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ORIGINAL ARTICLE

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The bioavailability and dose dependency of the deuterated anti-tumour agent 4,6-benzylidene-d_1-D-glucose in mice and rats

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Abstract The benzaldehyde derivative 4,6-benzylidene-D-glucose (BG) induces an inhibition of protein synthesis at otherwise non-toxic doses in cells grown in vitro. To increase the biological effect of BG, the hydrogen in the formyl group was exchanged with deuterium, resulting in 4,6-benzylidene-d_1-D-glucose (P-1013). In this study we compared the bioavailability of BG and P-1013, since both intraperitoneal and, especially, oral administration of the drugs would be a great advantage. We also examined whether or not P-1013 displays dose-dependent pharmacokinetics. Pharmacokinetics were studied by analysing plasma samples using reversed-phase high-performance liquid chromatography (HPLC). P-1013 was given at four different doses i.v. (60, 120, 145 and 230 mg/kg) and p.o. (60, 120, 170 and 230 mg/kg) to female Bom:NMRI-nu mice. The bioavailability was more than 50% for all doses. The results also indicate that P-1013 shows linear pharmacokinetics, with no change being observed in the half-life ($t_{1/2}$) with increasing dose and only a slightly more than proportional increase in the area under the concentration-time curve (AUC) occurring with increasing dose. A doubling in dose resulted in a 2.2-fold increase in the AUC. P-1013 and BG were also given i.v., p.o. and i.p. to female nu/nu-BALB/cABom mice and male Wistar rats. A high degree of bioavailability was found in both species, with 55–100% of the delivered dose being absorbed. Deuteration of BG does not seem to alter its bioavailability, as we found the same bioavailability for P-1013 as for BG. We conclude that the pharmacokinetics of P-1013 does not prevent its use as a cancer treatment drug given orally.

Key words Deuterated benzaldehyde derivative Pharmacokinetics · Benzylidene glucose

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Introduction

Benzaldehyde, isolated from figs used traditionally in the treatment of cancer [4, 21], has been shown to exert antitumour effects [5]. Various benzaldehyde derivatives have been tested as possible chemotherapeutic drugs. The first derivative reported to have clinical effect was a cyclodextrin benzaldehyde inclusion compound [5]. Another derivative, sodium benzylidene ascorbate, has been reported to be effective in patients with inoperable lung cancer [18] and advanced carcinoma [8]. The benzaldehyde derivative 4,6-benzylidene-D-glucose (BG) has also displayed tumour-inhibiting properties. Long-term treatment with BG has been reported to be successful in human clinical trials in Japan in patients with advanced solid malignancies of different types [23, 24]. Treatment with BG resulted in an anti-tumour response in 55.3% of 65 patients with inoperable carcinoma, with 10.7% showing a complete response [7]. In a Norwegian study on patients with colon cancer treated with BG for 2 months, however, no objective response was recorded [22]. BG induced tumour necrosis in rats having chemically induced hepatocellular carcinoma [13], but when it was tested against two human xenografts implanted into mice, no anti-tumour activity was observed [20]. The compounds were without significant clinical toxicity [6–8, 19, 22–24].

It has been shown using human cells in culture that benzaldehyde [10, 11], BG [12] and sodium benzylidene ascorbate [15] induce an inhibition of protein synthesis resulting in reduced cell-cycle progression. Protein synthesis inhibition seems to be their primary cellular effect, and cell inactivation follows as a result of prolonged inhibition of protein synthesis. The effect is reversible in the sense that when the drug is removed protein synthesis quickly returns to normal. A problem concerning these drugs is their relatively short biological half-life [1–3, 13], which makes continuous infusion unsuitable since the necessary treatment period could well be several months [15]. For benzaldehyde and sodium benzylidene ascorbate it has been reported that exchanging the hydrogen in the formyl group

with deuterium leads to increased effects on protein synthesis as well as to an increase in cytotoxicity [14, 15]. Since this exchange increases the cellular effects, it may also improve the tumour-inhibiting properties of benzaldehyde derivatives [1].

The deuterated analogue of sodium benzylidene ascorbate, zilascorb(^{2}H), given to mice with human tumour xenografts induced tumour necrosis and appeared considerably less toxic than many other anti-cancer agents [16]. In an earlier preclinical evaluation we performed comparative pharmacokinetics studies between BG and a deuterated analogue, 4,6-benzylidene-d_1-D-glucose (denoted P-1013), in mice, rats and dogs. No difference in the half-life ($t_{1/2}$) of BG vs P-1013 was observed in any of the three species [3]. Metabolism of BG and P-1013 leads to formation of undeuterated and deuterated 1,3-benzylidene-D-glucitol, respectively, as the only detectable metabolite [2, 3]. The half-life of this metabolite was measured and found to differ between rats given BG and those given P-1013. We observed no difference in the $t_{1/2}$ values obtained for undeuterated and deuterated benzylidene-glucitol in mice or dogs. The volume of distribution for these drugs is approximately 30 ml in mice (body weight, 25 g) [3]. This indicates that most of the drug accumulates in tissue and not in plasma. BG given to rats was distributed mainly in the kidney, and 40% of the dose was excreted within 24 h [24].

In the present study we continued the preclinical evaluation and investigated the bioavailability (defined as the fraction of the delivered dose that reaches the site of sampling, i.e. plasma) of BG and P-1013. Drugs were given i.v., orally and i.p. to mice and rats. Due to the long treatment period a high degree of bioavailablity is desired, since the drug may then be given in the form of tablets or another form suitable for oral administration. We also performed studies on the relationship between dose and pharmacokinetic parameters so as to determine whether or not P-1013 shows dose-dependent pharmacokinetics.

Materials and methods

Animals

Experiments were performed using female athymic mice (nu/nu-BALB/cABom or Bom:NMRI-nu) supplied by Bomholt Gård, Denmark, and male Wistar rats supplied by Charles River, Germany. An inbred and an outbred mouse strain were used in the experiments for purposes of comparison. Nude mice were used since in vivo effect studies were performed on xenografted mice. The animals were conditioned for at least 7 days before they were used in experiments. The animals were housed in air-conditioned rooms with controlled temperature (22 °–24 °C) and humidity (>50%) on a 12-h/12-h light/dark schedule. Tap water and a commercial, pelleted maintenance diet (RM1 and RM3, SDS Ltd., Great Britain) were fed ad libitum. Mice aged 8–16 weeks (body weight, 20–30 g) and rats aged 7–9 weeks (body weight, 150–180 g) were used in all experiments.

Pharmacokinetics studies

Unfasted and unanaesthetised animals were subjected to a single dose (i.v., p.o. or i.p.) of drug dissolved in isotonic saline at a concentration

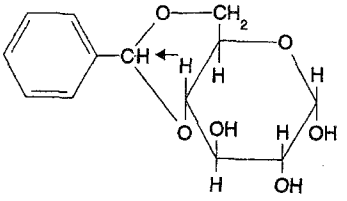

Fig. 1 The chemical structure of 4,6-benzylidene-D-glucose (BG). The α-isomer is shown, although the compound exists as both benzylidene-α- and benzylidene-β-D-glucose when in aqueous solution. The H in the acetyl group (*arrow*) is exchanged with deuterium in P-1013

of 10–17 mg/ml. The dose of BG resulting in 50% lethality (LD_{50}) in mice and rats is more than 400 mg/kg by i.v. injection, more than 3000 mg/kg by oral dosing and more than 1440 mg/kg by i.p. administration [24]. In clinical trials, daily doses on the order of 40 mg/kg have been given [22, 24]. A daily BG dose of 85 mg/kg given to rats was therapeutically effective [13]. On the basis of this result and the sensitivity of the high-performance liquid chromatography (HPLC) assay, doses ranging from 60 to 230 mg/kg were used for the studies in NMRI mice. BALB/c mice received doses of 120–272 mg/kg, and for the bioavailability studies in rats a dose of 85 mg/kg was used. Drugs were given i.v. via a tail vein. Oral administration was carried out by gastric intubation with a blunt steel canula. At selected time points [5, 10, 15, 20, 30, 45, 60, 90 and 120 min post-injection] mice were anaesthetised with propanidid (Sombrevin, Gedeon Richter Ltd., Hungary) and rats with halothane (1%–3%), and blood was immediately collected by cardiac puncture. Plasma was separated by centrifugation for 5 min and immediately frozen (−21 °C) for subsequent analysis.

Sample preparation and drug analysis

Previously reported methods for sample preparation and drug analysis by HPLC were applied [3]. Briefly, plasma was deproteinised by mixing with an equal volume of HPLC-grade acetonitrile (Rathburn Chemicals Ltd., Scotland), then centrifuged at 13,000 rpm (15,000 *g*) for 5 min and finally filtered through a 0.45-μm filter (Millipore, HV). Samples were then placed in an autosampler (Waters WISP 710B) programmed for two replicate injections of 20 μl each onto a 4.6- × 250-mm Supelco LC18-DB column (Supelco, USA). The HPLC column was eluted with 45% aqueous methanol (MilliQ water, Millipore; HPLC-grade methanol, Rathburn Chemicals Ltd., Scotland) delivered at 1 ml/min. BG and P-1013 were quantitated by fluorescence detection using a Perkin Elmer LS-5 spectrofluorometer set at excitation and emission wavelengths of 255 and 283 nm, respectively. The linear range for fluorescence detection of BG was between 0.005 and 2.5 m*M*. The lowest level of detection was 0.005 m*M*. Standard solutions of BG or P-1013 in mobile phase were used for calibration. Recovery of standard amounts of BG (0.25 m*M*) in human serum ranged between 95% and 102% (n = 10). Degradative loss of BG and P-1013 in plasma upon standing for 24 h at ambient temperature was insignificant. No interfering peak having a retention time similar to that of BG or P-1013 was found in blood drawn before drug administration.

Drugs

BG and P-1013 (Fig. 1) were provided by Norsk Hydro Research Centre, Porsgrunn, Norway. BG and P-1013 contain 0.1% benzaldehyde and when they are in aqueous solution two anomers [benzylidene-α- and benzylidene-β-glucose) are present at a ratio of 24.5% and 75.5%, respectively. The degree of deuteration in the acetal group of P-1013 was 99%. P-1013 was prepared from deuterated benzaldehyde dimethylacetate and D-glucono-δ-lactone as described elsewhere [1].

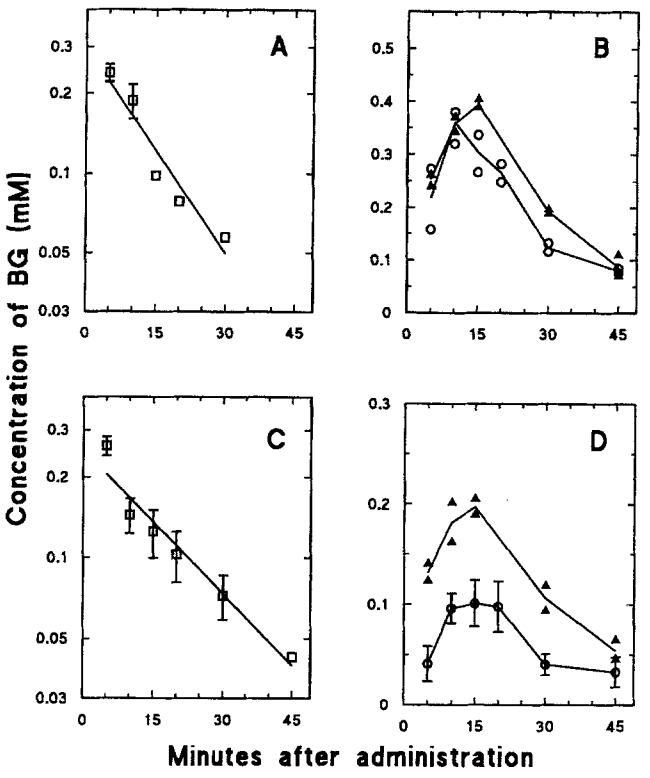

Fig. 2 **A** The concentration (m*M*) of BG in plasma as a function of time after i.v. injection of 120 mg/kg into to nu/nu-BALB/cABom mice (□). Each *data point* represents the average value for 4 mice. *Vertical bars* represent standard errors when these exceed the size of the symbols. The *solid line* represents the best-fit curve determined by linear regression. **B** The concentration (m*M*) of BG in plasma as a function of time after p.o. administration of 272 mg/kg (○) or i.p. administration of 233 mg/kg (▲) to nu/nu-BALB/cABom mice. Each *data point* represents 1 animal. The *solid lines* represent the curve drawn through the average plasma concentrations. **C, D** The concentration (m*M*) of BG in Wistar rat plasma as a function of time after treatment with 85 mg/kg given i.v. (□), p.o. (○) or i.p. (▲). For i.p. administration each *data point* represents 1 animal. For i.v. and p.o. administration each *data point* represents the average value for 4 rats. *Vertical bars* represent standard errors when these exceed the size of the symbols

Pharmacokinetic analysis of plasma concentration versus time data

The mean plasma concentration at each time point was calculated from the average determination made for two or four nu/nu-BALB/cABom mice, three Bom:NMRI-nu mice or two or four Wistar rats. New animals were used for each time point. Plasma concentration-time profiles were pharmacokinetically analysed by fitting an open, one-compartment model to the data. This model is described by the equation $C = Ae^{-kt}$, where A represents a concentration constant and k represents the elimination constant. When drugs were given i.v. the slope of the $\log_e$-concentration versus time curve was obtained by linear regression analysis of all time points and estimates k. In the case of oral or i.p. administration, a linear curve was fitted to the terminal linear portion [the elimination phase] of the $\log_e$-concentration versus time curve. Derived quantities such as the plasma elimination half-life ($t_{1/2}$) were calculated from the fitted curve using standard equations [18]. The goodness of fit was assessed by the squared correlation coefficient, r^2, which in our experiments was high (range 0.79–0.99). The AUC value for i.v. administration was calculated by analytical integration of the exponential equation (zero-to-infinity interval). The AUC values for oral and i.p. administration were computed according to the trapezoidal rule.

The drug bioavailability was computed as the ratio of the dose-corrected AUC values obtained after oral, i.p. and i.v. administration [18]:

$$\%\ \text{bioavailability} = \frac{\text{AUC e.v.}}{\text{AUC i.v.}} \times \frac{\text{Dose i.v.}}{\text{Dose e.v.}} \times 100,$$

where *e.v.* represents extravascular administration, which includes oral and i.p. dosing.

Results

Plasma concentration versus time curves generated for i.v., i.p. and p.o. administration in nu/nu-BALB/cABom mice and Wistar rats are shown for BG in Fig. 2 and for P-1013 in Fig. 3. The concentration of both BG and P-1013 in plasma decayed from 0.24 m*M* initially to undetectable amounts at 45 min after treatment with 120 mg/kg given i.v. to mice (Fig. 2 A and Fig. 3 A). At the highest dose given (264 mg/kg p.o.), P-1013 could not be detected in plasma at 60 min post-injection (Fig. 3 B). A one-compartment open model was fitted to the data from the i.v. studies, and after the initial absorption phase a one-compartment model was fitted to the i.p. and p.o. data.

The pharmacokinetic parameters determined by linear regression analysis of the experimental values are displayed in Tables 1 and 2 for nu/nu-BALB/cABom mice and in Tables 3 and 4 for rats. As can be seen in Tables 1–4, the $t_{1/2}$ values obtained for BG and P-1013 are similar, being independent of administration route, dose and animal species. From the data it can be seen that the bioavailability of BG and P-1013 is relatively high in both nu/nu-BALB/cABom mice and rats, with absorption being on the order of 55%–100%.

P-1013 was also given i.v. and p.o. to Bom:NMRI-nu mice at four different doses (Fig. 4). The smallest dose given was 60 mg/kg; for smaller doses it was difficult to obtain a concentration-time curve because the drug concentration in plasma would decrease below the detection limit soon after injection. When 60 mg/kg was injected i.v. the P-1013 concentration in plasma was 0.04 m*M* at 15 min post-injection and was undetectable at 20 min post-injection. The pharmacokinetic parameters derived from HPLC determination of the P-1013 content in plasma are shown in Table 5. It can be seen that the biological half-lives, $t_{1/2}$, obtained for the four different doses given i.v. are similar (approx. 7 min). The same appears to be the case for the data obtained for the oral results, with $t_{1/2}$ being approx. 11 min. The bioavailability of P-1013 given to Bom:NMRI-nu mice ranges from 52% to 85%.

No sign of toxicity was observed in the animals during the experiments. The highest BG dose given (272 mg/kg) was well below the LD_{50}. The highest plasma concentration measured was 0.6 m*M* in NMRI mice receiving 230 mg/kg i.v. This plasma concentration was well tolerated by the mice.

The relationship between the AUC and the dose is plotted in Fig. 5. The points representing oral administration (❑) are well fitted by linear regression ($r^2 = 0.96$), and the slope of

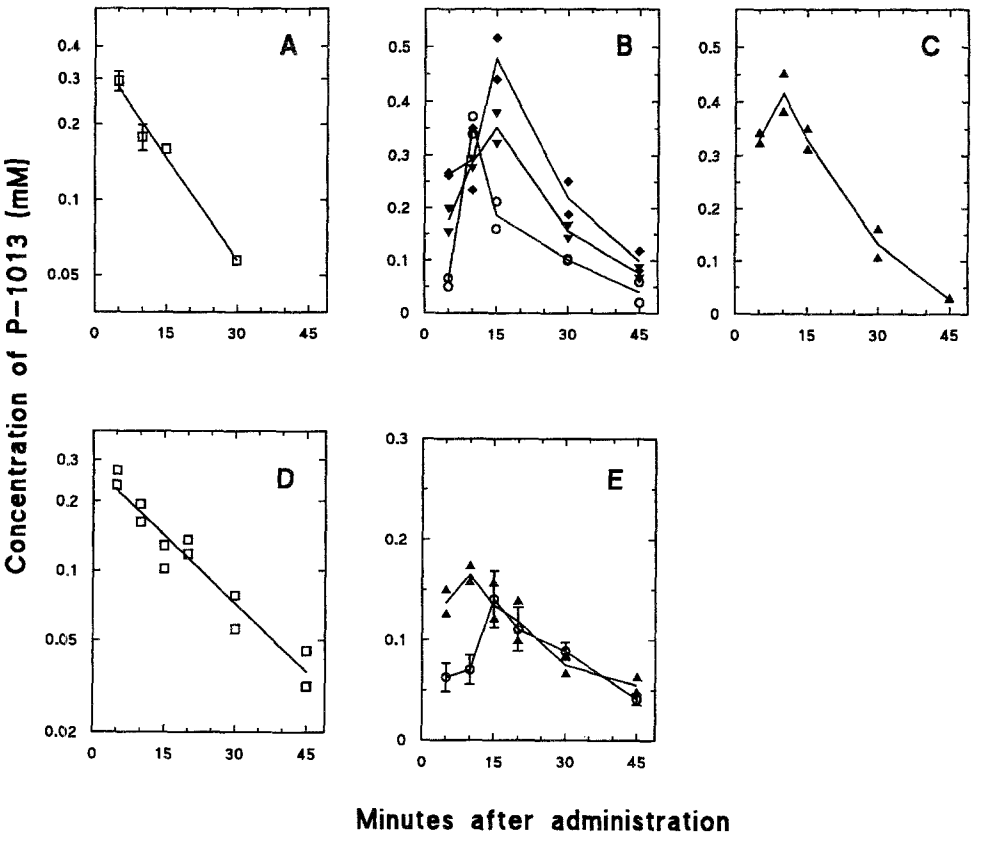

Fig. 3 **A** The concentration (m*M*) of P-1013 in plasma as a function of time after i.v. injection of 120 mg/kg into nu/nu-BALB/cABom mice (□) Each *data point* represents the average plasma concentration from 4 mice. *Vertical bars* represent standard errors when these exceed the size of the symbols. The *solid line* represents the best-fit curve determined by linear regression. **B, C** The concentration (m*M*) of P-1013 in plasma as a function of time after p.o. administration of 164 (○), 218 (▼) and 264 mg/kg (◆) and following i.p. administration of 227 mg/kg (▲) to nu/nu-BALB/cABom mice. Each *data point* represents 1 animal. The *solid line* represents the curve drawn through the average plasma concentrations. **D, E** The concentration of P-1013 (m*M*) in Wistar rat plasma after treatment with 85 mg/kg given i.v (□), p.o. (○) or i.p. (▲). For i.v. and i.p. administration each *data point* represent 1 animal. For p.o. administration each *data point* represents the average value for 4 rats. *Vertical bars* represent standard errors when these exceed the size of the symbols

Table 1 Pharmacokinetic parameters derived from the plasma level of P-1013 given by different routes of administration to nu/nu-BALB/cABom mice[a] (*n* Number of animals at each data point)

Route	Dose (mg/kg)	$t_{1/2}$ (min)	AUC (mg ml^{-1} min)	Bioavailability (%)	Cl (ml/min)	Vd (ml)	*n*
i.v.	120	11±1	1.6±0.3		1.8±0.3	29±3	4
p.o.	164	12±1	2.0±0.3	93±21	2.0±0.2	33±4	2
p.o.	218	14±2	2.6±0.5	93±21	1.5±0.3	28±4	2
p.o.	264	13±2	3.4±0.7	99±27	1.8±0.2	34±7	2
i.p.	227	9±1	2.3±0.3	80±16	1.5±0.2	19±2	2

[a] Data represent mean values ±SE

Table 2 Pharmacokinetic parameters derived from the plasma level of BG given by different routes of administration to nu/nu-BALB/cABom mice[a] (*n* Number of animals at each data point)

Route	Dose (mg/kg)	$t_{1/2}$ (min)	AUC (mg ml^{-1} min)	Bioavailability (%)	Cl (ml/min)	Vd (ml)	*n*
i.v.	120	11±1	1.4±0.2		2.0±0.3	33±3	4
p.o.	272	14±2	2.7±0.4	87±17	2.0±0.3	44±6	2
i.p.	233	14±1	3.1±0.2	118±18	1.6±0.2	32±2	2

[a] Data represent mean values ±SE

Table 3 Pharmacokinetic parameters derived from the plasma level of P-1013 given at 85 mg/kg by different routes of administration to Wistar rats[a] (*n* Number of animals at each data point)

Route	$t_{1/2}$ (min)	AUC (mg ml^{-1} min)	Bioavailability (%)	Cl (ml/min)	Vd (ml)	*n*
i.v.	14±2	1.6±0.3		9±2	169±19	2
p.o.	18±2	1.3±0.2	80±19	9±1	237±30	4
i.p.	17±3	1.7±0.4	100±35	8±1	198±46	2

[a] Data represent mean values ±SE

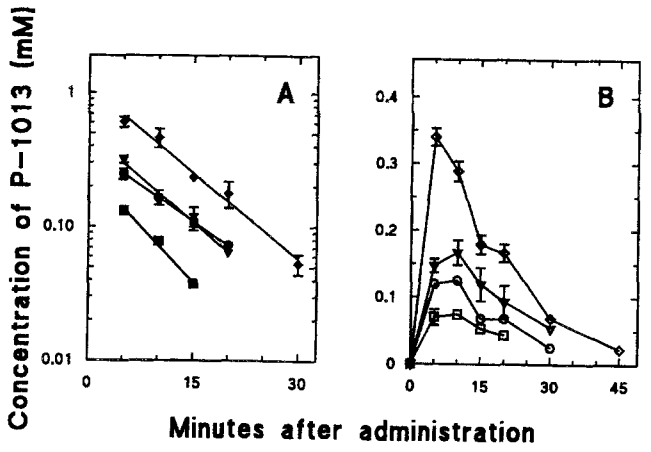

Fig. 4 A, B The concentration (m*M*) of P-1013 in plasma as a function of time as measured in Bom:NMRI-nu mice after treatment with P-1013 given **A** i.v. at 60 (■), 120 (●), 145 (▼) and 230 mg/kg and **B** p.o. at 60 (□), 120 (○), 170 (▽) and 230 mg/kg (◇). Each *data point* represents the average value for 3 animals. *Vertical bars* represent standard errors when these exceed the size of the symbols

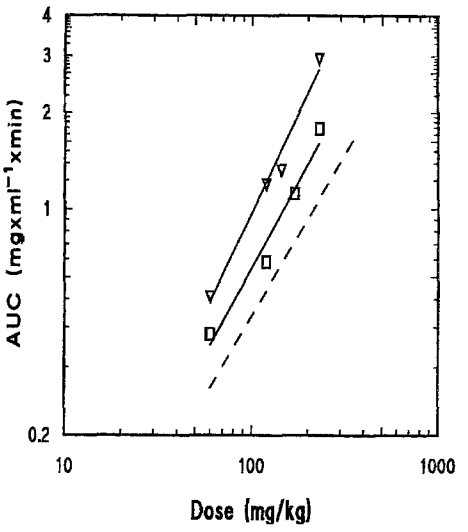

Fig. 5 Relationship of AUC to dose for P-1013 given i.v. (▽) and p.o. (□) to Bom:NMRI mice. The *dashed line* indicates the slope required for dose-independent pharmacokinetics. The slope of the regression line for oral administrationv is 1.07 ± 0.16 (r^2 = 0.96), and that for i.v. administration is 1.23 ± 0.16 (r^2 = 0.98)

Table 4 Pharmacokinetic parameters derived from the plasma level of BG given at 85 mg/kg by different routes of administration to Wistar rats[a] (*n* Number of animals at each data point)

Route	$t_{1/2}$ (min)	AUC (mg ml^{-1} min)	Bioavailability (%)	Cl (ml/min)	Vd (ml)	*n*
i.v.	14±2	1.6±0.3		9±2	186±26	4
p.o.	16±4	0.9±0.3	55±21	8±1	194±56	4
i.p.	15±4	1.8±0.3	113±35	9±1	200±61	2

[a] Data represent mean values ±SE

Table 5 Pharmacokinetic parameters derived from the plasma level of P-1013 given i.v. and p.o. to Bom: NMRI-nu mice[a] (*n* Number of animals at each data point)

Route	Dose (mg/kg)	$t_{1/2}$ (min)	AUC (mg ml^{-1} min)	Bioavailability (%)	Cl (ml/min)	Vd (ml)	*n*
i.v.	60	6±1	0.5±0.1		2.6±0.2	21± 3	3
p.o.	60	14±5	0.4±0.2	85±17	2.5±0.9	50±25	3
i.v.	120	8±1	1.2±0.2		2.4±0.2	30± 4	3
p.o.	120	9±1	0.7±0.1	52±14	2.5±0.3	32± 6	3
i.v.	145	7±1	1.3±0.2		2.8±0.2	27± 4	3
p.o.	170	12±3	1.1±0.2	68±18	2.6±0.2	45±11	3
i.v.	230	7±1	3.0±0.5		2.0±0.2	20± 3	3
p.o.	230	10±1	1.8±0.2	59±11	2.0±0.2	27± 2	3

[a] Data represent mean values ±SE

the regression line is 1.1 ± 0.2. For i.v. administration (▽) the corresponding regression curve has a slope of 1.2 ± 0.1 and a correlation coefficient (r^2) of 0.98.

Discussion

The main finding in the present study was that P-1013 is a highly available drug when given orally and i.p. and that it displays linear pharmacokinetics. The benzaldehyde derivative BG has attracted considerable attention due to its anti-cancer effects and its low toxicity. It is noteworthy that bone-marrow depression, gastrointestinal disturbances and other adverse effects almost inherent for the currently used anti-tumor agents have never been observed for BG, even when large doses were used for a considerable period [24]. As mentioned above, BG was deuterated to increase the biological effect. In a comparative pharmacokinetic study between BG and P-1013 we observed a deuterium isotope effect in rats but not in mice or dogs [3]. We therefore investigated the bioavailability of P-1013 and BG in both mice and rats. Due to the long treatment period needed with these drugs [16] it was of great importance to determine the

bioavailability of the drugs so as to achieve a clinically acceptable dosing form.

Absorption of the drugs appears to be rapid both from the gastrointestinal tract and the abdominal cavity in all animal models. The peak plasma concentration is usually reached within 10 min after injection. A parallel decline in the plasma concentration-time curves after the peak plasma concentration, indicating that the $t_{1/2}$ values were similar (Tables 1–4), implies that for both nu/nu-BALB/cABom mice and Wistar rats, drug disposition, not absorption, is rate-limiting. For Bom:NMRI-nu mice the $t_{1/2}$ value calculated from the p.o. results seems to be greater than that obtained for i.v. administration (11 ± 6 and 7 ± 2 min, respectively); however, this difference is not significant (Student's *t*-test, $P = 0.53$). It thus seems unlikely that there exists a compartment for P-1013 or that P-1013 undergoes enterohepatic cycling when given orally, since there would then be a supply of drug during the post-absorptive phase, and this would result in an increase in the $t_{1/2}$ value. A biodistribution study of benzaldehyde in rats indicated that the bile was not a major route of benzaldehyde excretion and that elimination appeared to occur mainly via the kidney [9].

Although the i.v. results obtained for the Bom:NMRI-nu mice may indicate a faster half-life for P-1013 in this model than in the Wistar rat and nu/nu-BALB/cABom mouse models (7 ± 2 and 12 ± 2 min, respectively), the difference is not significant (Student's *t*-test, $P = 0.16$).

The bioavailability of both BG and P-1013 is high when they are given orally and i.p. to mice and rats. Between 55% and 100% of the delivered dose is absorbed. There seems to be a small difference in bioavailability between the different animal models. The drugs seem to be less available to Wistar rats when given orally as compared with i.p. For nu/nu-BALB/cABom mice, over 90% of the delivered dose is absorbed following oral administration, but for Bom:NMRI-nu mice a smaller amount is absorbed (range, 52%–85%; Table 5). However, a considerable amount of the delivered drug is absorbed in all three animal models. This suggests that the drug enters the general systemic circulation with little pre-systemic elimination; thus, first-pass biotransformation or excretion to the bile must be slight.

Deuteration of BG does not seem to alter either absorption or bioavailability in any of the animal models, since the absorption of P-1013 is as rapid as that of BG and the bioavailability of P-1013 is about the same as that of BG. We have previously reported a comparison between pharmacokinetic data for BG and P-1013 [3], where we observed only one deuterium isotope effect. This was seen in rats in the metabolism of 1,3-benzylidene-D-glucitol, which is the first metabolite in the metabolism of BG, with a significant difference occurring in the $t_{1/2}$ value obtained for undeuterated versus deuterated glucitol. Other pharmacokinetic data were similar for BG and P-1013 in mice, rats and dogs.

Dose-dependent pharmacokinetics is reflected most commonly as an increase in the biological half-life of a drug with increasing dose and a greater than proportional increase in both the plasma concentration of the drug and the AUC with increasing dose [17].

A plot of the AUC against the delivered dose gives some interesting information (Fig. 5). For P-1013 given orally to Bom:NMRI-nu mice, the slope of the double logarithmic plot was 1.1 ± 0.2, meaning that a 2-fold increase in the dose would lead to a 2.2-fold increase in the AUC. When the drug was given i.v. the slope of the regression line was 1.2 ± 0.1. When the AUC was plotted against the delivered oral doses for nu/nu-BALB/cABom mice, the slope of the regression line was 1.1 ± 0.1 (results not shown, but data given in Table 1), indicating that there is no difference between inbred and outbred mice. Linear pharmacokinetics requires a slope of 1.0. Neither the slope for oral administration to Bom:NMRI-nu mice nor that for i.v. administration is significantly different from 1.0 (Student's *t*-test, $P = 0.81$ and $P = 0.56$, respectively). These results, together with the observation that the half-life of P-1013 does not change with different doses, indicate that P-1013 shows linear pharmacokinetics within the dose range tested (60–230 mg/kg). The apparent volume of distribution does not change with increasing dose, which also supports the notion of dose-independent pharmacokinetics. However, the data shown in Table 5 indicate a lower total body clearance at the highest dose given (230 mg/kg), which may suggest capacity-limited kinetics at such high doses. As the therapeutic doses of P-1013 will probably be far below this dose (approximately 40 mg/kg BG daily have been given in clinical trials [23]), capacity-limited kinetics probably will not be a problem in clinical use. For nu/nu-BALB/cABom mice a lower total body clearance at high doses is not observed (Table 1).

In conclusion the present results indicate that the pharmacokinetics of P-1013 does not prevent its use in cancer chemotherapy and show that this drug may be given orally to patients.

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