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Involvement of L-type amino acid transporter 1 in the transport of gabapentin into human placental choriocarcinoma cells

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ABSTRACT

Gabapentin (GBP) is a widely used antiepileptic drug, with potential for use in the treatment of epilepsy in pregnant women. Although studies have examined GBP transport mechanisms across the blood-brain barrier, kidney, and intestine, the mechanism in the placenta has not been fully elucidated. We previously reported that GBP accumulates at high concentrations in human placental choriocarcinoma BeWo cells. The purpose of this study was to examine the transport mechanism of GBP in placental choriocarcinoma cells (BeWo and JEG-3), and to identify the carrier involved. High concentrations of intracellular GBP accumulations were also found in JEG-3 cells. A kinetic analysis showed that a single carrier system was involved in the uptake of GBP. Furthermore, substrates for L-type amino acid transporter (LAT) and siRNAs targeted to LAT1 significantly decreased GBP uptake. Our observations from this study suggest that LAT1 is the main contributor to GBP transport in placental choriocarcinoma cells.

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1. Introduction

Epilepsy is a neurological condition that has been reported in 0.3-0.7% of all pregnant women [1]. Because epileptic seizures during pregnancy pose a risk to both the mother and the fetus, most pregnant women experiencing seizures are prescribed antiepileptic drugs [2]. However, exposure to certain antiepileptic drugs during pregnancy is associated with increased health risks to the fetus. For example, the risk of major congenital malformations increases with the use of certain earlier generation antiepileptic drugs such as valproic acid (VPA), phenobarbital (PB), and carbamazepine (CBZ) [2]. Furthermore, several studies have indicated that VPA is associated with increased risk of neurodevelopmental delay and autism spectrum disorder [3,4].

Gabapentin [1-(Aminomethyl) cyclohexaneacetic acid, GBP] is a widely used antiepileptic drug. It is also used in the management of neuropathic pain. Protein binding of GBP is low (<3%), and GBP is mostly excreted by the kidney in its unchanged form [5]. Newer antiepileptic drugs, such as lamotrigine (LTG), levetiracetam (LEV), topiramate (TPM), and gabapentin (GBP) are being increasingly used, even during pregnancy [6]. Although several epidemiological studies have addressed the risks of new antiepileptic drugs on the fetus, the safety and efficacy of these drugs during pregnancy have not been fully established. Fujii et al. [7] conducted a prospective cohort study and reported that GBP administration during pregnancy does not appear to increase the risk for major malformations. However, the study indicated the possibility of increased risk for low birth weight and preterm birth when GBP was administered. Recently, a case report described neonatal GBP withdrawal syndrome after prolonged *in utero* exposure [8]. Therefore, information on the transport mechanisms of antiepileptic drugs in placental cell models might contribute to estimate the adverse effect of these

Abbreviations: BCH, 2-Amino-2-norbornanecarboxylic acid; CBZ, carbamazepine; CLB, clobazam; CZP, clonazepam; GBP, gabapentin; LAT, L-type amino acid transporter; LC/MS/MS, liquid chromatography/tandem mass spectrometry; LEV, levetiracetam; LTG, lamotrigine; NZP, nitrazepam; OCTN, organic cation/carnitine transporter; PB, phenobarbital; PHT, phenytoin; T_3, 3,3′,5-triiodo-L-thyronine; T_4, L-thyroxine; TEA, tetraethylammonium; TPM, topiramate; VPA, valproic acid; ZNS, zonisamide.

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drugs on the fetus. However, little information is available on the transport mechanism of GBP in placental cell models.

Ohman et al. [9] reported that umbilical-to-maternal GBP plasma concentration ratios ranged from 1.3 to 2.1 (mean, 1.7), implying that the drug is actively transported across the near-term placenta. GBP is a zwitterion and the distribution coefficient (Log*D*, pH 7.4) has previously been reported to be −1.2 [10]. Therefore, a carrier-mediated mechanism might be involved in its transport across the plasma membrane. We previously reported that the accumulation of GBP in human placental choriocarcinoma BeWo cells occurred in higher concentrations than in other new-generation antiepileptic drugs such as LTG, LEV, and TPM [11]. Furthermore, the transport of GBP in BeWo cells showed saturation at high concentrations, suggesting that the transfer of GBP to choriocarcinoma cells is carrier-mediated.

Membrane transporters play a role in the pharmacokinetic and physiological processes in several organs, including the placenta. Over 400 membrane transporters have been identified and classified as ATP-binding cassettes (ABC) and solute carriers (SLC). While taking into consideration individual variation, it is crucial to examine the role of drug transporters in the placenta in order to prevent potential drug–drug interactions. In brain endothelial cells, GBP is transported by L-type amino acid transporter 1 (LAT1, *SLC7A5*) [10]. The organic cation/carnitine transporter (OCTN1, *SLC22A4*) contributes to the urinary excretion of GBP, with its genetic variant L503F resulting in decreased GBP transport [12]. Larsen et al. [13] suggest that in the intestine, a carrier-mediated system, which is inhibited by 2-Amino-2-norbornanecarboxylic acid (BCH), contributes to the transport of GBP in a rat model and in Caco-2 cells. System L transporter LAT1 catalyzes the sodium-independent transport of physiologically important substrates such as amino acids [14] and thyroid hormones [15], and is highly expressed in the placenta [16]. OCTN1 is an important transporter for various xenobiotics, and endogenous substrates such as L-carnitine, and is expressed in the placenta [17,18]. However, there is limited literature on studies investigating the involvement of these transporters in the uptake of GBP in placental cell lines.

In the present study, we investigated the uptake mechanism of GBP in human placental choriocarcinoma cells (BeWo and JEG-3) and the involvement of putative transporters in the transport process. The cell lines, BeWo and JEG-3, have been widely used to examine the transport mechanisms of xenobiotic in the placenta [19].

2. Materials and methods

2.1. Chemicals

Gabapentin (GBP), lamotrigine (LTG), levetiracetam (LEV), and topiramate (TPM) were purchased from Tokyo Chemical Industry (Tokyo, Japan). 2-Amino-2-norbornanecarboxylic acid (BCH), L-carnitine, L-thyroxine (T_4), clobazam (CLB), cimetidine, quinidine, and amino acids were purchased from Sigma-Aldrich (St. Louis, MO). 3, 3′, 5-Triiodo-L-thyronine (T_3) was purchased from Nacalai Tesque (Kyoto, Japan). Other chemicals were purchased from Wako (Tokyo, Japan).

2.2. Cell culture

BeWo cells were obtained from the Riken Cell Bank (Saitama, Japan). BeWo cells were cultured in Ham's F–12 K (Kaighn's modification) medium (Wako, Tokyo, Japan), supplemented with 15% fetal bovine serum and 1% penicillin-streptomycin (Sigma Aldrich, St. Louis, MO), at 37 °C under 5% CO_2. JEG-3 cells were purchased from DS Pharma Co. Ltd. (Osaka, Japan). JEG-3 cells were cultured in the Eagle's minimum essential medium (Wako), supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 1 mM sodium pyruvate, and 1% penicillin-streptomycin.

2.3. Uptake experiment

BeWo cells and JEG-3 cells were seeded on 24-well collagen-coated plastic plates. After the cells had grown to confluence, they were used for the uptake experiment. Once the growth medium was removed, cells were washed with a transport buffer and pre-incubated at 37 °C with 0.5 mL of transport buffer. The transport buffer consisted of Hank's balanced salt solution (HBSS) with 25 mM HEPES, maintained at a pH of 7.4. Uptake was initiated by adding the transport buffer containing the study drugs. To investigate the accumulation levels of GBP, LTG, LEV, and TPM, cells were incubated with 50 μM of each antiepileptic drug. The concentration of 50 μM is close to that of therapeutic levels of these drugs in blood. In plasma, reference concentration ranges are between 10 and 120 μM for GBP, 10 and 60 μM for LTG, 70 and 270 μM for LEV, and 15 and 60 μM for TPM [20]. For the time-dependent study, 5 μM of GBP was added to the transport buffer. The concentration-dependent study was conducted by adding GBP at concentrations ranging between 10 and 500 μM. The effects of various inhibitors on the GBP uptake were investigated by incubating the cells with transport buffer containing 100 μM of GBP in the presence or absence of inhibitors. To examine the concentration-dependent inhibition by BCH, the uptake of 10 μM GBP by cells was measured in the presence of increasing concentrations of BCH (~10 mM). The cells were incubated for the indicated time at 37 °C. After incubation, cells were immediately rinsed with ice-cold transport buffer. The cells were lysed with 200 μL of 1 N NaOH and the lysate was neutralized with HCl. The concentration of GBP was determined using liquid chromatography/electrospray ionization tandem mass spectrometry (LC/MS/MS) as previously described [11]. The uptake amount was normalized to the level of protein. The protein concentration was determined using a Pierce® BCA Protein Assay Kit (Thermo Scientific, Rockford, IL), in accordance with the manufacturer's instructions.

2.4. Estimation of intracellular water volumes of BeWo and JEG-3 cells

To investigate intracellular concentration of GBP, intracellular water volumes of BeWo and JEG-3 cells were estimated using modified methods described by Rottenberg et al. [21] and Shiraya et al. [22]. BeWo cells and JEG-3 cells were seeded on 24-well collagen-coated plastic plates. After removal of growth medium, cells were washed with a transport buffer and pre-incubated with 0.5 mL of buffer. The buffer was removed and 0.5 mL of buffer containing [^{3}H]-H_2O (1 μCi/mL) (PerkinElmer, Boston, MA), and [^{14}C]inulin-carboxyl (0.5 μCi/mL) (American Radiolabeled Chemicals, Inc. St. Louis, MO) was added to cells. The cells were incubated in the buffer for 10 min. The extracellular buffer was collected, and cells were solubilized with 1% sodium dodecyl sulfate (SDS)/0.2 N NaOH. The samples were mixed with 3 mL of scintillation cocktail (PerkinElmer) to measure radioactivity using a liquid scintillation counter.

The intracellular water volume was calculated using the following equation:

$$\text{Intracellularcellvolume} = V_s \times ({}^3H_c/{}^3H_s - {}^{14}C_c/{}^{14}C_s)$$

where V_s is the volume of extracellular buffer, ^3H_c is the ^{3}H counts in cells, ^3H_s is the ^{3}H counts in supernatant, $^{14}C_c$ is the ^{14}C counts in cells, and $^{14}C_s$ is the ^{14}C counts in supernatant.

The intracellular volume was expressed relative to protein.

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