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Elucidation of the structure of talinolol metabolites in man Determination of talinolol and hydroxylated talinolol metabolites in urine and analysis of talinolol in serum

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Abstract

The objective of this study was to determine the structure of talinolol metabolites formed and the amounts excreted in urine. Talinolol metabolites in urine were identified by comparing their HPLC retention times and their GC–MS profile with those of previously characterized reference compounds. The metabolites were quantified by HPLC with a normal-phase silica column, a single chloroform extraction and UV detection. Less than 1% of an administered dose was found in urine as hydroxylated talinolol. Other metabolites could be excluded. A sensitive method to determine talinolol in serum and a simple method for analysis of talinolol in urine are described. These methods were found to be precise and accurate for the measurement of talinolol in samples obtained from patients during chronic talinolol treatment as well as from healthy volunteers after a single dose of talinolol.

1. Introduction

Talinolol (Cordanum, Arzneimittelwerk, Dresden, Germany) is a cardioselective β_1-adrenoceptor antagonist which is frequently used in Germany and in Eastern Europe. Pharmacokinetic studies in man have shown that oral doses of talinolol are incompletely absorbed, and that urinary recovery of talinolol following i.v. administration is also incomplete [1–3]. It has been suggested that this could be due to either metabolism, biliary excretion, elimination in faeces or a combination of these factors.

The kinetics and the metabolism of several β-receptor blocking drugs have been described; considerable variations in kinetic parameters, in particular related to the bioavailability of the various compounds and the way in which they are excreted were found. Propranolol, for instance, is intensively metabolised in the liver and suffers from a substantial first pass effect following oral administration [4]. On the other hand, atenolol is excreted in urine virtually unchanged [5]. Knowledge of these factors is important in rational use of these drugs.

The objective of this study was to discover the structure of the metabolites formed and the amounts excreted in urine. The chemical formula of talinolol is 1-(4-cyclohexylureido-phenoxy)-2-hydroxy-3-*tert.*-butylaminopropane (Fig. 1). Sev-

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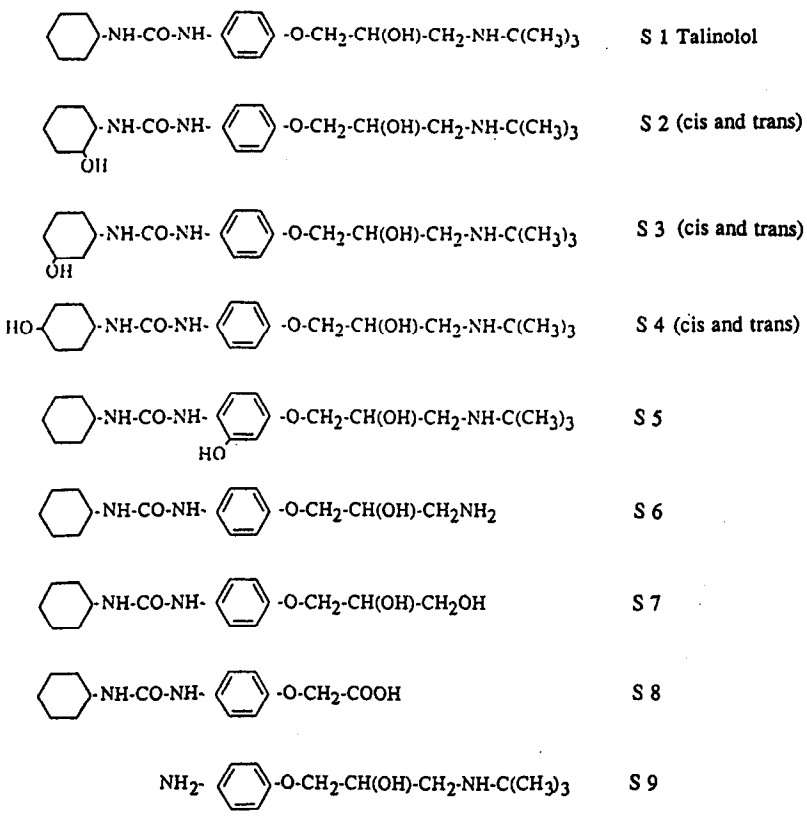

Fig. 1. Structures of reference compounds.

eral metabolic routes are possible: hydroxylation, degradation of the side chains and glucuronidation [4,6,7]. Theoretically, eight isomers of mono-hydroxylated talinolol are possible.

This paper presents the identification of talinolol metabolites in urine by comparing their high-performance liquid chromatographic retention times and gas chromatographic–mass spectrometric profile with those of previously characterized reference compounds and quantification of the metabolites by HPLC.

The limit of quantification of talinolol in serum reported in previous studies [8] was insufficient for new pharmacokinetic studies [2,3,9]. Therefore, a sensitive HPLC method (A) with a normal-phase silica column and a single extraction with diethyl ether to determine talinolol in serum and a simple HPLC method (B) with a reversed-phase column and a solid-phase extraction for quantification of talinolol in urine were developed.

2. Experimental

2.1. Chemicals

Talinolol, seven isomers of hydroxytalinolol and four hypothetic degradation products (see Fig. 1), and metoclopramide hydrochloride were kindly provided by Arzneimittelwerk (Dresden, Germany). 2,2,2-Trifluoro-N-methyl-N-trimethylsilyl-acetamide (for gas chromatography) (MSTFA), dichloromethane LiChrosolv (for chromatography), chloroform LiChrosolv (for chromatography), methanol LiChrosolv (for chromatography) and β-glucuronidase–arylsulphatase mixture (100 000 E/ml; from *Helix pomatia*) for biochemistry were purchased from

Merck (Darmstadt, Germany). Diethyl ether (HPLC grade) was obtained from ASID Bonz and Sohn (Böblingen, Germany). Pure water (20 MΩ) was obtained using ion-exchange system RS 40 E, SG Ionenaustauscher (Barsbüttel, Germany).

2.2. HPLC method A

Apparatus and chromatographic conditions

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-6A pump, a SPD-6A spectrophotometric detector, a SCL-6B controller, a SIL-6B autoinjector, equipped with a 50-μl loop, a column oven CTO-10A and a data system D450-MT2 (Kontron, Neufahrn, Germany). A normal-phase silica column (100 × 2.1 mm I.D., 5 μm particle size; Pye-Unicam, Boston, MA, USA) was used for the analysis and the system was equipped with a guard-column (30 × 2.1 mm I.D., 5 μm particle size; Pye-Unicam). The chromatographic column was maintained at a temperature of 35°C and equilibrated with the mobile phase. The mobile phase consisted of dichloromethane–methanol (87:13, v/v) and 0.5% concentrated ammonia. The solvent flow-rate was 1.0 ml/min and the detection wavelength was set at 242 nm.

Standard solutions

Stock solutions of talinolol, seven isomers of hydroxytalinolol and four hypothetic degradation products (see Fig. 1), and of the internal standard metoclopramide hydrochloride, were prepared by dissolving the salts in methanol to a final concentration of 1 mg/ml. Working solutions were obtained by further dilution of the stock solutions with methanol.

Extraction procedure for urine samples

This procedure involves a single extraction of the compounds together with an internal standard from alkalinized urine into chloroform. To 1-ml aliquots of urine in a 10-ml glass tube, 20 μl of metoclopramide hydrochloride solution (600 ng), 50 μl of saturated sodium carbonate solution and 4 ml of chloroform were added. The subsequent steps were the same as for the serum samples.

Extraction procedure for serum samples

This procedure involves a single extraction of the compounds from alkalinized serum into diethyl ether with an internal standard. To a 1-ml volume of serum in a 10-ml glass tube, 10 μl of metoclopramide hydrochloride solution (300 ng), 50 μl of a saturated sodium carbonate solution and 5 ml of diethyl ether were added. The mixture was shaken for 20 s (Heidolph mixer). After centrifugation for 10 min at 2500 *g* the organic phase was removed and evaporated to dryness. The residue was redissolved in 100 μl of methanol and a 75-μl aliquot was then injected in the 50-μl injection loop of the HPLC system.

2.3. HPLC method B

The equipment consisted of an autosampler Varian 9100 (Varian, Walnut Creek, CA, USA) injecting a 100-μl aliquot into a 50-μl sample loop. Water delivered by an HPLC pump LC 6A (Shimadzu, Kyoto, Japan) with a flow-rate of 1.4 ml/min is used to transfer the sample onto a solid-phase extraction cartridge (10 × 4 mm I.D.; Bischoff Manufit, Leonberg, Germany) filled with Perisorb A (30–40 μm particle size; Merck). This cartridge is fitted to a 10-port valve E C10W (Valco, Schenkon, Switzerland). After washing for 4 min the cartridge back-flushed with the eluent acetonitrile–phosphate buffer (0.05 mol/l, pH 4) (27:73, v/v) at a flow-rate of 1 ml/min. Thus talinolol and three reference compounds will be eluted from the extraction cartridge and brought onto the HPLC column for separation (RP 18, 125 × 4 mm I.D., 5 μm; endcapped, LiChroCART HPLC cartridge; Merck). The UV detector (Shimadzu LD 6A), set at a wavelength of 242 nm, is connected to a data system (MT2, Kontron).

2.4. Gas chromatography–mass spectrometry

Apparatus and chromatographic conditions

The gaschromatograph was a Varian 3400 (Varian) with an autosampler A200S (Finigan-MAT, San Jose, CA, USA). Chromatography was performed via a splitless injection on a Durabond 1 fused-silica capillary column (30 m × 0.25 mm I.D., film thickness 0.25 μm) (J and W Scientific, Folsom, USA). The carrier gas was helium with an inlet pressure of 70 kPa. The temperature program was as follows: 0.5 min isothermal at 80°C, 30°C/min to 170°C, 20°C/min to 280°C; followed by an isothermal period of 12 min at 280°C. The sample volume injected was 1 μl.

The samples were analysed using an ion-trap detector ITS 40 (Finigan-MAT). The mass spectrometer was operated in the electron-impact (EI) mode and the range from *m/z* 80 to *m/z* 510 was scanned.

Extraction procedure

A 1-ml sample of urine was mixed with 50 μl of a saturated sodium carbonate solution and 4 ml of diethyl ether in a 10-ml glass tube. The mixture was shaken for 20 s (Heidolph mixer). After centrifugation for 10 min at 2500 *g* the organic phase was discarded. A 4-ml volume of chloroform was added to the aqueous layer. The sample was shaken for 20 s (Heidolph mixer) and centrifuged for 10 min at 2500 *g*. Finally, the solvent was removed and evaporated to dryness.

Derivatization procedure

A 50-μl volume of MSTFA was added to the dried residue and mixed for 10 s. The sample was placed in an autosampler vial and after standing for two hours at ambient temperature, a 1-μl aliquot of this mixture was injected onto the gaschromatograph.

2.5. Samples

Analyses were carried out with serum and urine of young healthy volunteers after a single oral administration of 50 mg talinolol (Cordanum). Urine was collected over the intervals 0–4, 4–8, 8–12, 12–24 and 24–36 h. Moreover, urine of two patients who received 100 mg talinolol twice daily for more than three months, was collected over 24 h.

2.6. Hydrolysis

A 50-μl volume of an undiluted β-glucuronidase–arylsulphatase mixture from *Helix pomatia* was added to a 1-ml aliquot of urine and the samples were incubated at 37°C overnight (16 h). Quantitative measurements indicate that both the parent drug and the metabolites are not excreted in urine as either glucuronides or sulphates.

3. Results and discussion

3.1. Elucidation of the structure of talinolol metabolites

Determination of β-blocking drugs and identification of their metabolites is preferably performed using capillary gas chromatography–mass spectrometry after appropriate derivatization, such as a trimethylsilylation. Preliminary studies using talinolol and 3-trans-hydroxytalinolol (3t) as model drugs were carried out to identify derivatization products and chemical reactions in the GC–MS system. Thus two trimethylsilyl derivatives of each compound were identified (Fig. 2).

After injection of the mixture of the two trimethylsilyl derivatives dissolved in MSTFA, three characteristic compounds were obtained for talinolol and for compound 3t, respectively. These compounds could be identified by mass spectrometry (Fig. 2). Two of the reaction products of talinolol and compound 3t are identical: 1-(4-isocyan-phenoxy)-2-trimethylsilyloxy-3-*tert.*-butylaminopropane (I) and 1-(4-trimethylsilylamino-phenoxy)-2-trimethylsilyloxy-3-*tert.*-butylaminopropane (II). Using the other derivatives 1-(4-cyclohexylcarbodiimid-phenoxy)-2-trimethylsilyloxy-3-*tert.*-butylaminopropane (IIIa) and 1-(4-trimethylsilyloxy-cyclohexylcarbodiimid-phenoxy)-2-trimethylsilyloxy-3-*tert.*-butylaminopro-

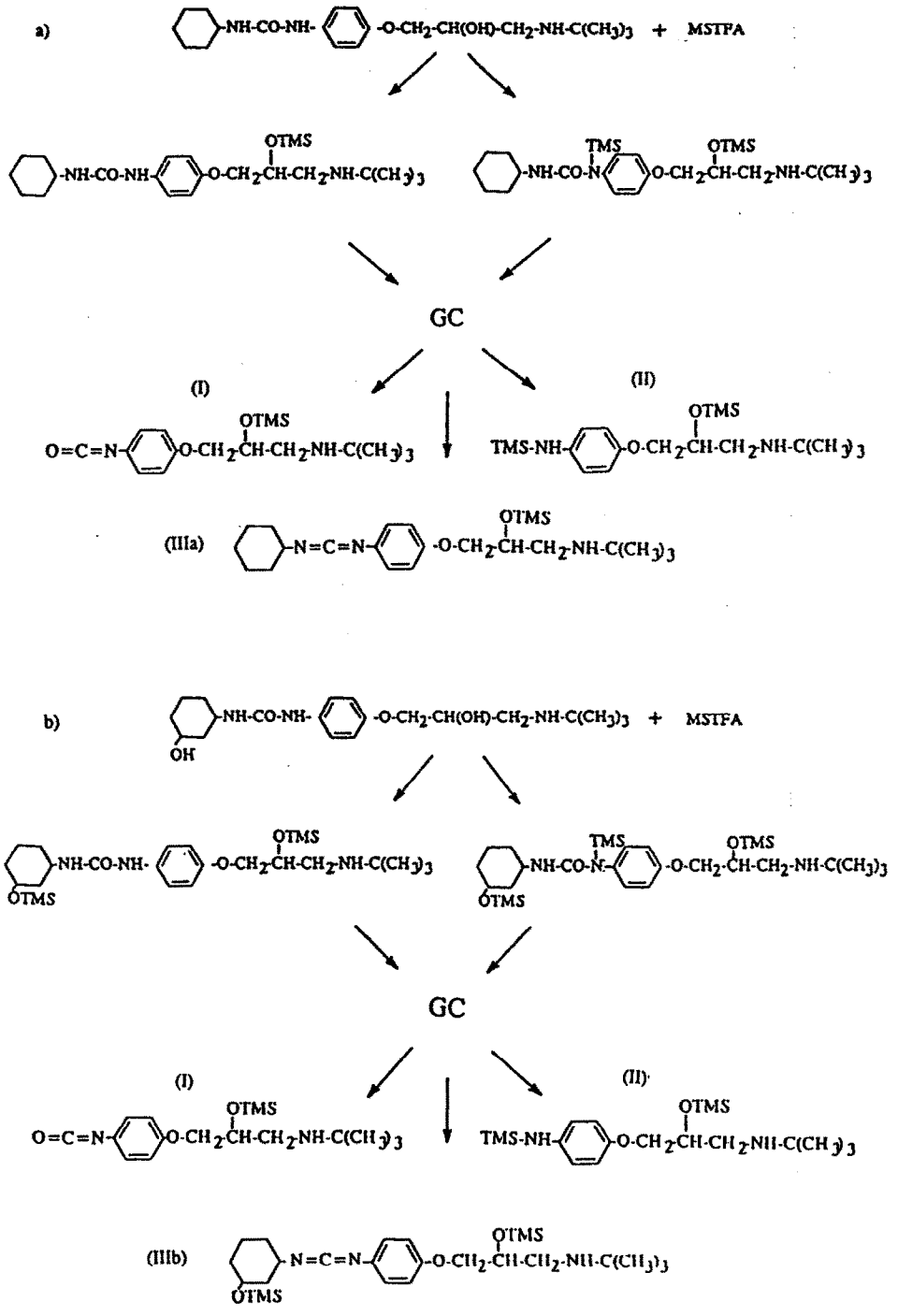

Fig. 2. Derivatization of talinolol (a) and compound 3t (b) with MSTFA and reactions of the derivatization products during gas chromatography.

pane (IIIb), respectively, it is possible to separate and analyse talinolol and the hydroxy metabolites, as shown in the chromatograms in Fig. 3. For the other reference compounds also three analogous characteristic compounds were obtained after derivatization and injection. The retention times of the obtained isocyanate, amine and carbodiimide compounds are shown in Table 1.

The described GC–MS method permits the simultaneous identification of talinolol and its possible metabolites. The minimum detectable concentrations, i.e. a signal-to-noise ratio of more than 3, for the studied substances were 20–500 ng/ml (Table 1).

Because of the occurrence of undesirable reactions during gas chromatography (as mentioned above), it was necessary to use a HPLC method to determine the metabolite concentrations.

Determination of talinolol metabolites with HPLC method A

With the HPLC method A (normal-phase conditions) it was possible to determine concentrations of the hydroxylated talinolol metabo-

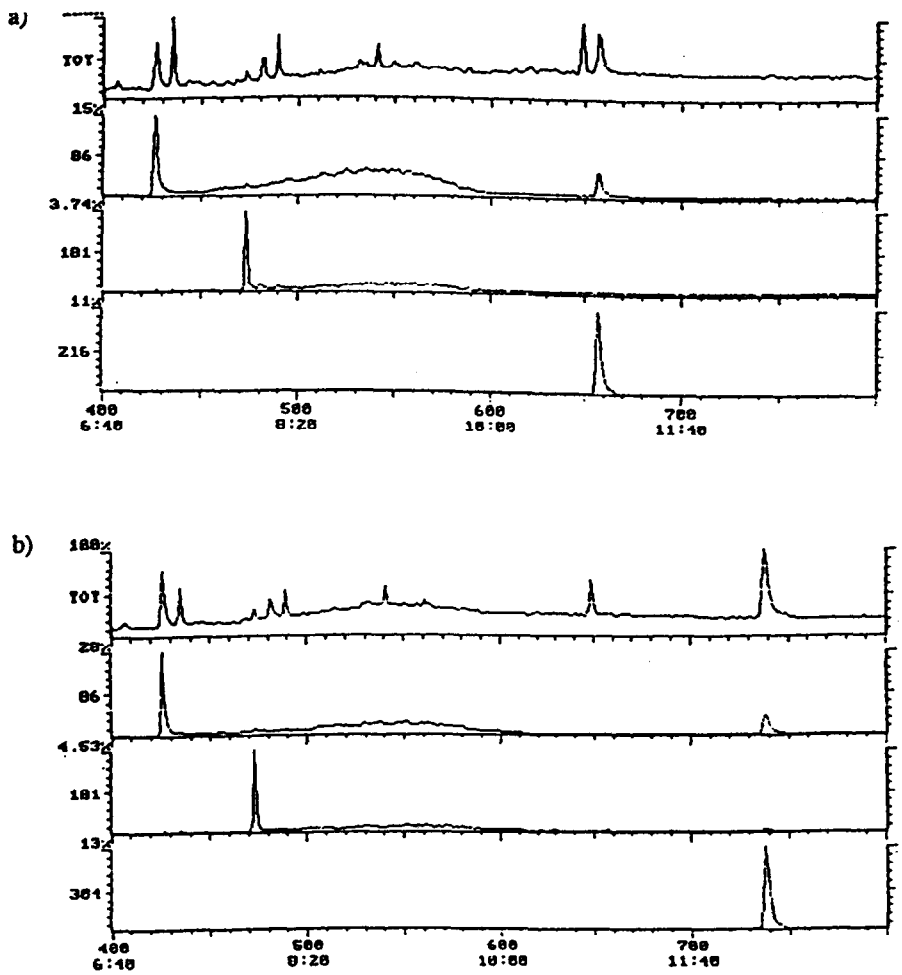

Fig. 3. Total-ion chromatograms and three characteristic single-ion chromatograms of (a) talinolol, (b) 3-*trans*-OH-talinolol (3t). The peak at *m/z* 86 is the basic peak of the isocyanate (I); *m/z* 181 is the basic peak of the amine (II), and *m/z* 216 and *m/z* 304, respectively are characteristic of the carbodiimide compounds (IIIa,b).

Table 1
Retention times of obtained isocyanate, amine and carbodiimide compounds and minimum detectable concentrations for the reference compounds with GC–MS

Compound	Retention time (s)			Minimum detectable concentration (ng/ml)
	Isocyanate	Amine	Carbodiimide	
1	426	474	659	10
2c	426[a]	474[a]	724	200
2t	426[a]	474[a]	732	20
3c	426[a]	474[a]	765	50
3t	426[a]	474[a]	740	20
4c	426[a]	474[a]	760	20
4t	426[a]	474[a]	780	20
5	495	509	704	20
6	427	472	654	500
7	408	455	630	20
8	343	395	562	20
9	–	474[a]	–	[b]

[a]Identical with the reaction products of talinolol.
[b]No separation possible.

lites and the hypothetical degradation product compound 9 (see Fig. 1). Symmetrical peaks were observed for the internal standard, talinolol and for the available isomers of mono-hydroxylated talinolol. Based on the GC–MS results the chromatographic conditions were optimized for the determination of the compounds 4t, 3c, 2c and 2t and 9 (Fig. 4).

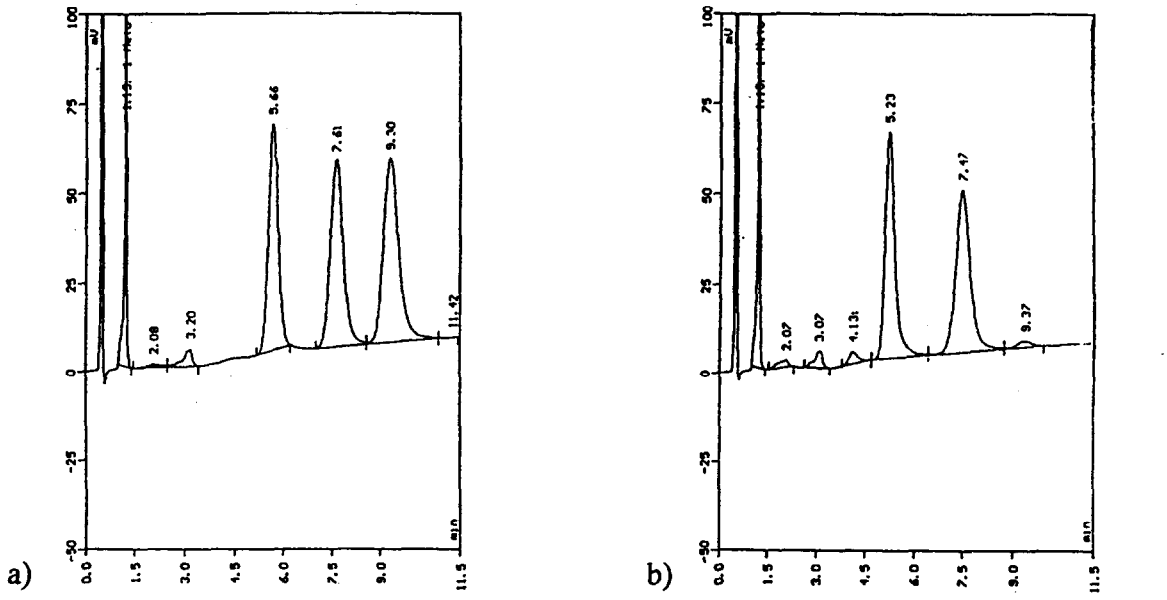

Fig. 4. Chromatograms of urine samples, HPLC method A. (a) Blank urine spiked with compounds 2t, 3t, 4t and metoclopramide (internal standard); (b) blank urine spiked with compounds 2c, 3c and metoclopramide. Peaks: 1.2 min, metoclopramide; 5.2 min, 2c; 5.7 min, 2t; 7.5 min, 3c; 7.6 min, 3t; 9.3 min, 4t.

In preliminary studies the metabolite 3t was not found in urine and only very small quantities of 4c were detected. Therefore a complete separation of all hypothetically possible isomers of mono-hydroxylated talinolol was not necessary.

The assay is reproducible and precise for talinolol, hydroxylated talinolol metabolites and compound 9 in human urine samples, as judged by a coefficient of variation of less than 20% at the lowest concentration examined ($n = 6$). The standard curves are linear over the 20–500 ng/ml range. The detection limit, i.e. a signal-to-noise ratio > 3, is 10 ng/ml and the lower limit of quantification, i.e. a coefficient of variation $< 20\%$ for 6 repeated measurements, is 20 ng/ml for each of the compounds using a 1.0-ml sample. This method was used to determine the talinolol metabolites in man. It was necessary to combine the results of HPLC and GC–MS to investigate the metabolism of talinolol because of (1) the low concentrations of talinolol metabolites, (2) the high concentrations of the parent drug, (3) the endogenous compounds in urine, and (4) very different blank urines (Fig. 5).

Determination of talinolol metabolites with HPLC method B

It was possible to separate talinolol and the hypothetical degradation products 6 and 7 under reversed-phase conditions. The assay is reproducible and precise for the compounds 6 and 7 in human urine samples. The standard curves are linear over 50–500 ng/ml. The lower limit of quantification (see above) is 50 ng/ml for both compounds using a 1.0-ml sample. This method was used to exclude substances 6 and 7 as talinolol metabolites in man.

3.2. Metabolism of talinolol in man

In our investigations less than 1% of the administered dose was recovered in urine as talinolol metabolites. The main metabolic pathway of talinolol is the hydroxylation of the cyclohexyl ring. The hydroxylation of the phenyl ring and degradation of the side chains could be excluded. Quantitative measurements indicate that both the parent drug and the metabolites

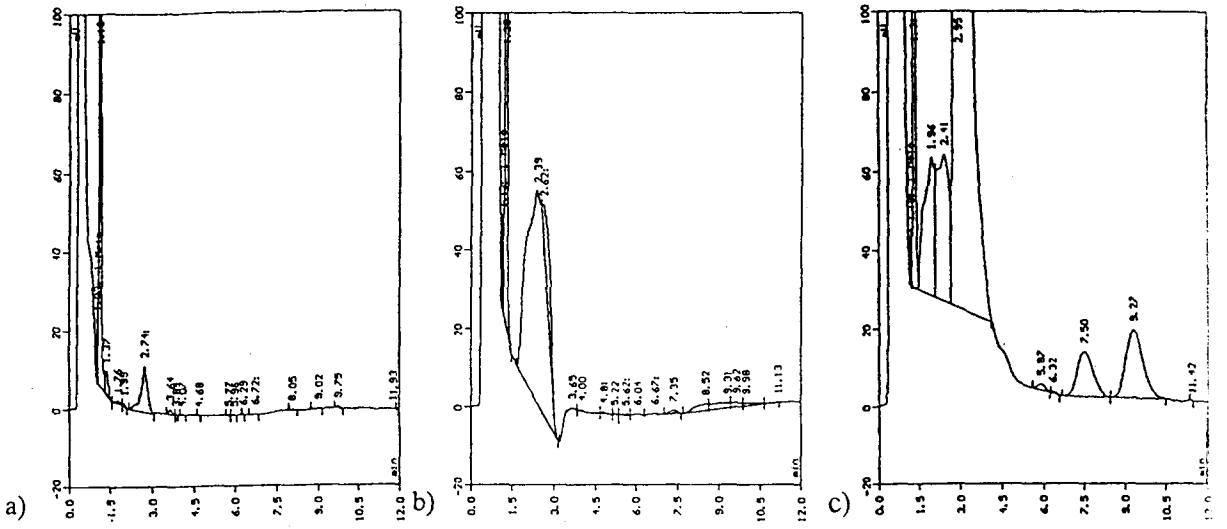

Fig. 5. Chromatograms of urine samples, HPLC method A. (a) and (b) Blank urine of several volunteers spiked with metoclopramide (internal standard); (c) urine of a patient, who received 200 mg talinolol daily for more than three months, collected over a 24-h period. Peaks: 1.2 min, metoclopramide (internal standard); 3.0 min, talinolol; 7.5 min, 3c; 9.3 min, 4t.

are not excreted as either glucuronides or sulphates.

The isomers 4t and the 3c were the main metabolites. In some cases two additional isomers (2t and 3t) were detected (Fig. 5). To illustrate the metabolism, the talinolol and metabolite concentrations and quantities in urine of a patient are shown in Table 2.

Less than 50% of the orally administered talinolol was eliminated in urine [1–3] and less than 1% was found as metabolite in urine. Therefore it has been suggested that the major part of the administered talinolol had been eliminated in faeces.

There were no striking differences in the amounts of the metabolites in urine formed by the patients (100 mg twice daily) and those formed by the volunteers (single 50-mg talinolol oral dose).

The described HPLC method A is also suitable to determine talinolol metabolites in serum. The limit of quantification for talinolol in serum is 5 ng/ml and for the metabolites 10 ng/ml. However, the expected concentrations of the metabolites–less than 3% of the parent drug concentration [1–3]–are below the detection limit. Consequently, hydroxylated talinolol was not found in human serum.

3.3. Determination of talinolol in serum with HPLC method A

For the determination of talinolol in serum a selective, sensitive and rapid HPLC method with a limit of quantitation (see above) of 5 ng/ml was developed. Serum samples obtained from patients during talinolol treatment (steady state) as well as from healthy volunteers during pharmacokinetic studies with a single dose of talinolol were extracted with diethyl ether. A normal-phase silica column was used for analysis.

Short retention times were found: 1.3 min for the internal standard (metoclopramide); 3.2 min for talinolol. No interfering peaks from endogenous compounds are found. Typical chromatograms are shown in Fig. 6.

The linearity and the precision were tested using spiked serum samples. The linearity of the method was confirmed in the range of 5–200 ng/ml. The precision of the method was assessed by determination of seven concentrations in six independent series of samples as shown in Table 3. Day-to-day precision data were obtained over a period of 22 working days by taking aliquots of serum spiked with 7 ng/ml, 85 ng/ml and 170 ng/ml talinolol, respectively and processing them daily. Low coefficients of variation were found: 16.9% (lowest concentration), 4.8% (middle concentration) and 4.8% (highest concentration).

This method was found to be selective, sensitive and rapid for the measurement of talinolol in serum.

3.4. Determination of talinolol in urine with HPLC method B

For the determination of talinolol in urine a selective, simple and rapid HPLC method with a limit of quantitation (see above) of 0.5 μg/ml

Table 2
Talinolol and metabolite concentrations and amounts excreted in a patient urine

Compound	Concentration (μg/ml)	Amount excreted (mg)	Percentage of dose
Talinolol	32.0	29.2	14.6
Metabolite 4t	0.225	0.205	0.10
Metabolite 3c	0.140	0.126	0.06

Urine was collected over a 24-h period, urine volume 912 ml.

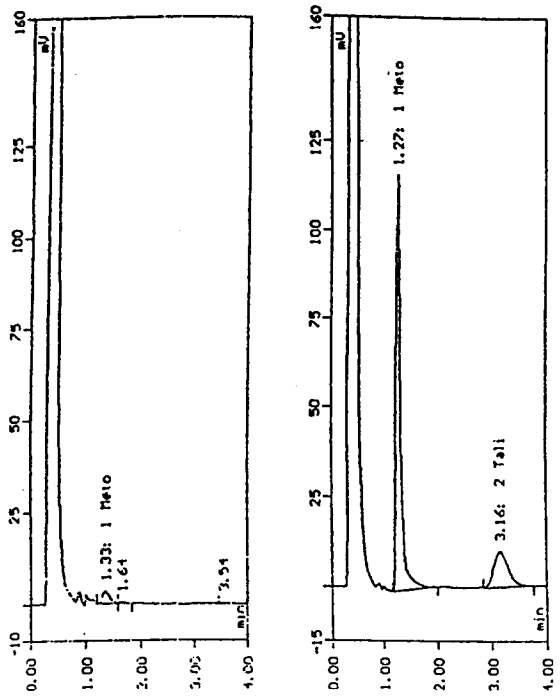

Fig. 6. Chromatograms of serum samples, HPLC method A. (a) Blank serum; (b) sample taken 2 h after oral administration of 50 mg of talinolol to a human (24 ng/ml). Peaks: 1.3 min, metoclopramide (internal standard); 3.2 min, talinolol.

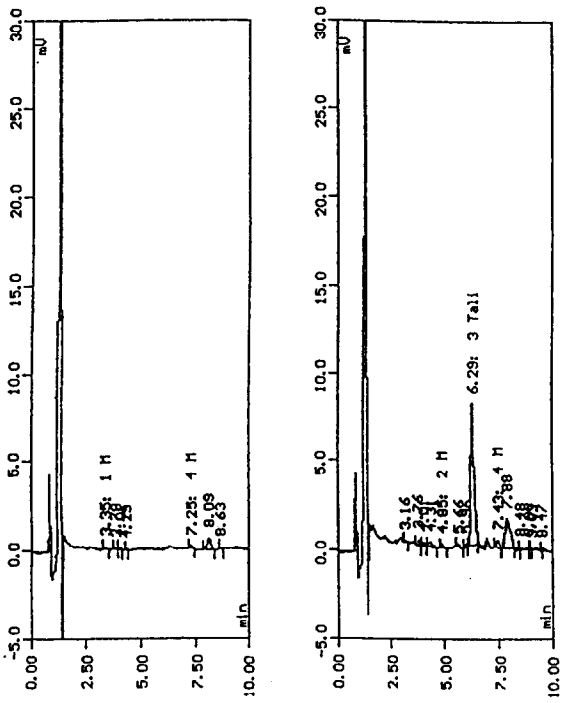

Fig. 7. Chromatograms of urine samples, HPLC method B. (a) Blank urine; (b) sample taken 2 h after oral administration of 50 mg talinolol to a human (2.94 μg/ml). Peak: 6.2 min, talinolol.

was developed. Samples were injected onto a solid-phase extraction cartridge in HPLC system B. After back-flushing talinolol was eluted from the extraction cartridge and brought onto the HPLC column for separation.

The linearity was tested over the range 0.5–8 μg/ml. Urine spiked with talinolol at levels of 0.5, 1, 2, 4 and 8 μg/ml was processed according to the complete procedure. Urine samples with higher concentrations were diluted with water. Typical chromatograms are shown in Fig. 7.

Table 3
Mean, standard deviation (S.D.) and coefficient of variation (C.V.) of six independent series of talinolol in serum determined with HPLC method A

Concentration added (ng/ml)	Concentration recovered (mean ± S.D.) (ng/ml)	C.V. (%)
5	5.6 ± 1.0	18.2
10	10.8 ± 0.9	8.8
20	20.0 ± 1.0	4.9
50	49.4 ± 3.0	6.2
100	98.6 ± 2.7	2.7
150	148.5 ± 2.8	1.9
200	202.0 ± 2.5	1.2

The precision of the method was assessed by determination of five concentrations in six independent series of samples as shown in Table 4. Day-to-day precision data were obtained over a period of 14 working days by taking aliquots of urine spiked with 1.70 μg/ml, 3.89 μg/ml and 7.00 μg/ml talinolol and processing them daily. Low coefficients of variation were found: 9.0%–10.5%.

4. Conclusions

In this study the metabolites of talinolol in urine of man could be identified. Because of the low concentrations of the talinolol metabolites as

Table 4
Mean, standard deviation (S.D.) and coefficient of variation (C.V.) of six independent series of talinolol in urine determined with HPLC method B

Concentration added (ng/ml)	Concentration recovered (mean ± S.D.) (ng/ml)	C.V. (%)
500	515 ± 58.3	11.3
1000	953 ± 46.7	4.9
2000	1926 ± 135.4	1.0
4000	4040 ± 171.2	4.2
8000	7994 ± 91.2	1.2

well as the high concentrations of the parent drug and of endogenous compounds in urine it was difficult to determine talinolol metabolites. On the one hand specificity of HPLC was not sufficient, and on the other hand talinolol and its metabolites could not be quantified by GC–MS due to the instability of their trimethylsilyl derivatives during gas chromatography. By combining the HPLC and GC–MS results the metabolism of talinolol was investigated. Less than 1% of the administered dose was found in urine as hydroxylated talinolol. Other metabolites could be excluded.

A sensitive method to determine talinolol in serum (A) and a simple method for its analysis in urine (B) are described. These methods were found to be precise and accurate for the measurement of talinolol in samples from patients and from pharmacokinetic investigations.

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References

[1] B. Terhaag, *Z. Klin. Med.*, 44 (1989) 119-124.
[2] B. Trausch, R. Oertel, K. Richter and T. Gramatté, *Br. J. Clin. Pharmacol.*, submitted for publication.
[3] B. Trausch, R. Oertel, K. Richter and T. Gramatté, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 349 (1994) 148.
[4] T. Walle and T.E. Gaffney, *J. Pharmacol. Exp. Therapeut.*, 182 (1972) 83-92.
[5] P.R. Reevers, J. McAinsh, D.A.D. McIntosh and M.J. Winrow, *Xenobiotica*, 8 (1978) 313-320.
[6] M.S. Lennard, *J. Chromatogr.*, 342 (1985) 199-205.
[7] J.G. Ridell, D.W.G. Harron and R.G. Shanks, *Clin. Pharmacokin.*, 12 (1987) 305-320.
[8] H. Pötter, M. Hülm and K. Richter, *J. Chromatogr.*, 241 (1982) 189-192.
[9] T. Gramatté, R. Oertel and K. Richter, *Klinische Pharmakologie Aktuell*, 4 (1993) 35.