In Vitro and *In Situ* Permeability of a 'Second Generation' Hydroxypyridinone Oral Iron Chelator: Correlation with Physico-Chemical Properties and Oral Activity

Nicholas Lowther,[1,6] Roy Fox,[1] Bernard Faller,[2] Hanspeter Nick,[2] Yi Jin,[2] Thomas Sergejew,[2] Yulia Hirschberg,[3] Rebecca Oberle,[3,4] and Harry Donnelly[5]

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Purpose. The *in vitro* and *in situ* transport of CGP 65015 ((+)-3-hydroxy-1-(2-hydroxyethyl)-2-hydroxyphenyl-methyl-1H-pyridin-4-one), a novel oral iron chelator, is described. The predictive power of these data in assessing intestinal absorption in man is described.

Methods. Caco-2 epithelial monolayer and *in situ* rat jejunum perfusion intestinal permeability models were utilized. *In vivo* iron excretion and preliminary animal pharmacokinetic experiments were described. Ionization constants and octanol/aqueous partition coefficients were measured potentiometrically. Solubilities and intrinsic dissolution rates were determined using standard procedures.

Results. Caco-2 cell ($P_{app} \sim 0.25 \times 10^{-6}$ cm.s^{-1}) and rat jejunum ($P_w \sim 0.4$) permeabilities of CGP 65015 were determined. The log D(pH 7.4) of CGP 65015 was 0.58 and its aqueous solubility was $<$ 0.5 mg.ml^{-1} (pH 3–9). The intrinsic dissolution rate of CGP 65015 in USP simulated intestinal fluid was 0.012 mg.min^{-1}.cm^{-2}. CGP 65015 promotes iron excretion effectively and dose dependently in animals.

Conclusions. Caco-2 and rat intestinal permeabilities predict incomplete oral absorption of CGP 65015 in man. Preliminary rat pharmacokinetics support this. Physico-chemical data are, also, in line and suggest that CGP 65015 may, in addition, be solubility/dissolution rate limited *in vivo*. Nevertheless, early animal pharmacological data demonstrate that CGP 65015 is a viable oral iron chelator candidate.

KEY WORDS: *in vitro* permeability; intestinal drug transport; iron chelator; solubility; lipophilicity; oral absorption.

INTRODUCTION

Iron overload continues to be treated with the parenterally effective (but orally inactive) chelator Desferal® (desferrioxamine-B, DFO). However, primarily because of the difficulty of patient compliance with the slow subcutaneous infusion regime required by Desferal®, the search is on to discover new orally active iron chelators (1,2). The development of an orally active, nontoxic iron chelator remains a challenge even though extensive research has been undertaken by both industry and academia.

3-Hydroxypyridin-4-ones (HPOs), a group of bidentate iron chelators first described over 15 years ago (3,4), are known to be orally active in animals (5–8) and man (9–11). The prototype of this family, 1,2-dimethyl-3-hydroxy-4-pyridinone (deferiprone, L1, CP20 or DMHP, Fig. 1), was introduced to the Indian market in 1995 (Kelfer®). However, the therapeutic margin of L1, and simple analogues, is narrow (12) so research efforts have continued to identify new, more complex, HPOs with an improved safety profile.

CGP 65015, ((±)-3-hydroxy-1-(2-hydroxyethyl)-2-hydroxyphenyl -methyl-1H-pyridin- 4-one, Fig. 1) represents such a new chemical entity resulting from Ciba's internal iron-chelation research program. Compared with L1, CGP 65015 has a higher affinity for iron; pM (a measure of the Fe-binding of a ligand under physiological conditions, allowing comparison between chelators of different chemical classes and denticities) is 21.3 for CGP 65015 *versus* 19.5 for L1 (13). In addition, the CGP 65015 iron complex is kinetically more stable than that of L1 under *in vitro* conditions. However, the question of oral activity remains. The current study has been carried out to assess the intrinsic permeability of CGP 65015 in both an *in vitro* cell model (Caco-2) and in an *in situ* rat intestinal perfusion model.

The Caco-2 cell model is an important tool in the *in vitro* assessment of oral drug permeability. Of particular interest is quantitative correlation of Caco-2 cell monolayers permeability with human drug absorption. This type of relationship is described by Artusson and Karlsson (14), who report a quantitative sigmoidal relationship between the percentage of dose absorbed in man of a series of drugs and the respective apparent drug permeabilities (P_{app}) through Caco-2 cell monolayers. This opens up the possibility to measure Caco-2 cell P_{app} values of research compounds and use them to predict the potential human oral absorption of these moieties using the drugs described in Artusson and Karlsson's paper as calibration compounds.

In addition to Caco-2 monolayer investigations, *in situ* animal perfusion models are of importance in the study of the intestinal transmucosal transport of drugs, and are extremely useful in determining whether low intestinal permeability may contribute to incomplete absorption of a test compound. Correlations between rat jejunal permeability (P_w) and the fraction of dose absorbed in man for a series of drugs have been developed by Amidon *et al.* (15) allowing the potential to use P_w values to predict oral human absorption of research compounds. The measured P_{app} and P_w permeabilities of CGP 65015 in this study are compared and correlated with physico-chemical measures of lipid membrane partitioning (log P, log D and Δ log P). Of course, membrane permeability is only one factor controlling the oral bioavailability of drugs. Aqueous solubility and dissolution processes are, also, extremely important elements influencing the biopharmaceutical classification of compounds (16). Hence, these parameters are also addressed in this study.

[1] Pharmaceutical and Analytical Development, Novartis Horsham Research Centre, Wimblehurst Road, Horsham, West Sussex RH12 5AB, United Kingdom.

[2] Iron Chelation Research, Novartis Pharma AG, CH-4002 Basel, Switzerland.

[3] Bioanalytics and Pharmacokinetics, Novartis Pharmaceuticals Corporation, Summit, New Jersey.

[4] Present address: R.W. Johnson Pharmaceutical Research Institute, 920 US Route 202, P.O. Box 300, Raritan, New Jersey 08869.

[5] Globepharm Ltd, P.O. Box 89C, Esher, Surrey KT10 9ND, United Kingdom.

[6] To whom correspondence should be addressed. (e-mail: nicholas.lowther@pharma.novartis.com)

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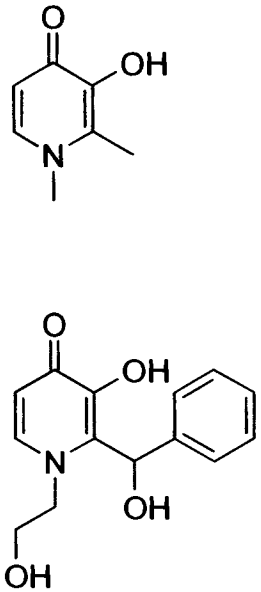

Fig. 1. Chemical structures of deferiprone (L1, top) and CGP 65015 (bottom).

Finally, early pharmacodynamic (Fe-excretion) and kinetic data in rodent and primate are presented and, using these results, the predictive capacity of the permeability and physico-chemical studies is assessed.

MATERIALS AND METHODS

Caco-2 Cell Study

Cell Culture

Caco-2 cells (passage 80–100) were cultured in flasks, then grown on Costar Transwell 4.71 cm^2 0.4 μm pore-size polycarbonate filters according to Nicklin *et al.* (17). Fourteen days after seeding onto filters, CGP 65015 was applied to the apical side of each well. The permeability study was carried out at 37°C.

Permeability Study (Dosing, Administration, and Sampling)

CGP 65015, at concentrations of 0.1, 0.3 and 1.0 $mg.ml^{-1}$, was administered in 2 ml apical medium consisting of Hanks balanced salt solution (HBSS) with 25 mM HEPES, 5 mM glucose and 0.1% w/v BSA, pH 7.0. At the highest CGP 65015 concentrations, it was necessary to add 1 % (v/v) DMSO to the test medium.

After 180 min. incubation with CGP 65015, the apical medium was replaced with equivalent medium containing 10^6 dpm [^{14}C]-mannitol (specific activity 2.11 $Gbq.mmol^{-1}$, Amersham International, Amersham, UK) as a permeability standard. Apical fluid was sampled prior to the addition to cells, and at the end of the incubation. Basolateral samples were generated by moving the filters to new plates containing 3 ml pre-warmed test media at 15, 30, 60, 90, 120 and 180 min, then at 195, 210 and 240 min for mannitol analysis.

Aliquots (100 μl and 1000 μl) of the apical and basolateral fluid, respectively were counted for [^{14}C]-mannitol radioactivity by liquid scintillation.

Analytical Procedures

Unchanged CGP 65015 in the test medium was measured by HPLC using a Prodigy ODS3 5 μm column (150 × 4.6 mm id, Phenomenex, UK), an isocratic mobile phase comprising 75/25 (% v/v) 10 mM sodium dihydrogen phosphate, pH 5.8/ acetonitrile, a flow rate of 1.0 $ml.min^{-1}$ and UV detection at 215 nm. Typical elution time was 5–6 min.

The HPLC method was found to be linear up to a CGP 65015 concentration of 0.5 $mg.ml^{-1}$. All basolateral samples analyzed were injected at a concentration within the assessed linearity boundaries. The estimated limit of quantification (LOQ) was 0.05 $\mu g.ml^{-1}$.

In Situ Jejunal Perfusion Study

Surgical Procedures

Fasted male Sprague-Dawley rats (ca. 300–380 g, Taconic Farms, Germantown, NY, US) were anaesthetized with a 1 $ml.kg^{-1}$ intraperitoneal injection of ketamine (100 $mg.ml^{-1}$): aceprozamine (10 $mg.ml^{-1}$): xylazine (20 $mg.ml^{-1}$) at 10:1:1 (v/v/v). Jejunal segments (mean length 11.3 cm) were isolated and cannulated *in situ* with Teflon tubing (3/16" o.d.) starting ca. 2 cm distal to the ligament of Treitz.

Intestinal Perfusion Procedures

MES buffer contained 150 mM NaCl, 10 mM MES and 5 mM KCl, pH 6.9. Stock solutions of the nonabsorbable markers, PEG 4000 (50 $mg.ml^{-1}$) and [^{3}H] PEG 4000 (specific activity 2.4 $mCi.g^{-1}$, DuPont NEN, Wilmington, DE, US, 1 $mg.ml^{-1}$), were prepared in 0.9% NaCl and stored at 4°C prior to use. The perfusion solution was prepared by adding CGP 65015 to MES buffer to give drug concentrations of 47 and 370 $\mu g.ml^{-1}$. An additional experiment was carried out with CGP 65015 at a loading of 1090 $\mu g.ml^{-1}$ in MES buffer containing 2% (w/ v) SDS. To allow for water flux corrections, PEG 4000 was added at a concentration of 0.1 $mg.ml^{-1}$, and [^{3}H] PEG 4000 at 0.004 $mg.ml^{-1}$. The mean osmolarity of the perfusion solution was 285 mOsm.

After a wash (25–40 min.) of the intestinal segment with MES buffer containing PEG 4000 (0.1 $mg.ml^{-1}$) at 0.167 $ml.min^{-1}$, CGP 65015 solution in MES buffer (or MES buffer containing 2% SDS) was perfused for 3–4 hr at 0.056–0.065 $ml.min^{-1}$. Samples of outlet perfusate were collected over 20 min. intervals.

Analytical Procedures

Comparative [^{3}H] PEG 4000 levels (cpm) in jejunal perfusate samples were assessed by liquid scintillation counting (100 μl aliquots). Mean inlet (cpm_{in}) and outlet (cpm_{out}) values were used to correct CGP 65015 concentrations for water absorption or secretion. CGP 65015 in perfusate samples was determined by HPLC at 45°C using a CapCell UG120 polymer C_{18} 5 μm column (150 × 2 mm id, Analytical Sales and Service, Mahwah, NJ, US), an isocratic mobile phase comprising 82:13:5 (v/v/v)

water:acetonitrile: methanol with 0.1 % phosphoric acid, a flow rate of 0.3 ml.min^{-1} and UV detection at 285 nm. Typical elution time was 3 min. Perfusate was diluted with mobile phase (5–20 fold) prior to injection (10 µl).

The HPLC method was found to be linear up to a CGP 65015 concentration of 0.1 mg.ml^{-1}. All samples analyzed were injected at a concentration within the assessed linearity boundaries.

Solubility Determinations

Solubilities were determined via spectrophotometric, or HPLC (method as described in the Caco-2 cell study), analysis of equilibrated, saturated, solutions of CGP 65015, using standard procedures. Constant ionic strength buffers were used to study the solubility of CGP 65015 *versus* pH.

Determination of Intrinsic Dissolution Rates

An adaptation of the method elaborated by Wells (18) was used. Tablets containing CGP 65015 (ca. 150 mg) were compressed (9 tons, 1 min. dwell time) using a 13 mm IR die. The (weighed) tablets were loaded onto a USP I basket holder using low melting paraffin wax BP, ensuring that only one face of the tablet (of surface area 1.33 cm^2) remained exposed. Excess wax was removed with a scalpel, as necessary. Dissolution rates were measured using a USP dissolution bath, stirrer speed 100 rpm, using either USP simulated gastric fluid (SGF, pH 1.2, 1 l), USP simulated intestinal fluid (SIF, pH 7.5, 1 l), or BP mixed phosphate buffer (pH 6.8, 1 l) as medium. The temperature was maintained at 37°C. Dissolved drug was monitored spectrophotometrically using a wavelength of 284 nm. The cumulative mass of CGP 65015 dissolved was plotted as a function of time. Least squares linear regression was performed on the initial portions of each plot, and intrinsic dissolution rates (IDRs) were derived from the respective slopes.

Sink conditions were maintained throughout these experiments, since the dissolved concentration was < 10 % of the respective equilibrium solubilities of CGP 65015 at 37°C.

Ionization Constants and log D Determinations

Ionization constants were measured by potentiometry using a PCA 101 automatic titrator (Sirius Analytical Instruments, Forest Row, UK). Experiments were performed at 25°C with 0.15 M KCl as background electrolyte.

Octanol/water partition coefficients were determined from dual-phase potentiometric titrations (19,20). Lipophilicity profiles were generated from pKa/log D values using pKa/LOGP for Windows software (Sirius Analytical Instruments Ltd, Forest Row, UK). Calculation of distribution coefficients from pKa/logP is well documented in the literature (19,21).

Iron Excretion in the Rat

Primary *in vivo* efficacy of CGP 65015 was carried out in the bile-duct cannulated, non iron-overloaded, rat model based on that described by Bergeron *et al.* (22). After compound administration the excretion of both biliary and urinary iron was monitored for 24 hours. Iron concentration in bile and urine was measured colorimetrically using the bathophenanthroline method (23).

Iron Excretion in the Marmoset

The *in vivo* efficacy of the test compound was determined in the marmoset (*Callithrix jacchus*) using an adaptation of the *Cebus apella* model of Bergeron *et al.* (24). Briefly, marmosets (300–450 g) were iron-overloaded by injections of iron dextran. For the iron-balance studies, animals were kept in metabolic cages and maintained on a low-iron diet in order to reduce faecal background. After compound administration the excretion of iron in urine and faeces was followed for 2 days. Urinary iron was measured colorimetrically and faecal iron by atomic absorption spectroscopy.

Pharmacokinetics in the Rat

Animal Studies

Male Fischer rats (200–300 g, Iffa Credo, France) and male Tif:RAI rats (Ciba, Sisseln, Switzerland) were used in this study. Animals were fasted over night prior to administration of the iron chelator.

Intravenous solution formulations of CGP 65015, in PBS containing 10% DMSO (v/v) and 30% propylene glycol (v/v), at a drug concentration of 10 mg.ml^{-1}, were prepared immediately prior to use. Rats were given a single bolus dose (2.5 ml.kg^{-1}) of the iv formulation to the tail vein. Three samples of blood (0.6 ml aliquots) were collected from each animal (retroorbital plexus). Plasma (200 µl) was separated from each sample and stored at −20°C prior to analysis.

Oral suspension formulations of CGP 65015 were prepared using vehicles comprising either 40% (v/v) aqueous Cremophor RH40, 0.5% (w/v) aqueous Klucel HF, or 0.5% (w/v) aqueous Klucel HF + 2% (w/v) SDS. The formulations (5 ml.kg^{-1}, 117.6 mg.kg^{-1} CGP 65015) were administered by gavage. Blood was collected as per the iv studies.

Analytical Procedures

Plasma concentrations of CGP 65015 were measured by reversed-phase HPLC, after protein precipitation (acetonitrile). Chromatography was carried out at room temperature using a Hypercarb PH graphitized carbon column (100 × 3.2 mm id, Shandon Scientific Ltd, Runcorn, UK). A mobile phase system comprising (A), 2.5 mM phosphate + 0.5 mM EDTA, pH 3.0 and (B), 80% acetonitrile + 20% (A) was used with a linear AB gradient (B increased from 15% to 70% over 30 min.). A flow rate of 0.5 ml.min^{-1} was used and detection was by UV at 285 nm. Typical elution time was 16 min.

The HPLC method was found to be linear over the range of CGP 65015 concentrations encountered in rat plasma. The estimated limit of quantification (LOQ) in plasma was ca. 0.2 µg.ml^{-1}.

RESULTS AND DISCUSSION

Caco-2 Cell Study

[^{14}C]-Mannitol Permeabilities

The mean cumulative radioactivity appearing in the basolateral well of cells treated with different concentrations (0.1–1.0 mg.ml^{-1}) of CGP 65015 *versus* time was measured. At each concentration it appears that the rate of mannitol movement

was slightly greater in the initial stages of the incubation (possibly due to the cells' reaction to a change in incubation medium). However, it was assumed that there is a linear relationship between accumulated activity and time and the slope of the regression was used to calculate apparent permeability, P_{app}. The calculated values for P_{app} (ca. 0.1×10^{-6} cm.s^{-1}) are within the expected range for mannitol permeability in intact Caco-2 monolayers (14). This indicates that the cell monolayers remained intact throughout the incubation with CGP 65015.

Iron Chelator Permeability

There was no evidence of metabolism, or other degradation products in the HPLC traces.

The mean cumulative appearances of drug *versus* time at each concentration of CGP 65015 were plotted and appended with the respective unweighted least-squares best fit regression lines. The quality of the curvefit is very good ($r^2 > 0.99$) in each case. The calculated P_{app} values are summarized in Table 1. There is no significant influence of drug concentration dosed to the apical compartment upon the resulting P_{app} values. This suggests that the permeability of CGP 65015 is not concentration dependent and the drug is passively absorbed.

The permeabilities of CGP 65015 have been compared with those of drugs of known absorption in man, using the data described by Artursson and Karlsson (14) as calibration compounds. A sigmoidal correlation curve was constructed from these data and used to predict the absorption of the test article. From this, CGP 65015 is predicted to exhibit incomplete absorption (ca. 46%) in man.

In Situ Jejunal Perfusion Study

Iron Chelator Permeability

Outlet (C_{out}) to inlet (C_{in}) concentration ratios of CGP 65015 were calculated after corrections for water flux. Effective permeability (P_{eff}) and jejunal permeability (P_w) were determined from C_{out}/C_{in}, according to Elliot *et al.* (25). The diffusion coefficient, D, was calculated by the method of Hayduk-Laudie (26). The rate of drug absorption at steady state, J_{ss}, is assumed to be equal to the difference between the rate into and out of the intestinal segment. A summary of the results appears in Table 2. The increase in J_{ss} is found to be proportional to the drug loading, and the respective intestinal permeability, P_w, values at low and high drug concentration are not statistically different ($p = 0.4$). This implies that the permeability of CGP 65015 is not concentration-dependent and the drug is passively absorbed, observations which are consistent with those found in the Caco-2 cell studies.

Table 1. Caco-2 Permeabilities to CGP 65015 at pH 7.0

CGP 65015 concentration [mg.ml^{-1}]	Iron chelator P_{app}[a] [cm.s^{-1} $\times 10^{-6}$]
0.1	0.215 ± 0.005
0.3	0.252 ± 0.008
1.0	0.259 ± 0.006

[a] The data are means of 6 filters ± SEM.

Using the data of Amidon *et al.* (15), a correlation curve can be constructed showing the relationship between rat intestinal permeability and the fraction of oral dose absorbed in man for a series of known drugs (calibration compounds). This curve has been used in this study to predict the fraction absorbed of CGP 65015 in man. The results in MES buffer predict incomplete absorption of the drug (ca. 40%), which corresponds very well with the Caco-2 cell predictions.

An approximately 4-fold increase in rate of drug absorption is observed when CGP 65015 is delivered as a saturated solution in MES buffer containing 2% SDS, *versus* a saturated solution in MES buffer alone. Although a precise fraction absorbed in man cannot be predicted, the results suggest that SDS might act as an absorption enhancer for CGP 65015.

Physico-Chemical Properties of CGP 65015

CGP 65015 has two ionizable functions of pKa 2.85 (pyridine-N) and 8.48 (OH) giving rise to a pH-species distribution profile with the unionized species dominating at physiological intestinal pH. The bell-shaped curve of the neutral species correlates with the log D *versus* pH distribution profile (Fig. 2), which demonstrates that, effectively, only the uncharged species partitions into the octanol phase. Distribution is essentially constant between pH 4 and 8, suggesting little influence of pH upon permeability under physiological conditions. The log D (octanol) within this pH range is only slightly positive (log D ca. 0.58) suggesting that lipid membrane permeability may be somewhat limited. The lipophilicity decreases at more acidic pH when the pyridine-N starts to protonate and at pH > 8 when the phenols start to ionize. So, one might predict lower *in vivo* membrane permeability of CGP 65015 outside the pH range 4–8, on the basis simple pH-partition theory.

Hydrogen-bond potential is known to be an important factor affecting membrane permeability. One way to measure the hydrogen-bond capability of a molecule experimentally is to compare its distribution in an amphiprotic (e.g. octanol) and an aprotic (e.g., cyclohexane) solvent. It has been reported that permeability decreases when Δ log P (octanol/cyclohexane) is higher than 3 (28). The log P of CGP 65015 in both aprotic solvents, e.g. cyclohexane, and proton-donor solvents, such as chloroform, is < -2, reflecting the poor solubility of the drug in nonamphiprotic media. Since the log P of CGP 65015 in octanol is 0.58, the Δ log P (octanol/cyclohexane) is likely to be close to, or exceed, 3. Hence, the hydrogen-bond acidity of the compound might be expected to limit its membrane permeability which, potentially, could result in incomplete oral absorption of the drug.

CGP 65015 is only moderately soluble in water (0.4 mg.ml^{-1}), which might suggest that absorption of the drug is likely to be dissolution rate limited (29). The pH/aqueous solubility profile of CGP 65015, although showing a strong dependence upon pH under acidic and basic conditions, remains < 0.5 mg.ml^{-1} between pH 3-9, which covers the physiological intestinal pH range. The intrinsic dissolution rates (IDRs), derived from the slopes of plots of CGP 65015 released *versus* time ($r^2 > 0.98$ in each case), are 0.58, 0.035 and 0.012 mg.min^{-1}.cm^{-2} in, respectively, USP simulated gastric fluid (SGF, pH 1.2), BP mixed phosphate buffer (pH 6.8) and USP simulated intestinal fluid (SIF, pH 7.5). The differences in IDR *versus* pH reflect the corresponding variation in equilibrium

Table 2. Intrinsic CGP 65015 Permeability in Rat Jejunum and Predicted Absorption in Man

Medium	MES buffer	MES buffer	MES buffer + 2% SDS
CGP 65015 concentration [mg.ml^{-1}]	0.047	0.37	1.09
J_{ss} [µg.hr^{-1}.cm^{-2}]	2.74 ± 0.77[b]	19.1 ± 4.4[b]	84.9 ± 24.5[b]
P_w	0.425 ± 0.15[b]	0.342 ± 0.084[b]	—[a]
P_{eff} [x 10^{-6} cm.s^{-1}]	1.80 ± 0.54[b]	1.52 ± 0.32[b]	—[a]
Predicted absorption in man [%]	43	36	—[a]

[a] P_w could not be determined since the diffusion coefficient in MES buffer + 2% SDS is unknown.
[b] The data are means ± SEM (n = 3–6).

solubility (the differences in result at pH 6.8 and 7.5 probably reflect variances in ionic strength of the respective media). Drugs having IDRs of < 1.0 mg.min^{-1}.cm^{-2} at 37°C frequently have bioavailability limitations due to low dissolution rate (29,30). In addition to solubility, the dose of a drug also has a significant influence upon biopharmaceutical classification (16). The proposed oral dose of CGP 65015 is high (ca. 20–50 mg.kg^{-1}). This, in conjunction with the only moderate aqueous solubility, gives rise to a high Dose Number, D_0, (16) which would be expected to exacerbate any solubility/dissolution limitations of the drug. Hence, at intestinal pH, both solubility and IDR of CGP 65015 are low enough to predict dissolution limited biopharmaceutics.

In Vivo Activity

The oral availability of CGP 65015 was assessed, indirectly, by its pharmacodynamic effect (induction of iron excretion) *in vivo*. It is well known that rodents (standard laboratory animals) differ substantially from primates in iron metabolism, i.e. uptake, pools, fluxes and excretion patterns (31,32) as well as in drug absorption, distribution, metabolism and excretion. For these reasons, CGP 65015 was evaluated in both rat and a primate model (*Callithrix jacchus*, commonly known as marmoset).

The effect of CGP 65015 is illustrated in Fig. 3. In both species CGP 65015 promotes iron excretion in a dose-dependent manner when given orally. In particular, the new drug is much

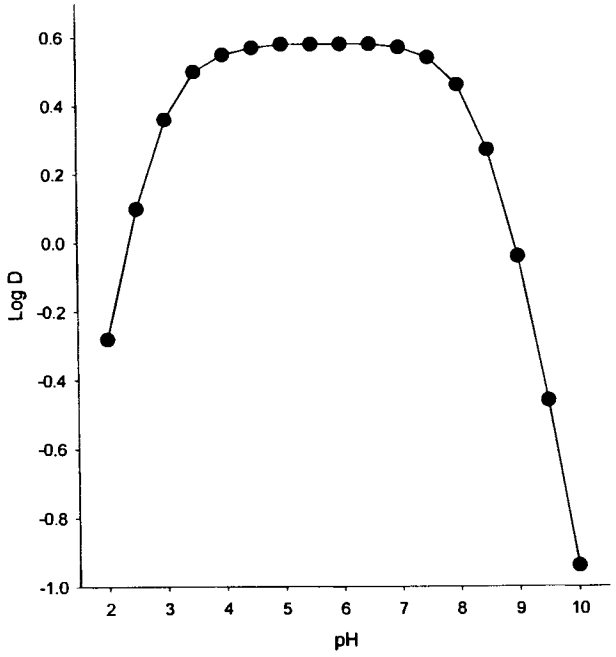

Fig. 2. Octanol/water distribution profile of CGP 65015.

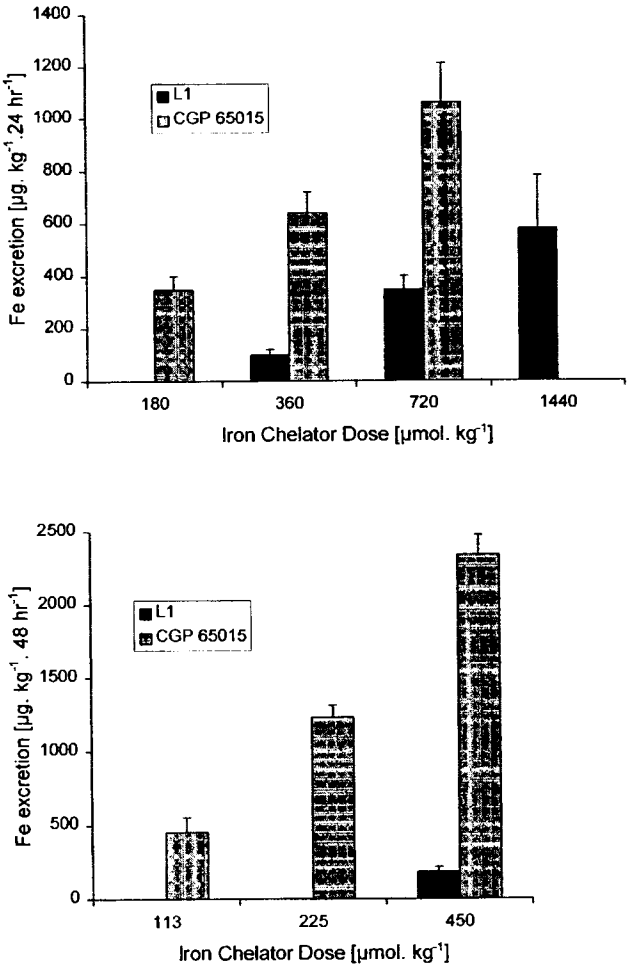

Fig. 3. Iron excretion in rats (top) and marmosets (bottom) induced by CGP 65015 and deferiprone (L1) given orally at the indicated dose (40 % aqueous Cremophor RH40 was used as vehicle; 5 ml.kg^{-1}). Each bar represents the mean of 3–8 animals (± SEM).

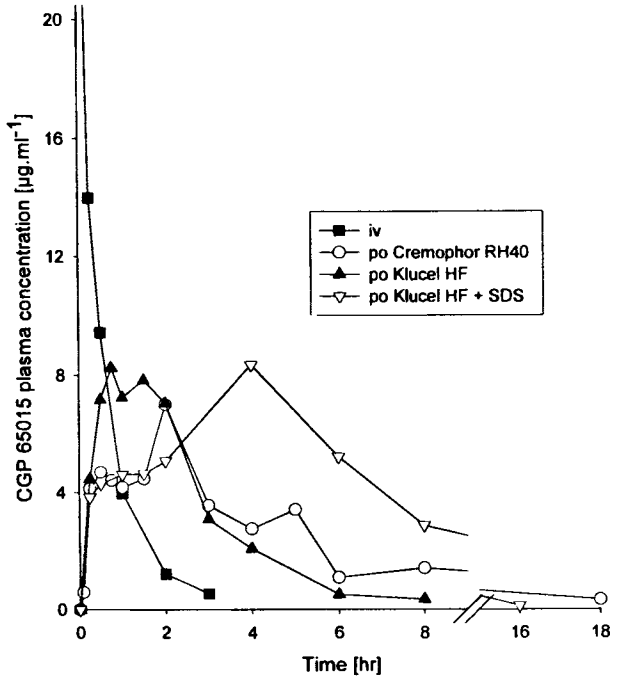

Fig. 4. Pharmacokinetic profiles after iv and po administration of CGP 65015 in rat (n = 3, error bars removed for clarity).

more effective than orally administered L1 in both rat and marmoset. In rat, it is estimated that an oral dose of about 70 mg.kg^{-1} CGP 65015 is equally effective as the standard subcutaneous dose of Desferal® (50 mg.kg^{-1}). In marmoset, at equivalent dose, the total iron excretion induced by CGP 65015 is more than 10 times higher than that of L1.

Preliminary pharmacokinetic profiles in rat appear in Fig. 4. After iv administration, distribution and elimination of CGP 65015 is best described by a two-compartment model. The compound is well distributed and the transfer between plasma and peripheral compartments is rapid. After oral application, absorption of drug is markedly slower than elimination, making it likely that absorption is the rate-limiting step in the overall pharmacokinetics. Computed bioavailabilities (BAV) confirm that CGP 65015 is incompletely absorbed when delivered orally in a simple aqueous vehicle (suspension in 0.5% Klucel HF, BAV = 39%). However, if 2% SDS is added to the dosing vehicle, the oral BAV increases to 89%. This correlates well with corresponding iron-excretion measurements (Table 3). Surfactants, such as SDS, are known to influence the absorption of drugs by wetting, intestinal permeability enhancement and increased drug solubility. In the case of CGP 65015, it is not yet certain which of these processes is the driving force behind the improved oral bioavailability in the presence of SDS. However, given that the *in vitro* data suggest that CGP 65015 potentially has both solubility and permeability limiting factors, it is possible that SDS has a beneficial effect on both of these counts.

Table 3. Iron Excretion After Oral Delivery of CGP 65015 (94 mg.kg^{-1}; 5 ml.kg^{-1}) in Rat

Vehicle	Iron excretion [µg.kg^{-1}.48 hr^{-1}]
0.5 % aqueous Klucel HF	425 ± 63
0.5 % aqueous Klucel HF + 2 % (w/v) SDS	773 ± 53

CONCLUSIONS

Preliminary pharmacodynamic experiments in rat and marmoset have confirmed that CGP 65015 effectively induces iron excretion in rat and marmoset and, hence, is a viable candidate as an oral iron chelator.

When compared with drugs of known *in vitro/in situ* permeability and human oral availability, the Caco-2 cell P_{app} and rat intestinal P_w predict that absorption of CGP 65015 in man is likely to be incomplete. These results are supported by preliminary pharmacokinetic experiments in rat demonstrating excellent *in vitro/in vivo* correlation and substantiating the predictive capacity of the permeability models. Lipophilicity measurements on CGP 65015 are in full accord with the observed moderate lipid membrane permeability. Solubility/dissolution processes are, also, predicted to contribute to incomplete oral bioavailability of CGP 65015.

Inclusion of a known solubilizer/absorption enhancer (SDS) into the vehicle used for administration of CGP 65015 has a beneficial effect upon the oral BAV of the compound. This provides some indicators for the design of an optimal formulation approach for CGP 65015.

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