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Analysis of 2β-carbomethoxy-3β-(4-fluorophenyl)-*N*-(3-iodo-*E*-allyl)nortropane in rat plasma
II. Pharmacokinetic profile in male and female Sprague–Dawley rats evaluated by capillary electrophoresis

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Abstract

This paper describes a pharmacokinetic study performed in Sprague–Dawley rats after i.v. administration of a single 6-mg/kg dose of 2β-carbomethoxy-3β-(4-fluorophenyl)-*N*-(3-iodo-*E*-allyl)nortropane (Altropane). Plasma samples were collected from the retro-orbital sinus at times up to 3 h after drug administration, extracted by solid-phase extraction, and the drug levels determined by capillary electrophoresis (CE). Pharmacokinetic parameters were determined by a standard noncompartmental model using WinNonlin version 1.5. The maximum plasma concentrations, clearances of the drug, and areas under the curve for male and female rats were 5.74 and 7.26 μg/ml, 135.7 and 98.5 ml/kg·min, and 44.23 and 60.92 μg·min/ml, respectively. The drug was cleared very rapidly from the systemic circulation, with a terminal $t_{1/2}$ of 7 to 10 min and a mean residence time of about 11 min for both sexes. The volume of distribution was approximately 1 l/kg. No metabolites were detected when the samples were analyzed individually. However, after samples were pooled and concentrated, traces of two unknown peaks that may represent metabolites were detected in concentrates from the last two timepoints. Part I of this work [J. Chromatogr. A, 895 (2000) 87] describes validation of CE methods for the analysis of aqueous and plasma samples of Altropane, including its solid-phase extraction from rat plasma. © 2001 Published by Elsevier Science B.V.

Keywords: Kinetic studies; Altropane; Carbomethoxy (4-fluorophenyl) iodoallylnortropane; Allylaltropane; Nicotinamide

1. Introduction

This research was conducted to evaluate the pharmacokinetic profile of 2β-carbomethoxy-3β-(4-fluorophenyl)-*N*-(3-iodo-*E*-allyl)nortropane (registered name Altropane, Boston Life Sciences, Boston, MA, USA) in rat plasma. The previously published Part I of this work [1] describes validation of a bioanalytical method to analyze Altropane by capillary electrophoresis (CE), including a solid-phase extraction (SPE) method. This paper, Part II, presents results of a pharmacokinetic study of Altropane in Sprague–Dawley rats. In an attempt to produce circulating metabolites, a high dose of nonradioactive Altropane was used in this study.

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Altropane is an ^{123}I-based nuclear medicine imaging agent that is being developed to diagnose two neurological disorders: Parkinson's disease (PD) [2–9] and attention deficit hyperactivity disorder (ADHD) [10]. PD is a chronic, irreversible, neurodegenerative disease for which there is no known cure [9], and it generally affects people over 50 years old. Inadequate production of dopamine in the brain causes PD. Its symptoms include resting tremor, muscle retardation, and rigidity. The dopamine transporters (DATs), which are found on the membranes of the dopamine-producing cells, regulate the amount of dopamine present in the nerve junctions or synapses. Altropane binds with high affinity and specificity to the DATs and thus allows visualization of the dopamine-producing nerve cells in the striatum. In PD, there is a marked decrease in the number of DATs in the striatal region of the brain.

Altropane was originally developed for early diagnosis of PD. The literature on Altropane reports preclinical research in primates [4–6], postmortem studies of normal and PD brain [5], and studies in healthy volunteers and patients with PD [8]. In these studies, Altropane has exhibited great promise as an imaging agent with single-photon emission computed tomography (SPECT) or positron emission tomography (PET), the two commonly used imaging methods. Effective detection of dopamine-containing neurons has been achieved using ^{123}I-labeled Altropane. This chemical was found to be a sensitive and selective marker of disease severity, and it may effectively visualize the degree of nerve functioning at various stages of the disease.

Altropane's potential to also diagnose ADHD was reported in a recent clinical study [10]. In this study, brain images of ADHD patients obtained using Altropane with SPECT have shown significantly elevated levels of DATs (as much as 70% higher than in healthy controls), which are associated with clinical symptoms of the disease. This is a promising breakthrough for diagnostic and drug development efforts for ADHD. ADHD affects between 3% and 5% of children in the USA, and in many cases, it continues into adolescence and adulthood.

Initial developmental studies on mammalian brain imaging with PET were performed using various cocaine analogs [11], followed by SPECT studies using Altropane [3]. The chemical structure of Altropane and, for comparison, that of cocaine are shown in Fig. 1. Altropane has been also referred to as (*E*)-*N*-iodoallyl-2β-carbomethoxy-3β-(4-fluorophenyl)tropane, *E*-2β-carbomethoxy-3β-(4-fluorophenyl)-*N*-(1-iodoprop-1-en-3-yl)nortropane, and E-IACFT in the literature.

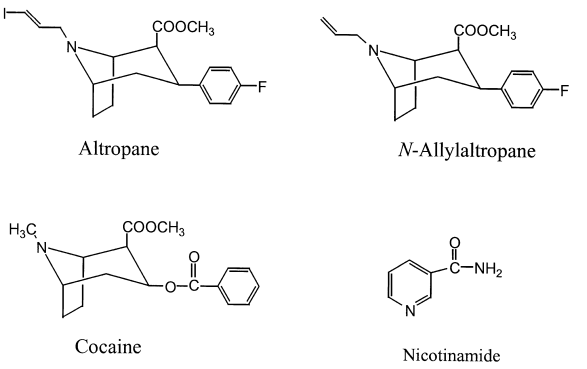

Fig. 1. Chemical structures of Altropane, *N*-allylaltropane, cocaine and nicotinamide.

Until the development of CE in the early 1990s, HPLC, GC, and gel electrophoresis were the mainstays among the separation-based analytical methods. While no CE methods for the analysis of Altropane previously existed in the literature, there are classic examples of CE applications for cocaine analysis, such as those by Weinberger and Lurie [12] and Wernly and Thormann [13]. Many other drug substances besides cocaine were included in the former study [12], and they were in the form of bulk drugs. The latter study [13] dealt with the analysis of common drugs of abuse and their metabolites in human urine. Both of these investigations achieved efficient separations for cocaine and a few of its derivatives, including benzoyl ecgonine (a metabolite of cocaine), by using micellar electrokinetic capillary chromatography (MECC). Capillary zone electrophoresis systems have also been successful for the analysis of cocaine [14] and analysis of the main tropane alkaloids from transformed *Hyoscyamus muticus* plants [15]. These researchers reported that CE gave better peak symmetry and selectivity, shorter run times [12], and more efficient separations [14] than HPLC.

These potential advantages prompted us to develop CE techniques to analyze Altropane in an aqueous medium and in rat plasma. Our earlier paper

[1] presented the CE method development and validation.

The objective of the study described here was to obtain data on the pharmacokinetic profile of Altropane in male and female Sprague–Dawley rats after a single i.v. administration.

2. Experimental

Prior to drug administration, a dosing formulation of Altropane was prepared and its concentration determined using the CE methods validated as described earlier [1]. The same methods were used to verify its homogeneity. A high dose was selected to increase the potential for detecting metabolites, if any. The pharmacokinetic study was performed using the same dosing formulation after verification. Plasma samples were extracted by SPE and analyzed by CE to measure Altropane and its possible metabolites, with nicotinamide used as the internal standard (IS) and sodium acetate as the electrophoretic run buffer. *N*-Allylaltropane was used to check the specificity of the CE system. The structures of nicotinamide and *N*-allylaltropane are also shown in Fig. 1. The validated methods described in our previous report [1] were used to analyze the plasma samples generated in the pharmacokinetic study.

2.1. Instrumentation

Two CE systems, a BioFocus 3000 and a BioFocus 2000 CE system (Bio-Rad, Hercules, CA, USA), were used in this study. The BioFocus 3000 system has a programmable fast-scanning UV–Vis detector that enables scanning of the UV spectra, and BioFocus 3000 software for system control and data collection and analysis, while the BioFocus 2000 system has only a single-wavelength UV–Vis detector. The CE capillaries were purchased from Bio-Rad.

The C_{18} Sep-Pak cartridges for SPE were purchased from Waters (Milford, MA, USA).

2.2. Reagents

Sodium acetate (99% pure) and nicotinamide (99+% pure) were purchased from Aldrich (Milwaukee, WI, USA). Methanol and ethyl acetate were HPLC grade, purchased from Mallinckrodt (St. Louis, MO, USA).

2.3. Samples

Altropane and *N*-allylaltropane samples investigated in this study were synthesized and supplied by Organix (Woburn, MA, USA).

All sample and buffer solutions were prepared in distilled, deionized (DD) water. Super-Q water was used for SPE.

2.4. Conditions

The CE experiments were performed using 30 cm×50 μm LPA (linear polyacrylamide coated) capillaries. The effective length (inlet to the detector) of the capillaries was 25.4 cm. The other conditions were 50 m*M* sodium acetate buffer, pH 4.1; ion migration from positive to negative polarity; run voltages of 20 or 18 kV; pressure injection of 20 p.s.i.s (1 p.s.i.=6894.76 Pa); detection wavelength of 210 nm; and capillary temperature of 20°C.

2.5. Animals: sex, strain and species

Male (age 7 weeks, mean body mass 221 g) and female (age 9 weeks, mean body mass 201 g) Sprague–Dawley rats were used in this study. They were purchased from Simonsen Labs (Gilroy, CA, USA). A total of 24 animals were assigned to the study.

2.6. Animal care

General procedures for animal care and housing were in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals* (1996) and the US Department of Agriculture through the Animal Welfare Act (7 USC 2131), 1985, and Animal Welfare Standards incorporated in 9 CFR Part 3, 1991. The quarantine period was 4 days. The animals were housed 1–5 per cage in 55.88×32.25×20.32 cm polycarbonate cages. The bedding was Sani-Chips hardwood bedding (P.J. Murphy Forest Products, Montville, NJ, USA). The light cycle was12 h light/12 h dark. The temperature

and humidity were maintained at 24–26°C and 40–49%, respectively. The animals had free access to Purina Certified Rodent Chow, No. 5002 (Richmond, IN, USA) and purified (filtered, deionized, and UV-treated) tap water.

The animals were assigned to groups (3/sex/group; 4 groups) 1 day before the study, randomized by weight sort, and individually identified by a unique ear punch number. All animals were sacrificed after the terminal blood collection.

2.7. Dosing solution preparation

The dosing solution intended for use in the animal experiments, 0.6 mg/ml Altropane at pH 5.0±0.1, was prepared 20 h prior to drug administration and stored in a refrigerator at 4°C until use.

A 48.48-mg aliquot of Altropane was dissolved in 2 ml of 0.1 *M* HCl, 60 ml of water previously acidified to pH 5.00 with 0.1 *M* HCl was added, and the mixture was well stirred. The pH of the resulting solution was 2.5. A few drops of 0.1 *M* NaOH was added until the pH reached 4.2, and then an aliquot of 18 ml water, pH 5.00, was added to bring the total volume of the solution to 80 ml. The pH of this solution was raised to 4.94 by carefully adding 0.05 *M* NaOH one drop at a time while thoroughly stirring. All precautions were taken to ensure that the pH of the mixture did not reach basic range, because prior experience has shown that basic conditions cause an irreversible change in the chemical constitution of Altropane.

Thus, the concentration of the dosing solution was 0.61 mg/ml (48.48 mg/80 ml), which was verified as described below (Section 3.1), and the pH was 4.94.

A portion of about 10 ml was separated for verification by CE analysis, and the remainder was used in the animal experiments to evaluate the pharmacokinetic profile and any potential metabolites.

2.8. Dose administration

Altropane was administered to male and female rats in a single i.v. dose of 6 mg/kg in a dosing volume of 10 ml/kg. This high dose was selected to increase the potential for detecting any metabolites of Altropane.

2.9. Sampling schedule and plasma collection for drug level determinations

Table 1 shows the blood collection schedule for each group of rats. Blood was collected from 3 male and 3 female rats predose and at 2, 5, 10, 20, 30 and 45 min and 1, 1.5, 2, 2.5 and 3 h after dose administration. Blood samples (approximately 1 ml) were collected from the retro-orbital sinus under CO_2/O_2 anesthesia. The samples were collected using EDTA as the anticoagulant, placed on wet ice until processed to plasma, and centrifuged at room temperature at 3000 rpm for 10 min to obtain plasma. Immediately after processing, all plasma samples were stored frozen at approximately −20°C until SPE and CE analysis. The red blood cells were discarded.

2.10. Pharmacokinetic analysis

The plasma data were analyzed by noncompartmental methods using a pharmacokinetic modeling program, WinNonlin, version 1.5 (Scientific Consulting, NC, USA). The following parameters were generated by the program: (i) terminal half-life, $(t_{1/2})_{\lambda z}$, calculated from the slope of the terminal phase, λ_z; (ii) area under the curve (AUC), where AUC was calculated to infinity according to the linear trapezoidal rule; (iii) area under the moment curve (AUMC), where AUMC was calculated to infinity according to the linear trapezoidal rule; (iv) maximum plasma concentration (Cp°) immediately after i.v. administration.; (v) total body clearance, where clearance=dose/AUC; (vi) volume of distribution calculated based on the terminal phase (V_z); (vii) volume of distribution at steady state (V_{ss}); and

Table 1
Allocation of animals and blood collection schedule

No. of rats		Timepoints	
3M/3F[a]	Predose	20 min	1.5 h
3M/3F[a]	2 min	30 min	2.0 h
3M/3F[a]	5 min	45 min	2.5 h
3M/3F[a]	10 min	60 min	3.0 h

[a] M=Male, F=female.

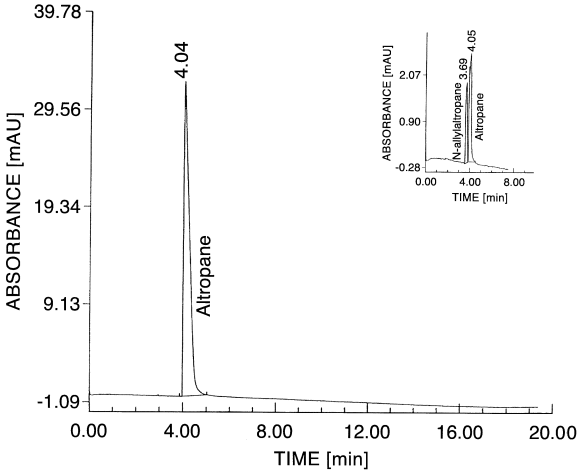

Fig. 2. An electropherogram of the dosing solution diluted 1:1 (Altropane 0.3 mg/ml in pH 4.9 hydrochloric acid, for conditions, see Section 2.4). Inset: Dosing solution spiked with *N*-allylaltropane.

(viii) mean residence time of drug (MRT), calculated using AUMC/AUC.

2.11. Metabolism profile

The metabolite profile of Altropane in plasma was examined by using plasma samples collected from individual rats at various timepoints and also by analyzing concentrated samples after pooling plasma samples collected at a few timepoints in the above experiments.

3. Results and discussion

3.1. Dosing solution verification

3.1.1. Qualitative observations

When electrophoresed alone, the dosing solution exhibited a single peak (Fig. 2). When it was spiked with *N*-allylaltropane, two closely migrating peaks were seen (Fig. 2 inset). This electrophoretic behavior was similar to what was observed in the specificity tests during methods development [1]. Furthermore, for the dosing solution, the UV spectrum scanned at the peak apex using the photodiode array detector of the CE instrument was identical to that recorded for a neat solution of Altropane (Figs. 3A and B). Also, the UV profile of Altropane is distinctly different from that of *N*-allylaltropane (Fig. 3C). These observations revealed that the dosing solution retained the qualitative identity and integrity of Altropane. The respective electropherograms from

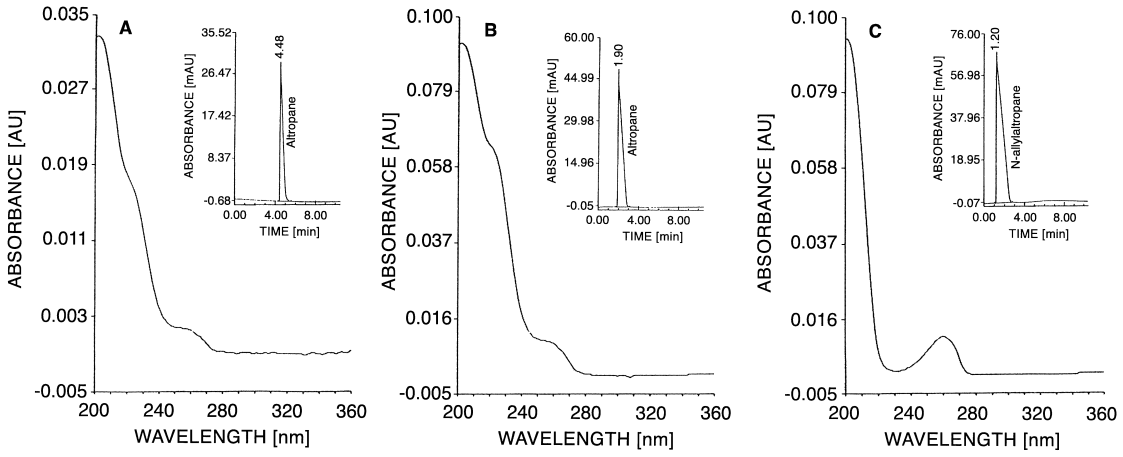

Fig. 3. The UV spectra scanned from the photodiode array detector. (A) Dosing solution 0.3 mg/ml (for conditions, see Section 2.4.). (B) Altropane neat solution 0.1 mg/ml (1 mg Altropane dissolved in 0.5 ml 0.5 *M* HCl and diluted to 10 ml with water). Conditions: 24 cm×50 mm capillary, pH 4 sodium acetate–acetonitrile buffer, 20 p.s.i. s load and 18 kV run voltage. (C) *N*-allylaltropane 0.1 mg/ml (1 mg *N*-allylaltropane dissolved in 0.5 ml 0.5 *M* HCl and diluted to 10 ml with water). Conditions: 24 cm×50 mm capillary, pH 4 sodium acetate–acetonitrile buffer, 20 p.s.i. s load and 18 kV run voltage.

which the UV spectra were scanned are shown as insets in Figs. 3A–3C.

3.1.2. Quantitative analysis

The dosing solution was assayed using five reference standard solutions of Altropane prepared as follows. A 1.076 mg sample of Altropane was accurately transferred into a 10 ml volumetric flask and dissolved in 0.05 *M* HCl. The flask was then filled up to the mark with more HCl to yield a 107.600 μg/ml solution of Altropane. By diluting aliquots from this reference solution, four more reference standard solutions (86.080, 60.256, 43.040, and 21.520 μg/ml) were prepared.

From each of the five reference solutions, a 0.5 ml aliquot was mixed with 0.5 ml of internal standard (I.S.) solution (2 mg nicotinamide/100 ml).

From the dosing solution, four aliquots of 0.5 ml, drawn from the top, middle, bottom and top of the solution, were pipetted out into 5 ml volumetric flasks and each flask was filled up to the mark with 0.05 *M* HCl. Then 0.5 ml of each resulting solution was mixed with 0.5 ml of I.S. solution.

3.1.3. Linearity and accuracy

The five reference solutions and four dosing solutions, after mixing with the I.S., were electrophoresed. To construct the standard curve, peak area ratios for the reference solutions were plotted against the concentration of Altropane in each solution. The linear equation for the curve, $y = 0.0243x + 0.037$, yielded an r^2 value of 0.9977. These results (Table 2) yielded an accuracy of 99.1% relative to the nominal concentrations prepared.

3.1.4. Concentration and homogeneity of the dosing solution

By substituting the peak area ratios for the four samples from the dosing solution in the linear equation for the curve, $y = 0.0243x + 0.037$, concentration of the solution was determined and its homogeneity verified. These results (Table 3) yielded a value of 594.23 μg/ml (0.59 mg/ml) for the dosing solution concentration. The standard deviation was 10.32 and the coefficient of relative variation (the standard deviation expressed as a percentage of the mean) for the four readings was 1.7%, and therefore, the dosing solution was considered homogeneous.

3.1.5. Precision

The method precision was determined by using a high concentration (107.600 μg/ml) and a low concentration (43.040 μg/ml) solution prepared to construct the above standard curve and electrophoresing each of the two solutions six times. The RSD values observed for these two solutions were 1.5 and 2.0%, respectively (Tables 4 and 5).

3.2. Pharmacokinetic analysis

3.2.1. Plasma drug levels

The levels of Altropane in plasma after SPE extraction and CE analysis are presented in Table 6. The drug concentrations were calculated using a

Table 2
Verification of dosing solution: standard curve

Reference solution no.	Reference solution (μg/ml)	Test solution (μg/ml)	Altropane peak area	I.S. peak area	Peak area ratio	Back-calculated concentration (μg/ml)	% Nominal
1	107.600	53.800	1 200 075	909 225	1.320	52.794	98.13
2	86.080	43.040	1 018 869	928 668	1.097	43.627	101.356
3	60.256	30.128	733 318	927 923	0.790	30.999	102.89
4	43.040	21.520	521 100	927 412	0.562	21.600	100.37
5	21.520	10.760	268 103	958 152	0.280	9.992	92.87
Average							99.12%

For conditions, see Section 2.4.
Equation: $y = 0.243x + 0.037$, $r^2 = 0.9977$.

Table 3
Verification of dosing solution: homogeneity and concentration

Solution[a] No.	Altropane peak area	I.S. peak area	Peak area ratio	Conc. of test solution (μg/ml)	Conc. of diluted dosing solution[a] (μg/ml)	Conc. of dosing solution (μg/ml)
1 (from top)	689 375	887 142	0.7771	30.456	60.911	609.11
2 (from middle)	722 287	953 195	0.7578	29.61	59.321	593.21
3 (from bottom)	699 413	933 040	0.7496	29.325	58.651	586.51
4 (from top)	698 532	929 462	0.7515	29.405	58.810	588.10
Mean						594.23
SD[b]						10.32
CRV[c]						1.7%

[a] Dosing solution diluted 1:10.
[b] SD = Standard deviation.
[c] CRV = Coefficient of relative variation (see Section 3.1.4).

Table 4
Verification of method precision at a high concentration (107.600 μg/ml Altropane solution)

Electrophoretic run No.	Altropane peak area	I.S. peak area	Peak area ratio
1	919 125	699 954	1.313
2	959 017	737 735	1.288
3	963 544	725 111	1.329
4	946 262	740 482	1.278
5	959 712	730 275	1.314
6	980 478	754 153	1.300
Average			1.304
SD			0.019
RSD (%)			1.5

For conditions, see Section 2.4.

Table 5
Verification of method precision at a low concentration (43.040 μg/ml Altropane solution)

Electrophoretic run No.	Altropane peak area	I.S. peak area	Peak area ratio
1	308 039	644 625	0.590
2	391 221	670 608	0.583
3	398 590	680 693	0.586
4	386 518	675 787	0.572
5	385 470	655 417	0.588
6	380 182	624 989	0.608
Average			0.588
SD			0.012
RSD (%)			2.0

For conditions, see Section 2.4.

Table 6
Altropane levels in rat plasma

Timepoint	Male		Female	
	Mean altropane conc. (μg/ml)[a,b]	SD[d]	Mean altropane conc. (μg/ml)[a,b]	SD[d]
Pre-dose	0.00	0.00	0.00	0.00
2 min	3.90	1.71	5.16	1.39
5 min	2.18	1.04	3.09	0.78
10 min	1.30	0.13	1.05 ($N=1$)	–
20 min	0.72	0.17	0.98	–
30 min	0.19	0.33	0.63	0.12
45 min	0.00	–	0.00	–
1.0 h	0.00	–	0.00	–
1.5 h	0.00	–	0.00	–
2.0 h	0.00	–	0.00	–
2.5 h	0.00	–	0.00	–
3.0 h	0.00	–	0 ($N=1$)	–

[a] $N=3$ rats/sex/timepoint unless otherwise noted.
[b] Reported as zero when no Altropane peak was observed (<LOD[c]).
[c] LOD = Limit of detection, 0.17 μg/ml.
[d] SD = Standard deviation.

standard curve (equation: $y=0.0501x$, $r^2=0.9972$) constructed using plasma spikes. Validation of this method was reported earlier [1]. Plasma samples collected prior to drug administration (predose) revealed no detectable Altropane levels. The Altropane levels in plasma after drug administration declined very rapidly, and all of the samples were below the limit of detection (LOD) by 45 min. Representative CE profiles of Altropane in male and female rat plasma at 2, 5, 10, 20, 30 and 45 min

timepoints after SPE and addition of the I.S. are presented in Figs. 4 and 5, respectively.

Noncompartmental analysis for Altropane was performed, for the male and the female rats separately. All calculations were performed using the mean plasma drug concentrations for each sex expressed in micrograms per milliliter and the sampling time in minutes. The pharmacokinetic parameters and constants calculated for both sexes are summarized in Table 7.

The parent compound was cleared very rapidly from the systemic circulation, with a mean residence time of 9.44 and 13.06 min for males and females, respectively. The maximum plasma concentrations of Altropane achieved immediately after 6 mg/kg i.v. administrations (Cp°) were 5.74 and 7.26 μg/ml for the male and female rats, respectively. The AUC values were also comparable: 44.23 and 60.92 μg·min/ml. The $t_{1/2}$ was also very short, 6.99 and 10.18 min for male and female rats, respectively. The short MRT and half-life values were consistent with the rate of clearance, with values of 135.7 and 98.5 ml/kg·min for the males and females, respectively. Although the pharmacokinetic parameters were similar for the two sexes, the plasma drug levels were consistently higher for the female rats. This was reflected in the pharmacokinetic parameters by a marginally higher AUC, larger $t_{1/2}$, and a slower rate

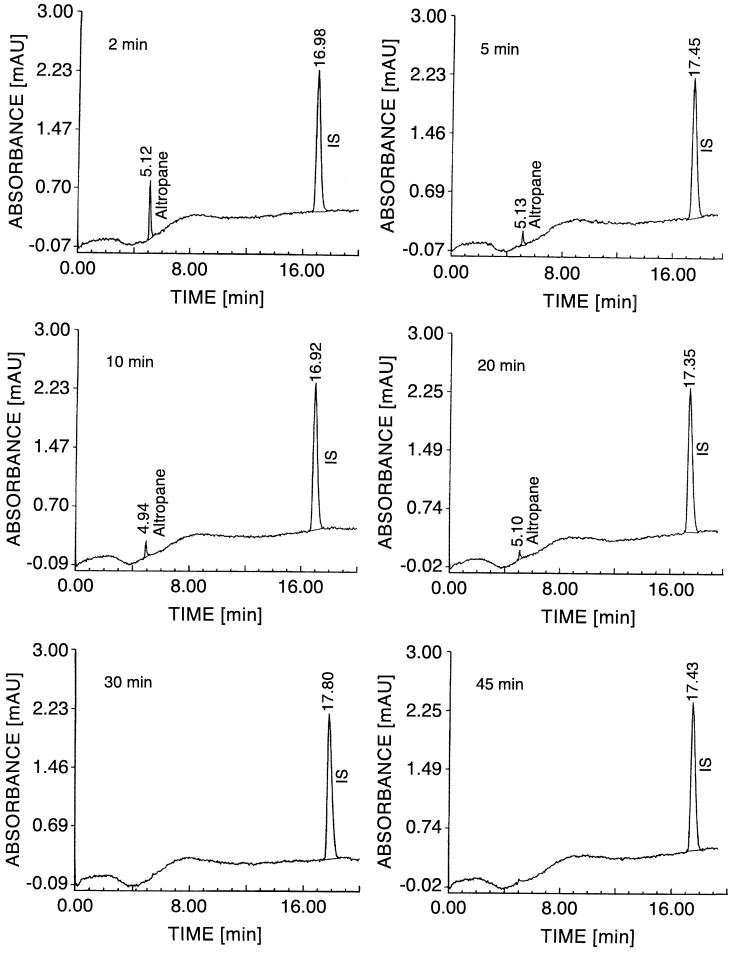

Fig. 4. CE profiles of Altropane in male rat plasma at 2, 5, 10, 20, 30 and 45 min timepoints after solid-phase extraction and addition of nicotinamide as an I.S.

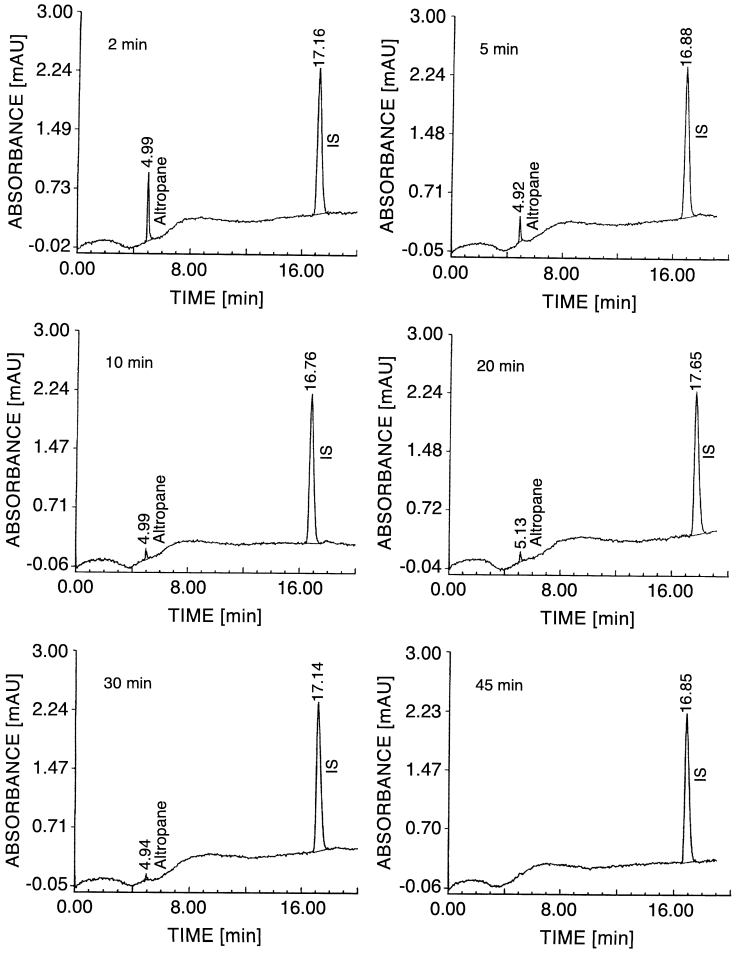

Fig. 5. CE profiles of Altropane in female rat plasma at 2, 5, 10, 20, 30 and 45 min timepoints after solid-phase extraction and addition of nicotinamide as an I.S.

of clearance for the female rats. The volume of distribution (V_z and V_{ss}) was approximately 1 l/kg for both males and females, suggesting distribution to total body water.

3.3. Metabolism profile

In the experiments performed for the pharmacokinetic evaluations, only the Altropane peak was found, and no potential metabolites were seen in the individual plasma samples. However, because CE is an inherently micro technique that allows injection of only a few nanoliters into the capillary, it is conceivable that metabolites might escape detection. To further examine the formation of potential metabolites, even as traces, the remaining plasma samples from four timepoints (5 min, 30 min, 2.5 h, and 3 h) were pooled and extracted using the same SPE method. The pooled samples for the 5- and 30-min time points were 3-fold concentrated compared to the individual samples. However, no peaks other than the I.S. and Altropane were detected. The pooled samples for the 2.5-h timepoint (also 3-fold concentrated) showed a trace of an early migrating peak, and the pooled sample for the 3-h timepoint (8-fold concentrated) showed traces of two early migrating unknown peaks.

The CE profiles of the pooled and concentrated

Table 7
Pharmacokinetic parameter estimates

Parameter[a]	Units	Male	Female
Dose	mg/kg	6.0	6.0
Cp^o	μg/ml	5.74	7.26
λ_z	min^{-1}	0.0992	0.0681
$(t_{1/2})_{\lambda z}$	min	6.99	10.18
$AUC_{0-infinity}$	μg·min/ml	44.23	60.92
$AUMC_{0-infinity}$	μg·min^2/ml	417.69	795.37
$MRT_{0-infinity}$	min	9.44	13.06
V_z	l/kg	1.37	1.44
V_{ss}	l/kg	1.28	1.29
Clearance	ml/kg·min	135.7	98.5

Cp^o: maximum serum concentration immediately after an i.v. administration; λ_z: rate constant for the terminal elimination phase; $(t_{1/2})_{\lambda z}$: terminal elimination half-life; $AUC_{0-infinity}$: area under the plasma concentration–time curve; $AUMC_{0-infinity}$: area under the moment curve; MRT: mean residence time of drug calculated using AUMC/AUC; V_z: volume of distribution calculated from the terminal phase; V_{ss}: volume of distribution at steady state; Clearance: total body clearance (Dose/AUC).

[a] Parameter estimates obtained by noncompartmental analysis of the data with uniform mass.

plasma samples from male and female rats are presented in Figs. 6 and 7. Because the quantities observed were below the limit of quantitation determined for Altropane, absolute values for the parent drug and the unknown peaks could not be calculated.

Also, because only trace amounts were found, characterization of these peaks was not possible and the peaks cannot be definitively identified as metabolites of Altropane.

4. Conclusions

Altropane is cleared very rapidly from the systemic circulation, with a MRT of about 10 to 13 min after i.v. administration. Despite the high dose of Altropane that was administered, no metabolites were detected when the plasma samples were analyzed individually. In pooled samples (approximately 3- and 8-fold more concentrated than individual samples), traces of two unknown peaks were detected at two timepoints only. Based on these results, it is concluded that metabolism is not a major factor in the disposition of Altropane in rats after i.v. administration.

The highest Altropane levels were observed at the

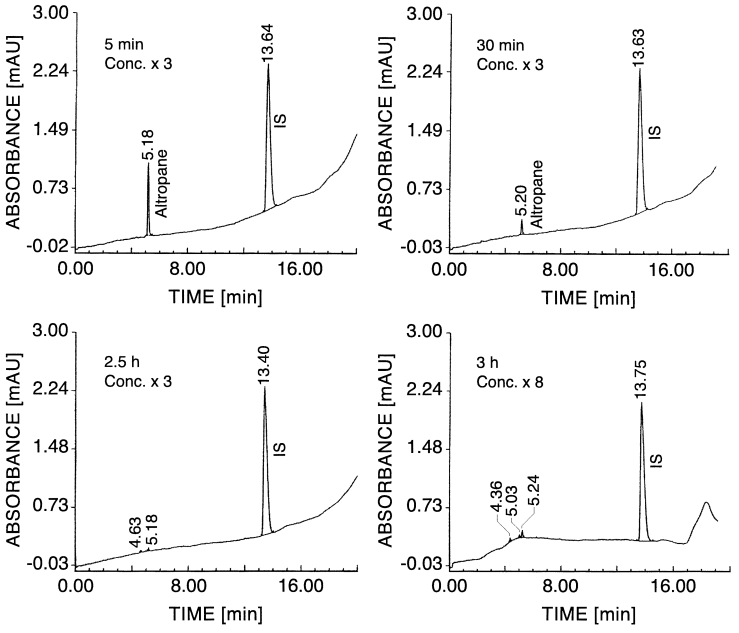

Fig. 6. CE profiles of Altropane from pooled and concentrated male rat plasma at 5 and 30 min and 2.5 and 3 h timepoints after solid-phase extraction and addition of nicotinamide as an I.S.

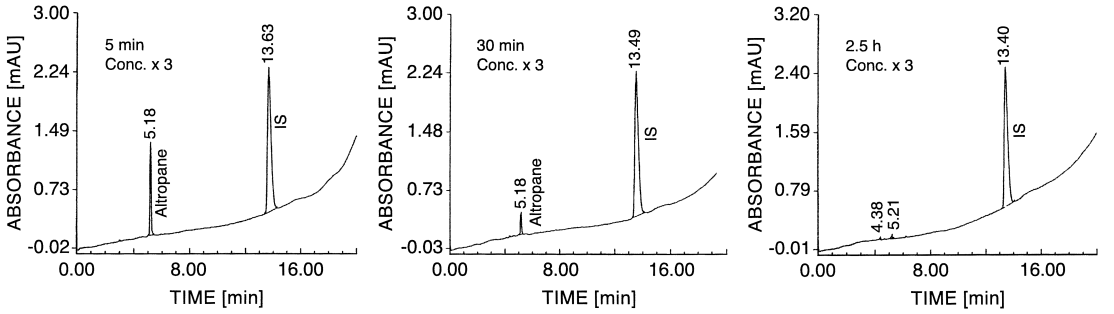

Fig. 7. CE profiles of Altropane from pooled and concentrated female rat plasma at 5 and 30 min and 2.5 h timepoints after solid-phase extraction and addition of nicotinamide as an I.S.

2-min timepoint in both sexes. However, after that, plasma levels declined very rapidly to less than the LOD for all of the samples by 45 min. The pharmacokinetic parameters as calculated by noncompartmental methods were comparable between the two sexes. The amount of drug available in the systemic circulation, as indicated by the AUC, was marginally higher for the female rats, and this was consistent with higher plasma levels and a lower rate of clearance as compared to the male rats.

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