Journal of Pharmaceutical and Biomedical Analysis 117 (2016) 276–288

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Urinary excretion and metabolism of miglustat and valproate in patients with Niemann–Pick type C1 disease: One- and two-dimensional solution-state ^{1}H NMR studies

Fay Probert[a], Victor Ruiz-Rodado[a], Xiaoyu Zhang[a,c], Danielle te Vruchte[b], Tim D.W. Claridge[d], Mark Edgar[e], Anna Zonato Tocchio[a], Robin H. Lachmann[f], Frances M. Platt[b], Martin Grootveld[a,*]

[a] Leicester School of Pharmacy, De Montfort University, The Gateway, Leicester LE1 9BH, UK
[b] Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT, UK
[c] School of Pharmacy, China Pharmaceutical University, Jiangning Campus, 639 Longmian Avenue, Jiangning District, Nanjing, Jiangsu 211198 China
[d] Chemistry Research Laboratory, Department of Chemistry, University of Oxford Mansfield Road, Oxford OX1 3TA, UK
[e] Department of Chemistry, Loughborough University, Loughborough, LE11 3TU, UK
[f] Charles Dent Metabolic Unit, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK

ARTICLE INFO

Article history:
Received 18 April 2015
Received in revised form 4 August 2015
Accepted 9 August 2015
Available online 13 August 2015

Chemical compounds:
Miglustat (CID 51634)
Valproate (CID 3121)
1-*O*-Valproyl-β-glucuronide (53477719)

Keywords:
NP-C disease
Miglustat
Valproate
Drug metabolism
NMR-based urinalysis
TOCSY/NOESY NMR techniques

ABSTRACT

Niemann–Pick type C1 (NP-C1) disease is a neurodegenerative lysosomal storage disease for which the only approved therapy is miglustat (MGS). In this study we explored the applications and value of both one- and two-dimensional high-resolution NMR analysis strategies to the detection and quantification of MGS and its potential metabolites in urine samples collected from NP-C1 disease patients ($n = 47$), and also applied these techniques to the analysis of the anticonvulsant drug valproate and one of its major metabolites in *ca.* 30% of these samples (*i.e.* from those who were also receiving this agent for the control of epileptic seizures). A combination of high-resolution 1D and 2D TOCSY/NOESY techniques confirmed the identity of MGS in the urinary ^{1}H NMR profiles of NP-C1 patients treated with this agent ($n = 25$), and its quantification was readily achievable *via* electronic integration of selected 1D resonance intensities. However, this analysis provided little or no evidence for its metabolism *in vivo*, observations consistent with those acquired in corresponding experiments performed involving an *in vitro* microsomal system. Contrastingly, the major valproate metabolite 1-*O*-valproyl-β-glucuronide was readily detectable and quantifiable in 14/47 of the urine samples investigated, despite some resonance overlap problems (identification of this agent was confirmed by experiments involving equilibration of these samples with β-glucuronidase, a process liberating free valproate). In order to facilitate and validate the detection of MGS in urine specimens, full assignments of the ^{1}H NMR spectra of MGS in both buffered aqueous (pH 7.10) and deuterated methanol solvent systems were also made. The pharmacological and bioanalytical significance of data acquired are discussed, with special reference to the advantages offered by high-resolution NMR analysis.

1. Introduction

Niemann–Pick type C disease (NP-C, OMIM 257220) is a neurodegenerative lysosomal storage disease caused by defects in either the *NPC1* or *NPC2* genes [1]. NP-C involves the altered storage and cellular trafficking of cholesterol and sphingolipids, coupled with diminished acidic store calcium levels [2]. Typically, this disease presents in childhood with clumsiness, ataxia, learning difficulties, vertical gaze palsy (prevalence >80%) and dysphagia (70%), together with cataplexy and epilepsy, which have a prevalence of ca. 50% [3]; hepatosplenomegaly is also common, and a number of early onset cases are fatal as a result of severe neonatal liver disease. Respiratory dysfunction may be a further clinical feature. Additionally, adult-onset disease can occur, and this can be associated with a neuropsychiatric presentation [1]. The pathologenic cascade in

Abbreviations: NP-C, Niemann–Pick type C disease; GSL, glycosphingolipid; MGS, miglustat; UD, puridine diphosphate; UDPGA, uridine 5'-diphosphoglucuronic acid trisodium salt.

* Corresponding author.
E-mail address: mgrootveld@dmu.ac.uk (M. Grootveld).

http://dx.doi.org/10.1016/j.jpba.2015.08.011
0731-7085/

NP-C disease includes neuroinflammation, neuronal apoptosis and oxidative stress, which are all likely to contribute towards the clinical phenotype [3]. In view of the high incidence of epilepsy in these patients, anti-epileptic drugs are employed for the control of seizures in some patients, including sodium valproate, lamotrigine and levetiracetam [4].

Currently, the only disease-modifying drug approved for NP-C disease treatment is the deoxynojirimicyn adduct miglustat (MGS, *N*-butyldeoxynojirimycin, (2R,3R,4R,5S)-1-butyl-2-(hydroxymethyl) piperidine-3,4,5-triol), which is an iminosugar derivative of a class of polyhydroxylated alkaloids which are extractable from some plants and micro-organisms. This therapeutic agent acts as an inhibitor of glucosylceramide synthase, and hence blocks the synthesis of all glucosylceramide-based glycosphingolipids (GSLs, including lactosylceramide and gangliosides) since the generation of glucosylceramide represents the first committed step in GSL biosynthesis [5]. The nature of this inhibition was first determined *via* the use of ceramide as an acceptor [6], and further investigations revealed that MGS was a competitive inhibitor of glucosyltransferase activity for this substrate, but a non-competitive inhibitor for uridine diphosphate (UDP)-glucose, an observation confirming that this inhibitory process involves molecular mimicry of the ceramide substrate [6].

MGS was first proposed for the treatment of NP-C disease in view of evidence available for slower disease progression and prolonged survival in animal models [7]. The clinical effectiveness of MGS has been confirmed in a randomised controlled clinical study conducted by Patterson et al. [8]. This investigation showed that treatment with this agent improved or stabilised several clinically-relevant markers of NP-C disease after 12 months of treatment (specifically horizontal saccadic eye movement velocity, an index correlated with disease progression). Moreover, further clinical studies have demonstrated the stabilisation of neurologic disease in both children and adults treated with this therapeutic agent [9]. Moreover, benefits regarding swallowing capacity, auditory acuity, and a diminished rate of ambulatory index deterioration were observed in patients who were older than 12 years. MGS can effectively cross the blood-brain barrier, and therefore has the potential to be effective in the treatment of lysosomal storage disorders with neurologic manifestations; indeed, an observational cohort study performed by Pineda et al. [10] indicates that MGS stabilises neurological disease in the majority of patients with NP-C disease. This therapeutic agent also appears to offer protection against oxidative stress which has been implicated in the aetiology of NP-C disease [11,12]. Moreover, beneficial effects on axonal degeneration have also been found in NP-C patients receiving this treatment [13], and hence life is prolonged, a therapeutic benefit primarily ascribable to an improved swallowing capacity, which leads to fewer cases of aspiration pneumonia [14].

MGS has also been found to offer valuable therapeutic benefits towards Sandhoff disease (specifically, a delayed symptom onset and an elevated life expectancy) [15].

Prior to the approval of MGS by the European Medicines Agency (EMA) in 2009 as a therapeutic agent for NP-C disease, it was approved by both this agency in 2002, and by the US Food and Drug Administration (FDA) in 2003 for the treatment of Type I Gaucher disease [16,17]. However, although its approval for NP-C disease treatment has been achieved in most countries, that in the USA remains outstanding. The recommended daily dose is 3×200 mg daily in adults, and this is adjusted according to body surface area in patients below 12 years of age (EMA). It has been shown to be rapidly absorbed, and maximal plasma concentrations are attained *ca.* 2.0–2.5 h. following dosing [18]. Moreover, renal clearance is *ca.* 150 ml/min., an observation indicating that the renal elimination mainly occurs through a filtration process, and also that the risk for interactions with alternative drugs eliminated *via* active secretion is low [18].

Previous approaches to the determination of MGS have only focused on its detection and quantification in human and mouse blood plasma [19], or human blood plasma and cerebrospinal fluid (CSF) [20], and have employed liquid chromatography-tandem mass spectrometric (LC–MS/MS) analytical techniques. In 2009 Guitton et al. utilised such a LC–MS/MS bioanalytical strategy for the rapid and sensitive identification and quantification of of MGS in plasma and CSF, in which sample preparation involved a simple protein precipitation step using a 75/25 (v/v) acetonitrile/methanol mixture. These researchers observed a satisfactory linear calibration throughout the concentration range of 125–2500 and 50–1000 ng/ml for blood plasma and CSF respectively, and an analytical accuracy of 98–106.5%. Subsequently, Spieker et al. [19] validated a highly sensitive LC–MS/MS method for the analysis of this drug, and separated it from matrix biomolecules on a Gemini C18 column with detection involving a triple stage quadrupole MS system operating in APCI mode, and achieved a sensitivity of only 10 ng/ml (45.6 nM). Therefore, this validated method offered an increased level of sensitivity and a reduced cycle time (<2 min. per chromatographic profile) when compared to alternative related methods available.

Despite the use of MGS in the treatment of GSL lysosomal storage diseases, very little has been reported on its solution-state structure using high-resolution NMR analysis. Although this NMR solution structure was previously reported by Butters et al. [21,22], and the chemical shift values for 1-butyl-3,4,5-tris(phenylmethoxy)-2-[(phenylmethoxy)-methyl], (2S,3R,4R,5S) [23], and the iminosugar derivatives *N*-butyl-*L-ido*-1-1-deoxynojirimycin [24] and *N*-butyl-*L*-altrostatin [25] have been previously described, to the best of our knowledge, the chemical shift assignments and associated coupling patterns and constants for MGS have not been previously reported in buffered aqueous media, alternative solvents or biofluids. Moreover, to date the urinary excretion of MGS in humans has not been previously investigated by ^{1}H NMR analysis.

Therefore, the major rationale for this investigation was to evaluate the abilities of both one- and two-dimensional high-resolution NMR analysis approaches to detect and quantitate MGS (together with any of its putative metabolic transformation products) in urine samples collected from NP-C1 disease patients receiving this drug (and further develop these novel methods), so that this strategy can be employed for the therapeutic monitoring of these agents in clinical laboratories. An additional rationale is the application of such techniques to explore the identification and quantification of further therapeutic agents that the patients involved may be receiving, as illustrated by valproate (for the control of epileptic seizures), together with their corresponding metabolites. Indeed, the multicomponent analytical abilities of the NMR techniques applied here offer major analytical advantages over alternative methods available for the urinalysis of drugs and their metabolites. Although less sensitive than corresponding LC–MS/MS methods, high-resolution ^{1}H NMR analysis of biofluids offers a number of bioanalytical advantages over the above LC–MS/MS methods. Firstly, there is only a small amount of sample preparation involved [simple addition of *ca.* 10% (v/v) D_2O as a field frequency lock, and with an internal standard solute such as TSP, where required], a process diminishing analytical throughput times. Secondly, it is a virtually non-invasive and non-destructive technique, *i.e.* samples analysed by this technique may subsequently be subjected to alternative forms of analysis, if desired. Thirdly, the ^{1}H NMR approach is particularly suited to the study and simultaneous, multicomponent analysis of drug metabolites, and prior separation and/or derivatisation reaction processes are not usually required for this purpose, as indeed they are for some LC methods. Hence, the NMR-based bioanalytical strategy employed here serves as the method