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Role of P-glycoprotein in tissue uptake of indinavir in rat

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Abstract

The effect of p-glycoprotein inhibition on tissue distribution of indinavir, an anti-HIV (human immunodeficiency virus) protease inhibitor drug, has been evaluated. Indinavir was co-administered intravenously in rats along with a p-glycoprotein inhibitor, PSC833, and the drug concentrations in plasma and various tissues were determined using a HPLC method. Additionally, initial uptake clearance of indinavir was evaluated in the brain and testes. The highest increasing effect of p-glycoprotein inhibition on the tissue uptake ratios of indinavir was found in central nervous system (CNS). The estimated tissue extraction the drug was indicative of (i) limited drug entry to brain parenchyma, which was increased significantly by p-glycoprotein inhibition, (ii) non-restricted drug entry to testes, heart and spleen, which was increased significantly in the case of heart and decreased in the case of testes and spleen as a result of p-glycoprotein inhibition, and (iii) drug accumulation in liver and small intestine and, to a lesser extent, kidney, which was not affected by p-glycoprotein inhibition. The uptake clearances of indinavir by brain parenchyma in PSC833-treated and control rats were 68.80±8.65 and 21.63±4.28 μl/min/g and the corresponding values for the testes were 39.84±4.90 and 36.65±2.54 μl/min/g. The difference was significant only in the case of brain parenchyma ($P<0.001$). These data showed that p-glycoprotein inhibition increases the CNS uptake of indinavir markedly and has some transient minor effects on drug uptake by some other tissues.

Keywords: P-glycoprotein; HIV protease inhibitors; Indinavir; Drug distribution; Tissue pharmacokinetics

Introduction

Human immunodeficiency virus (HIV) protease inhibitors, one of the most effective groups of antiretroviral drugs, are widely used as a part of the highly active antiretroviral therapy (HAART) in patients with acquired immunodeficiency syndrome (AIDS) (Eron, 2000). Indinavir is a potent HIV protease inhibitor with favorable virological, immunological, and clinical characteristics (e.g., Gulick et al., 1997). A concentration dependency has been demonstrated for anti-HIV activity of indinavir (Acosta et al., 1999), with sub-optimal concentrations of the drug in tissues, in particular central nervous system (CNS), leading to therapeutic failure as well as the emergence of drug-resistant viral strains despite adequate plasma concentrations and acceptable systemic antiviral efficacy indicators (Pialoux et al., 1997).

The limited penetration of HIV protease inhibitors to some tissues (i.e., CNS and testes) is attributed mainly to the expression of P-glycoprotein (Pgp) in blood–brain barrier (BBB) and blood–testis barrier (BTB) (Megard et al., 2002; Kim et al., 1998; Choo et al., 2000). Pgp is a 170-kDa energy-dependent plasma membrane efflux pump (Ling, 1997) encoded by the ABCB1 and ABCB2 genes in humans and mdr1a, mdr1b and mdr2 genes in rodents (Lee et al., 2001). As a member of ATP-binding cassette superfamily of transporters, Pgp is involved in the active efflux of a wide variety of compounds, including pharmacologically active agents, from organs such as CNS (e.g., Tatsuta et al., 1992; Lee et al., 2001) and testis (Holash et al., 1993; Choo et al., 2000) as well the hepatobiliary (Speeg and Maldonado, 1994; Watanabe et al., 1995; Yamazaki et al., 1996; Song et al., 1999; Matheny et al., 2001), renal (Speeg and Maldonado, 1994; Song et al., 1999; Matheny et al., 2001) and intestinal (Wacher et al., 1998; Matheny et al., 2001; Zhang and Benet, 2001) excretion of its substrates. In addition, there are some in vitro data supporting the involvement of multidrug resistance-associated proteins (MRPs), another family of the ATP-binding cassette transporters, in the tissue transport of indinavir (Srinivas et al., 1998; van der Sandt et al., 2001).

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At present, there is little in vivo data on the early-phase tissue distribution profile of indinavir, with most of the studies focused on the CNS distribution of this drug (Kim et al., 1998; Choo et al., 2000; Megard et al., 2002). In this study, the functional role of Pgp in the early-phase distribution (tissue uptake) of indinavir into different tissues has been evaluated using a highly potent and specific Pgp inhibitor, PSC833, which is a non-immunosuppressive cyclosporine D derivative (Twentyman and Bleehen, 1991).

Materials and methods

Materials

Indinavir sulphate (ethanol solvate form, MW 757.9) and PSC833 were kindly provided by Merck Research Laboratories (Rahway, NJ, USA) and Novartis Pharma (Basel, Switzerland), respectively. Verapamil hydrochloride (racemate form), triethylamine, phosphoric acid (assay 85.3%), and *tert*-butyl methyl ether were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada). Perchloric acid (assay 70%) was purchased from BDH Inc. (Toronto, Ontario, Canada). HPLC-grade water and acetonitrile were purchased from Caledon Laboratories Ltd. (Georgetown, Ontario, Canada). All other reagents used were of analytical purity grade and were purchased locally.

Animals

Male Sprague–Dawley rats (Charles River, St. Constant, Quebec, Canada) weighing between 280 and 300 g were used in this study. The animals were kept in standard cages with free access to water and standard rat chow ad libitum. A 12-h day–night cycle was used with lights on at 8:00 AM. The protocol for the animal experiments was reviewed and approved by the University of Toronto Animal Care Committee. The animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care.

Drug administration

The day before the experiments, the rats were anesthetized by intraperitoneal (i.p.) injection of a ketamine–xylazine cocktail (ketamine 100 mg/kg and xylazine 10 mg/kg) and a polyethylene–silicone rubber cannula was implanted in the right jugular vein. The rats were then caged singly and left overnight for complete recovery.

On the day of the experiments, a 5 mg/kg dose of indinavir sulphate (6.6 μmol) dissolved in a saline–propylene glycol–ethanol vehicle (5:4:1, v/v/v; 5 mg/ml) was injected intravenously to rats through the cannula. Two 5 mg/kg doses of PSC833 dissolved in a water–propylene glycol–ethanol vehicle (3:6:1, v/v/v; 12.5 mg/ml) were injected through the cannula 30 min before and concurrent with the indinavir dose to each rat. Another group of rats (vehicle control) received the same dosage schedule except that the same volume of PSC833-free vehicle was injected instead of PSC833 doses. The animals remained unrestrained during the entire drug administration and sampling time. At 1, 5 and 10 min following the indinavir dose, the animals were anesthetized by injection of an anesthetic cocktail (ketamine 10 mg/kg and xylazine 1 mg/kg) via the cannula and a 0.1-ml sample of cerebrospinal fluid (CSF) was withdrawn by direct cisternal puncture using a 25 G needle connected to a 1-ml syringe. Immediately after CSF sampling, a 1-ml blood sample was obtained via the cannula to pre-heparinized polypropylene microtubes. The animals were then immediately decapitated and the tissue samples including whole brain, heart, liver (an approximately 1-g piece of hepatic lobes), small intestine (an approximately 3-cm piece of duodenum), spleen, kidney and testis were harvested. The plasma was separated by centrifuging the blood samples at 1000 g for 10 min. The tissue samples were washed with normal saline. The CSF, plasma and tissue samples were kept frozen at −70 °C until drug assay time. A total number of 24 rats were used to collect four replicate biological samples at three time points from two study groups (i.e., 12 rats in each group).

Drug assay

A simple and validated reversed-phase high-performance liquid chromatography (HPLC) method was developed (Hamidi, 2006) and used for the determination of indinavir concentrations in biological samples from rats. The tissue samples were weighed and homogenized using a Brinkmann tissue homogenizer (Kinematica AG, Germany) while suspended in 2 ml ice-cold saline. For drug analysis, to 500 μl of the tissue homogenates or plasma samples, 100 μl of perchloric acid (70%) was added and, after vortex-mixing for 1 min, the resulting suspension was centrifuged at 18,000×*g* for 10 min. To 300 μl of plasma or tissue homogenate supernatants, prepared as described, or 100 μl of CSF (with no preparation step), 20 μl of verapamil hydrochloride (internal standard) aqueous solution (15 μM), 1 ml of KOH 4 M solution, and 3 ml of *tert*-butyl methyl ether were added and the resulting mixture was shaken at 1800 rpm for 15 min and then centrifuged at 5000×*g* for 10 min. The organic layer was separated by aspiration and evaporated to dryness using a vacuumed centrifuge (Centrivap Console; Labconco Co., Kansas City, MO, USA) in ambient temperature. Finally, the dried sample was re-dissolved in 100 μl of phosphoric acid 100 mM aqueous solution and 50 μl of the resulting solution was injected to the chromatograph. A mixture of phosphoric acid 50 mM aqueous solution and acetonitrile (65:35, v/v) with a final pH of 5.5, adjusted by the addition of triethylamine, was used as the mobile phase. The analyte separation was performed by a Synergi Hydro-RP column (150×4.6 mm, particle size 4 μm; Phenomenex Inc., Torrance, CA, USA) in ambient temperature. The eluent was delivered at a flow rate of 1.1 ml/min and the UV-detection wavelength was 215 nm. The method produced linear responses over the concentration range of 0.05–30 μM of indinavir in plasma and 0.05–2.5 μM both in CSF and brain with limits of detection of 12.5 nM in plasma and CSF and 6.25 nM in brain homogenate. The intra- and inter-run