European Journal of Pharmaceutical Sciences 114 (2018) 55–63

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Differential effects of hepatic cirrhosis on the intrinsic clearances of sorafenib and imatinib by CYPs in human liver

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ARTICLE INFO

Chemical compounds studied in this article:
Sorafenib (PubChem CID: 216239)
imatinib (PubChem CID: 5291)
midazolam (PubChem CID: 4192)
paclitaxel (PubChem CID: 36314)
tolbutamide (PubChem CID: 5505)
dextromethorphan (PubChem CID: 5360696)

Keywords:
Sorafenib biotransformation
Imatinib biotransformation
CYP3A4
CYP2C8
Hepatic cirrhosis

ABSTRACT

The tyrosine kinase inhibitors sorafenib and imatinib are important in the treatment of a range of cancers but adverse effects in some patients necessitate dosage modifications. CYP3A4 has a major role in the oxidation of sorafenib to its *N*-oxide and *N*-hydroxymethyl metabolites and also acts in concert with CYP2C8 to mediate imatinib *N*-demethylation. CYP3A4 expression and function are impaired in patients with advanced liver disease, whereas the functions of CYP2C enzymes are relatively preserved. We evaluated the biotransformation of sorafenib and imatinib in well-characterized microsomal fractions from 17 control subjects and 19 individuals with hepatic cirrhosis of varying severity. The principal findings were that liver disease impaired the microsomal oxidation of sorafenib to its major metabolites to 40–44% of control ($P < 0.01$), whereas the *N*-demethylation of imatinib was relatively unimpaired. The impairments in sorafenib biotransformation were correlated with decreased serum albumin concentrations and increased serum bilirubin concentrations in patients with liver disease, but not with the overall grade of liver disease according to the Child-Pugh system. In contrast, there was no relationship between imatinib *N*-demethylation and clinicopathologic factors in liver disease patients. These findings were accounted for in terms of the differential roles of CYPs 3A4 and 2C8 in the intrinsic clearance of the drugs. CYP3A4 has the major role in the intrinsic clearance of sorafenib but plays a secondary role to CYP2C8 in the intrinsic clearance of imatinib. In agreement with these findings CYP2C protein expression and CYP2C8-mediated paclitaxel 6α-hydroxylation were unimpaired in cirrhotic livers. This information could be adapted in individualized approaches such as in vivo CYP3A4 phenotyping to optimize sorafenib safety and efficacy in cancer patients with liver dysfunction.

1. Introduction

Tyrosine kinase inhibitors (TKIs) are a recently-developed class of targeted oncology drugs that have significantly improved the treatment of patients with a range of tumors. Sorafenib and imatinib are two important TKIs that are used in the treatment of liver and renal clear cell carcinomas and chronic myelogenous leukemia and gastrointestinal stromal tumors, respectively. Sorafenib targets the Raf kinase, vascular endothelial growth factor receptor and platelet derived growth factor receptor, while imatinib targets the TKs bcr-abl and c-kit (Demetri et al., 2002; Druker et al., 1996; Liu et al., 2006). However, although these agents are generally well tolerated, imatinib and sorafenib produce a range of toxicities in certain patients, including hypertension, edema or major dermal toxicities such as the hand-foot syndrome (Escudier et al., 2007; Ugurel et al., 2003). Such adverse effects may lead to the interruption or termination of TKI therapy.

The aim of personalized medicine is to tailor drug selection and dosage to the individual patient. Biotransformation enzymes, especially the hepatic cytochrome P450 (CYP) mixed-function oxidases, are major factors that influence the rate of drug elimination. Individuals in whom CYP oxidation capacity is high may clear drugs rapidly, which can result in treatment failure because therapeutic serum concentrations are not maintained, while low CYP oxidation may result in drug accumulation and toxicity at standard doses of drugs. Recent studies have demonstrated roles for the quantitatively important human hepatic CYP3A and CYP2C enzymes in the oxidation of the TKIs sorafenib and imatinib (Dutreix et al., 2004; Ghassabian et al., 2012; Lathia et al., 2006; Nebot et al., 2010).

Liver disease impairs CYP expression directly, which can decrease intrinsic drug clearance (CL_{int}), or indirectly as a result of

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https://doi.org/10.1016/j.ejps.2017.12.003
Received 14 June 2017; Received in revised form 19 October 2017; Accepted 4 December 2017
Available online 07 December 2017

pathophysiological changes that alter hepatic blood flow and the perfusion of hepatocytes. Hepatic cirrhosis is a major form of end-stage liver disease that is characterized by increased fibrotic infiltration and destruction of the hepatic architecture (Johnson et al., 2010). The obstruction of blood flow promotes portal hypertension as well as the development of portacaval shunts, ascites and gastrointestinal bleeding. Predictions of the effects of advanced liver disease on the biotransformation of drugs such as TKIs are of potential importance in the design of safer dosage regimen in patients (Johnson et al., 2010).

Clinical studies have indicated that the clearance of drugs that are substrates for CYP3A4, such as midazolam and erythromycin, is markedly decreased in advanced liver disease (Barre et al., 1987; Chalasani et al., 2001). Analogous biochemical studies have shown that the oxidative biotransformation of CYP3A4 substrates is impaired because the enzyme is down-regulated in liver disease (George et al., 1995; Guengerich and Turvy, 1991). The available information on alterations in CYP2C protein expression in patients with liver disease is more variable. As summarized by Johnson et al. (2010), the expression of CYP2C protein and activity ranged from unchanged in one study to 34–72% of control in two others. However, a likely contributing factor to this variation is the use of polyclonal antibodies that were unable to discriminate between CYP2C subfamily members. The use of monospecific antibodies and selective drug substrates may provide more detailed information on the impact of liver disease on individual CYPs 2C.

The biotransformation of TKIs like sorafenib and imatinib that are metabolized by CYP3A could complicate the treatment of cancer patients with hepatic dysfunction. Accordingly, the present study evaluated the biotransformation of these TKIs in well-characterized hepatic microsomal fractions from patients with cirrhotic liver disease, compared with control. The principal finding to emerge was that the dysregulation of CYP3A4 in cirrhosis markedly decreased sorafenib biotransformation, whereas imatinib oxidation, which also involves a significant contribution from the CYP2C8 enzyme, was relatively preserved. These findings may assist the design of dosage modification approaches in patients with liver disease and who receive TKIs that have a high dependence on CYP3A4 for clearance.

2. Materials and methods

2.1. Drugs and chemicals

Sorafenib (4-[4-([4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino)phenoxy]-*N*-methylpyridine-2-carboxamide) and its *N*-oxide and *N*-hydroxymethyl metabolites were synthesized as described previously (Gillani et al., 2015). Imatinib mesylate ((4-[(4-methylpiperazin-1-yl) methyl]-*N*-[4-methyl-3-[(4-pyridin-3-ylpyrimidin-2-yl)amino]phenyl] benzamide)), *N*-desmethylimatinib and imatinib-d_8 (internal standard) were gifts from Novartis (Basel, Switzerland). Midazolam, dextromethorphan, dextrorphan, and all biochemicals were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). Sorafenib-methyl-d_3 and 6α-hydroxypaclitaxel were purchased from Toronto Research Chemicals (North York, ON, Canada) and 1′-hydroxymidazolam was from Cerilliant (Round Rock, TX, USA). HPLC grade solvents were from LabScan (Lomb Scientific, Taren Point, NSW, Australia), and analytical grade reagents were from Ajax Chemicals (Sydney, NSW, Australia).

2.2. Liver donors and preparation of microsomal fractions

Experiments with human liver microsomes were approved by ethics committees of the Western Sydney Area Health Service and the University of Sydney, according to the Declaration of Helsinki. Liver tissue from 36 individual donors was obtained through the Queensland and Australian Liver Transplant Programs (Princess Alexandria Hospital, Brisbane, Queensland, and Royal Prince Alfred Hospital, Sydney, NSW, Australia, respectively). “Control” human liver was obtained as excess tissue during transplantation or as biopsies from the normal margin during resection of liver tumors. Cirrhotic tissue was obtained from individuals in whom the presence of ascites, hepatic encephalopathy and sepsis was recorded by the attending physician. The severity of liver disease was graded according to the Child-Pugh system which is calculated from the clinical assessment of ascites, encephalopathy, serum albumin and bilirubin concentrations, and prothrombin time. Each variable is graded between 1 and 3 based on the extent of impairment. The total Child–Pugh score ranges from 5 to 15, where 5 indicates that there is no hepatic decompensation and where 15 indicates severe liver failure (Christensen et al., 1984). The nutritional status of patients in the present study was assessed as normal, impaired or cachectic, based on the extent of muscle bulk and subcutaneous fat. Drug histories were collected where information was available.

In patients, venous blood samples were withdrawn after an overnight fast and used to determine serum bilirubin and albumin concentrations and prothrombin times. Blood was centrifuged and stored at − 70 °C until subjected to automated analysis (Department of Clinical Chemistry, Westmead Hospital, Westmead, NSW, Australia).

Liver tissue was obtained from the superior surface of the right lobe within 0.25 h of surgical removal, perfused with cold Viaspan solution (DuPont, Wilmington, DE, USA), snap frozen in liquid nitrogen and stored at − 70 °C. Liver microsomes were prepared by ultracentrifugation and stored at − 70 °C in potassium phosphate buffer (0.05 M, pH 7.4, containing 1 mM EDTA and 20% glycerol; Murray et al., 1985). Microsomal protein content was determined as described previously (Marcus et al., 1985).

2.3. Drug biotransformation by human liver microsomes

In all assays of drug biotransformation the linearity of product formation in incubations was established in relation to microsomal protein, substrate concentration and incubation time. Substrate concentrations were 2-11-fold of reported K_m, with the exception of the low-affinity substrate tolbutamide, which was studied around the K_m. To ensure enzyme saturation the substrate utilization was limited to < 15% in all cases.

Microsomal sorafenib biotranformation was determined as described previously (Ghassabian et al., 2012). Briefly, incubations (75 µg microsomal protein, 75 µM sorafenib) in 0.1 M potassium phosphate buffer (pH 7.4, 0.25 mL) at 37 °C were initiated using NADPH (1 mM). Reactions were terminated after 20 min with cold acetonitrile (0.5 mL), the precipitate was removed by centrifugation and the supernatant was dried under a stream of nitrogen gas; the internal standard was sorafenib-methyl-d_3. Sorafenib and its metabolites were quantified on a Thermo Scientific TSQ Quantum Access Max liquid chromatography-tandem mass spectrometry (LC-MS/MS) system using electrospray ionization (positive ion mode; San Jose, CA, USA) with separation on a Waters XTerra MS C18 3.5 µ column (150 × 2.1 mm I.D.; Waters, Rydalmere, NSW, Australia).

Microsomal imatinib biotranformation was determined as described previously (Nebot et al., 2010). Briefly, incubations (150 µg microsomal protein, 80 µM imatinib) in 0.1 M potassium phosphate buffer (pH 7.4, 0.2 mL) at 37 °C were initiated with NADPH (1 mM). Reactions were terminated after 10 min with 5% trifluoroacetic acid (5 µL) and applied to Varian Bond Elut Plexa solid-phase extraction cartridges (1 mL). Cartridges were eluted with methanol (3 × 1.25 mL aliquots) which was then evaporated to dryness; the internal standard was imatinib-d_8. Imatinib and *N*-desmethylimatinib were quantified on a Finnigan MAT TSQ 7000 LC-MS/MS system using electrospray ionization (positive ion mode; San Jose, CA, USA) with separation on an Altima C18 5 µ column (150 × 2.1 mm I.D.; Alltech Associates, Castle Hill, NSW, Australia).

CYP3A-mediated midazolam 1′-hydroxylation was determined as described previously (Ghassabian et al., 2009). Incubations (100 µg microsomal protein, 5 µM midazolam) in 0.1 M potassium phosphate