Biochemical Pharmacology 129 (2017) 85–95

Contents lists available at ScienceDirect

Biochemical Pharmacology

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

Inhibition of human UDP-glucuronosyltransferase enzymes by lapatinib, pazopanib, regorafenib and sorafenib: Implications for hyperbilirubinemia

John O. Miners [a,b,*], Nuy Chau [a], Andrew Rowland [a,b], Kushari Burns [a], Ross A. McKinnon [b], Peter I. Mackenzie [a,b], Geoffrey T. Tucker [c], Kathleen M. Knights [a], Ganessan Kichenadasse [b,d]

[a] Department of Clinical Pharmacology, Flinders University School of Medicine, Adelaide, Australia
[b] Flinders Centre for Innovation in Cancer, Flinders University School of Medicine, Adelaide, Australia
[c] Department of Human Metabolism, Medical and Biological Sciences (Emeritus), University of Sheffield, Sheffield, United Kingdom
[d] Department of Oncology, Flinders Medical Centre, Bedford Park, Australia

ARTICLE INFO

Article history:
Received 1 November 2016
Accepted 4 January 2017
Available online 6 January 2017

Chemical compounds studied in this article:
Bilirubin (PubChem CID: 5280352)
β-Estradiol (PubChem CID: 5757)
Lapatinib (PubChem CID: 208908)
Pazopanib (PubChem CID: 10113978)
Propofol (PubChem CID: 4943)
Regorafenib (PubChem CID: 178102514)
Sorafenib (PubChem CID: 216239)

Keywords:
UDP-glucuronosyltransferase
Enzyme inhibition
Kinase inhibitors
Hyperbilirubinemia
Drug–endobiotic interaction
Drug–drug interaction

ABSTRACT

Kinase inhibitors (KIs) are a rapidly expanding class of drugs used primarily for the treatment of cancer. Data relating to the inhibition of UDP-glucuronosyltransferase (UGT) enzymes by KIs is sparse. However, lapatinib (LAP), pazopanib (PAZ), regorafenib (REG) and sorafenib (SOR) have been implicated in the development of hyperbilirubinemia in patients. This study aimed to characterise the role of UGT1A1 inhibition in hyperbilirubinemia and assess the broader potential of these drugs to perpetrate drug–drug interactions arising from UGT enzyme inhibition. Twelve recombinant human UGTs from subfamilies 1A and 2B were screened for inhibition by LAP, PAZ, REG and SOR. IC_{50} values for the inhibition of all UGT1A enzymes, except UGT1A3 and UGT1A4, by the four KIs were <10 μM. LAP, PAZ, REG and SOR inhibited UGT1A1-catalysed bilirubin glucuronidation with mean IC_{50} values ranging from 34 nM (REG) to 3734 nM (PAZ). Subsequent kinetic experiments confirmed that REG and SOR were very potent inhibitors of human liver microsomal β-estradiol glucuronidation, an established surrogate for bilirubin glucuronidation, with mean K_i values of 20 and 33 nM, respectively. K_i values for LAP and PAZ were approximately 1- and 2-orders of magnitude higher than those for REG and SOR. REG and SOR were equipotent inhibitors of human liver microsomal UGT1A9 (mean K_i 678 nM). REG and SOR are the most potent inhibitors of a human UGT enzyme identified to date. *In vitro–in vivo* extrapolation indicates that inhibition of UGT1A1 contributes significantly to the hyperbilirubinemia observed in patients treated with REG and SOR, but not with LAP and PAZ. Inhibition of other UGT1A1 substrates *in vivo* is likely.

1. Introduction

Kinase signalling pathways regulate diverse cellular functions, including angiogenesis, apoptosis, differentiation and proliferation, and dysregulation of protein and lipid kinases is associated with a range of diseases [1,2]. In particular, mutations in the genes encoding protein and lipid kinases are linked to numerous malignancies in humans. Thus, there has been intense interest over the last two decades in the discovery and development of kinase inhibitors (KIs) for the treatment of cancer and other diseases. Thirty KIs have been approved since 2001, about two-thirds of these in the last five years [3].

Since the duration of treatment with KIs varies from weeks to years and polypharmacy is common in cancer patients (for the

Abbreviations: DDI, drug–drug interaction; β-EST, β-estradiol; HLM, human liver microsomes; IV–IVE, *in vitro–in vivo* extrapolation; KI, kinase inhibitor; 4MU, 4-methylumbelliferone; LAP, lapatinib; NSB, non-specific binding; PAZ, pazopanib; PRO, propofol; REG, regorafenib; SOR, sorafenib; UDP-GlcUA, UDP-glucuronic acid; UGT, UDP-glucuronosyltransferase.

* Corresponding author at: Department of Clinical Pharmacology, Flinders University School of Medicine, GPO Box 2100, Adelaide, SA 5001, Australia.

E-mail addresses: John.miners@flinders.edu.au (J.O. Miners), Nuy.chau@flinders.edu.au (N. Chau), Andrew.rowland@flinders.edu.au (A. Rowland), Kushari.burns@unisa.edu.au (K. Burns), Ross.mckinnon@flinders.edu.au (R.A. McKinnon), Peter.mackenzie@flinders.edu.au (P.I. Mackenzie), G.t.tucker@sheffield.ac.uk (G.T. Tucker), Kathie.knights@flinders.edu.au (K.M. Knights), Ganessan.kichenadasse@health.sa.gov.au (G. Kichenadasse).

http://dx.doi.org/10.1016/j.bcp.2017.01.002
0006-2952/

treatment of cancer and other co-morbidities) [4], patients receiving KIs are considered to be at high risk from drug–drug interactions (DDIs) [5]. Given the predominant role of cytochrome P450 (CYP) 3A4 and, to a lesser extent, other CYP enzymes (e.g. CYP1A2) in the metabolism of KIs, most studies investigating KIs as either victims or perpetrators of DDIs arising from enzyme inhibition have focussed on CYP3A4 inhibitors and substrates, respectively [6–9]. Further, both hepatic uptake (e.g. OATP1B1) and efflux transporters (e.g. P-glycoprotein and BCRP) are variably involved in KI disposition and DDIs may also arise from inhibition of transporter activity [10].

In addition to CYP- and transporter-mediated hepatic elimination, a number of KIs are known to be metabolised *via* glucuronidation [3,6,8]. Glucuronidation reactions involve UDP-glucuronosyltransferase (UGT) catalysed covalent linkage ('conjugation') of the substrate with glucuronic acid, which is derived from the cofactor UDP-glucuronic acid (UDP-GlcUA) [11]. UGT exists as a superfamily of enzymes [12]. The nineteen human UGT enzymes that utilise UDP-GlcUA as cofactor have been classified in the UGT 1A, 2A and 2B subfamilies. Of the hepatically expressed enzymes, UGT 1A1, 1A3, 1A4, 1A6, 1A9, 2B7 and 2B15 appear to be of greatest importance in drug and xenobiotic metabolism, with lesser contributions of UGT 2B4, 2B10 and 2B17 [13]. The individual UGT enzymes exhibit distinct, but overlapping, patterns of substrate and inhibitor selectivities. In particular, bilirubin is glucuronidated solely by UGT1A1 [13,14].

UGT1A9-catalysed glucuronidation contributes to the elimination of regorafenib (REG) and sorafenib (SOR) [9], and hence inhibition of UGT1A9 and other UGT enzymes by these KIs would not be unexpected. For example, dual inhibition of UGT1A1 and UGT1A9 by substrates of either enzyme has been demonstrated in this laboratory [15,16]. Consistent with this observation, inhibition of UGT1A1 by SOR *in vitro* has been reported [17,18]. Jaundice is a common adverse effect of both REG and SOR [19,20], and has also been reported as an adverse effect in patients treated with pazopanib (PAZ) and lapatinib (LAP) [21–23]. These observations are strongly suggestive of KI inhibition of UGT1A1-catalysed bilirubin glucuronidation since, as noted above, bilirubin is glucuronidated solely by this enzyme.

Data relating to UGT enzyme inhibition by KIs are sparse and hence understanding of the propensity of these drugs to precipitate drug–endobiotic interactions and DDIs is limited. Importantly, the FDA now recommends screening of investigational new drugs for inhibition of UGTs, while the EMA recommends at least screening for inhibition of UGT 1A1 and 2B7 [24,25]. Recent studies have demonstrated that inhibition kinetic studies with human liver microsomes (HLM) and recombinant proteins as the enzyme sources together with *in vitro–in vivo* extrapolation (IV–IVE) approaches predict the likelihood of a DDI or drug–endobiotic interaction arising from UGT enzyme inhibition *in vivo* [13,26–29]. Since hyperbilirubinemia is an important adverse effect in patients treated with LAP, PAZ, REG and SOR, this study aimed to characterise the role of inhibition of UGT1A1-catalysed bilirubin glucuronidation as the cause of jaundice. More broadly, the study additionally characterised the inhibition of human UGT 1A and 2B enzymes by LAP, PAZ, REG and SOR (see Fig. 1 for structures), to assess the potential role of these KIs as perpetrators of DDIs.

Importantly, inhibitor constants (K_i), which underpin IV–IVE, for LAP, PAZ, REG and SOR were corrected for microsomal binding and hence accurately reflect inhibition potential. Many KIs, but particularly REG and SOR, are highly lipophilic organic bases that bind extensively to the microsomal membrane [30]. Failure to account for non-specific binding (NSB) results in over-estimation of K_i, and K_m in the case of a substrate, and hence under-estimation of drug–endobiotic and DDI potential [13,31]. Notably, following correction for NSB, REG and SOR were identified as remarkably potent inhibitors of UGT1A1 with K_i values in the low nanomolar range making these compounds the most potent UGT inhibitors reported to date.

2. Materials and methods

2.1. Drugs and other chemicals

LAP, PAZ, REG and SOR were purchased from LC Laboratories, (Woburn, MA, USA); alamethicin (from *Trichoderma viride*), bilirubin, codeine, β-EST, β-estradiol-3-β-D-glucuronide, 4MU, 4MU β-D-glucuronide, PRO, and UDP-GlcUA (trisodium salt) were purchased from Sigma-Aldrich (Sydney, NSW, Australia); and codeine 6-O-β-D-glucuronide from Toronto Research Chemicals (North York, ON, Canada). Lamotrigine and lamotrigine N2-β-D-glucuronide were provided by the Wellcome Research Laboratories

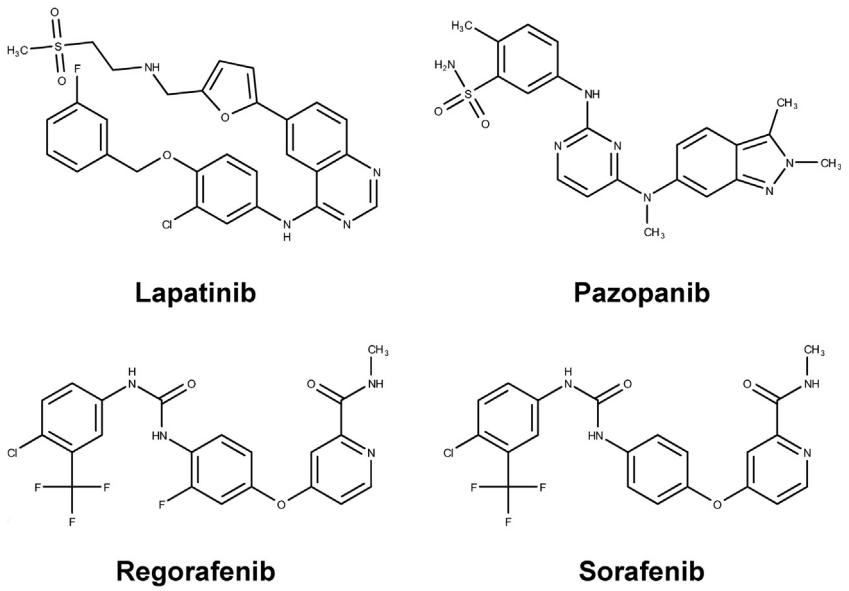

Fig. 1. Structures of lapatinib, pazopanib, regorafenib and sorafenib.