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Release and absorption characteristics of chlorphenesin carbamate sustained-release formulations: in vitro-in vivo and in vivo dog-human correlations

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Abstract

The bioavailability of chlorphenesin carbamate (CPC) in sustained-release (SR) formulations exhibiting different in vitro release characteristics in dogs and humans was examined using formulations tested previously in the in vitro dissolution method. The human-dog correlation of the bioavailability of SR formulations was examined under fasting conditions. Pharmacokinetic analysis using the Wagner-Nelson procedure revealed sustained-release absorption characteristics for the SR formulations, with the exception of the immediate-release (IR) formulation as control in dogs and humans. For each of the SR formulations tested, regression analysis results of the percentage of CPC absorbed in human against that in dogs, at corresponding times, indicated a high correlation. Moreover, the correlation of the dissolution rates and bioavailabilities of these formulations in humans was also examined. Although the in vitro CPC release profiles from the SR formulations were smooth and controlled, they were too rapid when compared with the in vivo human data. However, the rank order was exactly the same between in vivo and in vitro data, and a good relationship was found after time scaling of the release data. These data imply that the release characteristics of CPC after changing of the formulations could be evaluated using the in vitro dissolution method or dogs as an animal model in place of human studies.

Keywords: Chlorphenesin carbamate; Sustained-release formulation; In vitro-in vivo correlation; Human-dog correlation

1. Introduction

The sustained release of drugs in the gastrointestinal (GI) tract following oral administration is the intended rate-limiting factor in the absorption process which, in turn, determines the bioavailability and therapeutic response (Hendeles et al., 1984). It is therefore essential in the development stages of oral sustained-release (SR) dosage forms to evaluate formulations using the in vitro dissolution test and animal studies

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(Uchida et al., 1986; Yamakawa et al., 1986). However, the prediction of in vivo performance in humans, in particular the absorption rate and the bioavailability, by employing in vitro dissolution tests has been difficult. There is a need for in vivo animal studies that will provide data comparable to those obtained in human studies. Beagle dogs are easy to handle and have been extensively used for formulation studies. However, in many cases, the bioavailability of drugs after oral administration of SR dosage forms to dogs also differed considerably from that in humans, because of the discrepancy in GI physiology between the two species (Aoyagi et al., 1982; Ogata et al., 1982, 1984). If the absorption of a drug is not affected by the GI physiological factors showing wide variations between humans and dogs, a close correlation may be shown between the bioavailabilities in dogs and those in humans. Chlorphenesin carbamate (CPC), a muscle relaxant, has been used in the treatment of skeletal muscle trauma and inflammation (Stern, 1963; Abruzzi, 1964). The solubility of CPC is almost constant in water over the physiological pH range, and CPC also displays a high partitioning coefficient into the lipid layer, irrespective of the pH change. CPC is absorbed from all parts of the rat intestine, and the site of absorption of CPC is broad enough to justify the use of sustained-release preparations of CPC (Akimoto et al., 1993). Moreover, a good correlation between the bioavailabilities of CPC from SR formulations in the two species was obtained.

In the present study, the correlation between in vivo results in humans and in vitro dissolution rates was investigated. In vivo absorption profiles of CPC granules in dogs were also compared with those obtained in humans. From the results, the beagle dog study and in vitro dissolution test were evaluated as a substitute for human studies on CPC bioavailability.

2. Materials and methods

2.1. Dosage forms

The experimental SR formulations of CPC were prepared as follows. Sugar crystals were coated with aqueous hydroxypropyl cellulose solution, powdered corn starch and CPC by the tumbling granulation method, and dried to make drug-coated granules. The granules obtained were then coated with ethyl cellulose in a centrifugal fluidizing granulator (CF). In addition, an immediate-release (IR) formulation, which was in the form of uncoated granules, was also prepared. The details of the preparation method were the same as reported elsewhere (Takashima et al., 1989).

2.2. In vitro dissolution tests

The relevant in vitro experimental conditions have been described previously (Akimoto et al., 1993). According to the JP paddle method (JP XI), 900 ml of JP 1st (JP XI) fluid containing 0.01% polysorbate 80 as a medium was stirred at 100 rpm. The drug concentration in the medium was assayed spectrophotometrically at 227 nm.

The mean in vitro release plots and their simulation curves are shown in Fig. 1. In vitro release data were fitted using the Weibull equation (Huguenin et al., 1988):

$$F(t) = F_{\text{inf}}\left(1 - e^{-[(t-t_0)/T_d]^{\beta}}\right) \quad (1)$$

where $F(t)$ is the fraction dissolved at time t, F_{inf} represents the fraction dissolved after infinite time, t_0 is the lag time, T_d denotes the time parameter, representing the time for release of

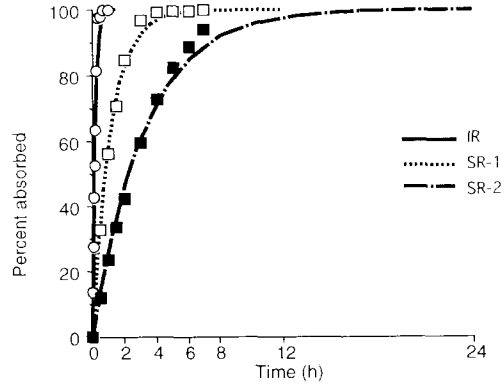

Fig. 1. In vitro dissolution profiles of CPC from experimental formulations together with the fitted curves corresponding to the Weibull function.

63.2% of the total dose, and β is the shape parameter. The parameters T_d, β and t_o were determined using FIT FUNCTION (RS/1 software package, 1988). Values for the ratio of SR formulations were calculated according to Eq. 2:

$$R = MDT_{vivo}/MDT_{vitro} \quad (2)$$

where MDT_{vitro} is the mean in vitro dissolution time, equal to the Weibull parameter T_d, for SR formulations, and MDT_{vivo} denotes an estimate of the mean in vivo dissolution time calculated according to Eq. 3:

$$MDT_{vivo} = MRT_{SR} - MRT_{REF} \quad (3)$$

where MRT_{SR} is the mean residence time of the SR formulations and MRT_{REF} represents the mean residence time for the reference dosage form (the solution or conventional tablet). Mean residence times (MRT) were obtained from the AUMC/AUC ratio. AUC, the area under the curve, and AUMC, the area under the first moment curve, were computed according to the trapezoidal rule (Yamaoka et al., 1978). Pharmacokinetic evaluation of the conventional tablet has been described and discussed elsewhere (Akimoto et al., 1993).

2.3. CPC analysis

Blood samples were collected in heparinized tubes. The samples were centrifuged and the plasma was kept frozen at −40°C until analysis. CPC concentration in plasma was determined by the normal-phase HPLC method. Procedural details of this method were described previously (Akimoto et al., 1993).

2.4. Pharmacokinetic studies

SR formulations, SR-1 and SR-2, and an IR formulation, IR, were evaluated pharmacokinetically in both dog and healthy human studies.

Five healthy human volunteers, aged 23–34 years and weighing 46–71 kg, participated in the studies. Each subject was determined to be in good health from their medical histories and physical examinations prior to beginning the study. All subjects gave their informed written consent. They were instructed to abstain from taking any medication and alcohol 1 week prior to starting and during the study. On the day of the experiment, each subject received two capsules containing each formulation in a randomized manner. Formulations were administered at 9:00 a.m. following overnight fasting. Food was withheld for 4 h after the administration of each formulation. No caffeinated drinks, beverages, or food were permitted from 24 h before the beginning until the end of each study. 2 weeks were allowed for the wash-out between treatment periods.

Four male beagle dogs, weighing between 12.5 and 13.8 kg, were administered a single CPC solution as a reference in the first week of the study. The SR formulations were administered to these dogs in a randomized manner at intervals of at least 2 weeks.

The method of Wagner and Nelson (1964) was used to calculate the cumulative percentage of the CPC dose absorbed from time 0 to t:

$$\% \text{ absorbed} = \frac{C_p(t) + K \cdot AUC_{0-t}}{K \cdot AUC_{0-\infty}}$$

where $C_p(t)$, K, AUC_{0-t} and $AUC_{0-\infty}$ are the plasma concentration of CPC at time t, the elimination rate constant, the dose normalized area under the CPC plasma concentration-time profile (AUC) from time 0 to t and the AUC from 0 to infinity, respectively, following oral administration of the conventional tablets or the aqueous solution. The AUC_{0-t} was determined according to the trapezoidal rule and $AUC_{0-\infty}$ was calculated according to the following equation:

$$AUC_{0-\infty} = AUC_{0-t_L} + C_p(t_L)/k$$

where t_L and $C_p(t_L)$ are time of last sampling and plasma concentration of CPC at t_L. These pharmacokinetic parameters were obtained from the data of our previous study (Akimoto et al., 1993).

2.5. Statistical analysis

Linear regression analysis was performed accordingly to the method of least squares using the

FIT LINE program (RS/1 software package, 1988). The correlation coefficients (r) between the percent absorbed and the percent in vitro released, and the percent absorbed in dogs and humans were respectively calculated by linear regression analysis.

3. Results

3.1. In vitro dissolution / in vivo absorption profiles

The percentages dissolved at different times using the paddle method (JP XI) are shown in Fig 1. The in vitro release profiles from SR formulations, SR-1 and SR-2, were smooth and continuous. Release from the IR formulation occurred more rapidly than that from SR formulations. The dissolution rates decreased with increasing amount of ethylcellulose as a coating material, in the following order: formulation IR, SR-1 and SR-2. The mean values of the doses dissolved within 7 h were almost 100% for both SR formulations.

The mean percentages absorbed vs time profiles following administrations to humans and dogs are illustrated in Fig. 2 and 3, respectively. The absorption of CPC after the administration of IR was almost completed in 4 h in both species. On the other hand, the absorption was also prolonged over 24 h after the administration of the SR formulations of CPC in dogs and humans.

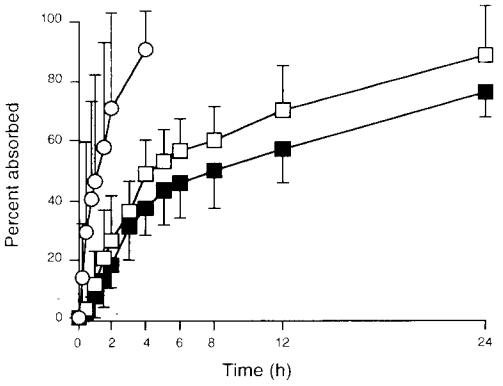

Fig. 2. Percent absorbed-time plot (mean ± S.D.) of CPC in dogs, after oral administration of IR (○), SR-1 (□) and SR-2 (■).

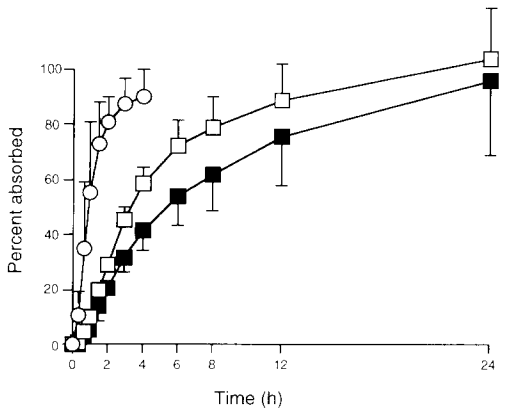

Fig. 3. Percent absorbed-time plot (mean ± S.D.) of CPC in humans, after oral administration of IR (○), SR-1 (□) and SR-2 (■).

In humans, the mean absorption profiles of CPC following the administration of all formulations were smooth. On the other hand, the mean absorption profiles following the administration of SR-1 and SR-2 showed a biphasic pattern in dogs; the profiles were smooth for the first 4 h and compared favorably with that in humans. Reduced rates were observed after the 4th hour. The means (±SD) of the dose absorbed in 4 h for SR-1 and SR-2 were 49.1 ± 11.0 and 37.9 ± 9.4% in dogs, and 58.8 ± 5.6 and 41.6 ± 7.6% in humans, respectively. The rate of CPC absorption was in decreasing order of formulations, IR, SR-1 and SR-2, as expected from the in vitro dissolution data.

3.2. In vitro-in vivo human correlations

Fig. 4 and Table 1 show the correlation results obtained from the linear regression analysis between the percentage absorbed in individual human subjects and the mean percentage released in vitro, at the corresponding times, from the same SR formulation. The correlation slope value and the correlation coefficient value (r) of SR-1 were 0.510 ± 0.045 and 0.921, respectively. In the same manner, a correlation slope value of 0.649 ± 0.036 and correlation coefficient of 0.954 were

Table 1
Correlation of absorption of CPC from SR formulations in humans with its dissolution rate and absorption in dogs, obtained by linear regression analysis

Formulation	In vitro-in vivo correlation				Dog-human correlation (dog in humans)	
	In vitro in dogs		In vitro in humans			
	Slope (±S.D.)	r (n)	Slope (±S.D.)	r (n)	Slope (±S.D.)	r (n)
SR-1	0.738 (0.094)	0.821 (32)	0.510 (0.045)	0.921 (25)	1.317 (0.060)	0.958 (45)
SR-2	0.584 (0.049)	0.910 (32)	0.649 (0.036)	0.954 (35)	1.323 (0.084)	0.923 (45)

The in vitro-in vivo, and dog-human correlation parameters for two novel experimental SR formulation were obtained by linear regression analysis.

obtained for SR-2. Although linear regression analysis of the data showed a good correlation between both rates, the in vitro release rates appeared to be faster than the in vivo release rates (absorption rate). This tendency was also observed in dogs.

Therefore, by linear transformation of the time base of one of the release curves, the equivalence of apparently different in vitro and in vivo release profiles can be tested. For both SR formulations, the MDT_{vitro} was calculated from the in vitro release data using the Weibull mathematical model. The mean values for MDT_{vitro} equal to the Weibull parameter T_d, for SR-1 and SR-2 were 1.16 and 3.18 h, respectively. Values for the ratio ($R = MDT_{vivo}/MDT_{vitro}$) for SR-1 and SR-2 were 3.31 and 2.31, respectively. The mean overall R value for both SR formulations calculated over all subjects was 2.81. The mean R values from the MDT analysis ($R = 2.81$) were used as time scaling (TS) factor. Fig. 5 depicts the in vitro release profiles which were recalculated using the TS factor for the experimental formulations with different release rates. The plots of the mean absorption vs time, together with the Weibull curves, are also shown in Fig. 5. Adequate correlations were found for both SR formulations.

3.3. In vivo dog-human correlation

The results of the correlation obtained from linear regression analysis between the percentage absorbed in individual human subjects and the mean percentage absorbed in dogs, at corre-

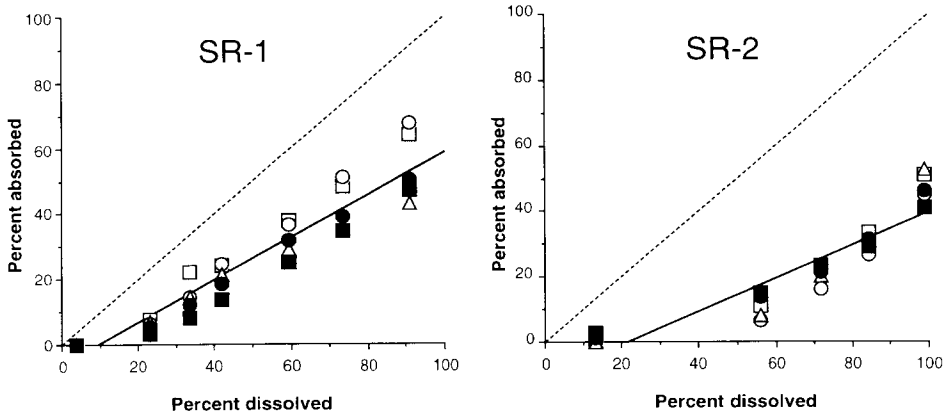

Fig. 4. Plots of percentage absorbed in individual humans vs the mean percentage released in vitro ($n = 3$) from sustained-release formulation (SR-1 and SR-2). The symbols each represent one human subject. The solid line denotes the best correlation, based on linear regression analysis and the dotted line represents the theoretical line, when the absorption of humans is equal to the in vitro dissolution of sustained-released formulations (SR-1 and SR-2).

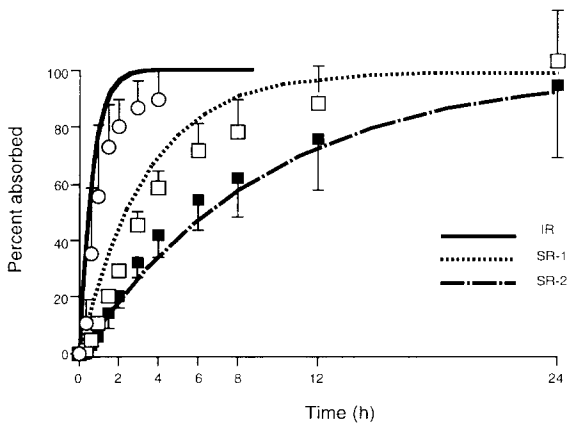

Fig. 5. Release of CPC from experimental formulations in vitro (fitted line) and in vivo (symbols) after time scaling (TS = 2.8) of the in vitro release profiles. Symbols represent mean ± S.D.

sponding times, from the same SR formulations, are presented in Fig. 6. A linear relationship was found between the in vivo percentage absorbed in dogs and that absorbed in humans.

The dog-human correlation parameters for two novel experimental SR formulations are given in Table 1. The correlation slope values between both SR formulations were almost the same, and the values for SR-1 and SR-2 were 1.317 ± 0.060 and 1.327 ± 0.084, respectively. The correlation coefficients of 0.958 and 0.923 were also obtained for SR-1 and SR-2, respectively. Linear regression analysis of the data showed a good correlation between the two species, with similar relationships for the different formulations.

4. Discussion

We have attempted to fit quantitatively the in vitro rates of CPC released from SR formulations to those absorbed in vivo as a function of time, derived using a modification of the method of Wagner and Nelson (1964). These plots are useful in studying relative absorption rates for a drug, with assuming a one-compartment model. A conventional, immediate-release tablet or aqueous solution of CPC, which are completely absorbed, were used as the reference products.

In humans, the rate of CPC absorption was in a decreasing order of formulations, as expected from the in vitro dissolution rates. In addition, a linear relationship was found between the in vivo percent absorbed under fasting conditions. Likewise, the correlation between the in vitro system and the in vivo evaluation system in dogs was also generally high, as shown in Table 1. These results suggest that changes in formulation release characteristics could be evaluated in vitro before going to humans because a correlation existed be-

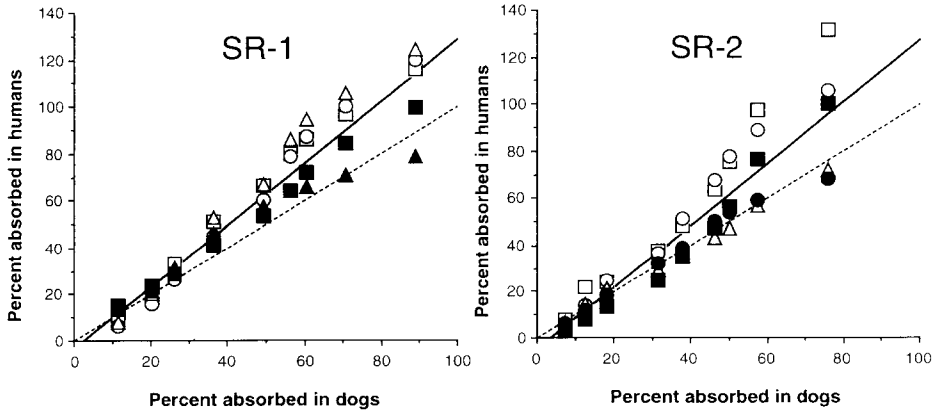

Fig. 6. Plots of percentage absorbed in individual humans vs the mean percentage absorbed in dogs from sustained-release formulations (SR-1 and SR-2). Symbols each represent one human subject. The solid line represents the best correlation, based on linear regression analysis and the dotted line denotes the theoretical line, when the absorption of humans is equal to that of dogs.

tween in vitro dissolution and in vivo bioavailability. Such a correlation might reduce the number of in vivo evaluations required to develop an acceptable SR formulation.

For all formulations, however, release in vitro was faster than that in vivo. Possibly, the conditions we selected for the in vitro dissolution tests were somewhat more extreme than those during fasting conditions in the GI tract of humans.

The CPC release from experimental formulations was not influenced by the pH of the medium, since the solubility was nearly constant within the physiological pH range (Akimoto et al., 1993). The experimental conditions, more or less, often affect the in vitro release characteristics. Previous experiments with some SR dosage forms showed that the mixing that occurs in the human GI tract was not as extensive as had been predicted (Levy et al., 1965). The apparent differences in dissolution rates when compared with the results of the in vivo study may be due to inappropriate stirring conditions.

Many efforts have been made to fit the in vitro and in vivo release characteristics of the SR formulations and to establish the predictive power of the in vitro experiments (Langenbucher and Mysicka, 1985; Mrhar et al., 1990; Kottke and Rhodes, 1991). Brockmeier (1986) proposed a TS factor to correlate in vitro and in vivo release data. Van Bommel et al. (1991) showed that an adequate correlation between the recalculated in vitro release profiles using TS factor and the mean in vivo release profiles was only found for acetoaminophen SR tablets. According to the method of Van Bommel et al. (1991), we also attempted to correlate in vivo and in vitro release data using the TS factor. The release of CPC from the SR formulations in vivo after time scaling (TS = 2.8) of the in vitro release profiles was similar to the in vivo profiles under the fasting condition in humans. The application of a TS factor also seems appropriate as a means of finding an in vitro release test which reflects the in vivo behavior of the formulation. However, this TS factor is not always the same, but depends on the in vitro dissolution conditions and on the type of dosage form.

To minimize initial human studies with an SR formulation, a suitable animal model for testing is generally preferable. Although the beagle dog has been widely used for the bioavailability study of many SR dosage forms, it has been shown that the dog may or may not be a good animal model for predicting the absorption of drug from SR dosage forms in humans (Morimoto et al., 1986). We recently demonstrated the pharmacokinetic parameters were similar in dogs and humans when the same formulation of CPC was studied (Akimoto et al., 1993). The present study also demonstrates that the rate and extent of CPC absorption in male dogs after the administration of the two SR dosage forms and the IR formulation decreased in the same order as in male humans under fasting conditions. In addition, a close linear relationship, as depicted in Fig. 5 and Table 1, might enable the prediction of the rate and extent of absorption in humans from dog studies.

Katori et al. (1991) reported that absorption of d-chlorpheniramine maleate from some types of controlled-release formulations in dogs terminated at about 3 h. Moreover, Cressman and Sumner (1970) found that the nondisintegrating SR tablet was expelled in the 6th hour in two of the four dogs in the study. In humans, however, the absorption of both drugs from SR products was maintained for more than 8 h.

The apparent in vivo absorption rates of CPC from the SR formulations were almost equivalent for the first 4 h in both species. The absorption in humans continued at a steady rate during the next 20 h, whereas absorption in dogs did not continue at a steady rate but instead slightly decreased, as shown in Fig. 2. The reduced rate of absorption under the fasting condition may be caused by the short time taken for the dosage forms to reach the colon. The mean oral-caecal transit time in dogs is considered to be shorter than that in humans (Morimoto et al., 1986). In general, the major site for drug absorption is considered to be the small intestine in the GI tract. The SR products may have passed through the main absorption site in dogs before the drug release was completed. As for CPC, colonic absorption has been demonstrated, although there it might proceed at a slow rate (Akimoto et al.,

1993). In addition, the drug disposition is considered to be comparable in dogs and humans; orally absorbed CPC is thought to be excreted mainly into urine as a glucuronide (Buhler, 1964). Therefore, it was believed that there was not a large discrepancy between the total extent of the bioavailability observed in dogs and that observed in humans. Moreover, linear regression analysis of the data showed a good correlation between the two species. Incidentally, several values of the percentages absorbed were beyond 100% in humans. The major reason for the overestimation problem in the percentage absorbed could be the intrinsic intra- and inter-individual variability of the drug; this problem in our case could be emphasized by the fact that the subjects were not the same as those involved in the reference formulation study (Akimoto et al., 1993). However, the plots were useful in comparing relative absorption rates between dogs and humans, since these overestimations were not considerable.

From the results described above, we suggest that by using an arbitrary dissolution system as a guideline, it is possible to distinguish two or more batches of formulation which release at different rates. However, to obtain reproducible correlations between in vitro dissolution curves and input functions appropriate use of time scaling factors or other mathematical functions is needed. On the other hand, the beagle dog, although not a perfect animal model for accurately predicting absolute bioavailability, appears to be a good model to predict absorption profiles of CPC from SR formulations in humans.

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