pH-Sensitive Nanoparticles: An Effective Means to Improve the Oral Delivery of HIV-1 Protease Inhibitors in Dogs

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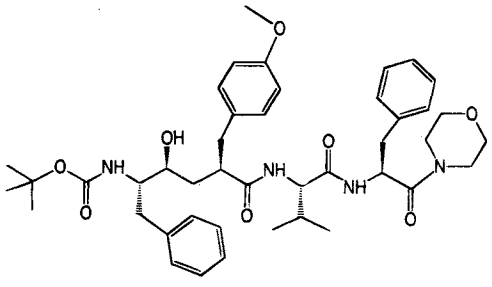
INTRODUCTION

Since it was found that HIV-1 protease was essential for HIV replication in cell culture, several highly potent peptide analogue inhibitors have been described (1). However, poor pharmacokinetic characteristics and low bioavailability appear to impair the clinical use of many HIV-1 protease inhibitors in the treatment of AIDS. It is thought that high lipophilicity, molecular weight above 500 and the numerous amide bonds of some compounds have a negative impact on the oral biovailability (2). The HIV-1 protease inhibitor (Fig. 1) is a peptidomimetic compound which is almost not ionizable, stable at physiological pH, highly lipophilic ($K_{n\text{-octanol/phosphate buffer pH 7.4}} = 10^4$) and practically insoluble in water (8.1 mg/l at pH 7.4). It was shown that the incorporation of the HIV CGP 57813 into polymeric pH-sensitive nanoparticles could substantially increase the bioavailability of the compound after oral administration to mice (3). The present study was conducted to determine if pH-sensitive nanoparticles loaded with CGP 57813 could provide plasma levels comparable to those of mice after oral administration to dogs. Nanoparticles made from polymers soluble in the upper part or the lower part of the gastrointestinal tract were evaluated.



$C_{43}H_{58}N_4O_8$ M_r: 758.96

Fig. 1. Chemical structure of CGP 57813.

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MATERIALS AND METHODS

Preparation and Characterization of Oral Formulations

The following methacrylic acid copolymers (Eudragit®, Röhm GmbH, Darmstadt, Germany) were chosen as pH-sensitive polymers: poly(methacrylic acid-*co*-ethylacrylate) with a monomer molar ratio of 1:1 (Eudragit® L100-55, USP/NF Methacrylic acid copolymer Type C), soluble from pH 5.5 upwards, and poly(methacrylic acid-*co*-methylmethacrylate) with a monomer molar ratio of 1:2 (Eudragit® S100, USP/NF Methacrylic acid copolymer Type B), soluble from pH 7 upwards. CGP 57813- loaded Eudragit® nanoparticles were prepared by the salting-out process as described previously (4): an aqueous gel (210 g) containing 10% (w/w) poly(vinyl alcohol) (PVAL) (Mowiol® 4–88; Hoechst, Frankfurt/M, Germany) and 60% (w/w) $MgCl_2 \cdot 6H_2O$ was added under stirring to an acetone solution (84 g) of 16% (w/w) Eudragit® and 2.82% (w/w) CGP 57813 (Ciba-Geigy, Basle, Switzerland) leading to the formation of an oil-in-water emulsion. Then, sufficient pure water was added (210 g) to allow the diffusion of acetone into the aqueous phase, with the result of forming nanoparticles. The nanoparticles were purified by cross-flow filtration using a Sartocon® Mini device (Sartorius, Göttingen, Germany) mounted with a polyolefin cartridge filter (100 nm pore size) as described elsewhere (4). For technical reasons, each final batch of Eudragit® nanoparticles was composed of 3 individual batches of purified suspensions which were first mixed and then freeze-dried as described previously (5). Freeze-dried batches were stored at −25°C until use. The size of the nanoparticles was determined by photon correlation spectroscopy with a Coulter® Nano-Sizer™ (Coulter Electronics, Harpenden, Hertfordshire, U.K.). The drug loading of Eudragit® nanoparticles was determined as follows: 25 mg of nanoparticles were dispersed and extracted in 50 ml of chloroform and CGP 57813 for 18 h under stirring. The suspension was filtered and assayed spectrophotometrically at 278 nm.

An aqueous suspension containing CGP 57813 in an amorphous form 2.0% (w/w), hydroxypropylmethylcellulose 1.8% (w/w) (Pharmacoat® 603, Shin-Etsu, Tokyo, Japan) and poloxamer 188 0.2% (w/w) (Pluronic® F68, BASF, Ludwigshafen, Germany) was evaluated as control formulation.

Animal Studies

Eudragit® nanoparticles were dispersed into water to give a concentration of 25 mg CGP 57813/ml and administered orally to beagle dogs (2 males, 2 females) by means of stomach tube. The animals reveived 100 mg/kg of CGP 57813. A first set experiment was performed with fasted dogs and another one with dogs fed (350 g Nafag 9405) 45 min before administration of the formulation. At the allotted times, 2.5 ml of blood were collected into heparinized tubes and centrifuged at 3,000 × g for 30 min. 500 µl of CH_3CN were added to 500 µl of plasma and the sample was allowed to stand on ice for 15 min. The sample was centrifuged 5 min at 10,000 × g. The supernatant was sampled, centrifuged 5 min at 10,000 × g and further analyzed by HPLC as described previously for CGP 57813 (6). The quantitation limit of the method was 0.1 µmol $l.^{-1}$ The area under the plasma concentration-time curve (AUC),

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the maximal plasma concentration (C_{max}) and the time of maximal plasma concentration (t_{max}) were determined. The animal studies were approved by the ethics committee of the Canton of Basle, Switzerland.

RESULTS AND DISCUSSION

The evaluation of the nanoparticulate formulations in dogs required an upscaling of the preparation and purification procedures. To our knowledge, the upscaling of the production of drug-loaded nanoparticles has been rarely addressed (7). By using the salting-out method followed by the cross-flow filtration of the raw suspension, it was possible to produce and purify relatively large amounts of Eudragit® nanoparticles (16 g). At the beginning of the filtration procedure (concentration step) (Fig. 2), the filtration rate dropped from 320 to 15 l m^{-2} $h.^{-1}$ This decrease of filtration rate can be attributed to the deposition of PVAL on the membrane surface, resulting in the formation of a gel layer which limits the flow of fluid trough the filter (8). During the diafiltration step (4) (addition of distilled water at the same rate the filtrate is removed) the gel layer was progressively removed and the filtration rate increased rapidly reaching more than 40 l m^{-2} $h,^{-1}$ in the case of Eudragit® S100 nanoparticles. The high filtration rates obtained herein, allow the extensive washing of large batches of particles in a raisonnable amount of time (< 3h), compared to other purification methods such as ultracentrifugation, which are cumbersome and adapted only to the purification of small laboratory scale batches (9).

The amount of nanoparticles recovered after preparation, purification and freeze-drying (batch yield) was always above 90%. De Labouret *et al.* (10) in a paper describing the preparation of Eudragit® nanoparticles by a precipitation method (11), were confronted with a massive particle loss as soon as the polymer concentration in the acetone phase exceeded 3%. In the case of the salting out method, the batch yield remained unaffected by the relatively high solid content (18.82%) of the organic phase. Accordingly, it was possible to obtain a good batch yield without having recourse to excessive volumes of acetone. The drug loadings of the nanoparticles are listed in table 1. They represent an almost 100% entrapment efficiency. The mean size of all batches of Eudragit® nanoparticles was around 250 nm.

Table 1. Pharmacokinetic Parameters of CGP 57813 Incorporated in Eudragit® Nanoparticles and Administered to Fasted (−) and Fed (+) Dogs; Mean (n = 4)

Eudragit®	Mean size (nm)	Drug loading (% w/w)	AUC_{0-8h} ± SEM (μmol l^{-1} h)	C_{max} ± SEM (μmol l^{-1})	t_{max} (h)
L100-55 −	246	14.44	7.71 ± 4.62	1.52 ± 1.08	1
L100-55 +	245	14.97	9.31 ± 4.06	2.04 ± 0.40	2
S100 −	254	14.34	n.d.	n.d.	n.d.
S100 +	264	14.70	14.21 ± 5.34	3.00 ± 1.02	3

n.d.: not determined, plasma concentrations below 0.1 μmol l^{-1}.

When CGP 57813 in an amorphous form was administered orally to fasted dogs as an aqueous suspension, no plasma levels of the compound could be detected (not shown). Therefore, no further investigations were carried with this formulation. As previously demonstrated with mice (3), the incorporation of CGP 57813 in Eudragit® nanoparticles substantially improved the bioavailability of the compound in dogs (Fig. 3 and Table I). In contrast to the results obtained with mice, the area under the plasma concentration-time curve (AUC) following the administration of Eudragit® L100-55 nanoparticles was relatively low. The AUC was slightly increased by the administration of food. It is possible that Eudragit® L100-55 nanoparticles dissolve too rapidly (dissolution starting at pH 5.5) in the intestinal fluids of dogs, leading to the precipitation of the compound. The presence of food may favor the absorption of CGP 57813 by increasing the intestinal bulk (dilution of the compound), slowing the GI transit and stimulating GI secretions. The positive effect of food was clearly observed with Eudragit® S100 nanoparticles. The administration of these particles to fasted dogs resulted in no detectable plasma levels of CGP 57813. The same particles administered to fed dogs gave the highest plasma concentrations. It has been demonstrated that unloaded

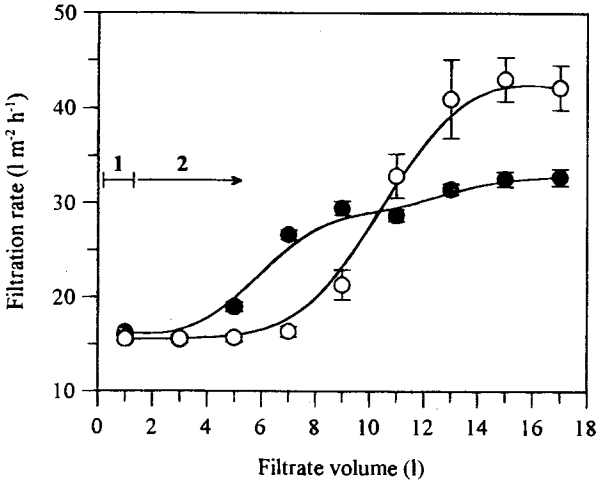

Fig. 2. Cross-flow filtration of Eudragit® L100-55 (-●-) and S100 (-○-) nanoparticles using 3 cartridge filters of 0.1 m^2 surace area; (**1**) concentration, (**2**) diafiltration; mean ± SEM (n = 5–6).

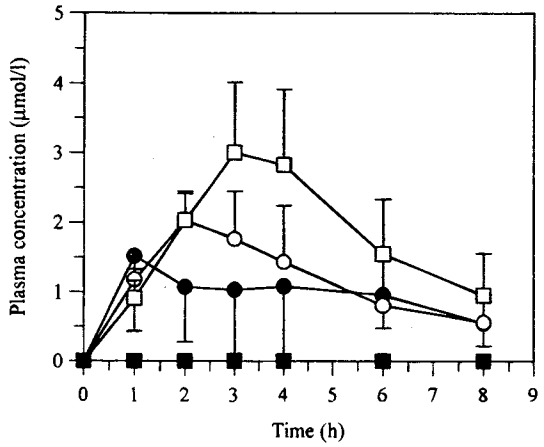

Fig. 3. Plasma concentration profiles following oral administration of CGP 57813 from Eudragit® L100-55 (-●- fasted, -○- fed dogs) and S100 (-■- fasted, -□- fed dogs) nanoparticles; mean ± SEM (n = 4). In vitro, the IC90 against HIV-1/LAV in MT-2 cells is 0.1 μmol l^{-1}.

Eudragit® S100 nanoparticles start to dissolve progressively at pH 6.6 (3). Therefore, in the absence of food, the nanoparticles finely dispersed in water may be eliminated too rapidly before starting to dissolve. By slowing down the GI transit, one can assume that the food favored the dissolution of the particles. In this case, a substantial difference in the AUC (4 fold) was observed between male and female dogs. Although the formulation was only evaluated on 4 animals, this may reflect a difference in the metabolism or the distribution of the compound. Other studies need however to be carried out to determine the influence of the animal sex on the pharmacokinetics of CGP 57813 in dogs.

Besides the administration conditions (food), this study has showed that the selection of an optimal pH-sensitive formulation strongly depends on the animal species. In a former study carried out with fed female mice (3), it was demonstrated that the highest AUC was obtained, not with Eudragit® S100 but with Eudragit® L100-55 nanoparticles. We confirm that, in the case of the dog model, pH-sensitive nanoparticles appear a promising formulation for HIV-1 protease inhibitors when selecting a polymer dissolving preferentially at a pH above 5.5.

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