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Short communication

Simultaneous determination of remacemide hydrochloride and desglycinylremacemide (AR-R12495XX) in brain tissue by high-performance liquid chromatography

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Abstract

Remacemide hydrochloride, a novel anticonvulsant agent, and its major active metabolite, desglycinylremacemide, were measured simultaneously in brain tissue by high-performance liquid chromatography with UV detection. Intra- and inter-assay variations for remacemide (1, 5, 10 μg/ml) were 5.1, 10.5 and 3.1% and 3.1, 4.0 and 1.3%, respectively. Intra- and inter-assay variations for desglycinylremacemide (1, 5, 10 μg/ml) were 4.2, 3.8 and 8.4% and 7.9, 8.8 and 3.1%, respectively. Limits of detection and quantification for both analytes were 4 and 31 ng/ml, respectively, with recovery consistently ≥85%. This reliable assay has applications in the pre-clinical neuropharmacokinetic and neuropharmacodynamic investigation of remacemide hydrochloride.

Keywords: Remacemide; Desglycinylremacemide

1. Introduction

Remacemide hydrochloride (RMD; (±)-2-amino-*N*-(1-methyl-1,2-diphenylethyl) acetamide hydrochloride; Fig. 1) is a novel, water-soluble compound with potential applications in epilepsy, Parkinson's disease and stroke. It is currently undergoing phase 3 clinical trials in epileptic patients with generalised tonic/clonic and complex partial seizures, in addition to preliminary investigations in cerebral ischaemia [1].

RMD undergoes desglycination to yield the more active metabolite AR-R12495XX (desglycinylremacemide; DGR; Fig. 1). Both compounds have been shown to inhibit the sustained repetitive firing of sodium-dependent action potentials in cultured

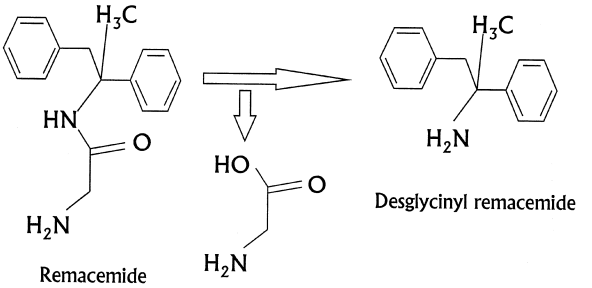

Fig. 1. Conversion of remacemide to desglycinylremacemide.

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neurones, with the metabolite being over 6-fold more potent than the parent compound [2]. In addition to an action on sodium channels, RMD and DGR have been proposed to possess weak channel blocking activity at the *N*-methyl-D-aspartate sub-type of glutamate receptor [3], a mechanism which may contribute to the reported cerebroprotectant properties of the drug [1]. Receptor binding studies have shown that both RMD and DGR displace [^{3}H]dizocilpine (MK-801) binding from synaptic membrane fractions of rat cerebral cortex and hippocampus [4], with DGR being 100-fold more potent in this respect.

The following high-performance liquid chromatography (HPLC) assay was developed as a tool for the pre-clinical investigation of concentration–effect relationships with remacemide and has been adapted from the previously published human plasma methodology [5,6].

2. Experimental

2.1. Equipment

The chromatography equipment consisted of a Gilson 231 sample injector and Gilson 401 dilutor (Anachem, Bedfordshire, UK), a Shimadzu SPD-6A UV spectrophotometric detector (218 nm), a Shimadzu LC-10AT liquid chromatograph and a Shimadzu C-R6A chromatopac recorder (all Shimadzu Europa, Milton Keynes, UK). The HPLC column was a Sphereclone C_6 (100×4.6 mm; 3 μm), obtained from Phenomenex (Cheshire, UK), and used at room temperature. Solid-phase extraction columns (100 mg; 1-ml columns) were obtained from IST (Mid Glamorgan, UK). Analysis of the results was carried out using MINITAB for Windows (version 10.1) on an Elonex PC-5120/1 microcomputer.

2.2. Reagents

RMD, DGR and internal standard, 2-(methylamino)-*N*-[1-phenyl-1-(phenylmethyl)propyl]-acetamide hydrochloride were obtained from AstraZeneca R&D Charnwood, UK. Solvents (HPLC grade) were obtained from Fisher Scientific (Loughborough, UK) and all other chemicals (reagent grade) were obtained from BDH (Leicestershire, UK).

2.3. Standards and solutions

Aqueous stock solutions of RMD (0.5 mg/ml), DGR (0.5 mg/ml) and internal standard (0.2 mg/ml) were prepared, aliquoted and stored at −70°C until required. Two monobasic potassium phosphate buffers (0.5 and 0.05 *M*) were prepared and the pH adjusted to 2.5 and 3.3, respectively, with 25% H_3PO_4. Mobile phase (acetonitrile–0.05 *M* potassium phosphate buffer, 29:71) and working standard solutions of RMD and DGR were prepared daily as required. Prior to use, the mobile phase was passed through an aqueous filter (pore size 0.45 μm; Millipore, France) and degassed with helium. The flow-rate of the mobile phase was 1 ml/min.

2.4. Tissue preparation

Naïve mouse brains (adult male ICR mice; 25–30 g; Harlan Olac, Bicester, UK) were homogenised at high speed (Polytron PT 1200 CL, Philip Harris Scientific, Staffordshire, UK) for 15 s in 15 volumes (v/w) of 1% perchloric acid. Thereafter, internal standard (50 μl of 0.2 mg/ml stock) was added. Homogenates were vortex mixed and then centrifuged at 800 *g* for 15 min. One ml of the supernatant was decanted into a tube containing 1.5 ml of 0.5 *M* potassium phosphate buffer and tubes mixed gently prior to extraction.

2.5. Solid-phase extraction

Benzenesulfonic acid (SCX) columns (pore size Å54) were used for the extraction procedure, as previously described [5]. The columns were conditioned immediately prior to use by 1 ml methanol, 1 ml elution fluid (acetonitrile–0.05 *M* sodium bicarbonate, 60:40) and 2 ml 0.05 *M* potassium phosphate buffer. Individual samples were added and washed with 1 ml 0.05 *M* potassium phosphate buffer. The columns were then rinsed with 1.5 ml methanol followed by 2 ml distilled water and allowed to dry. The analytes of interest were subsequently eluted from the columns into Pyrex borosilicate glass culture tubes (10×75 mm) with

3×0.25 ml elution fluid, the columns being allowed to dry between each application. Finally, the culture tubes were vortex mixed, contents transferred into glass auto-sampler vial inserts, and 75 μl injected into the chromatography system.

2.6. Validation

The method was validated on the basis of intra- and inter-assay variations. Pooled blank brain homogenate was spiked with either RMD (1, 5 or 10 μg/ml) or DGR (1, 5 or 10 μg/ml) and aliquoted appropriately. Five aliquots of each compound (at each concentration) were extracted and analysed on day 1 to determine intra-assay variation. Thereafter, one aliquot of each compound (at each concentration) was analysed each day for the next 5 days to determine inter-assay variation. Limits of detection and quantification were determined in pooled blank brain homogenates spiked with a range of concentrations (0.5–100 ng/ml) of RMD and DGR. The minimum concentrations, of both compounds, yielding visible and measurable peaks were acknowledged as the limits of detection and quantification, respectively.

Table 1
Intra- and inter-assay variations

	Spiked concentration (μg/ml)	Intra-assay variation (%)	Inter-assay variation (%)
RMD	1	5.1	3.1
	5	10.5	4.0
	10	3.1	1.3
DGR	1	4.2	7.9
	5	3.8	8.8
	10	8.4	3.1

3. Results

RMD, DGR and the internal standard showed good separation from each other and the solvent front (Fig. 2) with retention times for RMD, DGR and internal standard of approximately 6.0, 4.3 and 8.8 min, respectively. Recoveries from brain tissue were shown to be consistently ≥85%. Intra- and inter-assay variations were within an acceptable range (Table 1). Limits of detection and quantification for both RMD and DGR were found to be 4 and 31 ng/ml, respectively.

The method was employed to determine the

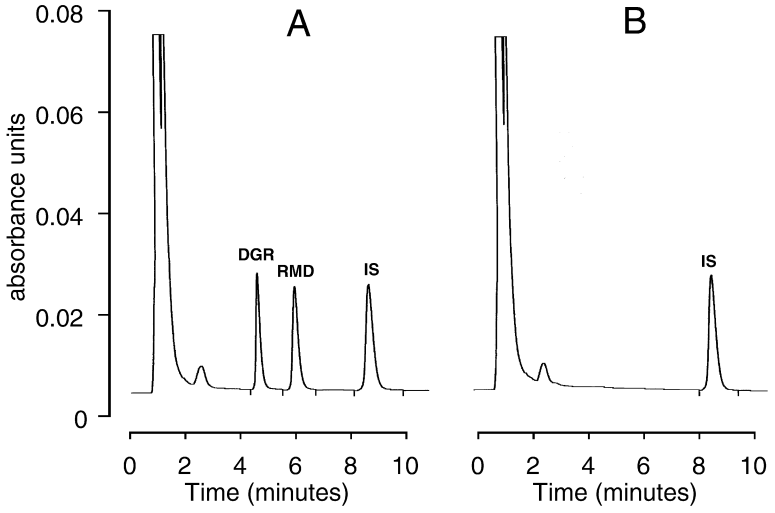

Fig. 2. (A) Sample chromatogram from an animal injected with 50 mg/kg remacemide (RMD) and sacrificed at 1 h post-injection. Mean (±S.E.M.) concentrations (n=4) of RMD and desglycinylremacemide (DGR) were 1.02 μg/ml (±0.04) and 1.65 μg/ml (±0.19), respectively. (B) Sample chromatogram from a non-treated control animal (I.S., internal standard).

concentrations of RMD and DGR in the brains of mice ($n=4$), sacrificed 1 h after intraperitoneal administration of 50 mg/kg RMD (in 0.9% saline). A sample chromatogram from one such animal is shown in Fig. 2. The mean concentrations of RMD and DGR (±S.E.M.) were found to be 1.02 μg/ml (±0.04) and 1.65 μg/ml (±0.19), respectively.

Assay selectivity was assessed by the addition of several experimental and antiepileptic agents that have the potential to be co-administered in future pre-clinical and clinical investigations of RMD. When injected directly into the HPLC system, peaks were observed with lamotrigine, carbamazepine, MK-801, sodium valproate, tiagabine, phenytoin and phenobarbitone. Following extraction only peaks from lamotrigine (3.1 min), carbamazepine (3.6 min) and MK-801 (3.8 min) remained (chromatograms not shown).

Table 2
Agents used in selectivity test

Agent	Concentration (μg/ml)
Carbamazepine	10
Lamotrigine	5
MK-801	1
Tiagabine	10
Gabapentin	10
Topiramate	20
Clobazam	1
N-methyl-D-aspartate	250
Phenobarbitone	50
Sodium valproate	50
Phenytoin	25

4. Discussion

The above method proved to be a simple, consistent and reliable method for the simultaneous measurement of RMD and its major active metabolite, DGR, in brain tissue. This procedure differs slightly from the previously published plasma methodology [5] in that silanization of the glassware was deemed unnecessary and centrifugation and subsequent extraction of the samples appears to have removed the majority of background interference. There are also differences in percentage recoveries, although this is hardly surprising when comparing plasma and tissue extractions of a partially protein bound compound [7,8].

Although similar, the method described above has applications beyond the previously published plasma/urine methodology. Pre-clinical studies of antiepileptic drugs often fail to consider the brain concentrations at which the candidate compounds exert their pharmacological effects. Determination of effective concentrations in experimental animals has important implications in the selection of suitable dose regimens in any future clinical development of the respective agent.

In the selectivity experiments, compounds were chosen on the basis that they have the potential to be co-administered in future investigations. The concentrations tested (Table 2) were chosen on the basis of expected experimental/therapeutic plasma and/or brain concentrations. Despite extraction, peaks were still observed with lamotrigine, carbamazepine and MK-801. However, these peaks did not interfere with the chromatography of the analytes of interest.

In conclusion, this simple and reliable method should prove to be a useful tool in the pre-clinical evaluation of RMD. Its applications in the investigation of both concentration–effect relationships and antiepileptic drug interactions may help to unravel the complexities of the mechanism(s) of RMD action [9] and shed further light on its likely clinical efficacy [10,11].

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