BIOCHEMICAL PHARMACOLOGY 76 (2008) 249–257

available at www.sciencedirect.com

ScienceDirect

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

In vitro characterisation of human renal and hepatic frusemide glucuronidation and identification of the UDP-glucuronosyltransferase enzymes involved in this pathway

Oranun Kerdpin [a,b], *Kathleen M. Knights* [a], *David J. Elliot* [a], *John O. Miners* [a,*]

[a] *Department of Clinical Pharmacology, Flinders University, Adelaide, Australia*
[b] *Department of Pharmacy Practice, Naresuan University, Phitsanulok, Thailand*

ARTICLE INFO

Article history:
Received 6 March 2008
Accepted 24 April 2008

Keywords:
Frusemide
Glucuronidation
UDP-glucuronosyltransferase
Human kidney
Human liver

ABSTRACT

In order to gain insights into the renal and hepatic glucuronidation of frusemide (FSM), this study: (i) characterised the kinetics of FSM glucuronidation by human liver microsomes (HLM) and human kidney cortical- (HKCM) and medullary- (HKMM) microsomes, and (ii) identified the human UDP-glucuronosyltransferase enzyme(s) involved in this pathway. HLM, HKCM and HLMM efficiently glucuronidated FSM. FSM glucuronide (FSMG) formation followed Michaelis–Menten kinetics in all tissues. While the mean K_m for FSMG formation by HKMM (386 ± 68 μM) was lower than the K_m values for HLM (988 ± 271 μM) and HKCM (704 ± 278 μM), mean V_{max}/K_m values were comparable for the three tissues. A panel of recombinant UGT enzymes was screened for the capacity to glucuronidate FSM. UGT 1A1, 1A3, 1A6, 1A7, 1A9, 1A10 and 2B7 metabolised FSM. Of the renally and hepatically expressed enzymes, comparison of kinetic parameters suggests a predominant role of UGT1A9 in FSM glucuronidation, although UGT1A1 may also contribute to FSMG formation by HLM. Consistent with these observations, the UGT1A selective inhibitors phenylbutazone and sulfinpyrazone decreased FSMG formation by HLM, HKCM and HKMM by 60–80%, whereas the UGT2B7 selective inhibitor fluconazole reduced FSM glucuronidation by $\leq$20%. The ability of HKCM and HKMM to form FSMG supports the proposition that the kidney is the main organ involved in FSM glucuronidation in vivo, although a role for hepatic metabolism remains a possibility in renal dysfunction. The data further demonstrate the potential importance of both the medulla and cortex in renal drug metabolism and detoxification.

1. Introduction

The anthranilic acid derivative frusemide (FSM) (Fig. 1) is a potent diuretic which acts via blockade of active sodium/potassium and chloride reabsorption in the thick ascending limb of the loop of Henle [1]. FSM, which is employed primarily in the treatment of oedema associated with renal impairment, congestive cardiac failure and hepatic cirrhosis, ranks amongst the most widely used clinical drugs. By way of example, in 2006 approximately 1.4 million and 10.5 million prescriptions for FSM were dispensed in Australia and England, respectively (http://www.medicareaustralia.gov.au; http://www.ic.nhs.uk/webfiles/publications/pca2006/). Available evidence indicates that

* *Corresponding author at*: Department of Clinical Pharmacology, Flinders University School of Medicine, Flinders Medical Centre, Bedford Park, SA 5042, Australia. Tel.: +61 8 82044131; fax: +61 8 82045114.
E-mail address: john.miners@flinders.edu.au (J.O. Miners).
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2008.04.014

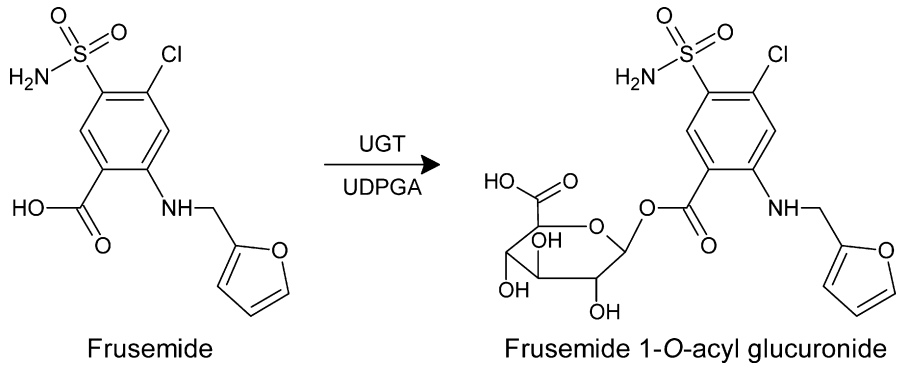

Fig. 1 – Structures of frusemide (FSM) and frusemide 1-O-acyl glucuronide (FSMG).

glucuronidation, resulting in the formation of FSM 1-O-acyl glucuronide (FSMG) (Fig. 1), is the major if not sole biotransformation pathway of FSM in humans [2]. Although renal excretion of unchanged drug is the predominant clearance mechanism of FSM in healthy subjects [3], the ratio of FSMG to FSM excreted in urine is significant ranging from 0.21 to 0.31 [4]. While it appears that FSM glucuronidation in humans and the rabbit occurs primarily in the kidney [2–6], renal dysfunction results in markedly increased fecal excretion of FSM (at the expense of urinary excretion of FSM and FSMG) [6,7]. Potentially, this may arise from biliary excretion of FSM itself or hepatically formed FSMG (with subsequent hydrolysis in the gastrointestinal tract).

Glucuronidation reactions are catalysed by the enzyme UDP-glucuronosyltransferase (UGT) and involve the covalent linkage of glucuronic acid, derived from the cofactor UDP-glucuronic acid (UDPGA), to a nucleophilic atom on the substrate. UGT exists as a superfamily of enzymes that exhibit distinct but overlapping substrate selectivities [8]. Nineteen human UGT proteins that utilize UDPGA as cofactor have been identified to date, and these have been classified into two families (UGT1 and UGT2) based on sequence identity [9]. The liver is the major site of glucuronidation. However, a number of extrahepatic tissues exhibit significant UGT activity [10]. Importantly, both UGT1A and UGT2B7 enzymes are known to be expressed in kidney cortex and medulla, including the proximal and distal convoluted tubules, the loops of Henle, and the collecting ducts [11]. Moreover, glucuronidation activity by human renal tissue has been demonstrated towards numerous xenobiotics and endogenous compounds [11–20].

Despite the widespread use of FSM and its clinical utilization over four decades, the comparative roles of kidney and liver in human FSM glucuronidation appear not to have been characterised. Furthermore, the UGT enzymes contributing to FSMG formation are yet to be elucidated. This study compared the kinetics of FSM glucuronidation by human liver microsomes (HLM) and both human kidney cortical- (HKCM) and medullary- (HKMM) microsomes to gain insights into hepatic and renal FSM metabolism. In addition, microsomal inhibition approaches and activity measurements with recombinant UGTs were employed to identify the human UGT enzyme(s) that potentially contribute to FSMG formation.

2. Materials and methods

Alamethicin (from *Trichoderma viride*), β-glucuronidase (from *Escherichia coli*), FSM, UDP-glucuronic acid (UDPGA; trisodium salt), phenylbutazone, sulfinpyrazone and zidovudine (3′-azido-3′-deoxythymidine) were purchased from Sigma–Aldrich (Sydney, Australia). Fluconazole was a gift from Pfizer Australia (Sydney, Australia). All other reagents and solvents were of analytical reagent grade.

2.1. Human liver and kidney microsomes

Human livers (HL 7, 10, 12, 29, and 40) were obtained from the human liver bank of the Department of Clinical Pharmacology, Flinders Medical Centre. Approval was obtained from the Flinders Medical Centre Clinical Research Ethics Committee and from the donor next-of-kin for the procurement and use of human liver tissue in xenobiotic metabolism studies. Microsomes were prepared by differential centrifugation, as described by Bowalgaha et al. [21], and stored at −80 °C until use. Similarly, human kidney tissues (HK 6, 7, 9, and 10) from subjects who had undergone radical nephrectomy for malignant disease were obtained from the joint Flinders Medical Centre/Repatriation General Hospital Tissue Bank. Approvals for tissue collection and in vitro xenobiotic metabolism studies were obtained from the Research Ethics Committees of the Repatriation General Hospital and the Flinders Medical Centre. Renal cortical and medullary tissue distant to the primary tumor were isolated from fresh kidneys immediately following surgery. HKCM and HKMM were prepared by differential centrifugation, as described by Tsoutsikos et al. [19], and stored at −80 °C until use. Renal and hepatic microsomes were activated by preincubation with alamethicin (50 μg/mg protein) on ice for 30 min prior to use in incubations [22].

2.2. Expression of UGT proteins

UGT 1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, 2B17, and 2B28 cDNAs were stably expressed in a human embryonic kidney cell line (HEK293), as described previously [23,24]. Cells were separately transfected with the individual UGT cDNAs cloned into the pEF-IRES-puro6 expression vector and incubated at 37 °C in Dulbecco's modified Eagle's medium, which contained puromycin (1 mg/l), 10% fetal calf serum, and