First-Pass Accumulation of Salicylic Acid in Gut Tissue After Absorption in Anesthetized Rat

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Purpose. The purpose of this paper is to report the study on the first-pass accumulation kinetics of salicylic acid (SA) in gut tissue after absorption by simultaneously analyzing drug contents in the lumen, gut tissue, and blood in anesthetized rats. ***Methods.*** Sodium salicylate (5.4 mg as SA) in 0.4 ml normal saline was administered into a closed 10-cm jejunal loop. Drained mesenteric blood from the loop area was collected every minute, while lost blood was replaced through infusion of oxygenated blood from donor rats. At 3, 10, 20, 40, or 60 min after dosing, SA remaining in lumen, accumulating in gut tissue, and appearing in blood were analyzed by HPLC. All the data were fitted into a linear two-consecutive (lumen and gut tissue) first-order kinetic model. ***Results.*** After absorption, significant amounts of SA accumulated in gut tissue before appearing in blood, *e.g.*, at 3 or 20 min after dosing, 74.4 or 54.4% of absorbed SA accumulated in gut tissue, respectively. Practically all administered SA was recovered. The estimated mean absorption time from the lumen and mean transit time in gut tissue of SA were 20.4 and 18.5 min, respectively. ***Conclusions.*** The above results indicate that gut tissue may act as a reservoir for drug accumulation during the first pass after oral absorption. Thus, the rate of transport of drug into blood circulation after oral administration may significantly differ from the true rate of absorption through the gut membrane. The potential transport resistance from gut tissue to blood should probably be considered in the modeling of GI absorption.

KEY WORDS: gastrointestinal absorption; salicylic acid; first-pass accumulation in gut tissue.

INTRODUCTION

The early rate of drug appearance in peripheral venous blood after oral administration may be important clinically or in bioavailability / bioequivalence studies (1,2). After absorption through the gut membrane, this rate can be reduced by the extent of first-pass metabolism as well as first-pass accumulation in gut tissue, liver, lungs, and the peripheral sampling tissue (3,4). The kinetics of first-pass accumulation in gut tissue and its implications in modeling of gut absorption (5–7) or in affecting early appearance rates in blood after oral administration (5–8) have received relatively little attention to date. This may be attributed to a common notion that once a drug molecule is absorbed through the membrane, it is instantaneously or rapidly carried away by the mesenteric blood (5–7).

The purpose of this paper is to report detailed results of our study on accumulation kinetics of salicylic acid (SA) in gut tissue using anesthetized rats as an animal model. In the present study, the amounts of SA in lumen, gut tissue, and mesenteric blood were simultaneously measured as a function of time after administration into a closed jejunal loop. To our knowledge, such a detailed kinetic study is the first of its kind ever reported.

MATERIALS AND METHODS

Drug Solution

Sodium salicylate solution containing about 13.5 mg/ml of SA USP (Malinckrodt, Inc., St. Louis, MO) was freshly prepared by dissolving an equal mole of SA and sodium hydroxide in normal saline. The drug solution was filtered through a 0.22 μm membrane filter (Alltech, Deerfield, IL). The exact concentration was assayed by high-performance liquid chromatography (HPLC).

Animals

Male Sprague Dawley albino rats (Harlan Sprague Dawley, Inc. Indianapolis, IN), weighing 310–390 g, were fasted overnight with water *ad libitum* prior to the experiment.

Surgical Procedures

The surgical procedure described by Hanson and Parson (9) was employed with some modification. Briefly, the rat was anesthetized with 20% (w/v) of urethane by S.C. (1.5 g/kg). The animal was kept warm with a heating pad and lamp. The abdomen was opened by midline incision. The entire intestine was then exposed. The organ was kept moist with normal saline (37°C). A 10-cm segment of jejunum, 30–40 cm from the pylorus, was identified. Blood vessels associated with the rest of the small intestine, cecum and the proximal colon were ligated. The jugular vein was cannulated and a 0.25 ml of heparin solution (heparin sodium injection USP, 1000 u/ml, Elkins-Sinn Inc., Cherry Hill, NJ) was injected. Superior mesenteric artery was cannulated. The heparinized blood (20–25 unit/ml) which had been oxygenated in the lungs of another rat was infused into the superior mesenteric artery. Blood flow was kept constant (0.45 ml/min) by means of a multistatic pump (Serial No. 62469, Buchler Instrument, Fort Lee, NJ). The hepatic side of the portal vein was ligated; a cannula (20-G Angiocath) was inserted at the distal end for blood collection. The positions and directions of the cannulas are shown in Figure 1.

Study Design

Sodium salicylate solution (5.4 mg as SA in 0.4 ml of normal saline) was injected as a bolus into the closed loop. Effluent blood was collected at 1-min intervals up to a certain time (3, 10, 20, 40 or 60 min); one preparation was used for each time period. The blood supply was then stopped and the closed loop segment was cut and removed. The exterior surface of the loop was rinsed with normal saline and blotted with tissue paper. The inside of the loop was rinsed with normal saline and blotted with tissue paper. The inside of the

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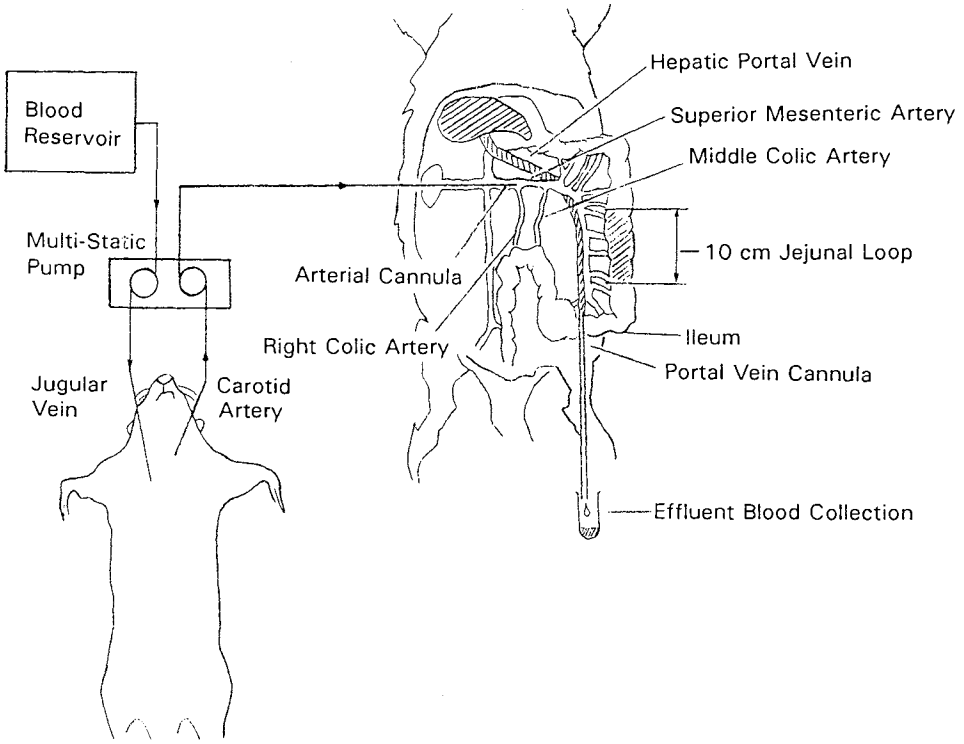

Fig. 1. Diagram of the *in situ* closed loop preparation. The blood vessels ligated and cannulated are shown.

loop was first washed with 7 ml (approximately seven times of the theoretical luminal volume) of saline and then flushed with about 50 ml of air. The drained luminal content was diluted to a final volume of 10 ml. After obtaining the wet weight, the loop tissue was chopped and homogenized with water in a 20-ml vial, and transferred into a 25-ml volumetric flask. The homogenate was brought to final volume (25 ml) with water and sonicated for 20 min. The collected blood and aliquots of both the luminal content and gut tissue homogenate were kept frozen until HPLC analysis.

HPLC Analysis

The HPLC method reported by B. E. Cham *et al.* (10) was used for determination of SA with some modification. Briefly, 200 μl of acetonitrile and 100 μl of 0.005 N hydrochloric acid were added to 100 μl of sample. After vortex mixing (Vortex-Genie, Scientific Industries, Bohemia, NY) at full speed for 30 sec, the mixture was centrifuged at 3000 rpm for 5 min. Thirty microliters of the clear supernatant was injected into the HPLC system. The HPLC system consisted of a reversed phase column (average particle size 10 μm, 30 cm × 3.9 mm, μBondapack C18, Waters, Milford, MA), a Waters Intelligent Sample Processor (WISP Model 712, Waters, Milford, MA), a solvent delivery module (Model 110B, Beckman Instruments, Berkeley, CA), a variable wavelength UV detector (SpectroMonitor III Model 1204A, Laboratory Data Control, Riviera Beach, FL) and a 10 mV potentiometric strip-chart recorder (Linear Instruments, Irvine, CA). The mobile phase was a mixture of glacial acetic acid, methanol and water (4:35:65 by volume). The flow rate of mobile phase was maintained at 1.5 ml/min. The SA separated from the sample was detected at 313 nm.

The standard curves for diluted luminal contents (5 to 100 μg/ml), homogenized tissues (5 to 100 μg/ml), and blood samples (2 to 100 μg/ml) were constructed separately. The mean response factor (peak height per unit concentration) was used for quantification (11).

Data Analysis

At the end of each study, the percent of dose remaining in the lumen or gut tissue was calculated by dividing the amount found in the lumen (X_l) or gut tissue (X_t) by the dose (X_o). The cumulative amount appearing in blood (X_b) was estimated by summation of SA found in the blood samples analyzed. Also, the rate of SA appearing in effluent blood (dX_b/dt) was obtained experimentally by multiplying the effluent blood flow rate by the concentration of SA in blood. The amount of drug disappearing from the lumen was assumed to be equal to the amount absorbed since SA is not degraded or metabolized in gut lumen or gut tissue (12).

A two consecutive first-order kinetic model (Figure 2) was used to describe the kinetics of absorption and accumulation of SA in the present study. According to the model, the X_l, X_t, and dX_b/dt can be described by following functions (5):

$$X_l = X_o \cdot e^{-k_l \cdot t} \tag{1}$$

$$X_t = \frac{k_l \cdot X_0}{(k_t - k_l)} (e^{-k_l \cdot t} - e^{-k_t \cdot t}) \tag{2}$$

$$\frac{dX_b}{dt} = X_l \cdot k_t \quad (3)$$

where k_l is the first-order transport rate constant from the gut lumen to gut tissue and k_t is the first-order transport rate constant from the gut tissue to the blood. To prevent bias of parameter estimates to one source of data, the X_l, X_t, and dX_b/dt data were simultaneously fitted into above three equations by PCNONLIN.

Mean absorption time from the lumen (MAT) and mean transit time in gut tissue (MTT) were estimated using following equations according to the model (Figure 2) (5):

$$\text{MAT} = \frac{1}{k_l} \quad (4)$$

$$\text{MTT} = \frac{1}{k_t} \quad (5)$$

MAT and MTT were also estimated by the following model-independent methods (5):

$$\text{MAT} = \frac{1}{X_0} \cdot \int_0^\infty X_l dt \quad (6)$$

$$\text{MTT} = \frac{1}{X_o} \cdot \int_0^\infty X_t dt \quad (7)$$

The integrated amount of SA in lumen or gut tissue from time zero to the last sampling time [$t(l)$] was calculated using the log trapezoidal rule during the descending phase and the linear trapezoidal rule during the ascending phase (13). The integrated amounts in the lumen or gut tissue from the last sampling time to infinity was estimated by (1,5):

$$\int_{t(l)}^\infty X_l dt = \frac{X_{lt(l)}}{k_l} \quad (8)$$

$$\int_{t(l)}^\infty X_t dt = \frac{X_{t,t(l)} \cdot (k_l \cdot e^{-k_t \cdot t(l)} - k_t \cdot e^{-k_l \cdot t(l)})}{k_l \cdot k_t \cdot (e^{-k_t \cdot t(l)} e^{-k_l \cdot t(l)})} \quad (9)$$

where $X_{l,t(l)}$ and $X_{t,t(l)}$ are the amounts of drug in lumen and gut tissue at the last sampling time, respectively. The value for k_t in Eq. 9 was based on that obtained from the curve fitting method. The contribution of the value from Eq. 9 to Eq. 7 is relatively minor, only about 17%.

RESULTS AND DISCUSSION

The simple HPLC assay for SA described above was found to be adequate for the present study. All the concentrations of SA in the samples were above the assay limit (less than 1 μg/ml). The coefficients of variation of response factors for diluted luminal, tissue and blood samples were 1.2%, 3.4%, and 6.9%, respectively.

Extensive accumulation of SA in the jejunal tissue was found in each of the five rats sacrificed at different time after dosing (Table I); the percentage of accumulation of the absorbed SA at 3, 10, 20, 40, and 60 min can be estimated to be 74, 80, 54, 42, and 15%, respectively. Consequently, the percentages of SA appearing in blood were much smaller than those disappearing from the lumen, especially during the first 40 min (Table I). Since SA is not metabolized in gut tissue, it is not surprising that nearly all the administered doses were recovered in the present study.

In earlier studies on cyclosporine in rabbits (14) and theophylline in rats (15), the amounts of drug appearing in blood during absorption were also much less than those absorbed. Although drug accumulation in gut tissue had been postulated as one of potential reasons to account for such a phenomenon (14,15), no actual demonstration of drug accumulation in tissue as shown here was reported.

The model shown in Figure 2 represents the simplest first-order kinetic model to account for absorption and accumulation of SA observed in the present study. The first order absorption is justified because SA has been shown to be absorbed from the rat lumen by an apparent first-order process (16). Similarly, treatment of gut or other tissue as a one compartment model with a first-order transit has been commonly employed in tissue disposition kinetic studies (1, 4, 5, 17). As shown in Figure 3A, the proposed model appears to adequately fit the limited data for absorption and accumulation. The MAT and MTT obtained by modeling analysis (Eqs. 4 and 5; MAT = 20.4 min and MTT = 18.5 min) are similar to those obtained by different methods (Eqs. 6 and 7; MAT = 27.4 min and MTT = 17.8 min). The above similarities support the appropriateness of the modeling analysis in the present experiment. The appropriateness of the model is further supported by the overall closeness between the model-predicted and observed appearance rates of SA in mesenteric blood (Figure 3B). The same model (Figure 2) has been employed earlier to account for the initial rising phase of the appearance rate of compounds in blood during a similar loop absorption study in rats (17). However, unlike the present study, no determination of the absolute amount of compounds present in gut tissue was made in that study.

The MTT of about 18 min in the jejunal tissue found in the present study indicates that an average absorbed molecule of SA will reside in jejunal tissue for about 18 min before being carried away by blood circulation under the conditions studied. Since the present study was carried out under anesthetic state with a reduced blood flow to the jejunal loop (0.47 ml/min/g wet tissue in the present study as compared

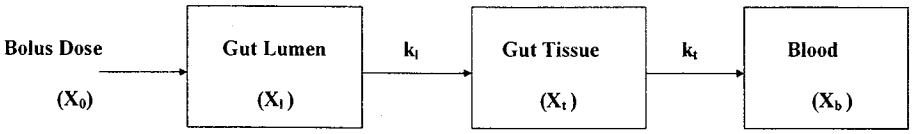

Fig. 2. A two consecutive first-order kinetic model. X_o, X_l, X_t, and X_b are the bolus dose, amounts of drug in the lumen, gut tissue, and blood, respectively. k_l and k_t are the first order transfer rate constants from the lumen to the gut tissue and that from the gut tissue to the blood, respectively.

Table I. Percentages of dose absorbed from the lumen, accumulating in gut tissue, and appearing in blood after administration of sodium salicylate into a 10 cm jejunal loop.

Time (min)	% absorbed[a]	% in gut tissue[b]	% in blood[c]	Model predicted % in blood[d]	Total recovery (%)
3	28.1	20.9 (74.4)	1.03 (n = 5)	1.1	94.2
10	38.9	31.4 (80.4)	8.3 (n = 4)	9.5	96.1
20	50.7	27.6 (54.4)	22.8 (n = 3)	27.5	92.1
40	71.3	29.8 (41.8)	48.0 (n = 2)	61.0	94.8
60	81.8	11.9 (14.5)	64.8 (n = 1)	81.4	100.0

[a] Calculated by 100 (Dose-X_l)/Dose, where X_l is the amount found in lumen.
[b] Values in parentheses indicating % of the absorbed drug accumulating in gut tissue, calculated by 100 X_t/(Dose-X_l), where X_t is the amount accumulating in gut tissue.
[c] Averaged value. Values in parenthesis is the number of rats used for calculation.
[d] Calculated by the integration of Eq. 3.

with 1.0–1.3 ml/min/g wet tissue in other study, ref. 18), the estimated MTT may be much higher than that in non-anesthetized normal animals. It is of interest to note that after oral absorption of aspirin in humans, a lag time of about 7 min has been estimated and attributed to the initial accumulation in gut tissue (19). In modeling oral absorption of amoxicillin in humans in a recent study (20), a single compartment to account for possible first-pass accumulation of drug in both gut tissue and liver (4) has been proposed in order to explain the delay in the appearance of drug in normally sampled peripheral blood.

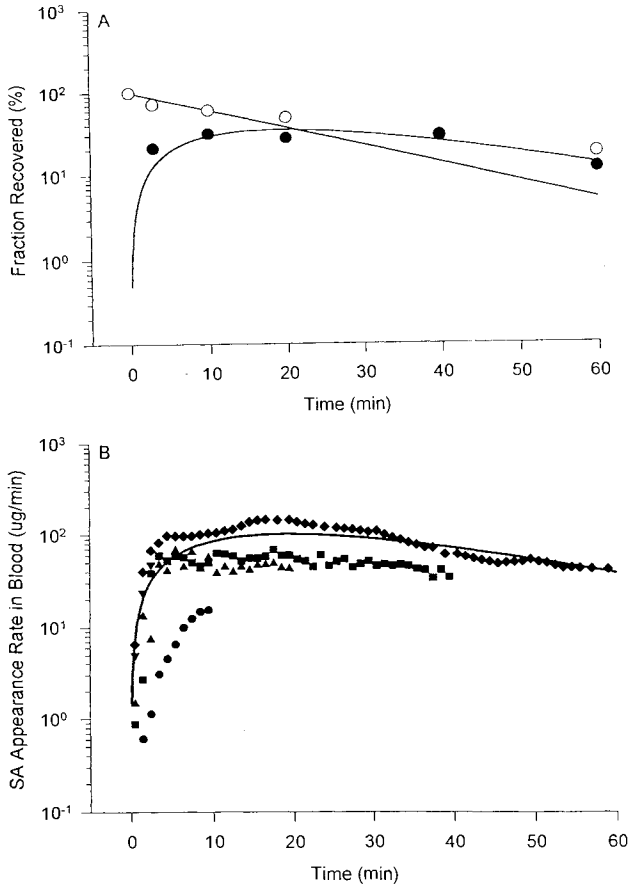

Fig. 3. (A) Percent of dose remaining in the lumen (○) and the gut tissue (●) following jejunal loop administration. Symbols are experimental data points (Table I) and lines are from the curve fitting based on the proposed model shown in Figure 2. (B) Appearance rates of salicylic acid in blood following jejunal loop administration. Symbols are experimental data points of appearance rate in blood from different studies (3 min, ▼; 10 min, ●; 20 min, ▲; 40 min, ■; 60 min, ♦) and the solid line is from the curve fitting based on the proposed model (Figure 2).

Although the site of accumulation has been postulated to be mainly in the intestinal membrane (16), it is likely that accumulation could also occur in other parts of the absorptive cell. For SA (pK_a = 3.0), mostly ionized at physiological pH, the observed accumulation is expected to be mainly in the cytosol. For carnitine, which is also mostly ionized at physiological pH, only 2% of the accumulation was found in the cellular membrane while 98% was found in the cytosol at 4 h after luminal administration (21).

The possible factors affecting the first-pass accumulation of drugs, such as gut tissue binding, and membrane permeability (expressed as octanol-water partition coefficient) of drug in gut tissue, as well as intestinal blood flow have been examined earlier in our laboratory for chloroquine, hydrochlorothiazide, desipramine and griseofulvin (5,22). The degree of accumulation appeared to be proportional to the degree of binding of drugs in gut tissue and inversely proportional to the permeability and blood flow. When both binding and permeability factors were simultaneously correlated with the accumulation of drugs tested, a good correlation (r = 0.956) was obtained. Comparison between this and earlier (5,22) studies could not be made because of differences in experimental design.

In some widely used intestinal absorption models, accumulation of drugs in gut tissue has not been considered (5–7). The significance of this and other potential issues in the determination of true wall or membrane permeability coefficients has been recently discussed (6,7). The conventionally determined wall permeabilities may only represent some hybrid constants. A term, intestinal absorptive clearance per unit surface area, has been recommended as an alternative to represent the values determined (6,7). It is also of interest to note that the role of unstirred water layer in absorption has been recently re-examined and its effect on the rate and extent of oral absorption *in vivo* has been shown to be generally clinically insignificant (23). Furthermore, intestinal permeabilities of drugs determined from *in situ* or *in*

vivo perfusion studies may markedly overestimate the extent of oral absorption *in vivo* (24).

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