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ENHANCEMENT OF SOLUBILITY, DISSOLUTION RATE, AND ORAL BIOAVAILABILITY OF RS-82856 BY COMPLEX FORMATION WITH CYCLODEXTRINS

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SUMMARY

RS-82856 is a new inotropic agent for treatment of congestive heart failure. Oral bioavailability was found to be very poor likely due to insufficient aqueous solubility (~ 4.4 mcg/ml) and slow dissolution rate. Inclusion complexes with cyclodextrins were shown to enhance the solubility, dissolution rate and thereby oral bioavailability of the drug. Maximum solubilities of the drug complexes with alpha-, beta-, and gamma-cyclodextrin were 14, 30 and 55 times, respectively, more soluble than the uncomplexed drug. Phase solubility studies revealed a 1:1 complexation constant of 136.5, 370.4, and 64.7 for alpha-, beta- and gamma-cyclodextrin complexes, respectively. The complexation between beta-cyclodextrin and the drug is apparently the strongest among the three cyclodextrins. Dissolution profiles of the beta-cyclodextrin complex indicated a dramatic increase in dissolution rate compared to that of the drug. However, a physical mixture of the beta-cyclodextrin and the drug gave an identical dissolution profile to that of the drug. The beta-cyclodextrin complex of the drug dissolves 90% within 20 minutes while the free base dissolves 25% within the same time interval in

water. In an acidic medium (pH 1.5) the beta-cyclodextrin complex and the free base dissolve 90% and 30% respectively within 10 minutes. In a single dose cross-over study in three dogs, the bioavailability of the beta-cyclodextrin complex was found to improve greatly over that of the drug. An increased C_{max} (2.5 times), and an increased AUC (2.5 times) were observed with the beta-cyclodextrin complex compared to the drug.

INTRODUCTION

The appeal of the cyclodextrin drug complex to pharmaceutical scientists is due not only to their ease of formation in aqueous systems, but also to their applications in solubilization of poorly water soluble compounds (1-5), enhancement of dissolution rate (6-8), oral bioavailability (9-10), and drug stability (11-14).

The inclusion behavior is described by a theory which proposes that a portion of the substrate which is less hydrophilic than the cyclodextrin may be enclosed within the cavity of the cyclodextrin. Because of enclosure of the hydrophobic moiety, aqueous solubility increases. The interaction between the enclosed substrate and cyclodextrin appears to be dependent on ligand size and substrate geometry (7). The binding forces consist of nonspecific interactions such as van der Waals and hydrophobic bonding. A covalent bond is not formed (7, 15).

RS-82856, N-cyclohexyl-N-methyl-4-(7-oxy-1,2,3,4,-tetra-hydroimidazo [2,1-6] quinazolin-2-one) (Fig.1), is a rod-shaped molecule with dimensions of 21.2 X 6.8 X 5.2 A, estimated from CPK molecule model. The hydrophobic cyclohexyl group at one end of the molecule appears to be a potential candidate for complex formation with cyclodextrins. Increased solubility, dissolution rate and thereby enhanced oral bioavailability may be anticipated for the inclusion complex.

The present study was undertaken to determine if the solubility could be increased by complex formation, and subsequently to determine if *in vivo* oral bioavailability of the complex would be improved.

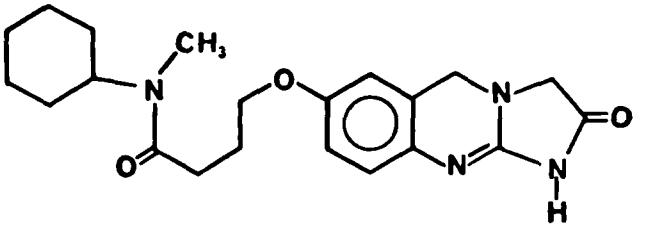

Fig. 1 Structure of RS-82856

MATERIALS AND METHOD

Material

Alpha, beta and gamma cyclodextrins (Aldrich, Milwaukee, WI 53233) were used as received. All solvents were analytical reagent grade. Double-distilled water was used throughout.

Solubility Studies

The phase solubility diagrams were obtained according to the method described by Higuchi and Connors (16). Excess amounts of drug were added to aqueous solutions containing various concentrations of cyclodextrins and the mixture was tumbled end over end at 25° ± 0.5°C. After equilibrium was attained (approx. 3 days) the supernate was pipetted off and filtered through a 0.2 micron filter. A portion of the filtrate was then diluted with 0.01M sodium phosphate solution (pH 6.8) and analyzed spectrophotometrically.

Preparation of Solid Complex

The beta-cyclodextrin/drug solid complex was prepared by mixing appropriate amounts of the cyclodextrin and free base in water. Amounts were calculated from the initial plateau point of the phase solubility diagram (see Fig. 2). Taking into account the magnitude of the stability

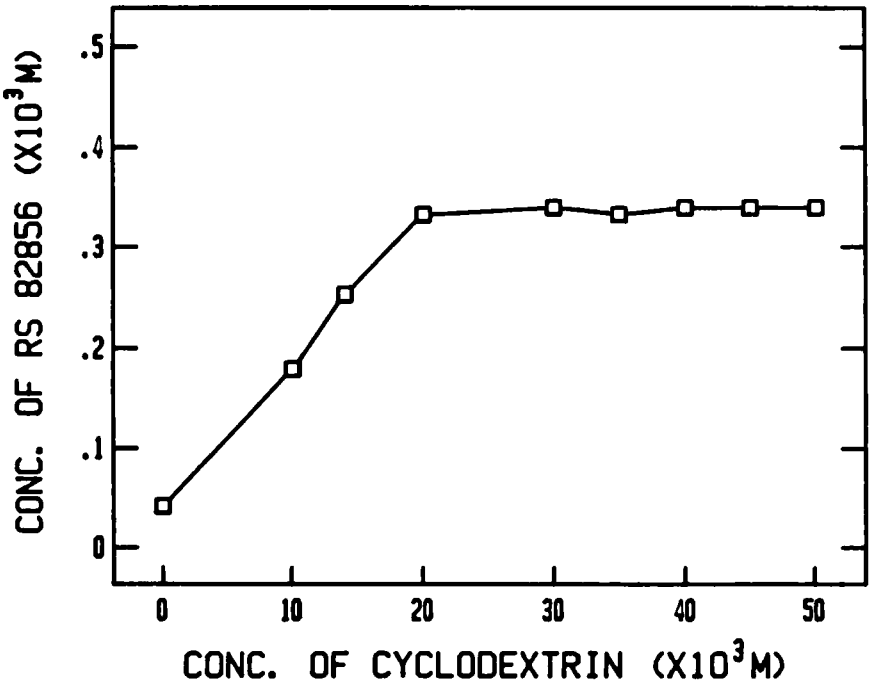

Fig. 2 Phase Solubility Diagram of RS-82856 Beta Cyclodextrin Complex

constant obtained from the phase solubility study; the concentration of the cyclodextrin (0.169 M) employed was largely in excess compared with that of the substrate (0.339 x 10^{-3}M). The aqueous mixture was stirred with a magnetic stirrer at 25°C for 1 day, then filtered through a sintered glass filter to remove insoluble material. The solution was evaporated to dryness under vacuum for approximately 3 hours to obtain the inclusion complex powder. The complex consisted of the drug and the beta-cyclodextrin complex in a molar ratio of 1:50 and contained 0.60% (W/W) of the free base of the drug.

Dissolution Studies

Dissolution data were obtained at 37°C ± 0.5°C using a HP 8451A diode array spectrophotometer to monitor the absorbance at 280 nm. Samples for

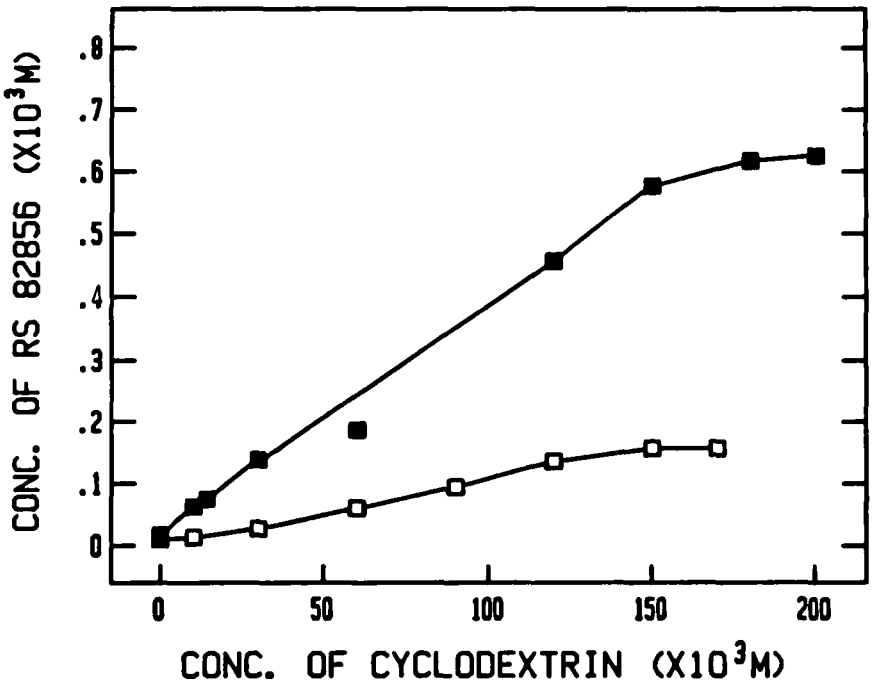

Fig. 3 Phase Solubility Diagram of RS-82856 Gamma (■) and alpha (□) cyclodextrin complex

dissolution analysis were prepared in duplicate by geometric dilution of the active with lactose (no lactose was added to the complex) and loose packing into a hard gelatin capsule (size #00, clear). The capsule weight (400 mg) and the free base equivalent of drug (2 mg/capsule) were identical for all samples. The dissolution media was 900 ml water, at 37°C and a pH of 6.25, or 900 ml simulated gastric fluid at 37°C and pH of 1.5.

RESULTS AND DISCUSSION

Figures 2 and 3 show the phase solubility diagrams obtained for the drug with alpha, beta and gamma-cyclodextrin. The data indicates that the solubility of the drug increases as a function of cyclodextrin

TABLE I

$K_{1:1}$ VALUES OF CYCLODEXTRIN COMPLEXES OF RS-82856

	Alpha-	Beta-	Gamma-
$K_{1:1}(M^{-1})$	136.5	370.4	64.7
MAX. SOL (mcg/ml)	62.5	130.7	240.0

concentration until the solubility limits of the cyclodextrins are reached. Beyond this point further addition of cyclodextrins does not lead to an increase in the concentration of the drug in solution. The cyclodextrin concentrations at which the plateau of the curves start are in good agreement with the reported solubility limits of the cyclodextrins (17).

The exact stoichiometric ratio of the complex could not be determined from the phase diagrams due to the Ap type curve (16). Nevertheless, an apparent constant calculated with the assumption of 1:1 stoichiometry was taken to describe the system as follows:

$$K_{11} = \text{Slope}/S_o\ (1\text{-Slope})$$

The calculated values of the constants (Table 1) decrease in the sequence of beta-, alpha-, and gamma-cyclodextrin. Apparently the inclusion complex formed with beta-cyclodextrin is the most stable one among the three. The cavity size of the cyclodextrin appears to be prevailing determinant for the "goodness of fit" between the guest molecule and the host cavity. The diameters of the cavities are 4.7-5.2 A, 6.0-6.4 A, and 7.5-8.3 A for alpha-, beta- and gamma-cyclodextrin, respectively (3), and the diameter of the drug (Fig. 1) is 5.2-6.8 A.

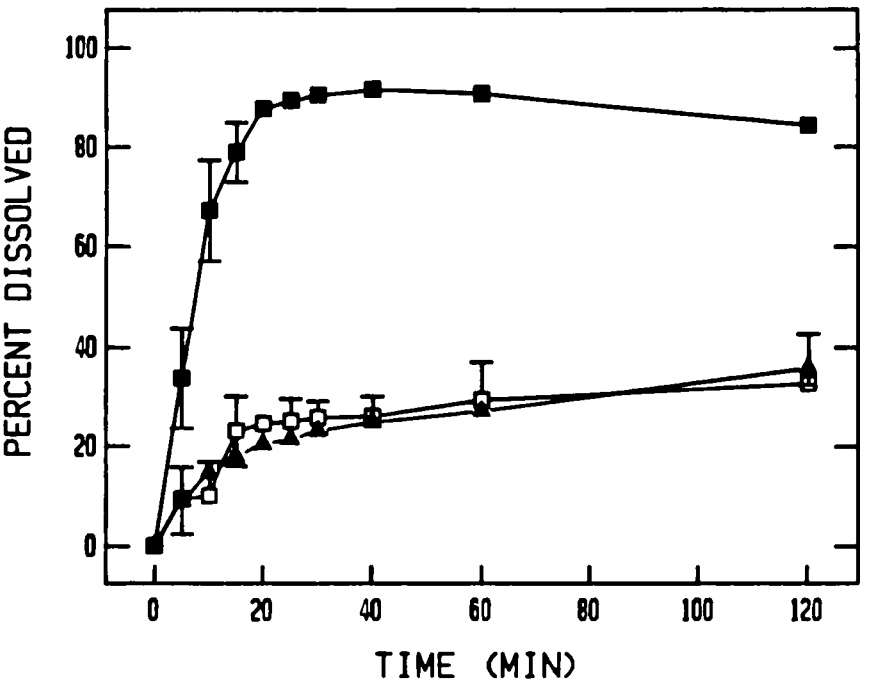

Fig. 4 Dissolution Behavior of RS-82856 free base (□), its beta cyclodextrin complex (■) and beta cyclodextrin physical mixture (▲) at the same concentration in water at 37°C.

The dissolution profiles (Figures 4 and 5) represent the mean of several determinations. Standard deviations are indicated by the bars. Reproducibility of the profiles is good in general. The largest standard deviation is about 12% (resulted with the free base).

It is evident that the dissolution rate for the beta-cyclodextrin complex of RS-82856 is significantly better than that of the drug itself. As shown in the dissolution profiles (Figure 4), within 20 min. 90% of the complexed drug was dissolved while only 22% of the free base and physical mixture were in solution at the same time period. The plateaus in these dissolution profiles (Figure 4) are only 1.5% and 13.3% of the respective equilibrium solubilities of the complex (130 mcg/ml) and the free base (5 mcg/ml). The "sink condition" is believed to have maintained throughout the experiment. However, it is not clear why the

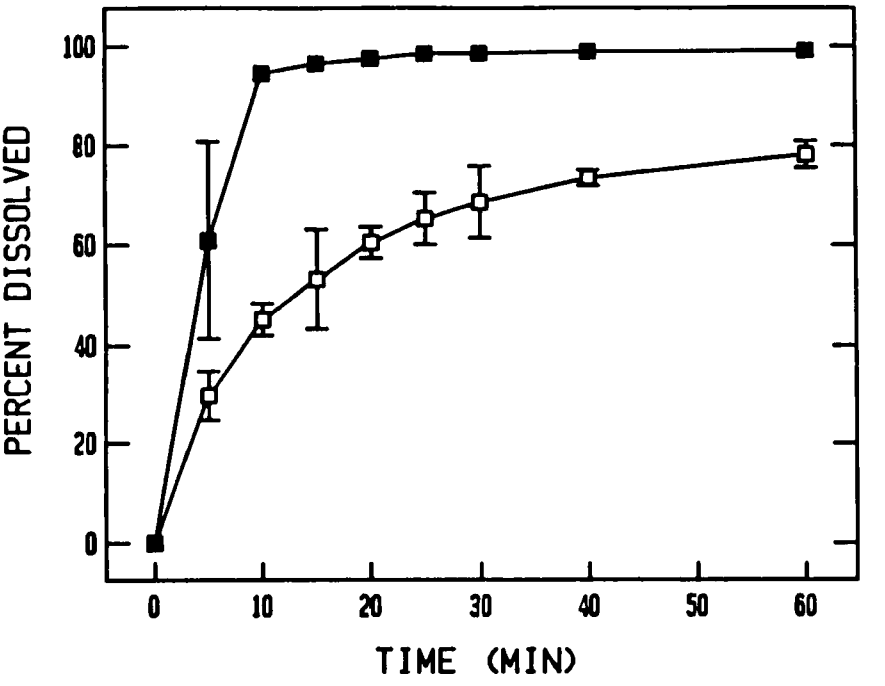

Fig. 5 Dissolution behavior of RS-82856 free base (□) and its beta cyclodextrin complex (■) at the same concentration at gastric pH.

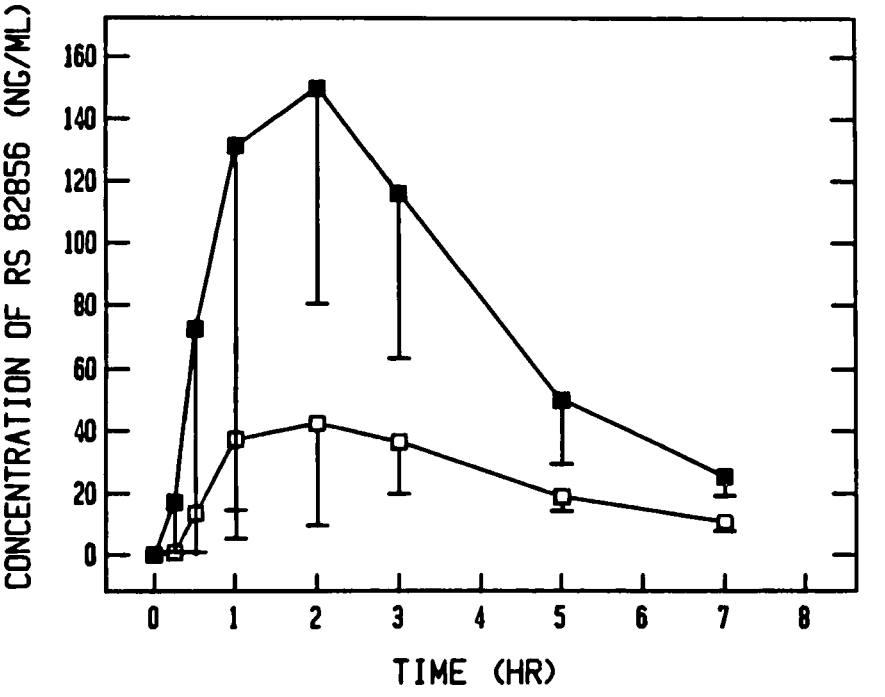

Fig. 6 Plasma concentrations of RS-82856 after oral administration of capsules to dogs.
Beta-cyclodextrin complex (■), free base (□).

TABLE II

Comparison of Pharmacokinetic Parameters of RS-8256 in Dogs
Given Single Oral Doses of RS-82856 in Two Different Formulations

Formulation	Dose (mg Free Base/ kg	Dog I.D.	RS-82856 AUC 0-7 hr. (ng/ml/hr.)	C_{max} (ng/ml)	T_{max} (hr)	T1/2 (hr)
		A	263.7	72.4	2.0	1.29
Free Base (A)	1.5	B	315.0	113.0	2.0	1.49
		C	134.9	37.8	2.0	1.39
		Mean	237.9	74.4	2.0	1.39
		±S.E.	± 53.6	±21.7	± 0	±0.06
Beta-Cyclodextrin	1.1	A	877.2	260.0	1.0	1.81
Complex (B)		B	499.0	144.0	3.0	1.50
		C	357.4	101.0	1.0	2.85
		Mean	577.9	168.3	1.67	2.05
		±S.E.	±155.2	47.5	±0.67	±0.41

$$\text{Mean Ratio} = \frac{AUC_{\text{Formulation B}}}{AUC_{\text{Formulation A}}} = 2.51 + 0.87$$

$$\text{Mean Ratio} = \frac{C_{max}\ (\text{Formulation B})}{C_{max}\ (\text{Formulation A})} = 2.51 + 1.17$$

dissolution rates for the free base and the physical mixture slowed down after 20 minutes of dissolution.

The slight decrease in the complex solubility after approximately 1 hour (Figure 4) may be attributed to the dissociation and subsequent precipitation of the free base. Similar dissolution behavior has been reported to occur with the PGE-gamma cyclodextrin complex (8).

At simulated gastric pH (Figure 5), the complex again showed improved dissolution rate over the free base. Ninety percent dissolution was reached within 10 minutes for the complex as compared to 41% free base. The pKa's of the drug are 3.5 and 11.25. It is therefore expected to dissolve faster in the simulated gastric fluid.

To evaluate the bioavailability of RS-82856 when administered in a solid form as the free base, or as the beta-cyclodextrin complex, three dogs were each given a single oral dose of the free base and the beta-cyclodextrin complex with a one week interval between doses in a randomized cross-over fashion. The free base was given at a dose of approximately 1.5 mg/kg and was administered as a mixture with lactose in a hard gelatin capsule. The beta-cyclodextrin complex was given at a dose of approximately 1.1 mg (free base equivalents)/kg and was also administered in a hard gelatin capsule. Samples of blood were taken from each dog at 0, 0.25, 0.5, 1.0, 2.0, 3.0, 5.0 and 7.0 hours following oral administration of each formulation. Plasma was separated from the whole blood by centrifugation and the concentrations of RS-82856 in the samples of plasma were determined by an HPLC method that has a sensitivity of 5 ng of RS-82856 per ml of plasma. The concentrations of RS-82856 in the plasma were used for the calculation of certain pharmacokinetic parameters for each formulation. The calculated pharmacokinetic parameters are shown in Table II.

The data in Table II show that although the beta-cyclodextrin complex was given at a dose of 1.1 mg free base/kg while the free base was given at a dose of 1.5 mg/kg, the beta-cyclodextrin complex gave rise to significantly higher AUC (2.51 $\pm$ 0.87 times that for free base) and C_{max} (2.51 $\pm$ 1.17 times that for free base). Therefore, it is evident that the superior dissolution property of the beta-cyclodextrin complex translated into an improved bioavailability of RS-82856.

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