DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 3(6), 507-522 (1977)

THE USE OF RADIO-LABELED DRUG IN EARLY DOSAGE FORM DEVELOPMENT TO PROVIDE A RELATION BETWEEN PHYSICAL DOSAGE FORM CHARACTERISTICS AND BIOAVAILABILITY

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ABSTRACT

A chemical compound in early drug development was used in its radio-labeled form to provide a relation to the physical dosage form characteristics and bioavailability. The ^{14}C-labeled compound, a dibenzthiepin acetic acid derivative, was recrystallized in the same manner as the cold compound. A procedure was developed such that the crystal type and size obtained was similar to that of the cold compound. The photomicrographs and specific surface area of both cold and ^{14}C-labeled material were equivalent. Capsules, prepared with both cold and ^{14}C-labeled material, were demonstrated to have equivalent in vitro dissolution profiles.

The capsules containing the ^{14}C-labeled material were found to be well absorbed when administered to cynomolgus monkeys. Assuming the monkey is a good absorption model for man, this study provided a preliminary assessment of the bioavailability of the compound in man. It also established

preliminary control parameters for the raw material and the dissolution of the capsules. These controls would then be used for monitoring capsules intended for early clinical studies.

INTRODUCTION

Ideally, the bioavailability of a solid dosage form should be known prior to its study in man or animals. The importance of bioavailability studies in drug development has previously been discussed [1 2]. In the early stages of the development of a new drug, this knowledge can be hindered by the time and expense required for the analytical method development for the drug analysis in biological fluids. Then when a method is available, it may be a time consuming and/or an expensive method to perform - yet this method is required to assess the drug bioavailability from the dosage form in question.

However, it may be possible to obtain the necessary information using another technique. The use of a radio-labeled drug in early drug development is generally used for metabolism and pharmacokinetic studies. It also may be used for absorption studies comparing an oral drug solution to an iv bolus injection. It generally has been thought difficult to relate a solid dosage form made with radio-labeled drug to its equivalent made with cold drug material. If, however, the parameters of the preparation of the crystals of a radio-labeled drug could be controlled so that the resulting product were similar in nature and could be related by physical measurements to the cold drug material, the radio-labeled drug could be utilized in bioavailability studies. The bioavailability study would provide absorption data on the radio-labeled drug capsules. This would be relative to the *in vitro* dissolution test and other tests (e.g., surface area) which then could be used to control capsule batches manufactured for early clinical studies.

The objective of this study was to examine this approach with a compound in the early drug development stage.

Experimental

Materials: The drug used in this study - 6,11-Dihydro-11-oxodibenzo [b,e] thiepin-3-acetic acid (compound I) - was used as received.[1] The ^{14}C-labeled compound I[1] was labeled at the carboxylic acid position. The ^{14}C-labeled compound I (specific activity 30.5 μCi/mg) was recrystallized with cold compound I from acetone/benzene to produce material with a specific activity of 1.25 μCi/mg. The recrystallization procedure used was identical to that used for production of cold batches of compound I.

All other materials were of USP grade.

Capsules: The capsule formulation used in these studies had been designed for optimal *in vitro* dissolution characteristics, as well as flow and compaction properties as required for an automated capsule filling machine. The compound I was present at 10 mg per capsule (standard hard gelatin). The lubricant, magnesium stearate, was present at 0.5% in the cold compound I formulated capsules and 0.6% in the radio-labeled compound I formulated capsules. The higher lubricant level in the radio-labeled compound I capsules was intended to provide a slightly slower *in vitro* dissolution rate, or at least insure that the *in vitro* dissolution rate would not be greater than that of the cold compound I formulated capsules.

Surface Area Determinations: Surface area determinations were made on compound I, both the cold and ^{14}C-labeled material. The surface area was determined using the single point BET method.[2] Also, photomicrographs were taken of each lot for comparison.

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[2] Quantasorb®, Quantachrome Corp., Greenvale, NY 11548

In Vitro Dissolution Apparatus: The in vitro dissolution determinations utilized a multiple spindle stirring drive with variable speed drive control.[3] This system allowed the monitoring of up to six dissolution determinations simultaneously. The description of the dissolution vessel, which is the critical part of an in vitro dissolution system, is found in Figure 1. The dissolution vessel was a round-bottom reaction kettle,[4] 1 liter. The stirring device was a polyethylene propeller,[5] which was rotated at 75 rpm. The receptor medium was 500 mls of deaerated, pH 7 phosphate buffer, 0.1 M, which was maintained at 37°C. Each capsule was located at the bottom of the dissolution vessel by use of a stainless steel wire, coiled loosely around the capsule. Samples of the dissolution medium were taken through a sintered glass filter stick. This consisted of a sintered glass filter[6] with the cup cut off at the glass frit. The filter stem then was replaced with a capillary tube (0.8 mm i.d. x 4.1 mm o.d.; 14 cm length) with 1.5 cm of the end ground to a smaller diameter (3.0 mm) to accept sampling tubing. 10 ml samples were withdrawn manually at 3 minute intervals from zero to 12 minutes and than at 16 and 20 minutes. At each sample time, the dissolution receptor was replaced with an equal volume of fresh buffer. After the last sample, the solution was stirred at 250 rpm for 20 minutes and sampled to determine maximum drug dissolution. The samples were analyzed by UV at 254 nm and for the labeled compound I capsules by liquid scintillation counting as well. The sample for liquid scintillation counting was prepared by pipetting 2 ml of dissolution sample into 15 ml of scintillation cocktail.[7]

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[3] Model 72R115 Hanson Research Corp., Northridge, CA 91324
[4] Kimax No. 33700-S1, Owens-Illinois, Inc., Toledo, OH 43666
[5] No. S-76680, Sargent-Welch, Anaheim, CA 92803
[6] Kimble No. 28400, Scientific Products, Menlo Park, CA 94025
[7] Oxyfluor-H_2O, New England Nuclear, Boston, MA 02118

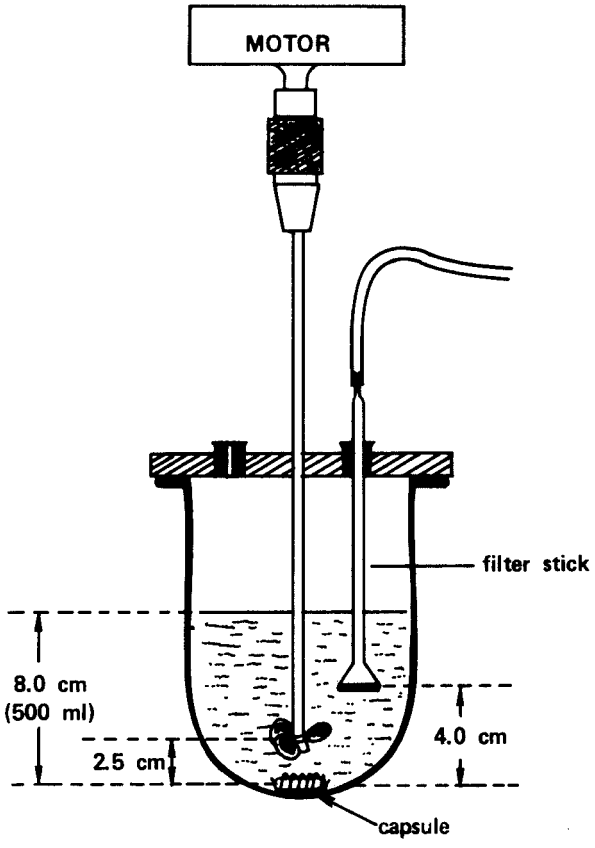


FIGURE 1. DISSOLUTION VESSEL AS SET FOR CAPSULE DISSOLUTION

Efficiency was determined by the standard method using ^{14}C toluene standard. The calculations for amount dissolved at each time were corrected for volume removed.

Since the UV and the liquid scintillation methods of analysis were shown to be equivalent, only the UV data were reported.

Capsule Bioavailability: Two normal adult male cynomolgus monkeys (*Macaca fascicularis*) #266 and #275 weighing 5.0

and 4.8 kg were used in this study. Both monkeys were in good health and were not used in other experiments for at least two weeks prior to this study or during the course of the study. Concomitant medication was not permitted during the course of the study. Each of these monkeys had previously received ^{14}C compound I either as an iv dose (#266) or an oral solution (#275) and were, therefore, ideal models in which to study the oral absorption characteristics of the drug from capsules.

Each monkey was fasted overnight (12 hours) prior to dosing, however, water as allowed *ad libitum*. Each monkey received a dose of two capsules which were placed deep in the throat of the monkey and washed down with about 20 ml of distilled water. Food was withheld for 4 hours after the dose, but water allowed *ad libitum* throughout the study.

Each monkey was set up with an indwelling intravenous catheter for sampling of blood during the 0 to 10 hour time period. After this, the catheter was removed and samples obtained from a suitable leg vein by venipuncture. Two to three ml blood samples were drawn into heparinized syringes prior to dosing (baseline) and at the following times after the dose: 0.25, 0.5, 1, 1.5, 2,3,4,6,8,10,24,28,72 and 96 hours. Complete urine collections were made just prior to dosing (baseline) and immediately after voluntary urination, following administration of the dose. The exact time and volume of urine collected was recorded. Urine was collected out to 96 hours. Complete fecal collections were made one or two days prior to dosing (baseline) and as soon as possible after voluntary defecation following administration of the dose. Fecal collections were carried out in 24 hour pools for 4 days following the dose.

Due to the instability of this compound in light, all samples were collected and stored in light resistant containers. In addition, all sample processing was carried out in a protected area, out of direct exposure to outside light or

artificial laboratory light. Blood samples were centrifuged immediately after collection and the plasma fraction separated and transferred to appropriate test tubes and placed in an ice-bath or refrigerator. The red blood cell fraction was discarded. As soon as possible after processing, duplicate aliquots of plasma (0.1 - 0.2 ml) were mixed with 15 ml of scintillation cocktail[8] in liquid scintillation vials and analyzed for total radioactivity. All urine samples were transferred to an ice-bath or refrigerator as soon as possible after collection. The urine collection was filtered through glass wool to remove debris, if necessary, and duplicate aliquots (0.05 - 0.2 ml) combined with 15 ml of scintillation coctail[8] and assayed for total radioactivity. Complete fecal collections were made as daily 24 hour samples and placed in tared containers. The collection was transferred directly to an oven (60°C) and completely dried. Dry weights were recorded and the entire sample blended and cuplicate 50 mg aliquots weighed and combusted for quantitation of total radioactivity.

Biological samples (plasma, urine and feces) were analyzed for total radioactivity only by the usual radio-assay and combustion procedures. All quench correction was by automatic external standardization. The data was then converted to equivalent amounts based upon the specific activity of the dose. Area under the plasma concentration versus time curve from 0 through 24 hours and fractional recovery of the dose in the urine from 0 through 96 hours was calculated. Absorption was estimated by comparing these parameters to those observed following intravenous dosing of compound I to cynomolgus monkeys. Urinary data was compared directly; however, plasma data had to be corrected for dose before comparing to the iv study due to the fact that exactly equivalent doses could not be given in the oral dose study.

RESULTS AND DISCUSSION

The procedure for the recrystallization of the ^{14}C-compound I required careful development. This was to ensure the production of crystals of the same type and size of the cold compound I and to minimize the loss of ^{14}C-compound I in the mother liquor. This may seem a trivial part of the experiment, yet without success here the remainder of the experiment could not proceed.

The specific surface area of both lots of compound I, ^{14}C-labeled and cold, Lot L and A respectively are reported in Table 1. At a significance level of $\alpha = .05$, the mean of one lot was not different from the other.

Photomicrographs were made of the two lots for visual comparison (Figure 2). The results confirm those of the surface area measurements. These measurements demonstrate the success of producing ^{14}C-compound I crystals with physical properties similar to the cold compound I crystals. This was necessary for the remainder of the experiment to bear significance.

Capsules containing the ^{14}C-compound I (Lot 126) and cold compound I (Lot 111A) were tested for their *in vitro* dissolution characteristics. These results are exhibited in Table 2 and Figure 3. The results are the mean of five individual capsule dissolution determinations.

TABLE 1

Comparison of Specific Surface Areas of Labeled and Cold Compound I

Lot	Specific Surface Area (m^2/gm) Determination:			
	1	2	3	Mean ± S.D.
Lot L, ^{14}C-Labeled Compound I	.15	.21	.16	.17 ± .03
Lot A, Compound I	.18	.19	.18	.18 ± .006

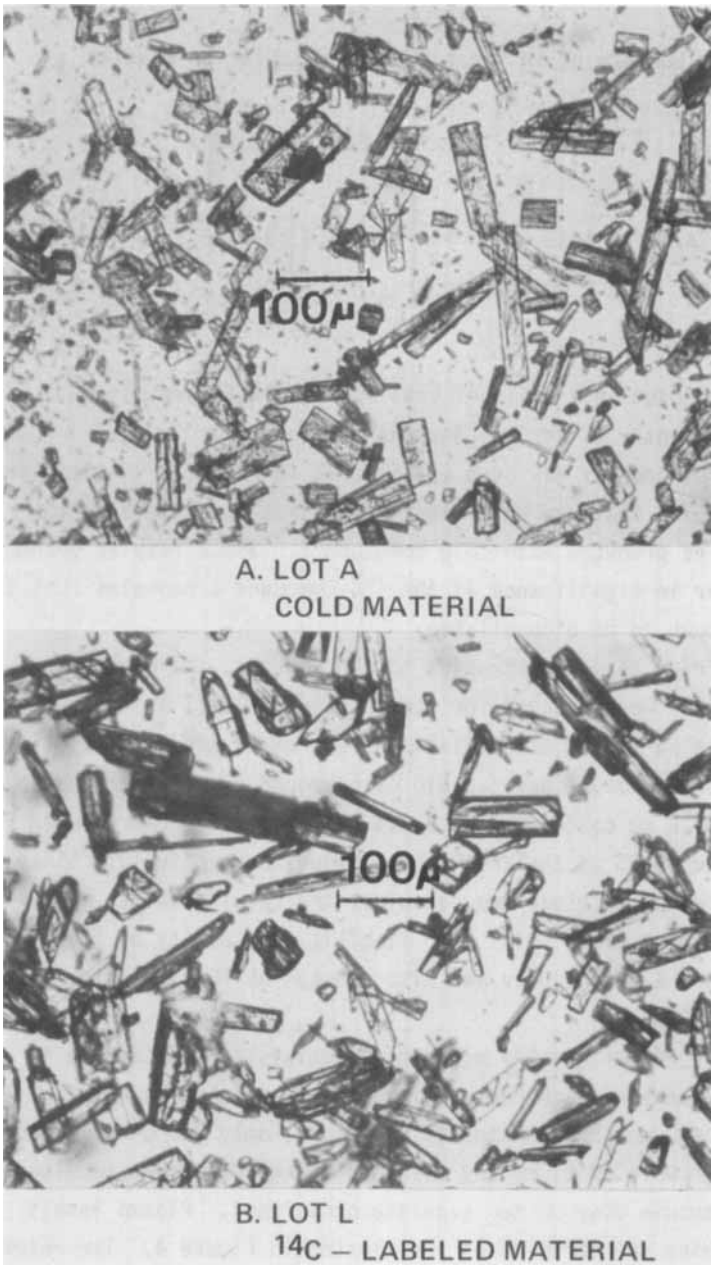


FIGURE 2. PHOTOMICROGRAPHS OF TWO LOTS OF COMPOUND I

TABLE 2

Summary of In Vitro Capsule Dissolution Results

Capsule Lot	Percent Dissolved ± S.D. with time (minutes)					
	3'	6'	9'	12'	16'	20'
Lot 111A	49 ± 15	77 ± 13	87 ± 6.9	91 ± 6.1	97 ± 5.1	99 ± 3.0
Lot 126	55 ± 28	86 ± 3.9	94 ± 4.1	96 ± 2.3	97 ± 2.0	100 ± 0

The dissolution results of Lots 126 and 111A were not significantly different [Student's t test, $\alpha = 0.05$].

Methodology has been established to produce ^{14}C-compound I capsules that are equivalent by physical measurements to capsules produced with cold compound I. These results become greater in significance if the ^{14}C-compound I capsules (Lot 126) are shown to be bioavailable.

Prior to administration to the monkeys, the capsules (Lot 126) were assayed for radioactive, as well as cold drug content by liquid scintillation spectrometry and UV analysis. The actual amount per capsule of compound I was determined to be 9.8 mg containing 11.9 μCi of label which represents 98% and 95.2% of the theoretical amount respectively. Since each monkey received two capsules, the total dose per monkey was 19.6 mg (23.8 μCi). The stability and purity of [^{14}C] compound I in the dose was also checked by TLC and determined to be >99%.

It was originally planned to administer the capsule to three monkeys which had received the drug intravenously in previous metabolism studies. However, only two of these monkeys were used, monkey #275 and monkey #266 (who received the capsule dose on two separate occasions). Plasma levels following the three doses are plotted in Figure 4. The relevant bioavailability parameters following the oral dose are listed in Table 3 and compared to iv data from a previous study.

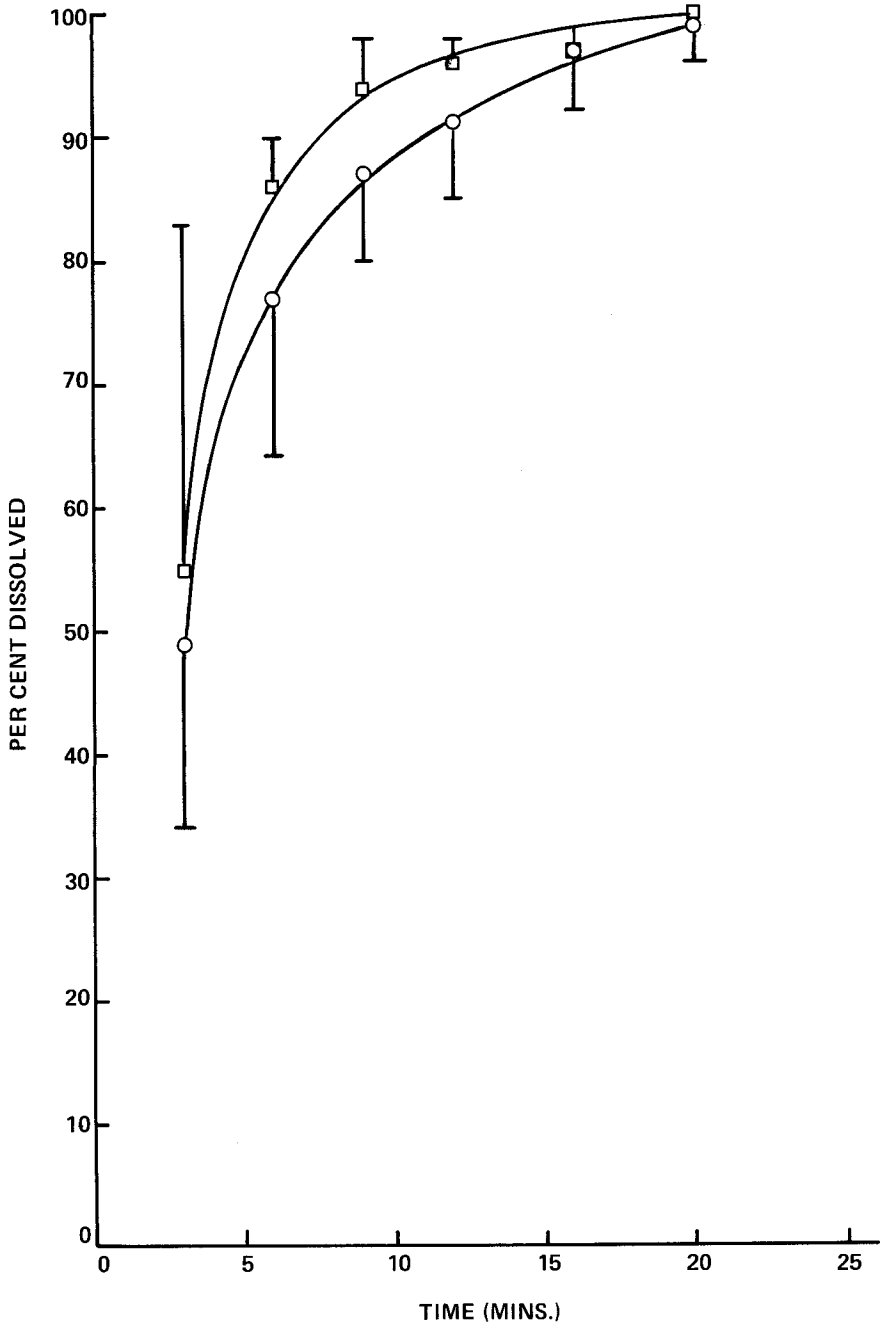
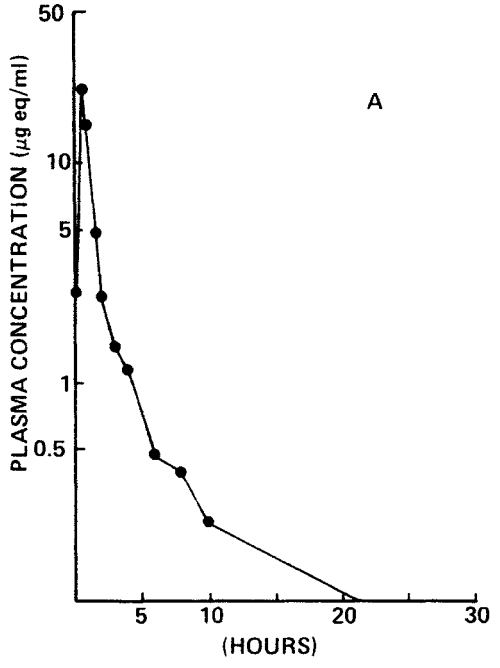
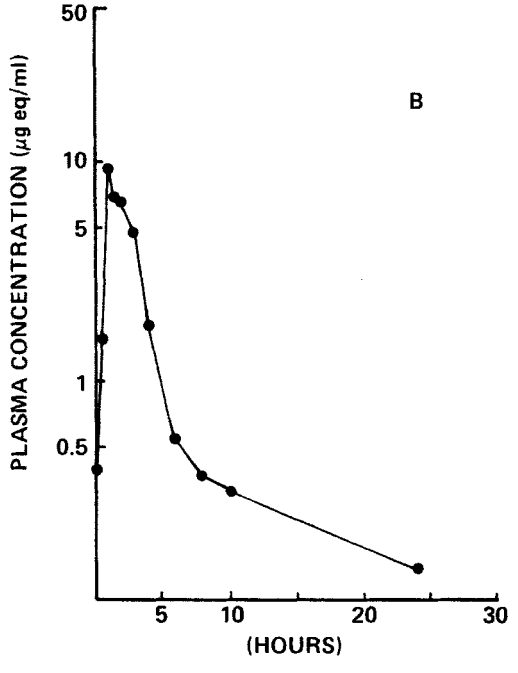


FIGURE 3. IN VITRO DISSOLUTION OF CAPSULES FROM THE TWO LOTS, ONE CONTAINING 10 mg OF COLD COMPOUND I AND THE OTHER 10 mg OF ^{14}C–COMPOUND I
○ LOT 111A; □ LOT 126; ERROR BARS ARE STD. DEV.





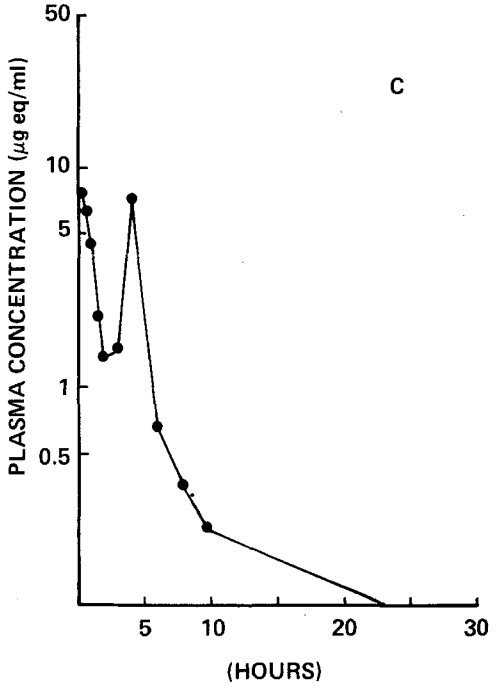


FIGURE 4. PLASMA LEVELS OF TOTAL EQUIVALENTS FOLLOWING AN ORAL DOSE OF ^{14}C–COMPOUND I (19.6 mg CONTAINING 23.8 μCi) TO CYNOMOLGUS MONKEYS (A) #266 FIRST DOSE, (B) #266 SECOND DOSE AND (C) #275.

Peak plasma levels ranged from 7.8 to 22.2 μg eq/ml while peak times ranged from 0.25 to 1.0 hours, as observed in Table 3. Monkey #275 was unusual in that two distinct peaks were observed at 0.25 and 4.0 hours. A possible explanation for this lies in the fact that two capsules were administered to each monkey. The capsules were administered one at a time, by depositing each in the monkeys throat followed by water and then closing the mouth allowing the monkey to swallow. It is conceivable that monkey #275

TABLE 3

Comparative Bioavailability Parameters Obtained Following Intravenous and Oral Dosing of ^{14}C-Compound I to Cynomolgus Monkeys

Subject	-[1]	#266(a)	#266(b)	#275
Dose route	iv	po	po	po
Dose rate (mg/kg)	5	3.9	3.8	4.1
Parameter:				
$AUC/_0^{24\ hr}$ (µg eq/ml. hr)	40.2	28.1	26.4	25.6
Cp, max (µg eq/ml)	-	22.2	9.1	7.8
T, max (hr)	-	0.5	1.0	0.25
Aexc, urine (% of dose)	88	88	86	79
Percent abosrption:				
-Urine analysis[2]	-	100	98	90
-AUC analysis[3]	-	90	86	78

[1] Data was averaged following intravenous doses of ^{14}C-compound I to cynomolgus monkeys.

[2] Percent absorption = $\frac{(\text{Aexc, urine})po}{(\text{Aexc, urine})iv} \cdot 100$

[3] Percent absorption = $\frac{AUCpo}{AUCiv} \cdot \frac{\text{Dose iv}}{\text{Dose po}} \cdot 100$

swallowed one of the capsules immediately and stored the other in the jowles until about 3 or 4 hours after dosing. Another possible explanation is that one of the capsules was retained in the stomach for a few hours due to delayed gastric emptying.

Area under the plasma concentration versus time curve (from 0 through 24 hours) was very reproducible ranging from 25.6 to 28.1 µg eq/ml hr. In comparing AUC after oral and iv dosing, the absorption of compound I was estimated at 78 to 90% as shown in Table 3. The percent of the dose excreted into the urine following oral capsules ranged from 79 to 88% which

compares closely to 88% excreted into the urine after iv dosing. Absorption estimates based upon urine excretion data ranged from 90 to 100%, as shown in Table 3. Less than 2% was found excreted in the feces.

Based upon the above data, it can be concluded that compound I is well absorbed from formulated capsules in the cynomolgus monkey. Assuming that the monkey is a good absorption model for man, equivalent capsules for studies in man should also be well absorbed and provide a meaningful study. The methodology employed in this study should prove to be very valuable in future studies with other formulations or drugs in man to insure that the time and resources spent will provide the necessary information.

CONCLUSIONS

1. This investigation has shown that radio-labeled material (compound I) was prepared with equivalent physical parameters to cold material, as measured by surface area, particle size and shape and dissolution rate of the material formulated in capsules.
2. The capsules with ^{14}C-labeled drug were administered to cynomolgus monkeys and plasma, urine, and fecal samples were monitored. Area under the curve, as well as urinary excretion data, indicated that the drug was well-absorbed (mean 90%, range 78 to 100%) from the capsules.
3. This procedure allows physical parameters of a drug substance and dissolution specifications on the dosage form (capsule) to be established early in development which have a meaningful relation to the bioavailability of the drug substance from a capsule dosage form.

ACKNOWLEDGEMENTS

The authors thank Dr. Howard Parnes of the Institute of Organic Chemistry, Syntex Research, for the synthesis of the ^{14}C-

labeled compound used in this study. The synthesis and biological activity of compound I used in the present study will be reported by Dr. Jack Ackrell, Dr. Joseph Muchowski and Mr. Wendel Rooks, all of Syntex Research, in a forthcoming publication.

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