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Enantioselective Determination of Ondansetron and 8-Hydroxyondansetron in Human Plasma from Recovered Surgery Patients by Liquid Chromatography–Tandem Mass Spectrometry

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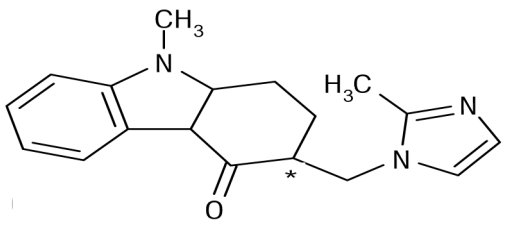
Abstract

A liquid chromatographic–mass spectrometric assay with atmospheric pressure chemical ionization for quantification of ondansetron and its main metabolite 8-hydroxyondansetron in human plasma was presented. The enantiomeric separation was achieved on a Chiralcel OD-R column containing cellulose tris-(3,5-dimethylphenylcarbamate). The validation data were within the required limits. The assay was successfully applied to authentic plasma samples. Quantitative results from postoperative patients receiving ondansetron demonstrated a great interindividual variability in postoperative plasma drug concentrations, the metabolites were not detected in their unconjugated form. A wide variation in the *S*-(+)-/*R*-(–)-ondansetron concentration ratio between 0.14 and 7.18 is indicative for a stereoselective disposition or metabolism. In further studies CYP2D6 and CYP3A4 genotype dependent metabolism of ondansetron enantiomers as well as of co-administered drugs and clinical efficacy of the medication should be tested.

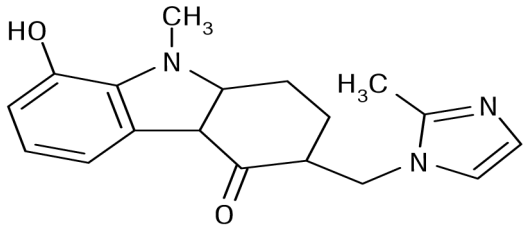
Introduction

Ondansetron, (±)-9-methyl-3-[(2-methylimidazol-1-yl)methyl]-2,3-dihydro-1*H*-carbazol-4-one (Figure 1), is a highly selective and potent 5-hydroxytryptamine type 3 (5-HT3) receptor antagonist. It is effective in the treatment of postoperative nausea and vomiting (PONV) as well as during cancer chemotherapy and radiotherapy, and it has reported anxiolytic and neuroleptic properties (1–4).

Ondansetron has a short plasma half-life of approximately 3 to 5 h (5,6). Between 70% and 76% of ondansetron is protein bound, and it is extensively metabolized in the liver by the hydroxylation of the indole ring to 7- and mainly 8-hydroxyondansetron (40%) followed by glucuronide or sulfate conjugation (7); 6-hydroxylation and demethylation are minor routes of metabolism. In terms of overall ondansetron elimination, CYP3A4 plays the predominant role, but primary hydroxylation is also done via CYP2A6 and 1A2 (8–10). Approximately 5% is excreted unchanged in the urine. Although some of the non-



Ondansetron (stereocenter marked with *)



8-Hydroxyondansetron

Figure 1. Structures of ondansetron and its main metabolite 8-hydroxyondansetron.

conjugated metabolites have pharmacological activity, their plasma concentrations are probably too low to contribute to the biological activity of ondansetron.

In pharmacokinetic studies ondansetron concentration was measured using achiral procedures. However, ondansetron is usually be marketed as a racemic mixture of the *R*-(–)- and *S*-(+)-enantiomers. Considering the parallel rightward displacement of the 2-methyl-5-HT concentration response curve at the longitudinal smooth muscle of the Guinea-pig ileum, the potency of *R*-(–)-ondansetron was approximately eightfold higher than that of *S*-(+)-ondansetron (11). Also, administration of *R*-(–)-ondansetron avoided adverse effects including headache, constipation, and increases in transaminase levels, which are associated with the administration of racemic ondansetron (12).

Besides a number of achiral procedures for the measurement of ondansetron in plasma samples (13–17), only a few chiral methods have been reported for the determination of *R*-(–)- and *S*-(+)-ondansetron up to now. These include high performance capillary electrophoresis with heptakis-(2,6-di-O-methyl)-β-cyclodextrin as a mobile phase modifier (18) as well as high-performance liquid chromatography coupled with ultraviolet-spectroscopy (HPLC–UV) using a Chiralcel OD column (19) or a Chiracel OD-R column (20). Recently a liquid chromatographic–tandem mass spectrometric (LC–MS–MS) procedure using an ovomucoid column was developed for the enantioselective measurement of ondansetron in human plasma samples (21).

The aim of the present work was the development of a chiral LC–MS–MS procedure for the quantitative determination of *R*-(–)- and *S*-(+)-ondansetron together with *R*-(–)- and *S*-(+)-8-hydroxyondansetron in postoperative patients receiving an antiemetic medication to proof possible stereoselective pharmacokinetic differences between both enantiomers.

Experimental

Chemicals

The (+)- and (–)-enantiomers of ondansetron and 8-hydroxyondansetron were a gift of GlaxoSmithKline (München, Germany). Racemic propanolol was purchased from LGC Promochem™ (Wesel, Germany). Triethylamine (TEA) was obtained from Fluka (Neu-Ulm, Gemany), ethanol absol. and sodium hydroxide were from Merck (Darmstadt, Germany), and 95% *n*-hexane and dichloromethane were from J.T. Baker (Deventer, Netherlands).

Standard solutions and calibration samples

A methanolic stock solution containing *R*-(–)- and *S*-(+)-ondansetron and *R*-(–)- and *S*-(+)-8-hydroxyondansetron as well as a stock solution containing (±)-propanolol were prepared in methanol in concentrations of 10 µg/mL. All solutions were stored at –20°C. For calibration mixed spiked plasma samples were created in following concentrations for each enantiomer: 2.5, 10, 25, 50, 75, 100, 200, and 250 ng/mL.

Sample preparation

A mixture of 0.2-mL plasma sample, internal standard solution (10 µL of propanolol in a concentration of 10 µg/mL methanol), and 0.2 mL of borate buffer (pH 9) was extracted with 1 mL dichloromethane. After centrifugation (4000 × *g* for 8 min), the organic phase was evaporated to dryness under a stream of nitrogen at 50°C. The residue was dissolved in 0.1 mL of HPLC mobile phase A, and a 10-µL aliquot was used for chromatography.

Chromatographic and MS equipment and conditions

An LC–MS–MS system consisting of an Agilent (Waldbronn, Germany) 1100 HPLC system (binary pump, degasser, and autosampler) coupled with an Applied Biosystems (Darmstadt, Germany) API 2000 triple-quadrupole MS was used for analysis performing atmospheric pressure chemical ionization (LC–APCI-MS). The enantiomeric separation was achieved on a Chiralcel OD-R column (10 µm, 250 × 46 mm; Daicel Chemical Industries, Illkirch, France) containing cellulose tris-(3,5-dimethylphenylcarbamate) as chiral selector. LC separation was performed using mobile phase A (water/acetonitrile, 98:2, 5 mM ammonium formate) and mobile phase B (water/acetonitrile, 10:90, 5 mM ammonium formate; pH 3.5) in a gradient program with a total flow of 400 µL/min (binary system): 0–15 min: 85% A; 15–16 min: 85% A → 0% A; 16–21 min: 0% A; 21–22 min: 0% A → 85% A; 22–30 min: 85% A. From the molecular ions ([M+H$^+$]) following transitions were detected in positive multiple reaction monitoring: ondansetron *m/z* 293.8 → 170.0, 184.0; 8-hydroxyondansetron *m/z* 309.9 → 185.9, 199.8; propanolol (IS) *m/z* 259.95 → 116.1, 182.9 (quantitation ions underlined).

For quantification, peak-area ratios of the analytes to the internal standard were calculated as a function of the concentration of the substances.

Validation protocol

Selectivity. For evaluation of method selectivity, blank plasma samples from different sources were prepared as described, but without adding any analyte or internal standard mix (n = 6). Additionally, six samples were analyzed from patients receiving the same medication with metoclopramide instead of ondansetron. Furthermore, blank samples were analyzed to check the absence of analyte ions in the respective peaks of the internal standard (n = 2).

Linearity. For calibration, plasma samples were spiked with the *R*-(–)- and *S*-(+)-enantiomers of ondansetron and 8-hydroxyondansetron in concentrations from 2.5 to 250 ng/mL and processed as described (n = 6). Using mean values, calibration curves were checked for variance homogeneity (F-Test) and for linearity (Mandel-Test).

Sensitivity. The LOD was defined as the analyte concentration at which the signal-to-noice ratio was 3. The LOQ was defined as three times the LOD.

Precision data and recoveries. Additionally, spiked quality control (QC) plasma samples (high = 100 ng/mL, middle = 50 ng/mL, and low = 10 ng/mL) were prepared and analyzed in each series. Absolute recoveries were calculated comparing the peak areas of all analytes after extraction with the results

after injection of the same concentration (50 ng/mL) directly without extraction, which was defined as 100%.

Matrix effects. Matrix-related ionization effects were evaluated by comparing analyte responses of post-extraction spiked samples to those of spiked samples representing 100% recovery. Five replicates at low (10 ng/mL) and high (100 ng/mL) concentration were analyzed.

Stability studies. For the evaluation of the enantiometic stability, blank plasma samples were spiked with 50 ng/mL (each enantiomer in single tests) and incubated at 25°C for 24 h, then extracted and analyzed as described. Furthermore long-term stability of all analytes was tested at –20°C for three months as well as the autosampler stability after extraction (ready-to-inject samples) at 20°C for 24 h. The freeze/thaw stability was evaluated after three complete freeze/thaw cycles on consecutive days. The analytes were considered stable when 85–115% of the initial concentration was found.

Application of the method

After approval by the local ethics committee and written informed consent plasma concentrations of the *R*-(–)- and *S*-(+)-enantiomers of ondansetron as well as of 8-hydroxyondansetron were analyzed in patients recovering from major abdominal surgery. General anesthesia was conducted using a standardized protocol: 2 mg/kg propofol, 0.2 mg fentanyl, and *cis*-atracurium for induction and remifentanil, isoflurane, and *cis*-atracurium for maintenance of anesthesia. About 30 min before termination of anesthesia, 4 mg ondansetron was given i.v. followed by an analgesic loading dose of dipyrone 1 g and piritramid 0.08 mg/kg.

Blood samples were obtained with ethylenediaminetetraacetic acid (EDTA) as anticoagulant between 30 and maximal 180 min after application of ondansetron. Whole blood was centrifuged and plasma was separated and frozen until analysis at –80°C.

Results and Discussion

The method's selectivity was tested by comparing the chromatograms of six different blank plasma samples with corresponding spiked plasma. Also, two blank samples were analyzed to check the absence of analyte ions in the respective peaks of the internal standard. Additionally, six samples were analyzed from patients recovering from major abdominal surgery and receiving the same medication with metoclopramide instead of ondansetron. No interferences due to matrix or other potentially interfering compounds were found.

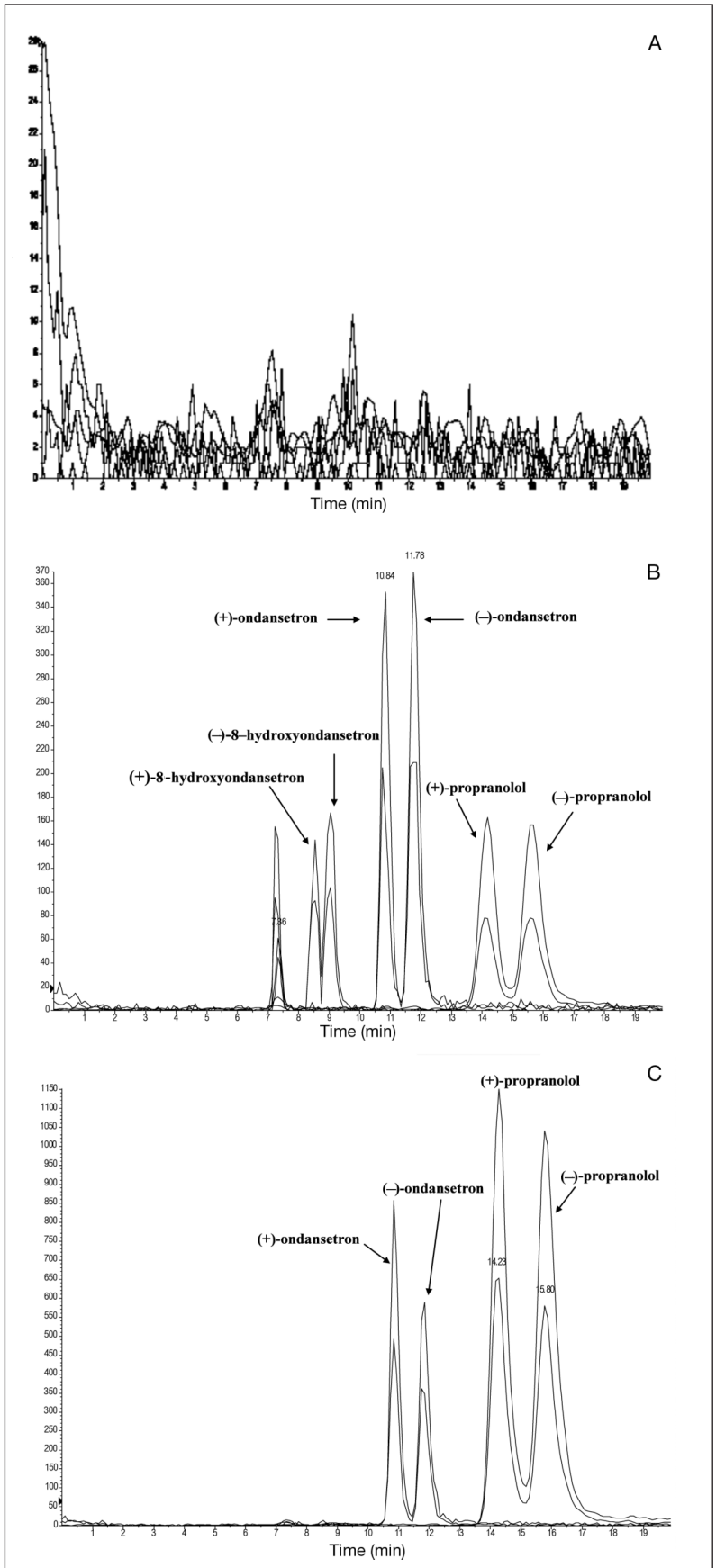


Figure 2. Chromatogram of a blank sample (A), a standard sample containing 100 ng/mL ondansetron and 8-hydroxyondansetron enantiomers (B), and finally of an authentic plasma sample containing *S*-(+)-ondansetron (55.2 ng/mL) and *R*-(–)-ondansetron (45.3 ng/mL) (C).

A complete enantiomeric separation of the *R*-(–)- and *S*-(+)-enantiomers of ondansetron and the racemic internal standard was obtained on a cellulose tris-3,5-dimethylphenylcarbamate OD-R column using an ammonium formate buffer/acetonitrile as mobile phase. Figure 2 shows chromatograms of a blank sample, an extracted standard sample with the *R*-(–)- and *S*-(+)-enantiomers of ondansetron and 8-hydroxyondansetron, and at least an authentic plasma sample extract only containing racemic ondansetron. The order of elution of the enantiomers was determined by separately injecting solutions of each enantiomer. The linear regression analysis yielded following equations:

R-(–)-ondansetron: $y = 0.106x + (–1.49)$ ($r = 1.0000$)
S-(+)-ondansetron: $y = 0.0555x + (–0.45)$ ($r = 0.9998$)
R-(–)-8-hydroxyondansetron: $y = 0.0164x + (–0.254)$ ($r = 0.9986$)
S-(+)-8-hydroxyondansetron: $y = 0.0342x + (0.148)$ ($r = 0.9991$)

The LODs of *R*-(–)- and *S*-(+)-ondansetron were 0.5 and 0.7 ng/mL with LOQs of 1.5 and 2.1 ng/mL. The LOD of *R*-(–)- and *S*-(+)-8-hydroxyondansetron was 0.6 ng/mL with an LOQ of 1.8 ng/mL. Intraassay ($n = 6$) and interassay precision ($n = 10$; two calibration curves during the study) showed acceptable values with relative standard deviations (R.S.D.) of less than 10 % for the high and middle control and less than 20% for the low control. For the ondansetron and 8-hydroxyondansetron enantiomers the recovery was between 85 and 91% ($n = 5$). Ion suppression and enhancement from plasma matrix was negligible under present conditions (84–95%). The enantiomers and metabolites were found to be stable in plasma at –20°C for 3 months, in the mobile phase at 20°C for 24 h, and during three freeze/thaw cycles. No chiral inversion was observed at 25°C for 24 h during storage, processing, and analysis. To sum up validation data were within international required limits and the assay was successfully applied to authentic plasma samples.

The analysis of 151 samples from patients routinely receiving intravenous 4 mg racemic ondansetron revealed positive results for *R*-(–)- and *S*-(+)-ondansetron in all analyzed samples. Without hydrolysis the main metabolite 8-hydroxyondansetron was not detected in any of the analyzed samples. Mean and median plasma concentrations, the concentration ranges, and the ratios *S*-(+)-/*R*-(–)-ondansetron are described in Table I.

Table I. Plasma Concentrations of *S*-(+)- and *R*-(–)-Ondansetron in 151 Plasma Samples from Postoperative Patients

	S-(+)-Ondansetron (ng/mL)	*R*-(–)-Ondansetron (ng/mL)	Sum *S*-(+)- + *R*-(–)-Ondansetron (ng/mL)	Ratio *S*-(+)-/*R*-(–)-Ondansetron
Mean	38.70	24.93	63.63	2.60
SD	11.59	21.21	22.57	1.52
Median	39.10	16.00	56.10	3.06
Min	8.04	4.15	33.95	0.14
Max	82.20	84.30	157.50	7.18

These present results from postoperative patients receiving ondansetron for postoperative treatment of PONV demonstrated a great interindividual variability in drug concentrations and, therefore, a wide therapeutic concentration range; the body mass index was not helpful to understand such differences in plasma concentrations. The measured concentrations of both ondansetron enantiomers (sums) are in the range described by others (13,22). According to previous reports free 8-hydroxyondansetron was not found in any case with an LOD of 0.6 ng/mL; a hydrolysis prior to analysis was not performed in the present study.

In general, large differences have been observed between individuals in the capacity and speed of drug metabolism. This variability may be due to concurrent diseases (e.g., renal failure, impaired liver function), co-medications, environmental pollutants that influence the enzymatic function of specific isoenzymes as well as other factors. Further well-recognized variables are genetic polymorphisms of metabolising enzymes or targets of drug action which can have a significant impact on pharmacokinetic/pharmacodynamic of respective drugs. Genetic variants of the cytochrome P450 (CYP) enzymes play a major role in pharmacokinetic variability and can affect outcome in drug therapy. Individuals carrying alleles reducing enzyme activity may have a phenotype that may metabolize a given drug poorly [poor metabolizer (PM)] or at an intermediate level [intermediate metabolizer (IM)] compared to extensive metabolizers (EM) carrying two wild-type alleles. A gene duplication or multiduplication can result in largely increased enzyme activity resulting in the ultrarapid metabolizer (UM) phenotype.

Considering ondansetron, there was no difference in the AUC, C_{max}, and $t_{½}$ in healthy subjects between EMs ($n = 6$) and PMs ($n = 6$) receiving a single dose (8 mg i.v.) (23). Otherwise, it was demonstrated that CYP2D6 UMs experienced more vomiting compared to EMs or PMs when given ondansetron in the treatment of chemotherapy-induced nausea and vomiting (24). But this difference was predictably more pronounced for topisetron than ondansetron because topisetron is primarily dependent on the CYP2D6 isoenzyme for metabolism. In a further study of 250 patients undergoing standardized general anesthesia UMs had increased therapeutic failure and a reduced response to ondansetron 4 mg (25). However, plasma concentrations were not determined in this study.

Data on enantiomeric disposition of ondansetron are sparse. Otherwise, stereoselective variations could be of interest in individual cases because of different pharmacodynamic properties of both enantiomers. In general a stereoselectivity can be assumed in the body distribution, the renal clearance, and also in the hepatic metabolism of drugs.

In a stereoselective pharmacokinetic study involving four healthy male Chinese, Liu et al. (21) found that the concentration of *R*-(–)-ondansetron was higher than of *S*-(+)-ondansetron at all the time points measured, implying a stereoselective disposition of ondansetron enantiomers. In contrast to this study, the present investigation enrolling a large collective of patients in a European hospital revealed interindividual differences in the concentration ratio of *S*-(+)-/*R*-(–)-

ondansetron between 0.14 and 7.18. These different findings could be explained by different study designs, volunteers without any co-medication versus patients receiving various drugs for anesthesia and suffering in part from diverse co-morbidity, as well as varying geographic distributions and frequencies of genetic variants in populations from different regions of the world (26).

Considering these preliminary results, CYP2D6 and CYP3A genotype dependent metabolism of ondansetron enantiomers might be of specific interest, because effects on efficacy of antiemetic treatment with ondansetron might be possible. Meanwhile, CYP dependent metabolism of a large amount of frequently used drugs is well described (27) with numerous reports describing lacking efficacy, side effects, or even toxicity of individual drugs. Multiple interactions in pharmacokinetics resulting in adverse drug reactions (ADRs) and outcome in drug treatment can be assumed (28).

In general, pharmacogenetics can help to individualize pharmacotherapy and to improve care by predicting the optimal dose and avoiding side effects and toxicity in individual patients (29). Screening for variations in the expression of drug metabolizing enzymes has been suggested as a potential tool for improving therapy regimes (30,31). Further candidate genes like receptors, transporters and other targets of pharmacotherapy are currently under investigation. Aspects of genetic differences influencing efficacy, side effects and adverse outcome of pharmacotherapy will be of importance for future medication regimes.

Conclusions

A quantitative determination of ondansetron in human plasma by enantioselective LC–MS–MS was achieved on a Chiralcel OD-R column containing cellulose tris-(3,5-dimethylphenylcarbamate). The method was fully validated and successfully applied to authentic plasma samples. Quantitative results from postoperative patients receiving ondansetron demonstrated a great interindividual variability in plasma drug concentrations and, therefore, a wide therapeutic concentration range. The main metabolite 8-hydroxyondansetron was not detected in its unconjugated form. A wide variation in the *S*-(+)-/*R*-(–)-ondansetron concentration ratio is indicative for a stereoselective disposition or metabolism. In further studies, CYP-dependent metabolism of ondansetron enantiomers and clinical efficacy of the medication should be tested.

Acknowledgments

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