DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 11(4), 909-916 (1985)

STUDIES DONE IN SINGLE RATS, SIMULTANEOUSLY COMPARING DISAPPEARANCE RATES OF COMPOUNDS FROM DIFFERENT REGIONS OF THE SMALL INTESTINE*

Joseph V. Swintosky and Elzbieta Pogonowska-Wala

College of Pharmacy

University of Kentucky

Lexington, Kentucky 40506

ABSTRACT

Presented here are a) a simple procedure for measuring regional permeability differences of compounds in the small intestine of a single rat, and b) preliminary study to test the procedure utilizing seven different benzoic acids, e.g. benzoic acid, o-hydroxybenzoic acid, 3,4,5-trihydroxybenzoic acid, p-hydroxybenzoic acid, m-hydroxybenzoic acid, 3,5-dinitrobenzoic acid, and 2,5-dihydroxybenzoic acid. Rates of disappearance (absorption) of these compounds were measured simultaneously in upper and lower segments of the small intestine using this variation of the in situ rat gut absorption study technique. Under the experimental conditions used, disappearance rate of a given

*Supported in part by grant funds from Rowell Labs., Baudette, Minnesota.

0363-9045/85/1104-0909$3.50/0

compound was no different from upper or lower segment for any of the compounds except for p-hydroxybenzoic acid. The latter disappeared about three times faster from the upper than lower segment. Not only is this experimental procedure of utility in differentiating regional permeability within the intestine. It offers the option of testing the effect of an additive or other environmental change on compound permeability, while the other segment serves as a control.

INTRODUCTION

In recent decades sustained release oral dosage forms were designed to release medication continuously over the entire length of the gastrointestinal tract in addition to release in the stomach. In this regard and for additional reasons it is of interest to determine whether the rate of disappearance (absorption) of drug or non-drug compounds varies significantly in different regions of the small intestine. In order to accomplish this objective, an experimental variation of the *in situ* rat gut technique was devised which allowed the utilization of a single rat per experiment for the simultaneous determination of disappearance rates from two gut segments, of a series of structurally related benzoic acids.

MATERIALS AND PROCEDURES

Seven compounds not previously studied as a group investigated and their pKa's are given in Table I. Types of rats used, methods of solution preparation and analyses, and general experimental procedures are as previously reported (1,2) from these laboratories.

TABLE 1

Disappearance Rates from Upper and Lower Segments of Small Intestines of Rats (*in situ*) after Administration of Various Benzoic Acid Derivatives at pH Values Corresponding to Their pKa's

Name of Compound	No. of Rats	Velocity Constant for Compound Disappearance from the Lumen [min^{-1}]			Absorption Half-Life [min]		pKa
		a	b	c	a	b	
benzoic acid	3	0.0435 ± 0.0048[d] (0.0490-0.0400)[e]	0.0529 ± 0.0157 (0.0630-0.0349)	NS	16	13	4.19
o-hydroxy-benzoic acid	4	0.0333 ± 0.0075 (0.0410-0.0260)	0.0356 ± 0.0097 (0.0440-0.00251)	NS	21	20	2.98
3,4,5-tri-hydroxy-benzoic acid	4	0.0054 ± 0.0017 (0.0068-0.0035)	0.0048 ± 0.0018 (0.0066-0.0030)	NS	128	144	4.33
p-hydroxy-benzoic acid	6	0.0241 ± 0.0086 (0.0302-0.0160)	0.0086 ± 0.0042 (0.0120-0.0045	p< 0.05	29	81	4.48
m-hydroxy-benzoic acid	4	0.0177 ± 0.0075 (0.0250-0.0090)	0.0222 ± 0.0072 (0.0320-0.0150)	NS	39	31	4.05
3,5-dinitro-benzoic acid	5	0.0250 ± 0.0098 (0.0373-0.0105)	0.0284 ± 0.0067 (0.0365-0.0210)	NS	28	25	2.82
2,5-dihydroxy-benzoic acid	3	0.0210 ± 0.0056 (0.0260-0.0150	0.0203 ± 0.005 (0.0250-0.0150)	NS	33	34	2.93

[a]Parameters from upper small intestine;
[b]Parameters from lower small intestine;
[c]Statistical Significance of Difference, NS-not signigicant (Student's test)
[d]Mean ± standard deviation
[e]Range

The present experiment was performed in a way that the only variable was an anatomical and functional difference between the two segments of intestine. For the purpose of standardization of the experimental conditions all of the tested compounds were dissolved in the buffer of the pH desired to attain 50% dissociation initially. The surface area to volume ratio was maintained essentially constant by using a uniform length of two segments of the small intestine, and a constant volume of the solutions introduced into them.

A midline incision was made to expose the rat small intestine; the bile duct was not ligated. The small intestine was divided into portions as follows: (a) upper section - beginning 2 cm distal to the ligament of Triets and (b) lower section - measured proximally beginning 2 cm proximal to the cecum. Both sections consisted of 30 cm lengths of the intestine. The selected regions were separated by double ligation and glass cannulae were inserted at both ends of the upper and lower segments of the intestine. Each of the cannulae were connected to 30 ml syringes fitted with stopcocks, as illustrated in Figure 1.

The intestinal lumen were washed gently with isotonic saline and five milliliters of air were introduced to expel residual saline and then were gently removed. Exactly 10 ml of drug solutions at $37^{o}C$, buffered at their respective pKa's, were introduced into upper and lower intestinal segments, and the intestine was returned to the abdominal cavity and covered with gauze. The intestine was kept moist and warm at about $37^{o}C$ during the procedure and care was taken to eliminate bleeding.

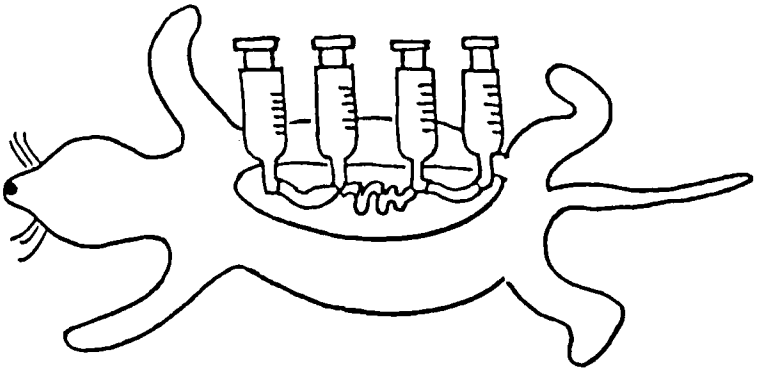

FIGURE 1

Modified *in situ* gut technique illustrating the method for simultaneously determining the rate of drug disappearance from two different portions of the gut in a single animal.

The solutions in the lumen were sampled immediately after introduction (time zero) and at 5 min intervals up to 65 minutes by aspirating solution into one of the syringes and withdrawing 0.1 ml samples prior to returning solution to the lumen. At the end of the experiment the remaining lumen solutions were collected, and their volumes and pH's were monitored.

RESULTS AND DISCUSSION

Despite the fact that the solutions introduced into the lumen were isotonic and buffered, they underwent some changes in volume and pH during the *in situ* experiments. After 65 minutes the volume of the lumen solutions were about 10% less than the initial values; however, variations in volume between the two intestinal segments were insignificant. Increase in the pH of the lumen solutions, sometimes as much as 1 pH unit, occurred during the experiment, but such variations from original conditions oc-

curred similarly in both upper and lower segments of the small intestine. The value of conducting the disappearance rate studies simultaneously, from different gut segments within a single rat, effectively overcomes the problems which might otherwise be introduced by such variations. The disappearance (attributed to absorption) of benzoic acid and its related derivatives followed approximate first order kinetics from both the upper and lower segments of the small intestine. The rate constants and half lives for disappearance from the gut lumen were calculated for the two different segments of the intestine. These are shown in the table. Half lives ranged from a low of about 13 minutes for benzoic acid to a high of about 144 minutes for 3,4,5-trihydroxybenzoic acid, under the conditions of this study. One can conclude that with the exception of one compound, p-hydroxybenzoic acid, there were not statistically significant differences between the absorption rate constants for the upper and lower intestinal segments. This is despite the fact that some differences in anatomy and physiology exist between segments of the intestine. The data suggests that absorption efficiency is probably similar in the upper and lower tract of the intestine for many compounds. On the other hand p-hydroxybenzoic acid illustrates the exception, and presumably there are other examples of exceptions which this simple experimental method can detect. The experimental method shows that disappearance from the lumen, of p-hydroxybenzoic acid, illustrated in Figure 2, is about three times faster from the upper than the lower gut segments. The method does not explain why this is so, and this study did not have that as a purpose.

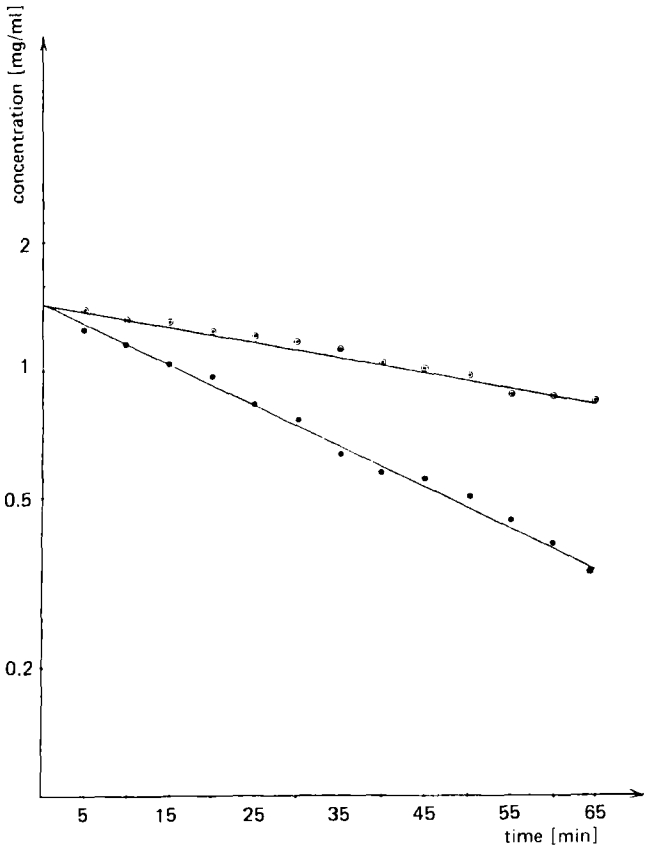

FIGURE 2

Plots for disappearance of p-hydroxybenzoic acid from the lumen of equal lengths of upper and lower small intestine of rat. Studies, done simultaneously in a single rat, indicate three times faster disappearance from upper than lower tract.

This technique for comparing absorption rates of a single compound from different segments of small intestine in a single rat is relatively fast and inexpensive. It lends itself to further simultaneous studies of different classes of compounds from different segments of the G.I. tract. Also for a given compound that is absorbed at identical rates from upper and lower

segments of the small intestine when lumen contents are similar, there is provided here, for example, an experimental method for testing the effect on absorption rate of changing the lumen content or pH in one segment and comparing it with the other.

REFERENCES

1. J. T. Doluisio, N. F. Billups, L. W. Dittert, E. T. Sugita, and J. V. Swintosky, J. Pharm. Sci. 58, 1196 (1969).
2. J. V. Swintosky and E. Pogonowska-Wala, Pharm. Intern., 3, 163 (1982).