Isosorbide-5-Nitrate Sustained-Release Pellets – An Example of Computer-Supported Drug Development

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Abstract: To achieve a fast onset and a sufficiently long duration of action in the long-term treatment of angina pectoris a composite dosage form was developed, consisting of a fast-release initial dose D_i and a slow-release maintenance dose D_m. The product is designed to be given once daily in the morning to achieve sufficiently high blood levels for clinical response during day-time with declining blood levels during night-time to avoid tolerance. In view of its pharmacokinetic properties, the antianginal drug isosorbide-5-nitrate (IS-5-N) was selected as the model substance. To minimize the influence of physiological factors such as GI-transit time and pH on the *in vivo* releasing properties, pellets with a membrane-controlled drug release appeared to be suitable. To investigate the *in vitro/in vivo* correlations, three variants of of this dosage form, differing in the D_i:D_m ratio and in the duration of the drug release, were prepared. The pharmacokinetics of these variants were tested in man, and their *in vitro* dissolution behaviour was characterized by their mean dissolution times (T_{Diss}). The *in vivo* performance was characterized by the mean residence time (MRT), the bioavailability (ba) relative to standard tablets, and the *in vivo* absorption rate by the method of Wagner and Nelson. The linear correlation coefficients were: ba vs. MRT, $r = -0.845$, $p < 0.01$; MRT vs. T_{Diss}, $r = 0.949$, $p < 0.001$, and ba vs. T_{Diss}, $r = -0.886$, $p < 0.05$. With the known pharmakokinetic and dissolution parameters, a prediction of the time course of the plasma level was attempted.

Recent investigations demonstrate a reversible development of tolerance during long-term antianginal treatment with organic nitrates. With a 40 mg ISDN q.i.d. dosage regimen, Blasini et al. (1) found an attenuation in the reduction of systemic and pulmonary artery pressures already after the first day. After 7 days, no effect was observed, even after additional sublingual administration of 10 mg ISDN. A complete recovery of response, however, was achieved after a drug-free interval of 36 h. Similarly, Parker et al. (2) showed that the initial vasodepressor response could be restored after a recovery phase of 21 h.

Most recently, Rudolph et al. (3) failed to observe tolerance under long-term treatment with 120 mg sustained-released ISDN s.i.d., with the reduction of exercise-induced ST-segment depression as the parameter for anti-ischemic activity. Under this dosage regimen, steady state ISDN plasma concentrations declined from 24 ng/ml after one hour to 1.7 ng/ml at 18 h and 0.4 ng/ml at 24 h after drug application. Its active main metabolite, isosorbide-5-nitrate (IS-5-N, Fig. 1) reached peak concentrations of approximately 440 ng/ml after 6 h and then declined to 140 ng/ml at 18 h and 60 ng/ml at 24 h. These findings led to the idea to establish a recovery period as part of the dosing interval when designing a dosage form for a once-daily administration.

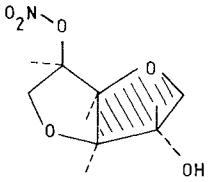

Fig. 1 Structural formula of isosorbide-5-nitrate.

Isosorbide-5-nitrate appeared to be the most promising compound in this respect. Its pharmacokinetics, pharmacodynamics, and therapeutic usefulness have been investigated in man since 1974. In contrast to the literature on the other organic nitrates, several investigators have obtained consistent results on the pharmacokinetic parameters of IS-5-N; its elimination half-life ranges between 4 and 6 h (4–10). The absolute bioavailability after administration of the fast-release standard tablets was found to be between 86 and 113 % (4, 5, 8, 9). The rather predictable *in vivo* behavior of IS-5-N and its long elimination half-life – the longest among all therapeutically used organic nitrates – make it a safe and reliable drug in angina pectoris prophylaxis. Therefore, IS-5-N was selected here as a model substance for the development of a new composite sustained-release formulation.

According to earlier investigations (4, 5, 7, 8, 10), the pharmacokinetics of IS-5-N follow a one-compartment open body model with first-order absorption and elimination, for which the plasma level/time courses are described by the Bateman function:

$$C(t) = C_0 \cdot \frac{k_1}{k_1 - k_2} \cdot (\exp(-k_2 t) - \exp(-k_1 t)) \quad (1)$$

with $C(t)$ = plasma concentration at time t (ng/ml)
t = time (h)
C_0 = dose/V (mg/l)
V = volume of distribution (l)
k_1 = absorption rate constant (h^{-1})
k_2 = terminal rate constant (h^{-1})

The plasma level-time course after 20 mg IS-5-N, described by eq. 1, is illustrated in Fig. 2.

Assuming the ideal case of an oral sustained-release formulation with zero-order release and rapid absorption, the plasma level vs. time function during absorption is given by eq. 2:

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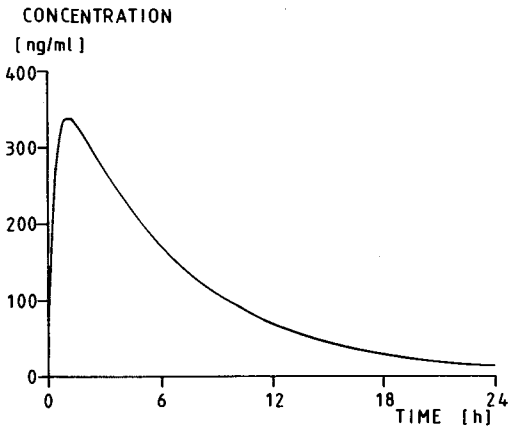

Fig. 2 Simulated plasma level-time course of IS-5-N after oral administration of a 20 mg standard tablet (k_1 = 2.8 h^{-1}, k_2 = 0.15 h^{-1}, c_0 = 400 ng/ml).

$$C(t) = \frac{k_0}{k_2 \cdot V} \cdot (1-\exp(-k_2 t)) \quad (2)$$

where k_0 = release rate (mg/h).

After the termination of the drug release, the plasma levels decline according to:

$$C(t) = C(t_v) \exp(-k_2(t-t_v)) \quad (3)$$

where t_v = time of the termination of the drug release.

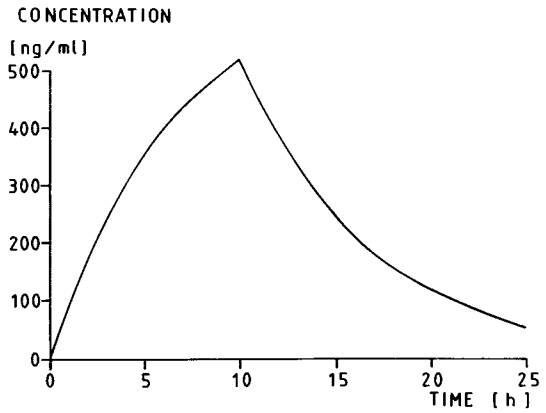

Fig. 3 Simulated plasma level-time course of IS-5-N after oral administration of a sustained-release formulation with zero-order release rate k_0 = 5 mg/h over a period of 10 h.

Fig. 3 shows such a course of the plasma level with a release rate of k_0 = 5 mg/h over a period of 10 h. The shape of this curve reveals two disadvantages.

1) Because of the slow rise in the drug plasma concentration, the hemodynamic effect is considerably delayed as compared to a fast-release tablet.

2) Independent of the release rate k_0, quasi-constant plasma concentrations cannot be reached within one administration interval. On the basis of these considerations a composite sustained-release form consisting of two fractions was designed, one rapidly released fraction and one liberated in zero-order fashion. For repeated administration with accumulation, the optimal ratio between the initial dose D_i and the maintenance dose D_m can be calculated according to eq. 4 (11):

$$D_i:D_m = \frac{1}{k_2 \cdot t_v} \cdot (1-\exp(-k_2(\tau-t_v))) \quad (4)$$

where τ = administration interval.

Simulated plasma level-time courses for a release period of 10 h, with various $D_i:D_m$ ratios, are shown in Fig. 4.

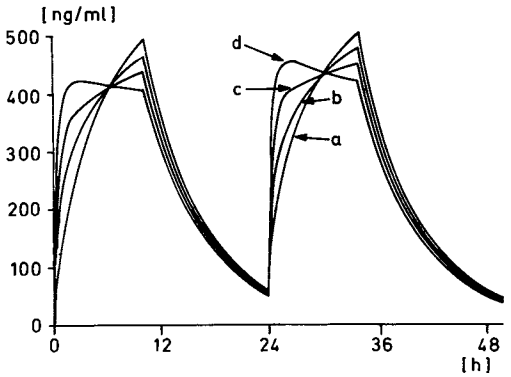

Fig. 4 Simulated plasma level-time courses of a composite formulation with various $D_i:D_m$ ratios, assuming a total dose D_{tot} = 50 mg and a release period t_v = 10 h ($D_i:D_m$ a) 0:1, b) 1:9, c) 1:3, d) 2:3).

With consideration of the physiological factors such as gastric emptying, intestinal transit time, motility, and pH, pellets appeared to be suitable as a multiple-unit dosage form. In comparison to single-unit preparations the drug release from pellets is less influenced by variations in transit times, as they are spread out over a considerable part of the intestine (12).

Lippold et al. (12) described the preparation of pellets by coating a drug-containing core with a layer consisting of a water-insoluble film-forming agent and water-soluble additives. Exposure of the film to an acceptor medium effects the formation of pores, and the drug release thus follows the principles of pore diffusion. Assuming a saturated solution in the pellets and ideal sink-conditions, the resulting zero-order release of the drug pellets with a defined porosity was shown to obey Fick's 1st law (12). The release thus depends on the permeability and thickness of the sustained-release layer, the pellet's surface area, and the water-solubility of the drug. In contrast to the erodible and matrix-controlled dosage forms, where the drug release depends on the hydrodynamics of the surrounding medium, the drug release from these pellets is not affected by agitation. On the basis of these considerations a pharmaceutical formulation of IS-5-N has been developed and is described here.

Materials and Methods

Materials: Isosorbide-5-nitrate (90 % with lactose, Sifa Chemicals); lactose (Brenntag, Mülheim); ethylcellulose (type N 7, Hercules); polyethylene glycol 6000 (Hoechst AG); hydroxypropylcellulose (Klucel LF, Hercules).

Equipment: W6 Wurster equipment (Kilian); pressure: 2.8 bar; inlet air temp.: 40° C; outlet air temp.: 25° C.
Preparation of drug formulations: The maintenance dose and the initial dose, calculated by eq. 4, were coated directly onto the pellets in a coating pan using an adhesive solution of 10 % hydroxypropylcellulose in water. The sustained-release layer (9:1 ethylcellulose/polyethylene glycol 10 % in 1:1 dichloromethane/ethanol) was again applied by the fluidized-bed technique. Three test formulations A, B, and C were prepared, each containing a total amount of 50 mg IS-5-N per capsule. The compositions and the structural characteristics are given in Table I.

Table I. Compositions and structural characteristics of formulations A, B, and C (for C smaller pellets were used)

Formulation	D_i (mg)	D_m (mg)	A (mm^2)	t_f (μm)
A	10.95	39.05	14.3	9.6
B	12.6	37.4	14.3	15.3
C	14.9	35.1	3.5	22.9

D_i = Initial dose
D_m = Maintenance dose
A = Surface area per pellet
t_f = Film thickness of the sustained-release layer

In vitro Tests

The release rate of the drug was determined by a modified rotating bottle method according to NF XIII. The amount of drug released was assayed by HPLC with UV detection at 200 nm. Additional experiments (14) showed that the *in vitro* release properties are model-independent and are not influenced by the hydrodynamics of the acceptor medium.

Pharmacokinetics

The *in vivo* performance of the three formulations A, B, and C has been examined on three occasions, twice in healthy volunteers and once in patients. Formulation A was investigated in a cross-over study versus 20 mg standard tablets. Blood specimens were taken before application and at 9 sampling times thereafter. The plasma IS-5-N concentrations were assayed by HPLC according to the method of Maddock et al. (15). Formulation B was investigated in a steady-state cross-over study, using 1 capsule of formulation B every day or 2 tablets of the 20 mg reference formulation per day. On the third day venous blood was withdrawn before the administration and at 9 to 10 times thereafter. Formulation C was tested in six patients (Group I), who received 1 capsule of formulation C once daily on two consecutive days. Blood samples were collected before the administration and at 9 sampling times during an administration interval on the first and the second day of treatment. Group II was treated with standard tablets containing 50 mg IS-5-N each, given every 8 h on two consecutive days. Blood sampling was carried out during the first and fourth dosing interval before the administration of the tablets and at 6 times thereafter. Plasma specimes were analyzed by capillary gas chromatography with an electron capture detector.

Calculations

In vitro dissolution: The dissolution behaviour of each test formulation was characterized by calculating the mean *in vitro* dissolution time T_{Diss}, following the method of Brockmeier (16, 17):

$$T_{Diss} = \frac{\int_o^\infty t \cdot dM(t)}{\int_o^\infty dM(t)} \quad (5)$$

where M(t) = amount of drug released at time t.

Pharmacokinetic parameters: The areas under the plasma level-time curves (AUC) were calculated by the trapezoid rule. After single doses, extrapolation to infinity was done by calculating the residual AUC fraction, i.e. C^*/k_2, where C^* is the last plasma concentration measured. In steady state, the AUC's were calculated over one dose interval. The relative bioavailabilities (ba) were calculated as the AUC ratios of the test and the reference formulations, correcting for the dose as appropriate:

$$ba = \frac{AUC\ (test\ formulation) \cdot Dose\ (reference)}{AUC\ (reference\ formulation) \cdot Dose\ (test)} \cdot 100\,\% \quad (6)$$

In the case of formulation C the relative bioavailability was computed as the ratio of the mean AUC's for the two groups of patients.

The mean residence time (MRT) was calculated by equation 7, extrapolating the plasma levels to values below the detection limit with the aid of the Bateman function, equation 1:

$$MRT = \frac{\int_o^\infty t \cdot C(t)dt}{\int_o^\infty C(t)dt} \quad (7)$$

In the steady-state study any plasma concentrations carried over from the preceding administration interval were subtracted, applying the Bateman function, equation 1.

In vivo/in vivo and in vivo/in vitro correlations: Any dependence of a) the mean residence time on the mean dissolution time, b) the relative bioavailability on the mean residence time, and c) the relative bioavailability on the mean dissolution time was determined by calculating the linear regression lines by least squares and the pertinent correlation coefficients r and then applying the null hypothesis to r.

In vivo absorption: In all these experiments the plasma level-time courses of IS-5-N could be described by one-compartment open body models, so that the rate and the extent of *in vivo* absorption can be suitable calculated by the Wagner-Nelson method (18). The method is based upon equation 8:

$$f = \frac{C(t)+k_2\int_o^t C(t)dt}{k_2\int_o^\infty C(t)dt} \quad (8)$$

where f = fraction absorbed.

Plasma-level simulations. Initially the plasma-level simulations started from the assumption of a zero-order release of the maintenance dose. In practice, this is not completely achievable with membrane-controlled diffusion (Fig. 5). A better approximation ($r > 0.999$) of the *in vitro* dissolution curve of the sustained-release fraction of the total dose is given by a 3rd order polynomial. The amount $D(t_n)$ of the drug released up to the time t_n can be described by:

$$D(t_n) = a + bt_n + ct_n^2 + dt_n^3 \quad (9)$$

where a, b, c, d are constants computed by third-degree regression. The amount released during a short time interval (t_{n-1}, t_n) is then $D(t_n)-D(t_{n-1})$. If this amount is absorbed *in vivo* with bioavailability F_m, and if the rate of absorption is regarded as constant during the interval (t_{n-1}, t_n), the plasma concentration C_1 (t_n) at the end of this interval will be:

$$C_1(t_n) = \frac{F_m(D(t_n)-D(t_{n-1}))}{V \cdot k_2(t_n-t_{n-1})} \cdot (1-\exp(-k_2(t_n-t_{n-1}))) \quad (10)$$

Any plasma concentration $C_2(t_n)$ remaining from the preceding interval will decay according to:

$$C_2(t_n) = C_2(t_{n-1}) \cdot \exp(-k_2(t_n-t_{n-1})) \quad (11)$$

so that the total plasma concentration $C_m(t_n)$ originating from the sustained-released maintenance dose D_m at time t_n is:

$$C_m(t_n) = C_1(t_n) + C_2(t_n) \quad (12)$$

The total drug plasma concentration $C(t_n)$ at time t_n consists of $C_m(t_n)$ and another fraction $C_i(t_n)$, originating from the initial dose D_i:

$$C_i(t_n) = \frac{F_i \cdot D_i}{V} \cdot \frac{k_1}{k_1 - k_2} \cdot (\exp(-k_2 t)-\exp(-k_1 t_n)) \quad (13)$$

where F_i = bioavailability of the initial dose.

Hence

$$C(t_n) = C_i(t_n) + C_m(t_n) \quad (14)$$

Scanning t_n from zero to infinity in sufficiently small steps results in a more subtle plasma level-time course simulation than one calculated by equations 1 to 3.

To match those simulations against measured data, an iterative trial-and-error approach was adopted, in which F_i was set to 100 % and F_m was calculated from the D_i:D_m ratio and the relative bioavailability measured.

Results

In vitro Performance

The cumulative dissolution curves of the test formulations are shown in Fig. 5. The mean *in vitro* dissolution times T_{Diss} were 3.55 h (formulation A), 5.14 h (formulation B), and 2.84 h (formulation C).

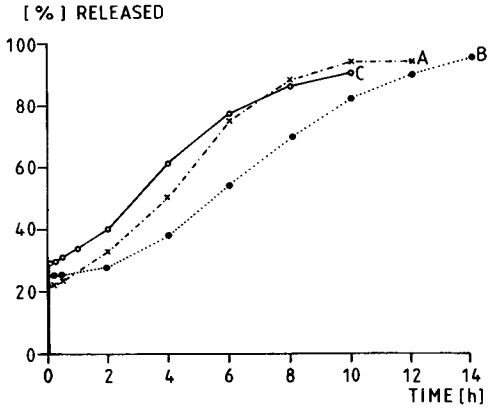

Fig. 5 Cumulative dissolution of formulations A, B and C.

In vivo Performance

The plasma level-time courses of IS-5-N after the administration of formulations A, B, and C are shown in Figs. 6 to 8. For

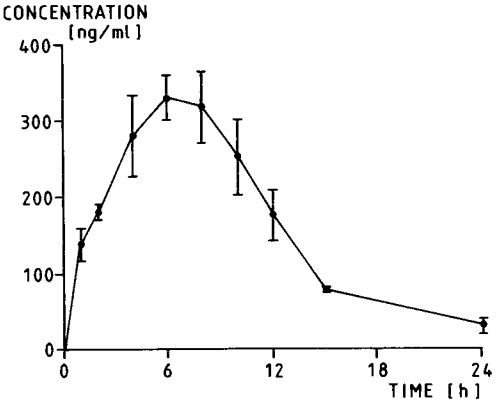

Fig. 6 Plasma level-time course after oral administration of 50 mg IS-5-N as formulation A (n = 3, means ± SEM).

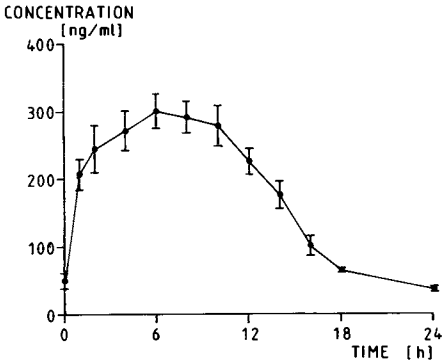

Fig. 7 Plasma level-time course after oral administration of 50 mg IS-5-N as formulation B (steady state, n = 12, means ± SEM).

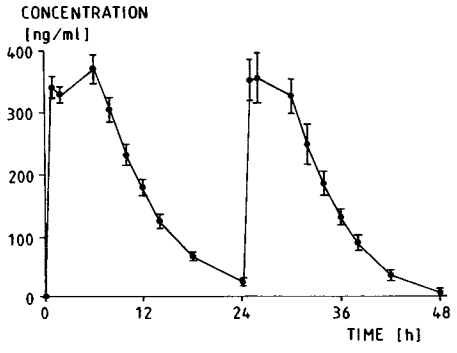

Fig. 8 Plasma level-time course after oral administration of the first and the second doses of 50 mg IS-5-N as formulations C (n = 6, means ± SEM).

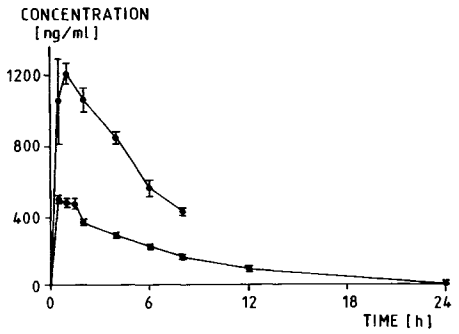

Fig. 9 Plasma level-time courses after oral administration of 20 mg (n = 12) and 50 mg (n = 6) IS-5-N as standard tablets (steady state; means ± SEM).

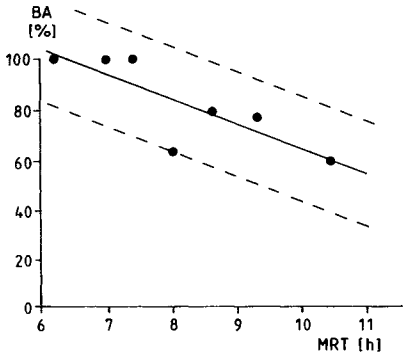

Fig. 10 Bioavailability ba as a function of the mean residence time MRT.

comparison, Fig. 9 represents the situation after the administration of the 20 and 50 mg standards. The principal pharmacokinetic data are listed in Table II.

Calculations

Graphs of linear regression lines and 95 % confidence intervals for
- relative bioavailability ba vs. mean residence time MRT
- mean residence time vs. mean dissolution time T_{Diss}, and
- relative bioavailability vs. mean dissolution time T_{Diss}

are given in Figs. 10 to 12. The correlation coefficients and corresponding levels of significance were calculated as − 0.845; $p < 0.01$ (ba vs. MRT), 0.949; $p < 0.001$ (MRT vs. T_{Diss}), and − 0.886; $p < 0.05$ (ba vs. T_{Diss}).

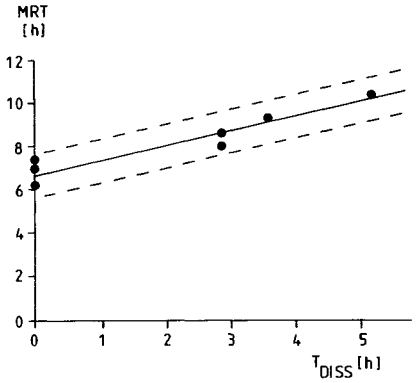

Fig. 11 MRT as a function of the mean dissolution time T_{Diss}.

Table II. Principal Pharmacokinetic Data of IS-5-N after p.o. Administration of Standard and Sustained Release Formulations

Formulation	Dose (mg)	Study design	n	MRT (h)	AUC (ng·h/ml)	$t_{1/2}$(e) (h)	C_{max} (ng/ml)	t_{max} (h)	ba (%)
Standard	20	Single dose	3	7.0	2112 ± 439[a]	4.83 ± 0.06	388 ± 134	1 (0.5–1.5)	100
Formulation A	50	Single dose	3	9.3	4070 ± 970[a]	3.72 ± 1.27	348 ± 71	6 (6–8)	76.9 ± 3.7
Standard	20	Multiple dose	12	7.4	2905 ± 373[b]	5.01 ± 1.26	541 ± 53	1 (0.5–1.5)	100
Formulation B	50	Multiple dose	12	10.4	4342 ± 1132[b]	7.3 ± 3.8	359 ± 99	8 (2–12)	60.1 ± 14.4
Standard	50	Single dose	6	6.2	5889 ± 899[a]	3.83 ± 1.35	858 ± 258	1 (0.5–4)	100
Formulation C	50	Single dose	6	8.6	4693 ± 506[a]	4.79 ± 0.90	376 ± 40	3.5 (1–6)	79.7[c]
Standard	50	Multiple dose	6	6.2	6399 ± 551[b]	4.18 ± 0.71	1384 ± 347	1.5 (0.5–2)	100
Formulation C	50	Multiple dose	6	8.0	4077 ± 564[b]	4.35 ± 0.67	386 ± 62	2 (1–6)	63.7[c]

[a] $AUC^{0-\infty}$ [b] $AUC^{0-\tau}$ [c] no intraindividual cross-over

n = Number of individuals
MRT = Mean residence time
AUC = Area under the plasma level/time curve (mean ± S.D.)
$t_{1/2}$ (e) = Terminal half-life (mean ± S.D.)
C_{max} = Peak plasma level (mean ± S.D.)
t_{max} = Time to peak plasma level (median and range)
ba = Relative bioavailability

Note added in proof:
Mean while another multiple dose bioavailability study in eighteen subjects using formulation C has been completed with following pharmacokinetic parameters: AUC = 5029±1272[b] ng·h/ml, $t_{1/2}$ (l) = 5.02±0.68 h, C_{max} = 488±129 ng/ml, t_{max} = 5 (2–8) h, and ba = 83.9±13.4 %.

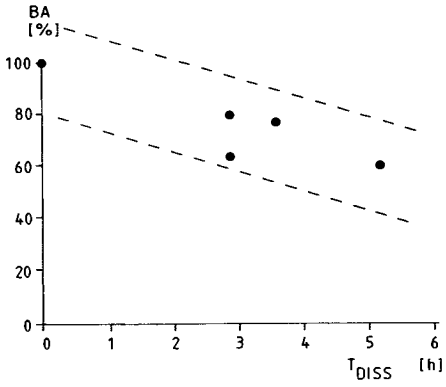

Fig. 12 ba as a function of T_{Diss}.

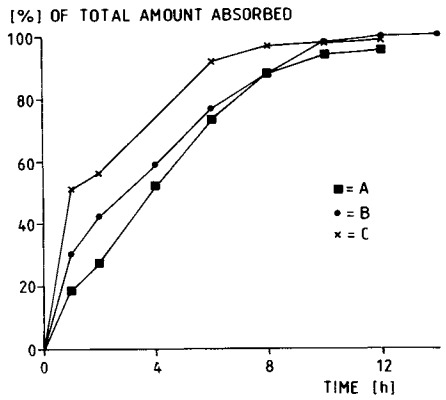

Fig. 13 *In vivo*-absorption of IS-5-N after the administration of formulations A, B, and C (Wagner-Nelson method).

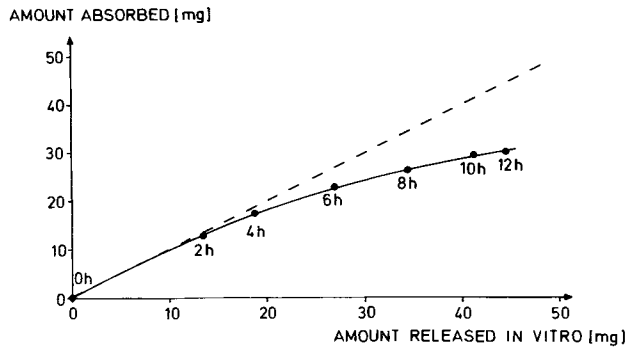

Fig. 14 Amount absorbed vs. corresponding amounts released *in vitro* in formulation B.

The drug absorption *in vivo* is characterized in Fig. 13 and compared drug released *in vitro* in Fig. 14. Fitting the cumulative drug release from the maintenance dose of formulation C to a 3rd order polynomial resulted in:

$$D(t_n) = 3.76 + 9.02\,t + 1.187\,t^2 - 0.1126\,t^3 \qquad (15)$$

The use of known values of D_i, F_i, F_m, k_1, k_2, and V and the time function of D_m, $D(t_n)$, for a plasma level/time course simulation according to equations 10 to 14 gave the graph plotted in Fig. 15.

Discussion

Comparison of Figs. 6 to 8 with Fig. 9 shows that steady-state values of C_{max} have been reduced from 540 ng/ml with the standard therapeutic dosage regimen (20 mg IS-5-N twice daily) to approximately 370 ng/ml. As desired, plateau plasma levels are reached 1 to 2 h after administration and are maintained over 6 to 12 h depending on the release properties of each formulation. The retard quotients R_Δ (19) are 1.75 (formulation A), 2.26 (formulation B), and 1.77 (formulation C). The MRT's increase from 7 h with the standard tablets to 8.6 to 10.4 h.

Although drug absorption from the lower parts of the intestine has been described (20), this will occur only to a limited extent. Thus, a decrease in relative bioavailability is observed with increasing MRT's. Fig. 10 suggests a linear correlation ($r = -0.845$; $p < 0.01$) between these two variables, which is supported by the *in vivo* absorption behavior as calculated by equation 8. It is evident from Fig. 13 that absorption continues over 10 h after administration. The relationship between release and absorption is clarified in Fig. 14, in which the amounts absorbed have been plotted versus the corresponding amounts released. Evidently, the *in vivo* absorption rate decreases continously relative to the *in vitro* release rate. This may be interpreted either by an impaired drug absorption in the lower parts of the intestine or by a continously decreasing *in vivo* release relative to the *in vitro* release. The linear correlation ($r = 0.949$; $p < 0.001$) of MRT vs. T_{Diss} is consistent with these findings. As expectet, the MRT increases with increasing T_{Diss}, but not to the same extent, as can be seen from the 0.69 slope of the regression line (Fig. 11) (21).

Since linear relationship of MRT vs. relative bioavailability ba and of MRT vs. T_{Diss} have been demonstrated, an analogous dependence of ba on the *in vitro* dissolution parameter T_{Diss} had to be tested. The plot of ba vs. T_{Diss} as shown in Fig. 12 might suggest such a relationship ($r = 0.886$; $p < 0.05$). On the basis of the correlation between the *in vitro* dissolution and the *in vivo* performance of the pharmaceutical formulations in this study, a prediction of the plasma level-time course after the administration of a particular formulation with a known mean dissolution time appears to be feasible. An example, calculated as described above, is given in Fig. 15. These results indicate that the combination of a drug with accurately known *in vivo* properties and with a formulation of reproducible biopharmaceutical properties yields a therapeutic agent of sufficiently predictable features.

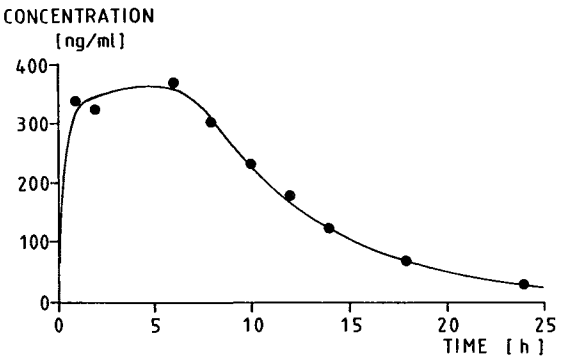

Fig. 15 Simulated (——) and measured (····) IS-5-N plasma levels after the administation of formulation C.

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Molecular Weights of Free and Drug-Loaded Nanoparticles

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Abstract: This study demonstrates that the molecular weight of polyalkylcyanoacrylate nanoparticles can be modified by the composition of the polymerization medium, the nature of the monomers and the drug to be linked to the carrier.
The influence of a surfactive agent is particularly important because polymers of very high molecular weight have been obtained. Likewise, polyalkylcyanoacrylate molecular weight distribution has been greatly modified after binding doxorubicin to nanoparticles. These long polymers could induce important changes in carrier degradation, in bound drug bioavailability and in polymer excretion rate. Some additional findings have been added concerning the state of polyalkylcyanoacrylate polymer during the degradation process.

Poor tissue specificity of pharmacologically active agents is a serious obstacle to their effective use. Formulations such as polyalkylcyanoacrylate nanoparticles could serve to improve the selectivity of these agents (1, 2). The use of nanoparticles increased the anticancer activity of dactinomycin against an experimental subcutaneous sarcoma of the rat (3). Furthermore, the possibility of significantly reducing the toxicity of doxorubicin by fixing it on nanoparticles has been demonstrated (4). More recently, whole body autoradiography performed on Lewis Lung carcinoma bearing mice showed an accumulation of the carrier in the tumoral tissue (5).

It is important to determine the molecular weights of the polymers forming nanoparticles, because the length of the polymer chain could greatly modify the body distribution of the carrier as well as the degradation rate of the polymer (6) and thus the bioavailability of the carried drug. Furthermore, it has been demonstrated that polyalkylcyanoacrylate nanoparticles undergo an enzymatic ester hydrolysis on the side chain without breaking the polymer (7). Therefore the polymers should retain the same number of monomeric subunits after dissolution. Heavier fractions are expected to remain longer in the body and the accumulation of such polymers of high molecular weight could also induce toxic side effects.

Some of the data presented in this paper partially confirm the preliminary results obtained by El-Egakey et al. (8). However, we have noted further unexpected results in our experiments. Moreover, this publication specifies the influence of all preparation conditions, the influence of the drug to be absorbed, and the state of the polyalkylcyanoacrylate polymer during the process of degradation.

Materials and Methods

Nanoparticle Preparation and Characterization

Free nanoparticles were prepared with two different monomers: isobutylcyanoacrylate (IBC)[1] and hexylcyanoacrylate

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