Anti-Cancer Drugs 1997, **8**, pp. 603–609

Pharmacokinetics of panomifene in healthy volunteers at phase I/a study

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Panomifene (PAN) /*E*/-1,2,-diphenyl-1-{4-[2-(2-hydroxyethyl-amino)-ethoxy]-phenyl}-3,3,3-trifluoropropene is a new original Hungarian compound and is a tamoxifen (TMX) analog. In the phase I/a study presented here the human tolerance, pharmacokinetics and endocrine effects of a single oral dose of panomifene were evaluated in healthy, post-menopausal, female volunteers. As to the dose escalation, pharmacokinetic studies were carried out at doses of 24, 48 and 96 mg in two volunteers, and 120 mg in one volunteer. To find a suitable dose or dose range, for further evaluation of the drug detailed pharmacokinetics were performed at a selected dose level (24 mg) in 10 volunteers. The pharmacokinetic study showed considerable interindividual variability of the parameters, and only a medium correlation between dose and AUC ($r = 0.876$). At the selected dose level (24 mg p.o.) the peak concentration of the plasma was 67.7 ± 17.4 ng/ml ($C_{max(meas)}$), the time to peak was 3.6 ± 1.8 h ($t_{max(meas)}$). The mean of the terminal half-life was 70.0 ± 23.1 h ($t_{1/2\beta}$) The area under the plasma concentration–time curve (AUC) calculated by the kinetic equation (AUC_{calc}) was 4814 ± 1172 and by the trapezoidal rule (AUC_{trap}) was 4612 ± 1357 (ng/ml) h.

Key words: Antiestrogen, panomifene, pharmacokinetics, phase I/a study, tamoxifen analog.

Introduction

Panomifene (PAN) /*E*/-1,2,-diphenyl-1-{4-[2-(2-hydroxyethyl-amino)-ethoxy]-phenyl}-3,3,3-trifluoropropene is a new original Hungarian compound and is a tamoxifen (TMX) analog (Figure 1). In preclinical *in vitro* and *in vivo* studies it was found to be a potent antiestrogen with strong inhibitory effect in estrogen-dependent tumors. The IC_{50} value of PAN on the binding of [^{3}H]estradiol to the estrogen receptors of rat uterus cytosol was found to be

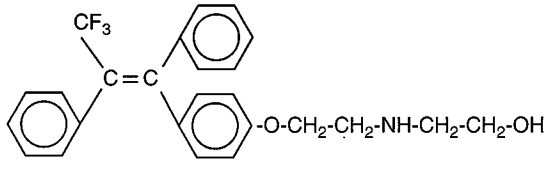

Figure 1. Chemical structure of PAN.

3×10^{-7} M, about 50% lower than that of TMX, while its relative binding affinity to the receptors was more than two times higher as compared to TMX.[1] In the *in vitro* studies the effect of PAN on the proliferation of the estrogen receptor and progesteron receptor positive MCF-7 breast cancer cells was investigated, and it was found that a 50% decrease of cell proliferation was achieved by a concentration 0.2 μM (2×10^{-7} M) of PAN.[2] The favorable properties led to the selection of PAN for clinical phase I trial.

In the placebo-controlled, partially double-blind phase I/a study the human tolerance, pharmacokinetics and endocrine effects of a single oral dose were evaluated in healthy post-menopausal female volunteers.[3] The present paper describes the clinical pharmacokinetics of the drug; the results of the endocrinological examinations were discussed in separate reports.[4,5]

Materials and methods

Design of the study

In the first part of the study dose escalation of the drug was performed including pharmacokinetics. Endocrinologic effects were measured after placebo and two selected doses (12 and 120 mg).[4,5]

Based on these results and in order to find a suitable dose or dose range for further evaluation, detailed pharmacokinetics were performed at a

This work was supported by a grant from EGIS Pharmaceuticals, Budapest, Hungary.

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selected dose level in 10 volunteers and further data were collected on tolerance.

The drug was received in capsules and was applied on an empty stomach. Standard breakfast was allowed 2 h post dose.

Participants

Altogether, 13 healthy volunteer women who were in post-menopause (at least 3 years after the last menstruation) entered the study. The mean age was 57.2 years. The body weight deviated not more than ±15% of the Broca index.

Ten volunteers participated in the first part of the study. Because of drop out of three volunteers in the second part, new persons were recruited to replace the missing ones.

The study protocol was approved by an independent ethical committee. The participants were fully informed about the nature of the study and its risk factors. Written informed consent was obtained from them. The study was performed under conditions that are in compliance with the Helsinki Declaration and the WHO recommendations for evaluation of drugs for use in man.

The reference numbers of the participants in the pharmacokinetic studies, the dose levels and treatment cycles where the pharmacokinetics were done are summarized in Table 1.

Treatment schedule 1: dose escalation

The starting total dose, selected on the basis of the preclinical toxicity data, was 3 mg. The following escalation steps were used: 0 (placebo), 3, 6, 12, 24, 48, 96 and 120 mg. The human tolerance study was performed according to a treatment matrix. The minimal period between the treatments was 4 weeks. All participants received at least one placebo cycle as control. Clinical (gynecologic, respiratory and opthalmologic), cardiovascular (blood pressure, cardiac rhythm and ECG parameters), hematological parameters and blood chemistry follow-up was done during every cycle and 4–6 weeks after the last cycle of the dose escalation since the next cycle for treatment schedule 2 was administered with a longer delay.

Representative pharmacokinetic studies were carried out at doses of 24, 48, 96 and 120 mg. Two volunteers entered the pharmacokinetic study at each dose level. Due to technical reasons, at 120 mg dose level the pharmacokinetics was performed only in one case.

Treatment schedule 2: investigations at the selected dose level

The second part consisted of human tolerance and pharmacokinetic studies at a selected dose level (24 mg).

Blood sampling

In the pharmacokinetic studies connected with dose escalation, blood sampling was planned at the following time points: before treatment (0), 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 24, 48, 72, 168 and 336 h after treatment. At the selected dose level (24 mg) blood samples were taken at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 24, 48, 72, 96 and 168 h.

Blood samples of 5 ml were collected in polypropylene tubes containing heparin.

Table 1. Treatment matrix

Cycle	Volunteer no.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1	3	0	3	0	3	0	0	0	6	6	24**	24**	24**
2	0	6	0	6	0	12	3	12	0	0			
3	12	12	12	12	12	24*	12	48*	96	12			
4	48	48*	24	24	48	48	24*	120	12	120*			
5	96*	0	48	96*	120	96	48	48	120	96			
6	120	(12)	120	120	24**	120	120		24**	120			
7	24**	120	120			24**	24**			24**			
8		24**											

The numbers in the table represent the applied dose of PAN given in mg.
Dose = endocrinological examination; dose* = pharmacokinetics for dose escalation; dose** = pharmacokinetics at the selected dose; (dose) = repeated dose for allergological examination.

Analytical method

As described previously,[6] an ion-pair chromatographic method was developed for the determination of PAN in the human plasma. TMX was used as internal standard (IS). Fluorescence detection was achieved by on-line photochemical conversion of the compounds under UV irradiation to highly fluorescent phenanthrene products using a post-column photoreactor included in the HPLC system.[6]

Pharmacokinetic evaluation

The pharmacokinetic data were analyzed by a non-linear estimation program using MedUSA software package (version 1.5/1.587) (Chemicro, Budapest, Hungary). The concentration versus time data were sequentially fitted to both one-compartment and two-compartment open models with a weighting of $1/(\bar{y} + y))^2$.[7] The best model was selected.

The equation describes the one-compartment p.o. model:

$$C(t) = \frac{Dk_a}{V(k_a - k_e)}[e^{-k_e(t-t_{lag})} - e^{-k_a(t-t_{lag})}] \quad (1)$$

The equation for the two-compartment p.o. model is

$$C(t) = A\,e^{-\alpha(t-t_{lag})} + B\,e^{-\beta(t-t_{lag})} + K\,e^{-k_a(t-t_{lag})} \quad (2)$$

where $K = -(A + B)$

The area under the plasma concentration–time curve (AUC) was calculated by integration of the equations describing the plasma concentration time course (AUC_{calc}) (method 1), by the trapezoidal rule according to the experimental data from zero to the last sampling time (AUC_t) (method 2) and by trapezoidal rule and the terminal elimination (AUC_{trap}) (method 3). The values for AUC obtained using method 1 and 3 were then compared by linear regression analysis.

The contribution of the absorption (k_a) and the α and β phases of PAN elimination to the total AUC was calculated using the following equation:

$$\%\text{ of AUC } [k_a, \alpha, \beta \text{ phase}] = \frac{\frac{x}{y}}{\frac{A}{\alpha} + \frac{B}{\beta} + \frac{K}{k_a}} \times 100$$

where

$$\frac{x}{y} = \frac{A}{\alpha}, \frac{B}{\beta} \text{ or } \frac{K}{k_a} \text{ respectively.}$$

The statistical analysis of the data was evaluated by a computer program: Pharmacologic Calculations (version 4.0).[8]

Results

Ten volunteers were participating in the dose escalation phase of the study. Altogether 69 cycles were given at seven different dose levels between 3 and 120 mg, as well as placebo. Pharmacokinetic monitoring was performed in seven cycles at four different dose levels between 24 and 120 mg of PAN.

The fitted and calculated pharmacokinetic parameters for individual volunteers are listed in Table 2. In four cases the two-compartment p.o. model and

Table 2. Pharmacokinetic parameters of the volunteers that participated in the dose escalation

Volunteer no. Dose	6 (24 mg)	7 (24 mg)	2 (48 mg)	8 (48 mg)	4 (96 mg)	1 (96 mg)	10 (120 mg)
$t_{1/2}$ (k_a) (h)	0.29	0.25	0.29	0.67	5.18	0.65	0.37
$t_{1/2}$ (α) (h)	1.54	1.51		1.28	6.96		
$t_{1/2}$ (β) (k_e) (h)	61.10	106.44	43.52	43.96	94.55	26.36	51.31
t_{lag} (h)	0.85	0.79	0.91	0.30	1.35	0.52	0.90
$C_{max(calc)}$ (ng/ml)	25.22	27.79	72.57	112.90	232.10	290.41	149.95
$C_{max(meas)}$ (ng/ml)	28.67	29.76	74.40	131.11	200.00	280.55	163.18
$t_{max(calc)}$ (h)	1.98	1.91	2.99	1.84	10.42	4.08	3.53
$t_{max(meas)}$ (h)	2.00	2.50	2.50	2.00	10.00	10.00	4.00
AUC_{calc} [(ng/ml) h]	1383	3084	4711	3010	9563	16403	11500
AUC_t [(ng/ml) h]	1453	2536	4101	3014	9573	17453	12770
AUC_{trap} [(ng/ml) h]	1472	2536	4101	3014	9573	17453	12770
Fit (r^2)[a]	0.958	0.957	0.970	0.944	0.977	0.945	0.920

[a]Observed versus fitted plasma PAN concentration.

in three cases the one-compartment p.o. model could be used to describe the pharmacokinetics of PAN.

The mean of plasma concentrations ± SD at each time-point determined in the 10 volunteers of the pharmacokinetic series at the selected dose level are shown in Figure 2. The data for six volunteers could be fitted to the one-compartment p.o. model and for four volunteers to the two-compartment model. The mean individual pharmacokinetic parameters and the ± SD values are presented in Table 3.

The AUC values were derived by integration and by the trapezoidal rule. The values given by methods 1 and 3 were compared by linear regression analysis and found to be in good agreement ($r^2 = 0.996$, slope = 1.109 ± 0.02, intercept = -676 ± 168, $n = 17$).

Fluctuations in plasma levels noted in volunteers may be attributed to enterohepatic recirculation of the drug.

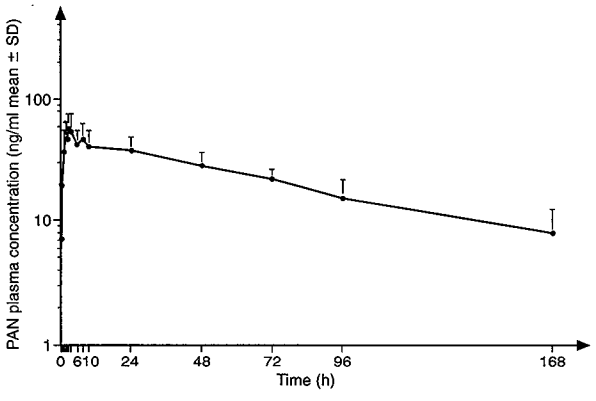

Figure 2. Mean plasma concentrations of PAN ± SD after oral administration of 24 mg ($n = 10$).

Table 3. Mean pharmacokinetic parameters at the selected dose level (dose: 24 mg p.o.)

	Mean ± SD	*n*
$t_{1/2}$ (k_a) (h)	0.76 ± 0.41	10
$t_{1/2}$ (α) (h)	18.1 ± 26.9	4
$t_{1/2}$ (β) (h)	69.6 ± 23.1	10
t_{lag} (h)	0.44 ± 0.28	10
$C_{max(calc)}$ (ng/ml)	55.2 ± 13.2	10
$C_{max(meas)}$ (ng/ml)	67.7 ± 17.4	10
$t_{max(calc)}$ (h)	4.90 ± 2.10	10
$t_{max(meas)}$ (h)	3.55 ± 1.77	10
AUC_{calc} [(ng/ml) h]	4814 ± 1172	10
AUC_t [(ng/ml) h]	3704 ± 788	10
AUC_{trap} [(ng/ml) h]	4612 ± 1357	10

The AUCs increased with increasing dose. The plot of AUC_{calc} versus dose showed, however, the considerable variation in pharmacokinetics of PAN at all dose levels over the dose range studied ($r = 0.876$) (Table 2 and Figure 3).

Statistical analysis of the pharmacokinetic parameters at the selected dose level showed an increase in the AUC with the successive treatment cycles of the study, according to the corresponding increase of the terminal half-lives (Figure 4). The mean AUC_{calc} ± SD in cycle 1–4 was 3370 ± 1645 (ng/ml) h, the corresponding mean terminal half-life was 65.5 ± 26.3 h, in cycle 6–8 the values were 5108 ± 938 (ng/ml) h and 77.2 ± 22.8 h, respectively.

The pharmacokinetic values of seven volunteers, where the plasma concentrations could be fitted to the two-compartment p.o. model, were also examined in detail (Table 4). In six cases the terminal elimination phase was the single most important contributor to the AUC_{calc} (91–99%), as it was in the pharmacokinetics fitted to the one-compartment p.o. model.

The probable causes and the possible consequences of the pharmacokinetic deviations were found as follows:

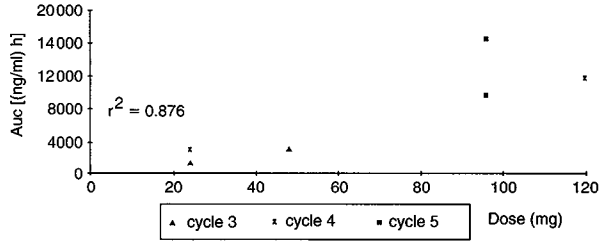

Figure 3. Dose dependence of AUC_{calc}.

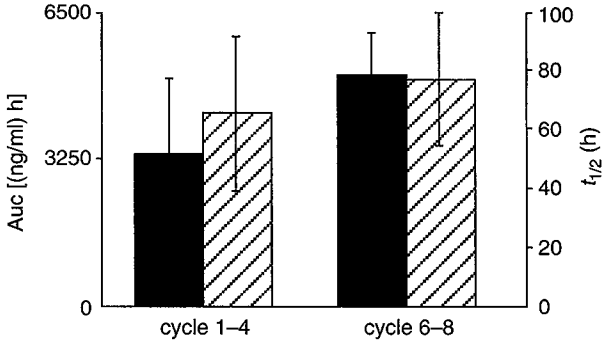

Figure 4. The mean AUC_{calc} (open bars) and terminal elimination (shaded bars) ± SD in cycle 1–4 ($n = 5$) and in cycle 6–8 at 24 mg of PAN ($n = 7$).

Table 4. Contribution of the absorption (k_a) and the α and β phases of PAN elimination to the total AUC

Volunteer no.	Dose (mg)	Cycle	$AUC_{calc.}$ [(nm/ml) h]	% of AUC		
				k_a phase	α phase	β phase
6	24	3	1383	−1	3	98
7	24	4	3084	−0.4	1.4	99
8	48	3	3010	−12	21	91
4	96	5	9563	−155	204	51
5	24	6	6470	−2	3	99
7	24	7	6939	−3	10	93
10	21	7	5202	−7	12	95
12	24	1	5698	−1	69	32

- In volunteer no. 7 the plasma samples were each time lipemic. In that volunteer the longest half-life of elimination was measured as well.
- In volunteer no. 8 peptic ulcer was diagnosed 2 months after cycle 5. She was therefore not eligible for the cycle given at the selected dose level. The volunteer was under gastroenterologic ambulance and her complaints ceased within a few days.
- Outstanding long apparent absorption half-life and lag time were evaluated in volunteer no. 4. As a possible explanation for this observation, the volunteer's bradycardia might come into consideration.
- Longer absorption and distribution phase occurred in volunteer no. 10. at the selected dose level. The volunteer was treated with 120, 96 and 120 mg of PAN in the previous cycles respectively. There was more than 1 month between the cycles.
- Mild lower abdominal pain was reported and slight spotting bleeding was present in volunteer no. 12.
- The chromatograms of the volunteer's plasma showed no extra peaks relating to the presence of any metabolite.

Discussion

PAN is a new trifluoromethyl-triphenylethylene type antiestrogen compound. In the present phase I/a study pharmacokinetics of the single oral dose were performed in healthy volunteers according to the dose escalation and at a selected dose level (24 mg).

As a consequence of the selected special age group (post-menopausal women), difficulties were encountered in finding suitable volunteers. From a clinical point of view, nine out of 10 volunteers tolerated the drug without complaints in the dose escalation study even at the highest dose level of 120 mg.

One of the volunteers had lower abdominal pain after 120 mg, which was even more severe when the cycle was repeated. It is not clear whether this dose might be considered the limit of tolerance of the drug, since this complaint might vanish within a few days during continuous administration (transitional agonistic effect). Lower abdominal pain occurred in two cases at the selected dose level (with one case of spotting bleedings) as well.

Hot flushes were reported in three volunteers but since their occurrence in time and their character were different, the relation to the drug administration was not clear. Other side effects occasionally encountered were: drowsiness, rashes, headache, sweating, heat sensation and dizziness. The side effects were not dose dependent.

No clear, important, repeatable changes were found in the clinical parameters. The changes were between the limits of the normal values. Long-term clinical, cardiovascular, gynecologic, respiratory and opthalmologic examinations did not reveal any important toxicity.

We compared our pharmacokinetic results with the data of TMX and TMX analogs (toremifene and droloxifene) obtained in similar studies.

The pharmacokinetics of TMX and TMX analogs have been studied by a number of research groups using a variety of methods to determine the concentrations of the antiestrogen compounds in the plasma. The comparison of the main pharmacokinetic data of TMX, droloxifene, toremifene and PAN is presented in Table 5.

The present pharmacokinetic study showed considerable interindividual variability of the parameters and only a medium correlation between dose and AUC ($r = 0.876$). Analysis of the plasma concentration versus time curves of PAN revealed that no single phase was responsible for this variability. For

Table 5. Comparison of pharmacokinetic data of TMX, droloxifen, toremifene and PAN in humans

Drug/reference	Dose (mg)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2\beta}$ (h)
TMX				
9	20	35–40	5	100
10	20	33	5	113
11	40	82	4.5	130
12	30	70	4	not published
Toremifene				
13	20	not published	1.5–2.5	175
Droloxifene				
14	20	24.8	1.4	36.7
14	40	63.7	2.6	29.2
PAN				
–	24	67.7	3.6	69.6

most of the volunteers, however, the terminal elimination phase was the major contributor to the total AUC. A wide variation in elimination half-lives was seen. The elimination half-lives and the corresponding AUCs increased with the successive doses.

Several authors reported that wide individual variation occurred in the subjects treated with TMX.[15–17] In the study by Adam *et al.*[9] after three widely separated single doses of TMX (at least 28 days) a reversible increase in elimination and in AUC was demonstrated. The authors suggested a reversible and limited inhibition of the hepatic metabolism of the drug.

We failed to detect biotransformation products for PAN in volunteers by HPLC. Hence, there is no evidence that metabolism significantly accounts for the interindividual variability in PAN clearance, or biliary excretion could play an important role, or both. Further studies are warranted.

The maximal plasma concentrations of PAN achieved in the healthy volunteers (25.2–290.4 ng/ml) were dependent on the dose applied. These concentrations were in the same range which caused 50% proliferation inhibition on MCF-7 cells (2×10^{-7} M = 85.4 ng/ml).[2]

PAN seems to be a safe TMX analog, the single oral dose did not exert any noteworthy toxic side effects in healthy women. The trial using the repeated drug administration schedule (phase I/b) will be performed in cancer patients.

Acknowledgments

We gratefully acknowledge the skilful technical assistance of Mrs Elisabeth Rozsnyay-Czapko. This study would not have been possible without the dedicated work of the assistants of the Clinical Pharmacology Department. We thank Mr Attila Nagy for his help in the preparation of the manuscript.

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(Received 18 February 1997; revised form accepted 15 May 1997)