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Absorption and elimination of imatinib through the rat intestine *in vitro*

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ABSTRACT

Imatinib is a potent selective inhibitor of tyrosine kinases and is used primarily in the treatment of chronic myeloid leukemia and the gastrointestinal stromal tumour. Although, it is well established that imatinib is a substrate of several transport proteins which are also active in the intestinal mucosa, the mechanisms of imatinib intestinal absorption and elimination were not systematically investigated yet. To do that, we used a Sweetana-Grass type of diffusion chambers with segments of rat intestine as a model of the intestinal mucosa, measured the permeability coefficients of imatinib and its major metabolite (N-desmethyl imatinib) in both directions with and without specific and general inhibition of active transport, and calculated the efflux ratios. The results show that the good bioavailability of imatinib is highly likely achieved by its active absorption from the intestine and that its active elimination through the intestinal mucosa is mediated by a synergistic activity of organic cation transporter 1 in the basolateral membrane and the added activity of two efflux proteins (P-glycoprotein and breast cancer resistant protein) in the apical membrane of enterocytes of the rat ileum. Interestingly, it was found that N-desmethyl imatinib is only transported by P-glycoprotein.

1. Introduction

Imatinib (Gleevec, Glivec or STI571) is a potent and selective inhibitor of three tyrosine kinases–Bcr-Abl1, platelet derived growth factor receptor (PDGFR) and steam cell factor/c-kit (Manley et al., 2002). It is clinically used to treat chronic myeloid leukemia (CML), gastrointestinal stromal tumour (GIST), and a number of other malignancies. More than ten years' of experience with imatinib, established that it is easy to administer, safe, and well tolerated and is thus, despite the development of other more potent drugs, still the standard of care in CML and GIST (Druker et al., 2006; O'Brien et al., 2003).

Imatinib is taken orally in recommended doses ranging between 400 and 800 mg a day in both indications (Cortes et al., 2010). According to several pharmacokinetic studies and clinical trials (Cortes et al., 2010; Gschwind et al., 2005; Judson et al., 2005; le Coutre et al., 2004; Peng et al., 2004, 2005; Schmidli et al., 2005), it is characterized by high bioavailability of 98%. Imatinib is also a substrate of CYP3A4, and its major metabolite N-desmethyl imatinib (NDI) is also active against the BCR-ABL1 tyrosine kinase. The parent drug and its metabolites are mainly eliminated in the faeces (Gschwind et al., 2005). The high interindividual variability in the systemic exposure, sometimes ranging from 40 to 60%, results in highly variable plasma drug concentrations and consequently, also in different treatment responses (Eechoute et al., 2011). In fact, the interindividual variability in pharmacokinetics is thought to be one of the reasons for resistance to imatinib therapy (Gorre et al., 2001). According to many studies of imatinib transport and metabolism published in recent years, there is no doubt, that active transport of imatinib into and out of the leukemic cell is an important issue in drug efficacy (Thomas et al., 2004). Several studies have shown that imatinib interacts with various multi drug resistance proteins, including P-glycoprotein, breast cancer resistance protein (BCRP), and multi drug resistance protein 1 (MRP1) as well as with polyspecific organic cation transporter 1 (OCT1), being the major active influx protein responsible for the transport of imatinib into the cancer cell (Thomas et al., 2004). It has been noticed that the majority of CML patients who have a suboptimal response to imatinib either have low OCT 1 activity (Engler et al., 2008; White et al., 2008a, b) or exhibit overexpression of *ABCB1*, the gene coding for Pgp (Mahon et al., 2003). On the other hand, conflicting findings have been published regarding the type of imatinib interaction with BCRP transporter. According to the available literature, imatinib could be an inhibitor (Houghton et al., 2004; Jordanides et al., 2006) or also a substrate (Brendel et al., 2007; Burger et al., 2004; Nakanishi et al., 2006) of this transporter. Nakanishi et al. also

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claim that imatinib itself could attenuate the resistance of target cells by suppressing the expression of BCRP (Nakanishi et al., 2006).

As all these transporters are active also in the gastrointestinal tract (GIT), they might not just play a role in cellular resistance or susceptibility of tumour cells to imatinib therapy, but also influence the gastrointestinal absorption and elimination of imatinib (Eechoute et al., 2011). A reduction in drug systemic exposure over prolonged time periods is one indication of possible changes in pharmacokinetic processes during treatment (Yoo et al., 2012). However, very few studies have directly studied the behavior of imatinib in the GIT until now. Oostendorp et al. showed the effect of Pgp and BCRP and to a lesser extent of OCT1/2 on imatinib absorption and elimination by comparing the bioavailability of the drug in wild type and Pgp/BCRP or OCT1/2 knockout mice. They also proposed that another, still unidentified transporter might be involved in imatinib absorption (Oostendorp et al., 2009). Experiments performed on different cell lines showed that some other intestinally and hepatic expressed transporters, for example organic anion transporting polypeptides (OATP1A2 and OATP1B3) (Hu et al., 2008) and organic cation/carnitine transporter (OCTN2) (Eechoute et al., 2011; Hu et al., 2008; Yamakawa et al., 2011), might also have the impact on imatinib pharmacokinetics. On the other hand, Kwon et al. believe that the passive transport of imatinib is fast and therefore, none of the transporters that interact with imatinib could significantly affect the drug's bioavailability (Kwon et al., 2004).

All these, somehow conflicting reports and the evident absence of any evaluation of imatinib intestinal permeability properties in an established *in vitro* model for biopharmaceutical classification of drug permeability, led us to conduct a study on isolated segments of rat small intestine to better understand the effects of major transporters known to be involved in the process of absorption and excretion of imatinib from the GIT wall and their possible impact on drug systemic bioavailability.

2. Materials and methods

Verapamil, corticosterone, and procainamide as well as all salts used for the buffers and incubation salines were supplied by Sigma–Aldrich Chemie (Germany). PSC-833 was purchased from Tocris Bioscience (UK), imatinib and prazosin were from Sequoia Research Products (UK), N-desmethyl imatinib (NDI) was from SAS Alsachim (France), and Ko143 was from Solvo Biotechnology (Hungary).

2.1. "In vitro" permeability studies

Rat jejunum from Sprague-Dawley rats (200–300 g) was obtained, prepared, and mounted in side-by-side diffusion chambers (Easy Mount; Physiologic Instruments, San Diego, CA, USA). The animals were denied access to food and had water *ad libitum* 18 h before they were sacrificed by decapitation. The intestine was quickly excised and placed in the ice-cold oxygenated Ringer buffer. Small intestine was used in the experiments. For duodenum, segments up to 6 cm from the pyloric sphincter were used; segments of jejunum were excised from the region 20 cm distally from the pyloric sphincter and 30 cm distally from the ileo-caecal junction; while, the tissue segments of ileum were excised from the region up to 20 cm distally from the ileo-caecal junction. Two to three centimeters long segments of rat jejunum excluding the Payer's patches were stretched on the inserts of diffusion chambers with apertures of 1.0 cm^2. The experiments conform to the law for the protection of animals (Republic of Slovenia), and the use of the tissue samples from previously sacrificed animals for experimental purposes is registered at the Veterinary Administration of the Republic of Slovenia.

Ringer buffer of 2.5 mL with 10 mM d-glucose or 10 mM mannitol on serosal and mucosal side of the tissue, respectively, was used as an incubation saline. The tissue was kept at 37 °C during the experiments except when the permeability during general inhibition of all active transport was measured at 4 °C. The pH of the incubation salines was always 7.4 on both sides of the intestinal tissue. Incubation salines were oxygenated and circulated by bubbling with carbogen gas mixture containing 95% O_2 and 5% CO_2 for experiments at 37 °C or (to maintain appropriate pH value of the incubation salines due to higher solubility of CO_2) 98% O_2 and 2% CO_2 at 4 °C. After the tissue segments were placed into the diffusion chambers, 25 min was always allowed for equilibration. The experiments started by the addition of stock solutions of imatinib or NDI in the donor compartment to provide the final 100 μM donor concentrations. This concentration was determined in preliminary experiments (data not shown) as optimal because the rat intestinal mucosa is not viable *in vitro* at higher imatinib concentrations and because lower concentrations were not sufficiently stable due to the drug binding to the tissue and the diffusion chambers. The stock solutions were added alternatively to the mucosal and to the serosal compartment to obtain bidirectional permeability measurements. Inhibitors of active transport were included in the incubation salines either on the mucosal or on the serosal side depending on the location of their target transporter protein to minimize their interaction with the transporters based on the opposite side of the intestinal mucosa. Their concentrations are given in Tables 1 and 2. On the mucosal side, verapamil was used to concomitantly inhibit the activity of Pgp and BCRP transporter proteins, 10 μM PSC-833 was used for specific inhibition of Pgp, and 5 μM KO-143 specifically inhibited the activity of BCRP. The OCT inhibitors employed on the serosal side in this study were previously used for the research of imatinib active transport in the leukocytes. Prazosin was used as an OCT1 inhibitor and partial OCT3 inhibitor. Prokainamide was used to inhibit the activity of OCT2 transporters, although, it also inhibits OCT1 but to a lesser extent. Corticosterone was used because it specifically inhibits OCT3 transporters (Thomas et al., 2004; White et al., 2007). Seven samples of 250 μL were withdrawn from the acceptor compartment in 25 min intervals. This volume was replaced by the appropriate fresh incubation saline. The rat jejunal tissue viability and integrity were controlled throughout the experiments by monitoring the trans-tissue potential difference and the short circuit current with a multi channel voltage-current clamp (model VCC MC8, Physiologic Instruments) (Polentarutti et al., 1999; Soderholm et al., 1998; Žakelj et al., 2004). At the end of the experiments, the trans-tissue potential difference after the addition of d-glucose to the mucosal compartment (final concentration was 25 mM) was also measured. The tissue segments in the diffusion chambers were considered viable if the negativity of trans-tissue potential measured on the mucosal side with the serosal side potential set to zero (standard procedure for diffusion chambers) was at least −1.1 mV after the addition of d-glucose to the mucosal side. The tissue integrity and viability *in vitro* were also evaluated by the trans-tissue electrical resistance which was calculated according to the Ohm's law from the short circuit current and the trans-tissue potential difference. As the trans-tissue potential difference values are zero at 4 °C, the trans-tissue electrical resistance was calculated from the electrical current necessary to clamp the potential difference to 2.0 mV. The tissue integrity was considered to be good when the measured trans-tissue electrical resistance was greater than 18 Ωcm^2 during the entire experiment. At 4 °C only the trans-tissue electrical resistance criterion can be applied because there is no active ion transport at this temperature. Only the measurements obtained