Application of Propranolol to the Keratinized Oral Mucosa: Avoidance of First-Pass Elimination and the Use of 1-Dodecylazacycloheptan-2-one (Azone) as an Absorption Enhancer of Bioadhesive Film-Dosage Form

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The bioavailability of propranolol applied to the oral mucosa was examined in the hamster. The capacity of hamster cheek pouch, used as a model of keratinized oral mucosa, to metabolize propranolol in vitro is enormously lower than that of the liver. Significant amounts of propranolol absorbed from the small intestine were metabolized to naphthoxylactic acid and 4-hydroxypropranolol (4HP) during the passage through the intestinal wall, and then the greater portion of unchanged propranolol and almost all 4HP were subsequently metabolized by hepatic first-pass elimination in vivo. The systemic bioavailabilities of propranolol after the intra-small-intestinal loop and the intra-cheek-pouch administrations were 8.4% and 88.5%, respectively. The bioavailability of propranolol was improved further (to 97.1%) by a 1-h pretreatment of the cheek pouch with 5% 1-dodecylazacycloheptan-2-one (Azone)-emulsion. Bioadhesive film-dosage forms of propranolol were prepared with hydroxypropylcellulose. Both the in vitro permeation and the in vivo absorption of propranolol across the cheek pouch were enhanced by the incorporation of Azone to the film-dosage form.

Keywords — propranolol; first-pass elimination; oral mucosa; hamster cheek pouch; bioavailability; absorption; 1-dodecylazacycloheptan-2-one (Azone); hydroxypropylcellulose; bioadhesive dosage-form

Introduction

Propranolol is a commonly used β-adrenergic receptor blocking drug. However, the bioavailability of orally administered propranolol is low and varies from individual to individual. The variations in blood levels as well as the low bioavailability from oral doses have been attributed to extensive drug metabolism during absorption and first passage through the liver. To avoid the presystemic elimination of propranolol, some delivery routes such as nasal, rectal and transdermal were examined and improved bioavailabilities were reported.

The oral-mucosal route is one of the nonparenteral administration routes and drugs absorbed from the oral mucosa can avoid both the intra-alimentary canal and the hepatic first-pass eliminations. We have been investigating the drug absorption from the oral mucosa using a hamster cheek pouch as a model of the keratinized oral mucosa and recently reported that 1-dodecylazacycloheptan-2-one (Azone), an enhancer of transdermal permeation, enhanced both the in vitro permeation and the in vivo absorption of salicylic acid in hamster cheek pouch.

This report presents our results on (1) the improvement of the bioavailability of propranolol by oral-mucosal administration and (2) the enhancing effect of Azone on the oral-mucosal absorption of propranolol applied as a film dosage form.

Materials and Methods

Materials — Propranolol was used as its hydrochloride salt, purchased from Nakarai Chemicals (Kyoto, Japan). 4-Hydroxypropranolol and naphthoxylactic acid were kindly supplied by Imperial Chemical Industries plc (Macclesfield, England). Azone was a gift from Nelson Research (Irvine, CA). Polysorbate 20 was obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Hydroxypropylcellulose (HPC-SL) was supplied by Nippon Soda Co. (Tokyo, Japan).

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All the other chemicals were reagent grade products obtained commercially. Isotonic buffer solutions used were citric acid-Na$_2$HPO$_4$ (pH 3.0-5.0), NaH$_2$PO$_4$-Na$_2$HPO$_4$ (pH 6.0-8.0) and NaHCO$_3$-Na$_3$CO$_3$ (pH 9.0).

**In Vitro Metabolism of Propranolol** — The liver and the cheek pouch from male golden hamsters (100-140 g) were surgically removed after decapitation, cut into the small pieces with a razor blade and homogenized in 3 volumes of ice-cold 1.15% KCl-20 mM Tris-HCl buffer solution (pH 7.4) with a Teflon-glass homogenizer. The 9000 × g supernatant was obtained by centrifugation of the homogenate for 30 min at 4°C. The 9000 × g supernatants of both the liver and the cheek pouch (0.7 ml each) were incubated in a medium containing nicotinamide adenine dinucleotide phosphate (NADP) (0.126 μmol), glucose-6-phosphate (5.60 μmol), MgCl$_2$ (14.0 μmol), nicotinamide (2.10 μmol), glucose-6-phosphate dehydrogenase (1 U) and propranolol (2.8-140 μmol) in 0.7 ml of 1.15% KCl-20 mM Tris-HCl buffer solution (pH 7.4). Incubation was carried out at 37°C for 15 min and the reaction was terminated by the addition of 1 N NaOH solution. The alkalinized reaction mixture was extracted with CHCl$_3$, and an aliquot of the organic extract was re-extracted with 0.1 N HCl. An aliquot of the resulting aqueous layer was neutralized by the addition of 0.1 N NaOH solution, and was filtered through a 0.45 μm pore-size membrane filter (SJHV, Nihon Millipore Kogyo, Yonezawa, Japan) for the measurement of propranolol by high-pressure liquid chromatography (HPLC). Propranolol metabolism was determined by measuring the disappearance of the drug during the incubation.

**In Vivo Metabolism of Propranolol** — Male golden hamsters (100-140 g) were used under urethane anesthesia (1.5 g/kg, i.p.). Both kidneys of the hamster were surgically ligated to exclude renal excretion of the drug and its metabolites.

1) i.v. Administration: Propranolol dissolved in saline was administered into the femoral vein (20 μmol/ml/kg) of the hamster. Blood samples were collected by cardiac puncture 10 min after drug administration, and the plasma was separated immediately by centrifugation.

2) Intra-cheek-pouch Administration: Propranolol dissolved in isotonic buffer solution (pH 9.0) was administered into hamster cheek pouch (20 μmol/10 ml/kg) as described previously. Blood samples were collected by cardiac puncture 30 min after drug administration, and the plasma was separated immediately by centrifugation.

3) Intra-small-intestinal Loop Administration: Propranolol dissolved in isotonic buffer solution (pH 7.4) was administered into the loop of the whole small-intestine (20 μmol/10 ml/kg). Blood samples from the mesenteric vein and the heart were collected separately 10 min after drug administration, and the plasma was separated immediately by centrifugation.

Propranolol and 4-hydroxypropranolol in plasma were extracted with ether under alkaline conditions and the plasma concentrations of these compounds were determined by HPLC. Naphthoxylactic acid in plasma was extracted with ether under acidic conditions and the plasma concentration of this compound was determined by HPLC.

**Absorption of Propranolol from Hamster Cheek Pouch** — The absorption experiments were carried out as described previously. Propranolol dissolved in an isotonic buffer solution (pH 3.0—9.0) was administered (20 μmol/10 ml/kg) into the cheek pouch. The luminal contents were combined with the washings and the remaining amount of propranolol was determined by HPLC.

**Pretreatment of the Cheek Pouch with Azone-Emulsion** — The pretreatment of the cheek pouch with 5% Azone-emulsion (in 0.1% polysorbate 20 solution, pH 7.0) was carried out for 1 h in the same manner as described previously. Then the drug absorption experiments were carried out as described above.

**Pharmacokinetic Analysis of the Plasma Propranolol** — Under urethane anesthesia, the carotid artery of the hamster was cannulated with polyethylene tubing (o.d. 0.8 mm, i.d. 0.5 mm; Dural Plastics, Australia). Propranolol solutions in saline, pH 9.0 isotonic buffer solution and pH 7.4 isotonic buffer solution were administered (20 μmol/kg) into the femoral vein, the
cheek pouch and the small-intestinal loop, respectively. Blood samples (0.15 ml each) were periodically collected from the cannula and the plasma concentration of propranolol was determined by HPLC. Pharmacokinetic evaluations were carried out by non-compartmental analysis of the plasma concentration-time data based on the statistical moment theory. The moments were calculated by the trapezoidal method with a monoexponential extrapolation of the terminal phase.

The mean absorption time (MAT) of the intra-cheek-pouch and the intra-small-intestinal loop administrations were estimated by applying the following equations:

\[
\text{MAT}_{\text{icp}} = \text{MRT}_{\text{icp}} - \text{MRT}_{\text{iv}}
\]
\[
\text{MAT}_{\text{isi}} = \text{MRT}_{\text{isi}} - \text{MRT}_{\text{iv}}
\]

where MRT is the mean residence time of propranolol in plasma, and icp, iv and isi are the subscripts for the intra-cheek-pouch, the intravenous and the intra-small-intestinal loop administrations, respectively.

The systemic bioavailabilities (F) of propranolol were calculated by applying the following equations:

\[
F_{\text{icp}}(\%) = \left(\frac{\text{AUC}_{\text{icp}}}{\text{AUC}_{\text{iv}}}\right) \times 100
\]
\[
F_{\text{isi}}(\%) = \left(\frac{\text{AUC}_{\text{isi}}}{\text{AUC}_{\text{iv}}}\right) \times 100
\]

Preparation of Film-Dosage Forms Containing Propranolol — A mixture of 3.2 g of HPC-SL and 83.2 mg of propranolol hydrochloride was dissolved in 40 ml of ethanol, and 1.2 ml of isotonic buffer solution (pH 9.0) was added to the solution for the preparation of Control-film. The pH of the resulting ethanolic solution was 7.9—8.1. To prepare an Azone-film, 0.16 g of Azone was further added to the resulting solution. The ethanolic solutions of HPC containing propranolol were separately moulded into a Teflon tray, and were dried at 60 °C for 24 h. Film-dosage forms thus obtained were nearly transparent and approximately 0.3 mm thick. The apparent concentration of propranolol in either preparation was 3.1 μmol/cm².

In Vitro Permeation of Propranolol across Hamster Cheek Pouch from Film-Dosage

Forms — A glass cell having an available diffusion area of 1.13 cm² was employed. The design and the dimensions of the cell are shown in Fig. 1. A film-dosage form containing propranolol was clamped on carefully-excised tissue of hamster cheek pouch. The receptor compartment was filled with 18.5 ml of isotonic phosphate buffer solution (pH 7.4) and was stirred continuously at 600 rpm. The experiment was carried out in a temperature-controlled chamber at 37 °C. The concentrations of propranolol in the receptor fluid were periodically determined by HPLC and the cumulative amount transferred into the receptor compartment were calculated as described previously.

Absorption of Propranolol from the Film-Dosage Forms — A rectangular piece of film-dosage from containing propranolol (20 μmol/6.45 cm²/kg) was inserted into the cheek pouch and both surfaces of the piece were put between the cheek pouch mucosa. The dosage form adhered tightly to the mucosa and swelled gradually to a gel-like state. The plasma concen-
Propranolol Absorption from Oral Mucosa

Table I. Michaelis-Menten Parameters Estimated for Propranolol Degradation in the 9000 x g Supernatants of the Liver and the Cheek Pouch of Hamster

<table>
<thead>
<tr>
<th>Tissue</th>
<th>$K_m$</th>
<th>$V_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$m</td>
<td>($\mu$mol/h/g tissue)</td>
</tr>
<tr>
<td>Liver</td>
<td>3.43</td>
<td>2.65</td>
</tr>
<tr>
<td>Cheek pouch</td>
<td>7.69</td>
<td>7.98 x 10^{-2}</td>
</tr>
</tbody>
</table>

Determinations of propranolol were periodically determined in a similar manner to that described above.

**Analytical Methods** — A high-pressure liquid chromatograph (LC-5A, Shimadzu, Kyoto, Japan) equipped with a fluorescence HPLC monitor (RF-535, Shimadzu) was used in a reversed-phase mode with a Nucleosil 5C$_{18}$ column (4.6 i.d. x 150 mm, Chemo Scientific Co., Osaka, Japan). A mixture of acetonitrile: 20 mM NH$_4$Cl: 0.05% phosphoric acid (3:2:2 by volume) was used as the mobile phase at a flow rate of 0.8 ml/min. The excitation and the emission wavelengths for determination of propranolol, 4-hydroxypropranolol and naphthoxylactic acid were Ex. 314 nm—Em. 340 nm, Ex. 310 nm—Em. 380 nm and Ex. 285 nm—Em. 340 nm, respectively.

**Statistical Analysis** — Results were expressed as the mean ± standard error. The statistical analysis was carried out by the use of Student’s t-test.

**Results and Discussion**

**Metabolizing Activity of Hamster Cheek Pouch and Liver in Vitro**

The activity to metabolize propranolol in the 9000 x g supernatant of the cheek pouch homogenate was compared with that in the same fraction of the liver homogenate in the hamster. The kinetic parameters, the apparent $K_m$ and $V_{max}$ values, of propranolol-degrading enzymes obtained from the double-reciprocal plots are listed in Table I, where the apparent velocity of the metabolic degradation of propranolol was expressed as the disappearance of the drug during the incubation. The parameters for propranolol degradation in the hamster liver were roughly

![Fig. 2. Plasma Concentrations of Propranolol (PPL), Naphthoxylactic Acid (NLA) and 4-Hydroxypropranolol (4HP) after Intravenous, Intra-Cheek-Pouch and Intra-Small-Intestinal Loop Administrations of Propranolol (20 $\mu$mol/kg) in Kidney-Ligated Hamsters](image-url)

The time and the site of blood collection are presented in parentheses where C and P represents the cardiac plasma and the portal venous plasma, respectively. ND: not detected. Results are expressed as the mean of two experiments.
compatible with the values reported by Shand and Oates in rat liver. The maximal degradation velocity, $V_{\text{max}}$, of the cheek pouch was approximately one-thirtieth of that of the liver, and the apparent $K_m$ of the former was approximately twice that of the latter. This means that the activity to metabolize propranolol in the cheek pouch is enormously lower than that in the liver.

Metabolic Pathways of Propranolol in Vivo

To clarify the administration-route dependent metabolism of propranolol in vivo, we determined the plasma concentrations of propranolol and its major metabolites, naphthoxylic acid and 4-hydroxypropranolol, after intravenous, intra-cheek-pouch and the intra-small-intestinal loop administrations. The results are shown in Fig. 2. When propranolol was administered into the femoral vein or into the cheek pouch, a small fraction of naphthoxylic acid besides propranolol was found in the systemic circulation in either case. However, the systemic propranolol/naphthoxylic acid ratio was reversed after the intra-small-intestinal loop administration, while in this case, the portal venous level of propranolol was higher than that of naphthoxylic acid and considerable mounts of 4-hydroxypropranolol could also be detected in the portal venous plasma.

As to the presystemic elimination of propranolol, Iwamoto and Watanabe reported that there was no gastrointestinal first-pass metabolism of propranolol in rats, since the same systemic availability was obtained after oral and intraportal administration. Hayes and Cooper demonstrated in dogs and monkeys that the metabolic pathways of propranolol were different between oral and intravenous dosings, and that 4-hydroxypropranolol, a pharmacologically active metabolite, was present in the plasma at concentrations comparable to that of propranolol itself after oral dosing but not after intravenous dosing. Similar results were reported by de Leede et al., who investigated the urinary recovery of propranolol and its metabolites after oral, rectal and i.v. administration in rats. However, as shown in Fig. 2, the presystemic elimination of propranolol in hamsters differed somewhat from these findings. Our observations can be summarized as follows: (1) a considerable amount of propranolol absorbed from the small intestine is metabolized to naphthoxylic acid and 4-hydroxypropranolol by the intestinal first-pass elimination, (2) then a large portion of unchanged propranolol and almost all 4-hydroxypropranolol are subsequently metabolized by the hepatic first-pass elimination, and (3) presystemic elimination after the intra-cheek-pouch administration was almost negligible compared with that after the intra-small-intestinal loop administration.

Absorption of Propranolol from Hamster Cheek Pouch

The buccal absorption characteristics of propranolol were investigated by Schürmann and Turner in humans by using the human buccal absorption test. Their findings are in general agreement with the pH-partition theory. However, the epithelial thickness and the degree of keratinization vary with the regions of the oral mucosa and these regional differences affect the mucosal permeability. So, we investigated the absorption of propranolol more precisely, i.e., not from the whole oral cavity but from the keratinized oral mucosa using a hamster cheek pouch as a model mucosa. As shown in Fig. 3, the absorption of propranolol, a basic compound ($pK_a=9.45$), from the cheek pouch was pH-dependent and increased with increasing pH.

![Figure 3. pH-Absorption Profile of Propranolol from Hamster Cheek Pouch](image)

Results are expressed as the mean ± S.E. of 4 to 7 experiments.
suggesting the preferential absorption of the unionized form. Approximately 40% of the administered dose disappeared from the lumen of the cheek pouch in 1 h at pH 9.0. The absorption seems not to be affected by the buffer solutions dissolving propranolol under the present conditions (pH 3.0—9.0), since the oral-mucosal absorption of phenacetin and that of atenolol were not altered from pH 3.0 to 7.0\textsuperscript{44} and from pH 5.0 to 10.0,\textsuperscript{25} respectively.

**Avoidance of First-Pass Elimination of Propranolol**

Recently, Kondo and Sugimoto\textsuperscript{29} reported that the hepatic first-pass elimination of nifedipine was more efficiently bypassed by buccal dosing than by rectal and percutaneous dosings in rats, and that the systemic availability of nifedipine was improved from 56% (intraduodenal) to 80% (buccal). To confirm the avoidance of the first-pass elimination of propranolol, plasma concentrations of propranolol were periodically determined after administration (20 μmol/kg) into the femoral vein, into the cheek pouch and into the small-intestinal loop, and the results are shown in Fig. 4. In the intra-cheek-pouch administration study, the effect of a 1-h pretreatment of the cheek pouch with 5% Azone-emulsion on the plasma concentration-time profile was also investigated. As is evident from the figure, plasma concentration of propranolol after intra-cheek-pouch administration was much higher than that after intra-small-intestinal loop administration. Moment analysis was carried out and the pharmacokinetic parameters estimated are listed in Table II. The systemic bioavailability of propranolol (\(F\)) was improved significantly to 88.5% after the intra-cheek-pouch administration compared with that after the intra-small-intestinal loop administration (8.4%). Moreover, Azone treatment improved the bioavailability after the intra-cheek-pouch administration to a level almost equal to that after intravenous administration, and the mean absorption time (MAT) was also shortened to

**TABLE II. Pharmacokinetic Parameters for Propranolol in the Hamster Estimated by Moment Analysis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intravenous</th>
<th>Intra-small-intestinal loop</th>
<th>Intra-cheek-pouch</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N^c)</td>
<td>4</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>(AUC) (nmol·h/ml)</td>
<td>3.155±0.180</td>
<td>0.266±0.006</td>
<td>2.792±0.096</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>1.97 ± 0.06</td>
<td>2.97 ± 0.14</td>
<td>8.34 ± 0.39</td>
</tr>
<tr>
<td>MAT (h)</td>
<td>-</td>
<td>1.00</td>
<td>6.37</td>
</tr>
<tr>
<td>(F) (%)</td>
<td>100</td>
<td>8.4</td>
<td>88.5</td>
</tr>
</tbody>
</table>

Results are expressed as the mean value ±S.E.

\(a\) Propranolol was administered into the cheek pouch without any pretreatment.

\(b\) Propranolol was administered into the cheek pouch pretreated with 5% Azone-emulsion for 1 h.

\(c\) Number of experiments.
approximately one-half by this pretreatment. These results agree well with out previous findings that the pretreatment with Azone enhanced the absorption of salicylic acid from the cheek pouch\textsuperscript{19} by lowering the barrier function of the stratum corneum layer.\textsuperscript{18} These findings that the elimination of propranolol during the absorption process in the oral mucosa is almost negligible and that the absorption from the keratinized oral mucosa can be enhanced by Azone treatment seem to be useful for the development of new dosage forms of poorly available drugs such as propranolol.

**Absorption of Propranolol from Film-Dosage Form**

The application of hydroxypropylcellulose (HPC) to bioadhesive dosage forms has been attempted to obtain suitable adhesion properties to the wet surface of the mucosa.\textsuperscript{30,31} Yotsuyanagi \textit{et al.}\textsuperscript{32} reported that some clinical improvements were noted by the application of a thin HPC film containing analgesics and antibiotics to patients with serious aphthae. In the present study, two film-dosage forms containing propranolol, \textit{i.e.}, Control-film and Azone-film, were prepared with HPC-SL, the least viscous grade available, since a film prepared with HPC-SL showed the fastest drug release in our preliminary study. Both films prepared were translucent, which means that propranolol and Azone incorporated were homogeneously solubilized in these preparations. The pliability of Azone-film was somewhat superior to that of Control-film.

Figure 5 shows the permeation of propranolol across the excised hamster cheek pouch from the film-dosage forms \textit{in vitro}. The permeation was significantly enhanced by the incorporation of Azone into the HPC film and the cumulative amount of propranolol transferred into the receptor compartment within 3 h from Azone-film was approximately 2-fold larger than that from Control-film. Further, the enhanced absorption of propranolol from Azone-film was also confirmed by the plasma concentration-time profiles shown in Fig. 6. The enhancing effect of Azone on propranolol absorption from the film-dosage form applied on the keratinized oral mucosa was observed soon after application, while a considerable lag-time was reported in the effect of Azone on percutaneous absorption.\textsuperscript{33} \textit{AUC} from time zero to 3 h for Azone-film was approximately 1.6-fold larger than that

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**Fig. 5.** Effect of Azone Incorporation on the Permeation of Propranolol across Excised Hamster Cheek Pouch from Film-Dosage Forms \textit{in Vivo}

Cumulative amounts of propranolol transferred into the receptor compartment from Control-film (□) and Azone-film (■) were determined. Results are expressed as the mean ± S.E. of 4 experiments. Significantly different from Control-film: \textit{a)} \textit{p} < 0.05; \textit{b)} \textit{p} < 0.01.

**Fig. 6.** Plasma Concentration of Propranolol after Intracheck-Pouch Administration of Film-Dosage Forms

Rectangular films containing 20 \textmu mol/kg propranolol were applied on the surface of the cheek pouch mucosa: Control-film (□); Azone-film (■). Results are expressed as the mean ± S.E. of 3 experiments. Significantly different from Control-film: \textit{a)} \textit{p} < 0.05; \textit{b)} \textit{p} < 0.01.
Propranolol Absorption from Oral Mucosa

for Control-film. However, we could not recognize a significant difference in the in vitro release profiles from these two-film preparations determined preliminarily in the same manner except for the use of a 0.45 mm thick glass filter instead of the cheek pouch tissue. Therefore, it is suggested that Azone molecules incorporated in the film affect not the releasing process but the permeation process of propranolol across the keratinized oral mucosa.

The absorption rate of a drug applied as a film-dosage form is slower than that of a drug applied as a solution in general because of the slow swelling rate of the film and of the slow diffusion rate of the drug molecules in the gel-like swollen film. So bioadhesive HPC film is a candidate for an oral-mucosal dosage form which provides prolonged action. The present finding that the drug absorption from the HPC film-dosage form applied on the keratinized oral mucosa can be effectively enhanced by the incorporation of Azone in the film is quite significant.

In conclusion, the use of the oral mucosa, as a drug application site where the hepatic first-pass elimination can be avoided, and Azone as an effective enhancer at this site may enable us to develop a new dosage form which would assure improved systemic drug delivery.

References

21) D. G. Shand and J. A. Oates: Metabolism of propranol-


