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Short communication

Analysis of unbound plasma concentration of oxcarbazepine and the 10-hydroxycarbazepine enantiomers by liquid chromatography with tandem mass spectrometry in healthy volunteers

Natalícia de Jesus Antunes [a], Lauro Wichert-Ana [b], Eduardo Barbosa Coelho [b], Oscar Della Pasqua [c,d], Veriano Alexandre Junior [e], Osvaldo Massaiti Takayanagui [e], Maria Paula Marques [a], Vera Lucia Lanchote [a,*]

[a] *Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil*
[b] *Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil*
[c] *Division of Pharmacology, Leiden Academic Centre for Drug Research, Leiden University, Leiden, The Netherlands*
[d] *University College London, School of Life and Medical Sciences, London, UK*
[e] *Departamento de Neurociências e Ciências do Comportamento, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil*

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ABSTRACT

This study describes the development and validation of a method for the analysis of unbound plasma concentrations of oxcarbazepine (OXC) and of the enantiomers of its active metabolite 10-hydroxycarbazepine (MHD) [S-(+)- and R-(−)-MHD] using liquid chromatography with tandem mass spectrometry (LC–MS/MS). Additionally, the free fraction of the drug is described in healthy volunteers (n = 12) after the oral administration of 300 mg OXC/12 h for 5 days. Plasma aliquots of 200 μL were submitted to ultrafiltration procedure and 50 μL of the ultrafiltrate were extracted with a mixture of *tert*-butyl methyl ether:dichloromethane (2:1, v/v). OXC and the MHD enantiomers were separated on a OD-H chiral phase column. The method was linear in the range of 4.0–2.0 μg/mL for OXC and of 20.0–6.0 μg/mL plasma for the MHD enantiomers. The limit of quantification was 4 ng for OXC and 20 ng for each MHD enantiomer/mL plasma. The intra- and inter-day precision and inaccuracy were less than 15%. The free fraction at the time of peak plasma concentration of OXC was 0.27 for OXC, 0.37 for S-(+)-MHD and 0.42 for R-(−)-MHD. Enantioselectivity in the free fraction of MHD was observed, with a higher proportion of R-(−)-MHD compared to S-(+)-MHD.

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1. Introduction

Oxcarbazepine (OXC) is considered a prodrug and part of its anticonvulsant effect depends on its active 10-hydroxycarbazepine (MHD) metabolite which is formed by the rapid presystemic reduction of OXC. MHD contains a chiral center at position 10, but the R-(−)- and S-(+)-MHD enantiomers exert similar anticonvulsant effects in animal models [1–5]. The kinetic disposition of the MHD metabolite is enantioselective in healthy volunteers after administration of a single oral dose of OXC, with an area under the plasma concentration versus time curve (AUC) S-(+)/R-(−) ratio of 3.8 [6].

The binding of drugs to plasma proteins affects different pharmacokinetic and pharmacodynamic parameters since only the free concentration is available for distribution, elimination and receptor interaction [7,8]. Plasma protein binding of chiral drugs can be enantioselective, affecting the pharmacological activity and pharmacokinetic profile of these drugs [9]. In patients with trigeminal neuralgia, the percentage of plasma protein binding was approximately 59% for OXC and 39% for the MHD metabolite [10]. Plasma protein binding of MHD administered as enantiomeric mixture to epileptic patients was 40% using equilibrium dialysis and 45% using ultrafiltration [11], while *in vitro* studies report values of 30% for both MHD enantiomers in rat and human plasma [12].

* Corresponding author at: Faculdade de Ciências Farmacêuticas de Ribeirão Preto-USP Departamento de Análises Clínicas, Toxicológicas e Bromatológicas, Avenida do Café s/n, Campus da USP, 14040-903, Ribeirão Preto, SP, Brasil.
E-mail address: lanchote@fcfrp.usp.br (V.L. Lanchote).

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Table 1
Validation of the analysis method of unbound oxcarbazepine in plasma.

	Unbound oxcarbazepine
Linearity	4.0 ng/mL–2.0 μg/mL
Equation of the line	Y = 2.2194x + -0.0009
r	0.9981
Limit of quantitation (ng/mL)	4.0
Precision (CV%, n = 10)	10.52
Accuracy (% Inaccuracy)	8.06
Intra-assay precision (CV%)	
4.0 ng/mL (n = 5)	9.4
12.0 ng/mL (n = 5)	5.79
0.6 μg/mL (n = 5)	3.40
0.96 μg/mL (n = 5)	7.93
1.6 μg/mL (1:1) (n = 5)	9.69
Interassay precision (CV%)	
4.0 ng/mL (n = 5)	9.18
12.0 ng/mL (n = 5)	7.70
0.6 μg/mL (n = 5)	9.50
0.96 μg/mL (n = 5)	7.59
1.6 μg/mL (1:1) (n = 5)	9.16
Intra-assay accuracy (RSE%)	
4.0 ng/mL (n = 5)	9.37
12.0 ng/mL (n = 5)	−12.17
0.6 μg/mL (n = 5)	−9.8
0.96 μg/mL (n = 5)	−12.62
1.6 μg/mL (1:1) (n = 5)	−14.16
Interassay accuracy (RSE%)	
4.0 ng/mL (n = 5)	10.17
12.0 ng/mL (n = 5)	−10.82
0.6 μg/mL (n = 5)	−3.55
0.96 μg/mL (n = 5)	−13.48
1.6 μg/mL (1:1) (n = 5)	−11.66

Coefficient of variation (CV) = [(SD/mean) × 100]; r = linear correlation coefficient; % Relative Standard Error (RSE) = $[(C_{obs} - C_{nominal})/C_{nominal}] \times 100$.

Table 2
Validation of the analysis method of unbound MHD enantiomers in plasma.

	Unbound R-(−)-MHD	Unbound S-(+)-MHD
Linearity	20.0 ng/mL–6.0 μg/mL	20.0 ng/mL–6.0 μg/mL
Equation of the line	y = 0.4039x + 0.0019	y = 0.4286x + 0.0012
r	0.9988	0.9992
Limit of quantitation (ng/mL)	20.0	20.0
Precision (CV%, n = 10)	6.84	9.20
Accuracy (% Inaccuracy)	−9.83	−3.28
Intra-assay precision (CV%)		
20.0 ng/mL (n = 5)	7.71	10.20
60 ng/mL (n = 5)	14.52	8.48
3.0 μg/mL (n = 5)	7.52	8.09
4.0 μg/mL (n = 5)	12.3	8.74
8.0 μg/mL (1:1) (n = 5)	12.29	8.16
Interassay precision (CV%)		
20.0 ng/mL (n = 5)	1.89	1.90
60 ng/mL (n = 5)	5.44	1.25
3.0 μg/mL (n = 5)	3.05	2.37
4.0 μg/mL (n = 5)	13.69	1.42
8.0 μg/mL (1:1) (n = 5)	4.19	4.53
Intra-assay accuracy (RSE%)		
20.0 ng/mL (n = 5)	7.71	10.20
60 ng/mL (n = 5)	14.52	4.45
3.0 μg/mL (n = 5)	−7,61	−5.91
4.0 μg/mL (n = 5)	−6.98	−2.93
8.0 μg/mL (1:1) (n = 5)	−13.51	−12.76
Interassay accuracy (RSE%)		
20.0 ng/mL (n = 5)	1.27	6.50
60 ng/mL (n = 5)	9.23	9.91
3.0 μg/mL (n = 5)	2.89	2.59
4.0 μg/mL (n = 5)	13.50	10.94
8.0 μg/mL (1:1) (n = 5)	1.55	−1.31

Coefficient of variation (CV) = [(Standard deviation/mean) × 100]; r = linear correlation coefficient; % Relative Standard Error (RSE) = $[(C_{obs} - C_{nominal})/C_{nominal}] \times 100$.

The methods for the separation of the unbound concentration of OXC and MHD described so far have used equilibrium dialysis or ultrafiltration [11,12], followed by HPLC with ultraviolet (UV) detection, for OXC and MHD as enantiomer mixture [10,11] or for the MHD enantiomers [12]. Regarding the methods for analysis of total concentration of oxcarbazepine and MHD enantiomers in plasma using LC–MS/MS, the quantification limit reported ranged from 12.5 to 50.0 ng for OXC and of 31.5 ng to 50 ng for each MHD enantiomer/mL plasma [13–15].

There are no clinical data on the plasma protein binding of individual MHD enantiomers. The present study describes the development and validation of a method for the sequential analysis of the unbound concentration of OXC and MHD enantiomers in plasma using ultrafiltration and liquid chromatography coupled to mass spectrometry (LC–MS/MS). The method showing a quantification limit of 4.0 ng for OXC and of 20.0 ng for each MHD enantiomer/mL plasma, so far the most sensitive one, was used for analysis of the free fraction of the drug in plasma samples collected at the time of peak plasma concentration (t_{max}) from healthy volunteers after the oral administration of 300 mg OXC/12 h for 5 days.

2. Materials and methods

2.1. Analysis of the unbound concentration of OXC and of the MHD enantiomers

2.1.1. Standard solutions and reagents

Oxcarbazepine (99.6%) was purchased from USP (Rockville, USA) and the racemic MHD metabolite (98%) from Toronto Research Chemicals (North York, Canada). 4-Methylprimidone (internal standard, IS) was purchased from Sigma (St. Louis, MO, USA). The solvents dichloromethane and *tert*-butyl methyl ether were obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA), hexane, methanol and ethanol from Panreac Química SAL (Barcelona, Spain), and isopropanol from Tedia Way (Fairfield, USA). All solvents were of chromatographic grade. Ammonium acetate was obtained from Mallinckrodt Baker (Phillipsburg, Xalostoc, Mexico). The water used in the experiment was purified with the Sinergy UV® system (Millipore, Molsheim, France).

Stock solutions were prepared in methanol at a concentration of 100 μg OXC/mL and 200 μg MHD/mL. Dilutions were then prepared to obtain the working solutions at concentrations of 0.008, 0.02, 0.04, 0.2, 0.4, 0.8, 1.6, 2.4 and 4 μg OXC/mL methanol and of 0.08, 0.2, 0.4, 2.0, 4.0, 8.0, 16, 24 and 40 μg MHD/mL methanol. The 4-methylprimidone solution was prepared at a concentration of 200 μg/mL methanol and diluted to 40 μg/mL.

2.1.2. Sample preparation

Aliquots (200 μL) of blank plasma or plasma samples were transferred to a Centrifree® ultrafiltration device (Millipore, Carrigtwohill, Ireland). The plasma ultrafiltrate was obtained by centrifugation of the samples at 1875 g for 40 min in a centrifuge with a fixed-angle rotor (angle of 36°) (model NT 825, Nova Técnica, Piracicaba, Brazil) refrigerated at 4 °C.

Aliquots (50 μL) of the ultrafiltrate were spiked with 25 μL of the IS and extracted with 2 mL of a mixture of *tert*-butyl methyl ether:dichloromethane (2:1, v/v). The tubes were shaken for 40 min in a horizontal shaker (Marconi desktop reciprocating shaker, model MA 139/CTF) and then centrifuged for 10 min at 1275 g (Hitachi® refrigerated centrifuge, model CF8DL, Tokyo, Japan). The organic phases were separated and concentrated in a vacuum evaporation system (Christ®, model RVC 2-25 CD plus, Funkentstörungsgrad, Germany). The residues obtained were

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