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Determination of talinolol in human plasma by high performance liquid chromatography–electrospray ionization mass spectrometry: Application to pharmacokinetic study

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Abstract

A rapid and sensitive method for determination and screening in human plasma of talinolol is described using propranolol as the internal standard. The analytes in plasma were extracted by liquid–liquid extraction using methyl *t*-butyl ether. After removed and dried the upper organic phase, the extracts were reconstituted with a fixed volume of buffer of ammonium acetate and acetonitrile (60:40, v/v). The extracts were analyzed by a HPLC coupled to electrospray ionization mass spectrometry (HPLC–MS/ESI). The HPLC separation of the analytes was performed on a Phenomenex C18 (250 mm × 4.6 mm, 5 μm, USA) column, with a flow rate of 0.85 mL/min. The complete elution was obtained within 5.5 min. The calibration curve was linear in the 1.0–400.0 ng/mL range for talinolol, with a coefficient of determination of 0.9996. The average extraction recovery was above 83%. The methodology recovery was between 101% and 102%. The limit of detection (LOD) was 0.3 ng/mL for talinolol. The intraday and inter-day coefficients of variation were less than 6%. This HPLC–MS/ESI procedure was used to assess the pharmacokinetics of talinolol. A single oral 50 mg dose of talinolol tablet was administered to 12 healthy Chinese volunteers, the main pharmacokinetic data are as follows: C_{max} was 147.8 ± 63.8 ng/mL; t_{max} was 2.0 ± 0.7 h; $t_{1/2}$ was 12.0 ± 2.6 h. The method is accurate, sensitive and simple for the pharmacokinetic study of talinolol.

Keywords: Talinolol; Pharmacokinetics; HPLC–MS/ESI

1. Introduction

Talinolol is frequently used in Germany and east Europe for the treatment of arterial hypertension, coronary heart failure (CHF), and tachydysrhythmias[1,2]. The drug is a long-acting, highly selective β1-adrenoceptor antagonist with moderate lipophilic properties, without partial agonist activity and non-specific membrane-stabilizing effect [3]. In the therapeutic dosage range (50–300 mg/day), β1 selectivity of talinolol is superior (factor 20) to atenolol (factor 13), comparable to metoprolol (factor 15–20) and smaller than bisoprolol (factor 75). Talinolol has a moderate affinity for β1-adrenoceptors and a higher and longer degree of β1-adrenoceptor occupancy than propranolol. Talinolol has a half-life of approximately 12 h, longer than bisoprolol, atenolol and metoprolol and is suitable for once daily administration [1]. Studies conducted up till now have clearly shown that talinolol does not exert a negative influence on atherogenic lipid profile with some positive tendencies and a neutral effect on glucose metabolism. Recent reports suggest that talinolol exerts antioxidant effects (significant decrease of plasma lipid peroxides and neutrophil activation) in smokers [4] and in patients with coronary heart disease [5]. In a single-center controlled study it has been shown that this drug can improve left ventricular function and exercise capacity after 6 months of treatment in 46 patients with CHF [5]. The effects of talinolol were similar to those of metoprolol and suggest that this benefit is a class-effect of β1-adrenoreceptor blockade.

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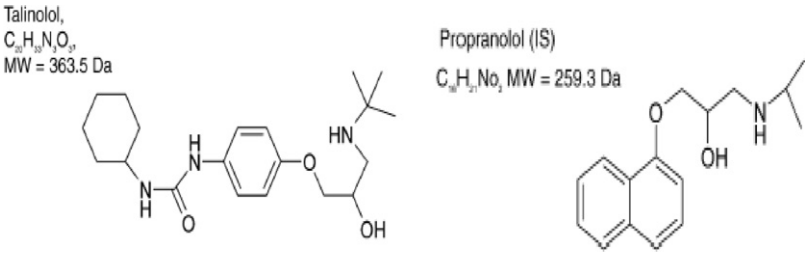

The chemical structure of talinolol and propanolol (I.S.)

The advantages of the therapeutic profile of talinolol have led to an increasing use in treatment of patients. However, even if this compound has fewer undesirable side effects, it can lead to typical side effects, such as bradycardia, hypotension, bronchoconstriction, aggravation of cardiac failure, etc., overdose of beta-blockers may lead to life-threatening situations [6–8]. In conditions of renal impairment or comedication with inhibitors or inducers of *p*-glycoprotein [9–15], routine therapeutic drug monitoring (TDM) seemed to be useful, furthermore, TDM is a useful tool for determination of noncompliance. So, the development of a rapid and specific method allowing the screening and the determination of this compound in biologic fluids could be of great interest either in therapeutic drug monitoring use or in toxicologic screening in the case of suicide involving this compound [3].

At present, various assays of talinolol were developed, mainly based on normal-phase or reversed-phase separation followed by UV, fluorescence or tandem mass spectrometric detection. Oertel et al. and Zhang et al. [10,16–18] described an LC–UV assay where basified serum was extracted with diethylether. Metoclopramide was used as the internal standard (I.S.). The analyte was separated on a normal-phase column and the detection was performed at 254 nm. The assay was found to be linear in the range 5–200 ng/mL using a 1 mL serum aliquot. The other group [19] achieved better sensitivity by tandem mass spectrometric detection using online solid-phase extraction system combined with atmospheric pressure chemical ionization (APCI). The analyte was isolated from plasma using solid-phase extraction cartridges as a column-switching device. The assay was found to be linear in the range 2.5–200 ng/mL using 0.25 mL plasma aliquot with a LOQ of 2.5 ng/mL. Reversed-phase liquid chromatography followed by fluorimetric detection (252 nm extinction, 332 nm emission) was also applied to determine talinolol in serum [20,21]. The assay was found to be linear in the range of 5–1000 ng/mL using a 0.5 mL serum aliquot. The analyte was isolated from serum using liquid–liquid extraction with diethyl ether and separated on a reversed-phase standard bore column, using propranolol as the internal standard. In order to provide more guidance to the reasonable use of this compound, a simpler method that would be suitable for routine analysis is required. For this purpose, we have established the HPLC–MS/ESI method for the determination of talinolol in human plasma, using propranolol as internal standard. We also applied this method to assess the pharmacokinetics of talinolol tablet (Cordanum®-50 mg tablet, from Arzneimittelwerk Dresden GmbH, Dresden, Germany). A single 50 mg dose of talinolol tablet was administered to 12 healthy Chinese volunteers. The specimen collection from human subjects was approved by the Ethical Committee of XiangYa Second Hospital of Central South University (Changsha, P.R. China). The method is accurate, sensitive and simple for routine therapeutic drug monitoring (TDM) as well as toxicologic screening, and for the study of the bioavailability of talinolol.

2. Experiment

2.1. Equipments and reagents

A system of HPLC (Waters 2690, USA)–MS with a Micromass ZQ mass spectrometer (Wythenshawe, Manchester, UK) equipped with an electrospray ionization (ESI) ion source was used. COMPAQ Deskpro and MassLynxTM 3.5 Workstation software were utilized. Auto Science® AP-01P Vacuum Pump (Automatic Science Instrument Co., LTD, TianJin) was utilized.

Talinolol reference standard (>99.8%) and tablet (Cordanum®-50 mg tablet, lot number: 5C057A) were generously donated by Arzneimittelwerk Dresden GmbH (Germany); propranolol (>99.8%) was purchased from SIGMA (Steinheim, Germany).

HPLC grade reagents (methanol, acetonitrile and methyl *t*-butyl ether) were obtained from Tedia Company Inc. (Fairfield, America). Other AR grade reagents (ammonium acetate, sodium hydroxide, formic acid) were obtained from Chemical Reagent Factory of Hunan (Changsha, Hunan, China). Blank human blood was collected from healthy drug-free volunteers. Plasma was obtained by centrifugation of blood treated with the anticoagulant sodium heparin. The samples were stored at −70 °C in ultra cold freezer up to the time of analysis.

2.2. Standard solutions

The primary stock solutions of talinolol (1.16 mg/mL) and propranolol (1.5 mg/mL, I.S.) were prepared by dissolving appropriate amount of pure substance in methanol. Working solutions were obtained by diluting the stock solutions with methanol. All the standard solutions were stored at −20 °C.

Routine daily calibration curves were prepared in drug-free plasma. Appropriate volumes of working solutions and drug-free human plasma were added to each test tube. Final concentrations were 1, 2, 5, 10, 25, 50, 100, 200 and 400 ng/mL. Quality control

samples that were run in each assay, were prepared in the same way, and final concentrations were 2, 50, 200 ng/mL.

2.3. Chromatographic conditions

The analytes were separated on a Phenomenex C18 (4.6 mm × 250 mm, 5 μm, USA) column with column temperature 40 °C. The mobile phase was buffer (formic acid: 1‰, NH_4Ac: 10 mmol/L)–acetonitrile (60:40, v/v) and was filtered using 0.45 μm filters in a Millipore solvent filtration apparatus and was never recirculated. The flow-rate was 0.85 mL/min, and the postcolumn splitting ratio was 3:1.

2.4. MS/ESI detection conditions

The compounds were ionized using electrospray ionization (ESI) ion source in the positive ion mode of the mass-spectrometer. Selected ion monitoring (SIM) was used for quantitation by the protonated molecular ions of each analyte. The detection conditions were as follows—capillary voltage: 3.00 kV; cone voltage: 33.0 V for talinolol, 28.0 V for propranolol (I.S.); extractor voltage: 3.0 V; source temperature: 110 °C; desolvation temperature: 280 °C; cone gas flow: 100 L/h; desolvation gas flow: 400 L/h.

2.5. Sample preparation

All frozen human plasma samples were previously thawed at ambient temperature and centrifuged at 9500 rpm for 5 min to precipitate solids. The sample (1.0 mL) was spiked with 100 μL of I.S. solution 0.5 μg/mL and 100 μL of sodium hydroxide 0.1 mol/L and vortex-mixed for 10 s, then 5 mL of *t*-butyl methyl ether was added. The samples were vortex-mixed for approximately 2 min, centrifuged at 3000 rpm for 5 min. The upper layer of organic phase was transferred to another set of clean glass tubes and evaporated to dryness under a stream of nitrogen at 60 °C. The residue was reconstituted in 100 μL mobile phase and vortex-mixed for 10 s; 20 μL solution was injected for analysis through auto-sampling injector.

2.6. Validation of the method

The extraction recoveries were determined at three concentration levels by comparing the analyte peak areas obtained from the quality control samples ($n = 5$) after extraction to those obtained from the corresponding unextracted reference standards prepared at the same concentrations. The methodology recoveries were calculated according to the equation:

$$\text{Methodology recovery (\%)} = \left(\frac{\text{concentration}_{\text{measured}}}{\text{concentration}_{\text{theoretical}}}\right) \times 100$$

Precision assays were carried out five times using three different concentrations on the same day and over five different days.

Calibration was performed by a least-squares linear regression of the peak-area ratios of the drugs to the I.S. versus the respective standard concentration, the lowest concentration of the calibration was LOQ (S/N > 9), the lowest concentration detectable was LOD (S/N = 3).

The selectivity (the absence of interferences from endogenous components in the biological matrix or exogenous components from the isolation procedure) was assessed by extracting control blank plasma samples in each validation run. The lack of interfering peaks at the same analyte retention time was considered as acceptable selectivity.

Stability of the quality control plasma samples and stock solutions (2, 50 and 200 ng/mL) were subjected to short-term (12 h, 25 °C) room temperature, four freeze/thaw (−20 to 25 °C) cycles and long-term (30 days, 25 °C) stability tests. Subsequently, the concentrations were measured in comparison to freshly prepared samples.

Matrix effect was investigated using the combination of extraction recovery and suppression (overall analyte recovery).

2.7. Pharmacokinetic study

The method was applied to evaluate the pharmacokinetics of talinolol tablet (Cordanum®-50mg tablet, from Arzneimittelwerk Dresden GmbH, Dresden, Germany). The study was an open study in 12 healthy male Chinese volunteers with a mean age of 22 ± 1 years and their mean height and weight were 175.1 ± 5 cm and 68.6 ± 5.2 kg, respectively. After screening and washout period (of at least 2 weeks), the individuals who were qualified were confined for approximately 60 h. Study schedule: pre-study period: medical history, general physical examination, electrocardiogram, clinical laboratory examination; confined period: a 4 mL blood sample was collected before 50 mg dosing and 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0, 48.0 and 60.0 h post-dosing; post-study period: general physical examination, electrocardiogram, clinical laboratory examination.

3. Results and discussions

3.1. HPLC–MS/ESI

The HPLC–MS/ESI using selected ion monitoring in the positive ion mode provided a highly selective method for the determination of talinolol and propranolol (I.S.). The experiment results showed that formic acid could improve the separation and increase the MS sensitivity. When the formic acid ratio reached to 1‰, the analytes could be separated completely from the endogenous substances in the plasma. At the same time, the retention times of the analytes were obviously shortened, the retention times of talinolol and the I.S. were approximately 3.95 and 4.29 min, respectively; no endogenous substance was observed in the chromatograms of blank plasma. Compared with the published methods [10,16–18,20,21], the chromatographic run of this method was shortened, the complete elution was obtained within 5.5 min. The selected ion monitoring in the positive ion mode in control human plasma were identified at *m/z* 364.3 for $[\text{talinolol} + H]^+$, 260.3 for $[\text{I.S.} + H]^+$. The chromatograms of samples were shown in Fig. 1.

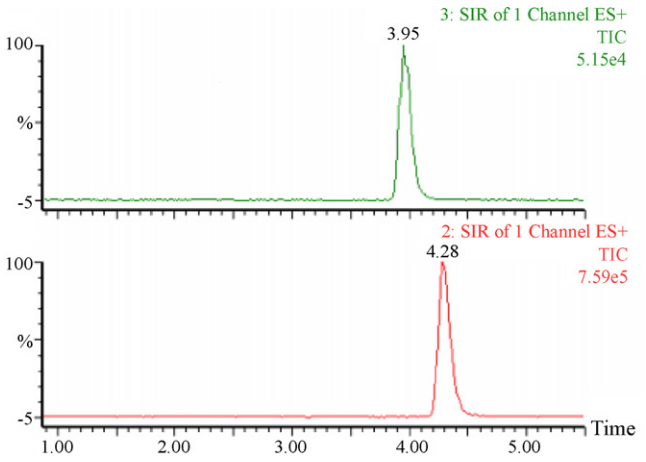

Fig. 1. The chromatograms of samples. Channel 2:propranolol (I.S.), $m/z = 260.3$, 518 ng/mL. Channel 3:talinolol, $m/z = 364.3$, 5.1 ng/mL.

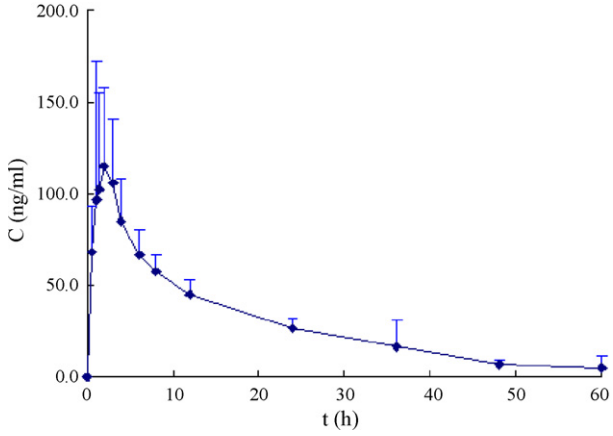

Fig. 2. The main plasma concentration vs. time profiles after a single oral dose of 50 mg talinolol ($n = 12$).

We compared two solvents (ether and methyl *t*-butyl ether) for extraction from plasma of talinolol, and finally we chose methyl *t*-butyl ether as the solvent for liquid–liquid extraction, because it has an appropriate recovery (83%) with a low variability (<12%) in comparison with ether (mean extraction recovery was 69% with a variability of 16%). As demonstrated in this assay, this method is sensitive, specific, allowing for analyzing samples in batches and perfectly suitable for a high-throughput routine such as pharmacokinetic studies.

3.2. Linearity

The calibration curve followed the regression equation $y = a + bx$ over the concentration range from 1.0 to 400.0 ng/mL (regression equation: $y = 0.00607658c + 0.00254951$, $r = 0.9996$). A linear least-squares regression with a weighting index of $1/x$ was carried out on the peak area ratios of talinolol and I.S. versus talinolol concentrations of the nine human plasma standards (in duplicate) to generate a calibration curve.

3.3. Accuracy, precision and sensitivity

The mean extraction recoveries (means ± S.D.), methodology recoveries (means ± S.D.), intra-day and inter-day precision for the analyte are shown in Tables 1 and 2. The average extraction recovery for talinolol was above 83%. The average methodology recovery was between 101% and 102%. The intra-day and inter-day R.S.D. were less than 6%. The limit of quantification (LOQ) validated was 1 ng/mL (S/N > 9) defined as the lowest concentration at which the R.S.D. was below 20%.

3.4. Selectivity

The intercepts of the calibration graph did not significantly differ from zero, underlining the selectivity of the assay. Based on the analysis of drug free plasma from control plasma and blank samples included in each validation run, endogenous or chemical components did not interfere with the drug and internal standards over the concentration range. Analysis of study predose samples did not show any significant interference.

3.5. Stability

Talinolol concentrations of plasma samples and stock solutions appeared to be stable after short-term (12 h) room temperature, four freeze/thaw (−20 to 25 °C) cycles and long-term (30 days) stability tests (Table 3).

3.6. Matrix suppression

Matrix suppression or enhancement during the ionization process can be a severe problem when developing LC–MS assays [22]. One of the fundamental issues in bioanalysis is that method validation is performed with spiked matrix samples. Despite that the same matrix, plasma for example, is used for preparing calibration and QC samples, significant differences can be observed between these samples and the study samples. It is therefore important to identify possible suppression or enhancement issues and several approaches have been proposed [22]. One of the approaches is to investigate the suppression in various lots of plasma, the parameter that can be easily determined, is the combination of extraction recovery and

Table 1
Mean extraction recoveries (±S.D.), methodology recoveries (±S.D.) and R.S.D.

Added drug	Conc. (ng/mL)	Mean extraction recoveries (%, $n = 5$)		Mean methodology recoveries (%, $n = 5$)		
		Mean recoveries ± S.D.	R.S.D. (%)	Found ± S.D.	Recoveries (%)	R.S.D. (%)
Talinolol	2	86 ± 0.06	7	2.0 ± 0.06	101	3
	50	83 ± 0.08	12	51 ± 1	102	2
	200	84 ± 0.1	10	202 ± 6	101	3

Table 2
Intra-day and inter-day precision

Added drug	Conc. (ng/mL)	Intra-day precision (%, $n=5$)			Inter-day precision (%, $n=5$)		
		Found ± S.D.	R.S.D. (%)	R.E.* (%)	Found ± S.D.	R.S.D. (%)	RE* (%)
Talinolol	2	2.0 ± 0.06	3	3	2 ± 0.1	6	5
	50	51 ± 1	2	3	50 ± 5	6	8
	200	202 ± 6	3	2	204 ± 12	5	5

R.E.*: Relative error.

Table 3
Stability test (short-term stability, freeze-and-thaw stability, long-term stability tests)

	Short-term stability test, values after 12 h (ng/mL, 25 °C)			Freeze-and-thaw stability test, values after 4 times (ng/mL, −20 to 25 °C)			Long-term stability tests, values after 30 days (ng/mL, 25 °C)		
Reference values	2	50	200	2	50	200	2	50	200
Mean ± S.D.	2.0 ± 0.09	51 ± 4	202 ± 12	2.0 ± 0.2	52 ± 5	204 ± 18	2.0 ± 0.1	50 ± 5	199 ± 9
R.S.D. (%)	8	8	6	9	10	9	9	9	4

suppression (overall analyte recovery). In the present work the overall analyte recovery for the plasma was found to be 83.5% for talinolol (50 ng/mL, $n=5$, R.S.D. = 12%) and 85% for I.S. (0.5 ug/mL, $n=5$, R.S.D. = 3%). Study samples from different subjects may behave differently regarding matrix suppression. The absolute peak area of the I.S. was used as a marker to monitor possible suppression effects with study samples. The R.S.D. of I.S. peak area was found to be less than 8% for batches of more than 200 samples including samples from different subjects, calibration and QC samples. It was therefore concluded that the matrix suppression for this assay is not an issue.

3.7. Analysis of pharmacokinetics

All values are presented as arithmetic mean and standard deviation (S.D.). The Rank Sum Test according to Wilcoxon for paired and unpaired comparisons was used to calculate the difference in pharmacokinetic parameters. The significant level was set at $p<0.05$ (two sided).

This pharmacokinetic study is conducted in a well-selected, compliant group of healthy subjects. All study procedures have been standardized as well as possible with regard to duration of fasting, physical activity, and body position during the period of absorption. Under these conditions, the pharmacokinetic results of the study (AUC, C_{max}, t_{max}, $t_{1/2}$) were similar to the dose-adjusted results with single oral 100 mg talinolol by other authors [11,18]. The mean plasma concentration versus time profiles after a single oral dose of 50 mg of talinolol is shown in Fig. 2. The main pharmacokinetic parameters of talinolol were calculated and summarized in Table 4. The maximum plasma concentration (C_{max}) of talinolol was 147.8 ± 63.8 ng/mL; the time to it (t_{max}) was 2.0 ± 0.7 h; the elimination half-life ($t_{1/2}$) of talinolol was 12.0 ± 2.6 h. As previously described, a double peak during absorption was evident in 7 of the 12 individual concentration-time curves. Its nature needs to be elucidated.

Absorption of drugs from the gastrointestinal tract is a complex process the variability of which is influenced by many physicochemical and physiologic factors [23,24]. There is no relation between the double-peak phenomena of talinolol and enterohepatic recycling and fractionated gastric emptying [21,25,26].

There is ample evidence that talinolol passes the apical membrane of intestinal enterocytes rapidly. This uptake is counteracted by p-gp leading to reduced availability in the jejunum/ileum and rectum. The total extent of talinolol absorption can be decreased most likely by binding of non-absorbed talinolol (and/or re-secreted by p-gp) to bile acids resulting in double-peaks in the plasma curves after overnight fasting [21]. We have administered talinolol after overnight fasting, food was eaten in our study 1.5 h and 5 h after administration, that is, talinolol was able to form complexes with bile acids under fasting condition for at least 1.5 h, result in double-peaks in the plasma curves.

The directional transport of drugs along the presystemic enterohepatic pathway requires the presence and coordinate function of drug uptake as well as efflux transporters on the apical and basolateral membranes of the enterocytes and hepatocytes such as MDR1, MDR3, MRP1-6, and BCRP of the ATP binding cassette transporter family, OATP1A2, OATP1B1, OATP1B3, and OATP2B1 of the organic anion transporting polypeptide family, or OCT1-3 of the organic cation transporter family [27–30]. The complex interplay between intestinal uptake and kick-back processes along the apical/luminal surface, the transit throughout the highly compartmented enterocyte fol-

Table 4
Pharmacokinetic parameters of talinolol in plasma of 12 volunteers after a single oral 50 mg dose

Parameters	Talinolol (mean ± S.D.)
$AUC_{0\to60}$ (ng h/mL)	1763.7 ± 377.5
$AUC_{0\to\infty}$ (ng h/mL)	1860.0 ± 377.9
C_{max} (ng/mL)	147.8 ± 63.8
T_{max} (h)	2.0 ± 0.7
$T_{1/2}$ (h)	12.0 ± 2.6
CL/F (L/h)	27.9 ± 5.5
V/F (L)	481.5 ± 117.9

lowed by secretion along the basolateral membrane, as well as the processes involved in hepatic uptake and secretion may lead to irregularities in plasma concentration curves such as shoulders of multiple peaks. Consequently, noncoordinate influence as caused by nutrients or transporter modulating drugs may lead to irregularities in extent and rate of talinolol absorption.

4. Conclusions

Compared with other methods, HPLC–MS/ESI improved the specificity and sensitivity, shortened the analytical time of the samples. The main aim of the study was to establish a HPLC/MS method that was suitable for the study of pharmacokinetics of talinolol in human plasma. The method described here has been found to be specific and accurate in practical application. To the best of our knowledge, this method meets the request of the present pharmacokinetic studies and also suits for the analysis of samples in batches when undertaking TDM or bioavailability.

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