Enhanced Delivery of Drugs to the Liver by Adenovirus-Mediated Heterologous Expression of the Human Oligopeptide Transporter PEPT1

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ABSTRACT

To explore the feasibility of drug delivery to the liver by the use of adenovirus-mediated human oligopeptide transporter (hPEPT1) gene transfer, we examined the accumulation of L-[3H]carnosine in the hepatoma cell line (HepG2 and WIFB9) and mouse liver. We constructed a recombinant adenovirus encoding hPEPT1-enhanced yellow fluorescent protein (EYFP) fusion gene (AdhPEPT1-EYFP). In vitro uptake of L-[3H]carnosine was determined in HepG2 and WIFB9 cells transduced with AdhPEPT1-EYFP. In vivo, the accumulation of L-[3H]carnosine in mouse liver was evaluated after transduction of AdhPEPT1-EYFP. At pH 6.0, the uptake of L-[3H]carnosine by HepG2 and WIFB9 cells transduced with AdhPEPT1-EYFP was increased 15- and 2-fold, respectively, compared with the cells without transduction. At pH 7.4, uptake of L-[3H]carnosine in AdhPEPT1-EYFP transduced HepG2 cells was 3 times greater than that of nontransduced cells. In the presence of carnosine or glycylsarcosine as an inhibitor at 20 mM, the uptake of L-[3H]carnosine was reduced to a level comparable to that of nontransduced cells. At 30 min after intravenous administration of L-[3H]carnosine to mice transduced with AdhPEPT1-EYFP at 1 \times 10^{10} plaque-forming units/mouse, the tissue-to-plasma concentration ratio (Kp) of L-[3H]carnosine in the liver was significantly increased to 7 times that of nontransduced mice. In contrast, the Kp value of [14C]inulin, a marker for extracellular fluid space, remained unchanged after adenoviral transduction suggesting minimal pathological damage of tissues. hPEPT1-EYFP was localized at both the basolateral and apical membranes in HepG2 cells, WIFB9 cells, and mouse liver. In conclusion, our results suggest that delivery of oligopeptide to the liver by adenovirus-mediated heterologous expression of hPEPT1 in vivo is feasible.

Many drugs reach high concentrations in the liver due to rapid uptake from the bloodstream by passive, receptor- or transporter-mediated processes. At the sinusoidal membrane of hepatocytes, Na+-dependent taurocholate-cotransporting polypeptide (NTCP; Ntcp in rodents), organic anion transporting polypeptide (OATP; oatp in rodents), and organic cation transporters (OCT; Oct in rodents) are expressed (Lecureur et al., 2000). Among them, members of the OATP family can mediate the hepatic uptake of various drugs and xenobiotics, such as the organic anion bromosulfophthalein (Cui et al., 2001; Kullak-Ublick et al., 2001), benzylpenicillin (Tamai et al., 2000a), and the 3-hydroxy-3-methylglutaryl-CoA-reductase inhibitor pravastatin (Hsiang et al., 1999).

Oligopeptide transporters PEPT1 and PEPT2 accept not only dipeptides and tripeptides as substrates but also peptide-mimetic drugs, such as β-lactam antibiotics (Saito et al., 1995; Miyamoto et al., 1996; Sai et al., 1996; Tamai et al., 1997), angiotensin-converting enzyme inhibitors (Hu and Amidon, 1998), the antiviral drug valacyclovir (Balimane et al., 1998), and the anticancer drug bestatin (Saito and Inui, 1997). It is well known that transport activity of oligopeptide transporters is pH-dependent (Fei et al., 1994; Liang et al., 1995). The physiological role of both transporters lies in the (re)absorption of peptides from the intestinal and renal tubular lumen (Daniel, 2000). In the liver, however, it has been reported that the ability to take up small peptides from the circulation was negligible (Lombardo et al., 1988). From these reports, the liver seems to lack expression of PEPT1 or PEPT2 at the sinusoidal membrane of hepatocytes.

ABBREVIATIONS: OATP, organic anion transporting polypeptide; hPEPT1, human intestinal H+/peptide cotransporter; FCS, fetal calf serum; GlySar, glycylsarcosine; RT-PCR, reverse transcription-polymerase chain reaction; EYFP, enhanced yellow fluorescent protein; bp, base pair; PFU, plaque-forming units; MOI, multiplicity of infection; HBSS, Hank’s balanced salt solution; MES, 4-morpholineethanesulfonic acid; GFP, green fluorescent protein; PBS, phosphate-buffered saline; Kp, tissue-to-plasma concentration ratio; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
There have been several experimental trials aimed at using the endogenously expressed oligopeptide transport activity for improving oral bioavailability (Tamaì et al., 1998) or using cultured cells, such as the human fibrosarcoma cell line HT-1080 (Nakanishi et al., 1997) and the human pancreatic cell lines AsPC-1 and Capan-2 (Gonzalez et al., 1998) that express oligopeptide transport activity for tumor targeting. From this point of view, we previously examined the feasibility of tumor-selective delivery of dipeptides or peptide-mimetic drugs by using the oligopeptide transport activity (Nakanishi et al., 2000). To our knowledge, however, there has been no experimental trial on drug delivery using the activity of oligopeptide transporter in the liver since these transporters are unlikely to be expressed in the liver.

Recombinant adeno virus is an attractive method for gene transfer in vitro and for in vivo gene therapy (He et al., 1998). It is believed that high levels of transgene expression can generally be obtained in comparison with other viral or non-viral vectors, such as retrovirus or lipofectamine, respectively (Sato et al., 2000). In addition, since it has the advantage of having little integrated into the genome, it is possible to minimize insertional mutagenesis (Jaffe et al., 1992). Therefore, we attempted to explore a new concept of drug delivery of oligopeptide drugs into the liver by the use of adenovirus-mediated heterologous expression of oligopeptide transporter.

In the present study, to deliver small peptides to the liver selectively, we constructed a recombinant adeno virus encoding a fusion gene of human PEPT1 and enhanced yellow fluorescent protein (AdhPEPT1-EYFP) fusion gene. Then, oligopeptide transport activity was assessed in the polarized hepatoma cell lines HepG2 and WIFB9 transduced with or without AdhPEPT1-EYFP in vitro. After transduction of AdhPEPT1-EYFP into mice, the distribution of L-[^3]H)carnosine, a substrate of the oligopeptide transporter hPEPT1, into the liver was evaluated in vivo.

**Experimental Procedures**

**Materials.** HepG2 cells were purchased from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). HeLa cells stably expressing hPEPT1 were previously prepared in our laboratory (Nakanishi et al., 2000). WIFB9 cells were used as described previously (Sai et al., 1999). Dulbecco’s modified Eagle’s medium, fetal calf serum (FCS), and nonessential amino acids were obtained from Invitrogen (Carlsbad, CA). Modified Eagle’s medium was used to grow cells at 37°C and 5% CO2. Cells were grown in modified Eagle’s medium with Earle’s balanced salt solution (HBSS) (0.952 mM NaCl, 0.385 mM Na2HPO4, 25 mM D-glucose, and 10 mM MES) adjusted to pH 6.0 with NaOH. Osmolality of the HBSS was measured in Hanks balanced salt solution (HBSS) (0.952 mM CaCl2, 5.36 mM KCl, 0.441 mM KH2PO4, 0.812 mM MgSO4, 136.7 mM NaCl, 0.385 mM NaHPO4, 25 mM d-glucose, and 10 mM MES or HEPES) adjusted to pH 6.0 with NaOH. Osmolality of the HBSS was adjusted to 310 mosm/kg. To quantify [3H]GlySar or L[^3]H)carnosine in the cells, the washed cells were solubilized by the addition of 5 N NaOH (0.25 ml), followed by shaking for 2 h. The resultant lysates were neutralized with 5 N HCl and mixed with 4 ml of the liquid scintillation cocktail Clearsol-I (Nakarai, Kyoto, Japan). Radioactivity was determined using a liquid scintillation counter (LSC-1000; Aloka Co. Ltd., Tokyo, Japan). Protein determinations were done using the protein dye binding method with bovine serum albumin as a standard (Bradford, 1976).

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Laboratory Animals of Takara-machi Campus of Kanazawa University. Male ddY mice (6–8 weeks old; Nippon SLC, Hamamatsu, Japan) were used in this study. All animals received standard mouse chow and water ad libitum. Mice were anesthetized with intramuscular administration of ketamine/xylazine (140.8 mg/kg). AdhPEPT1-EYFP and AdhGFP were injected through the right jugular vein using a 0.5-ml syringe with a 29.5-gauge needle. The total amount of adenovirus injected per mouse was 1 × 10⁷ or 1 × 10¹⁰ PFU for AdhPEPT1-EYFP. AdhGFP was given at a dose of 2.4 × 10⁴ PFU. The dose of AdhGFP was selected to let fluorescence expression level of GFP comparable to that of hPEPT1-EYFP in mouse liver received 1 × 10¹⁰ PFU AdhPEPT1-EYFP. GFP expression level was confirmed by fluorescent microscopy.

**Tissue Preparation for Analysis of hPEPT1-EYFP Transgene Expression.** After sacrifice of the mice, tissues were immediately dissected and washed with PBS, then quickly frozen in liquid nitrogen and stored at −80°C. For fluorescence analysis of hPEPT1-EYFP expression, resected tissues were embedded in optimal cutting temperature compound (OCT; Sakura Finetchnical Co., Ltd., Tokyo, Japan). Serial 10-μm tissue sections were prepared with a Cryostat HM505E (Carl Zeiss, Inc.) at −20°C. Fluorescence images of sections were obtained using a Zeiss Axiosvert 100 microscope (Carl Zeiss, Inc.).

**Reverse Transcriptase-Polymerase Chain Reaction.** Total RNA was extracted from frozen tissue using RNaseasy Mini-Kit (QIAGEN, Chatsworth, CA) according to the manufacturer’s instructions. Total RNA was reverse-transcribed and amplified by PCR in the presence of specific primers for hPEPT1-EYFP. The primer pair consisting of 5′-AACTGTAACCCGCTTAG-3′, and the downstream primer was 5′-CCAAGGGATGATGATGTGCTG-3′, yielding a 1379-bp fragment. cDNA synthesis and predenaturation were performed at 48°C/30 min and 94°C/2 min. Amplifications consisted of 1 cycle at 94°C for 2 min followed by 40 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 90 s, with an extension step at 72°C for 10 min. To demonstrate the integrity of the RNA, we measured glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression using primers 5′-CCCTCTACAAGTTGTGATGCGT-3′, yielding a 983-bp fragment. cDNA synthesis and predenaturation were performed at 48°C/30 min and 94°C/2 min. PCR was performed in a total volume of 50 μl at 94°C 30 s, 60°C 90 s, 72°C 90 s (40 cycles), and 72°C/10 min for extension.

**Western Blot Analysis.** Mouse liver membranes were prepared as previously described (Tamai et al., 2000b). Briefly, 800 μl of the homogenate was mixed with 750 μl of 1.17 M KCl solution containing 58.3 mM tetrasodium pyrophosphate and centrifuged at 230,000g for 75 min. The resultant pellet was suspended in 10 mM Tris-HCl and 1 mM EDTA, pH 7.4, and centrifuged at 230,000g again. The obtained pellet was suspended in 600 μl of 10 mM Tris-HCl and 1 mM EDTA, pH 7.4, and dispersed ultrasonically. After the addition of 200 μl of 16% SDS solution, the solution was mixed and centrifuged at 15,000g for 2 min, and the resultant supernatant was used for Western blot analysis. Protein quantification of samples was performed using the Lowry assay. Each sample was separated on 12% SDS-polyacrylamide gel, proteins were transferred to polyvinylidene difluoride membranes (Immobilon, Millipore, Bedford, MA), and the membrane was incubated in buffer consisting of 20 mM Tris, 137 mM NaCl, and 0.1% Tween-20, pH 7.5, containing 10% skim milk. The membrane was incubated with primary anti-EGFP antibody (Living Colors-A. v. Peptide Antibody; BD Biosciences Clontech) for 1 h, rinsed with the above buffer without skim milk three times, and incubated with secondary antibody (donkey anti-rabbit IgG, horseradish peroxidase-linked whole antibody; Amersham Pharmacia Biotech UK, Little Chalfont, Buckinghamshire, UK). The membrane was washed with the above buffer without skim milk, and the proteins were detected by enhanced chemiluminescence detection method using an ECL Plus Western blotting detection system (Amersham Pharmacia Biotech UK, Ltd.).

**Immunofluorescence Analysis.** Serial 10-μm liver sections were prepared with a cryostat at −20°C and fixed in methanol for 1 min. Sections were treated with 0.3% Tween-20 in PBS for 20 min and blocked for 30 min with 3% blocking agent (Amersham Pharmacia Biotech UK, Ltd.) in PBS. Then their samples were incubated with primary C219 anti-mdr1 antibodies (Signet Pathology Systems, Inc., Dedham, MA) at a 1:10 dilution for 1 h at room temperature. The incubation was followed by three 5 min washes in PBS. Secondary antibody Alexa Fluor 594 goat anti-mouse IgG (Molecular Probes, Inc., Leiden, The Netherlands) diluted with 3% blocking agent in PBS at 1:200 was added, and incubation continued for 30 min. The slides were washed as described above. They were mounted in VECTASHIELD (Vector Laboratories, Inc., Burlingame, CA) and observed under a fluorescence microscope.

**L-Carnosine Disposition Using Anesthetized Mice Transduced with AdhGFP.** Mice were anesthetized with intramuscular injection of ketamine/xylazine (140/8 mg/kg) 2 days after virus injection. A 100-μl aliquot of saline solution containing test compounds (L-[³H]carnosine, 5 μCi; L-[³H]glycyl-L-carnosine, 5 μCi/mouse; [¹⁴C]ulinin, 0.5 μCi/mouse/25 g) was injected through the external jugular vein. Blood (30–40 μl) was collected at the indicated time with heparinized microhematocrit capillary tubes. At 30 min after administration, mice were sacrificed, and then tissues were quickly isolated and rinsed with ice-cold PBS. The tissues were solubilized by SOLUENE-350 (Packard BioScience B.V., Groningen, The Netherlands) for 3 h, and radioactivity was measured using a liquid scintillation counter.

**Results**

**Uptake of [³H]GlySar by HeLa Cells Transduced with AdhPEPT1-EYFP.** To examine whether the dipeptide transport activity is induced by AdhPEPT1-EYFP transduction, we evaluated [³H]GlySar uptake in HeLa cells. When HeLa cells were transduced with AdhPEPT1-EYFP, uptake 20 to 30 times higher than that of nontransduced HeLa cells was observed at 15 min (Fig. 1). The uptake of dipeptide was higher than that by HeLa cells stably transfection with hPEPT1-encoding plasmid vector. When HeLa cells were infected with AdhPEPT1-EYFP at an increasing MOI from 40 to 400, 1.5-fold increase of [³H]GlySar uptake was observed. Expression level of hPEPT1-EYFP protein in HeLa cells was increased with MOI (data not shown).

**Expression of hPEPT1-EYFP in HepG2 and WIFB9 Cells.** The expression of hPEPT1-EYFP was examined by fluorescence microscopy in HepG2 and WIFB9 cells. Those cells were transduced by AdhPEPT1-EYFP at MOI 40. As shown in Fig. 2, fluorescence of hPEPT1-EYFP was observed in both HepG2 and WIFB9 cells. In addition, the expression was observed at both basolateral and apical membranes of HepG2 and WIFB9 cells.

**Uptake of L-[³H]Carnosine in HepG2 and WIFB9 Cells Transduced with AdhPEPT1-EYFP.** To examine whether the dipeptide transport activity was induced in AdhPEPT1-EYFP-transduced HepG2 and WIFB9 cells, uptake of L-[³H]carnosine was measured for 15 min. As shown in Fig. 3, AdhPEPT1-EYFP-transduced HepG2 and WIFB9 cells (MOI 40) showed 10 and 2 times higher uptake, respectively, than nontransduced controls. When AdGFP was transduced in HepG2 cells as a control vector at MOI 40, uptake of L-[³H]carnosine was similar to nontransduced cells (data not shown).

Two oligopeptides were included in the uptake buffer to
test whether L-[3H]carnosine uptake was inhibitable or not (Fig. 4). Concentration of unlabeled inhibitor was used above apparent $K_m$ (12.9 mM) of carnosine for PEPT1. Twenty millimolar L-carnosine inhibited over 80% of the uptake of L-[3H]carnosine in AdhPEPT1-EYFP-transduced HepG2 cells (MOI 40), leaving an uptake comparable with that of nontransduced HepG2 cells. In the presence of 20 mM GlySar, a similar reduction of uptake was obtained.

To confirm the pH-dependent nature of oligopeptide transport activity in adenovirus-transduced HepG2 cells, uptake of L-[3H]carnosine was compared between pH 6.0 and 7.4 (Fig. 4). In AdhPEPT1-EYFP-transduced cells, uptake of L-[3H]carnosine was 6 and 3 times greater than that of nontransduced cells at pH 6.0 and 7.4, respectively. The uptake of L-[3H]carnosine at pH 6.0 was 2 times greater than that at pH 7.4. These results were consistent with the reported activity of H+/peptide cotransporter (Fei et al., 1994; Liang et al., 1995).

Expression of hPEPT1-EYFP in Mouse Liver after AdhPEPT1-EYFP Transduction. To investigate whether hPEPT1-EYFP transcript was expressed in vivo after intravenous administration of AdhPEPT1-EYFP into mice, RT-PCR of hPEPT1-EYFP was conducted. At the virus dose of $1 \times 10^9$ PFU/mouse, mRNA of hPEPT1-EYFP was detected only in the liver (Fig. 5). At the virus dose of $1 \times 10^{10}$ PFU, the hPEPT1-EYFP transgene was predominantly found in the liver, and low levels were present in the kidney and the spleen. To evaluate whether the hPEPT1-EYFP protein was
induced in vivo, expression in the liver was examined by fluorescence microscopy and Western blot analysis. As shown in Fig. 6A, at the virus dose of $1 \times 10^9$ PFU/mouse, hPEPT1-EYFP fluorescence was very low (Fig. 6A) despite the presence of mRNA signals (Fig. 5A). However, when the dose was increased to $1 \times 10^{10}$ PFU/mouse, fluorescence of hPEPT1-EYFP markedly increased in the liver (Fig. 6A, c) but was not detected in the kidney or the spleen (data not shown). On the other hand, Western blot analysis (Fig. 6B) indicated the expression of the fusion protein with a molecular mass of about 130 to 140 kDa in the membrane of the liver after transduction with AdhPEPT1-EYFP. In addition, the amount of hPEPT1-EYFP protein increased with an increase of the viral dose from $1 \times 10^9$ to $1 \times 10^{10}$ PFU/mouse. No expression of hPEPT1-EYFP was observed in nontransduced liver.

Drug Disposition in AdhPEPT1-EYFP-Transduced Mice. To evaluate whether the disposition of dipeptides was improved after AdhPEPT1-EYFP transduction of mice, the tissue distribution of $\text{L-[3H]carnosine}$ was measured at 30

Fig. 4. Effect of inhibitor and pH on the uptake of $\text{L-[3H]carnosine}$ by HepG2 cells transduced with AdhPEPT1-EYFP. A, competition for $\text{L-[3H]carnosine}$ uptake by inhibitor in HepG2 cells with or without AdhPEPT1-EYFP transduction at MOI 40. Uptake of $\text{L-[3H]carnosine}$ (100 nM) by HepG2 in the absence or presence of inhibitor (20 mM carnosine or 20 mM glycylysarcosine) was measured at 37°C by incubating cells in HBSS at pH 6.0 for 15 min. B, uptake of $\text{L-[3H]carnosine}$ in HepG2 cells with (closed columns) or without (open columns) AdhPEPT1-EYFP transduction at pH 6.0 or 7.4 using a 15-min incubation period at 37°C. Data are presented as means ± S.E.M. of four experiments.

Fig. 5. RT-PCR analysis of mRNA levels of hPEPT1-EYFP in various organs after adenovirus-transduction of mice. Expression of hPEPT1-EYFP mRNA in mouse transduced with AdhPEPT1-EYFP at the dose of $1 \times 10^9$ PFU/mouse (A) or $1 \times 10^{10}$ PFU/mouse (B) was detected by RT-PCR. Total RNA extracted from liver, kidney, and spleen of transduced mouse was subjected to RT-PCR using primer pairs specific for hPEPT1-EYFP or GAPDH. The RT-PCR products were analyzed by agarose gel electrophoresis and stained with ethidium bromide. GAPDH was used as a control for RNA integrity.
In vivo expression of hPEPT1-EYFP fusion protein in liver after adenovirus-transduction of mice. A, fluorescence microscopic images of cryosections of AdhPEPT1-EYFP-transduced mouse liver. Nontransduced liver of mice was used as a control (a). The liver tissues of transduced mice were obtained 2 days after i.v. injection of AdhPEPT1-EYFP at a dose of $1 \times 10^9$ PFU/mouse (b) or $1 \times 10^{10}$ PFU/mouse (c) (original magnification, 200×). B, Western blotting analysis of the expression of hPEPT1-EYFP fusion protein in the liver. Nontransduced mouse liver was used as a control (a). Mice were transduced with AdhPEPT1-EYFP at a dose of $1 \times 10^9$ PFU/mouse (b) or $1 \times 10^{10}$ PFU/mouse (c). Each lane was loaded with 20 μg of protein. Molecular masses are indicated.

Fig. 6. In vivo expression of hPEPT1-EYFP fusion protein in liver after adenovirus-transduction of mice. A, fluorescence microscopic images of cryosections of AdhPEPT1-EYFP-transduced mouse liver. Nontransduced liver of mice was used as a control (a). The liver tissues of transduced mice were obtained 2 days after i.v. injection of AdhPEPT1-EYFP at a dose of $1 \times 10^9$ PFU/mouse (b) or $1 \times 10^{10}$ PFU/mouse (c) (original magnification, 200×). B, Western blotting analysis of the expression of hPEPT1-EYFP fusion protein in the liver. Nontransduced mouse liver was used as a control (a). Mice were transduced with AdhPEPT1-EYFP at a dose of $1 \times 10^9$ PFU/mouse (b) or $1 \times 10^{10}$ PFU/mouse (c). Each lane was loaded with 20 μg of protein. Molecular masses are indicated.

In the current study, we attempted to induce expression of the oligopeptide transporter hPEPT1 in mouse liver by using a recombinant adenovirus to examine the feasibility of this approach for drug delivery. A recombinant adenovirus encoding hPEPT1-EYFP was constructed. EYFP was used to monitor the expression level of hPEPT1. The molecular mass of hPEPT1-EYFP protein is 130 to 140 kDa by Western blot analysis (Fig. 6B). Basu et al. (1998) reported that immuno-blotting of a Caco-2 membrane protein preparation using the anti-peptide antibody against hPEPT1 revealed a positive band with an apparent molecular mass of ~110 kDa. The anti-EGFP antibody recognized the EYFP tag in hPEPT1-EYFP protein and showed a 30-kDa band in the control GFP vector-transduced liver (data not shown). This result shows that hPEPT1-EYFP protein expressed in the liver is about 30 kDa larger than hPEPT1 alone. The function of this protein was confirmed by uptake studies in AdhPEPT1-EYFP-transduced cell lines (Figs. 1, 3, and 4). Uptake of $[^3]$Hcarnosine by AdhPEPT1-EYFP-transduced HepG2 cells was time-and pH-dependent and inhibited by dipeptides. Thus, the functional expression of hPEPT1-EYFP protein as an oligopeptide transporter protein was successfully achieved by adenoviral transduction.

In the case of delivery of drugs from the systemic circulation to the liver under physiological conditions, the pH of the systemic circulation is 7.4. In our study, uptake of $[^3]$Hcarnosine in AdhPEPT1-EYFP-transduced HepG2 cells was observed at pH 7.4 (Fig. 4B) and pH 6.0. Transport activity of hPEPT1 at neutral pH has also been reported by other
The localization of hPEPT1-EYFP protein is critical for delivery of peptide drugs from the systemic circulation to the liver. Our results demonstrate that hPEPT1-EYFP protein was expressed at apical and basolateral membranes in HepG2 or WIFB9 cells (Fig. 2) and mouse liver (Fig. 7). In our experiment, the duration of expression of hPEPT1-EYFP protein in the apical and the basolateral membranes differs with viral dosage. In the liver of mice given the dose of 10^9 PFU, hPEPT1-EYFP may localize mainly at the apical membrane, as observed in intestinal epithelial cells (Sai et al., 1996; Walker et al., 1998), and consequently, the uptake of carnosine by hPEPT1-EYFP at the basolateral membrane is low. When a high dose of 10^{10} PFU was injected, hPEPT1-EYFP protein was expressed both in the apical and basolateral membranes (Fig. 7), resulting in a large increase of K_p value in the liver. Therefore, to control drug disposition in the liver, the dose of adenovirus must be optimized according to the required drug concentration.

Duration of transgene expression has been examined by many investigators. Jaffe et al. (1992) reported that intraperitoneal infusion of Ad-α1AT (human α1-antitrypsin cDNA) produced a detectable serum level of human α1AT for 4 weeks. Recombinant adenoavir expression of human pancreatic lipase gene was sustained for 7 days (Kuhel et al., 2000). In our experiment, the duration of expression of AdGFP was 4 weeks in mouse liver (data not shown), whereas that of hPEPT1-EYFP is unknown. Recombinant adenovirus has advantages as a vector system in minimizing insertional mutagenesis and affording transient expression of the transgene. Therefore, duration of gene expression must be considered to optimize the concentration in the liver.

**TABLE 1**

<table>
<thead>
<tr>
<th>AdhPEPT1-EYFP</th>
<th>K_p Value</th>
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<tbody>
<tr>
<td>Control</td>
<td>1.42 ± 0.23</td>
</tr>
<tr>
<td>1 x 10^9</td>
<td>1.83 ± 0.64</td>
</tr>
<tr>
<td>1 x 10^{10}</td>
<td>7.02 ± 0.64</td>
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* Significantly different from the control by Student’s t test (p < 0.05).
PEPT1 is a low-affinity oligopeptide transporter (e.g., parent $K_m$ for carnosine is 12.9 mM), whereas PEPT2 is the high-affinity one (Ramamoorthy et al., 1995), so PEPT1 is expected not to be saturated by substrates in the circulation. Thus, we preferred PEPT1 as a candidate for drug delivery from the systemic circulation to the liver. Because PEPT1 accepts a wide range of peptide and peptide-like substances as substrates (Saito and Inui, 1993; Saito et al., 1995; Miyamoto et al., 1996; Sai et al., 1996; Tamai et al., 1997; Hu and Amidon, 1998; Balimane et al., 1998), other peptide drugs may be transported. This system is also applicable to chemically modified substrates that are recognized by PEPT1. Candidates include l-valyl ester prodrugs of zidovudine and acyclovir. Indeed, the absorption of acyclovir in the intestine was improved by peptide-mimetic derivation (Soul-Lawton et al., 1995).

In conclusion, we have demonstrated a novel strategy for drug delivery to the liver by means of adenovirus-mediated heterologous expression of an oligopeptide transporter gene. Further studies on regulation of hPEPT1-EYPFP in the liver may allow accurately controlled delivery of small peptides.

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References


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