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VOLUME AND CONCENTRATION EFFECTS ON ABSORPTION PARAMETERS OF A SUBSTITUTED BENZIMIDAZOLE ANTHELMINTIC AFTER SUBCUTANEOUS INJECTION

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ABSTRACT

It is important to understand and optimize the delivery of a drug substance. The present study examines the effect of concentration and volume at a fixed dose rate of a solution of a substituted benzimidazole (I) on its absorption after subcutaneous administration to rabbits. Plasma levels and excretion profiles were determined by measuring the isotopically labeled Compound I. As well, residue concentrations at the injection site were determined. The results indicate that Compound I was completely absorbed after injection at the three concentrations administered. Absorption rate, as measured by average peak plasma level was found to be directly proportional to the relative surface area of the volume injected. This deviation from the result predicted by diffusion controlled absorption may be explained by the Compound I precipitation at

the interface of the injected solution and surrounding tissue. Subsequent dissolution of Compound I would yield the observed result.

INTRODUCTION

In order to obtain the practical objective of optimizing delivery of a drug, it is fundamental that the parameters limiting and controlling the delivery process be delineated and understood. Previous studies have independently quantitated the effects that changes in drug concentration and the dose volume (1-5) exert on the rate of absorption of subcutaneously or intramuscularly administered drugs. The experiments described in this study were designed to examine the effects that dose volume and concentration had on the absorption efficiency of the reputed benzimidazole anthelmintic (6) (5-((2-Methylpropyl)-sulfinyl)-1H-benzimidazol-2-yl) carbamic acid, methyl ester (I). This objective was approached by administering isotopically labeled material to rabbits at a fixed dose. Three concentrations of solution were administered, the volume varying inversely to the strength of the solution. The rates of absorption and excretion of the subcutaneously administered doses were evaluated by measuring and comparing the time profiles for the plasma, urine and fecal drug equivalents concentrations. Injection site residue concentrations were determined to provide additional evidence to substantiate the conclusions that were reached from the plasma and excretory products analyses.

BACKGROUND AND THEORY

The role of diffusion in drug absorption from a parenteral injection site has been discussed previously (7). From that discussion the

following form of Fick's Law for the drug diffusion model was developed:

$$\frac{dN}{dt} = \left(\frac{DAK}{dV_s}\right) A_s \qquad \text{Eq 1}$$

where dN/dt is the flux of drug from the injection site, defined as the drug amount per unit time; K is the equilibrium distribution coefficient of the soluble drug between the adjacent membrane and aqueous phase at the injection site; A is the area exposed to the absorption process; D is the diffusion coefficient in the membrane; d is the diffusional distance; V_s is the volume injected; A_s is the amount injected. As presented the equation is in the derivative or rate form of a first order process. The rate, as defined, is the drug amount per unit time as in contrast to other rate designations, concentration or percentage per unit time. Since the amount of drug absorbed determines plasma levels and absorption rate, this definition is a more meaningful expression to use in comparing the affect of different factors on drug absorption from the injection site.

A quantitative comparison of the effects of volume and concentration on the rate of drug absorption from a subcutaneous depot requires certain assumptions. These are that D, K and d are constant values and that the pharmacological effects of the drug do not alter them. Also, if the volume injected may be assumed to be either a sphere, hemisphere or disc, the following relationship exists between the relative area (RA) and relative volume (RV):

$$RA = (RV)^{2/3} \qquad \text{Eq 2,}$$

where relative area is the area of the injection volume exposed to the absorption process of interest divided by the area of a reference

injection volume and relative volume is the volume of injection of interest divided by a reference volume of injection.

The effects of injection volume, drug concentration and total amount of drug injected on the absorption rate and rate constant was examined for the following three cases:

Case I. In this case the injected volume was constant with concentration increasing and hence, total drug amount injected. The model then predicted an increase in absorption rate directly proportional to the amount injected and no change in the rate constant (RC), defined as $(DAK)/dV_s$.

Case II. The injected volume increased with the concentration remaining constant which resulted in an increased amount injected. The rate constant was proportional to A/V_s. Upon substitution of RA for A and RV for V_s and introducing the relationship between them, yielded the RC proportional to $RV^{-1/3}$ or $RA^{-1/2}$. The rate then became proportional to $RV^{2/3}$ or to RA, since RV may be substituted for A_s in the expression.

Case III. The drug amount injected was held constant, hence concentration and volume varied inversely. The RC was proportional to $RV^{-1/3}$ or $RA^{-1/2}$ as in Case II, however, the rate was also proportional to these expressions, since in this case A_s was a constant.

The following table summarizes these three cases.

	CONSTRAINT ON:			PROPORTIONATELY TO, OF	
CASE	VOL.	CONC	AMT	RATE CONSTANT	RATE
I	Const.	↑	↑	No Change	A_S
II	↑	Const	↑	$RV^{-1/3}$ or $RA^{-1/2}$	$RV^{2/3}$ or RA
III	↑	↓	Const	$RV^{-1/3}$ or $RA^{-1/2}$	$RV^{-1/3}$ or $RA^{-1/2}$

The situation as exists in Case I has been reported (3). Absorption of isonicotinamide and isoniazid administered IM in rats in a concentration range of 17-250 mM, was a first order process with a constant rate constant over the concentration range studied. Using the definition of rate presented here, the rate observed was proportional to the amount injected. These results, then, support Fick's diffusion model. Similar results have been reported with IM administered sucrose solutions ranging in concentration from 0.19-9.6 mg/ml (8).

Experiments establishing the effect of volume on the absorption of mannitol or sucrose from IM depots have been performed (8). These experiments conformed to the parameters as set in Case II. If the data is treated according to first order absorption kinetics, as postulated by the diffusion model, a rate constant and a rate at a specified time may be calculated. The initial rate for mannitol, calculated for the two injection volumes, 6 and 42 µl, relate to one another as 1:4.1 compared to a theoretical ratio of 1:3.7. For sucrose, the initial rate ratio was

calculated to be 1:8.5 versus a theoretical ratio of 1:6.35. Although the data is limited, it would appear that the theory for diffusional controlled absorption is satisfied in both examples. Other experiments (1,2) employing injection volumes of isonicotinamide solutions less than or equal to 20 µl resulted in no change in the first order depletion rate constant. The authors explained this discrepancy by stating that the absorbing area does not vary significantly in the case of injection volumes in the range, 5 through 20 µl.

The conditions described for Case III do not appear to have been studied such that observed absorption might be compared with that predicted by the model.

In this study, it was deemed of interest to determine the effects of concentration and volume on parenteral drug absorption when the amount of drug administered is fixed (i.e., dosage scaled to the weight of the animal) and to test Case III theory. Although the protocol meets the requirements for Case III, two experimental conditions may lead to results that vary with those predicted on the basis of theory. The two conditions are 1) the use of a non-aqueous but water miscible vehicle; 2) the use of a salt of a drug which as the free base has poor solubility at physiologic conditions.

EXPERIMENTAL

FORMULATION

Solutions of carbon 14 labelled Compound I as the hydrochloride salt were made in propylene glycol, USP at 25, 50 and 75 mg/ml, equivalent to the free base. The specific activity of each concentration was 1.43 µCi/mg of free base.

IN VIVO ADMINISTRATION

Three groups, each of four mature female New Zealand white rabbits (2.0-3.7 kg), were administered I at 15 mg/kg free base equivalent by subcutaneous injection. The different concentrations, 25, 50 and 75 mg/ml were administered in groups I, II and III, respectively. Each animal was maintained in a separate metabolism cage. The experiment duration was eight days. Blood, urine and feces were collected at the time points indicated in the table below. Each injection site was excised at the time of sacrifice. All samples were frozen until analyzed.

DATA COLLECTION TABLES

Day	Blood (hr)	Urine (hr)	Feces (hr)
1	0, 2, 4, 8	0-8, 8-24	0-24
2	24, 32	24-32, 34-48	24-48
3	48	48-56, 56-72	47-72
4	72	72-80, 80-96	72-96 Sacrifice two animals from each group
5	96	96-104, 104-120	96-120
6	120	120-128, 128-144	120-144
7	144	144-152, 152-168	144-168
8	168	Sacrifice remaining animals	

ANALYSIS

Total radioactivity in the plasma and urine samples were analyzed by liquid scintillation counting (LSC) methods. Results are reported as drug equivalents. Feces were combusted[1] after drying and the carbon-14 carbon dioxide flushed into Carbosorb®[2] with subsequent analysis by LSC.

[1]Model 306, Packard Instrument Co., Inc., Downers Grove, IL 60515
[2]Packard Instrument Co., Inc., Downers Grove, IL 60515

Each injection site tissue was weighed and digested in 3N KOH at 60°C for 96 hours. After final volume measurement, duplicate aliquots were neutralized and analyzed by LSC.

TABLE I

PERCENT DOSE RECOVERED FROM URINE AND FECES FROM EACH ANIMAL

Group Number	Animal[1] Number	Sacrifice (day)	Dose Recovery (%)		
			Urine	Feces	Total
I	1	4	85.3	9.4	94.7
I	2	8	81.4	13.4	94.8
I	3	4	90.7	12.1	102.8
I	4	8	74.2	21.8	96.0
II	5	8	75.8	12.5	88.3
II	6	4	82.7	8.2	90.9
II	7[2]	-	-	-	-
II	8	8	82.9	6.2	84.1
III	9	4	81.5	13.0	94.5
III	10	4	67.1	2.9	70.0
III	11	8	83.7	5.3	89.0
III	12[3]	8	-	-	-

[1]Group I 25 mg/ml; Group II 50 mg/ml; Group III 75 mg/ml
[2]Animal 7 died before 72 hours after injection.
[3]Animal 12 did not receive full dose due to faulty injection.

RESULTS AND DISCUSSION

The total percent dose recovered from urine and feces from each animal is listed in Table I. The cumulative percent dose recovered in urine and feces as a function of time is plotted in Figure 1. These data

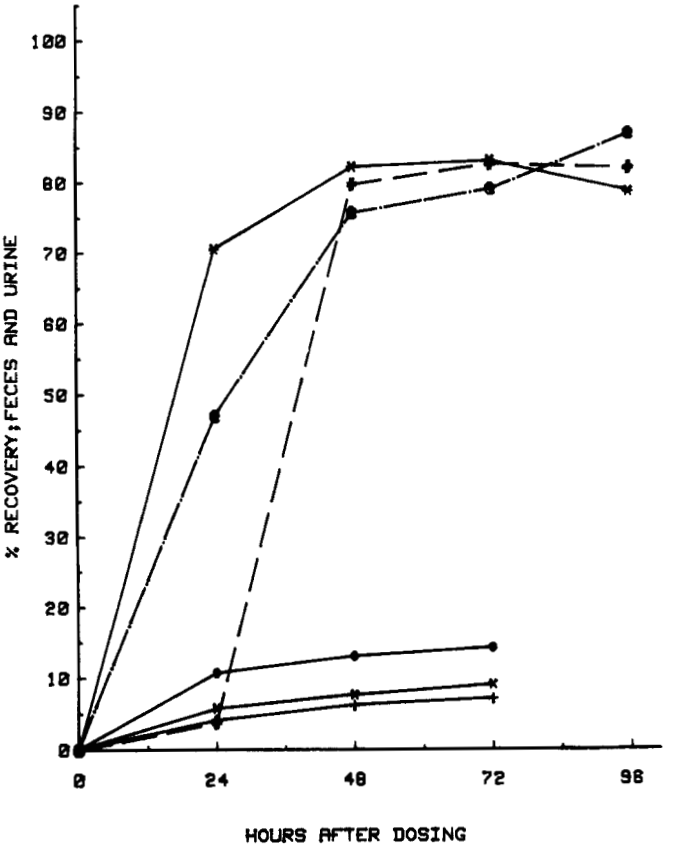

Figure 1. Cumulative percent dose recovered in the urine and feces from rabbits injected with Compound I. Key: Urine; ● , group I, 25 mg/mL; #, group II, 50 mg/mL; @ , group III, 75 mg/ml. Feces; O , group I; x, group II; +, group III.

indicate that the majority of dose has been excreted by 72 hours and the largest portion was excreted in the urine. The data also suggests that there was minimal difference in percent dose recovered among groups, hence, the concentration of Compound I in the formulation did not appear to affect total absorption.

The results on the residue remaining at the injection site (Table II) also indicated there was no difference between groups. The residue

TABLE II

RESIDUE REMAINING AT THE INJECTION SITES

Group Number	Animal[1] Number	Sacrifice (day)	Injection Site Residue (ppm)	
I	1	4	0.37	
I	2	8	0.24	
I	3	4	0.28	
I	4	8	0.09	$\bar{x}$ = .25 ± .12 (s.d.)
II	5	8	0.1	
II	6	4	0.1	
II	7[2]	-	-	
II	8	8	0.16	$\bar{x}$ = .12 ± .03 (s.d.)
III	9	4	0.13	
III	10	4	0.19	
III	11	8	0.08	
III	12[3]	8	-	$\bar{x}$ = .13 ± .06 (s.d.)

[1]Group I 25 mg/ml; Group II 50 mg/ml; Group III 75 mg/ml
[2]Animal 7 died before 72 hours after injection.
[3]Animal 12 did not receive full dose due to faulty injection.

remaining was very low indicating good Compound I absorption from the injection site. The recovery data from the urine and feces support this conclusion.

The plasma level of total radioactivity converted to mcg Compound I equivalents per ml of plasma from the three groups are plotted versus time in Figure 2. The average data for this figure is found in Table

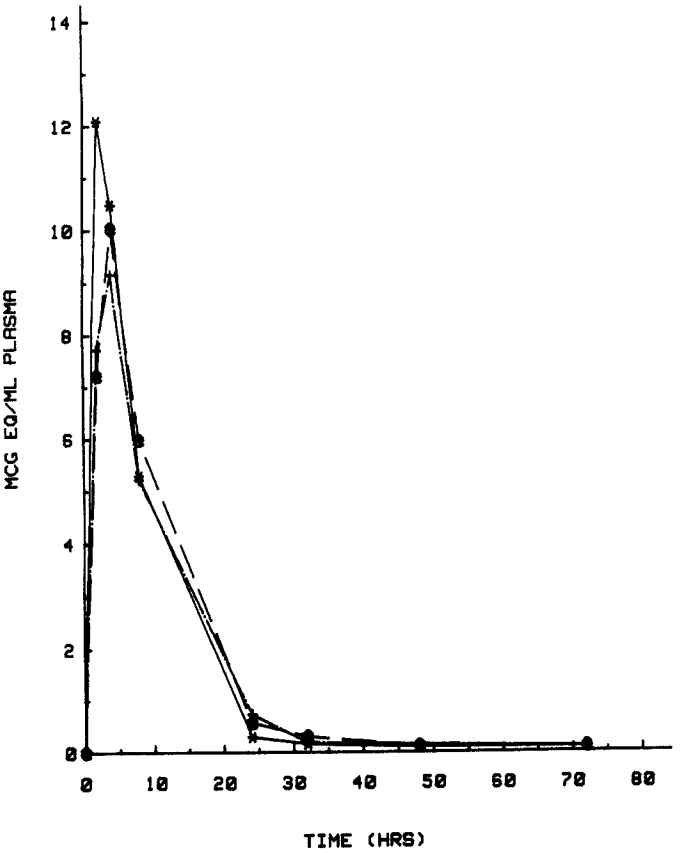

Figure 2. Total radioactivity expressed as drug equivalents of I per mL plasma as a function of time after subcutaneous administration of I at three concentrations. Key: * , group I; @ , group II; +, group III.

III. These data indicate a decrease in the absorption rate as concentration increases and injected volume decreases. According to the diffusion theory set forth in Case III, the reverse of this should be true with the rate or a measure thereof proportional to $RV^{-1/3}$ or $RA^{-1/2}$. Using average peak plasma level as a measure of absorption rate a linear relation was found as a function of relative surface area of the injection volume (Figure 3). For surface area to be a function of absorption rate leads to speculation that compound precipitation occurs at the interface of the injection solution and tissue fluids. This would not be totally unexpected due to low aqueous solubility of Compound I at

TABLE III

SUMMARY OF AVERAGE PLASMA DRUG LEVELS FOR RABBITS IN GROUPS I, II AND III REPORTED AS mcg EQUIVALENTS/ml PLASMA

Time Point (hr)	Group I (25 mg/ml)[2]	Group II (50 mg/ml)[2]	Group III (75 mg/ml)[2]
2	12.09	7.22	7.72
4	10.48	10.03	9.16
8	5.29	5.98	5.22
24	0.293	0.569	0.731
32	0.160	0.305	0.193
48	0.104	0.123	0.154
72[1]	0.0897	0.096	0.095

[1]Assay of blood plasma from animals after 72 hours indicated drug plasma levels were not significantly grater than background isotope levels.

[2]Each animal was administered 15 mg/kg free base equivalent and 21.4 μCi/kg of the carbon 14 labeled compound I.

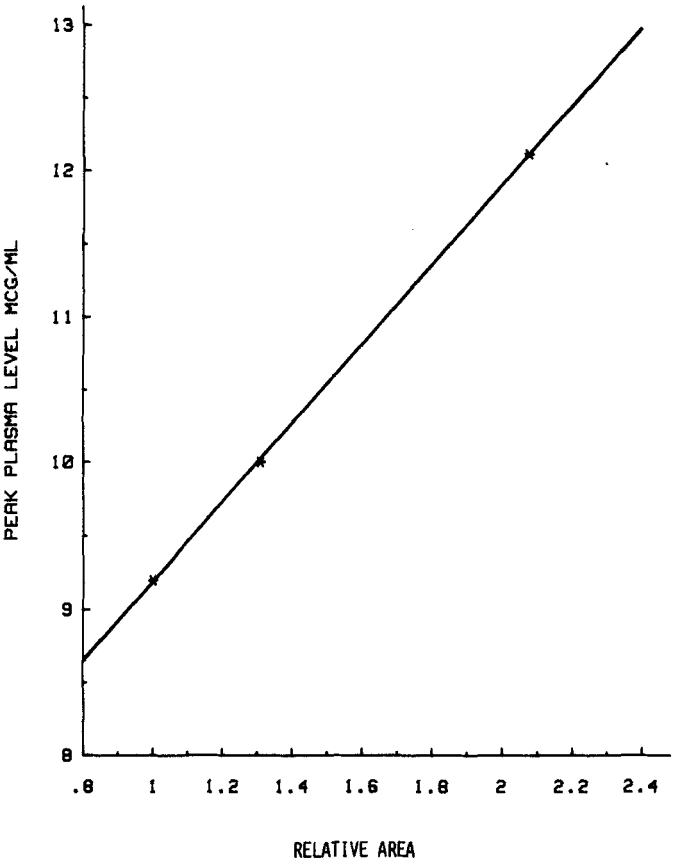

Figure 3. Relationship between the relative area of the volume injected to the peak plasma level.

physiological pH. If this occurred, the subsequent dissolution of the precipitated Compound I would control the absorption rate and the rate would then become a function of the relative surface area. This hypothesis, while it explains the results obtained in this study, still requires verification with a more statistically adequate number of animals.

In summary, Compound I was well absorbed after subcutaneous injection at the three concentrations as indicated by plasma profiles, injection site residue and recoveries from urine and feces. The data indicate that the absorption process may involve compound precipitation followed by dissolution controlled absorption, contrary to a purely diffusion controlled absorption process. Even though compound I absorption appeared complete after subcutaneous administration in rabbits, extrapolation to larger animals must be done with caution due to the large difference in amount injected.

REFERENCES

1. K. Kakemi, H. Sezaki, K. Okumura, H. Kobayashi, and S. Furusawa, Chem. Pharm. Bull. (Tokyo) 20(3), 443 (1972).

2. K. Kakemi, H. Sezaki, K. Okumura, and C. Takada, Chem. Pharm. Bull. (Tokyo), 19, 2058 (1971).

3. K. Kakemi, H. Sezaki, K. Okumura, and S. Ashida, Chem. Pharm. Bull. (Tokyo) 17, 1332 (1969).

4. S. Feldman, Bull. Parenteral Drug Assoc., 28, 53 (1974).

5. J. Schou, Pharmacol. Rev. 13, 441 (1961).

6. L. R. Cruthers, R. D. Haugwitz, M. Haslanger, B. V. Maurer, J. Watrous and W. H. Linkenheimer, Experientia, 34, 1574 (1978).

7. B.E. Ballard, J. Pharm. Sci., 57, 357 (1968).

8. R. B. Sund and S. Schou, Acta. Pharmacol. et Toxicol., 21, 313 (1964).