Note

Effect of Glycerol-Related Compounds on Carrier-Mediated Glycerol Uptake in HCT-15 Human Colon Cancer Cell Line

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Summary: The effect of several compounds, which are structurally analogous to glycerol, on carrier-mediated glycerol uptake was examined in HCT-15 cells to help clarifying the functional characteristics of the glycerol transport system. The carrier-mediated uptake of glycerol conformed to the Michaelis-Menten kinetics with a Michaelis constant of 21.1 µM and the tested compounds were all suggested to inhibit it competitively with the values of the inhibition constant (Ki) in the increasing order as follows: monobutyrin (41.0 µM) ≤ monoacetin (54.6 µM) < diglycerol (154 µM) < 1,2-propanediol (1650 µM). Therefore, they all may possibly be substrates of the carrier-mediated glycerol transport system, for which the glycerol esters (monoacetin and monobutyrin) have the highest affinities among them. It was also found that S-(+)-enantiomer of 1,2-propanediol (Ki = 484 µM) has a higher affinity than its R-(−)-enantiomer (Ki = 19100 µM), indicating enantioselective recognition. These results support the suggestion that a specific carrier protein is involved in glycerol uptake in HCT-15 cells. It would be of interest to identify the carrier, which may be present also in some organs, and further investigate the possibility that glycerol ester derivatives of drugs might be delivered via the carrier.

Keywords: glycerol; carrier-mediated transport; competitive inhibition; substrate specificity; enantioselectivity; HCT-15 cell

Introduction

The HCT-15 human colon cancer cell line has a Na+-dependent carrier-mediated transport system for glycerol uptake.1,2) A similar transport system has been suggested to be present also in the small intestine and is of interest for its role in the absorption of glycerol and structurally analogous compounds.3–6) Such transport systems would be of interest not only for their roles in the disposition of glycerol and analogs but also for utilization as possible pathways of drug delivery.

The uptake of glycerol was in both HCT-15 cells and the rat small intestine highly saturable, Na+-dependent and inhibited by metabolic inhibition by 2,4-dinitrophenol, indicating the involvement of Na+-dependent secondary active type of carrier-mediated transport systems.1–6) Also, it was in the both specifically inhibited by some compounds structurally analogous to glycerol, such as glycerol 3-phosphate and 1,2-propanediol (propylene glycol), indicating that the transport systems are specific for glycerol and analogs. However, there was found a large difference in the Michaelis constant (Km) of glycerol transport, being about 50-fold greater in the rat small intestine (770 µM) than in HCT-15 cells (15.0 to 23.4 µM). This difference in Km may be accounted for by the presence of homologous carriers with different affinities in each animal rather than by a species difference between a carrier in the human and its orthologous counterpart in the rat. Thus, they may represent two isoforms of a group of Na+-dependent glycerol carriers, although their molecular entities have not been identified yet. These carrier-mediated transport systems are presumed to be involved in the absorption of glycerol as a nutritional substance from the small intestine and its uptake from the systemic circulation into some organs for utilization in various physiological processes.7,8)

To help clarifying the functional characteristics of such glycerol transport systems, we examined in the present study the effect of several compounds, which are structural-
Effect of Glycerol-Related Compounds on Glycerol Uptake

Materials and Methods

Materials: [2-3H] glycerol (74.0 GBq/mmol) was purchased from GE Healthcare Biosciences Co. (Piscataway, NJ, U.S.A.), unlabeled glycerol was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and Clear-sol II, a scintillation fluid, was from Nacalai Tesque, Inc. (Kyoto, Japan). All other reagents were of analytical grade and commercially available.

HCT-15 cells, a human colon cancer (adenocarcinoma) cell line, were obtained from Cell Resource Center for Biomedical Research, Tohoku University.

Uptake Experiments in HCT-15 cells: HCT-15 cells were seeded at the density of 5 × 10^4 cells/well (1 mL/well) in 24-well plates (Techno Plastic Products AG, Trasadingen, Switzerland) and cultured in RPMI 1640 medium (Sigma-Aldrich Co., St. Louis, MO, U.S.A.) containing 10% FBS and 1% penicillin/streptomycin under an atmosphere of 95% air - 5% CO2 at 37°C for 48 h.

Test solutions were prepared in Hanks’ solution (0.952 mM CaCl2, 5.36 mM KCl, 0.441 mM KH2PO4, 0.821 mM MgSO4·7H2O, 136.7 mM NaCl, 0.385 mM Na2HPO4·12H2O, 25 mM D-glucose, 10 mM HEPES, pH 7.5). To test solutions were added [3H]glycerol (0.4 μM) and, when required, also unlabeled glycerol to adjust the concentration. The cells in each well were preincubated in glycerol-free Hanks’ solution (1 mL) for 30 min at 37°C. After removing the preincubation solution, uptake was initiated by adding 0.25 mL of Hanks’ solution containing [3H]glycerol at 37°C. To stop uptake, 1.5 mL of ice-cold Hanks’ solution was added, and the cells were washed twice with ice-cold Hanks’ solution (2 mL). To determine the amount of [3H]glycerol taken up by the cells, the cells in each well were solubilized in 0.2 M NaOH containing 0.5% SDS (0.5 mL) for 2 h and transferred to a counting vial, and then 5 mL Clear-sol II, a scintillation fluid, was added for liquid scintillation counting of radioactivity. The cellular protein content was determined by the method of Lowry et al.

Data Treatment: The uptake was estimated by subtracting the amount initially adsorbed to the cells. For kinetic analysis, the uptake rate (J) was calculated by dividing the uptake by time during the initial uptake phase (10 min), where the uptake was previously shown to be proportional to time. The uptake clearance (CL_up) was calculated by dividing J by the concentration in the medium (Cm), and a minor, passive (nonsaturable) component of CL_up was subtracted to estimate the clearance of uptake by carrier-mediated (saturable) transport (CL_up,c). The passive component of CL_up was estimated as the CL_up at a high concentration of 10 mM, where carrier-mediated transport was saturated and negligible. The carrier-mediated uptake of glycerol was analyzed by assuming a single Michaelis-Menten type transport component, where CL_up,c is expressed as a function of Cm, using the maximum transport rate (Jmax) and the Michaelis constant (Km), as follows:

\[
CL_{\text{up,c}} = \frac{J_{\text{max}}}{K_m + C_m}
\]

The kinetic parameters of Jmax and Km were estimated by fitting Eq. 1 to the experimental data of CL_up,c versus Cm profile using a nonlinear regression program, WinNonlin (Pharsight Co., Mountain View, CA, U.S.A.), and the reciprocal of variance as the weight. The expression of CL_up,c in the presence of a competitive inhibitor is as follows:

\[
CL_{\text{up,c}} = \frac{J_{\text{max}}}{K_m(1 + C/K_i) + C_m}
\]

where C, Ci, and Ki are the concentration of the inhibitor and the inhibition constant, respectively. With the values of Jmax and Km fixed at those determined in the absence of the inhibitor, Ki was estimated by fitting Eq. 2 to the experimental data of CL_up,c versus Cm profile for a given Ci.

Statistical Analysis: Differences between groups were examined for statistical significance by using analysis of variance (ANOVA) followed by Dunnnett’s test.

Results and Discussion

In the initial set of experiments, we examined the effect of test compounds, with increasing the concentration, on glycerol uptake (Fig. 1). They were all found to significantly inhibit the uptake of [3H]glycerol (0.4 μM). Glycerol es...
Fig. 2. Effect of glycerol-related compounds on the concentration-dependent profile of carrier-mediated glycerol uptake in HCT-15 cells

Data represent the mean ± S.E. (n = 4). The uptake of \[^3\text{H}\]glycerol was evaluated at 37°C and at 10 min in the presence of a test compound (●) or in its absence (○). The clearance of carrier-mediated uptake was estimated by subtracting the passive transport component evaluated at 10 mM glycerol from the total uptake clearance. Tested compounds were monoacetin (0.1 mM, A), monobutyrin (0.1 mM, B), diglycerol (0.1 mM, C), 1,2-propanediol (1 mM, D), S(+)-1,2-propanediol (1 mM, E), R(−)-1,2-propanediol (10 mM, F). The solid lines represent the computer-fitted profiles. Shown in the insets are the Eadie-Hofstee plots, where \(J_c\) and \(C_m\) represent the carrier-mediated uptake rate (pmol/min/mg protein) and concentration in the medium (μM), respectively. Kinetic parameters are shown in Table 1.

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ers (monoacetin and monobutyrin) were the most potent inhibitors, inhibiting glycerol uptake almost completely at the highest concentration of 10 mM and also by about 40% even at the lowest concentration of 0.05 mM. Diglycerol, an ether type of glycerol derivative, also showed a potent inhibitory effect. The remaining uptake in the presence of these esters and ether at 10 mM was close to the passive (nonsaturable) uptake component (15% of total uptake) estimated in the presence of 10 mM glycerol, indicating almost complete inhibition of carrier-mediated transport. Inhibition by 1,2-propanediol was weaker, being significant only at the highest concentration of 10 mM. Interestingly, an enantioselective characteristic was suggested for inhibition by 1,2-propanediol, as its S(+)-enantiomer inhibited glycerol uptake more extensively than its R(−)-enantiomer.

According to kinetic analyses (Fig. 2 and Table 1), the carrier-mediated (saturable) component of glycerol uptake conformed to the Michaelis-Menten kinetics with a Michaelis constant of 21.1 μM and the profiles of \(CL_{up,c}\) versus \(C_m\) in the presence of inhibitors could be described using the competitive inhibition model with the \(K_i\) values in the increasing order as follows: monobutyrin (41.0 μM) < monoacetin
Table 1. Inhibition constants ($K_i$) for the inhibition of carrier-mediated glycerol uptake by glycerol-related compounds in HCT-15 cells

<table>
<thead>
<tr>
<th>Tested compounds</th>
<th>$K_i$ ($\mu$M)</th>
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<tbody>
<tr>
<td>Monoacetin</td>
<td>54.6 ± 3.8</td>
</tr>
<tr>
<td>Monobutyrin</td>
<td>41.0 ± 4.3</td>
</tr>
<tr>
<td>Diglycerol</td>
<td>154 ± 18</td>
</tr>
<tr>
<td>1,2-Propanediol</td>
<td>1650 ± 390</td>
</tr>
<tr>
<td>S(+)-1,2-Propanediol</td>
<td>484 ± 36</td>
</tr>
<tr>
<td>R(−)-1,2-Propanediol</td>
<td>19100 ± 6400</td>
</tr>
</tbody>
</table>

Data represent the computer-fitted parameters with S.E. by the analyses of the concentration-dependent profiles of carrier-mediated glycerol uptake in Figure 2. The values of $J_{max}$ and $K_m$ were 56.5 ± 7.0 pmol/min/mg protein and 21.1 ± 2.9 $\mu$M, respectively.

(54.6 $\mu$M)<diglycerol (154 $\mu$M)<1,2-propanediol (1650 $\mu$M). Therefore, they all may possibly be substrates of the carrier-mediated glycerol transport system, for which the glycerol esters (monoacetin and monobutyrin) have the highest affinities among them. The $K_i$ values for monoacetin and monobutyrin were relatively close to the $K_m$ of glycerol transport (21.1 $\mu$M), indicating their high affinities comparable with that of glycerol. Interestingly, the $K_i$ for the $S(+)$-enantiomer of 1,2-propanediol (484 $\mu$M) was estimated to be two orders of magnitude smaller than that for its $R(−)$-enantiomer (19100 $\mu$M), evidently indicating enantioselective recognition, favoring the $S(+)$-enantiomer, by the transport system. However, even the $S(+)$-enantiomer was suggested to have a much lower affinity than the ester and ether derivatives as well as glycerol, as indicated by the greater $K_i$. These results suggest that esterification or etherification of a hydroxyl group at the end of the carbon backbone of glycerol alters the affinity for the transport system little or only moderately, while the substitution of a hydroxyl group with a hydrogen reduces it drastically. Thus, an oxygen, or a polar moiety, at that position seems to play an important role in recognition by the transport system. It should also be noted that having two hydroxyl groups, one at an end of the carbon backbone and another at the middle, seems to be prerequisite to be recognized by the transport system because 1-propanol, 2-propanol and 1,3-propanediol did not show any inhibitory effect at a concentration as high as 10 mM, as we reported previously.

The Eadie-Hofstee plot for the inhibition of glycerol uptake by $S(+)\text{-}1,2\text{-propanediol}$ (Fig. 2E) indicates an alternative possibility that $J_{max}$ might be reduced as a result of a noncompetitive mode of inhibition. However, it is not evident with inhibition by racemic 1,2-propanediol (Fig. 2D), in which the $S(+)$-enantiomer is presumed to be the major inhibitory component, and either with inhibition by the $R(−)$-enantiomer (Fig. 2F). Particularly for the racemate, the data point in its presence at the highest $C_m$ where $C_{lim} = \frac{C_m}{J_{lim}}$ ($J_{lim}$) is smallest and $J_c$ is greatest and close to the predicted $J_{max}$ is almost overlapped with that in its absence. We, therefore, assumed that the mode of inhibition by the $S(+)\text{-}enantiomer$, as well as the racemate and the $R(−)$-enantiomer, is more likely to be competitive than noncompetitive, although we cannot exclude the possibility that it might be noncompetitive or mixed.

Aquaglyceroporins are a group of channels, which belong to aquaporin (AQP) water channels and through which glycerol is known to be able to permeate. Although the selectivities of aquaglyceroporins for solutes have not been fully elucidated, AQP3 is reportedly permeable to 1,2-propanediol as well as glycerol, with a reflection coefficient close to null. AQP3 is reported to be also permeable to 1,2-ethanediol (ethylene glycol) with a negligible reflection coefficient, even though the inhibition of glycerol uptake by this compound in HCT-15 cells was previously shown to be smaller than that by 1,2-propanediol, indicating its even lower affinity for the carrier-mediated glycerol transport system than 1,2-propanediol. Thus, the characteristics of substrate recognition by the transport system, which favors glycerol much more than 1,2-propanediol and 1,2-ethanediol, seem to be quite different from the solute selectivity of AQP3.

It has been reported that glycerol uptake by rat hepatocytes is mediated by a carrier-mediated mechanism with a $K_m$ of 100 $\mu$M, which is an order of magnitude greater than that in HCT-15 cells (15.0 to 23.4 $\mu$M in this and previous studies). The hepatic glycerol transport is reportedly also inhibited by monoacetin competitively with a $K_i$ of 500 $\mu$M, which is an order of magnitude greater than that in HCT-15 cells (54.6 $\mu$M) in accordance with the difference in $K_m$. Thus, the carrier-mediated glycerol transport system in the liver seems to be different from the one in HCT-15 cells as the affinities for substrates are quite different, but they may have some common features in substrate recognition mechanism as the $K_i$ for monoacetin in hepatocytes is shifted, compared with that in HCT-15 cells, by an extent similar to that for the shift of the $K_m$ of glycerol transport. It should also be noted that the transport system in the liver is presumed to be of facilitated diffusion type, while the one in HCT-15 cells has been suggested to be of Na$^+$-dependent and secondary active type.

In conclusion, monoacetin, monobutyrin, diglycerol and 1,2-propanediol were suggested to competitively inhibit carrier-mediated glycerol uptake in HCT-15 cells. Therefore, all these glycerol-related compounds may possibly be substrates of the carrier-mediated glycerol transport system. Among them, glycerol esters (monoacetin and monobutyrin) were suggested to have the highest affinities, which are relatively close to that of glycerol. Enantioselective characteristic was also suggested for recognition by the carrier. These results further support the suggestion that a specific carrier protein is involved in glycerol uptake in HCT-15 cells. It would be of interest to identify the carrier, which may be present also in some organs, and further investigate the possibility that glycerol ester derivatives of drugs might be delivered via the carrier.
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References


