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Pharmacokinetics of 2-(α-thenoylthio)-propionylglycine (TTPG) in healthy volunteers — an oral dose-proportionality investigation

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Abstract: The dose linearity of 2-(α-thenoylthio)-propionylglycine (TTPG) pharmacokinetics after a single oral administration at three different TTPG doses (180, 540 and 1080 mg) was evaluated in 12 healthy volunteers according to an open, randomized, cross-over study with a 1-week wash-out period between each administration. The duration of the study, for each subject, was 4 weeks.

Plasma concentration and urinary excretion of TTPG and its two systemic metabolites, namely propionylglycine (tiopronin) and thiophenecarboxylic acid (TCA) were assayed by a previously well validated HPLC method. Due to differences in the physical and chemical properties of these compounds, two assays were needed, one to measure TTPG and TCA as such, and one to measure derivatized tiopronin. Both used UV detection. TTPG, tiopronin and TCA were quickly detected in plasma, suggesting that the drug administered is rapidly absorbed and biotransformed, in part, in the systemic circulation into the two metabolites noted above. Time-to-peak for all three analytes showed a trend to increase with increasing doses of TTPG, being: 0.42, 0.40 and 0.67 h ($P < 0.01$) with TTPG; 0.53, 0.47 and 0.73 h ($P < 0.05$) with TCA; and 1.33, 2.13 and 2.58 h ($P < 0.01$) with tiopronin. C_{max} showed the opposite behaviour with values (ng ml^{-1}) normalized to the dose of 540 mg: 1235, 905 and 513 ($P < 0.001$) with TTPG; 888, 547 and 383 ($P < 0.001$) with TCA; and 7290, 6950 and 5170 ($P < 0.01$) with tiopronin. Normalized $AUC_{(0-t)}$ was poorly affected by the dose of TTPG; it showed no dose relationship with TTPG, a modest increase with the highest dose with TCA ($P < 0.5$) and a modest decrease with the highest dose of tiopronin ($P < 0.05$). Half-life was evaluated only for tiopronin and did not show any statistically significant change between the three doses administered. As shown by the AUC values, tiopronin is the most representative circulating compound; in addition, it possesses the longest $t_{½}$ value and a significant renal clearance, 63% of the dose administered being excreted in urine. The results demonstrate that with TTPG the rate of absorption is dose-related.

Keywords: *2-(α-Thenoylthio)-propionylglycine (TTPG); propionylglycine (tiopronin); thiophenecarboxylic acid (TCA); oral dose proportionality; pharmacokinetics; metabolism.*

Introduction

2-(α-Thenoylthio)-propionylglycine (TTPG, Streptonin, Broncoplus®) is a drug endowed with mucolytic activity both in laboratory animals and in man. Previous pharmacokinetic investigations of this drug have focused only on the parent drug (TTPG) and its active metabolite TCA [1, 2]. They are both easily analysed as such with HPLC and UV detection, while tiopronin, the main active metabolite, which requires chemical derivatization, was not detected.

Studies carried out in man, rat, dog and monkey ascertained that TTPG, at least in part, is absorbed as such, since it was detected in plasma for several hours after oral administration. The hydrolysis of thiocarboxylate ester producing TCA and tiopronin, thus seems to be a relatively slow process which occurs partly in the presystemic circulation and in liver and partly in the systemic blood and tissues, as a result of non-specific hydrolytic enzyme catalysis. The further stages of metabolism steps described in the literature consist of a glycine conjugate of TCA which is excreted in urine [3], and mercaptopropionic acid formed from the amide hydrolysis of tiopronin [4, 5].

As TTPG and tiopronin have different physical and chemical properties, the first being more lipophilic than the second, more extensive investigations were planned in order

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to define the absorption and excretion kinetics, with measurement in plasma and urine of TTPG, TCA and tiopronin.

More recently the authors have validated a new LC method for evaluating these three analytes in biological samples, and have investigated their kinetics after oral administration of TTPG at a single dose of 540 mg in 12 healthy subjects [6]. From this investigation, tiopronin proved to be the most relevant and long-lasting circulating compound [6].

The aim of this investigation was to check the absorption and excretion processes of TTPG, TCA and tiopronin after oral administration of three different doses of TTPG (180, 540 and 1080 mg) in 12 healthy volunteers. These doses were selected in order to cover the recommended daily therapeutic regimen, which ranges from 540 to 1080 mg, divided into two or three daily doses.

Experimental

Study design

The study was carried out with 12 healthy volunteers, from 20 to 29 years old (six males and six females). Table 1 shows the main data coded for these volunteers, who were carefully informed about TTPG, its activity and possible side-effects, and were enrolled only according to their free decision whether to take part in the trial and after giving their informed consent. Subjective and objective examinations, as well as blood chemical analyses, were performed on the volunteers before the treatment in order to ascertain their health status. The experimental protocol had been previously approved by an Ethical Committee.

It was an open, randomized study. TTPG sachets, each containing 180 mg of drug, were used. All subjects received a single oral administration of each of the three doses of TTPG (180, 540 and 1080 mg) according to a cross-over design after 10 h fasting (Table 1). The duration of the study for each subject was 4 weeks, consisting of a 1-week run-in period, followed by three single administrations of TTPG, with a 1-week wash-out period between each administration, and a 1-week follow-up period after the last administration.

Sampling

Heparinized blood samples were collected from all subjects at the following times: 0 (a baseline sample), 10, 20, 30, 45 min, 1, 1.25, 1.50, 2, 3, 4, 6, 9, 12 and 15 h after administration. Urine was collected cumulatively, for each subject, at the following intervals: 0 (a baseline sample), 0–6, 6–12 and 12–24 h after administration.

Assay methods for plasma and urine

TTPG, tiopronin and TCA were evaluated in plasma and urine using an HPLC method previously optimized and validated [6]. TTPG and TCA were analysed as such, whereas tiopronin was derivatized; in all cases UV detection was used for quantification.

Table 1
Main data of the volunteers enrolled

Volunteer no.	Age (y)	Body weight (kg)	Height (cm)	Randomization of treatment*
1	26	66.8	177	ABC
2	24	62.0	174	BCA
3	29	77.3	180	CAB
4	20	70.1	183	CBA
5	20	56.4	172	ACB
6	20	46.8	166	BAC
7	22	75.0	182	ABC
8	21	59.6	168	BCA
9	21	61.6	171	CAB
10	23	51.4	167	CBA
11	22	60.0	168	ACB
12	22	87.0	192	BAC
Mean	23	64.5	175	
SD	3	11.4	8	

*Treatment A: one 180 mg TTPG sachet; treatment B: three 180 mg TTPG sachets; treatment C: six 180 mg TTPG sachets.

Pharmacokinetic and statistical analysis method

C_{max} and t_{max} were obtained by direct inspection of the data. AUC was evaluated by the trapezoidal rule. Half-life ($t_{½}$) was evaluated by linearizing the log of concentration vs time of the β-phase using the linear regression method. Mean values and SD were obtained by standard procedures.

The C_{max}, AUC and $t_{½}$ were compared by the Latin square analysis of variance method in order to detect any statistically significant effect for doses, subjects and periods. The non-parametric Friedman test was selected for t_{max}, as this parameter is appropriate for timed blood sampling.

Results

Table 2 and Fig. 1 show the mean plasma concentrations of TTPG, TCA and tiopronin after three doses of TTPG administered to the volunteers. The plasma concentration of TTPG as such and of TCA, appeared on average at the first sampling time (0.17 h), reached a peak in the range 0.40–0.73 h and decreased to undetectable levels after 3 h, for doses of 180 and 540 mg, and after 6 h for a dose of 1080 mg. Plasma concentration of tiopronin was detectable at all sampling times, peaking in the range 1.33–2.58 h for the three doses investigated.

Table 3 shows mean values of the main non-compartmental pharmacokinetic parameters, namely t_{max}, C_{max} and $AUC_{(0-t)}$ evaluated from the raw data of plasma concentrations of TTPG, TCA and tiopronin, after the three doses of TTPG in the same volunteers. In the case of tiopronin $AUC_{(0-\infty)}$ and the elimination half-life were also determined. C_{max} and AUC are also reported as normalized values with reference to the intermediate dose of 540 mg, in order to allow a direct evaluation of whether the kinetics are linearly related to the scalar doses administered.

The plasma concentration–time behaviour of TTPG and TCA did not allow any evaluation of the β-phase and consequently the $AUC_{(0-\infty)}$ for these two analytes was not evaluated. The time-to-peak increased at the highest dose, reaching statistically significant

Table 2
Plasma concentration of TTPG, TCA and tiopronin measured in plasma samples collected after single oral administration of three doses (180, 540 and 1080 mg) of TTPG. Mean values of 12 findings in ng ml^{-1}; SD in brackets

	TTPG			TCA			Tiopronin		
Time (h)	180 mg	540 mg	1080 mg	180 mg	540 mg	1080 mg	180 mg	540 mg	1080 mg
0.17	223 (104.7)	445 (256.1)	390 (215.2)	137 (55.8)	265 (138.9)	297 (220.4)	377 (228.9)	945 (607.9)	1089 (782.0)
0.33	397 (115.4)	770 (335.7)	758 (326.2)	273 (89.5)	472 (200.4)	584 (292.4)	1459 (414.6)	2441 (973.8)	3017 (1350.7)
0.50	344 (87.2)	843 (258.5)	910 (273.5)	265 (89.5)	530 (185.3)	693 (264.1)	1754 (366.2)	3665 (1390.0)	4229 (1825.4)
0.75	286 (90.1)	602 (207.9)	936 (267.0)	237 (89.5)	439 (144.7)	705 (209.6)	1953 (409.8)	3999 (1411.6)	5690 (1938.2)
1	241 (85)	436 (127.4)	721 (241.1)	214 (86.8)	355 (128.1)	591 (153.7)	2060 (452.5)	3938 (1437.0)	5753 (1897.0)
1.25	230 (94.0)	506 (263.6)	551 (155.6)	205 (92.6)	382 (176.8)	477 (100.9)	2183 (556.9)	4107 (1526.9)	5612 (2318.9)
1.50	165 (70.0)	683 (498.4)	500 (286.7)	184 (76.0)	525 (321.5)	452 (174.6)	2292 (615.0)	5525 (2184.3)	5289 (1940.1)
2.00	64 (45.0)	430 (266.0)	822 (674.5)	96 (38.9)	439 (202.8)	777 (620.0)	1877 (451.5)	5915 (2110.7)	6854 (3891.1)
3.00	4 (15.4)	176 (233.6)	643 (518.9)	12 (29.0)	285 (316.4)	903 (433.4)	1132 (292.3)	4493 (1771.4)	9060 (4127.6)
4.00	*	*	90 (75.7)	*	*	317 (225.0)	696 (158.0)	2588 (804.3)	5393 (2433.2)
6.00	*	*	4 (14.9)	*	*	70 (98.9)	376 (84.9)	1104 (296.9)	2451 (957.7)
9.00	*	*	*	*	*	*	235 (50.9)	569 (109.1)	1222 (495.0)
12.00	*	*	*	*	*	*	160 (55.8)	418 (85.6)	853 (414.1)
15.00	*	*	*	*	*	*	125 (63.7)	350 (78.1)	584 (489.8)

*Sampling not planned.

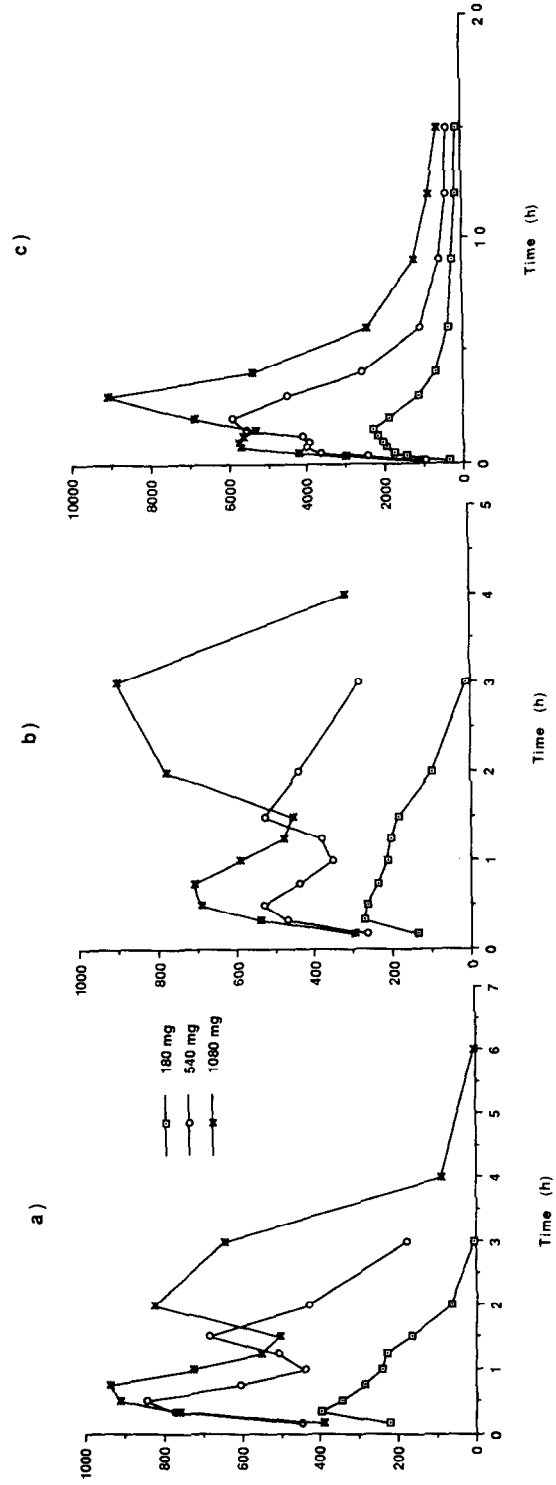

Figure 1
Plasma concentration–time behaviour of (a) TTPG, (b) TCA and (c) tiopronin in 12 healthy volunteers treated orally with 180, 540 and 1080 mg of TTPG. Mean values in ng ml^{-1} of plasma.

Table 3
Pharmacokinetic parameters of TTPG, TCA and tiopronin after a single administration of three TTPG doses in healthy volunteers. Mean values of 12 findings; SD in brackets

TTPG dose		180 mg (=*A*)	540 mg (=*B*)	1080 mg (=*C*)	*P**
TTPG	t_{max} (h)	0.42	0.40	0.67	*A* − *B* NS *A* − *C* < 0.01
		(0.13)	(0.11)	(0.18)	*B* − *C* < 0.01
	C_{max} (ng ml^{-1})	412	905	1027	
		(110.7)	(276.3)	(273.5)	—
	C_{max}* (ng ml^{-1})	1235	905	513	*A* − *B* < 0.001 *A* − *C* < 0.001
		(332.2)	(276.3)	(136.7)	*B* − *C* < 0.01
	$AUC_{(0-t)}$ (ng ml^{-1} h)	439	1390	2391	
		(117.7)	(498.0)	(833.2)	—
	$AUC_{(0-t)}$* (ng ml^{-1} h)	1316	1390	1196	
		(353.2)	(498.0)	(416.6)	NS
TCA	t_{max} (h)	0.53	0.47	0.73	*A* − *B* NS *A* − *C* < 0.05
		(0.21)	(0.15)	(0.17)	*B* − *C* < 0.01
	C_{max} (ng ml^{-1})	296	547	767	
		(92.0)	(185.6)	(236.1)	—
	C_{max}* (ng ml^{-1})	888	547	383	*A* − *B* < 0.001 *A* − *C* < 0.001
		(276.1)	(185.6)	(118.1)	*B* − *C* < 0.01
	$AUC_{(0-t)}$ (ng ml^{-1} h)	393	1196	2846	
		(135.0)	(347.6)	(965.4)	—
	$AUC_{(0-t)}$* (ng ml^{-1} h)	1181	1196	1423	*A* − *B* NS *A* − *C* < 0.05
		(405.0)	(347.6)	(492.7)	*B* − *C* < 0.05
Tiopronin		1.33	2.13	2.58	*A* − *B* < 0.01 *A* − *C* < 0.01
	t_{max} (h)	(0.57)	(0.51)	(0.44)	*B* − *C* < 0.05
	C_{max}	2430	6950	10340	
	(ng ml^{-1})	(520)	(1480)	(3830)	—
	C_{max}*	7290	6950	5170	*A* − *B* NS *A* − *C* < 0.001
	(ng ml^{-1})	(1550)	(1480)	(1920)	*B* − *C* < 0.01
	$AUC_{(0-t)}$	8950	25470	42670	
	(ng ml^{-1} h)	(1860)	(5200)	(15140)	—
	$AUC_{(0-t)}$*	26840	25470	21340	*A* − *B* NS *A* − *C* < 0.01
	(ng ml^{-1} h)	(5570)	(5200)	(7570)	*B* − *C* < 0.05
	$AUC_{(0-\infty)}$	10000	27540	47000	
	(ng ml^{-1} h)	(2000)	(5590)	(16200)	—
	$AUC_{(0-\infty)}$*	30000	27540	23500	*A* − *B* NS *A* − *C* < 0.01
	(ng ml^{-1} h)	(6000)	(5590)	(8100)	*B* − *C* < 0.05
	$t_{1/2}$ (h)	4.90	4.12	4.14	
		(1.33)	(0.58)	(1.08)	NS

* Value normalized to the 540 mg dose.

*P** was evaluated by the latin square ANOVA. When a statistically significant effect for the treatment was encountered, detailed *P* values for the doses were given.

NS = effect not statistically significant.

levels with all the analytes. With tiopronin t_{max} showed an increasing trend for the three doses of TTPG administered.

Normalized C_{max} showed the opposite behaviour, in that it decreased with increasing dose of TTPG. This was a progressive trend for TTPG and TCA, whereas tiopronin showed lower, statistically significant values only with the highest dose.

$AUC_{(0-t)}$ in the case of TTPG did not show any relevant or statistically significant dose-related trend, whereas with the highest dose of TTPG, TCA reached values higher, and tiopronin lower, than those detected with the doses of 180 and 540 mg, the difference being statistically significant. Half-life did not show any relevant or statistically significant difference. No difference was detected related to the period.

Table 4 shows the mean cumulative urinary excretion (CUE) of TTPG, TCA and tiopronin observed in the volunteers treated with the three oral doses of TTPG. CUE was very poor with TTPG and with TCA, which are cleared from the body by biotransformation [2], whereas for tiopronin it ranged from 57 to 75% of the dose administered, clearly showing no dose dependence trend.

No statistically significant effect was encountered with cumulative urinary excretion of the three analytes, related to both doses administered and periods. The tolerance of TTPG was found to be very good in all the volunteers.

Discussion

The data obtained during this investigation demonstrate that TTPG given orally is absorbed in the gut and is transformed into its two metabolites, namely TCA and tiopronin. As TTPG is detected as such in plasma, one should conclude that this metabolic transformation occurs, in part, in presystemic circulation, and in part, in systemic tissues and blood. Among the compounds assayed, tiopronin appears to be the most relevant compound, as it shows the highest value of C_{max} and of AUC, together with an elimination $t_{½}$ ranging on average from 4 to 5 h.

The problem of whether the kinetics of TTPG are linearly related to doses administered needs more detailed discussion. TTPG enteral absorption is clearly affected by the dose administered, mainly as regards the rate and only marginally as regards the extent. The most dose-related parameters are in fact t_{max} and C_{max}, the first being directly and the second inversely related to the dose. As tiopronin is kinetically the most relevant and the primary active compound, its behaviour should be regarded as the most predictive of linearity, mainly for the kinetic-dynamic relationships. The close constant value of $t_{½}$ and the cumulative urinary excretion of tiopronin leads to the conclusion that its elimination rate is not dose related. Carlsson *et al.* [7] have investigated the pharmacokinetic of tiopronin after IV bolus injection of a dose of 250 mg in healthy volunteers and have encountered cumulative urinary excretion of this drug to the extent of 75% of the dose, a value not very different from that found in this study (i.e. 63%) as a mean value over the three doses investigated. Even if the experiments were carried out in two different facilities and on different subjects, the comparison between the two values would lead to an absolute bio-

Table 4
Cumulative urinary excretion of TTPG, TCA and tiopronin after single oral administration of three doses (180, 540 and 1080 mg) of TTPG in healthy volunteers. Mean values of 12 determinations (in mg) and as a percentage of the dose administered; SD in brackets

	TTPG			TCA			Tiopronin		
Time (h)	180 mg	540 mg	1080 mg	180 mg	540 mg	1080 mg	180 mg	540 mg	1080 mg
0–6 h	1.53	4.65	3.60	0.01	0.00	0.00	61.78	237.30	340.22
(mg)	(0.78)	(3.97)	(3.94)	(0.02)	(0.00)	(0.00)	(25.51)	(66.19)	(113.47)
6–12 h	0.00	0.02	0.08	0.02	0.08	0.10	0.00	5.16	16.93
(mg)	(0.00)	(0.05)	(0.22)	(0.14)	(0.15)	(0.26)	(0.00)	(5.16)	(9.00)
12–24 h	0.00	0.00	0.28	0.06	0.02	0.08	0.00	0.00	11.42
(mg)	(0.00)	(0.00)	(0.96)	(0.14)	(0.07)	(0.13)	(0.00)	(0.00)	(39.56)
Total	1.53	4.67	3.96	0.09	0.10	0.18	61.78	242.45	368.57
(mg)	(0.78)	(3.96)	(3.86)	(0.18)	(0.18)	(0.33)	(25.51)	(68.14)	(109.91)
Total % (mg)	0.85	0.86	0.37	0.11	0.04	0.04	57.48	75.20	57.16

availability of tiopronin after oral administration of TTPG of about 80%.

The results obtained in this investigation lead the authors to conclude that with TTPG an inverse dose-relationship clearly appears to apply, with respect to rate and, less evidently, to the extent, of enteral absorption, whereas no dose-relationship is detected in the excretion process of its active metabolite tiopronin, which is cleared from the body mainly via urine.

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