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Research Paper

Site-specific contribution of proton-coupled folate transporter/haem carrier protein 1 in the intestinal absorption of methotrexate in rats

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Abstract

Objectives Methotrexate is reportedly a substrate for proton-coupled folate transporter/haem carrier protein 1 (PCFT/HCP1) and reduced folate carrier 1 (RFC1). In this study, we examined the contribution of PCFT/HCP1 and RFC1 in the intestinal absorption of methotrexate in rats.

Methods Western blot analysis was carried out to evaluate the protein levels of PCFT/HCP1 and multidrug resistance-associated protein 2 in brush-border membrane of rat small intestine. Mucosal uptake of methotrexate was studied in the rat everted small intestine and an in-situ intestinal perfusion study of methotrexate was also carried out in rats.

Key findings In transport studies using everted intestine, the mucosal methotrexate influx rate in proximal intestine at pH 5.5 was significantly greater than that at pH 7.4. Coadministration of folate or its analogues, such as folinate and 5-methyltetrahydrofolate, substrates for both PCFT/HCP1 and RFC1, significantly suppressed the methotrexate influx at pH 5.5, whereas thiamine pyrophosphate, an inhibitor for RFC1 alone, exerted no significant effect. Western blot analysis showed higher PCFT/HCP1 expression in proximal than distal small intestine. In distal small intestine, methotrexate influx rate was low and was not pH dependent. Also, folate and its analogues exerted no significant effect on methotrexate absorption.

Conclusions Based on the present and our previous results, the site-specific contributions of various transporters including PCFT/HCP1 in methotrexate intestinal absorption were discussed. The variation in luminal pH and the involvement of multiple transporters in methotrexate absorption may cause variation in oral bioavailability among patients.

Keywords ABC transporters; intestinal absorption; methotrexate; PCFT/HCP1; site-specific absorption

Introduction

Methotrexate, a folic acid antagonist, is an inhibitor of dihydrofolate reductase (DHFR) and is widely used as a chemotherapeutic agent to treat neoplastic disease and autoimmune disease such as rheumatoid arthritis.[1–3] In the chemotherapy of neoplastic disease, such as non-Hodgkin's lymphomas, methotrexate is administered systemically as an early high-dose regimen.[4,5] In the treatment of rheumatoid arthritis, methotrexate is administered orally as a low-dose intermittent regimen.[3,6–8] Though the mean oral bioavailability of methotrexate at a low dose is relatively high (approximately 75%), the plasma concentrations of methotrexate exhibit wide variation in clinical practice.[9–13] The variability of oral bioavailability of methotrexate was not ascribed either to food intake or to renal failure (low glomerular filtration rate).[14,15] In contrast, the divided oral administration of methotrexate (e.g. 30 mg split dose separated by 8 h, weekly), significantly increased the oral bioavailability in adult patients.[12]

The lower and scattered oral bioavailability of methotrexate at a higher oral dose and the higher oral bioavailability at a lower oral dose may come from the involvement of multiple transporters in methotrexate intestinal absorption. Methotrexate is known as a substrate of ATP-binding cassette (ABC) efflux transporters such as multidrug resistance-associated proteins (MRPs) and breast cancer resistance protein (BCRP).[16–19] Previously, we examined the contribution of MRP2, MRP3 and BCRP in methotrexate absorption in

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rats,[20,21] in which MRP2 was expressed abundantly in the proximal small intestine, as compared with distal small intestine, and suppressed methotrexate absorption significantly in the proximal small intestine. In contrast, MRP3 localized in basolateral membranes was abundantly expressed in the distal small intestine, as compared with proximal intestine, and significantly facilitated methotrexate absorption from the distal intestine. The role of MRP3 in facilitating methotrexate absorption has also been demonstrated by using *Abcc3*$^{-/-}$ and wild-type mice.[22] BCRP expressed in brush-border membrane along the whole small intestine lowered methotrexate absorption in both proximal and distal small intestine. Like this, the involvement of multiple ABC transporters in methotrexate intestinal absorption was observed to depend on their site of expression.[20,21]

In addition, methotrexate is also recognized by solute carrier (SLC) transporters such as reduced folate carrier 1 (RFC1), proton-coupled folate transporter/haem carrier protein 1 (PCFT/HCP1), organic anion transporting polypeptide 1A2 (OATP1A2) and organic anion transporter 3 (OAT3).[23–28] RFC1, an anion exchanger, is highly expressed along the entire intestinal brush-border membrane in rats,[29] and the RFC1-mediated transport has a neutral pH optimum and specificity for reduced folate.[29–31] PCFT/HCP1 is expressed on the brush-border membranes of proximal intestine and transports both oxidized and reduced folate by using a proton-gradient as a driving force. The PCFT/HCP1-mediated folate transport is saturable and methotrexate inhibits the folate transport extensively. Also, the transport of folate and of methotrexate are optimum at pH 5.5 and are negligible at pH 7.0.[27,32–37] Thus, in this study, we examined the contribution of PCFT/HCP1 mainly, as well as RFC1 and MRP2, to determine the site-specific and multiple transporter-mediated intestinal absorption of methotrexate in rats.

Materials and Methods

Materials

Methotrexate was obtained from Wako Pure Chemicals (Osaka, Japan). Folate, folinate, 5-meythyltetrahydrofolate, thiamine pyrophosphate and benzbromarone were purchased from Sigma Chemical Co. Ltd (St Louis, USA). A rabbit polyclonal antibody to PCFT/HCP1 (ab25134) and M_2III-6, a mouse anti-MRP2 monoclonal antibody, were obtained from Abcam (Cambridge, UK) and Chemicon International, Inc. (Temecula, USA), respectively. Peroxidase-labelled affinity antibody to rabbit IgG (H + L) and peroxidase-labelled affinity antibody to mouse IgG (H + L) were from Kirkegaard & Perry Laboratories, Inc. (Gaithersburg, USA). All other chemicals used were of the highest purity available.

Animals

Male Sprague-Dawley (SD) rats, 7–9 weeks old, were fasted overnight with free access to water before the experiments. Experiments with animals were performed in accordance with the 'Guide for Animal Experimentation' from the Committee of Research Facilities for Laboratory Animal Sciences, Hiroshima International University, which is in accordance with the 'Guidelines for proper conduct of animal experiments' from the Science Council of Japan.

Expression analysis of PCFT/HCP1 and MRP2 in rat small intestine

Western blot analysis was carried out to evaluate the protein levels of PCFT/HCP1 and MRP2 in brush-border membrane of rat small intestine. The whole small intestine was excised and divided into two parts in an equal length, and the brush-border membranes of proximal and distal small intestine were prepared by a magnesium/EGTA precipitation method in the same manner as described previously.[38] Detection of PCFT/HCP1 and MRP2 proteins was carried out using a rabbit polyclonal antibody to PCFT/HCP1 (1 : 60 dilution) and M_2III-6 (1 : 50 dilution) as the primary antibody, respectively.[38] As the secondary antibody, peroxidase-labelled affinity antibody to rabbit IgG (H + L) and peroxidase-labelled affinity antibody to mouse IgG (H + L) were used for PCFT/HCP1 and MRP2, respectively. The optical density of immunoreactive proteins was estimated by a computer-aided densitometer with NIH Image (the public domain program developed at the US National Institutes of Health, Bethesda, USA).

Mucosal uptake of methotrexate in rat everted small intestine

Everted small intestinal sacs (5 cm length) were prepared by using proximal and distal small intestine. Methotrexate was dissolved in isotonic pH 5.5 and pH 7.4 incubation media (in mM: 0.4 KH_2PO_4, 140 NaCl, 5 KCl, 25 glucose, 1 $CaCl_2$, 0.5 $MgCl_2$ and 10 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) for pH 5.5 or 10 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) for pH 7.4) at appropriate concentrations. The closed everted sacs were immersed in 5 ml of substrate-free incubation medium prewarmed at 37°C and pre-oxygenated with 5% CO_2–95% O_2 gas for 15 min. Bubbling of the incubation medium with a CO_2–O_2 gas was continued throughout the uptake study. The serosal side (inside of the everted sac) was filled with 0.5 ml of substrate-free incubation medium, and the sac was immersed in 5 ml incubation medium containing methotrexate. Each sac was incubated at either 37°C or 4°C and the serosal fluid of the sac was collected at designated time intervals (up to 120 min maximum) to measure the mucosal-to-serosal transport of methotrexate. The sac was then washed quickly and carefully with ice-cold, substrate-free incubation medium to remove possibly adsorbed methotrexate from the surface of the sac. The mucosal tissue of the sac was collected by scraping with a cover glass to measure methotrexate concentration. In the intestinal transport and uptake studies, the concentration of methotrexate in the incubation medium was varied from 0.1 to 200 μM. In the inhibition study, folate, folinate, 5-meythyltetrahydrofolate or thiamine pyrophosphate was added to the mucosal incubation medium at a final concentration of 200 μM as an inhibitor. In this inhibition study, the incubation of everted sac with methotrexate was carried out for 10 min, and serosal fluid and mucosal tissue

were collected to estimate the initial transport and uptake rates of methotrexate.

Eadie–Hofstee plots were made for mucosal-to-serosal transport rates and mucosal uptake rates of methotrexate in everted proximal intestine, in which the rate of methotrexate (v) was plotted against the clearance (v/[S]) of methotrexate. Eadie–Hofstee plots revealed the contribution of two saturable components in the mucosal-to-serosal transport of methotrexate, and a single saturable component with a simple diffusion in the mucosal uptake of methotrexate. Accordingly, the plots of mucosal-to-serosal transport rates of methotrexate against methotrexate concentrations were analysed using Equation 1:

$$v = V_{max1}[S]/(K_{m1} + [S]) + V_{max2}[S]/(K_{m2} + [S]) \quad (1)$$

where v is the initial transport rate, [S] is the initial methotrexate concentration, V_{max1} and V_{max2} are the maximum transport rates for the high- and low-affinity components, respectively, and K_{m1} and K_{m2} are the Michaelis constants for the high- and low-affinity components, respectively. The plots of mucosal uptake rate of methotrexate against methotrexate concentrations were analysed using Equation 2:

$$v = V_{max}[S]/(K_m + [S]) + K_d[S] \quad (2)$$

where v is the initial uptake rate, [S] is the initial methotrexate concentration, V_{max} is the maximum uptake rate and K_m and K_d are the Michaelis constants and the coefficient of simple diffusion, respectively. Curve fitting analysis was performed using KaleidaGraph program (Version 3.501, Synergy Software, Reading, USA).

In-situ intestinal perfusion study of methotrexate

Rats were anaesthetized with pentobarbital (30 mg/kg, i.p. injection) and affixed supine on a surface kept at 37°C to maintain the body temperature at approximately 36°C. The lumen of proximal intestine (a 20-cm long segment from 5 cm below the bile duct opening) was perfused in a re-circulating manner at a flow rate of 1 ml/min with 20 ml of incubation medium (either pH 5.5 or 7.4) containing methotrexate (1 μM) and 4% dimethyl sulfoxide (DMSO), in which DMSO (4%) was used to aid the solubility of benzbromarone. In the inhibition study, folate and benzbromarone were used as inhibitors and added to the perfusate at a concentration of 200 or 400 μM, respectively. The intestinal perfusate was collected periodically to determine the concentration–time profile of methotrexate in the perfusate. CDNB was dissolved at a concentration of 1 μM in pH 6.0 isotonic phosphate-buffered saline (PBS) containing 4% DMSO.

Data analysis

The biological fluid samples containing methotrexate were diluted appropriately with 20% perchloric acid. Intestinal mucosa samples were homogenized in an equal volume of 20% perchloric acid. These biological samples were kept on ice for at least 30 min, and centrifuged at 3000 rev/min for 10 min. The concentration of methotrexate in the supernatants of various biological samples was determined by HPLC using a column of YMC-pack ODS-AM (50 × 4.6 mm; YMC Inc., Wilmington, USA). The mobile phase was a mixture of 0.1 M acetate buffer (pH 6.0) and acetonitrile (90 : 10, v/v), or a mixture of acetonitrile, methanol and 1% acetic acid in a ratio of 5 : 10 : 85 (v/v), depending on the biological samples. The flow rate of the mobile phase was 1 ml/min, and detection of methotrexate was made at a wavelength of 304 nm. The detection limit of methotrexate under this analytical condition was approximately 1 nM.

Differences among group mean values were assessed by Kruskal–Wallis or analysis of variance tests followed by a post-hoc test (Dunn's test) or Student's t-test. $P < 0.05$ was considered statistically significant.

Results

Western blot analysis for PCFT/HCP1 and MRP2 expression in rat small intestine

Whole small intestine was divided into two equal parts, the proximal and distal small intestine, and expression levels of PCFT/HCP1 and MRP2 proteins in the intestinal brush-border membranes were evaluated by Western blot analysis (Figure 1). Bands of approximately 51 and 190 kDa, corresponding to the molecular size of PCFT/HCP1 and MRP2, respectively,[36,39] were observed in the brush-border membranes from both proximal and distal small intestine. The band densities of PCFT/HCP1 and MRP2 in proximal intestine were 4.3-fold and 2.8-fold stronger than those in distal small intestine, respectively.

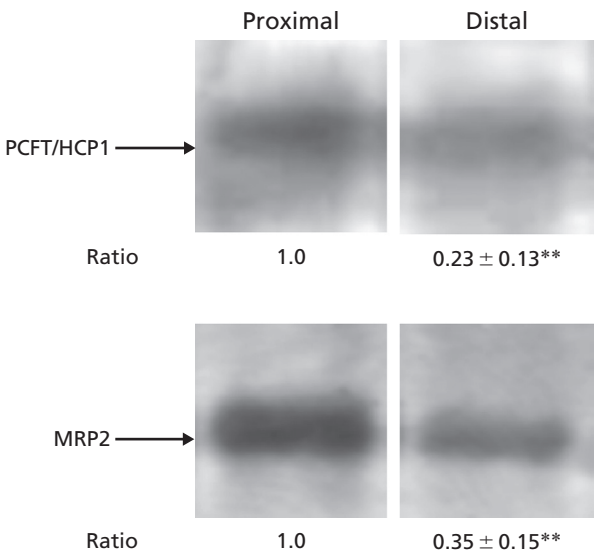

Figure 1 Western blot analysis for PCFT/HCP1 and MRP2 proteins and the relative density of staining intensity for these proteins in the brush-border membrane of proximal and distal small intestine of rats. Each value of relative staining intensity (Ratio) represents the mean ± SD of results from three rats. $^{**}P < 0.01$, compared with the value for proximal intestine.

Kinetic analysis of mucosal methotrexate influx in rat everted small intestine

The mucosal-to-serosal transport and mucosal uptake of methotrexate were measured at pH 5.5 using rat everted intestine (Figure 2), since folate transport is pH dependent and optimal at pH 5.5.[27,36] The mucosal-to-serosal transport of methotrexate followed a zero-order rate fashion with no lag time in either proximal or distal small intestine; the rate in proximal intestine was approximately 1.4-fold higher than that in distal intestine. The concentrations of methotrexate in the mucosal membrane of proximal and distal intestine increased with time up to 30 min and then reached an almost steady level. The steady mucosal methotrexate concentration in proximal small intestine was approximately 1.8 times that in distal small intestine.

The initial rate of mucosal-to-serosal transport and mucosal uptake of methotrexate was estimated over a concentration range of 0.1–200 μM of methotrexate. Both the transport rate across everted intestinal sacs and the mucosal uptake rate of methotrexate were concentration dependent (Figure 3). Eadie–Hofstee plots of these data suggested that there are two saturable components in the mucosal-to-serosal transport process of methotrexate. The estimated K_m and V_{max} values, by fitting the plots of transport rates at different methotrexate concentrations, were 9.5 ± 0.3 μM and 45 ± 6.0 pmol/min per 5 cm intestine for high affinity and 97 ± 8.5 μM and 187 ± 6.0 pmol/min per 5 cm intestine for low affinity components, respectively. The mucosal uptake of methotrexate was analysed using a single saturable component and a single non-saturable component (K_d). The estimated K_m and V_{max} values were 4.2 ± 1.1 μM and 57 ± 13 pmol/min per g intestine, respectively. The K_d value was 4.5 ± 0.2 μl/min per g intestine.

Characteristics of methotrexate influx in rat proximal small intestine

Addition of folate, a substrate for both PCFT/HCP1 and RFC1, to the mucosal incubation medium at pH 5.5 significantly suppressed the rates of mucosal-to-serosal transport and mucosal uptake of methotrexate in rat proximal small intestine (Figure 4). Similarly, folate analogues, such as folinate and 5-methyltetrahydrofolate (5-MTHF), both PCFT/HCP1 substrates, suppressed the methotrexate influx in proximal intestine. In contrast, thiamine pyrophosphate (TPP), an inhibitor for RFC1 alone, did not show any significant effect on methotrexate influx. Suppressive effects of folate and its analogues were not observed in distal small intestine. In addition, methotrexate influx in proximal small intestine, but not in distal small intestine, was markedly reduced by the low temperature (4°C) and neutral pH (pH 7.4) of the incubation medium.

Contribution of influx and efflux transporters in methotrexate absorption

The contribution of SLC influx and ABC efflux transporters in methotrexate absorption was examined using pH 5.5 and pH 7.4 perfusate in in-situ intestinal perfusion studies. Methotrexate disappeared from the intestinal perfusate in a zero-order rate manner. The disappearance rate, or intestinal absorption rate, of methotrexate at pH 7.4 was significantly lower, by approximately 55%, than that at pH 5.5 (Figure 5). The addition of folate to the pH 5.5 intestinal perfusate decreased the disappearance rate of methotrexate by approximately 54% of the control. Benzbromarone, an MRP inhibitor, increased the disappearance rate of methotrexate significantly by approximately 1.3 fold at pH 5.5. The mixture of folate and benzbromarone decreased the disappearance rate to the same level of folate alone. At pH 7.4, the absorption rate of methotrexate was approximately half of that at pH 5.5, and co-administration of folate, benzbromarone, or both, exerted no significant effect on methotrexate absorption.

Discussion

Methotrexate is used as a chemotherapeutic agent to treat neoplastic disease and autoimmune disease such as rheumatoid arthritis.[1–3] The scattered oral bioavailability of methotrexate, however, limits the clinical use for rheumatoid

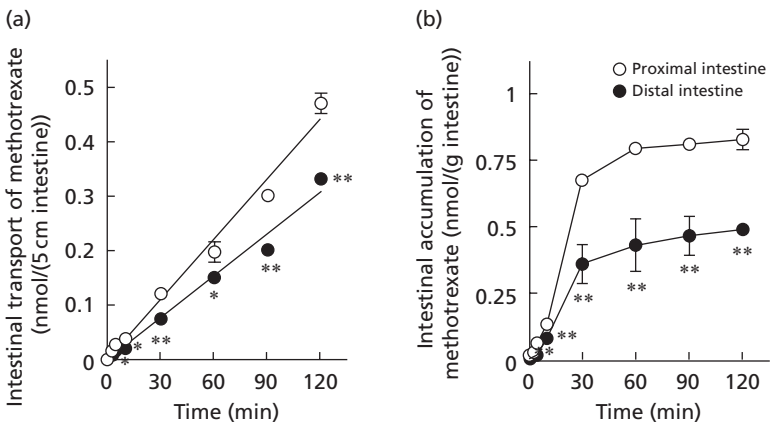

Figure 2 Time course for mucosal-to-serosal transport (a) and mucosal uptake (b) of methotrexate in everted proximal and distal small intestine of rats. Methotrexate was applied at a concentration of 1 μM to the mucosal side of the everted sac at 37°C and pH 5.5. Each value represents the mean ± SD of results from three rats. *$P < 0.05$, **$P < 0.01$, compared with the value for proximal intestine.

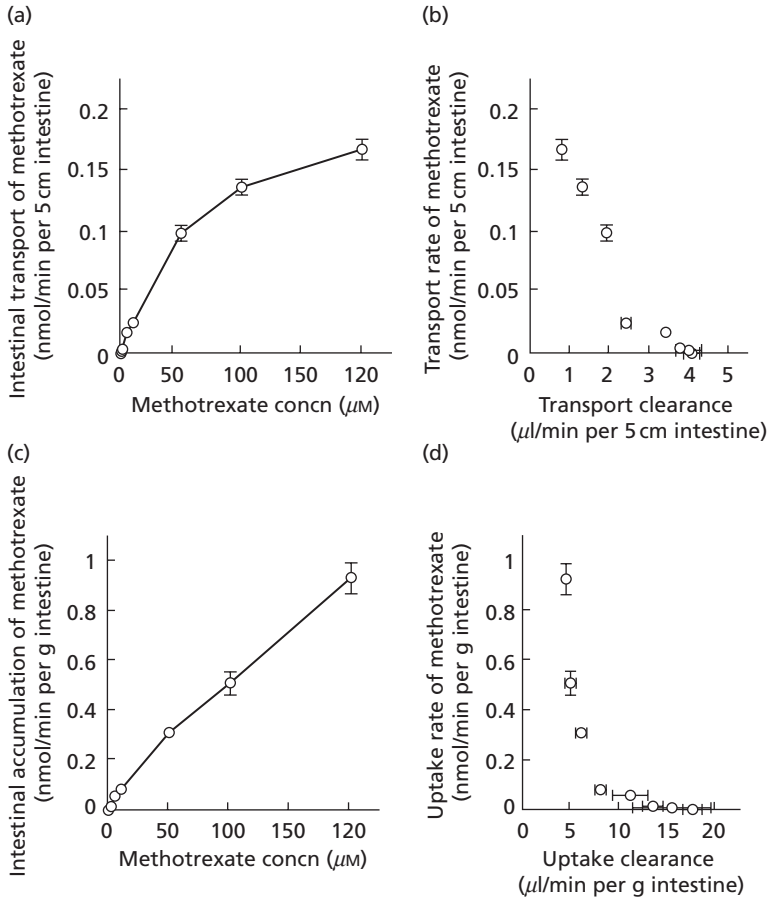

Figure 3 Concentration dependency in mucosal-to-serosal transport (a) and mucosal uptake (c) of methotrexate in everted proximal small intestine of rats. The corresponding Eadie–Hofstee plots are presented in (b) and (d), respectively. Methotrexate was applied at a concentration of 1 μM to the mucosal side of the everted sac at 37°C and pH 5.5. Each value represents the mean ± SD of results from three rats.

arthritis patients. Methotrexate is a hydrophilic compound having a carboxyl and amino group of pK_a 4.84 and 5.51, respectively.

In this study, we investigated the contribution of SLC influx transporters, such as PCFT/HCP1 and RFC1, in addition to MRP2, in methotrexate intestinal absorption at two different pH conditions (pH 5.5 and 7.4). The protein levels of PCFT/HCP1 and MRP2 in brush-border membrane of rat proximal small intestine were significantly higher than those in the distal small intestine, in good agreement with previous reports (Figure 1).[22,36,40] Though the level of RFC1 protein was not determined in this study, RFC1 is reportedly expressed on the brush-border membrane of whole small intestine, as well as BCRP.[22,41] In accordance with the site-specific expression of PCFT/HCP1 (Figure 1), the mucosal-to-serosal transport rate and mucosal influx rate of methotrexate in proximal small intestine were significantly higher than those in distal small intestine (Figure 2). Eadie–Hofstee plots of methotrexate influx rates (Figure 3) suggested that there are two saturable components in the mucosal-to-serosal transport process, and a single saturable and a single non-saturable component in the mucosal uptake process of methotrexate. The estimated K_m values for high-affinity transport and for mucosal uptake of methotrexate in proximal intestine were 4.2–9.5 μM in this study (Figure 3), and this value is comparable to the K_m value (5.8 μM) reported for PCFT/HCP1-mediated methotrexate influx.[27] Folate, folinate and 5-methyltetrahydrofolate, substrates for PCFT/HCP1, significantly suppressed the mucosal methotrexate influx in the proximal small intestine at pH 5.5, but not in the distal small intestine. In contrast, thiamine pyrophosphate, an inhibitor of RFC1 alone, did not show any significant effect on methotrexate influx in either the proximal or the distal small intestine, irrespective of the expression of RFC1 along the whole small intestine.[22,41] The contribution of PCFT/HCP1, in addition to MRP2, to methotrexate absorption in the proximal small intestine was also observed in the in-situ perfusion study (Figure 5). These results suggest that PCFT/HCP1, but not RFC1, mediates methotrexate absorption in the proximal small intestine under acidic condition (<pH 7). Taken together, the intestinal absorption is mediated by PCFT in the proximal small intestine at pH 5.5 and the contribution of PCFT to total methotrexate absorption is approximately 50%, though the contribution of PCFT is dependent on methotrexate concentration. In contrast, at pH 7.4 including the distal intestine, the contribution of PCFT to methotrexate absorption is considered to be negligible. MRP2 lowers the

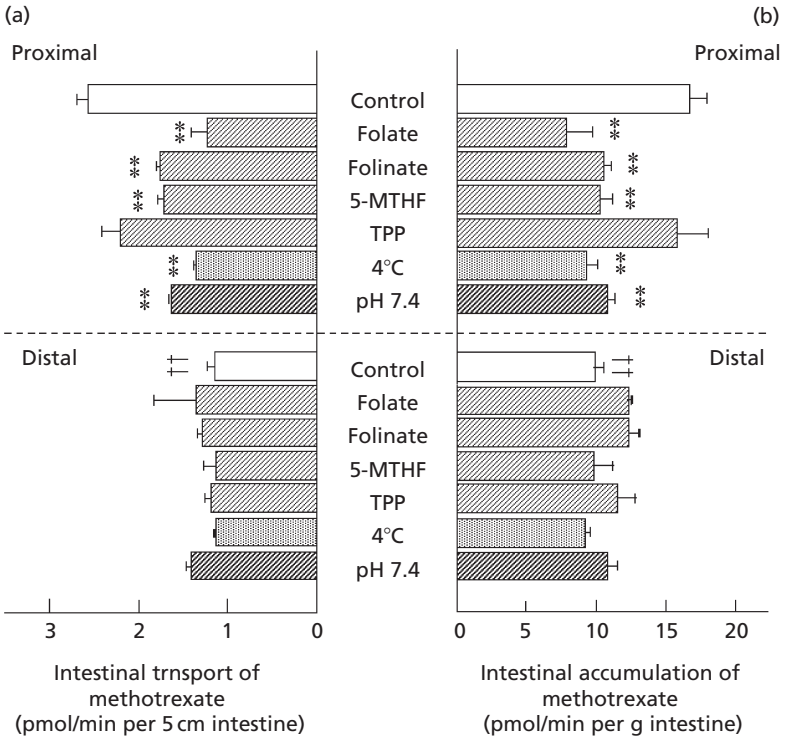

Figure 4 Effect of co-administration of folate-related compounds, medium temperature and medium pH on mucosal-to-serosal transport (a) and mucosal uptake (b) of methotrexate in everted proximal and distal small intestine of rats. Methotrexate was applied at a concentration of 1 μM to the mucosal side of the everted sac. The concentration of each folate-related compound was 200 μM. 5-MTHF, 5-methyltetrahydrofolate; TPP, thiamine pyrophosphate. For the pH 5.5 incubation, the incubation medium was kept at 37°C or 4°C and pH 5.5; for the pH 7.4, the incubation medium was kept at 37°C and pH 7.4. Each value represents the mean ± SD of results from three rats. $^{**}P < 0.01$, compared with the value for control (at 37°C and pH 5.5); $^{\dagger\dagger}P < 0.01$, compared with the value for control proximal intestine.

intestinal methotrexate absorption in the proximal small intestine to some extent but, usually, the inhibitory effect of MRP2 is not considered to be significant because the mean oral bioavailability of methotrexate at a low dose is relatively high (approximately 75%).[13]

In the mucosal-to-serosal transport process, the brush-border membrane transport and basolateral membrane transport are involved, and it is reported that a single carrier-mediated transport system is involved in the basolateral transport of folate in the intestine.[42,43] The K_m value

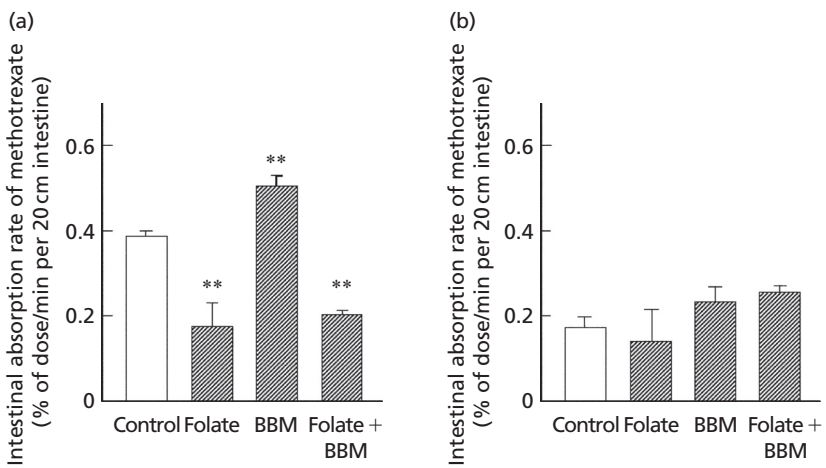

Figure 5 Effect of folate and benzbromarone (BBM) on intestinal absorption of methotrexate at pH 5.5 (a) and pH 7.4 (b) in in-situ perfusion study using a 20-cm-long proximal small intestine of rat. The amount of methotrexate absorbed was assumed to be of the same magnitude as the amount disappearing from the perfusate. The concentration of folate and BBM in the perfusate was 200 μM and 400 μM, respectively. Each value represents the mean ± SD of results from three rats. $^{**}P < 0.01$, compared with the value for control.

of 97 μM for the low-affinity component, estimated in this study (Figure 3), may represent some carrier-mediated basolateral transport system of methotrexate, as for folate transport.[42,43] Recently, Hamid *et al.*[44] also reported the presence of carrier-mediated folate transport across intestinal basolateral membrane, with pH optimum at 7.0, besides exhibiting Na^+ independence, and reported that the decreased basolateral transport activity in a rat model of alcoholism was associated with down-regulation of RFC, which resulted in decreased RFC protein levels in intestinal basolateral membrane. Further study will be necessary to clarify the carrier-mediated transport system involved in the basolateral transport of folate analogues including methotrexate.

It is reported that the transport of methotrexate is optimal at pH 5.5, decreases with increasing pH and becomes almost negligible at pH 7.0.[27,36] The gastrointestinal pH in normal human subjects was found to be pH 1.0–2.5 in the stomach, pH 6.6 ± 0.5 in the proximal small intestine and pH 7.4–7.5 in the mid and distal small intestine, as evaluated by using a pH-sensitive radiotelemetry capsule in 66 normal subjects.[45] Accordingly, the contribution of proton-coupled PCFT/HCP1-mediated transport in methotrexate absorption may be limited in the proximal small intestine, irrespective of the expression level of PCFT/HCP1 in the distal small intestine. So, the luminal pH is considered to be an important factor in determining the extent of oral bioavailability of various compounds mediated by PCFT/HCP1, as well as peptide transporter PEPT1.[46] Regarding PEPT1, it is reported that co-administration of a proton-releasing polymer that supplies the driving force enhanced proton-coupled PEPT1-mediated intestinal absorption of peptide compounds such as cefixime.

As described already, methotrexate is a substrate of multiple transporters, including RFC1, PCFT/HCP1, OATP1A2, OAT3, MRP2, MRP3 and BCRP.[23,24,26–28] All of these transporters are reportedly expressed in rodent and human intestine, and we established the contribution of PCFT/HCP1, MRP2, MRP3 and BCRP in methotrexate absorption in our previous and present studies.[21] These results suggest the site-specific contribution of multiple transporters in methotrexate absorption. Among various transporters, the contribution of PCFT/HCP1 to methotrexate absorption was mostly observed in proximal intestine, in good agreement with the site-specific expression levels of PCFT/HCP1 and physiological pH condition along the small intestine.

Conclusions

In this study, we examined the contribution of PCFT/HCP1, as well as RFC1 and MRP2, to figure out the site-specific and multiple transporters-mediated intestinal absorption of methotrexate in rats. PCFT/HCP1 was expressed in the proximal small intestine abundantly, and it mediated methotrexate influx under acidic conditions in the proximal small intestine, but not in the distal small intestine. Coadministration of benzbromarone increased the intestinal absorption of methotrexate, indicating the contribution of MRP2 to the intestinal absorption of methotrexate in the proximal small intestine. In contrast, RFC1 was not observed to contribute to methotrexate absorption throughout the whole small intestine. Like this, a marked site-specific and pH-dependent intestinal absorption of methotrexate was observed. The variation in luminal pH and the contribution of site-specific multiple transporters, including SLC and ABC transporters such as PCFT/HCP1, MRP2, MRP3 and BCRP, to methotrexate absorption may cause variability in methotrexate absorption among patients.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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