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IN-VITRO/IN-VIVO CORRELATION OF DRUG LIBERATION WITH AN EXTENDED RELEASE PERORAL DOSAGE FORM FOR ILOPROST IN MAN

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ABSTRACT

Iloprost is a chemically stable prostacyclin analogue for which therapeutic efficacy was demonstrated after i.v. infusion treatment in several vascular diseases. In order to facilitate drug therapy and to enlarge therapeutic usability an peroral dosage form for non-hospitalized patients was developed on the basis of an extended release (ER) pellet formulation mimicking therapeutic plasma levels as obtained after i.v. infusion in man. Modified drug liberation was pH-independent and showed an in-vitro release of 75 to 95 % of dose over 3 h at pH 7.4 using the basket method. In 12 PAOD-patients the pharmacokinetic profile of the ER-formulation in capsules (SH K 529 I/M) was characterized after repeated administration of 150 µg iloprost as compared to a standard i.v. infusion treatment with iloprost (Ilomedin®). Peroral treatment resulted in highly reproducible plasma level profiles in the therapeutic range (> 50 pg/ml) for approx. 6 h, i.e. a similar time period as obtained after i.v. infusion. Half-value duration was approx. 4 h. AUC accounted for 0.8 $\pm$ 0.2 ng·h/ml as compared to 1.0 $\pm$ 0.2 ng·h/ml (i.v.), bioavailability was 19 $\pm$ 5%. A level A correlation (1:1 correlation) of in-vitro liberation and in-vivo absorption could be set up based upon individual plasma level data by means of the Wagner-Nelson method. Linear correlations with a slope of approx. 1 were obtained when plotting dose fraction released in-vitro vs. dose fraction absorbed in-vivo. Half-lives of liberation in-vitro and in-vivo were similar. A lag-time for the onset of in-vivo absorption was observed caused by disintegration of the dosage form and liquid mediated formation of ER diffusion membrane on the pellets.

The present ER-dosage form of iloprost provides long-lasting plasma levels of iloprost in the therapeutic range and thus might be a clinically effective equivalent of i.v. infusion, which can easily be used by ambulant patients. The intraindividual reproducibility and the correlation of in-vitro and in-vivo performance of the modified release preparation are prerequisites for efficacy, safety and patient compliance.

INTRODUCTION

Prostacyclin (PGI_2) is a very potent inhibitor of platelet aggregation and is characterized by a number of different interesting pharmacodynamic properties [1, 2]. Since the compound itself is chemically and metabolically unstable due to its enolether structure, molecular modifications were made to obtain PGI_2-mimetics which can be used pharmacotherapeutically. Iloprost (Figure 1) is such a stabilized analogue which maintain the pharmacological properties of the endogenous precursor [3-6]. Although the chemical stability was remarkably increased the structural formula clearly reveals that the upper side chain is still suitable for ß-oxidative metabolism. The pharmacokinetics of the compound was characterized after i.v. dosing in man by a biphasic decline of plasma levels with half-lives of 3 - 5 min for the distribution phase and approx. 0.5 h for the terminal phase. Total clearance accounted for approx. 15 - 20 ml/min/kg. Metabolism mainly affected the α-chain and resulted in pharmacologically inactive tetranor-metabolites generated by consecutive ß-oxidation [7, 8]. After peroral administration of iloprost as a solution the compound was immediately absorbed from the GI-tract and rapidly cleared from the central compartment with rate constants similar to i.v. The absolute bioavailability was approx. 15 - 20 % [7, 8] due to first-pass metabolism.

Based upon these characteristics iloprost was initially developed for the treatment of PGI_2-mimetic responsive disease states by i.v. infusion administration with dosages in the range of 1 - 2 ng/kg/min given over 4 - 6 h daily, which were therapeutically effective in peripheral arterial occlusive disease (PAOD), M. Raynaud and thrombangiitis obliterans [9-12]. The pharmacokinetics of i.v. iloprost (Ilomedin®) in patients were similar to volunteers [13].

Although the beneficial effect of iloprost has to be considered a therapeutic step forward, the need for hospitalization for a considerable time period to administer the i.v. infusion treatment led to efforts to optimize drug therapy by providing an peroral dosage form. Such a formulation can be easily taken by ambulant patients and can either be used as an initial therapeutic treatment scheme or in continuation of an i.v. infusion treatment. The initial intention was to mimic the effective plasma level profiles after i.v. infusion over a similar period by a suitable peroral dosage form. Due to its pharmacokinetic characteristics, iloprost per se did not look as a promising candidate with a bioavailability of approx. 20 % and a terminal half-lfe of 0.5 h. Different retardation concepts were tested in animal models and in man and finally an extended release (ER) pellet preparation in capsules was profiled for further development [14-16]. The ER profile was obtained by coating materials with pH-independent liberation. This formulation was able to maintain therapeutic plasma levels for a period of 4 to more than 6 h after single or twice daily administration.

In case of retarded drug liberation by pharmaceutical measures, as realized in all kind of modified release preparation, it is of interest to investigate whether there is any correlation between in-vitro liberation profiles and in-vivo data. From plasma

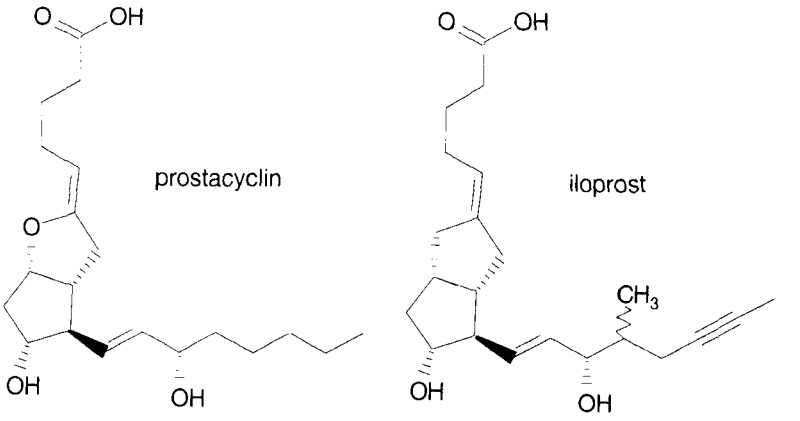

Figure 1: Structural formula of prostacyclin and iloprost

level profiles the amount of drug absorbed at a certain time point can be calculated according to Wagner-Nelson [19] and Loo-Riegelman [20] or by alternative methods. Correlations of in-vivo and vitro data can be obtained at different degree of reliability and complexity [17, 18]. According to USP the highest degree of correlation is attributed as level A and describes a complete linear function between dose fraction released in an appropriate in-vitro model and dose fractions absorbed in-vivo. Other correlations consider different parameters, e.g. mean dissolution and mean residence time [18]. A level A correlation is considered to enable predictions from in-vitro dissolution data for in-vivo performance, which is of high importance in case of change of manufacturing site or equipment to avoid complete in-vivo bioequivalence studies.

The present paper describes the evaluation of a dose-titration study in PAOD patients with the ER preparation of iloprost which included an i.v. reference treatment. Part of the study results were analyzed with regard to setting up an in-vivo/in-vitro correlation of iloprost absorption and release. Due to the different character of the present formulation (in contrast to normal retard preparation a continuous plasma level profile over 24 h was not intended) and the determinant drug liberation, which governs the in-vivo elimination phase, the present correlation could not be simply achieved by applying routine methods.

MATERIALS AND METHODS

Drug substance and formulation

Iloprost complexed by ß-cyclodextrine (ß-CD) to improve stability and feasibility of drug handling. The ß-CD clathrate was formulated as membrane coated micro-

pellets with a pH-independent ER profile (ER-formulation), which were filled in hard gelatine capsules containing either 50 µg (SH K 529 I) or 100 µg (SH K 529 M) of iloprost for individual dose adaption. For the pharmacokinetic study an i.v. reference medication was used consisting of Ilomedin® [0.1 mg iloprost/ml Tris-HCl buffer (pH 8.3) containing 1 % ethanol, manufacturer: Schering AG, Berlin] which was diluted in physiological saline for i.v. infusion.

In-vitro liberation study

The in-vitro liberation profile was characterized according USP with apparatus 1 (basket). A sample of pellets was added to 900 ml of dissolution medium (0.066 M phosphate buffer pH 7.4, Sörensen), which was maintained at 37 ± 0.5° C with a rotation speed of 50 rpm. Samples were withdrawn after suitable time intervals and analysed by HPLC for determination of released amount of iloprost. pH-independence of release was tested by subjecting capsules to testing at pH 1.2 (0.1 N hydrochloric acid) and pH 5.4 (0.066 M phosphate buffer), thus covering physiological rates of the GI-tract. As iloprost is sensitive to acid media at elevated temperatures in diluted solutions pellet material of the dissolution test was subjected to HPLC-determination of residual content of iloprost to obtain the amount of iloprost released from the formulation. Influence of the rotation speed was investigated at 50 rpm and 100 rpm. For the final test procedure a pH of 7.4 was selected which was given by the above mentioned phosphate buffer.

In-vivo liberation study

The pharmacokinetics of peroral iloprost given as ER formulation was characterized in 12 PAOD patients (7 f, 5 m, age: 64 ± 13 yrs.; body weight: 74 ± 8 kg, size: 1.72 ± 0.06 m), who had given their written informed consent to participating in the study. The study protocol was approved by an Ethics Committee. The clinical part of the study was performed by Prof. Loose at the Klinik Dr. Guth in Hamburg, Germany. Only parts of the pharmacokinetic results of the study will be reported for the in-vivo correlation purpose.

The patients were initially treated with an i.v. infusion of iloprost (Ilomedin ®, Schering AG, Berlin) starting with 0.5 ng/kg/min for 0.5 h, followed by 1.0 ng/kg/min for 0.5 h and (according to subjective tolerability) a final infusion rate of 1.5 ng/kg/min, which was maintained for 5 h. In total the infusion lasted for 6 h with a maximum dose of 0.495 µg/kg (first day of study).

The peroral treatment study part consisted of an individual dose titration in 50 µg-steps from 100 to 300 µg iloprost with the ER-formulation starting on the second day. Dose titration was performed according to individual tolerability as single dose experiment for each dose level with dose increase every day. Medication took place in the morning on an empty stomach. Capsules were taken together with 100 ml non-sparkling water or caffeine-free tea. Afterwards absence of food and drinks was obeyed for 3 h. Thereafter a standardized hospital breakfast (bread, jam, butter, water or caffeine-free tea) was served and approx. one hour later a normal lunch.

Plasma samples were taken prior to and at 0.5, 1.0, 1.5, 3.0, 6.0, 6.08, 6.17, 6.25, 6.5, 7.0 and 24 h after start of infusion on day 1. After peroral administration plasma samples were obtained at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8 and 24 h postdose. Samples were immediately frozen at -18° C until analysis.

Analytical measurements

In-vitro liberation

Dissolution samples from batches of SH K 529 I and M used for the in-vitro/in-vivo correlation experiment were analysed by an HPLC-method using a Hypersil ODS column (125 x 4.6 mm, 5 µm). The eluent consisted of acetonitrile/water/phosphate buffer pH 7.4 (Sörensen) /tetrabutylammonium-perchlorate (300 ml/ 630 ml/ 70 ml/ 2.0 g). The method was performed with a flow rate of 2 ml/min. UV-detection at 205 nm lead to peak areas of iloprost that were calculated using an external standard calibration curve for quantitation. The system was operated at ambient temperature [14].

Influence of rotation speed and pH of dissolution medium (including determination of residual content in pellet material) were investigated with an HPLC-method using a Spherisorb ODS II column (125 x 4.6 mm, 3 µm). The eluent consisted of acetonitrile/water/ß-cyclodextrin (330 ml/670 ml/8 g) that was adjusted to pH 2.0 with concentrated phosphoric acid. HPLC was performed with a flow rate of 1.0 ml/min at a temperatureof 15°C)and peak areas from UV-Detection at 200 nm were calculated using an external standard calibration curve for quantitation.

Bioanalyses of iloprost

Plasma levels of iloprost were measured by a sensitive and specific scintillation proximity assay (SPA) [22], which was a modification of a conventional RIA [21]. In short, 0.2 ml of plasma was extracted after acidification to pH 2.0 by 2.5 ml diethylether. After centrifugation and phase separation by solidification the organic layer was taken to dryness and redissolved in 0.25 ml BSA-buffer (phosphate buffer pH 7.0 (0.067 M), NaCl (0.15 M), Na_3N (0.1 %, w/v) and bovine serum albumine (0.1 %, w/v). 0.21 ml were pipetted by a Microlab 2200 (Hamilton) into the cavities of t-trays (LKB) and mixed with 0.05 µl tracer solution (iloprost-^{3}H-methylester in BSA-buffer at 4000 - 5000 cpm/0.05 ml), 0.05 ml antiserum dilution (1:15000 in BSA-buffer; antiserum raised against a BSA-conjugate of iloprost-9-pentenyl carboxylic acid in rabbits) and 0.06 ml anti-rabbit SPA suspension in BSA-buffer (Amersham, UK). After shaking overnight samples were analyzed in a LKB/Wallac 1205 Betaplate counter. Calibration curves and quality control samples were prepared from plasma of untreated subjects and treated as decribed before. The limit of quantitation was in the 10 - 20 pg/ml range.

Pharmacokinetic evaluation

Pharmacokinetic parameters were calculated from plasma level profiles applying a model-independent approach by means of TOPFIT [23]. This program offers a Wagner-Nelson approach for in-vivo liberation calculation, which was used taking

into account the terminal half-lives of iloprost as obtained after i.v. dosing. A rationale for this approach is given in the Result section. Level A correlations were obtained by plotting in-vitro vs. in-vivo release characteristics in linear graphs [24].

RESULTS

In-vitro liberation

The in-vitro liberation profile of the ER preparations SH K 529 I and M were specified as depicted in Figure 2. Actual release data of the batches tested are displayed as solid line and the specification limits are given in dotted lines.

Further studies proved the absence of significant influence from rotation speed. Dissolution profiles of one batch tested at 50 and 100 rpm, respectively, showed only slight accelerated release rates for the 100 rpm-level. Results from both rotation speeds met actual specification limits. The rotation speed of 50 rpm was chosen for the final testing procedure in order to discriminate batches with insufficient dissolution performance. Figure 3 depicts release data of one batch tested at 50 and 100 rpm with specification limits in dotted lines.

Dissolution profiles generated at different pH-values (i.e. 1.2; 5.4 and 7.4) spanning the physiological rate indicated a pH-independent release of iloprost from the ER formulation. Dissolution data at pH 1.2 were derived from analysis of residual content of iloprost in the pellet material only. Release rates at pH 5.4 showed no statistically significant differences for the data obtained with residual content determination as compared to results from analysing samples of dissolution medium as usual. For comparative purposes pH 7.4 results were added obtained from the standard dissolution test. Figure 4 shows dissolution profiles of the different pH-values in a 3-dimensional plot.

Pharmacokinetics in PAOD-patients

After i.v. infusion with dose titration from 0.5 to 1.0 and 1.5 ng/kg/min in 0.5 h intervals and maintenance of the final infusion rate for 5 h steady state iloprost plasma levels accounted for 210 ± 31 pg/ml. AUC(0-7 h) was calculated as 1.0 ± 0.24 ng·h/ml. The terminal half-life of iloprost was 0.32 ± 0.13 h. Plasma levels above 50 pg/ml lasted for 6.4 ± 0.9 h.

Following p.o. administration of 150 µg iloprost as ER-formulation on two consecutive treatment days, plasma levels of iloprost were nearly superimposible (Figure 5). Mean individual peak plasma levels were 185 ± 40 pg/ml (1st adm.) and 191 ± 52 pg/ml (2nd adm.) observed at 1.6 ± 0.5 h and 1.7 ± 0.8 h postdose. However these data do not contribute much to the characterization in case of an ER-dosage form intending to provide a plateau-like plasma level profile. The half-value duration was 4.0 ± 1.0 h and 3.8 ± 0.7 h and plasma level in the anticipated therapeutic range (i.e. above 50 pg/ml) were maintained for 6.7 ± 1.5 h and 5.5. ± 1.2 h. AUC(0-8 h) accounted for 0.79 ± 0.19 ng·h/ml and 0.76 ± 0.20 ng·h/ml

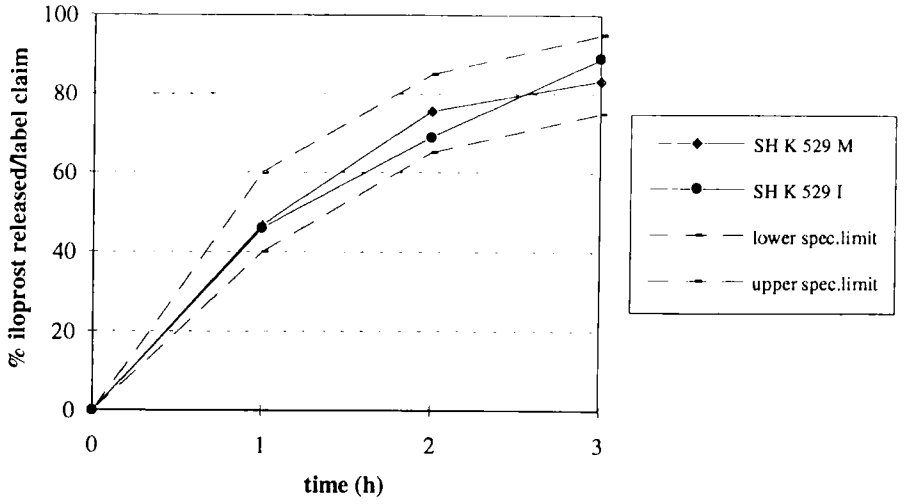

Figure 2: In-vitro liberation of iloprost from the ER pellet preparation SH K 529 I/M tested according to USP by the basket method at pH 7.4

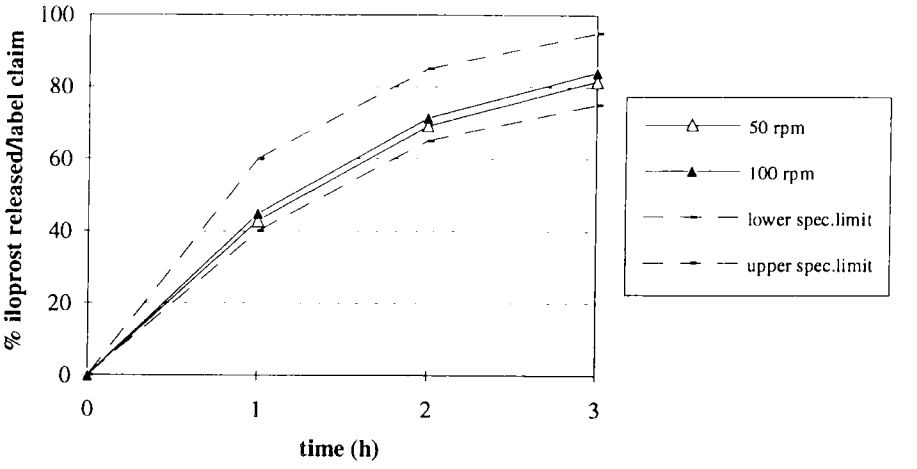

Figure 3: In-vitro liberation of iloprost from the ER pellet preparation SH K 529 according to USP by the basket method at pH 7.4 with rotation speeds of 50 and 100 rpm

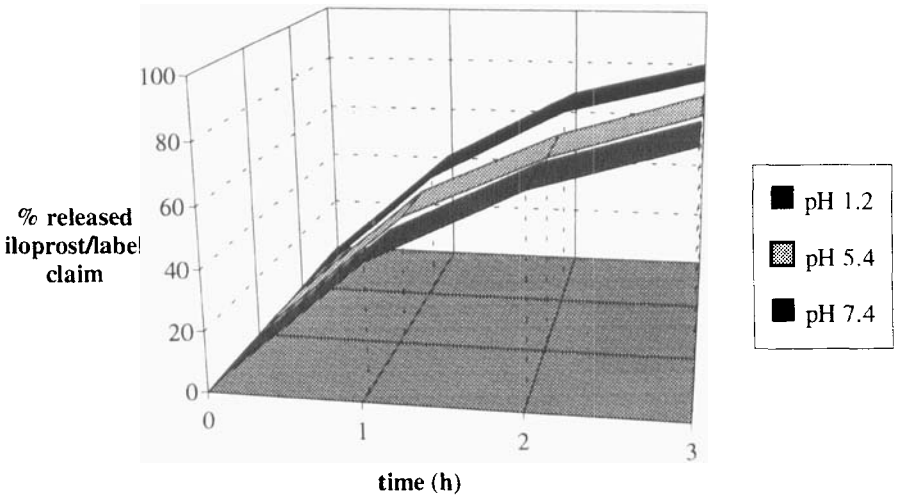

Figure 4: In-vitro liberation of iloprost from SH K 529 according to USP by the basket method at different pH-values

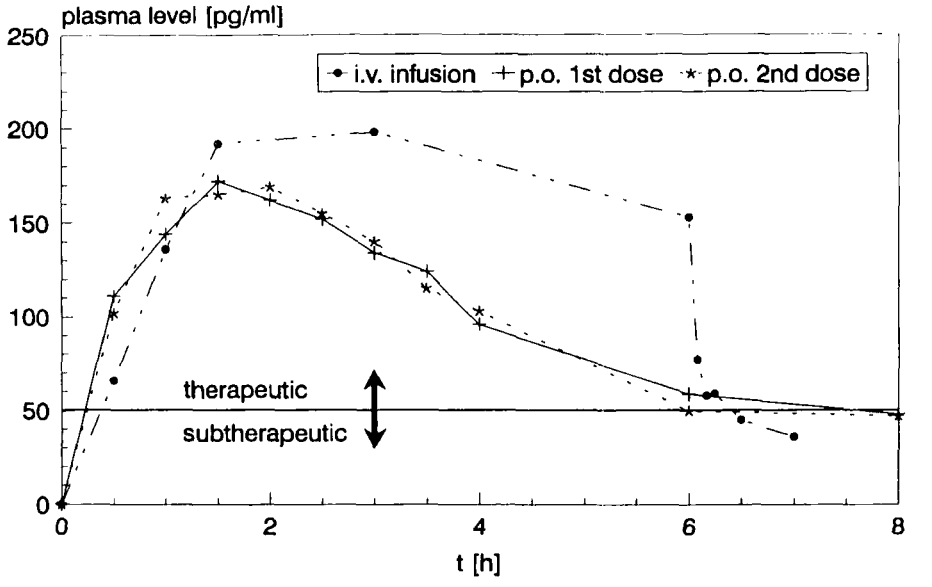

Figure 5: Mean plasma levels of iloprost in PAOD-patients after i.v. infusion treatment with dose titration [0.5 ng/kg/min (0.5 h), 1 ng/kg/min (0.5 h) to 1.5 ng/kg/min (5 h)] given as Ilomedin® and p.o. administration of iloprost as ER-formulation SH K 529 I/M at a dose of 150 µg iloprost (given on two consecutive days)

after both treatments. The absolute bioavailability of iloprost was 19 ± 5 %. The decline of plasma levels was monophasic and could be characterized by an apparent half-life of 2.4 ± 0.6 h and 2.3 ± 0.6 h after first and second treatment.

In-vivo liberation

From the individual plasma level profiles the percent of dose absorbed was calculated by the Wagner-Nelson method using the individual terminal half-life as obtained after i.v. dosing. The reason for this approach was twofold. First, although it is know that iloprost exhibits a biphasic disposition profile (with half-lives of approx. 0.08 and 0.5 h) the distribution phase could not be sufficiently characterized in the test subjects after i.v. dosing. Due to the short half-life of this initial phase it was anticipated that this phase was of minor importance after p.o. administration of the ER-dosage form. This concept was encouraged by the p.o. data, which revealed an apparent terminal half-life of iloprost of approx. 2.4 h. This result demonstrated that the decline of plasma level profiles was determined by the extended in-vivo release and absorption of the drug from the dosage form and that a short distribution phase could be neglected. In order to use the correct pharmacokinetic parameter for the Wagner-Nelson approach the ER-formulation mediated apparent half-lives were disregarded in favour of the original individual terminal half-lives after i.v. dosing.

The in-vivo absorption could be characterized by a dose fraction of approx. 4 - 5 % at 0.5 h postdose. Increasing to a mean of 32 % at 1.5 h and reaching 72 - 74 % after 4 h. The data obtained from both 150 µg-dosages are summarizes in the following Table 1. Figure 6 displays the individual time courses after the first administration.

Both data sets exemplify the excellent reproducibility of the in-vivo liberation performance of the investigated ER-formulation in PAOD-patients, which was also seen from individual and mean plasma level profiles.

In-vitro/in-vivo correlation

For this purpose the available mean in-vitro liberation profile of the pellet batch used in the experiment was plotted versus the individual in-vivo absorption data and showed an excellent linear level A correlation for all test subjects (Figure 7). Within the referable time interval the slope of the correlation lines was approx. 1. The x-axis intercept was due to the delay of the in-vivo release and absorption. This multi-step process is assumed to start with the disintegration of the normal gelatine capsule followed by the release of the pellet preparation into the GI-tract. Afterwards the coating material swell by uptake of GI liquid and capillaries are formed as channels for the release of iloprost, which will dissolve in the GI liquid according to a concentration gradient to be maintained after absorption via GI surface into the blood. Taking into account this cascade a 1:1 in-vitro/in-vivo correlation with a corresponding in-vivo intercept is obvious. This result was also obtained, when the time for in-vitro liberation and in-vivo absorption of defined dose fractions were compared (Figure 8). The dotted lines gave the lag-time window ranging from approx. 0.2 to 1.2 h.

Table 1: Estimation of in-vivo iloprost absorption [% of dose] after p.o. administration of 150 µg iloprost as ER-formulation SH K 529 I/M in PAOD-patients on two study days by the Wagner-Nelson method

	first day				second day			
	in-vivo absorption [% of dose]							
t [h]	mean	sd	min	max	mean	sd	min	max
0	0	0	0	0	0	0	0	0
0.5	10.3	3.7	4.0	14.5	10.9	7.5	4.6	31.7
1.0	21.8	6.5	9.4	30.2	23.4	9.1	15.5	47.9
1.5	32.0	6.1	20.5	41.3	32.5	4.8	25.3	39.5
2.0	42.4	5.6	32.4	51.0	47.1	10.2	35.3	72.5
2.5	51.3	6.5	39.4	62.5	55.9	9.5	47.4	80.4
3.0	59.8	5.7	50.4	68.8	64.3	10.3	53.7	90.8
3.5	66.3	5.5	59.2	77.7	70.6	11.2	57.4	100.0
4.0	72.1	5.1	64.0	79.4	73.8	5.1	67.1	85.8

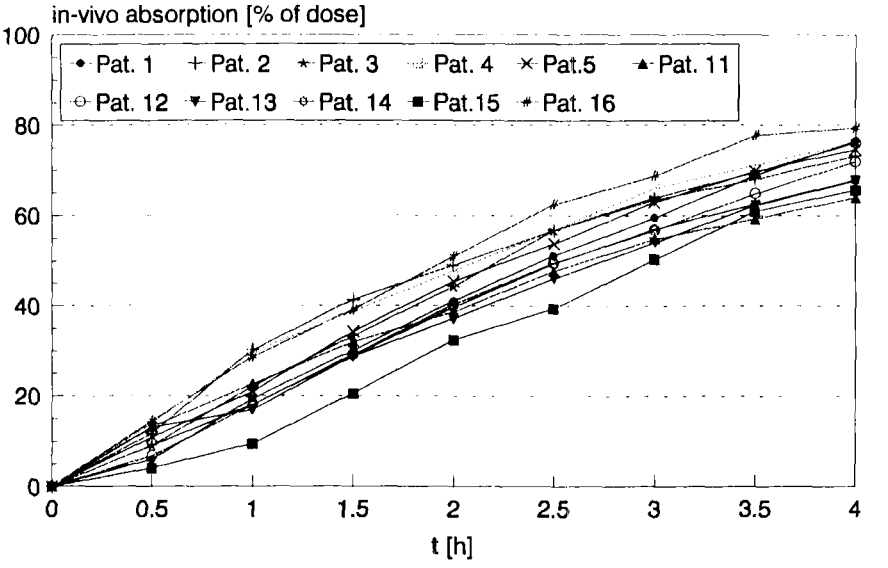

Figure 6: In-vivo absorption of iloprost in PAOD-patients after single p.o. administration of 150 µg as ER-formulation SH K 529 I/M (first day)

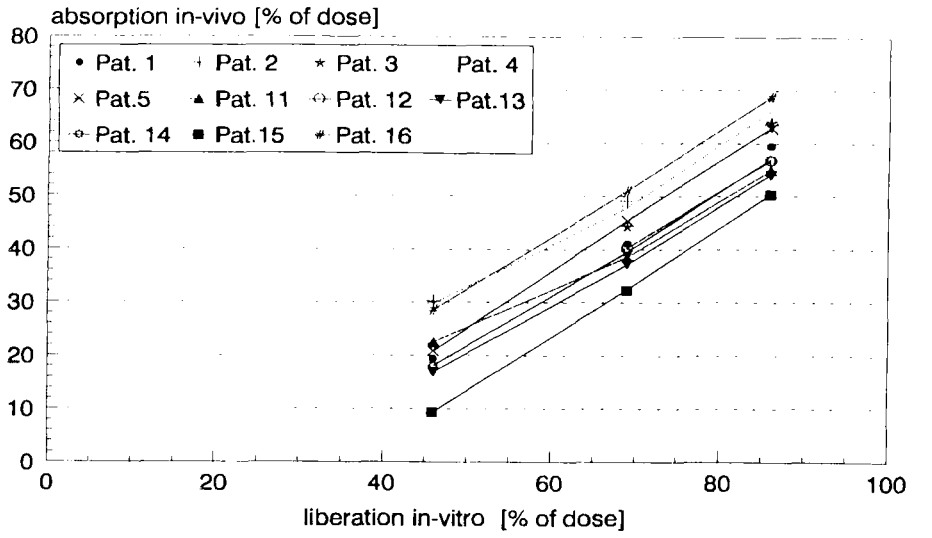

Figure 7: In-vitro/in-vivo correlation of iloprost liberation from the ER-formulation SH K 529 I/M

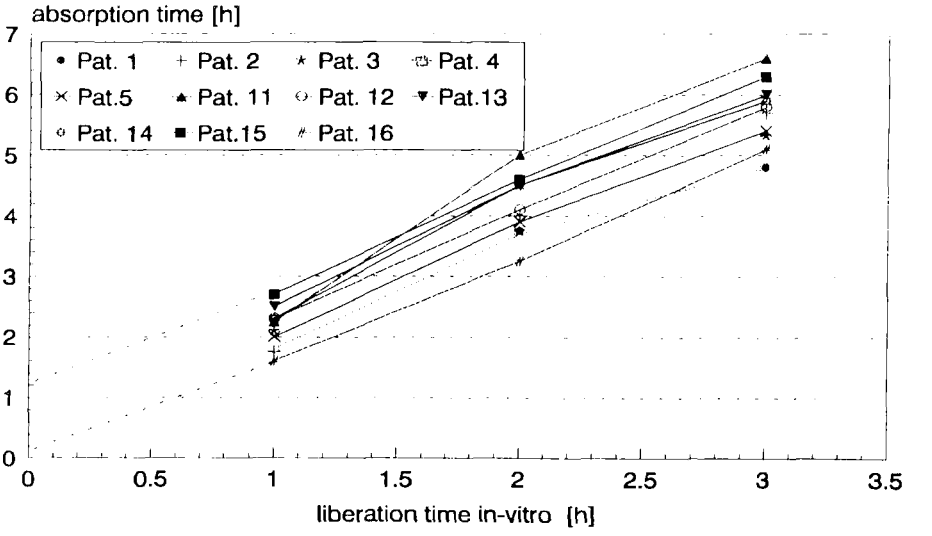

Figure 8: In-vitro/in-vivo correlation of liberation and absorption time of iloprost from ER-formulation SH K 529 I/M

DISCUSSION

In the present paper a level A in-vitro/in-vivo correlation was set up for the ER-pellet formulation SH K 529I/M containing iloprost, which is subject to clinical testing at present. Based upon in-vitro liberation data obtained at pH 7.4 with the basket method and in-vivo data generated in PAOD-patients after peroral administration of 150 µg iloprost.

It could be demonstrated that the ER-formulation exhibited a highly reproducible in-vivo liberation profile as verified by nearly superimposable plasma level profiles after repeated administration in one of the therapeutic target population. The bio-availability of iloprost was not reduced by applying a modified release concept.

The in-vitro liberation took place over a time period of at least 3 h. In-vivo additional processes, e.g. the initial disintegration of the capsule followed by swelling of pellet coating material prior to drug release, are needed prior to drug absorption. Together with the observed plasma level profiles this information implies that iloprost can be absorbed both from the stomach and the upper parts of the intestines, because under fasted conditions the gastric transit time is certainly shorter (approx. 1 h with a range from 16 - 137 min [25, 26]). From p.o. administration of iloprost solutions an immediate and complete absorption from the stomach had been shown [7, 8], which is in agreement with the pH-hypothesis. Iloprost as a weak carbonic acid (pk_a: 4.9) should be preferentially absorbable under acidic conditions of the stomach (pH: 0.5 - 4.0) due to the presence of its unionized free acid form. However, apart from pH considerations the differences in the surface for absorption may also be important. A certain dose fraction of iloprost is present unionized in the upper part of the intestines making absorption there also explainable.

In-vitro data demonstrated the pH-independent drug release from the ER-formulation. The in-vitro/in-vivo correlation can be considered as a principle in-vivo proof for this observation, because all individuals exhibited a slope of approx. 1. As it is rather unlikely that all test persons had identical pH-conditions (as well as liquid volumes) in the GI-tract, an in-vivo pH-independence could be inferred from the data. Individual differences in liquid volumes and transit times might be the reason for varying lag-times prior to the onset of in-vivo absorption. The uniform slope of the time curves displayed in Figure 8 demonstrated the similarity of in-vitro liberation and in-vivo absorption half-lives.

In summary, the present ER-formulation of iloprost exhibits a level A in-vitro/in-vivo correlation as demonstrated in a target patient population. The extended relase profiles provided therapeutic drug levels for a time period similar to i.v. infusion treatment. Therefore this peroral formulation should be a therapeutic equivalent, which can be individually titrated according to PGI_2-mimetic tolerability and easily be used by ambulant patients. The intraindividual

reproducibility of therapeutic plasma levels is a prerequisite for efficacy, safety and patient compliance.

Acknowledgement

In this paper parts of the results of a clinical trial were reported. The authors wish to express their gratitude for the excellent performance of this trial to Prof. Loose and his co-workers at the Klinik Dr. Guth, Hamburg, Germany. Furthermore the help of colleagues in the function Clinical Development of Schering AG Germany to realize this study is highly appreciated.

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