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RESEARCH PAPER

Rectal Absorption of Vigabatrin, a Substrate of the Proton Coupled Amino Acid Transporter (PAT1, Slc36a1), in Rats

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ABSTRACT

Purpose To investigate the rectal absorption of vigabatrin in rats, based on the hypothesis that PAT1 (Slc36a1) is involved.

Methods Male Sprague–Dawley rats were dosed rectally with five different gels, varying in buffer capacity, the amount of vigabatrin, and co-administration of proline or tryptophan. Western blotting was used to detect rPAT1 in rat rectal epithelium. *X. Laevis* oocytes were injected with *SLC36A1* cRNA for the expression of hPAT1, prior to two-electrode voltage clamp measurements.

Results rPAT1 protein was present in rat rectal epithelium. Approximately 7%–9% of a 1 mg/kg vigabatrin dose was absorbed after rectal administration, regardless of the formulation used. Increasing the dose of vigabatrin 10-fold decreased the absolute bioavailability to 4.2%. Co-administration of proline or tryptophan changed the pharmacokinetic profile, indicating a role of PAT1 in the rectal absorption of vigabatrin. Transport of vigabatrin via hPAT1 expressed in *X. Laevis* oocytes had a K_m of 5.2 ± 0.6 mM and was almost completely inhibited by tryptophan.

Conclusions Although vigabatrin is a PAT1 substrate and the rPAT1 protein is expressed in the rectum epithelium, vigabatrin has low rectal absorption in rats.

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ABBREVIATIONS

GABA	γ-amino butyric acid
hPAT1	proton-coupled amino acid transporter human isoform
mPAT1	proton-coupled amino acid transporter murine isoform
Pro	L-proline
rGAPDH	rat glyceraldehyde 3-phosphate dehydrogenase
rPAT1	proton-coupled amino acid transporter rat isoform
rSlc36a1	gene encoding rat solute carrier member 36a1
SLC36A1	gene encoding human solute carrier member 36A1
TEVC	two-electrode voltage clamp
Vig	vigabatrin

INTRODUCTION

Vigabatrin has been used in the treatment/regulation of infantile spasms in more than 50 countries for the past two decades and has recently been approved for this use in the United States (1,2). Infantile spasms are seen in 1 of 2000 infants (2) and is one of the most treatment-resistant pediatric epilepsy syndromes (3). Vigabatrin is a synthetic derivative of GABA and is an irreversible GABA transaminase inhibitor (4). Two bioequivalent oral formulations are available on the US market, tablets and sachets of powder for oral solution, with an absolute bioavailability of 60%–70% (5,6). Vigabatrin has a linear dose relationship over the therapeutic dose range (7,8) and the pharmacokinetic parameters of vigabatrin do not change in adult patients who had eaten a breakfast of approximately 600 kcal, 1 h before administration of 1 g vigabatrin (9). The pediatric dose is from 50 to 150 mg/kg/day (10).

Vigabatrin has a log P of -0.1 (11) and, considering its relative high oral bioavailability, this suggests that absorptive transporters are involved in the intestinal absorption of vigabatrin. A study of vigabatrin transport across the Caco-2 cell CLEFF9-clone monolayers without a pH-gradient, indicated a low intestinal permeability of vigabatrin from lumen to blood (12). In Caco-2 cells, vigabatrin inhibited the apical uptake of amino acids, which are substrates of the proton-coupled amino acid transporter, hPAT1, encoded by the gene *SLC36A1* (13). The intracellular pH decreased following application of 10 mM vigabatrin (Na^+-free solution, pH 5.5) to the apical side of Caco-2 cell monolayers (13). These data indicate that vigabatrin is transported across the apical membrane via a proton-coupled transporter (13). Abbot *et al.* showed that 10 mM vigabatrin (Na^+-free solution, pH 5.5) induced inward currents in hPAT1-expressing *X. Laevis* oocytes (13). Vigabatrin is not transported via OCTN2 (14) and the transport studies in the Caco-2 cell CLEFF9-clone indicated that vigabatrin is not a substrate of the efflux transporters P-gp and MRP (12). In summary, hPAT1 seems to be the main candidate transporter in mediating intestinal vigabatrin absorption. PAT1 is an intestinal transporter located in the apical membrane, transporting proline and inhibited by tryptophan (15,16). *SLC36A1* has been detected at the mRNA level in the human gastrointestinal tract from esophagus to the rectum (15) and in the rat small intestine (17).

We have recently characterized the expression of *rSlc36a1* in mucosal samples from rat intestine. *rSlc36a1* was expressed all along the intestine with maximal expression in the jejunum, whereas the expression level in the ileum and colon was minimal. Surprisingly, a high *rSlc36a1* expression in the rectum was observed (18). Considering the possible rectal expression of PAT1 and its proposed involvement in intestinal vigabatrin transport, we hypothesized that rectal vigabatrin administration could be a clinically attractive administration route for pediatric use.

The purpose of the present study was to investigate the *in vivo* absorption of vigabatrin in rats after rectal administration in the presence or absence of proline and tryptophan and to measure the amount of the rPAT1 protein in rat rectum. We also characterized the transport of vigabatrin via hPAT1 in *X. Laevis* oocytes, and investigated if tryptophan is an inhibitor of hPAT1-mediated vigabatrin transport.

MATERIALS AND METHODS

Materials

Vigabatrin was supplied by H. Lundbeck A/S (Valby, Denmark). The internal standard, 6-amino hexanoic acid, methylcellulose (2% in water produces a 4000 cP solution at 20°C), L-tryptophan and L-proline were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, methanol and 2-propanol (HPLC grade) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). The mMESSAGE mMACHINE T7 mRNA-capping kit was obtained from Ambion (Austin, TX, USA). The *Xenopus laevis* frogs were from the African Reptile Park (Tokai, South Africa). Deionized water was generated using a water purification system (Elga Labwaters, UK). All other chemicals were of analytical grade.

Two-Electrode Voltage Clamp Measurements

Two-electrode voltage clamp (TEVC) measurements were performed as described previously (19). *X. laevis* handling and oocyte extraction procedures were performed according to national guidelines and approved by the Danish Animal Experiments Inspectorate. *SLC36A1* cRNA was synthesized by *in vitro* transcription using the mMESSAGE mMACHINE T7 mRNA-capping Kit according to the protocol supplied by the manufacturer. *X. Laevis* oocytes were injected with 9–18 nL diluted *SLC36A1* cRNA (2 μg/μL) and TEVC measurements were performed 4–6 days post-injection. Oocytes were voltage clamped at -60 mV and continuously perfused with Ringer's solution, pH 6.0 (in mM: NaCl, 115; KCl, 2.5; $CaCl_2$, 1.8; $MgCl_2$, 0.1; MES, 10). Compounds were dissolved in Ringer's solution, and added by full bath application, while the change in membrane current was monitored. Non-injected oocytes served as negative controls.

Protein Preparations

Caco-2 cells expressing hPAT1 were used as a positive control. Briefly, Caco-2 cells were grown in DMEM supplemented with 10% foetal bovine serum, non-essential amino acids and antibiotics at 37°C in a 5% CO_2 atmosphere. One week before protein preparation Caco-2 cells were seeded on Transwell™ filter inserts (Corning) to induce cell differentiation and PAT1 protein expression. Caco-2 cells were trypsinized, collected by centrifugation and washed twice with PBS.

Rat rectal epithelium was collected from male Sprague–Dawley rats (Charles River, Sulzfeld, Germany). Mucosal scrapings from excised intestinal tissue of approximately 1 cm^2 were re-suspended in 5 volumes of the tissue in 0.01 M Tris–HCl (pH 8.0), 0.14 M NaCl, 1% Triton X-100, 0.1% Na-deoxycholate, 0.1% SDS, 2.5 μg/ml proteinase inhibitors, 1 mM PMSF and 10 mM Na-pyrophosphate and incubated for 30 min at 4°C. The epithelium samples were then homogenized by sonification (Branson Sonifier Cell disruptor B15), following

which, the supernatant was collected after centrifugation for 10 min at maximum speed at 4°C.

Western Blot Analysis and Antibodies

SDS-PAGE and western blot analysis were performed as previously described (20). Protein concentration was determined by the Bradford assay. Briefly, prior to SDS-PAGE, equal amounts of total protein from the different cell lysates were mixed with one volume of 2 x Laemmli-SDS-sample buffer and heated at 95°C for 10 min. The following primary antibodies were used: N-cadherin (H-63), E-cadherin (H-108), Villin (H-60) and GAPDH (FL-335) (Santa-Cruz) and human and rat polyclonal anti-PAT1 (custom manufactured). Secondary antibody used for western blot analysis was polyclonal goat anti-rabbit IgG (Molecular Probes). Proteins were visualized via chemiluminescence (ECL-Plus, Amersham Biosciences) and visualized using Multiimage III (Alpha Innotech). All antibodies were used according to the manufacturer's instructions.

Vigabatrin Formulations

The solution for intravenous injection contained 1 mg/mL of vigabatrin and was made isotonic with mannitol. 2% (w/v) methylcellulose solutions were prepared by adding mannitol and methylcellulose to 70°C deionized water or 50 mM phosphate buffer and mixed for 30 min on a magnetic stirrer (see Table I). The heating was turned off and stirring was continued until ambient temperature was reached, after which 1 mg vigabatrin/mL was dissolved in the gel and the pH adjusted to 6.0±0.1 with HCl/NaOH.

In Vivo Study

The protocol was approved by the Animal Welfare Committee, appointed by the Danish Ministry of Justice. All animal procedures were carried out in compliance with EC Directive 86/609/EEC, Danish law regulating experiments with animals, and the NIH guidelines on animal welfare. Male Sprague–Dawley rats were purchased from Charles River (Sulzfeld, Germany). All animals were acclimatized 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 20–40 h and randomly assigned to receive one of the treatments. During recovery animals were allowed free access to water.

The conscious animals (273–307 g) were dosed with 1 mL/kg of either the intravenous formulation or the rectal gel containing 1 mg vigabatrin/mL. Blood samples of approximately 200 μL were obtained by individual vein puncture of the tail vein after 5, 15, 30, 45 and 60 min and 2, 3, 4 and 6 h into EDTA coated tubes. Plasma was harvested immediately by 15 min of centrifugation at 4°C, 2765 × g (Centrifuge Multifuge 1 S-R, Heraeus, Hanau, Germany), and stored at -80°C until analyzed. After the experiment the animals were euthanized by gas.

Bioanalysis of Vigabatrin

The method used an Agilent 1100 system with two binary pumps, two vacuum degassers and a column compartment, and a CTC HTC PAL autosampler. The system was coupled to a Micromas Quattro Ultra tandem mass spectrometer. Data acquisition software was MassLynx version 4.0.

A Bio Basis AX (Thermo-Hypersil, Keystone) guard column (2.1 × 10 mm, 5 μm) and an Asahipak NH_2P-50 2D 150 × 2 mm (Shodex, Japan) HPLC column were connected via the imbedded Valco 6-port 2-position electrically actuated switching valve in the Agilent 1100 binary pump. The injection was directed onto the pre-column with a flow of 0.25 mL/min. After 0.64 min, the valve was switched directing the analyte onto the analytical column, eluted with 0.25 mL/min. The flow on the pre-column was increased to 0.5 mL/min flushing sample debris to waste until 3.49 min where the flow was reduced to 0.25 mL/min and the valve was switched and the system equilibrated for 5 min before the next injection. The valve was programmed to conduct a "front cut" of the effluent to shunt of injection

Table I Content of the 2% (w/v) Methylcellulose Rectal Formulations. The pH of all Gels was Adjusted to 6.0

Formulation	Vigabatrin dose (mg/kg)	Viscosity (cP)	Phosphate buffer (mM)	Tryptophan (mM)	Proline (mM)
IV	1	nm*			
Rectal gel 1	1	4000			
Rectal gel 2	1	4000	50		
Rectal gel 3	1	4000		20	
Rectal gel 4	1	4000			20
Rectal gel 5	10	4000			

* nm: not measured

residuals from the analytical column as well as phospholipids, who are known be retained strongly on the applied analytical HPLC providing massive ion-suppression as late eluters. The columns were operated at a flow rate of 0.25 mL/min with a mobile phase composed of 65% acetonitrile and 35% ammonium acetate (20 mM, pH 4). The run time was 8 min.

The mass spectrometer was connected to the HPLC system via an electrospray interface and was operated in the positive ionization mode. The detection of the analytes was based on multiple reaction monitoring of the protonated molecules $(M\text{-}H)^+$ and their major collision-induced fragments (m/z 129.8→112.9 for vigabatrin and m/z 131.8→113.9 for 6-aminohexanoic acid (internal standard)). The dwell time for both channels was 600 ms. The Cone and the Capillary voltages settings were 35 V and 3.2 kV.

Calibration standards were prepared as pools of 2.5 mL to cover the assay range of 10–500 ng/mL by adding 50 µL of 500, 1000, 2500, 5000, 12500 and 25000 ng/mL working standards of vigabatrin to 2450 µL aliquots of rat EDTA control plasma, corresponding to plasma concentration of 10, 20, 50, 100, 250 and 500 ng/mL. Quality control (QC) samples were prepared in a similar way with analyte concentrations of 400 ng/mL (High QC), 75 ng/mL (Middle QC) and 30 ng/mL (Low QC). Calibration and QC pools were stored in polypropylene tubes at -80°C until use.

100 µL aliquots of a rat EDTA plasma sample were transferred to a 96-well, 1 mL deep well plate and spiked with 300 µL refrigerated acetonitrile, containing 100 ng 6-aminohexanoic acid/mL as internal standard. Zero and blank samples were prepared by adding 300 µL refrigerated acetonitrile with and without internal standard to 100 µL rat EDTA control plasma. Calibration standards and QCs were prepared in the same way as the study samples. Plates were sealed, whirled for 10 min and subsequently centrifuged at 5000 × g for 20 min at 10°C. 300 µL of the supernatant was transferred to a new 96-well plate and evaporated to dryness with N_2 at 45°C and reconstituted in 150 µL of methanol/acetonitrile (70/30, v/v). A 25 µL aliquot of the final extract was injected into the LC-MS/MS system. The signal to noise ratios should be >6 for vigabatrin and internal standards and >30 for the LLOQ standard. The method was operated as a qualified method, i.e., without formal pre-study validation but with sufficient in-study validation to ensure reliable, quantitative results.

Data Analysis

The K_m-value for the electrogenic transport of vigabatrin via hPAT1 was estimated from the concentration-dependent inward currents measured by TEVC after application of vigabatrin. Data were fitted to the Michaelis-Menten equation:

$$I = \frac{I_{\max} \cdot [S]}{K_m + [S]} \quad (1)$$

where I is the inward current measured relative to the current induced by 20 mM Pro, $I_{\max}$ is the maximal inward current, K_m is the Michaelis constant in mM and $[S]$ is the concentration of vigabatrin in mM.

The pharmacokinetic parameters were calculated using WinNonlin Professional version 5.2 (Pharsight Corporation, Mountain View, CA, USA). The plasma concentration-time profiles of vigabatrin after intravenous dosing were fitted to a two compartment model where after the drug clearance (CL), the volume of distribution (Vd), the mean residence time (MRT) and half-life ($t_{½}$) were estimated. A non-compartmental model was used to analyze the data obtained following rectal administration. The area under the curve ($AUC_{0\to\infty}$) was determined using the linear trapezoidal method and extrapolation of the last measured plasma concentration to infinity for animals dosed intravenously. The area under the curve for vigabatrin after rectal administration (AUC_{0-6}) was calculated using the linear trapezoidal rule from time zero to the last measured plasma concentration at 6 h post-dose. The total bioavailability (F_a) of vigabatrin following rectal administration was calculated for the individual animal using the following equation:

$$Bioavailability, F_a = \left(\frac{AUC_{0\to\infty}}{AUC_{0\to 6}}\right) \cdot \left(\frac{Dose_{IV}}{Dose_{rectal}}\right) \quad (2)$$

$AUC_{0\to\infty}$ is the area under the curve following intravenous vigabatrin administration and $AUC_{0\to 6}$ following rectal administration.

Statistical Analysis

The software Sigma Stat for Windows version 3.5 from Systat Software Inc. (Richmond, CA, USA) was used for the statistical calculations. Differences between formulations were assessed using one-way ANOVA. P-values < 0.05 were considered statistically significant.

RESULTS

Vigabatrin is a Substrate for hPAT1, Its Translocation is Inhibited by Tryptophan

In order to verify that vigabatrin is a substrate of hPAT1 the translocation of vigabatrin via hPAT1 was determined in voltage clamped *SLC36A1* cRNA injected oocytes using the

TEVC technique. Changes in membrane current relative to changes in current induced by 20 mM of the PAT1 substrate proline are shown in Fig. 1a. Concentration-dependent currents were determined and the resulting Michaelis constant (K_m) was determined to 5.2±0.6 mM (n=9), while the I_{max} was 92.7±4.0% of the current induced by 20 mM proline (n=9). No changes in the membrane current were induced in non-injected oocytes. Furthermore, the ability of the non-translocated hPAT1 inhibitor tryptophan to inhibit proline- or vigabatrin-induced current was measured. As evident from Fig. 1b, 10 mg/mL tryptophan (corresponding to a molar concentration of 49 mM) did not induce any changes in the membrane current of hPAT1 expressing oocytes, while 1 mg/mL proline (corresponding to a molar concentration of 9 mM) and 1 mg/mL vigabatrin (corresponding to a molar concentration of 8 mM) induced changes of approximately 70 nA and 45 nA, respectively. Co-application of proline and vigabatrin did not induce any additional current compared to proline alone, indicating that the two compounds are translocated via the same transporter. Co-application of tryptophan with proline or vigabatrin resulted in a smaller change in the membrane current, compared to application of proline or vigabatrin alone. Tryptophan inhibited the current induced by proline approximately 75%, while the current induced by vigabatrin was inhibited approximately 85%.

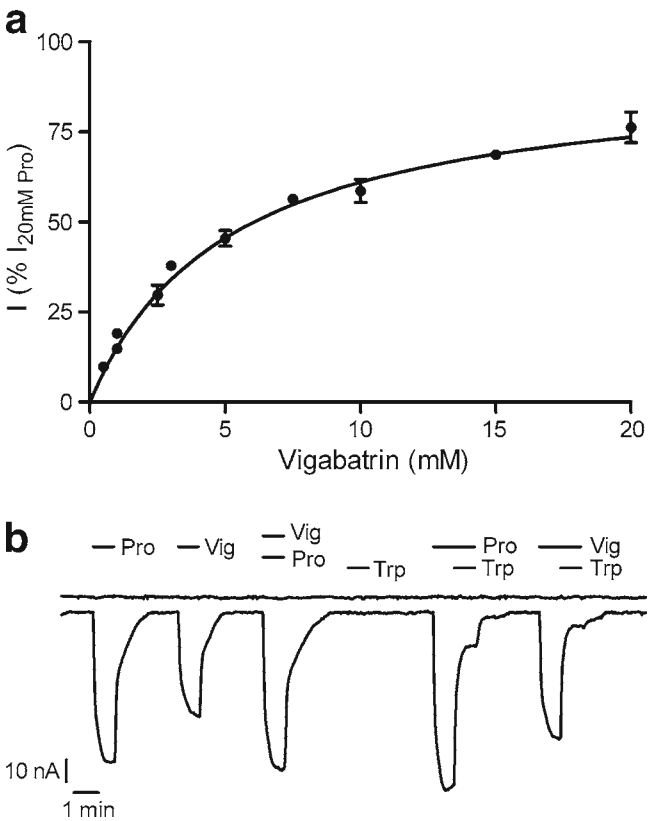

Fig. 1 (**a**) Concentration-dependent hPAT1-mediated current induced by vigabatrin in *SLC36A1* cRNA injected *X. laevis* oocytes. The current was measured relative to the current induced by 20 mM proline (Pro). The pH was buffered at 6.0 and oocytes were voltage clamped at -60 mV. Each data point represents the mean ± S.E.M. (n=9). Oocytes were obtained from 2 different frogs. (**b**) Representative traces showing changes in membrane current obtained by application or co-application of the indicated compounds. The concentrations of the compounds were as follows: proline (Pro): 1 mg/mL (≈9 mM), vigabatrin (Vig): 1 mg/mL (≈ 8 mM), tryptophan (Trp): 10 mg/mL (≈49.0 mM). Upper trace: non-injected *X. laevis* oocyte; lower trace: *SLC36A1* cRNA injected *X. laevis* oocyte. The pH of the buffer was 6.0 and oocytes were voltage clamped at -60 mV. Experiments on at least 4 different oocytes showed similar results.

rPAT1 Protein is Expressed in Rat Rectal Epithelium

The presence of rPAT1 protein in adult rat rectum samples from three different rats was shown by western blot analysis (Fig. 2). Immunolabeling of rPAT1 between 37 and 53 kDa was observed. The PAT1 specificity of the antibody was verified by testing with two different antibodies raised against either the human N-terminal of hPAT1 or an internal amino acid sequence of rat and mouse rPAT1/mPAT1. Both antibodies recognized a protein of similar size. The rPAT1 level in the rectal epithelium samples was similar to that measured in Caco-2 cells cultured for one week on Transwell™ filters.

Vigabatrin is Absorbed Rectally, but Proline and Tryptophan Have Limited Effect on Pharmacokinetic Profile

The plasma concentration profile of vigabatrin following intravenous injection of 1 mg/kg is presented in Fig. 3. The data was fitted to the bi-exponential Eq. (3):

$$C_{pl} = 6637 \cdot e^{-8.28t} + 1765 \cdot e^{-1.00t} \quad (3)$$

where C_{pl} is the concentration of vigabatrin (in ng/mL) in plasma and t is time (in hours).The pharmacokinetic parameters $AUC_{0\rightarrow\infty}$, CL, Vd, MRT and $t_{½}$ following intravenous administration to rats are listed in Table II. The vigabatrin plasma concentration *versus* time profiles following rectal administration of 5 different rectal gels are presented in Fig. 4 and the non-compartmental pharmacokinetic data in Table III. Vigabatrin was absorbed quickly from all the rectal formulations, as indicated by the t_{max}. Vigabatrin was absorbed significantly faster from rectal gel containing 50 mM phosphate buffer at pH 6 (gel 2) than gel containing proline (gel 4), whereas no significant differences were found between the other formulations.

For the rectal gels containing vigabatrin (1 mg/kg), the highest C_{max} was obtained for the gels containing 50 mM phosphate buffer (gel 2) and 20 mM proline (gel 4), and the lowest C_{max} was seen for the gel containing 20 mM tryptophan (gel 3). However, none of these differences were

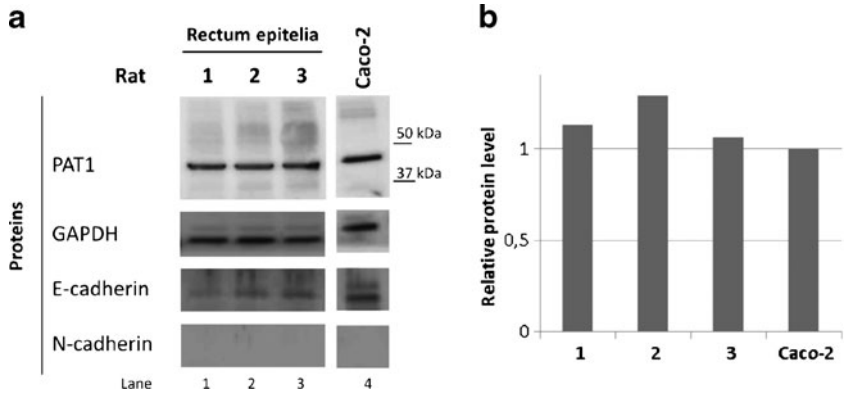

Fig. 2 rPAT1 protein level in adult rat rectum epithelium. (**a**) Western blot analysis of rPAT1, rGAPDH, N- and E-cadherin in 10 μg protein from 3 different rats (lane 1-3) and Caco-2 cells (lane 4). (**b**) The histogram illustrates the rPAT1 protein level in rat rectum epithelia relative to the hPAT1 level in Caco-2 cells.

statistically significant. The animals dosed with vigabatrin (10 mg/kg) had a significantly higher C_{max} than the groups dosed with 1 mg/kg.

The AUC and hence the absolute bioavailability, F_a, was within the same range for all the gels containing vigabatrin 1 mg/kg demonstrating only a limited effect of the additional components. The animals dosed with 10 mg/kg had a significant higher AUC than the low dose groups, but also a significantly lower F_a, demonstrating a lack of dose proportionality over the investigated dose range after rectal administration.

DISCUSSION

In the present study, the potential for rectal administration of vigabatrin via the proton-coupled amino acid transporter rPAT1 was investigated. hPAT1 has been suggested to be an absorptive transporter relevant for the absorption of several drugs, including vigabatrin (13), gaboxadol (21), δ-aminolevulinic acid (22), various active proline derivatives (16) and β-guanidinopropionic acid (23). Transport of these substrates has been characterized primarily with *in vitro* studies. Small natural amino acids such as proline, glycine and alanine (24) have also been identified as hPAT1 substrates and recently two dipeptides, Gly-Gly and Gly-Sar, were shown to be translocated via hPAT1 (19). On the other hand tryptophan, 5-hydroxy-L-tryptophan (5-HTP) and serotonin have been shown to be inhibitors of hPAT1/mPAT1-mediated transport (16).

Despite the accumulation of *in vitro* data on PAT1 substrates, very few *in vivo* studies have been published. To our knowledge, the only *in vivo* studies addressing the impact of PAT1 are those by Larsen *et al.* investigating the impact of tryptophan and 5-HTP on oral gaboxadol absorption in dogs and rats, respectively (18,21,25). These studies indicated a role of dog PAT1/rPAT1 in the oral absorption of gaboxadol, where a concentration-dependent effect of tryptophan on the maximal plasma concentrations was evident. In rats, 5-HTP caused an altered clearance, making this inhibitor unsuitable for *in vivo* studies, since high doses are required in order to saturate PAT1. Thus, in the present study, tryptophan was used as an inhibitor of rPAT1-mediated transport and proline as a competitive inhibitor. The present study used *in vitro* studies of hPAT1 to confirm the findings of

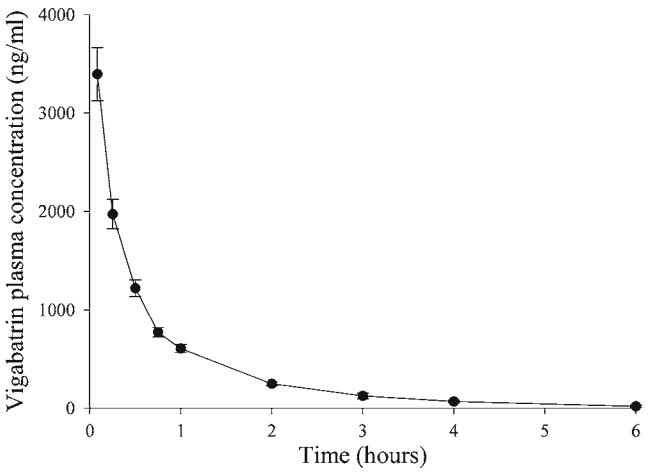

Fig. 3 Vigabatrin plasma concentration *versus* time profile following intravenous administration of vigabatrin (1 mg/kg) to rats. Data represents mean ± S.E.M. (n=6).

Table II Pharmacokinetic Parameters of Vigabatrin (1 mg/kg) Following Intravenous Administration. The Data were Fitted to a Two Compartment Model, the Results are Mean ± S.E.M. (n=6)

Pharmacokinetic parameter	Obtained value
$AUC_{0\rightarrow\infty}$	2364 ± 258 ng·h/mL
CL	427 ± 41 mL/kg/h
V_d	378 ± 68 mL/kg
MRT	0.87 ± 0.15 h
$t_{½}$	0.6 ± 0.1 h

Table III Pharmacokinetic Parameters of Vigabatrin Following Rectal Administration, the Results are Shown as mean ± S.E.M. of 3-6 Animals (n=3–6). The Brackets Show Significant Differences Among the Pharmacokinetic Data Obtained for the Administered Rectal Gels

Formulation	C_{max} (ng/mL)	T_{max} (hours)	AUC (ng·hour/mL)	F_a (%)
Rectal gel 1	71 ± 11 (5)	0.5 ± 0.1	179 ± 29 (5)	7.6 ± 1.2 (5)
Rectal gel 2	87 ± 15 (5)	0.3 ± 0.1 (4)	177 ± 22 (5)	7.5 ± 0.9 (5)
Rectal gel 3	57 ± 9 (5)	0.5 ± 0.1	171 ± 29 (5)	7.3 ± 1.2 (5)
Rectal gel 4	87 ± 25 (5)	0.7 ± 0.1 (2)	212 ± 63 (5)	9.0 ± 2.6 (5)
Rectal gel 5	562 ± 43 (1,2,3,4)	0.4 ± 0.1	1140 ± 160 (1,2,3,4)	4.2 ± 0.6 (1,2,3,4)

Abbot *et al.* that vigabatrin is a substrate of hPAT1. In addition the K_m (5.2 mM) was determined. This is a fairly good affinity compared to other PAT1 substrates. Moreover, it was found that tryptophan was able to almost completely inhibit the inward current mediated by vigabatrin in hPAT1-expressing oocytes. *In vitro* it is therefore possible to reduce the hPAT1-mediated transport of vigabatrin by applying tryptophan to the *in vitro* system. The possibility of rectal absorption of vigabatrin was investigated based upon published data demonstrating the presence of *SLC36A1* mRNA in the rectum of humans (24) and *rSlc36a1* mRNA in the rectum of rats (18). Since the presence of mRNA in a tissue does not necessarily mean that the protein is expressed, we confirmed the expression of rPAT1 protein in rat rectal epithelial samples in the present study. Caco-2 cell monolayers have been shown to constitutively express hPAT1 (24) and were therefore used as a positive control. The level of the reference protein rGAPDH was monitored as a loading control. The presence of N- and E-cadherin verified the presence of epithelial cells and the potential presence of smooth muscle cells from the intestinal wall (26–28).

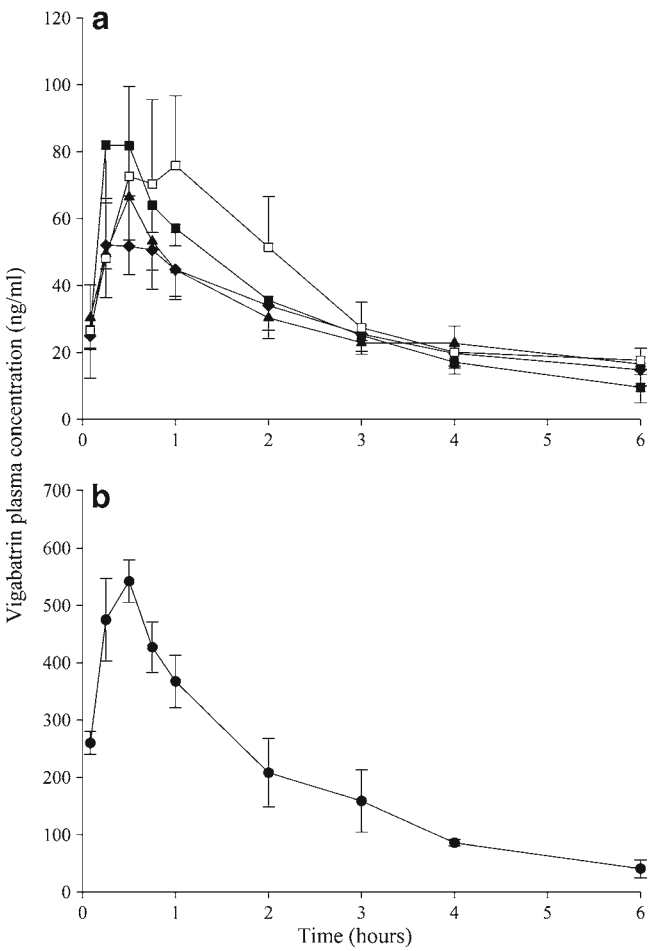

Fig. 4 Vigabatrin plasma concentration *versus* time profile following rectal administration of vigabatrin to rats (**a**); rectal gel 1 (▲); 2 (■); 3 (♦) and 4 (□); (**b**) rectal gel 5. Data represents mean ± S.E.M. (n=3–6). The composition of the different gels may be found in Table I.

Collectively, these data support the rationale of targeting PAT1 in the rectal epithelium to facilitate the absorption of vigabatrin. Upon rectal administration, the absorbed fraction of vigabatrin was approximately 8%. The compound has a logP of -0.1 (11), so passive absorption must be considered to be very low. Co-administration of vigabatrin and the PAT1 inhibitor tryptophan or the competitive inhibitor proline caused no changes in the fraction of vigabatrin absorbed. Larsen *et al.* also saw no effect of tryptophan on the oral absorption fraction of the PAT1 substrate, gaboxadol, administered to dogs (21). These observations could be due to insufficient amounts of inhibitor or species differences between dogs and rats.

Transport by PAT1 is dependent upon a proton gradient and when phosphate buffer was added to maintain the pH at 6, vigabatrin was absorbed faster. The opposite, a longer t_{max}, was observed when an inhibitor (20 mM proline) was added. The K_m of hPAT1 for proline has been reported to be 1.6 mM (16), hence the effect on the pharmacokinetic profile of vigabatrin could be interpreted as a competitive effect on the transporter, leading to a prolonged absorption profile. Only a trend towards a slightly prolonged absorption time was seen in the animals co-administered with

49 mM tryptophan. However, since the K_m of hPAT1 for tryptophan is 5–8 mM (16,21), this may be due to insufficient inhibition of the transporter. Even though species differences exist for transporters and metabolizing enzymes we are not aware of any publications specifically showing this for PAT1. The substrate selectivity seems to be similar for hPAT1 and rPAT1 (15). Moreover, the K_m values reported in the literature for proline are 0.87 ± 0.4 mM for rPAT1 (29), 2.8 ± 0.1 mM for mPAT1 (30) and generally 1.6–3.6 mM for hPAT1. For GABA similar variations in the values have been reported; 0.499 ± 0.135 mM for rPAT1 (31), 3.1 ± 0.2 mM for mPAT1 (30) and 1.95 ± 0.78 mM for hPAT1 (30). Considering the normal laboratory to laboratory and method to method variation in affinity values there is no clear indication that species variations in PAT1 substrate recognition and kinetics itself are present. Although the effect of both proline, buffer and, to a lesser extent, tryptophan seems to point toward an involvement of rPAT1 in transporting vigabatrin following rectal administration, the involvement of other transporters cannot be excluded.

When the administered dose of vigabatrin was increased from 1 to 10 mg/kg, a lack of dose proportionality was evident, though the dose was much lower than the therapeutic doses in the pediatric use of the compound. The low capacity of the transporter in the rectum seems to be a limitation for the absorption of vigabatrin in rats and needs to be evaluated in larger animals in future studies.

CONCLUSIONS

In the present study, it was demonstrated that vigabatrin is transported via recombinant hPAT1 in a concentration-dependent manner. The rPAT1 protein was detected in rat rectal epithelium and following rectal administration, 4-9% of the dose was absorbed, depending on the dose and formulation. Minor alterations in the pharmacokinetic profile were observed in the presence of the PAT1 inhibitor tryptophan, but the bioavailability remained relatively constant. The expression of rPAT1 protein in the rectum does, however, suggest that targeting the PAT1 in rectum could be a drug delivery strategy for the pediatric use of vigabatrin.

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