Deconvolution Analysis for Absorption and Metabolism of Aspirin in Microcapsules

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We have previously proposed a novel deconvolution method, which can estimate first-pass metabolism of orally administered drugs. In the present study, we examined whether this deconvolution method is useful for evaluating oral dosage forms. The absorption and first-pass metabolism of orally administered aspirin formulated in several forms were analyzed. Two types of microcapsules consisting of Eudragit L100 alone and Eudragit L100/ethylcellulose (4:6) were prepared as sustained release formulations, for comparison with aspirin in powder form. The deconvolution analysis revealed that absorption of aspirin was sustained by encapsulating it in microcapsules. Interestingly, it also revealed that the percentage metabolized during absorption was different among the three types of formulations. Thus, the deconvolution method has enabled a comprehensive analysis of orally administered drugs. This method is believed to contribute to the evaluation of oral drug formulations.

Key words deconvolution; oral absorption; first-pass metabolism; microcapsule; aspirin

Several approaches have been used for the determination of the kinetics of orally administered drugs, including compartment analysis, moment analysis, and deconvolution analysis. Compartment analysis gives more detailed kinetics but requires specific assumption on the absorption process.1,2 Moment analysis, although relatively simple to use, provides only average values that reflect a certain time-course.3,4 Contrary to this, deconvolution analysis is able to evaluate the dynamics of drug absorption without the assumption of proper kinetic models.5–7 This method has also been used to develop and evaluate in vitro-in vivo correlations for drug release from oral dosage forms.8–11

From a pharmaceutical point of view, it is important to evaluate the first-pass metabolism of a drug, as well as its absorption. We have proposed a novel deconvolution method that can quantitatively assess the first-pass metabolism of orally administered drugs.12 This method successfully described the kinetics of orally administered aspirin12 and fibrinogen receptor antagonist ester prodrugs.10

In the present study, we analyzed the absorption and first-pass metabolism of orally administered aspirin formulated in several dosage forms, using this deconvolution method. The main objective is to demonstrate the usefulness of our deconvolution method for evaluating oral dosage forms. Two types of microcapsules containing aspirin were prepared as sustained release formulations. These microcapsules were encapsulated in PVCaps, which have been designed for preclinical studies by Capsugel, Inc., and orally administered to rats, for comparison with aspirin in powder form. Plasma concentration profiles of aspirin and its metabolite (salicylic acid) were analyzed using the deconvolution method.12

MATERIALS AND METHODS

Materials Aspirin, salicylic acid, and aluminium tris(phenolate) were obtained from Nacalai Tesque, Inc., Kyoto, Japan. o-Toluic acid was obtained from Kanto Chemicals, Tokyo, Japan. Eudragit-L100 and ethylcellulose (8.1—11.0 cp) were supplied from Röhm Pharma GmbH, Darmstadt, Germany and Shintetsu Chemicals, Tokyo, Japan, respectively. PVCaps (size 9 capsule) were kindly supplied by Capsugel, Inc. (Greensboro, SC). Other chemicals were special grade reagents or HPLC grade.

Preparation of Aspirin Microcapsules Two types of microcapsules consisting of Eudragit L100 alone and Eudragit L100/ethylcellulose (4:6) were prepared according to the solvent evaporation method.13 The polymers (2.1 g) and aspirin (0.7 g) were dissolved in 30 ml of acetone at 10 °C. The solution was poured into 100 ml of liquid paraffin containing 0.25 g aluminium tris(phenolate) at 10 °C. The temperature was gradually elevated to and maintained at 20 °C with stirring at 250 rpm for 10 h, then gradually elevated to 57 °C and kept there for 1 h. The microcapsules were taken, washed five times with 50 ml of hexane, and allowed to dry in a desiccator under reduced pressure. The microcapsules ranging 300—600 μm size were used for the following experiments.

Dissolution Experiment The procedure and apparatus were essentially the same as described in Japan Pharmacopeia XIII. The sample containing 20 mg aspirin equivalent was gently spread over the surface of 900 ml of the dissolution medium (No. 2 solution, pH 6.8) at 37 °C and stirred at 100 rpm. At predetermined time intervals, 0.3 ml of sample was withdrawn with a syringe. The solution was filtered through a polycarbonate membrane (0.45 μm) and subjected to HPLC assay.14 An equivalent volume of fresh medium was added to maintain the volume of the dissolution medium.

In Vivo Experiment Male Wistar-strain rats (SLC Inc., Shizuoka, Japan), weighing 180—210 g, were fasted for 12 h with free access to water, prior to the experiments. Under anesthesia with ether, a polyethylene tube (0.28 mm, I.D., 0.61 mm, O.D.) was inserted into the right femoral artery of the rat. The rat was placed in a Bollman cage and allowed to recover from anesthesia for more than 1 h. PVCaps capsules, filled with powder or microcapsules of aspirin, were orally administered to the rat stomach via a sonde at a single dose of 4 mg aspirin equivalent. At given time periods, 0.25 ml of blood was taken through the cannulated tube and immedi-
ately chilled. The plasma was separated by centrifugation at 4°C, and frozen with liquid nitrogen, and stored at -80°C until HPLC assay. After each sampling, the reduced blood volume was supplemented with an equal volume of saline containing 100 IU/ml heparin. Water was supplied to each rat 1 h after dosing. In several groups of rats, aspirin or salicylic acid in ethanol/PEG400/water (1:4:5, v/v/v) was injected through the cannulated tube into the blood at a single dose of 0.4 or 4 mg/body.

**HPLC Analysis** Aspirin and salicylic acid in plasma were determined by HPLC. The HPLC analysis was carried out using a Shimadzu LC-6A HPLC system (Kyoto, Japan), equipped with SPD-6A UV-VIS detector and SIL-6B autoinjector. The detection wavelength was set at 237 nm. Separation of aspirin and salicylic acid was made using a Cosmosil 5C18 packed column (4.6 × 150 mm, Nacalai Tesque, Inc., Kyoto, Japan.) at the temperature of 40°C. The mobile phase consisted of 720 ml water, 280 ml acetonitrile, and 0.978 µl orthophosphoric acid (85%); the flow rate was 1 ml/min.

**Deconvolution Analysis** Deconvolution analysis for orally administered aspirin was made according to our previous report. Provided that the plasma concentration of aspirin and its metabolite (salicylic acid) after bolus injection of aspirin are \(G_d(t)\) and \(G_m(t)\), and that the plasma concentration of salicylic acid after bolus injection of salicylic acid is \(G_m(t)\). the plasma concentration profiles of the drug \((O_d(t))\) and its metabolite \((O_m(t))\) after oral administration of the drug are given as,

\[
O_d(t) = \int_0^\infty I_d(\theta)G_d(t-\theta)d\theta
\]

\[
O_m(t) = \int_0^\infty I_d(\theta)G_m(t-\theta)d\theta + \int_0^\infty I_m(\theta)G_m(t-\theta)d\theta
\]

where \(I_d(t)\) and \(I_m(t)\) express the rates of the drug and its metabolite transported into the systemic circulation. Therefore, \(I_d(t)\) and \(I_m(t)\) can be estimated by the following successive calculations:

1. Estimate \(I_d(t)\) by deconvoluting \(O_d(t)\) with \(G_d(t)\).
2. Calculate the first term in Eq. 2 by convoluting \(I_d(t)\) with \(G_m(t)\).
3. Subtract the first term from \(O_m(t)\) to calculate the second term in Eq. 2.
4. Estimate \(I_m(t)\) by deconvoluting the second term with \(G_m(t)\).

Deconvolution and convolution calculations were performed by a point-area method. \(G_d(t)\), \(G_m(t)\), and \(G_m(t)\) were approximated as poly-exponential functions, through curve-fitting to plasma concentration profiles after bolus injection of aspirin and salicylic acid, using a nonlinear least-squares regression program MULTI.15

**Statistical Analysis** Statistical analysis were performed by unpaired Student’s t test.

**RESULTS**

Figure 1 shows the dissolution profiles of aspirin powder and microcapsules in pH 6.8 buffer solution at 37°C. The dissolution rate of aspirin in microcapsules, especially in Eudragit L100/ethylcellulose (4:6), was slower than that in the powder form. The dissolution from Eudragit L100/ethylcellulose (4:6) microcapsules was only 14% after 8 h.

Figure 2 shows plasma concentration profiles of aspirin and salicylic acid after bolus injection at a dose of 0.4 and 4 mg/body. When aspirin was injected to the blood, the drug was rapidly metabolized to salicylic acid. Elimination of salicylic acid from the systemic circulation was much slower than aspirin. Table 1 summarizes the pharmacokinetic parameters of aspirin and salicylic acid. The pharmacokinetic parameters of aspirin at a high dose were almost the same as those at a low dose. The elimination rate constant of salicylic acid was reduced by about 60% when the injected dose increased from 0.4 to 4 mg. Within the narrow ranges of the plasma level, the elimination of salicylic acid apparently followed linear kinetics.

Figure 3 shows the plasma concentration profiles after oral administration of aspirin in various dosage forms. The plasma level of the intact form was extremely low, especially in Eudragit L100/ethylcellulose (4:6) microcapsules. It took 4 h for the concentration of the metabolite to reach the maximum value in both microcapsule formulations, while it took less than 2 h in the powder form. The maximum concentration of the metabolite achieved was highest in the powder form, followed by Eudragit L100 microcapsules and Eudragit L100/ethylcellulose (4:6) microcapsules in this order.

The fractions of aspirin and its metabolite reaching the systemic circulation were estimated by the deconvolution method (Fig. 4). In the powder form, the input of the intact form into the systemic circulation ceased within 2 h after dosing. The fractions of intact and metabolite forms reaching the systemic circulation by 8 h were 0.31±0.05 and 0.63±0.05 (mean±S.E.), respectively. In Eudragit L100 microcapsules, the input of both intact and metabolite forms into the systemic circulation lasted 8 h. The ratio of the metabolite to the total amount reaching the systemic circulation was slightly lower, although statistically not significant, in Eudragit L100 microcapsules than in the powder form. The fractions of intact and metabolite forms reaching the systemic circulation by 8 h were 0.40±0.10 and 0.40±0.11, respectively. As compared with others, the input rate was slower in Eudragit L100/ethylcellulose microcapsules. The fractions of the intact and metabolite forms reaching the systemic circulation by 8 h were 0.039±0.005 and 0.29±0.02,
Fig. 2. Plasma Concentration Profiles of Aspirin (○) and Salicylic Acid (□) after Bolus Injection at a Dose of 0.4 and 4 mg/body. Data are the mean ± S.E. of three experiments.

Table 1. Mono- or Bi-exponential Equations Describing Plasma Concentration Profiles of Aspirin and Salicylic Acid after Bolus Injection to Systemic Circulation

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/body)</th>
<th>Form</th>
<th>( C_p = A \times \exp(-\alpha t) )</th>
<th>( \alpha (\text{h}^{-1}) )</th>
<th>( \beta (\text{h}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>4</td>
<td>Intact aspirin</td>
<td>0.328</td>
<td>16.7</td>
<td>—</td>
</tr>
<tr>
<td>Aspirin</td>
<td>0.4</td>
<td>Salicylic acid</td>
<td>-0.545</td>
<td>16.7</td>
<td>0.166</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>4</td>
<td>Intact aspirin</td>
<td>0.0348</td>
<td>14.9</td>
<td>—</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>0.4</td>
<td>Salicylic acid</td>
<td>-0.0540</td>
<td>14.9</td>
<td>0.394</td>
</tr>
</tbody>
</table>

Fig. 3. Plasma Concentration Profiles of Aspirin (○) and Salicylic Acid (□) after Oral Administration of Aspirin in Powder, Eudragit L100 Microcapsule and Eudragit L100/Ethylcellulose (4:6) Microcapsule Forms. Data are the mean ± S.E. of at least three experiments.
respectively. Unlike the case of Eudragit L100 microcapsules, the ratio of the metabolite was higher than in the powder form ($p<0.05$).

**DISCUSSION**

We have developed a deconvolution method for estimating the first-pass metabolism of orally administered drugs and demonstrated the validity of this method. The present investigation revealed that, when aspirin in the powder form was orally administered, the sum of intact and metabolite forms absorbed into the systemic circulation was estimated to be nearly unity. This might indicate that the analysis of the real time course is possible.

In the present investigation, the absorption characteristics of aspirin in different dosage forms were compared. Encapsulation of aspirin into microcapsules resulted in sustained absorption of the drug. It was more remarkable with the Eudragit L100/ethylcellulose (4:6) microcapsules, due to a water-insoluble nature of ethylcellulose. It should also be noted that the dissolution rate of aspirin from Eudragit L100/ethylcellulose (4:6) microcapsules was much faster in vivo than in vitro. It might be due to the difference in wetting effect. Bile salts are known to lower the surface tension at the solid/liquid interface, presumably accelerating wetting and penetration of the fluid into the microcapsule particles.

The ratio of metabolite to the total amount reaching the systemic circulation was different among the dosage forms tested. This might be related to their different release rates. There have been several reports indicating that nonspecific esterases found along the entire gastrointestinal tract are responsible for some loss of aspirin, in addition to metabolism in the liver. Since esterase activities are saturable, the first-pass metabolism would be greater as the dissolution rate is slower. This might be the case of Eudragit L100/ethylcellulose (4:6) microcapsules, having a slower release rate of aspirin. However, the first-pass metabolism of aspirin in the microcapsules consisting of Eudragit L100 alone was less extensive than that in the powder form. Thus, factors other than the release rate of the drug are involved. It is likely that differences at the site of disintegration might affect the first-pass metabolism. Since Eudragit L100 is a pH-sensitive enteric polymer, the microcapsules disintegrate only in the intestine. Taken together with the fact that the metabolism of aspirin occurs in both stomach and intestine, encapsulation of aspirin in the microcapsules would, at least, suppress its metabolism in the stomach.

Despite the dose-dependent elimination of salicylic acid, it was assumed for the sake of the deconvolution analysis that the elimination of salicylic acid followed a linear kinetic process. Having considered that the elimination of salicylic acid apparently followed linear kinetics within the narrow range of its plasma level and that the elimination rate constants were determined based on its plasma level, this assumption seems reasonable.

In conclusion, we demonstrated that the absorption and first-pass metabolism of aspirin varied according to the dosage forms through the deconvolution analysis that we developed. Thus, this deconvolution method is believed to contribute to the evaluation of oral drug formulation.

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**REFERENCES**


