Effect of absorption enhancers on the absorption of FD-4 as a poorly absorbable marker macromolecule from the liver surface in rats

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The purpose of this study is to improve the rate at which drugs are absorbed from the liver surface by utilizing absorption enhancers such as extracted saponin from Saponaria officinalis (saponin), sodium caprate (Na-Cap), sodium salicylate (Na-Sal), and disodium ethylenediaminetetraacetic acid. Fluorescein isothiocyanate-dextran with molecular weights of 4400 (FD-4) as a poorly absorbable marker macromolecule was applied to the rat liver using a cylindrical diffusion cell, in the presence or absence of the absorption enhancers. After a 15-min pre-treatment with or the co-administration of saponin or Na-Cap, the absorption ratios of FD-4 and plasma area under the curve were significantly increased, compared to control values. On the other hand, the absorption of FD-4 was increased by a 30-min pre-treatment with Na-Sal. The absorption of FD-4 applied to the rat liver was promoted by absorption enhancers. This approach could have therapeutic use for poorly absorbable compounds.

Key words: Absorption enhancer – Liver surface – FD-4 – Saponin – Absorption ratio – Sodium caprate.

Tissue selective drug delivery to the liver is useful because the normal treatment of liver diseases with intravenous and oral administration routes have been complicated by inadequate drug delivery to target site in the liver because of almost equal distribution via the bloodstream. We have demonstrated that the liver surface application could achieve a site-selective delivery of drugs, including 5-fluorouracil (5-FU), FD-4 [1]. From the result of this distribution experiment, drug availability in the liver was expected to be much higher compared to systemic intravenous administration. In addition, we have found the liver surface application to be useful for drugs such as biologically active compounds and gene [2-4]. We have clarified the dependency of the absorption rate on the molecular weight and poor absorption of macromolecules [5,6]. Accordingly, absorption of biologically active macromolecules and nucleic acid medicines from the liver surface needs to be enhanced for clinical application.

Absorption enhancers have been added to drug solutions to improve bioavailability of poorly absorbable drug [7]. These absorption enhancers include surfactants, fatty acids, bile salts and chelating agents [8,9]. Many researchers have studied the mechanisms of such enhancers on drug absorption across various membranes. These mechanisms include an increase in membrane fluidity, interaction with the ability of calcium ions to maintain the dimensions of intracellular space, solubilization of mucous membrane [10-14]. However, few studies have applied an absorption enhancer to the membrane of peritoneal organ. In addition, effects of absorption enhancers on drug absorption across the surface of a particular organ, such as the liver, are unknown. Our experimental system is useful for restricting the area of application and examining absorption-enhancing effects. Previously, we suggested the possibility of controlling the intrarenal distribution of a drug with the absorption enhancer saponin on the rat kidney surface [15].

In this study, we chose several compounds as absorption enhancers, extracted saponin from Saponaria officinalis (saponin), sodium caprate (Na-Cap), sodium salicylate (Na-Sal), and disodium ethylenediaminetetraacetic acid (Na₂-EDTA). We examined their effects on drug absorption from the rat liver utilizing fluorescein isothiocyanate-dextran with molecular weights of 4400 (FD-4) as a model for poorly absorbable compounds, the absorption of which from the liver surface has been previously studied [5].

I. MATERIALS AND METHODS

1. Chemicals

FD-4 and Na-Cap were obtained from Sigma Chemical Co. (St. Louis, MO, United States) and Katayama Chemical Ind. Co., Ltd. (Tokyo, Japan), respectively. Saponin, Na-Sal and Na₂-EDTA were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were reagent grade products.

2. Animal experiments

All animal procedures in the present study conformed to the Guidelines for Animal Experimentation in Nagasaki University.

Male Wistar rats (240-290 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and the left femoral artery was cannulated with a polyethylene tube (i.d. 0.5 mm, o.d. 0.8 mm, Dural Plastics, Dural, Australia). The middle abdomen was cutted open about 3 cm. The body temperature of the rats was kept at 37 °C with a heat lamp during the experiment. A cylindrical diffusion cell (i.d. 9 mm, area 0.64 cm²) was attached to the liver with an adhesive chemical, Aron Alpha (Daiichi Sankyo Co., Ltd., Tokyo, Japan).

The FD-4 solution (10 mg/mL) was prepared in an isotonic phosphate-buffered saline (PBS, pH 7.4). The drug solution (0.1 mL) was added to the diffusion cell directly. After application of the drug solution, a 200-µL blood sample was collected at selected times from the heparinized cannula inserted into the femoral artery over 6 h and centrifuged at 15,000 rpm for 5 min. The solution remaining in the diffusion cell was withdrawn up to 10, 15, 30, 45, 60 and 90 min after drug application.

3. Treatment method for absorption enhancers

Absorption enhancers were administered in two different ways (pre-treatment or co-administration). In the co-administration ex-
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1. Effect of absorption enhancers on FD-4 under different treatment conditions

Figures 1A-1D show the plasma concentration profiles of FD-4 until 6 h after its application to the liver surface in rats under different treatment conditions. Table I lists the ratios of absorption and moment parameters compared to control (48.5 % of dose). As shown in Figures 1A-1B, the enhanced absorption from the liver surface was corroborated by an overall increase in the plasma concentration explained by an increased AUCp following the 15-min pre-treatment or co-administration of saponin (Figures 1A) or Na-Cap (Figures 1B). However, no significant changes in the absorption ratio (Table I) or AUCp were recognized after the 30-min pre-treatment. In the case of Na-Sal, the 15-min pre-treatment did not have an absorption-enhancing effect (Table I), while significant increases in the absorption rate and AUCp of FD-4 were seen after the 30-min pre-treatment. However, no significant changes in the absorption of FD-4 compared to the control were recognized on treatment with Na2-EDTA, as shown in Table I and Figures 1D.

2. Effect of the concentration of absorption enhancer on the absorption of FD-4 from rat liver surface

Table II lists the ratios of absorption of FD-4 6 h after its application to the rat liver following pre-treatment for 15 min and the co-administration of different concentrations of absorption enhancers. On pre-treatment for 15 min with saponin and Na-Cap, the absorption of FD-4 increased at concentrations from 0.01 to 0.1 %. Further increases did not enhance the absorption of FD-4 from the liver surface. In the case of the co-administration of absorption enhancers, a significant increase in the absorption of FD-4 was recognized by increasing the concentration of saponin from 0.1 to 0.5 %. A similar trend was observed for Na-cap at concentrations from 0.1 to 0.25 %. In both cases, the absorption of FD-4 was not increased further by raising the concentration of saponin and Na-Cap to 1 or 0.5 %.

3. Rate of absorption FD-4 from the rat liver in the presence of enhancers

Figure 2 shows semi-log plots of the amount remaining in the dif-
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Table II - Absorption ratio in 6 h after the application of FD-4 to the rat liver with several concentrations of absorption enhancers as a pre-treatment for 15 min or co-administration.

<table>
<thead>
<tr>
<th>Absorption enhancers</th>
<th>Concentration (% w/v)</th>
<th>Absorption ratio (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>48.5 ± 1.2</td>
</tr>
<tr>
<td>15 min pre-treat.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saponin</td>
<td>0.01</td>
<td>51.1 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>54.7 ± 0.8*</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>52.7 ± 1.3</td>
</tr>
<tr>
<td>Na-Cap</td>
<td>0.01</td>
<td>48.2 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>54.6 ± 1.4*</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>40.9 ± 2.7</td>
</tr>
<tr>
<td>Co-administration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>49.1 ± 0.7</td>
</tr>
<tr>
<td>Saponin</td>
<td>0.1</td>
<td>56.9 ± 2.0**</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>59.2 ± 1.6***</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>49.8 ± 0.4</td>
</tr>
<tr>
<td>Na-Cap</td>
<td>0.1</td>
<td>56.9 ± 2.4**</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>59.1 ± 1.1***</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>47.2 ± 0.9</td>
</tr>
<tr>
<td>Na-Sal</td>
<td>0.1</td>
<td>53.4 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>55.2 ± 0.9***</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>56.9 ± 0.8***</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE for at least three experiments. Significantly different from the control (*P < 0.05, **P < 0.01, ***P < 0.001).

Figure 2 - Semi-log plots of the amount of FD-4 remaining in the diffusion cell after application to the liver surface. Each point represents the mean ± SE for at least three experiments. Significantly different from control (*P < 0.05, **P < 0.01).

Table III - Absorption ratio in 6 h after the application of FD-4 to the rat liver at different intervals following a 15-min pre-treatment with 0.1 % w/v saponin or Na-Cap.

<table>
<thead>
<tr>
<th>Absorption enhancers</th>
<th>Interval time after pre-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Saponin</td>
<td>48.5 ± 1.2</td>
</tr>
<tr>
<td>Na-Cap</td>
<td>54.7 ± 0.8*</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE for at least five experiments. Significantly different from control (*P < 0.05).

4. Possibility of reversible effects of absorption enhancers on the surface membrane

Table III shows the effect of time (0, 0-30, 0-60 min) following the 15-min pre-treatment with 0.1 % saponin or Na-cap on the ratio of absorption of FD-4 until 6h from the rat liver surface. When FD-4 was applied immediately after the enhancer (0 min), the absorption was significantly enhanced under both treatment conditions. The difference in the absorption ratio was insignificant 30 min after the removal of saponin or Na-cap. Furthermore, a 60-min-interval resulted in almost the same absorption ratio as the control.

III. DISCUSSION

We examined the absorbability of macromolecules from the liver surface and its dependence on molecular weight, and demonstrated clearly that FDs with different molecular weights as model macromolecules were absorbed from the liver surface at different rates [5]. The purpose of this study is to improve the rate at which drugs are absorbed from the liver surface by utilizing absorption enhancers such as saponin, Na-Cap, Na-Sal, and Na-EDTA. We examined their effect on drug absorption from the rat liver utilizing FD-4 as a model for poorly absorbable compounds.

As shown in Figures 1A-1B, by the 15 min pre-treatment with or the co-administration of saponin or Na-Cap, the absorption of FD-4 over 6 h was significantly increased compared to control. The absorption ratios in 6 h calculated from the amount of FD-4 remaining in the diffusion cell and plasma area under the curve were significantly increased, compared to control values (Tables I and III). The absorption-enhancing mechanism for saponin and Na-Cap is considered to involve membrane perturbation and the transcellular route [12, 14]. Saponin and Na-Cap could interact with membrane lipids and protein, causing membrane perturbation. Membrane perturbation might be one of the important factors in absorption-enhancing effect from the rat liver.

On the other hand, the absorption of FD-4 was increased by a 30-min pre-treatment with Na-Sal. Na-Sal is considered to interact with membrane proteins via the transcellular route [10]. This implies a different absorption-enhancing mechanism such as the transcellular route and optimal treatment. Na-EDTA is considered to have a calcium-chelating effect (loosening of tight junctions) and paracellular effect [11]. Na-EDTA did not have an effect under any treatment conditions. The ratio of absorption of FD-4 increased by changing the concentration of absorption enhancers following pre-treatment for 15 min and the co-administration (Table II). The co-administration of absorption enhancers, saponin, and Na-cap, significantly increased the absorption of FD-4 by raising the concentration. However, the absorption of FD-4 was not increased further by raising the concentration of saponin and Na-cap to 1 or 0.5 %. This indicates the existence of optimal concentrations of saponin and Na-cap for the absorption-enhancing effect [12, 13]. On the other hand, a dose-dependent increase in the absorption of FD-4 over 6 h was observed in the case of 0.1-1.0 % Na-Sal.

The liver is covered by a serous membrane containing a monolayer of squamous epithelial cells, considered a barrier to absorption [17, 18]. Absorption enhancers need to achieve an effect without causing severe damage or local irritation to the epithelial cells of the liver. The barrier function of the epithelial cells recovered to the control level. No significant effect on the absorption of FD-4 was seen when there was a 30-min interval after the application of saponin and Na-cap for 30 min (Table III). The clear recovering of FD-4 absorption following removal of the enhancers implies no severe effect on the biological
membrane of liver epithelial cells and reversible effect of the absorption enhancers. However, this indirect method is not sufficient. Further studies are needed to clarify this point.

With respect to strategies for drug administration methods on the liver surface, we reported that liver site-selective drug accumulation by continuously instilling [19]. On the other hand, implantable infusion pumps [20] and sheet-shaped materials [21-23] have been developed and applied for the treatment of several diseases. Moreover, remarkable progress has been made in endoscopic [24], laparoscopic operation techniques [25]. Taking these findings into consideration, the suitable combination of drug administration methods and medical skills could make possible the clinical application of drugs onto peritoneal organ surface.

In conclusion, the absorption of FD-4 applied to the rat liver was promoted by absorption enhancers. The approach could have therapeutic use for poorly absorbable compounds.

REFERENCES


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