NaCl, 125; KCl, 5; CaCl₂, 1.4; NaH₂PO₄, 1.2; NaHCO₃, 10; and 200 mg/dl of d-glucose. The chambers were placed in a temperature-controlled box to maintain the solution at 37 °C. Ringer solution was adjusted to pH 7.4 at 37 °C prior to the experiment.

Electrical Parameters—The transmural potential difference (PD) and the short-circuit current (Iₛₑ) of the jejunal membrane were measured at 10 min intervals. The membrane resistance (Rₘ) was calculated by applying Ohm’s law, taking into account the resistance of the bathing solution. Details were described in our previous report. Transmural Flux of Sulfanilic Acid (SA)—SA-containing Ringer solution (10 mm) was introduced to the mucosal side of the membrane after preincubation with normal Ringer solution for 25 min. SA-containing Ringer solution was prepared to have the same osmolality and the same sodium concentration as normal Ringer solution. A sample (1 ml) was taken every 10 min from the serosal side for 1 h and the volume of the bathing solution was kept constant by the addition of fresh Ringer solution. The concentration of SA in the sample solution was estimated spectrophotometrically as reported previously. The mucosal-to-serosal flux rate of SA was calculated from the rate of increase in the serosal concentration of SA.

Voltage-Clamp Experiment—After introducing SA-containing Ringer solution, PD was clamped immediately to an arbitrary value (−20—+30 mV) by applying electric fields externally, and this voltage-clamp condition was maintained throughout the experiment. Flux rates of SA were measured under various levels of applied PD and the obtained values were analyzed according to the equations described in detail in Results.

Blood Urea Nitrogen (BUN)—After removal of the jejunum, a suitable amount of blood was taken from the jugular vein and BUN was determined by a diacetylmonoamine method. A standard solution (BUN 30 mg/dl; Kanto Chemical Co., Japan) was used to estimate the concentration of BUN.

Materials—All reagents used in these experiments were of reagent grade and were used without further purification.

Results

Transmural Flux of SA

Figure 1 shows the cumulative amount of SA transferred from the mucosal side to the serosal side across the jejunal membrane as a function of time. It is clear that the transferred amount of SA increased significantly when the membrane obtained from GL or NP rats was used. Since the regression lines became linear after a lag time of 10—20 min in all cases, SA flux rates were calculated from the linear portion of each plot and the mean values are listed in Table I with the corresponding values of BUN. The SA flux rate was increased significantly (about 1.5—1.6 fold) in the state of acute renal failure, regardless of the procedure used for induction. Also, BUN was significantly increased, though the increase in the NP group was less than in the GL group. Even in the group of GL rats, there were large variations in the level of BUN (80—230 mg/dl), while no significant correlation was observed between SA flux rate and the level of BUN.

Electrical Parameters

Figure 2 shows the time courses of PD, Iₛₑ and Rₘ of the jejunal membrane obtained from control and GL rats. Similar patterns were observed in PD and Iₛₑ, which were somewhat higher in the NP group than in the control throughout the experimental period. On the other hand, Rₘ was somewhat lower in the NP group. However, there was no significant
Table 1. Effects of Acute Renal Failure on the Flux Rate of SA across the Jejunal Membrane and BUN

<table>
<thead>
<tr>
<th>Group</th>
<th>SA flux rate (nmol/cm² min)</th>
<th>BUN (mg/100 ml)</th>
</tr>
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<tbody>
<tr>
<td>Control (6)</td>
<td>2.05 ± 0.07</td>
<td>12.7 ± 1.2</td>
</tr>
<tr>
<td>Glycerol-treated (10)</td>
<td>3.26 ± 0.15&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>161.1 ± 14.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5/6 Nephrectomized (4)</td>
<td>2.94 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42.7 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are expressed as the mean ± S.E. with the number of experiments in parentheses. a) \( p < 0.01 \), b) \( p < 0.001 \), compared with each control. c) \( p < 0.001 \), between glycerol-treated and nephrectomized groups. SA flux rate was not significantly different between these two groups.

Voltage-Clamp Experiments

According to Schultz and Zalusky,<sup>17</sup> the transmural total flux of ionized molecules \( J_t \) can be expressed as the sum of two individual fluxes, i.e., the flux through the transcellular pathway \( J_m \) and that through the paracellular pathway \( J_d \) as:

\[
J_t = J_m + J_d
\]

Further, \( J_d \) is thought to be exponentially dependent on \( PD \) and can be expressed as:
Table II. Transcellular and Paracellular Flux Rate of SA

<table>
<thead>
<tr>
<th>Group</th>
<th>SA flux rate (nmol/cm²·min)</th>
<th>Transcellular</th>
<th>Paracellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.23 ± 0.32</td>
<td>1.58 ± 0.24</td>
<td></td>
</tr>
<tr>
<td>Glycerol-treated</td>
<td>1.02 ± 0.28*</td>
<td>1.97 ± 0.22</td>
<td></td>
</tr>
</tbody>
</table>

Values are derived from the least square fit of each line in Fig. 3 using the MULTI program, and their ranges of error are expressed as ± S.D. * Significantly changed by the glycerol treatment (p < 0.01 by analysis of variance).

Fig. 3. Effect of Externally Applied PD on the Flux Rate of SA across the Jejunal Membrane

○, control; ●, glycerol-treated groups. The intercept (transcellular flux) and the slope (paracellular flux) of each line are summarized in Table II.

\[ J_d = \phi J_d \cdot \exp(-zF \cdot PD/2RT) \]  \hspace{1cm} (2)

where \( J_d \) refers to the paracellular flux at the short-circuited condition and \( z, F, R \) and \( T \) have their usual meanings. Assuming that \( J_m \) is insensitive to \( PD \), Eq. 3 is obtained:

\[ J_t = J_m + \phi J_d \cdot \xi \]  \hspace{1cm} (3)

where \( \xi = \exp(-zF \cdot PD/2RT) \). Based on this equation, the SA flux rate was measured under various levels of applied \( PD \) and plotted against \( \xi \) (Fig. 3). Straight lines which have high regression coefficients (\( R = 0.97 \) and 0.83 for control and GL groups, respectively) were obtained. From the least-squares fit of each line, the intercept on the ordinate (transcellular flux) and the slope of the line (paracellular flux) could be estimated for both groups and the results are summarized in Table II. It is clear that acute renal failure caused an increase in the intestinal permeability mainly in the transcellular pathway and no significant change occurred in the paracellular one.

Discussion

The reduced barrier function of the intestinal membrane in the state of acute renal failure, demonstrated in our previous report,\(^{10,11}\) has been reconfirmed in the present study using the rat jejunal membrane mounted between Ussing-type chambers. Since the rate-determining step in the intestinal absorption of polar and poorly-absorbable drugs such as SA is thought to be the permeation through the epithelial layer, the increase in SA flux rate shown in Table I should correspond to the increase in SA absorption from the intestinal tract in vivo. Thus, it is reasonable to consider that the increase in intestinal absorption of polar drugs observed in in situ experiments was perhaps due to the enhanced permeability of the intestinal membrane itself, but not due to alterations in other physiological factors. It is also apparent that the increase in the intestinal permeability is not dependent on the means employed to induce the disease, since membranes obtained from GL and NP rats showed similar increases in SA flux rate, in spite of significantly higher levels of BUN in the GL group. This indicates that the enhanced permeability of the membrane arises not from the direct effect of injected glycerol or other artificial factors, but from the renal failure state.
In order to characterize the change in the membrane permeability, SA flux rate was measured under various levels of applied PD and divided into two individual components, paracellular and transcellular fluxes. It has been suggested that, in leaky tissues such as small intestine or gallbladder, low-molecular-weight and water-soluble substances can pass through the paracellular tight-junctional pathway of the epithelium.\textsuperscript{18,19} We have also demonstrated that SA penetrates the jejunal membrane mainly via the paracellular pathway\textsuperscript{20} and a similar result was obtained in the control group of this study (Table II). However, in the GL group, only the transcellular flux of SA increased significantly, indicating that the cellular membrane of the intestinal epithelial cell became permeable even to this poorly-lipophilic drug during acute renal failure. Since the major ionic conductance pathway in leaky tissues is thought to be the paracellular one, it is reasonable to consider that the values of $R_m$ measured by our method represent the electrical resistance of the paracellular pathway.\textsuperscript{21,22} Thus, since no significant change in $R_m$ was observed in acute renal failure, we can conclude that there is no significant change in the permeability of the paracellular pathway to ions. These electrophysiological results are consistent with the results of a morphological study described in our previous report.\textsuperscript{11} The electronmicroscopic observations showed the formation of blebs at the tip of the microvilli after the induction of acute renal failure, suggesting the denaturation of cellular membranes of absorptive cells. On the other hand, no change was detectable in the structure of junctional complexes. It can be considered that those microscopical alterations in the intestinal epithelium cause the permeability change in only the transcellular pathway. In addition, we have demonstrated that brush border membrane vesicles prepared from the intestinal mucosa of rats with renal failure possess high permeability to mannitol compared with the vesicles prepared from control rat.\textsuperscript{11} These data also indicate the increased permeability of the cellular membranes to polar substances. In this way, the electrophysiological techniques which make it possible to measure drug fluxes across two individual pathways can provide direct evidence to characterize the changes in the barrier functions of the intestinal membrane.

If the alteration in the permeability of cellular membranes seen in the enterocyte also occurs in other tissues during renal failure, it is likely that the permeability change causes variations in drug metabolism and/or distribution in various ways. For example, when the membrane permeability of the hepatocyte is altered, the extent of drug metabolism including the first-pass effect seems to be influenced. It has been reported that not only lowered efficiency of renal drug excretion but also changes in metabolizing enzyme activity or in drug binding to serum protein cause variations in the drug disposition in patients with renal failure.\textsuperscript{1,2} Therefore, it is important to clarify whether the membrane permeability to drugs is altered during renal failure in various tissues and, if it is so, to consider the effects on drug disposition in the body.

No significant changes were observed in PD and $I_{sc}$ of the jejunal membrane of rat with acute renal failure. $I_{sc}$ is thought to represent the electrogenic flow of ions across the membrane\textsuperscript{23} and, in the small intestine, most of the $I_{sc}$ is attributed to the active transport (mucosal-to-serosal) of Na\textsuperscript{+}, especially the co-transport with glucose under these conditions.\textsuperscript{17,24,25} When $R_m$ is not altered, PD shows a similar pattern to $I_{sc}$, as shown in Fig. 3. Thus, it is clear that the function of the intestinal membrane to transport Na\textsuperscript{+} or glucose actively is not influenced by the disease state. In practice, an in vitro study using everted rings of the small intestine failed to show any change in the absorption of amino acids, dipeptides and glucose after partial nephrectomy.\textsuperscript{7} Further, Wizemann \textit{et al.}\textsuperscript{26} assumed that amino acid absorption from the small intestine is undisturbed or even enhanced during renal failure. This is consistent with our findings presented here.

Basic investigations concerning the effect of various disease states, not only renal failure, but also diabetes, hypertension and so on, on the functions of the gastrointestinal tract are
thought to be important in deciding the oral dosage schedule for each patient, and also when considering the reason for the occurrence of adverse drug reactions. Electrophysiological studies are clearly useful in this area.

References