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ORIGINAL ARTICLE

Intestinal absorption of the antiepileptic drug substance vigabatrin is altered by infant formula in vitro and in vivo.

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Abstract

Vigabatrin is an antiepileptic drug substance mainly used in pediatric treatment of infantile spasms. The main source of nutrition for infants is breast milk and/or infant formula. Our hypothesis was that infant formula may affect the intestinal absorption of vigabatrin. The aim was therefore to investigate the potential effect of coadministration of infant formula with vigabatrin on the oral absorption in vitro and in vivo. The effect of vigabatrin given with an infant formula on the oral uptake and transepithelial transport was investigated in vitro in Caco-2 cells. In vivo effects of infant formula and selected amino acids on the pharmacokinetic profile of vigabatrin was investigated after oral coadministration to male Sprague–Dawley rats using acetaminophen as a marker for gastric emptying. The presence of infant formula significantly reduced the uptake rate and permeability of vigabatrin in Caco-2 cells. Oral coadministration of vigabatrin and infant formula significantly reduced C_{max} and prolonged t_{max} of vigabatrin absorption. Ligands for the proton-coupled amino acid transporter PAT1, sarcosine, and proline/L-tryptophan had similar effects on the pharmacokinetic profile of vigabatrin. The infant formula decreased the rate of gastric emptying. Here we provide experimental evidence for an in vivo role of PAT1 in the intestinal absorption of vigabatrin. The effect of infant formula on the oral absorption of vigabatrin was found to be due to delayed gastric emptying, however, it seems reasonable that infant formula may also directly affect the intestinal absorption rate of vigabatrin possibly via PAT1.

Abbreviations

Ala, alanine; ANOVA, analysis of variance; AUC, area under the curve; CL, clearance; Gly, glycine; HBSS, Hanks balanced salt solution; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; IV, intravenously; MES, 2-(N-morpholino) ethanesulfonic acid; PO, oral gavage; Pro, proline; QC, quality control; Sar, sarcosine; Tau, taurine; TEER, transepithelial electrical resistance; Trp, tryptophan.

Introduction

Vigabatrin is approved for treatment of infantile spasms in infants and children between the age of 1 month and 2 years (The European Agency for the Evaluation of Medicinal Products 1999; U.S. Department of Health and Human Services 2010). It is administered orally to infants and children using sachets of powder dissolved in a suitable fluid, which is often suggested to be milk or infant formula (Danish Health and Medicines Authority 2013; Drug information online 2013). Vigabatrin seems to be transported from the intestinal lumen into the intestinal epithelial cell via the proton-coupled amino acid transporter, PAT1 (Abbot et al. 2006; Holm et al. 2012; Nøhr et al. 2014). PAT1 is a proton-coupled amino acid transporter involved in the intestinal transport of proline

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(Pro), alanine (Ala), glycine (Gly), taurine (Tau) and sarcosine (Sar) (Metzner et al. 2006; Anderson et al. 2009) and substances such as gaboxadol, δ-amino levulinic acid, and GABA (for reviews see [Frolund et al. 2013; Thwaites and Anderson 2011]), whereas L-tryptophan (Trp) is a well-known inhibitor of PAT1 (Metzner et al. 2005). The transepithelial transport of vigabatrin across an in vitro model of the small intestine (Caco-2 cells) likewise showed that vigabatrin transport was mainly PAT1-dependent (Nøhr et al. 2014). It has previously been demonstrated that the absorption of gaboxadol, another PAT1 substrate, was altered in the presence of amino acids both in vitro and in vivo and it was thus concluded that PAT1 is involved in the intestinal transport of gaboxadol both in vitro and in vivo (Larsen et al. 2009, 2010). Moreover, some dipeptides have been shown to reduce gaboxadol and vigabatrin transport in PAT1 expressing *Xenopus Laevis* oocytes (Frolund et al. 2012). Since most low-immunogenic infant formula contains extensively hydrolyzed protein that is amino acid and small peptides, we hypothesize that these infant formulas could interfere with vigabatrin absorption in the intestine. The aim of this study was, therefore, to investigate the effect of coadministration of infant formula on the uptake and transport of vigabatrin in Caco-2 cells and on the pharmacokinetic profile of vigabatrin using rats as an in vivo model.

Materials and Methods

Composition of infant formula

The infant formula, Nutramigen Lipil was prepared in a concentration of 150 mg mL^{-1}, which is the amount given to infants. This concentration equals the dose of 1500 mg kg^{-1} administered here to rats. Infant formula solution contains (per liter); 21 g hydrolyzed casein, L-cystine, L-tyrosine, and Trp, 37.5 g fat, 7.3 g linoleic and linolenic acid, 82.5 g carbohydrate and a variety of vitamins and minerals, and nutrients such as choline, inositol, carnitine (19 mg), Tau (45 mg) (Mead Johnson Nutrition 2013).

Cell cultivation

Caco-2 cells were cultured as previously described (Nielsen et al. 2001; Larsen et al. 2008). The experiments were performed on cells in passages 10 through 18. Polycarbonate membranes (1.12 cm^2, 0.4 μm pore size) of Transwell™ (Corning Life Sciences, Tewksbury, MA) inserts or 24-well cell culture plates (1.90 cm^2) were used as supports for cell cultivation. The cells were seeded at a density of $8.9 \cdot 10^4$ cells cm^{-2}. Experiments performed on cells cultured on 24-well plates were performed 6 days after seeding and for cells seeded on Transwell™ inserts the experiments were performed 20 days post seeding.

Caco-2 cell experiments

Hanks balanced salt solution (HBSS) buffer (in mmol/L: $CaCl_2$, 1.26; $MgCl_2$, 0.49; $MgSO_4$, 0.41; KCl, 5.33; KH_2PO_4, 0.44; Na_2HPO_4, 0.34; D-Glucose, 5.56; $NaHCO_3$, 4.5) supplemented with 0.05% bovine serum albumin (BSA) was used during cell experiments. The sodium chloride (NaCl) content was either 138 mmol/L (denoted HBSS) or 48 mmol/L (denoted NaCl-reduced HBSS). The solutions were buffered to 6.0 or 7.4 using 10 mmol/L MES (2-(N-morpholino)ethanesulfonic acid) or HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) as buffer systems, respectively. The experimental solutions had osmolarities between 270 and 336 mOsmol L^{-1}. A tissue resistance measurement chamber (Endohm) using a voltmeter (EVOM) achieved from World Precision Instruments (Sarasota, FL) was used to measure the transepithelial electrical resistance (TEER) of the Caco-2 cell monolayers cultured on Transwell™ inserts. The TEER was measured after equilibration to room temperature and measurements were in the range of 394–633 Ωcm^2.

The uptake experiments were performed as previously described (Nøhr et al. 2014). Infant formula solutions was prepared in H_2O buffered with 10 mmol/L MES or HEPES in order to keep the osmolality of the solution approximately iso-osmotic (310–336 mOsmol L^{-1}). The infant formula solution (150 mg mL^{-1}) was prepared by incubation at 37°C for 15 min with occasional agitation. Hereafter the infant formula suspension was centrifuged at 4°C for 15 min at 18000*g* to remove particles and lipids. The supernatant was centrifuged again to remove the lipids. The centrifuged infant formula solution will be denoted "low fat infant formula" in the following. The infant formula solution had a sodium concentration of 360.0 mg L^{-1} and a chloride concentration of 720.0 mg L^{-1}. NaCl-reduced HBSS had a sodium concentration of 1125.3 mg L^{-1} and a chloride concentration of 2025.0 mg L^{-1}.

In the transport studies across Caco-2 cell monolayers the concentration of vigabatrin was 1.0 mmol/L. The transport was measured in the apical to basolateral (A–B) direction. The experiments were performed as previously described (Nøhr et al. 2014).

The ability of infant formula, Trp, Gly-Gly, Gly-Sar, 2-amino-2-norbornanecarboxylic acid (BCH) and Ile to inhibit the vigabatrin transport in the A–B direction was investigated. NaCl-reduced HBSS containing 1.0 mmol/L vigabatrin and 35.0 mmol/L Trp, 20 mg mL^{-1} Gly-Gly (151.4 mmol/L), Gly-Sar (136.9 mmol/L), BCH (128.9 mmol/L) or Ile (152.5 mmol/L), with the osmolarity

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adjusted to 270–310 mOsmol L^{-1} with mannitol, or H_2O containing 1.0 mmol/L vigabatrin and low fat infant formula (150 mg mL^{-1}), with an osmolarity of 310–336 mOsmol L^{-1} was applied to the apical side of the cells. As a control the vigabatrin transport in the absence of excess compound was measured in both HBSS and NaCl-reduced HBSS buffer.

The paracellular flux was experimentally determined by measuring the A–B transport of [^{14}C]-mannitol after the vigabatrin transport experiments as previously described (Nøhr et al. 2014).

In vivo study

The in vivo 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 were approved for Lundbeck A/S by the Animal Welfare Committee appointed by Danish Ministry of Food, Agriculture and Fisheries. Male Sprague–Dawley rats were obtained from Charles River (Sulzfeld, Germany) and acclimatized for a minimum of 5 days prior to the experiment. During this time the rats were fed standard diet and were allowed free access to water. Prior to the experiment the animals were fasted 16–20 h and randomly assigned to receive one of the formulations (Table 1). The male Sprague–Dawley rats weighed 276–335 g on the day of dosing. After administration 200 μL blood samples were drawn from the tail vein at time points 0, 5, 10, 15, 20 and 40 min and 1, 2 and 4 h into ethylenediaminetetraacetic acid (EDTA)-coated tubes. Plasma was separated by 15 min of centrifugation at 4°C, 2765*g*, and stored at −80°C until analysis. During the experiment animals were allowed free access to water but were restricted from food. After the experiment animals were euthanized using CO_2.

A total of 96 animals divided into 16 groups each consisting of 6 animals were assigned to the experiment. Animals were dosed by oral gavage (PO) or intravenously (iv) in the tail vein with one of the formulations presented in Table 1. Each rat was dosed 10 mL kg^{-1} body weight of the formulations containing either 1.0 or 10.0 mg vigabatrin kg^{-1}. The formulations were adjusted with NaCl to osmolarities of 260–312 mOsmol L^{-1}.The low fat infant formula (150 mg mL^{-1}) was prepared as described above, but was centrifuged at 3960*g* to remove lipids. The osmolarities of the solutions containing infant formula were in the range of 313–408 mOsmol L^{-1}. The pH of the formulations was adjusted to 7.30 ± 0.15 with NaOH.

Quantitative analysis

The sample preparation and quantification of vigabatrin in samples from in vitro studies was performed as previously described (Nøhr et al. 2014).

Plasma samples were protein precipitated and spiked with internal standard (rac-vigabatrin ^{13}C,d_2) and diluted 120 times in mobile phase A. Vigabatrin was quantified by hydrophilic interaction chromatography followed by MS/MS detection. The LC-MS/MS system was obtained from Waters (Milford, MA) and consisted of an Acquity UPLC system coupled to Xevo TQ MS system. Data acquisition

Table 1. Overview of the formulations administered to Sprague–Dawley rats.

Formulation	Route of adm.	Dose, mg kg^{-1}	Dose, mg kg^{-1}		Dose, mg kg^{-1}				
		Vigabatrin	Infant formula	Low fat infant formula	Pro	Trp	Sar	BCH	Acetaminophen
0	iv	1	–	–	–	–	–	–	–
1	PO	1	–	–	–	–	–	–	–
2	PO	1	1500	–	–	–	–	–	–
3	PO	1	–	1500	–	–	–	–	–
4	PO	1	–	–	100	100	–	–	–
5	PO	1	–	–	–	–	200	–	–
6	PO	1	–	–	–	–	–	200	–
7	PO	10	–	–	–	–	–	–	–
8	PO	10	1500	–	–	–	–	–	–
9	PO	10	–	1500	–	–	–	–	–
10	PO	10	–	–	–	–	200	–	–
00	iv	–	–	–	–	–	–	–	120
11	PO	10	–	–	–	–	–	–	120
12	PO	10	1500	–	–	–	–	–	120
13	PO	10	–	1500	–	–	–	–	120
14	PO	10	–	–	–	–	200	–	120

The doses listed correspond to concentrations of 86.9 mmol/L Pro, 49.0 mmol/L Trp, 224.5 mmol/L Sar, 128.9 mmol/L BCH, and 79.4 mmol/L acetaminophen in the solutions administered.

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software was Unifi version 1.5.1.013. The mobile phases used were 5.0 mmol/L ammonium formate and 0.25% formic acid in water/acetonitrile (10:90) (mobile phase A) and in water (10:90) (mobil phase B). 5 μL of sample was injected onto an Acquity UPLC BEH Amide column (2.1 × 100 mm, 1.7 μmol/L) at a column temperature of 60°C. The analysis was run in gradient mode with a start concentration of 95% mobile phase A and with a flow rate of 0.600 mL min^{-1}. A linear gradient was run within the first 2.10 min, running from 95% to 50% mobile phase A. Until 2.30 min the gradient was 50% mobile phase A. In the time interval 2.30–2.40 min the initial gradient at 95% mobile phase A was re-established. The total runtime was 2.80 min. The HPLC system was connected to the mass spectrometer via an electrospray interface and was operated in the positive ionization mode. Vigabatrin was detected using multiple reaction monitoring (MRM) of the protonated molecules $(M\text{-}H)^+$ and their major collision-induced fragments (*m/z* 130.10 → 113.10 for vigabatrin and *m/z* 133.10 → 116.10 for the internal standard, rac-vigabatrin $^{13}C,d_2$). The dwell time for both channels was 0.020 sec and the Cone and the Capillary voltages were 15 V and 7 eV. Calibration standards were prepared in the range 15–15000 ng mL^{-1} vigabatrin in blank plasma from Sprague–Dawley rats. Quality control (QC) samples were prepared in the same way with resulting vigabatrin concentrations of 45, 450 or 12000 ng mL^{-1}.

The quantification of acetaminophen was performed using an acetaminophen analysis kit (Cambridge Life Sciences Ltd., Cambridge, UK) according to the protocol provided by the manufacturer. The range of detection was 1.5–378 μg/mL.

Data treatment

The vigabatrin flux (J, nmol min^{-1} cm^{-2}) was calculated as the amount (Q, nmol) of vigabatrin transported across the area (A, cm^2) of a Caco-2 cell monolayer over a fixed time period (t, min) (eq. 1):

$$J = \mathrm{d}Q/\mathrm{d}t \cdot 1/A. \quad (1)$$

The vigabatrin transport was in steady state for 10–150 min. The apparent permeability (P_{app}, cm sec^{-1}) was calculated from the steady-state fluxes (J) and the initial vigabatrin concentration (C_o, mmol/L) (eq. 2):

$$P_{app} = J/C_0. \quad (2)$$

The pharmacokinetic parameters were estimated using Phoenix 64 WinNonlin. The plasma concentration time profile of vigabatrin after intravenous administration (iv) was fitted to a two compartment model. The plasma concentration time profile derived after iv administration of acetaminophen was fitted to a one-compartment model. A non-compartmental model was used to fit the plasma concentration time profiles after oral administration of vigabatrin or acetaminophen. The area under the curve (AUC_∞) and elimination rate (k_e) was estimated by linear regression of the last three time points and extending to infinity, as an estimate for the elimination. The bioavailability F_{abs} was estimated for each individual animal (x) by the following equation (eq. 3):

$$F_{abs} = [AUC_x/AUC_{iv}] \cdot [Dose_{iv}/Dose_x] \quad (3)$$

The AUC_x indicates the AUC after oral administration of a certain dose of acetaminophen or vigabatrin ($Dose_x$) to each individual animal and AUC_{iv} indicates the AUC after iv administration of $Dose_{iv}$.

The mean cumulative fraction of absorbed acetaminophen and vigabatrin was calculated by numerical deconvolution using the Längenbucher approach (Längenbucher 1982). The input data for the deconvolution curves were calculated by equation 4–7:

$$I_1 = (R_1/T)/W_1 \quad (4)$$

$$I_2 = (R_2/T - I_1W_2)/W_1 \quad (5)$$

$$I_3 = (R_3/T - I_1W_3 - I_2W_2)/W_1 \quad (6)$$

$$I_n = (R_n/T - I_1W_n - I_2W_{n-1} - \ldots - I_nW_2)/W_1 \quad (7)$$

The input function $I(t)$ represents the absorption process, R(t) is the response function, here equal to the plasma concentration after oral administration, W(t) is the weighted function and represents the plasma concentration obtained after iv administration of the drug (vigabatrin or acetaminophen). T was fixed at 5 min. Deconvolution curves were used to estimate the absorption rate constant after oral administration of acetaminophen representing an estimation of the rate of gastric emptying (k_{empty}) (eq. 8).

$$Q(t) = Q_{max} \cdot (1 - e^\wedge(-k_{empty}t)) \quad (8)$$

$Q(t)$ represents the amount of acetaminophen absorbed at a given time (t) and Q_{max} is 100%. The R^2 values for the fitted curves were between 0.71 and 0.97. The k_{empty} values estimated from each separate animal were individually used to estimate the absorption rate constant (k_a) of vigabatrin. This was done following two different approaches in order to estimate k_a for vigabatrin. The deconvolution curves after oral vigabatrin administration were fitted to equation (9) and the plasma concentration time profiles of vigabatrin were fitted to equation (10):

$$Q(t) = 100 \cdot (1 - k_{empty}/(k_a - k_{empty}) \cdot (e^\wedge(-k_{empty}t) - e^\wedge(-k_at)) - e(-k_{empty}t)) \quad (9)$$

$$Q(t) = F_{abs} \cdot D \cdot (1 - k_{empty}/(k_a - k_{empty}) \cdot (e^\wedge(-k_{empty}t) - e^\wedge(-k_at)) - e(-k_{empty}t)) \cdot e^\wedge(-k_et) \quad (10)$$

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$Q(t)$ represents the amount of vigabatrin (in% eq. 9 or in ng mL^{-1} eq. 10) at a given time (t). F_{abs} represent the absorption fraction of vigabatrin and D is the vigabatin dose (ng mL^{-1}). k_e was estimated to 2.2 h^{-1} from the plasma concentration time profile after iv administration of 1.0 mg kg^{-1} vigabatrin (formulation 0). The plasma concentration time profiles of vigabatrin were fitted with R^2 values between 0.70 and 0.97, except the curve for Sar at 1.0 mg kg^{-1} vigabatrin which had values between 0.45 and 0.92. The deconvolution profiles of vigabatrin were fitted to equation (10) resulting in R^2 values in the range 0.80–0.99.

Statistical analysis

The statistical analysis was performed in GraphPad Prism software version 6.00 (GraphPad Software, Inc., La Jolla, CA). Differences between means were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. $P > 0.05$ was considered non-significant (ns); *denotes a level of significance of $P < 0.05$. Results are presented as mean ± SEM.

Materials

Vigabatrin was provided by H. Lundbeck A/S (Valby, Denmark). Acetonitrile gradient grade was from Vetec Química Fina Ltda (Rio de Janeiro, Brazil) or LC-MS grade from Sigma-Aldrich (St. Louis, MO, USA). Rac-vigabatrin $^{13}C,d_2$ was purchased from Toronto Research Chemicals (Toronto, Canada). BSA was from Biotech Line (Slangerup, Denmark). Infant formula Nutramigen Lipil was obtained from Mead Johnson Nutrition (Nijmegen, The Netherlands). D-[1-^{14}C]-mannitol (58.8 Ci·$mmol^{-1}$) and Ultima Gold scintillation liquid were purchased from PerkinElmer (Boston, MA).The acetaminophen assay kit was purchased from Cambridge Life Sciences Ltd. (Cambridge, UK). Caco-2 cells were obtained from German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany). Cell culture plasticware was obtained from Corning Life Sciences (Tewksbury, MA). Water used for the experiments was obtained from a Milli-Q water purification system. All other chemicals were from Sigma-Aldrich (St. Louis, MO).

Results

Transepithelial vigabatrin transport across Caco-2 cell monolayers was decreased in the presence of infant formula and dipeptides

To investigate the effect of infant formula on vigabatrin transport in vitro, Caco-2 cells were used as an in vitro model. Infant formula, amino acids, and dipeptide contribute to increased solution osmolarity, and the transport of vigabatrin was, therefore, initially investigated in HBSS and NaCl-reduced HBSS. The transport of vigabatrin in the apical to basolateral direction was unaffected by the lowered amount of NaCl in the isosmotic transport medium (see Fig. 1). The NaCl-reduced HBSS buffer was subsequently used to dissolve Trp, BCH, Ile, Gly-Gly, and Gly-Sar. Infant formula was dissolved in buffered water, as NaCl is a constituent of infant formula itself. The apparent permeability (P_{app}) of vigabatrin was significantly decreased in the presence of low fat infant formula,

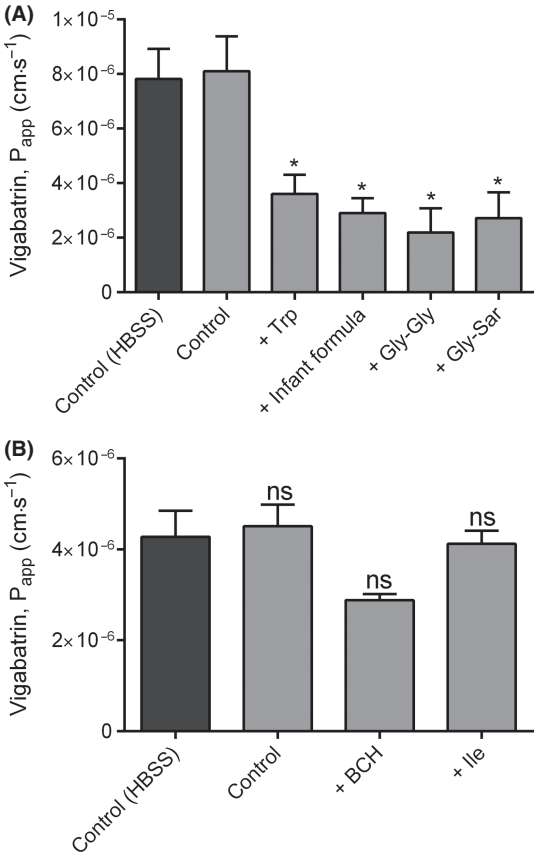

Figure 1. Apparent permeability of transepithelial vigabatrin (1.0 mmol/L) transport across Caco-2 cell monolayers in the presence or absence of infant formula. (A) The permeability (P_{app}) was measured in the presence or absence of 35.0 mmol/L Trp, infant formula or 20 mg mL^{-1} (151.4 mmol/L) Gly-Gly or Gly-Sar (136.9 mmol/L). (B) Vigabatrin permeability in the absence or presence of 20 mg mL^{-1} BCH (128.9 mmol/L) or Ile (152.5 mmol/L). Permeability was measured across Caco-2 cells cultured for 20 days on transwell filters. Dark gray control is the vigabatrin permeability in HBSS buffer, light gray columns are in NaCl-reduced HBSS. Columns represent mean ± SEM of permeability measured in 3–4 passages. ANOVA followed by Dunnett's multiple comparison test. *Significant difference from both controls ($P < 0.05$).

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Gly-Gly and Gly-Sar as well as in the presence of the PAT1-inhibitor Trp ($P < 0.05$, $n = 3$–4) (Fig. 1A). The presence of high concentrations of non-PAT1 ligands BCH and Ile did not significantly reduce the P_{app} of vigabatrin across the Caco-2 cell monolayers, although the control permeability, due to passage variation, was lower than in Figure 1A ($P > 0.05$, $n = 3$–4) (Fig. 1B). The paracellular mannitol permeability was constant ($1.6 \cdot 10^{-7} \pm 0.20 \cdot 10^{-7}$ cm sec^{-1}), regardless of the solution used. The decrease in vigabatrin permeability induced by the added compounds was not due to impaired cell integrity and indicates that transepithelial transport of vigabatrin was mainly facilitated by PAT1. The apical vigabatrin uptake rate was significantly decreased in the presence of low fat infant formula or Gly-Gly at an apical pH of 6.0 ($P < 0.05$, $n = 3$–4). The control value was 1.01 ± 0.11 nmol min^{-1} cm^{-2}, whereas the uptake rate was reduced to 0.53 ± 0.09 nmol min^{-1} cm^{-2} in the presence of low fat infant formula or 0.48 ± 0.07 nmol min^{-1} cm^{-2} in the presence of Gly-Gly. At an apical pH of 7.4 the uptake of vigabatrin was reduced to 0.31 ± 0.04 nmol min^{-1} cm^{-2}, consistent with a proton-dependent uptake, and neither low fat infant formula or Gly-Gly affected the cellular uptake rate of vigabatrin ($P > 0.05$, $n = 3$–4) under these conditions. The uptake rate at pH 7.4 was 0.28 ± 0.06 or 0.25 ± 0.03 nmol min^{-1} cm^{-2} in the presence of low fat infant formula or Gly-Gly, respectively. The in vitro investigation of the interaction between infant formula and vigabatrin transport showed that infant formula, dipeptides and a known PAT1-inhibitor, Trp, decreased the permeability of vigabatrin, whereas PAT1-non-ligands did not. Furthermore, the uptake rate of vigabatrin was reduced by infant formula and Gly-Gly. Collectively, this indicates that vigabatrin and possibly free amino acids and dipeptides from the infant formula interact with the apical PAT1 carrier.

Vigabatrin absorption in Sprague–Dawley rats was decreased after coadministration with infant formula and PAT1-ligands

In order to investigate whether the in vitro findings were predictive for the in vivo effect of infant formula on the absorption of vigabatrin, the vigabatrin absorption was investigated in Sprague–Dawley rats. The vigabatrin plasma concentration profiles after oral administration of 1.0 or 10.0 mg kg^{-1} vigabatrin showed a rapid absorption phase with the maximal plasma concentration (C_{max}) appearing 0.25–0.33 h after oral administration (Fig. 2). The time to reach C_{max} (t_{max}) after administration of 1.0 and 10.0 mg kg^{-1} vigabatrin was significantly increased in the presence of infant formula and low fat infant formula (Fig. 2, Tables 2 and 3). C_{max} of vigabatrin was significantly decreased after oral coadministration of infant formula and low fat infant formula (Fig. 2A, Tables 2

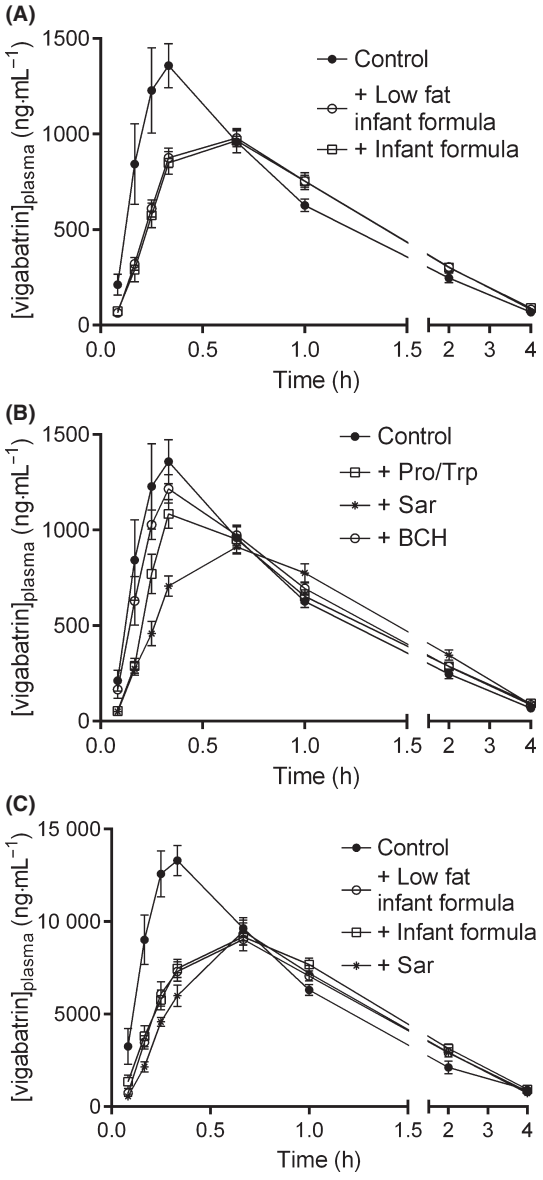

Figure 2. The plasma concentration time profile of vigabatrin after oral administration of 1.0 or 10.0 mg kg^{-1} vigabatrin to male Sprague–Dawley rats. (A) The rats received solutions containing 1.0 mg kg^{-1} vigabatrin alone or coadministered with 1500 mg kg^{-1} infant formula or low fat infant formula. (B) The rats received solutions containing 1.0 mg kg^{-1} vigabatrin alone or coadministered with 100 mg kg^{-1} (86.9 mmol/L) Pro and (49.0 mmol/L) Trp, 200 mg kg^{-1} (224.5 mmol/L) Sar or (128.9 mmol/L) BCH. (C) The rats received solutions containing 10.0 mg kg^{-1} vigabatrin alone or coadministered with 1500 mg kg^{-1} infant formula or low fat infant formula or 200 mg kg^{-1} Sar. Data points represent mean ± SEM from six different animals.

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Table 2. Estimated pharmacokinetic parameters after administration of 1.0 mg kg^{-1} vigabatrin to rats.

Formulation	0	1	2	3	4	5	6
Route of adm.	iv	PO	PO	PO	PO	PO	PO
Dietary component	None	None	Infant formula	Low fat infant formula	Pro/Trp	Sar	BCH
$AUC_{0-\infty}$ (h ng mL^{-1})	2421 ± 182	1747 ± 96	1751 ± 59	1781 ± 37	1770 ± 86	1763 ± 70	1839 ± 68
t_{max} (h)		0.25 [0.25; 0.33]	0.67 [0.67; 0.67]*	0.67 [0.58; 0.66]*	0.33 [0.33; 0.33]	0.67 [0.67; 0.75]*	0.33 [0.33; 0.33]
C_{max} (ng mL^{-1})		1403 ± 124	965 ± 64*	984 ± 45*	1084 ± 75*	920 ± 31*	1215 ± 73
CL(mL h^{-1} kg^{-1})	426 ± 34	581 ± 30	574 ± 19	563 ± 12	571 ± 26	572 ± 23	548 ± 20
k_e (h^{-1})	2.0 ± 0.2	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.1
F_{abs} (%)	100	72 ± 4	72 ± 2	74 ± 2	73 ± 4	73 ± 3	76 ± 3

C_{max}, $AUC_{0-\infty}$, CL, k_e, and F_{abs} are expressed as mean ± SEM, t_{max} is expressed as the median [Q1; Q3] (25% and 75% percentile) from six rats.
*Significant difference from oral administration of vigabatrin 1.0 mg kg^{-1} ($P < 0.05$).

and 3), indicating an effect of infant formula on the vigabatrin absorption. Pro/Trp or Sar also significantly decreased the C_{max} of vigabatrin after oral coadministration (Fig. 2B). Sar also had a significant prolonging effect on t_{max} of vigabatrin absorption (Table 2). Sar had a similar effect on t_{max} and C_{max} at 10.0 mg kg^{-1} vigabatrin (Fig. 2C). To verify that only PAT1-ligands were able to affect the pharmacokinetic parameters of vigabatrin absorption, BCH was coadministered with vigabatrin. BCH did not significantly alter the pharmacokinetic parameters of vigabatrin absorption after oral administration of 1.0 mg kg^{-1} vigabatrin (Fig. 2B, Table 2). For all the treatments investigated the AUC was similar to the relative controls (1.0 or 10.0 mg kg^{-1}). The elimination constant (k_e) and clearance (CL) also remained unchanged (Tables 2 and 3). These findings indicate that infant formula and PAT1-ligands alter t_{max} and C_{max} of vigabatrin after oral coadministration to rats and potentially affect the absorption profile of vigabatrin from the small intestine, but not the fraction absorbed.

Acetaminophen absorption was altered after oral coadministration with infant formula

The absorption of acetaminophen was investigated in order to evaluate the effect of infant formula, low fat infant formula and Sar, on the rate of gastric emptying. The plasma concentration time profile of acetaminophen showed a rapid increase in the plasma concentration (Fig. 3). Coadministration with infant formula and low fat infant formula decreased the absorption of acetaminophen, whereas Sar did not (Table 4). The t_{max} of acetaminophen absorption was significantly increased and the C_{max} of acetaminophen was decreased after oral coadministration with infant formula, whereas low fat infant formula or Sar did not alter these parameters. Infant formula did not significantly alter the AUC, CL or k_e of acetaminophen. These findings indicate that administration of infant formula has an effect on the rate of gastric emptying.

Correcting vigabatrin absorption for gastric emptying

The mean cumulative fraction of absorbed acetaminophen was calculated according to equations (4–7) and is depicted as a function of time as deconvolution profiles in Figure 4A. Acetaminophen was rapidly absorbed with about 90% absorbed after 30 min. The absorption constant for acetaminophen (k_{empty}) was calculated by fitting the deconvolution profiles to equation (8). The resulting k_{empty} values are presented in Figure 4B. The k_{empty} for

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Table 3. Estimated pharmacokinetic parameters after oral administration of 10 mg kg^{-1} vigabatrin to rats.

Formulation	7	8	9	10
Route of adm.	PO	PO	PO	PO
Dietary component	None	Infant formula	Low fat infant formula	Sar
$AUC_{0-\infty}$ (h ng mL^{-1})	17810 ± 808	17699 ± 533	16460 ± 267	15946 ± 816
t_{max} (h)	0.33 [0.25; 0.33]	0.67 [0.67; 1.00]*	0.67 [0.58; 0.67]*	0.67 [0.67; 0.67]*
C_{max} (ng mL^{-1})	13567 ± 953	9237 ± 731*	9087 ± 301*	9485 ± 738*
CL (mL h^{-1} kg^{-1})	567 ± 24	568 ± 18	608 ± 10	635 ± 32
k_e (h^{-1})	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0
F_{abs} (%)	74 ± 3	73 ± 2	68 ± 1	66 ± 3

C_{max}, $AUC_{0-\infty}$, CL, k_e and F_{abs} are expressed as mean ± SEM, t_{max} is expressed as the median [Q1; Q3] (25% and 75% percentile) from six rats.
*Significant difference from oral administration of vigabatrin 10.0 mg kg^{-1} ($P < 0.05$).

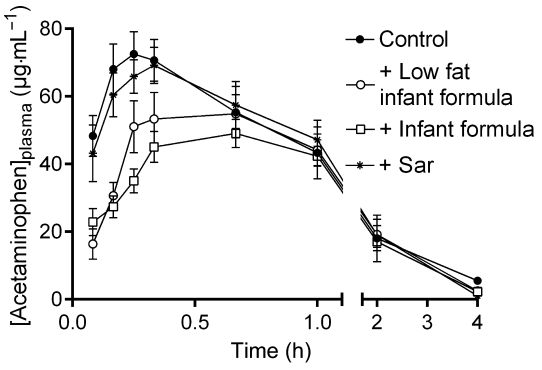

Figure 3. The plasma concentration time profile of acetaminophen after oral administration of 120 mg kg^{-1} acetaminophen to male Sprague–Dawley rats. The rats received solutions containing 120 mg kg^{-1} acetaminophen and 10.0 mg kg^{-1} vigabatrin alone or coadministered with 1500 mg kg^{-1} infant formula, low fat infant formula or 200 mg kg^{-1} Sar. Data points represent mean ± SEM from six different animals.

acetaminophen absorption showed a huge variation in the control group, whereas a much smaller variation was observed in the presence of additional coadministrations. k_{empty} was significantly decreased in the presence of infant formula or low fat infant formula, whereas Sar did not affect the k_{empty}. This indicates that infant formula, but not Sar, decreases the rate of gastric emptying.

Since infant formula decreases the rate of gastric emptying attempts were made to estimate the absorption constant (k_a) for vigabatrin under the different experiment conditions. This was done using two different approaches: The k_a values were estimated by fitting the deconvolution curves calculated for vigabatrin to equation (9), composed of 2 first-order processes that is gastric emptying and intestinal absorption. In the same way plasma concentration time profiles of vigabatrin were fitted to equation (10) to estimate the k_a values, assuming that gastric emptying is a prerequisite for intestinal absorption. The resulting k_a values are presented in Table 5. The k_a values were generally quite comparable between approaches and doses. However, k_a was significantly lower after coadministration of vigabatrin and Sar, moreover, coadministration of vigabatrin and Pro/Trp significantly decreased the k_a of vigabatrin based on the plasma concentration profile. There is a tendency that the k_a for vigabatrin was lower after coadministration with infant formula and low fat infant formula, however, the effect was not significant. This is probably due to the high variation observed within the control group. The deviation reflects the great variation in k_{empty} in the control situation and the great variation in plasma profiles of vigabatrin within the control group. The two approaches for estimating k_a for vigabatrin gives similar results and the overall impression is that Sar and possibly Pro/Trp affects the absorption profile of vigabatrin also after taking the effects of changed gastric emptying into consideration. There is, hence, a tendency toward an effect of the infant formula on vigabatrin absorption profile as indicated by the decreased k_a.

Discussions and Conclusions

Vigabatrin is mainly used for treating infantile spasms in infants and young children. The recommended pediatric dose is between 50 mg kg^{-1} day^{-1} and 150 mg kg^{-1} day^{-1} divided into two daily doses given as an oral solution (Drug information online 2013). Each dose thus contains 25–125 mg kg^{-1}, compared to the dose of 1.0 or 10.0 mg kg^{-1} administered to rats in this study. It is recommended by the U. S. Food and Drug Administration (FDA) that vigabatrin solutions are administered to infants with or without food, whereas in Denmark the product resume suggest that vigabatrin is dissolved in milk or infant formula (Danish Health and Medicines Authority 2013). This raises the question if infant formula alters intestinal vigabatrin absorption in infants supported by infant formula. Vigabatrin is a substrate of PAT1 and the transepithelial transport across

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Table 4. Estimated pharmacokinetic parameters after oral administration of 120 mg kg^{-1} acetaminophen to rats.

Formulation	00	11	12	13	14
Route of adm.	iv	PO	PO	PO	PO
Dietary component	None	None	Infant formula	Low fat infant formula	Sar
$AUC_{0-\infty}$ (h μg mL^{-1})	155 ± 4	104 ± 20	90 ± 6	99 ± 13	105 ± 14
t_{max} (h)		0.21 [0.15; 0.33]	0.67 [0.50; 0.67]*	0.33 [0.33; 0.67]	0.33 [0.21; 0.22]
C_{max} (μg mL^{-1})		76 ± 6	49 ± 4*	58 ± 7	72 ± 5
CL (mL h^{-1} kg^{-1})	775 ± 22	1413 ± 277	1354 ± 87	1318 ± 174	1294 ± 223
k_e (h^{-1})	1.1 ± 0.0	2.2 ± 0.6	1.4 ± 0.3	1.6 ± 0.3	2.8 ± 0.8
F_{abs} (%)	100 ± 3	67 ± 13	58 ± 4	64 ± 8	67 ± 9

C_{max}, $AUC_{0-\infty}$, CL, k_e and F_{abs} are expressed as mean ± SEM, t_{max} is expressed as the median [Q1; Q3] (25% and 75% percentile) from six rats.
*Significant difference from oral administration of 120 mg kg^{-1} acetaminophen ($P < 0.05$).

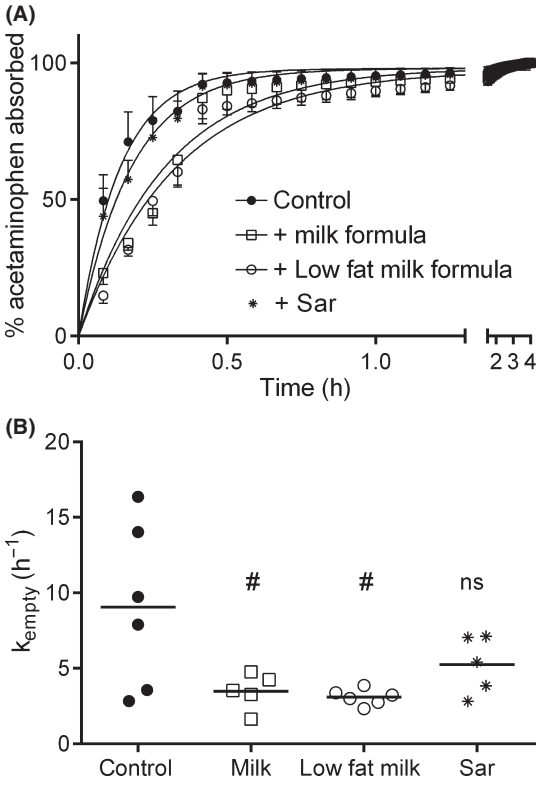

Figure 4. (A) Deconvolution profiles of acetaminophen absorption after oral administration to male Sprague–Dawley rats. Deconvolution profiles of 120 mg kg^{-1} acetaminophen alone or coadministered with 1500 mg kg^{-1} infant formula, low fat infant formula or 200 mg kg^{-1} Sar. Data represents mean ± SEM from five to six different animals. The data were fitted to equation (8) to estimate the absorption rate constant for acetaminophen (k_{empty}). (B) Distribution of k_{empty} values of acetaminophen estimated from the individual animals. ANOVA followed by Dunnett's multiple comparison test. #Significant difference from control ($P < 0.05$).

Caco-2 cell monolayers is mainly dependent on the transport function of PAT1 (Abbot et al. 2006; Holm et al. 2012; Nøhr et al. 2014). Here we found that the apparent absorptive permeability of vigabatrin across Caco-2 cells was decreased in the presence of low fat infant formula and Gly-Gly and Gly-Sar. For the infant formula investigated here the protein source is extensively hydrolyzed casein supplemented with three free amino acids (L-cystine, L-tyrosine, and Trp), however, the actual concentrations are not known. Taurine is present in a concentration of 0.36 mmol/L, which is too low to reduce vigabatrin absorption. It is a possibility that dipeptides and amino acids from the infant formula are responsible for the inhibition of vigabatrin uptake and transport and thus it seems plausible that the reduction in vigabatrin absorption may be due to a direct interaction with PAT1 due to inhibition via components of the infant formula for example free amino acids or dipeptides resulting from hydrolysis of casein.

Table 5. k_a values calculated from vigabatrin deconvolution curves (DC) and plasma concentration time profiles (PC), using the estimated k_{empty} values and fitting to eqs. (9) or (10), respectively.

Vigabatrin (mg kg^{-1})	1		10	
Profile fitted	PC	DC	PC	DC
Control	3.3 ± 0.4	7.9 ± 1.9	3.4 ± 0.4	8.1 ± 2.3
Milk	2.8 ± 0.3	5.3 ± 0.9	2.6 ± 0.3	4.1 ± 0.5
Low fat milk	2.6 ± 0.1	5.2 ± 0.3	2.6 ± 0.1	5.0 ± 0.3
Sar	1.6 ± 0.1*	2.9 ± 0.2*	1.7 ± 0.1*	3.2 ± 0.2*
Pro/Trp[a]	1.9 ± 0.2*	4.0 ± 0.6		
BCH[a]	2.4 ± 0.2	5.8 ± 1.7		

*Significant difference from control ($P < 0.05$).
ak_a values were estimated on the basis of k_{empty} values for the control situation.

In vitro findings may not always be transferable to the in vivo situation, which is why this study includes an investigation of the interaction between infant formula and vigabatrin in vivo in rats. The maximal plasma concentration and time to reach this was decreased and prolonged, respectively, at both vigabatrin concentrations

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investigated when the compound was coadministered with the infant formula. In comparison the PAT1 substrate Sar had similar effects and also Pro/Trp affected the C_{max}, while the non-PAT1-ligand BCH did not alter C_{max} or t_{max}. The coadministration with infant formula, Pro/Trp, Sar or BCH did not alter other pharmacokinetic parameters such as k_e, CL or AUC and the absorption fraction F_{abs} remained unchanged, but clearly the absorption profile was affected. It is well known that intake of high calorie meals decreases the gastric emptying rate and it has previously been shown that milk protein slows the rate of gastric emptying in humans (Calbet and MacLean 1997). We, therefore, investigated if the altered pharmacokinetic profile of vigabatrin was a result of delayed gastric emptying and thereby if the initially observed effects on vigabatrin absorption were merely caused by a calorie effect from the coadministered components. For this purpose, acetaminophen was used as a marker for gastric emptying (Willems et al. 2001). The assumption is that the intestinal absorption of acetaminophen is faster than the gastric emptying, that is the compound is absorbed instantly when emptied from the stomach. Infant formula and low fat infant formula significantly decreased the rate of gastric emptying. The coadministration of Sar with acetaminophen did not alter the rate of gastric emptying (k_{empty}) of acetaminophen compared to acetaminophen administered alone, which is in accordance with a previous study showing that the presence of Trp or mannitol did not alter the pharmacokinetic parameters of acetaminophen after oral administration to rat or dog (Larsen et al. 2009, 2010). Since Sar or Trp do not alter k_{empty}, but do decrease vigabatrin plasma C_{max}, this study provides the first evidence for an actual involvement of PAT1 in the absorption of vigabatrin in vivo, as both Pro, Trp and Sar has previously been shown to decrease vigabatrin uptake and transepithelial transport in Caco-2 cells (Nøhr et al. 2014). Furthermore, we show here that the PAT1-non-ligands BCH and Ile did not significantly alter the apparent permeability of vigabatrin in Caco-2 cells which was in agreement with the in vivo data as BCH did not significantly affect the plasma concentration time profile of vigabatrin in Sprague–Dawley rats. Infant formula decreased vigabatrin absorption by affecting k_{empty}. We also investigated if k_a was affected, by using two different pharmacokinetic strategies. First, we estimated the k_a from vigabatrin deconvolution curves using k_{empty} derived from acetaminophen deconvolution curves. Second, we estimated k_a from the vigabatrin plasma concentration time profiles using k_{empty} and the k_e constant obtained after iv administration of vigabatrin. Both approaches are dependent on k_{empty}, which was found to be highly variable in fasted animals. When additional caloric content was applied together with vigabatrin and acetaminophen the variation decreased. Even with this variation in mind, both approaches consistently showed that Sar reduced vigabatrin k_a, supporting the notion that Sar decreases vigabatrin intestinal absorption rate via a direct competitive inhibition of the PAT1-mediated vigabatrin transport. After oral administration of vigabatrin to humans there is generally a great variation in plasma concentrations (Sanchez-Alcaraz et al. 1996; Erdal et al. 1999; Lindberger et al. 2003), which is consistent with the large variations observed after oral administration to rats. The estimates of vigabatrin k_a after coadministration with infant formula and low fat infant formula yields lower rate constants than the controls at both doses and both estimation approaches. However, with the variation in the control group the effect on the absorption step cannot be shown with statistical certainty. The doses of vigabatrin administered to the rats were lower than the clinical relevant doses administered to infants; however, the effect of infant formula and Sar seemed to be similar at both 1.0 and 10.0 mg kg^{-1} vigabatrin in rats, and similar effects of infant formula may thus be expected after coadministration with clinical relevant doses of vigabatrin to infants.

In conclusion, we provide experimental evidence for an in vivo role of PAT1 in the intestinal absorption of vigabatrin. We also show a food–drug interaction between infant formula and vigabatrin absorption in vitro as well as in vivo. The effect of infant formula was due to a delayed gastric emptying, but it also seems likely that infant formula affect the intestinal absorption rate of vigabatrin.

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Disclosure

None declared.

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