Original article

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Comparison of the transcellular transport of FDG and D-glucose by the kidney epithelial cell line, LLC-PK$_1$

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Objectives **Almost all D-glucose (GLU) filtered through the glomerulus is reabsorbed by the renal proximal tubules, whereas a high portion of 2-[^{18}F]fluoro-2-deoxy-D-glucose [(^{18}F)FDG] is excreted through the urine. However, [^{18}F]FDG is not entirely excreted in the urine suggesting that it may be partially reabsorbed by the proximal tubules. The purpose of this study was to compare the time course of transcellular transport of administered [^{14}C] labeled FDG ([^{14}C]FDG) with that of [^{14}C] labeled GLU ([^{14}C]GLU) using the kidney epithelial cell line, LLC-PK$_1$.**

Methods **Transcellular transport of [^{14}C]FDG and [^{14}C]GLU by LLC-PK$_1$ cells was measured in Na^+-containing or Na^+-free Dulbecco's phosphate-buffered saline [PBS(+) and PBS(−), respectively] in the presence or absence of phlorizin, phloretin, probenecid, or tetraethylammonium bromide inhibitors that predominantly inhibit sodium-dependent glucose transporters (SGLTs), sodium-independent glucose transporters, organic anion transporters, and organic cation transporters, respectively.**

Results **When assayed in PBS(+), less [^{14}C]FDG than [^{14}C]GLU was reabsorbed by the proximal tubular cells over the entire incubation time. Reabsorption of [^{14}C]FDG was mediated mainly by SGLT at early time points in the incubation, whereas high reabsorption of [^{14}C]GLU was mediated by both SGLT and glucose transporter over 90 min of incubation. Secretion of [^{14}C]FDG also tended to be slightly higher than that of [^{14}C]GLU over 90 min of incubation.**

Conclusion **Transcellular transport of [^{14}C]FDG over time by LLC-PK$_1$ cells was clarified. The polarized distribution of transcellular transporters of [^{14}C]FDG and [^{14}C]GLU in LLC-PK$_1$ cells differs. *Nucl Med Commun* 31:141–146 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

The 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG)-positron emission tomography (PET) is commonly used for the diagnosis, staging, measurement of treatment response, and management of patients with various types of malignancies [1–3]. The mechanism and biochemical pathway of FDG uptake in cancer cells has been extensively studied both *in vitro* [4] and *in vivo* [5,6]. [^{18}F]FDG, which is an analog of D-glucose (GLU), is taken up into cells through the cell membrane through sodium-independent glucose transport (GLUT) proteins [7,8], and is then phosphorylated to [^{18}F]FDG-6-phosphate by hexokinase inside the cell [9,10].

Glucose transport into mammalian cells is accomplished by two classes of transporters: sodium-dependent GLUTs (SGLTs) and facilitative GLUTs. SGLTs consist of 14 transmembrane domains, and six subtypes (SGLT1–SGLT6) of SGLTs have been identified [11]. The family of facilitative GLUTs comprises 13 members (GLUT1–GLUT13) [12]. The expression levels of SGLTs and GLUTs have been shown to differ between certain tumor and normal cells. The SGLTs and GLUTs display a polarized distribution in renal proximal tubule cells that have an apical Na^+-glucose cotransport system [13,14] that involves SGLTs [15,16] and a basolateral Na^+-independent facilitated diffusion system that involves GLUTs [17,18].

A model of the glucose transport systems in the proximal tubule of kidney epithelial LLC-PK$_1$ cell line derived from pig is shown in Fig. 1. This cell line has been useful for studying the polarized distribution of the transcellular transporters SGLTs and/or GLUTs [15,19–21]. Thus, this cell line exhibits a basolateral Na^+-independent GLUT-1

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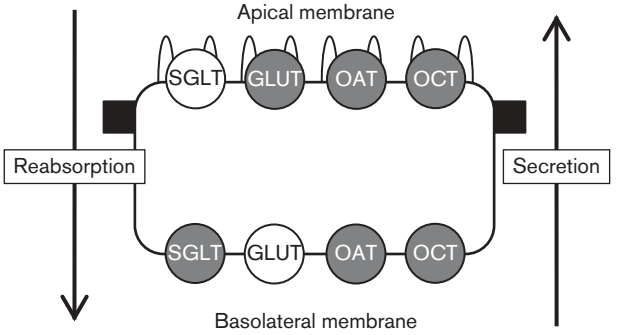
Fig. 1



Transport systems in the kidney epithelial LLC-PK_1 cell line. The transporters that were assayed for transcellular transport of [^{18}F]FDG included the sodium-dependent glucose transporter (SGLT), the sodium-independent glucose transporter (GLUT), the organic anion transporter (OAT), and the organic cation transporter (OCT). The white and gray circles indicate high and moderate transporter expression levels, respectively.

system for sugar efflux [15,19] and an apical SGLT-1 system for Na^+-glucose entry [15,20–22]. GLUT-1 is the only GLUT isoform that has been detected in LLC-PK_1 cells. In addition to SGLTs and GLUT-1, LLC-PK_1 cells also express other transporters typical of kidney proximal tubules such as organic anion transporters (OATs) [23] and organic cation transporters (OCTs) [24], on both the apical and basolateral sides of the cell. OATs and OCTs are believed to be important for kidney function as they have extremely broad substrate selectivity and their substrates include not only endogenous anions and cations but also several clinically important drugs [25,26].

In clinical studies, elimination of [^{18}F]FDG through the urine interferes with the visualization of both urologic diseases around the pelvis and abdominal abnormalities. In an earlier study using rats, phlorizin (PHZ), an SGLT inhibitor, improved urinary excretion of [^{18}F]FDG [27]. However, as many transporters reside on the apical and basolateral sides of the proximal tubules, other transporters besides SGLT may also be associated with transcellular transport of [^{18}F]FDG in the kidney. The mechanism of transcellular transport of [^{18}F]FDG in the proximal tubules has not been fully elucidated. In this study, the transcellular transport of [^{14}C] labeled FDG ([^{14}C]FDG), over time, was investigated by the proximal tubules using the LLC-PK_1 cell line and [^{14}C]FDG transport was compared with that of [^{14}C] labeled GLU ([^{14}C]GLU). The potential contribution of OATs and OCTs, which are the main transporters in the proximal tubules, to [^{14}C]FDG transport was investigated in addition to that of SGLT and GLUT.

Materials and methods

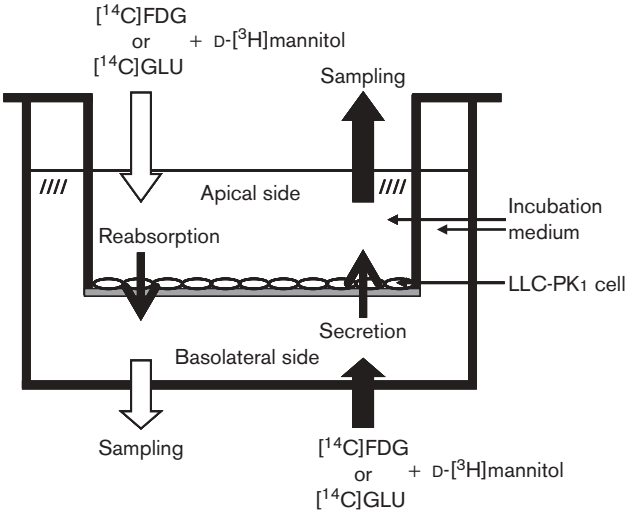
Materials

[^{14}C]FDG, [^{14}C]GLU, and D-[^{3}H]mannitol (100 µmol/l, 37 MBq/ml) were obtained from American Radiolabeled Chemicals Inc. (St Louis, Missouri, USA). PHZ, phloretin (PHT), and probenecid, (which predominantly inhibit SGLT, GLUT and OAT, respectively) were acquired from Sigma-Aldrich (Tokyo, Japan). Tetraethylammonium bromide (which inhibits mainly OCT) was purchased from Nacalai Tesque (Kyoto, Japan). LLC-PK_1 cells were obtained from Dainippon Pharmaceutical Co. (Osaka, Japan).

Cell culture and monolayer preparation

Cell studies were carried using the modified methods described by Shikano *et al.* and Kiyono *et al.* [28–30]. Briefly, LLC-PK_1 cells were maintained by serial passage in 25 cm^2 cell culture flasks. The cells were fed with Dulbecco's modified Eagle's medium (Sigma-Aldrich) supplemented with L-glutamine (2 mmol/l) and 10% fetal bovine serum without antibiotics, in an atmosphere of 5.0% CO_2/95.0% air at 37°C (pH 7.4). The cells were subcultured every 5 days using 0.02% ethylenediamine tetraacetic acid solution and 0.05% trypsin. For the preparation of a cell monolayer on a micropore support, the cells were seeded at a density of 5×10^5 cells/cm^2 on a polycarbonate membrane filter (growth surface area: 1.12 cm^2, membrane pore size: 3 µm) in Transwell cell chambers. The volume of medium inside and outside the Transwell chamber was approximately 0.5 and 1.5 ml, respectively. The medium was replaced with fresh medium every 2 days. The cells were used 4–6 days after harvesting.

Fig. 2



Outline of transport experiments using the LLC-PK_1 cell line. LLC-PK_1 cells were grown as a monolayer on a permeable support in a Transwell cell chamber. [^{14}C] fluoro-2-deoxy-D-glucose ([^{14}C]FDG) or [^{14}C] D-glucose ([^{14}C]GLU), together with D-[^{3}H]mannitol, were added to either the apical or the basolateral side. Aliquots of the incubation medium were taken from both sides at specified times, and the radioactivity was measured.

Measurement of transcellular transport using LLC-PK$_1$ cells

Transcellular transport between LLC-PK$_1$ cells, grown in Transwell chambers, was investigated according to the scheme illustrated in Fig. 2. Paracellular fluxes and extracellular trapping of [^{14}C]FDG and [^{14}C]GLU were calculated using D-[^{3}H]-mannitol, a compound that is not transported through the cells. The incubation medium was Dulbecco's phosphate-buffered saline [PBS(+); pH 7.4, adjusted by the addition of HCl or NaOH solution] containing 137 mmol/l NaCl, 3 mmol/l KCl, 8 mmol/l Na_2HPO_2, 1 mmol/l $CaCl_2$, and 0.5 mmol/l MgCl. The Na^+-free medium [PBS(−)] used was PBS in which NaCl and Na_2HPO_4 were replaced with choline chloride and K_2HPO_4, respectively. For transport studies, the culture medium was removed from both sides of the cell monolayer and the cell monolayer was first preincubated with 0.5 ml PBS(+) or PBS(−) on the apical side and with 1.5 ml PBS(+) or PBS(−) on the basolateral side for 10 min at 37°C. After preincubation, [^{14}C]FDG (10 kBq; 0.278 mmol/l) or [^{14}C]GLU (10 kBq; 0.250 mmol/l) together with [^{3}H]mannitol were added to the incubation medium of the apical or the basolateral side. The inhibitors, PHZ, PHT, probenecid, and tetraethylammonium bromide, were also added at a final concentration of 0.1 mmol/l in PBS(+) concomitantly with each tracer addition. For transport measurements, an aliquot (50 µl) of the incubation medium from the opposite side of the cell to which the tracer had been added was obtained at 10, 30, 60, and 90 min after the start of the tracer incubation, and the radioactivity of each sample was measured. An LSC-51000 liquid scintillation counter (Aloka) was used to determine the ^{14}C and ^{3}H radioactivity of the collected incubation media as well as that of the cell monolayer that was solubilized using 0.5 ml of 1 N NaOH.

Statistical analysis

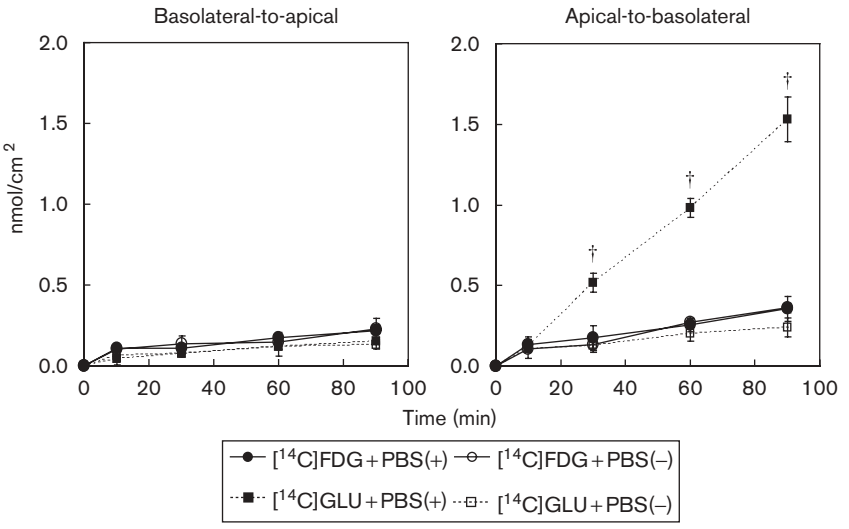
Measurements are expressed as means ± SD. Statistical analyses were performed using an unpaired *t*-test. Probability values were 0.01 or 0.05 ($P < 0.01$, 0.05).

Results

The transcellular transport of [^{14}C]FDG and [^{14}C]GLU, assessed in Transwell experiments using LLC-PK$_1$ cells, is shown in Fig. 3. Measurements of basolateral-to-apical and apical-to-basolateral transport were carried out in PBS(+) and PBS(−), and D-[^{3}H]mannitol was assayed to correct for paracellular flux. Transport of both [^{14}C]FDG and [^{14}C]GLU gradually increased over 90 min after the administration of each radiotracer. The apical-to-basolateral transport of [^{14}C]GLU in PBS(+) was significantly higher than that in PBS(−) from 30 min of incubation onward. The transport of [^{14}C]GLU in PBS(+) was significantly higher than that of [^{14}C]FDG in either PBS(+) or PBS(−) and was approximately four-fold higher than [^{14}C]FDG at 90 min of incubation. Although the basolateral-to-apical transport of [^{14}C]FDG in PBS(+) or PBS(−) was not significantly different from that of [^{14}C]GLU, the basolateral-to-apical transport of [^{14}C]FDG tended to be slightly higher than [^{14}C]GLU at all sampling time points.

The effects of the inhibitors of transcellular transport on the transport of [^{14}C]FDG and [^{14}C]GLU, in PBS(+) at 10 min of incubation, are shown in Fig. 4. In the absence

Fig. 3

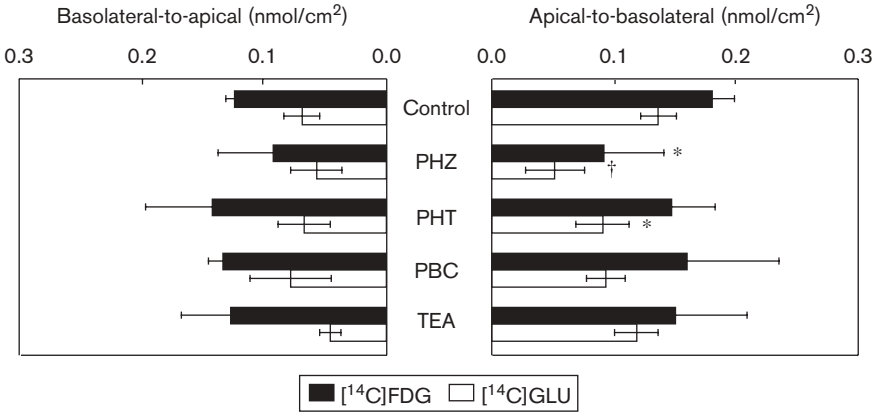


Time-course of transcellular transport of [^{14}C] fluoro-2-deoxy-D-glucose ([^{14}C]FDG) and [^{14}C] D-glucose ([^{14}C]GLU) by LLC-PK$_1$ cells. Transcellular transport of [^{14}C]FDG (solid line) and [^{14}C]GLU (dashed line) in phosphate-buffered saline (PBS)(+) (solid symbols) or PBS(−) (open symbols) after paracellular flux correction using D-[^{3}H]mannitol is shown. Each point represents the mean ± SD ($n = 4$ monolayers). †$P < 0.01$, significant difference between the sample and the control in PBS(+) and PBS(−).

of inhibitors, the transport of [^{14}C]FDG in either direction was slightly higher than that of [^{14}C]GLU. Apical-to-basolateral transport of [^{14}C]FDG decreased significantly after PHZ treatment compared with the control ($P < 0.05$) whereas apical-to-basolateral transport of [^{14}C]GLU was significantly lower than the control after treatment with either PHZ or PHT ($P < 0.01$, $P < 0.05$, respectively). No marked differences in the inhibition of basolateral-to-apical transport were observed between [^{14}C]FDG and [^{14}C]GLU.

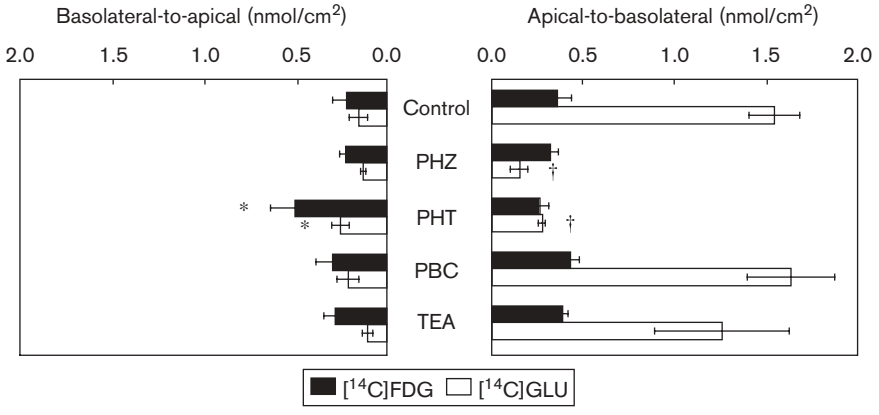
The effects of the inhibitors on the transcellular transport of [^{14}C]FDG and [^{14}C]GLU in PBS(+) at 90 min of incubation are shown in Fig. 5. PHT was the only inhibitor that had any marked effect on the transport of [^{14}C]FDG. However, apical-to-basolateral transport of [^{14}C]GLU was decreased significantly compared with the control after treatment with either PHZ or PHT ($P < 0.01$ for both inhibitors). PHT significantly increased the transcellular transport of both [^{14}C]FDG and [^{14}C]GLU in the basolateral-to-apical direction

Fig. 4



Transcellular transport of [^{14}C] fluoro-2-deoxy-D-glucose ([^{14}C]FDG) and [^{14}C] D-glucose ([^{14}C]GLU) after treatment with the indicated inhibitors or a control. In the figure, the transport of [^{14}C]FDG and [^{14}C]GLU were shown using black bars and white bars, respectively. Inhibitors used were: Phlorizin (PHZ), phloretin (PHT), probenecid (PBC) and tetraethylammonium bromide (TEA) that predominantly inhibit sodium-dependent glucose transporters (SGLTs), sodium-independent glucose transporters (GLUTs), organic anion transporters (OATs) and organic cation transporters (OCTs), respectively. Transport was assessed at 10 min of incubation in phosphate-buffered saline (+) with or without each inhibitor and paracellular flux was corrected for using D-[^{3}H]mannitol. Each point represents the mean ± SD ($n = 4$ monolayers). $^{\dagger}P < 0.01$ and $^{*}P < 0.05$, significant differences between control and inhibitor for [^{14}C]FDG or [^{14}C]GLU transcellular transport.

Fig. 5



Effect of transport inhibitors on transcellular transport of [^{14}C] fluoro-2-deoxy-D-glucose ([^{14}C]FDG) and [^{14}C] D-glucose ([^{14}C]GLU) by LLC-PK$_1$ cells at 90 min of incubation. The effect of transporter inhibitors on the transcellular transport of [^{14}C]FDG (black bars) and [^{14}C]GLU (white bars) was assayed as described in the legend to Fig. 4, except that the incubation time was for 90 min. PBC, probenecid; PHT, phloretin; PHZ, phlorizin; TEA, tetraethylammonium bromide. $^{\dagger}P < 0.01$ and $^{*}P < 0.05$, significant differences between control and inhibitor for [^{14}C]FDG or [^{14}C]GLU transcellular transport.

($P < 0.05$). The effect of the inhibitors over 30–90 min of incubation showed a similar trend to that observed at 90 min of incubation.

Discussion

We have characterized the renal transcellular transport (reabsorption and secretion) of FDG in the kidney proximal tubules using a well-established in-vitro model, the LLC-PK_1 cell line, as well as inhibitors of specific transporters and multiple assay time points for comparison with GLU transport. Although there are many transporters in the renal proximal tubules, to date only the use of an SGLT inhibitor has been reported to increase the excretion of [^{18}F]FDG in the urine [31]. Our study did not include an assay of amino acid transporters that have been proposed to transport [^{18}F]FDG [32,33]. This study is the first report of the potential of transporters other than SGLT and amino acid transporters on [^{18}F]FDG transport and extends the earlier data obtained with the PHZ inhibitor by including an early (10 min) time point in a detailed time-course study of its effect on [^{18}F]FDG transport over 90 min. This provides further finding to an earlier report whose first measurement of the reabsorption of filtered [^{18}F]FDG in the proximal tubules after diuretic or PHZ treatment was at 0–30 min.

Our data on the PHZ inhibitor at early time points are consistent with the role of SGLT in [^{14}C]FDG transport suggested by an earlier rat study. In this study, 49% of [^{14}C]FDG in PBS(+) was reabsorbed by LLC-PK_1 cells at 10 min of incubation. The reabsorption of [^{14}C]FDG was mediated predominantly by SGLT, whereas [^{14}C]GLU was reabsorbed through SGLT and GLUT (Fig. 4). Reabsorption of GLU, but not FDG, by GLUT is consistent with earlier reported results that substitution of a hydroxy group at the second position of GLU with a fluorine atom modulates its ability to be transported by the glucose carrier [33]. Therefore, FDG will be transported only by SGLT and not by GLUT, resulting in substantially greater urinary excretion of [^{14}C]FDG. In the rat study, 56% of the filtered [^{18}F]FDG was reabsorbed in the proximal tubules during the first 30 min of diuretic or PHZ treatment [27]. The amount of [^{14}C]FDG reabsorbed by the LLC-PK_1 cells after PHZ treatment was slightly lower than that reported in the rat study. This lower reabsorption is likely because of the fact that PHZ treatment of rats influences not only the kidneys but also the entire body.

This study showed little reabsorption of [^{14}C]FDG over 30–90 min after the administration of the radiotracer, whereas [^{14}C]GLU levels increased dramatically during this time by reabsorption through SGLT and GLUT (Fig. 5). In addition, only the SGLT inhibitor, PHT, significantly increased the secretion of both [^{14}C]FDG and [^{14}C]GLU over 30–90 min of incubation ($P < 0.05$). As we observed a remarkable reduction in the cellular accumulation of both radiotracers at the basolateral membrane over this time period (data not shown), the observed effect of PHT might be influenced by the leakage of the accumulated radiotracer to the apical side of the proximal tubules because of the inhibition of PHT GLUT. The other kidney transporters, OAT and OCT, showed little association with the transcellular transport of [^{14}C]FDG and [^{14}C]GLU. This could potentially be because of a lower specificity of these transporters for FDG and GLU. On account of the described effects of PHT, FDG was reabsorbed through SGLT at early time points after the administration of PHT, whereas GLU was reabsorbed through SGLT and GLUT over the entire incubation time after the administration of PHT administration. Although neither tracer showed high secretion mediated by a specific transporter, the secretion of FDG was higher than that of GLU.

Our data are important for the clinical use of [^{18}F]FDG. Currently, the clinical use of [^{18}F]FDG-PET is an unsatisfactory method for the detection of malignant neoplasms around urinary organs because the radioactivity excreted into the urine hampers interpretation of the PET images. If SGLT is inhibited at an early phase after the administration of [^{18}F]FDG in a clinical study, kidney reabsorption could be decreased and urinary excretion promoted. This effect has been shown in an earlier rat study [25]. That is, GLUT mainly mediates the uptake of [^{18}F]FDG into the tumor, as well as the excretion of [^{18}F]FDG from the tumor, whereas SGLT predominantly mediates kidney reabsorption of [^{14}C]FDG. In addition, if GLUT is inhibited in the delayed phase after [^{18}F]FDG administration in a clinical study, the secretion of [^{18}F]FDG may be increased. Our data suggest that inhibitors of GLUT will have little or no influence on tumor accumulation of [^{18}F]FDG at the delayed phase after the administration of [^{18}F]FDG and may also provide a decrease in the background level of [^{18}F]FDG in the body. However, it should be noted that there are species differences between pig and human kidney and our findings need to be confirmed in a human system. Nevertheless, this study may have important implications for the improvement of the image quality around urinary organs and for the reduction of radiation exposure in clinical [^{18}F]FDG-PET studies.

Conclusion

The transcellular transport of [^{14}C]FDG, over time, by LLC-PK_1 cells was clarified and differences in the polarized distribution of transcellular transport between [^{14}C]FDG and [^{14}C]GLU were shown. Thus, less reabsorption of [^{14}C]FDG than [^{14}C]GLU, in PBS(+), was observed in the proximal tubular cells. Reabsorption of [^{14}C]FDG was mediated mainly by SGLT at early incubation time points, whereas marked reabsorption of [^{14}C]GLU was mediated mainly by SGLT and GLUT. The secretion of [^{14}C]FDG also tended to be slightly higher than that

of [^{14}C]GLU at all sampling times. The PHT inhibitor significantly elevated the secretion of [^{14}C]FDG and [^{14}C] GLU in the proximal tubular cells over 30–90 min of incubation.

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References

1 Som P, Atkins HL, Bandoypadyay D, Fowler JS, MacGregor RR, Matsui K, *et al*. A fluorinated glucose analog, 2-fluoro-2-deoxy-D-glucose (F-18): nontoxic tracer for rapid tumor detection. *J Nucl Med* 1980; **21**:670–675.
2 Kumar R, Jana S. Positron emission tomography: an advanced nuclear medicine imaging technique from research to clinical practice. *Methods Enzymol* 2004; **385**:3–19.
3 Hustinx R, Benard F, Alavi A. Whole-body FDG-PET imaging in the management of patients with cancer. *Semin Nucl Med* 2002; **32**:35–46.
4 Burgman P, Odonoghue JA, Humm JL, Ling CC. Hypoxia-induced increase in FDG uptake in MCF7 cells. *J Nucl Med* 2001; **42**:170–175.
5 von Forstner C, Egberts JH, Ammerpohl O, Niedzielska D, Buchert R, Mikecz P, *et al*. Gene expression patterns and tumor uptake of ^{18}F-FDG, ^{18}F-FLT, and ^{18}F-FEC in PET/MRI of an orthotopic mouse xenotransplantation model of pancreatic cancer. *J Nucl Med* 2008; **49**:1362–1370.
6 Nguyen XC, So Y, Chung JH, Lee WW, Park SY, Kim SE. High correlations between primary tumours and loco-regional metastatic lymph nodes in non-small-cell lung cancer with respect to glucose transporter type 1-mediated 2-deoxy-2-F18-fluoro-D-glucose uptake. *Eur J Cancer* 2008; **44**:692–698.
7 Yamamoto T, Seino Y, Fukumoto H, Koh G, Yano H, Inagaki N, *et al*. Over-expression of facilitative glucose transporter genes in human cancer. *Biochem Biophys Res Commun* 1990; **170**:223–230.
8 Younes M, Lechago LV, Somoano JR, Mosharaf M, Lechargo J. Wide expression of the human erythrocyte glucose transporter Glut1 in human cancers. *Cancer Res* 1996; **56**:1164–1167.
9 Weber G. Enzymology of cancer cells. (second of two parts). *N Engl J Med* 1977; **296**:541–551.
10 Monakov NK, Neistadt EL, Shavlovskii MM, Shvartsman AL, Neifakh SA. Physicochemical properties and isoenzyme composition of hexokinase from normal and malignant human tissues. *J Natl Cancer Inst* 1978; **61**:27–33.
11 Medina RA, Owen GI. Glucose transporters: expression, regulation and cancer. *Biol Res* 2002; **35**:9–26.
12 Wood IS, Trayhurn P. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br J Nutr* 2003; **89**:3–9.
13 Sacktor B. Sodium-coupled hexose transport. *Kidney Int* 1989; **36**:342–350.
14 Silverman M. Glucose transport in the kidney. *Biochim Biophys Acta* 1976; **457**:303–351.
15 Ohta T, Isselbacher KJ, Rhoads DB. Regulation of glucose transporters in LLC-PK$_1$ cells: effects of D-glucose and monosaccharides. *Mol Cell Biol* 1990; **10**:6491–6499.
16 Hediger MA, Coady MJ, Ikeda TS, Wright EM. Expression cloning and cDNA sequencing of the Na^+/glucose co-transporter. *Nature* 1987; **330**:379–381.
17 Carruthers A. Facilitated diffusion of glucose. *Physiol Rev* 1990; **70**:1135–1176.
18 Cheung PT, Hammerman MR. Na^+-independent D-glucose transport in rabbit renal basolateral membranes. *Am J Physiol* 1988; **254**:F711–718.
19 Miller JH, Mullin JM, McAvoy E, Kleinzeller A. Polarity of transport of 2-deoxy-D-glucose and D-glucose by cultured renal epithelia (LLC-PK$_1$). *Biochim Biophys Acta* 1992; **1110**:209–217.
20 Mullin JM, Weibel J, Diamond L, Kleinzeller A. Sugar transport in the LLC-PK1 renal epithelial cell line: similarity to mammalian kidney and the influence of cell density. *J Cell Physiol* 1980; **104**:375–389.
21 Rabito CA, Ausiello DA. Na^+-dependent sugar transport in a cultured epithelial cell line from pig kidney. *J Membr Biol* 1980; **54**:31–38.
22 Rabito CA. Localization of the Na^+-sugar cotransport system in a kidney epithelial cell line (LLC PK$_1$). *Biochim Biophys Acta* 1981; **649**:286–296.
23 Imaoka T, Kusuhara H, Adachi-Akahane S, Hasegawa M, Morita N, Endou H, Sugiyama Y. The renal-specific transporter mediates facilitative transport of organic anions at the brush border membrane of mouse renal tubules. *J Am Soc Nephrol* 2004; **15**:2012–2022.
24 Urakami Y, Kimura N, Okuda M, Masuda S, Katsura T, Inui K. Transcellular transport of creatinine in renal tubular epithelial cell line LLC-PK1. *Drug Metab Pharmacokinet* 2005; **20**:200–205.
25 Pritchard JB, Miller DS. Mechanisms mediating renal secretion of organic anions and cations. *Physiol Rev* 1993; **73**:765–796.
26 Sekine T, Watanabe N, Hosoyamada M, Kanai Y, Endou H. Expression cloning and characterization of a novel multispecific organic anion transporter. *J Biol Chem* 1997; **272**:18526–18529.
27 Moran JK, Lee HB, Blaufox MD. Optimization of urinary FDG excretion during PET imaging. *J Nucl Med* 1999; **40**:1352–1357.
28 Shikano N, Kawai K, Nakajima S, Kubodera A, Kubota N, Ishikawa N, Saji H. Transcellular transport of radioiodinated 3-iodo-α-methyl-L-tyrosine across monolayers of kidney epithelial cell line LLC-PK$_1$. *Ann Nucl Med* 2004; **18**:227–234.
29 Shikano N, Kawai K, Nakajima S, Kubodera A, Kubota N, Ishikawa N, Saji H. Transcellular transport of 4-iodo-L-meta-tyrosine via system L across monolayers of kidney epithelial cell line LLC-PK$_1$. *Nucl Med Biol* 2004; **31**:477–482.
30 Kiyono Y, Yamashita T, Doi H, Kuge Y, Katsura T, Inui K, *et al*. Is MIBG a substrate of P-glycoprotein? *Eur J Nucl Med Mol imaging* 2007; **34**:448–452.
31 Kosuda S, Fisher S, Wahl RL. Animal studies on the reduction and/or dilution of 2-deoxy-2-[18F]fluoro-D-glucose (FDG) activity in the urinary system. *Ann Nucl Med* 1997; **11**:213–218.
32 Thorens B. Glucose transporters in the regulation of intestinal, renal, and liver glucose fluxes. *Am J Physiol* 1996; **270**:G541–53.
33 Kleinzeller A, McAvoy EM, McKibbin RD. Active renal hexose transport structural requirements. *Biochim Biophys Acta* 1980; **600**:513–520.