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Is oral absorption of vigabatrin carrier-mediated?

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ABSTRACT

The aim of the study was to investigate the intestinal transport mechanisms responsible for vigabatrin absorption in rats by developing a population pharmacokinetic (PK) model of vigabatrin oral absorption. The PK model was used to investigate whether vigabatrin absorption was carrier-mediated and if the proton-coupled amino acid transporter 1 (PAT1) was involved in the absorption processes. Vigabatrin (0.3–300 mg/kg) was administered orally or intravenously to Sprague Dawley rats in the absence or presence of PAT1-ligands L-proline, L-tryptophan or sarcosine. The PK profiles of vigabatrin were described by mechanistic non-linear mixed effects modelling, evaluating PAT1-ligands as covariates on the PK parameters with a full covariate modelling approach. The oral absorption of vigabatrin was adequately described by a Michaelis–Menten type saturable absorption. Using a Michaelis constant of 32.8 mM, the model estimated a maximal oral absorption rate (V_{max}) of 64.6 mmol/min and dose-dependent bioavailability with a maximum of 60.9%. Bioavailability was 58.5–60.8% at 0.3–30 mg/kg doses, but decreased to 46.8% at 300 mg/kg. Changes in oral vigabatrin PK after co-administration with PAT1-ligands was explained by significant increases in the apparent Michaelis constant. Based on the mechanistic model, a high capacity low affinity carrier is proposed to be involved in intestinal vigabatrin absorption. PAT1-ligands increased the Michaelis constant of vigabatrin after oral co-administration indicating that this carrier could be PAT1.

1. Introduction

Over the recent years the roles of transporters in drug absorption have received increasing attention. One of the proposed interactions is between vigabatrin and the proton-coupled amino acid transporter 1, PAT1. This interaction was originally proposed by Abbot et al., who showed induction of currents in mPAT1-expressing oocytes and intracellular acidification in Caco-2 cells expressing hPAT1 endogenously when exposed to vigabatrin (Abbot et al., 2006). These observations suggests that the cellular transport step across the luminal membrane of enterocytes is mediated by PAT1, which was recently confirmed by Nøhr et al. using a direct measurement of the amount of vigabatrin transported into Caco-2 cells (Nøhr et al., 2014a). Furthermore, Nøhr and coworkers demonstrated that the transepithelial vigabatrin transport across Caco-2 cells was dependent on the interaction between hPAT1 and vigabatrin (Nøhr et al., 2014a). Vigabatrin is not metabolized to any significant degree in rat, dog, monkeys and human (Gibson et al., 1990) and eliminated via the kidney in unchanged form (Durham et al., 1993; Gibson et al., 1990; Haegele and Schechter, 1986; Rey et al., 1992; Saletu et al., 1986). Considering this and the hydrophilic (aqueous solubility of 452 g/l) and zwitterionic nature of vigabatrin ($\mathrm{Log}\,D_{7.0} = -2.16$, $pK_a = 4.02$ and 9.72 (Henczi et al., 1995; Rey et al., 1992)) this would suggest that PAT1 plays a major role in the oral absorption of vigabatrin and may determine the

Abbreviations: BCH, 2-amino-2-norbornanecarboxylic acid; BSV, between subject variability; cGI, GI concentration; CL, systemic clearance; *F*, oral bioavailability; F_{max}, maximum oral bioavailability; GI, gastrointestinal; GOF, goodness-of-fit; IV, intravenous; K_m, Michaelis constant; $K_{m,app}$, apparent Michaelis constant; k_{tr}, transit rate constant; k_{GIe}, gastrointestinal elimination rate constant; OFV, Objective Function Value; PAT1, proton-coupled amino acid transporter 1; PO, oral (*per os*); Pro, L-proline; Q2, inter-compartmental clearance; Sar, sarcosine; Trp, L-tryptophan; V1, central volume; V2, peripheral volume.

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bioavailability of vigabatrin. However, conflicting results have been obtained in studies aimed to show the impact of the interaction between PAT1 and vigabatrin *in vivo* (Holm et al., 2012; Nøhr et al., 2014b,c). Broberg et al. showed *PAT1* mRNA expression and functional PAT1 activity in the rat small intestine and rectum, but none in the colon (Broberg et al., 2012). Therefore, Holm et al. investigated the potential of rectal administration of vigabatrin targeting PAT1 in the rat rectum (Holm et al., 2012). However, this study did not provide evidence for a role of PAT1 in rectal vigabatrin absorption (Holm et al., 2012). In contrast, Nøhr et al. reported a decreased maximal vigabatrin plasma concentration and an increased time to reach this after oral co-administration of vigabatrin and the PAT1-substrate sarcosine (Sar) to rats (Nøhr et al., 2014c), suggesting that PAT1 could be involved in the intestinal absorption of vigabatrin. Surprisingly, in contrast to the findings in rats Sar did not affect intestinal vigabatrin absorption in Göttingen mini-pigs (Nøhr et al., 2014b). There is therefore a need to clarify if the interaction between PAT1 and vigabatrin, which has been substantiated by a number of *in vitro* studies (Abbot et al., 2006; Holm et al., 2012; Nøhr et al., 2014a), has any *in vivo* relevance for intestinal vigabatrin absorption.

To obtain a mechanistic understanding of the absorption processes involved in vigabatrin transport into the systemic circulation, it was decided to use a model development strategy to describe the pharmacokinetic (PK) data. Traditional non-compartmental description of PK data, like maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}) and area under the plasma concentration–time (AUC), suffer from a lack of mechanistic insight into the underlying processes (e.g. changes in T_{max} can both arise from changes in factors such as systemic clearance, absorption delay, changes in the absorption rate constant or changes in central volume of distribution). Using a population modelling approach the current study provides a detailed opportunity to investigate mathematical hypotheses underlying the absorption processes of vigabatrin and impact of dose and PAT1-substrate or -inhibitor on absorption delay, absorption rates and absolute oral bioavailability. Furthermore, the population modelling approach allows simultaneous analysis of all plasma concentration–time profiles after oral and intravenous (IV) administration, strengthening the mechanistic understanding of drug absorption and disposition (Mould and Upton, 2012).

The aim of the study was thus to investigate if drug transporters, notably PAT1, are involved in the absorption of vigabatrin *in vivo*. In order to investigate this, we followed two strategies. Firstly, we investigate the dose-dependent absorption of orally administered vigabatrin. Deviation from a dose-independent oral bioavailability or absorption rate would suggest the involvement of a drug carrier, since passive diffusion is known to be proportional to the dose administered. Secondly, we aimed at investigating the involvement of PAT1 in the oral absorption of vigabatrin using an inhibitor-based strategy, where vigabatrin was co-administered with a PAT1-substrate i.e. Sar or a combination of a PAT1 substrate and inhibitor, i.e. L-proline (Pro) and L-tryptophan (Trp).

2. Material and methods

2.1. Materials

Vigabatrin was provided by H. Lundbeck A/S (Valby, Denmark). Acetonitrile LC-MS grade, 2-amino-2-norbornanecarboxylic acid (BCH), Pro, Sar and Trp were purchased from Sigma–Aldrich (St. Louis, MO, USA). Mannitol was from Unikem (Copenhagen, Denmark). Ammonium formate was purchased from Fluka, Sigma–Aldrich (Steinheim, Switzerland); formic acid was from Merck KGaA (Darmstadt, Germany). Rac-vigabatrin $^{13}C,d_2$ was purchased from Toronto Research Chemicals (Toronto, Canada). Water used for the experiments were obtained from a Mili-Q water purification system.

2.2. Methods

2.2.1. Compound selection

L-proline (Pro) is a commonly used substrate for investigation of PAT-mediated transport (Foltz et al., 2005; Frolund et al., 2010; Metzner et al., 2004, 2006; Thwaites et al., 1993). L-tryptophan (Trp) is an inhibitor of PAT1-mediated transport (Metzner et al., 2005) and Sar is a PAT1-substrate (Boll et al., 2003; Metzner et al., 2004, 2006). In the following substrates and inhibitors will be denoted ligands as a collective term. 2-amino-2-norbornanecarboxylic acid (BCH) is a specific L-type transporter ligand (Kim et al., 2010), but does not interact with PAT1 (denoted non-PAT1-ligand). We therefore chose the PAT1-ligands Pro, Trp and Sar as positive controls and BCH as a negative control for the study.

2.2.2. In vivo study

The study was conducted in accordance with the Danish law regulating animal experiments, EC Directive 2010/63/EU and the NIH guidelines on animal welfare. The protocol and procedures performed was approved by the institutional animal ethics committee monitored by Danish Ministry of Food, Agriculture and Fisheries. Male Spraque Dawley rats were purchased from Charles River, Germany (Sulzfeld, Germany). All animals were acclimatised and maintained on standard feed with free access to water for a minimum of 5 days prior to the experiment. Before entry into the experiment the animals were fasted for 16–20 h with free access to water and were randomly assigned to receive one of the treatments.

2.2.3. Vigabatrin formulations

The content of the administered formulations is presented in Table 1. Each rat was given 10 ml/kg of the formulation. The doses for IV injection were 1.0 mg/kg vigabatrin alone or combined with 100 mg/kg Pro and 100 mg/kg Trp. IV vigabatrin was dosed alone or in the combination with Pro and Trp administered IV or orally (PO) (dosing regimen 1, 2 or 3, respectively). The PO doses were 0.3, 1, 3, 30 or 300 mg/kg vigabatrin alone or in combination with 100 mg/kg Pro and 100 mg/kg Trp, 200 mg/kg Sar or 200 mg/kg BCH. The formulations given IV were filtered through a sterile 0.22 μm filter prior to administration. The osmolarity of the formulations (dosing regimen 1–12) was adjusted with NaCl to 266–291 mOsmol/kg (the osmolarity of the solution containing 300 mg/kg was 385 mOsmol/kg) and the pH to 7.3 ± 0.05 with 1 M HCl or NaOH. The formulations in dosing regimen 13–14 were made isotonic with mannitol and the pH was adjusted to 6.0 ± 0.1.

2.2.4. Plasma sampling

The animals (273–332 g on the day of dosing) were dosed conscious with 10 ml/kg either intravenously (into the tail vein) or by oral gavage according to the dosing regimens presented in Table 1. Blood samples of 100–200 μl were obtained by individual vein puncture of the tail vein and collected into EDTA coated tubes (Microvette 500 K3E, Sarstedt, Nümbrecht, Germany). Eight blood samples were drawn serially from each rat in the time interval 1 min–4 h (dosing regimen 1–11, Table 1) or 10 plasma samples within 5 min–6 h (dosing regimen 12–13, Table 1). Plasma was harvested immediately by 10 min of centrifugation at 4 °C, 2765*g* and stored at −80 °C until analysis. The animals were euthanized by CO_2 after the experiment.

In each dosing regimen 1–12 (Table 1) 5 rats were dosed at time point 0, except for dosing regimen 5 where 10 rats were included. The plasma samples were drawn from each rat according to the