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INTERSPECIES COMPARISON OF PHARMACOKINETIC PARAMETERS OF AN ORAL SUSTAINED RELEASE PREPARATION OF ILOPROST

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ABSTRACT

Iloprost is a chemically stable, pharmacologically highly potent PGI_2-mimetic for which therapeutic efficacy was proven after iv infusion treatment in PAOD-patients. The development of an oral, therapy facilitating preparation was mainly based on the imitation of plasma levels as obtained after iv to provide an equieffective dosage form. Due to the short half-life in plasma a modified release preparation was formulated. In the present set of experiments the pharmacokinetics of sustained release iloprost in animals and man was investigated after species specifically different dosages. After normalization for the bioavailable fraction of a 150 μg dose several mean pharmacokinetic parameters were similar in all species with peak plasma levels of 162, 223 and 160 pg/ml (pig, dog, man), t_{max}-values of 1.5, 1.9 and 1.6 h, AUC-values of 651, 730 and 763 pg·h/ml. The half-value duration, representing the time of half-maximal plasma levels and thus describing retardation, accounted for 2.8, 2.5 and 2.4 h. For all species a correlation between in-vitro liberation data of the dosage form and drug amount absorbed in-vivo could be shown. Despite differences in gastrointestinal conditions pharmacokinetics was able to demonstrate an interspecies comparability of systemic iloprost levels after intragastric treatment with an extended release preparation of iloprost, which helped to select an optimal formulation variant and to extrapolate toxicological tolerability data for the administration to man. By this strategy a promising oral dosage form could be selected which might be therapeutically equivalent to iv infusion after individual dose titration in patients.

INTRODUCTION

Iloprost (INN for 5-{(E)-1S,5S,6R,7R)-7-hydroxy-6-[(E)-(3S,4RS)-3-hydroxy-4-methyl 1-octen-6-ynyl]-bicyclo[3.3.0]oct-3-ylidene}-pentanoic acid) is a chemically stable, highly potent PGI_2-mimetic [1-3, 13]. Since this compound exhibits similar pharma-codynamic activity as its endogenous precursor, inhibition of thrombocyte aggrega-tion is one of the main therapeutic applications. Therefore iloprost was successfully tested in patients suffering from peripheral arterial occlusive disease (PAOD) [4-6]. The pharmacokinetics of iloprost are mainly characterized by a total clearance of 15 - 20 ml/min/kg in man,

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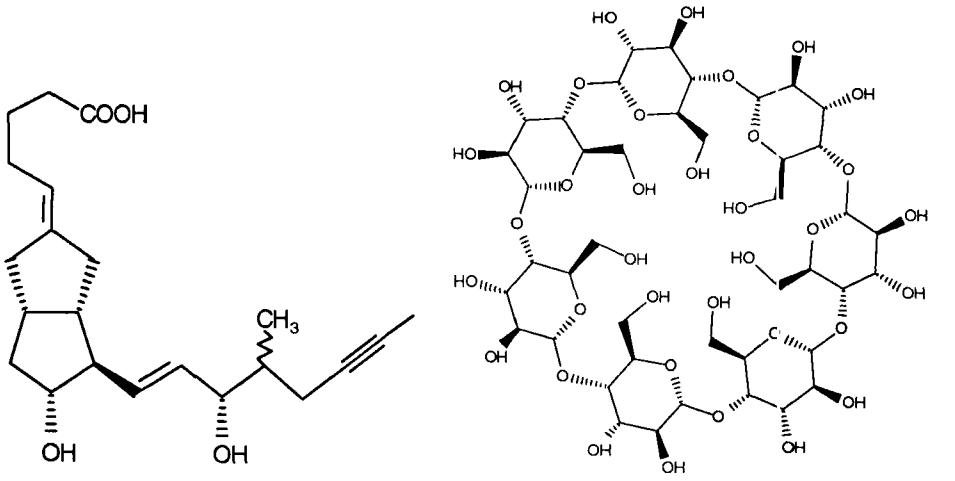

FIGURE 1:

Structural formula of iloprost ß-cyclodextrin clathrate

biphasic disposition in plasma with half-lives of 5 min and 20-30 min, an absolute bioavailability of 15-20% and complete metabolic degradation, mainly via ß-oxidation, to pharmacologically inactive tetranor-metabolites which are excreted with the urine and feces at a ratio of 2:1 [7-10]. These characteristics make iloprost an ideal candidate for iv infusion treatment, which, however, is limited to hospitalized patients. PAOD with its different stages often needs long-term treatment of patients.

Before this background it was desirable to develop an oral dosage form which could be used for ambulant therapy. Due to low bioavailability and short disposition half-lives a sustained release dosage form was formulated with the ß-cyclodextrin inclusion compound of iloprost (Fig. 1), aiming primarily at the imitation of therapeutically effective plasma level profiles known from iv infusion of dosages of 1-2 ng/kg/min over 6 h. Therefore, the most important selection criterion was the pharmacokinetic profile provided by the sustained release dosage form. The screening of various formulations in healthy volunteers could be avoided by the establishment of an appropriate pig-model [11]. This animal species exhibits quite similar pharmacokinetic parameters of iloprost especially in terms of total clearance and at least equally important much similarity in terms of the physiology and anatomy of its gastro-intestinal tract.

The development of an oral sustained release dosage form does not only include the selection of an appropriate preparation, characterization in man but also toxicological testing of the formulation. In this field mostly other animal species, e.g. dog and monkey apart from rodents, were used for systemic tolerance testing. But also for toxicological risk assessment based on animal experiments an important prerequisite is the transferability of results for extrapolation to man. Especially in case of a sustained release dosage form this aim creates several problems. Firstly an intact capsule or tablet preparation can not be administered to rats or mice and secondly disposition data of the drug must be comparable or at least extrapolatable to man.

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The aim of the present paper is to describe an experimental approach to the characterization of a sustained release dosage form of iloprost in animals and man on the basis of pharmacokinetics, to compare the corresponding data in the different species and to establish an example of a pharmacokinetic extrapolation from animal data to man.

MATERIALS AND METHODS

Dosage Form

Iloprost was used as its ß-cyclodextrin clathrate to prepare pellets coated with a diffusion membrane that liberates the active ingredient independent of pH-conditions by diffusion after the formation of channels in the presence of gastro-intestinal fluid. For oral administration the pellets were filled in normal hard gelatine capsules which contained either 50 or 100 µg of iloprost [11]. The experimental formulations were called K 529 I and K 529 M depending on drug contents.

Animal Experiments

Pig-studies

Male castrated pigs (German Landrace x Large White, body weight: approx. 26 kg) were used. For drug administration an initial experiment, using gelatine capsules filled with dye, indicated that the insertion of a corresponding number of hard gelatine capsules into chopped apple resulted in an intact swallowing of the dosage form. Animals were given approx. 500 µg of iloprost by peroral administration and blood sampling was performed at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 24 h postdose by a chronically implanted central venous catheter.

Dog studies

Each four male and female beagle dogs (breeder: Winkelmann, Germany, body weight: 7-10 kg) were given oral iloprost by capsules K 529 I and/or K 529 M at dosages of 37.5, 75 and 150 µg/kg once daily by peroral administration. Blood collection was performed at 0, 1, 2, 3, 4 and 6 h postdose.

Study in healthy Volunteers

Nine healthy male volunteers (age: 21 to 45 yrs., body weight: 57 to 91 kg) were given 150 µg iloprost as each one capsule K 529 I and K 529 M on fasted state by peroral administration. Blood samples were obtained at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8 and 10 h postdose.

In all experiments plasma was obtained from blood samples immediately after collection and stored frozen at - 18 ^{o} C until analyzed.

Bioanalyses

Iloprost was measured in plasma by a sensitive and specific radioimmunoassay, which has been described in detail [12]. In short, plasma samples (0.2 ml) were extracted after acidification to pH 2 - 4 by 2.5 ml of diethylether. After solidification of the aqueous layer

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in methanol/dry ice, the organic phase was dried and redissolved in BSA-buffer. Iloprost-^{3}H-methylester (specific activity: 2475 GBq/mmol) was used as tracer. The antiserum had been raised in rabbits against iloprost-9-pentenyl-BSA. After addition of tracer and antiserum samples equilibrated and separation of bound and unbound tracer was obtained by the charcoal method. At 50 and 100 pg/ml intra- and inter-assay coefficient of variation were 8 and 2% and 17 and 12%. The limit of determination was approx. 10 - 20 pg/ml. The assay had been validated against a GC/MS method for bioanalysis of iloprost [12].

Pharmacokinetic Evaluation

Pharmacokinetic parameters were calculated by model-independent approach using TOPFIT (Gödecke, Schering, Thomae; Germany). The half-life of iloprost was calculated from the terminal linear phase and will be called apparent half-life ($t_{1/2\,app}$) because of the influence of dosage form on disposition in plasma; i.e. drug elimination is determined by drug absorption. Peak plasma levels (c_{max}) and its time points (t_{max}) were given as measured. As a criterion for retardation the half-value duration ($dt_{c\ max/2}$), i.e. the duration of half-maximal plasma levels, was obtained directly from individual concentration vs. time profiles. Bioavailability was roughly evaluated based upon iv data obtained in the respective species in separate experiments. By means of the Wagner-Nelson method the dose fractions absorbed at definite time points were calculated from plasma level profiles.

RESULTS

Pharmacokinetics of Iloprost in Pig Plasma

After peroral administration of iloprost as capsules to male pigs in chopped apple the maximum plasma levels of 70 - 130 pg/ml occurred within 1 to 2 h postdose. Plasma levels were quite similar in the animals tested. The apparent half-life in plasma was 3.0 ± 0.9 h and the mean residence time was 3.4 ± 0.3 h. Half-maximal plasma levels lasted for 2.4 to 3.4 h; while plasma levels above 50 pg/ml (the envisaged therapeutic range in man) lasted for approx. 2 - 4 h. With AUC-values of the active parent compound of 308 to 484 pg·h/ml the bioavailable dose fraction accounted for 5.1 to 8.1 %

Pharmacokinetics of Iloprost in Dog Plasma

In dogs oral iloprost was tested at dosages of 37.5, 75 and 150 μg/kg. Peak plasma levels increased dose dependently from 739 ± 92 pg/ml to 1300 ± 108 pg/ml and 1979 ± 127 pg/ml. T_{max} ranged from 1.0 to 4.0 (1.9 ± 1.1) h. The half-value duration was lower in the 37.5 μg/kg-group with 1.5 ± 0.2 h as compared to 2.9 ± 1.4 h and 3.1 ± 0.9 h in the other two groups. Similar to c_{max} also AUC-values were strictly dose-dependent with 2156 ± 487 pg·h/ml, 4375 ± 1332 pg·h/ml and 7261 ± 1226 pg·h/ml. The apparent half-live of iloprost disposition was 0.84 ± 0.12 h, the mean residence time amounted to 2.5 ± 0.4 h. Taking into account a mean iloprost clearance of 50 ml/min/kg (as known from earlier studies in dogs) the bioavailable dose fraction in that animal species was approx. 16 ± 4 %. Dose dependency of mean iloprost levels in dogs following peroral administration is illustrated in Fig. 2.

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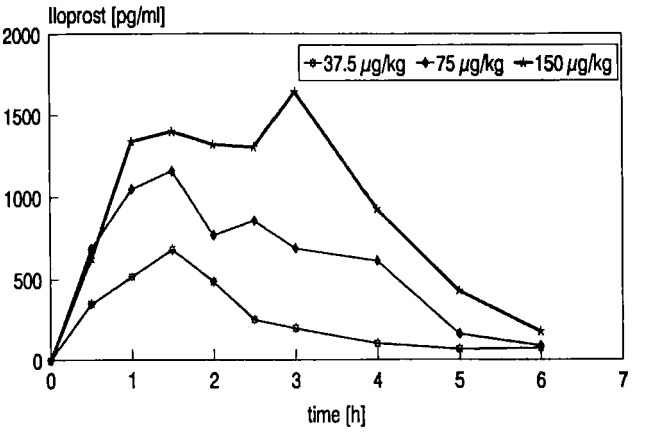

FIGURE 2:

Plasma levels of iloprost in beagle dogs after peroral administration of SH K 529 I/M

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Pharmacokinetics of Iloprost in Human Plasma

After peroral ingestion of 150 µg iloprost as sustained release preparation K 529 I/M by nine healthy volunteers peak plasma levels of 89 to 231 (160 ± 42) pg/ml were observed after 1 to 3 (1.6 ± 0.7) h postdose. The apparent half-life of iloprost in plasma was 1.6 ± 0.8 h and mean residence accounted to 3.0 ± 0.7 h. The duration of half-maximal plasma levels lasted for 1.8 to 3.4 (2.4 ± 0.6) h and therapeutic plasma levels were obtained for 2.1 to 5 (3.3 ± 1.0) h. Total AUC ranged from 547 to 1185 (763 ± 222) pg·h/ml. Based upon a mean AUC of 1006 pg·h/ml (obtained by iv infusion of 35 µg for 6 h in man in a separate experiment) rough estimates of the bioavailable dose fractions would result in 12.7 to 27.5 % with a mean of 17.7% (Fig. 3).

Interspecies Comparison of Pharmacokinetic Parameters

The following table 1 gives a numerical comparison of pharmacokinetic parameters (normalized for the bioavailable fraction of a dose of 150 µg per individuum in case of c_{max} and AUC) obtained after peroral administration of sustained release iloprost in pig, dog and man. Normalization for dose and respective bioavailable dose fraction led to comparable peak plasma levels and AUC-values in pig, dog and man (Fig. 4). Bioavailability data were in the same range for oral iloprost in man and dog as compared to pig which exhibited approx. 3 times lower values. Time points of peak plasma levels and half-value duration as characteristics of the dosage form administered were similar concerning mean values and range in all species investigated.

A second approach to compare pharmacokinetic data in the three species under investigation is the correlation of in-vivo and in-vitro data characterizing the extended release preparation. A dissolution profile of the active ingredient was optained in-vitro in pH 7.4 buffer solution because the dosage form does not exhibit pH-dependent release of iloprost. From plasma level profiles the dose fractions absorbed at definite time points

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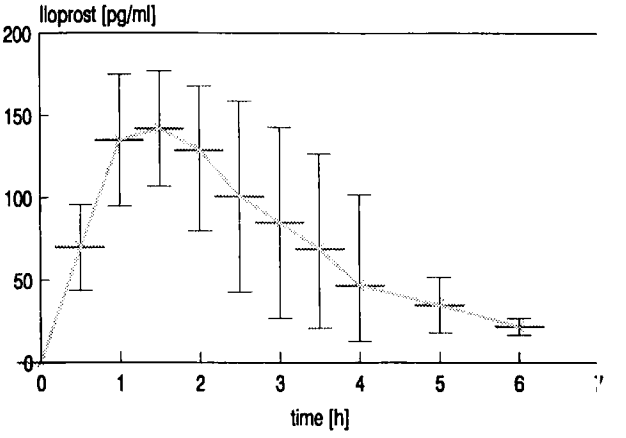

FIGURE 3:

Plasma levels of iloprost in volunteers after peroral administration of 150 μg iloprost as sustained release preparation K 529 I/M (mean ± sd of n = 9)

TABLE 1:

Interspecies comparison of pharmacokinetic data obtained in pig, dog and man after peroral administration of a sustained release preparation of iloprost (mean and range)

SPECIES	PIG	DOG	MAN
n	3 - 4	8	9
dose [μg]	250	375 - 1500	150
$c_{max\ d}$ [pg/ml]	162 (120 - 222)	223 (164 - 293)	160 (89 - 231)
t_{max} [h]	1.5 (1.0 - 2.0)	1.9 (1.0 - 4.0)	1.6 (1.0 - 3.0)
AUC_d [pg·h/ml]	651 (529 - 829)	730 (526 - 1032)	763 (547 - 1185)
f [%]	6.3 (5.1 - 8.1)	16 (12 - 23)	18 (13 - 28)
$\Delta t_{c\ max/2}$ [h]	2.8 (2.4 - 3.4)	2.5 (1.3 - 4.3)	2.4 (1.8 -3.4)

abbreviations:

$c_{max\ d}$	dose normalized (150 μg) peak plasma level
t_{max}	time point of peak plasma level
AUC_d	dose normalized (150 μg) area under the plasma level vs. time curve
f	bioavailability
$\Delta t_{c\ max/2}$	half-value duration

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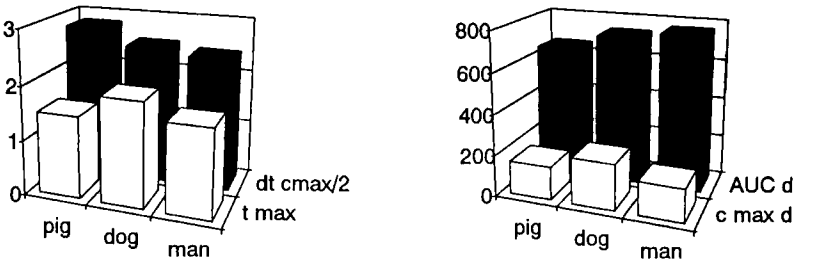

FIGURE 4:

Comparison of normalized pharmacokinetic parameters of iloprost after peroral administration in pig, dog and man

The y-axis was not named as it reads [pg/ml] for c_{max}, [pg·h/ml] for AUC, [h] for t_{max} and $\Delta t_{c\ max/2}$.
AUC and c_{max}-values were normalized for the bioavailable dose fraction and a dose of 150 μg.

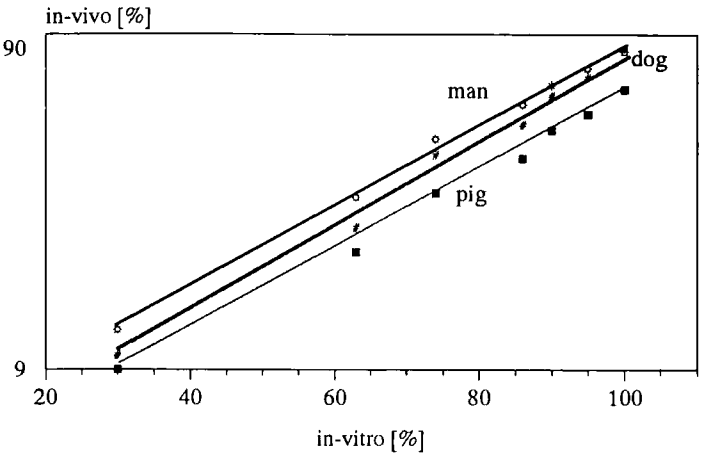

FIGURE 5:

Correlation of iloprost dose fractions released in-vitro (dissolution testing) and absorbed in-vivo (calculated by Wagner-Nelson equations) from extended release dosage forms

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were calculated according to the Wagner-Nelson method. A comparison of in-vitro and in-vivo data of drug released and absorbed is illustrated in fig. 5 and demonstrates a clear-cut correlation.

DISCUSSION

In the present paper the pharmacokinetics of iloprost administered as an extended release preparation were compared in pig, dog and man. The data demonstrated that the prognostic power of animal experiments can be substantially improved by monitoring systemic drug levels and pharmacokinetic evaluation. This holds true to two directions:

1. the selection of an appropiate animal model to avoid a large scaled screen of various formulations in man and

2. to validate results obtained in toxicological risk assessment in respect of administration to man.

The formulation investigated exhibited a pH-independent drug liberation which might be the main reason for the comparability of disposition parameters. Although anatomic-physiological conditions in the GI-tract were quite similar in pig and man they differ remarkable in the dog. This species is characterized by a higher mucos production, increased pH-values and different transit times. Our results, however, verified that all these factors do not influence the prognostic value of pharmacokinetic information. It can be deduced from the data that the absorption of iloprost is not only limited to the stomach, where due to pH-conditions the uncharged molecule is readily reaching systemic circulation. By intragastric administration of iloprost solutions immediate peak plasma levels were obtained in several species. By extended liberation of iloprost which exceeded the gastric residence time of the dosage form an absorption in the upper parts of the intestines has to be concluded. Taking into account the pk-value of iloprost of 4.9 even under less acidic conditions a sufficient portion of the prostacyclin mimetic is available to absorption processes in unionized form. A second favourable factor in the upper parts of the intestines is the increase of surface. Due to the non dependency of liberation on pH a homogenous picture of plasma level profiles was obtained in the three animal species, which was also reflected by pharmacokinetic parameters, i.e. normalized AUC-, c_{max}-, MRT- and $\Delta t_{c\ max/2}$-values. For all experiments rate describing data, like half-lives and residence times, were much higher than known from oral iloprost administered as solution. In the latter case terminal half-lives were in the range of 10 - 30 min and MRT was below 1 h. Therfore it can be concluded that the formulation used modified the disposition towards liberation being the rate-determining step. Another indication of the pH-independent in-vivo performance of the formulation was obtained from the in-vivo in-vitro comparison of dose fractions absorbed and released, which correlated for all data sets.

In contrast to allometric or physiologically based approaches for interspecies extrapolation of pharmacokinetic data a more simplistic strategy was used to compare pharmacokinetics of iloprost after intragastric adminsitration as extended release form in three species. By means of bioanalyses of drug levels a rationale could be established for the extrapolation from animal experiments to man both in terms of toxicological risk evaluation and selection of formulation.

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