Folate malabsorption is associated with down-regulation of folate transporter expression and function at colon basolateral membrane in rats

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Abstract
Folates, an essential component (important B vitamin) in the human diet, are involved in many metabolic pathways, mainly in carbon transfer reactions such as purine and pyrimidine biosynthesis and amino acid interconversions. Deficiency of this micronutrient leads to the disruption of folate-dependent metabolic pathways that lead to the development of clinical abnormalities ranging from anaemia to growth retardation. Folate deficiency due to alcohol ingestion is quite common, primarily due to malabsorption. The present study dealt with the mechanistic insights of folate malabsorption in colonic basolateral membrane (BLM). Wistar rats (n 12) were fed 1 g/kg body weight per d ethanol (20%) solution orally for 3 months and folate transport was studied in the isolated colonic BLM. The folate exit across colon BLM shows characteristics of carrier-mediated process with the major involvement of reduced folate carrier (RFC). The chronic ethanol ingestion decreased the uptake by decreasing the affinity by 46 % (P<0·01) and the number of transport molecules by 43 % (P<0·001) at the colon BLM. The decreased uptake was associated with down-regulation of proton-coupled folate transporter (PCFT) and RFC expression at mRNA and protein levels. The extent of decrease was 44 % (P<0·01) and 24 % (P<0·05) for PCFT and 23 % (P<0·01) and 57 % (P<0·01) for RFC at mRNA and protein levels, respectively. Moreover, folate transporters were associated with lipid rafts (LR) of colon BLM, and chronic alcoholism decreased the association of these transporters with LR.

Key words: Rats; Proton-coupled folate transporter; Reduced folate carrier; Alcohol; Lipid rafts; Colon; Folate

Folate is an essential micronutrient, the primary function of which is as a carrier of single-carbon units for the biosynthesis of thymidylate, purines, methionine and glycine(1,2). Maintenance of intracellular folate homeostasis is vitally important; particularly for rapidly replicating cells such as colonic epithelial cells. Indeed, perturbed 1-carbon transfer reactions resulting from folate depletion and disrupted folate metabolism predispose normal colonic epithelial cells to neoplastic transformation through aberrant DNA synthesis, integrity, repair and methylation(3–5). A number of epidemiological and clinical studies linking decreased folate status with risk of colorectal cancer support this. Folate is absorbed in the proximal small intestine after the polyglutamate chain is hydrolysed by glutamate carboxypeptidase II. The hydrolytic step is followed by membrane transport of monoglutamyl folate into cells by folate transporters(6–8). Whereas monoglutamates are the only circulating forms of folate in blood and the only forms of folate that are transported across the cell membrane, once taken up into cells, cellular folate exists primarily as polyglutamates(9). Intracellular folate is converted to polyglutamates by folyopolyglutamate synthase(10). The polyglutamylation of cellular folates is a form of metabolic trapping, allowing the retention of folate that would otherwise be lost to efflux from cells(10). In the colon, folate – exogenous as well as synthesised by normal microflora – undergoes the initial step of transport across the brush border membrane (BBM) and then the exit of folate across basolateral membrane (BLM) into portal circulation. The mechanism of absorption of dietary folate in the BBM and BLM of colon has been investigated and the existence of pH-dependent, 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS)-sensitive, carrier-mediated uptake system has been demonstrated with the involvement of reduced folate carrier (RFC) in the exit of folate out of colonocytes(7,11). However, the role of proton-coupled folate transporter (PCFT) still remains silent.

Abbreviations: BBM, brush border membrane; BLM, basolateral membrane; BLMV, basolateral membrane vesicles; CAM, colon apical membrane; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LR, lipid rafts; MES, 3-(N-morpholino)ethanesulfonic acid; PCFT, proton-coupled folate transporter; rPCFT, rat proton-coupled folate transporter; rRFC, rat reduced folate carrier; RFC, reduced folate carrier.

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Deficiency of folate is highly prevalent throughout the world and alcohol ingestion has been the major contributor\textsuperscript{12,13}. Approximately 60–70% of binge drinkers are folate deficient\textsuperscript{14}. Regardless of the cause, folate deficiency leads to a variety of clinical abnormalities like megaloblastic anaemia, growth retardation etc., whereas optimisation of folate homeostasis prevents certain disorders like neural tube defects\textsuperscript{15}. Earlier, we have reported the derangement of PCFT and RFC across colon apical membrane (CAM) and of RFC on intestinal membrane surfaces during chronic alcohol ingestion in rats\textsuperscript{7,13,16–18}. However, no studies have been attempted so far to delineate the distribution of folate transporters to lipid microdomains and activity of folate transport system in BLM under conditions of folate malabsorption. For this, the present work was sought to characterise the folate transport across the colon BLM and the role of the folate transporters therein during alcoholism. We used rats as an experimental model of chronic alcoholism based on the published studies demonstrating their resemblance and utility as a model in order to relate to humans on the basis of blood and intestinal alcohol concentration as well as plasma folate levels\textsuperscript{18–20}. Such mechanistic insights could lead to strategies for deducing folate transport regulation in diverse cellular microenvironments and will be important for designing therapeutic targets involving derangements in folate transport systems in primary absorptive epithelia.

Materials and methods

Chemicals

Radiolabelled 5-\textsuperscript{14}C-methyltetrahydrofolate, potassium salt with specific activity 2400 Ci/mmol (88.8 x 10\textsuperscript{10} Bq/mmol) were purchased from Amersham Pharmacia Biotech (Hong Kong). Color burst\textsuperscript{TM} electrophoresis marker (molecular weight 8000–220 000) was purchased from Sigma Chemical Company (St Louis, MO, USA). Total RNA Extraction Kit was purchased from Taurus Scientific (Cincinnati, OH, USA). Moloney Murine Leukemia Virus RT (RevertAid\textsuperscript{TM} from Ambion, Inc. (Austin, TX, USA). Primary antibodies rabbit anti-rat reduced folate carrier (rfC) and anti-rat proton-coupled folate transporter (rpCFT) polyclonal antibodies were raised in rabbits in our laboratory\textsuperscript{21}. Horseradish peroxidase labelled goat anti-rabbit-IgG secondary antibodies were purchased from G Biosciences (St Louis, MO, USA). Enhanced Chemiluminescence Detection Kit was purchased from BioRad Laboratories (Hercules, CA, USA). Enhanced percoll gradient method\textsuperscript{22} was employed to prepare the basolateral membrane vesicles. The mucosa was scraped from the proximal colon. The scrapings were homogenised in ice-cold buffer containing 250 mM-mannitol and 12 mM-HEPES-Tris, pH 7.4 and centrifuged at 2500 g for 20 min. The supernatant was then centrifuged at 22 000 g for 25 min and the resultinguffy layer of the pellet resuspended in the same buffer followed by homogenisation in glass Teflon homogeniser. The resulting homogenate was mixed with percoll at a concentration of 15.4% and centrifuged at 48 000 g for 20 min. A distinct band of BLMV was seen at the upper one-third of the percoll gradient. The band was aspirated by a syringe and suspended in buffer consisting of 100 mM-mannitol, 120 mM-HEPES-Tris, pH 7.4 and centrifuged at 48 000 g for 30 min. The pellet obtained was resuspended in loading buffer containing 280 mM-mannitol and 20 mM-HEPES-Tris, pH 7.4 and centrifuged at 48 000 g for 20 min twice in order to wash out the residual percoll from membrane preparation. The final pellet representing purified BLMV was suspended in loading buffer (280 mM-mannitol, 20 mM-HEPES-Tris, pH 7.4) at 5 mg/ml protein concentration. Purity of the membrane preparations was checked by measuring the specific activities of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase in BLMV and in original homogenate.

Transport of 5-\textsuperscript{14}C-methyltetrahydrofolate

Uptake studies were performed at 37°C using the incubation buffer (100 mM-NaCl, 80 mM-mannitol, 10 mM-HEPES, 10 mM-3-(Nmorpholino)ethanesulfonic acid (MES), pH 7.0). A quantity of 10 μl of vesicles (50 μg protein) was added to the incubation buffer containing 5-\textsuperscript{14}C-methyltetrahydrofolate at a concentration as specified. The initial rate of transport was determined by stopping the reaction after 20s by adding ice-cold stop solution containing 280 mM-mannitol, 20 mM-HEPES-Tris, pH 7.4 at 5 mg/ml protein concentration. Purity of the membrane preparations was checked by measuring the specific activities of Na\textsuperscript{+}, K\textsuperscript{+}-ATPase in BLMV and in original homogenate.

RT-PCR analysis

Total RNA was isolated from the colon by using total RNA extraction kit and complementary DNA synthesis was carried out using random primers. The cDNA was stored at −20°C until use. Primers for folate transporters were selected from the published literature and are given in Table 1. A detailed procedure of RT-PCR was reported earlier\textsuperscript{23}.

Preparation of colon basolateral membrane vesicles

Basolateral membrane vesicles (BLMV) from colon was prepared by the self-generating percoll gradient method\textsuperscript{24} as described earlier\textsuperscript{22}.

Animals

Young adult male albino rats (Wistar strain) weighing 100–150 g (2–3 months old) were obtained from the Institute’s Central Animal House. The animals were housed in clean wire mesh cages with controlled temperature (23 ± 1°C) and humidity (45–55%) and had a 12 h dark–12 h light cycle throughout the study. The rats were randomised into two groups of twelve animals each. The rats in group I were given 1 g ethanol (20% solution)/kg body weight per d and those in group II received isonergic amount of sucrose (36% solution) orally by Ryle’s tube daily for 3 months. The rats were fed \textit{ad libitum} commercially available pellet diet (Ashirwad Industries, Ropar, India) and water. Animals from both the groups were killed under anaesthesia using sodium pentothal.

The protocol of the study was approved by ‘Institutional Animal Ethical Committee’ and ‘Institutional Biosafety Committee’.

Folate malabsorption in rat colon 801
out from the purified and intact total RNA according to the manufacturer's instructions. Expression of RFC, PCFT and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was evaluated using sequence-specific primers corresponding to the sequence in the open reading frame. PCR mixture (20 μl) was prepared in 1 × PCR buffer consisting of 0·6 U of Taq polymerase, 2 μM of each primer for rGAPDH, rPCFT and rRFC along with 200 μM of each deoxyribonucleotide triphosphate. In optimized PCR, the initial denaturation step was carried out for 2 min at 95°C. The denaturation, annealing and elongation steps were carried out, respectively, for 1 min at 94°C, 45 s at 64°C (PCFT) or 56°C (GAPDH) and 1 min at 72°C for thirty-five cycles. In the case of RFC denaturation, annealing and elongation steps were carried out, respectively, for 30 s at 94°C, 30 s at 52·1°C, 30 s at 72°C for thirty-five cycles. The final extension step was carried out for 10 min at 72°C. The primers were designed using Primer3 Input (version 8.0.4; http://primer3.sourceforge.net). The sequences of the primers used were as follows: 5′-CATGCTAAGGCACTGGTGA-3′ (sense) and 5′-TTTCCACAGGACATGGACA-3′ (antisense) for RFC, AAGCCAGTTATGGGCACAG (sense) and GGATAGGCTGTGGTCAAGGA (antisense) for PCFT. The expected PCR products of size 120, 300 and 400 bp were obtained for rRFC, rPCFT and rGAPDH, respectively when electrophoresed on 1·2% agarose gel. The densitometric analyses of products were determined by using 'Scion image' software (Scion Image, Frederick, MD, USA).

Western blot analysis

For protein expression studies, colon BLMV (100 μg) were resolved on 10% SDS-PAGE and transferred to polyvinylidene fluoride membrane for 20 min at 15 V. Western blotting was performed using the procedure described by Towbin et al. (24) using polyclonal primary antibodies as rabbit anti-rat RFC (1:800 dilutions) raised against the specific region of rat RFC synthetic peptide corresponding to amino acids 494–512 (25). The polyclonal antibodies against PCFT (1:1000 dilutions) were raised against the specific region of rat PCFT synthetic peptide corresponding to amino acids 442–459. Secondary antibodies used were goat anti-rabbit IgG-horseradish peroxidase (1:200). Presence of antibody at specific site(s) was revealed using freshly prepared 3,3′-diaminobenzidine (anti-peroxidase) and H2O2 at room temperature for 3–5 min and counter-staining with haematoxylin.

Immunohistochemical analysis

Freshly removed colon was cut followed by fixing in sufficient amount of 10% formalin (26). Paraffin sections of 4 μm thickness on poly-l-lysine-coated slides were baked overnight at 57°C. Endogenous peroxidase was quenched by pretreatment with 1% H2O2 in methanol for 20 min followed by washings in PBS. Slides were put in primary diluted antibody (rabbit polyclonal anti-rat RFC and PCFT (1:200) for 2 h at 37°C followed by secondary antibodies as goat anti-rabbit IgG-horseradish peroxidase (1:200)). Presence of antibody at specific site(s) was revealed using freshly prepared 3,3′-diaminobenzidine (29) and H2O2 at room temperature for 3–5 min and counter-staining with haematoxylin.

Estimation of folate by microbiological assay

The folate estimations were determined by microtitre plate assay using L. casei as described earlier (26). For intracellular folate concentrations in colon, a 10% homogenate of colon was made in phosphate buffer of pH 6·3 containing 5 mg/ml ascorbate. The homogenate was incubated at 110°C for 10 min followed by centrifugation at 300 rpm for 10 min. Then, 0·1 ml of the supernatant was treated with 0·02 ml of rat plasma conjugase in 0·375 ml of phosphate buffer of pH 4·5. The free folate was then determined by a standard microbiological microtitre plate assay using L. casei. All the steps were carried out in aseptic conditions.

Statistical analysis

The data were computed as means and standard deviations. Group means were compared by using the Student’s t test and ANOVA was used wherever necessary. The acceptable level of significance was less than 5% for each analysis. The power of the study was 0·80.

Results

Folate levels in colon

As the present study dealt with folate malabsorption during alcoholism, the determination of folate levels was of prime importance. The results showed that a significant reduction (P < 0·01) in colonic tissue folate levels (the levels were 8·8 (sd 0·09) and 4·2 (sd 0·05) nmol/g tissue for control and ethanol fed, respectively) in chronic alcoholism, confirming the association of alcoholism with folate deficiency.
Folate malabsorption in rat colon

Expression of the proton-coupled folate transporter and reduced folate carrier in colon; association with lipid rafts

In order to elucidate the mechanism of reduced folate transport in chronic alcoholism, transcriptional and translation regulations of the RFC and PCFT were studied. The relative mRNA for the PCFT was approximately 1.8 fold (44%) lower (P<0.01) while that for the RFC was approximately 1.3 fold (23%) lower (P<0.01) in the ethanol-fed group (Fig. 3(a) and (b)).

Further, to investigate the effect of chronic alcohol feeding on the level of expression of the PCFT and the RFC protein, we performed Western blotting on the colon BLM protein of both the groups of rats (Fig. 3(c) and (d)). The results showed a significant decrease of 1.3 fold (24%) in the level of expression of the PCFT (P<0.05) and 2.3 fold (57%) in the levels of RFC (P<0.01) proteins in ethanol-fed rats (Fig. 3).

LR are the specialised microdomains of the plasma membrane that are essential for the normal functioning of various membrane transporters. We sought to determine whether the folate transporters (PCFT and RFC) are associated with lipid microdomains of the colon basolateral membrane of rat. We had validated this technique earlier by measuring the specific activity of alkaline phosphatase (well-known marker for LR) in all the fractions collected from gradient

**Fig. 1.** (a) Time course of folate uptake in the colon basolateral membrane vesicles (BLMV). Uptake of 5-[14C]-methyl-tetrahydrofuran (THF; 0.5 μM) was measured in a buffer of pH 7.0 (100 mM-NaCl, 80 mM mannitol, 10 mM-HEPES, 10 mM-2-(N-morphinolino)ethanesulfonic acid (MES), pH 7.0) for 10–240 s. (b) Uptake of 5-[14C]-methyl-THF in the colon BLMV as a function of pH optimum. Uptake was measured by varying the pH of incubation buffer (100 mM-NaCl, 80 mM-mannitol, 10 mM-HEPES, 10 mM-MES) from 5.0 to 8.0, keeping intravesicular pH 7.4 at 0.5 μM substrate concentration for 20 s. Values are means and standard deviations of four separate uptake determinations. Mean values were significantly different from those of control (---). *P<0.05, **P<0.01, ***P<0.001. ——, Ethanol fed.
using CAM\textsuperscript{(7)}. The pattern of specific activity of alkaline phosphatase in all these fractions had revealed a gradient with considerable activity in the top floating fractions (1–4), indicating that these fractions contained LR. So the top fractions isolated from the gradient using colon BLM were subjected to Western blotting for the PCFT and the RFC (Fig. 3(e) and (h)). We found the presence of the PCFT and RFC protein in the floating fractions on the top five fractions (20–30%) with negligible or no expression thereafter of Optiprep density gradient. Together, these data provide strong evidence that the majority of the PCFT and the RFC pool are associated with the lipid raft microdomains. Moreover, chronic alcoholism leads to a decreased association of both the PCFT and the RFC to the LR (Fig. 3(e) and (h)).

The extent of decrease was 1·23- to 1·41-fold for PCFT ($P<0.01$ and $<0.05$) and 1·12- to 1·21-fold for RFC ($P<0.01$, $<0.05$), respectively, which is in accordance with the decreased levels of these transporters in the colon BLM.

**Localisation of proton-coupled folate transporter and reduced folate carrier in colon**

As there was a significant decrease in RFC and PCFT expression across colon BLM, we studied the localisation of these transporters in colon by immunohistochemistry (Fig. 4). The localisation of the RFC and PCFT was seen at both the apical and basolateral side membranes of colon. In ethanol-fed rats, there was marked reduction in the intensity of RFC and PCFT positive cells in colon (Fig. 4).

**Discussion**

The observed reduced folate levels in the colon of ethanol-fed rats suggested the association of chronic alcoholism with colonic folate malabsorption. After the transport of folate across the CAM, the folate is transported across colon BLM to portal circulation. The higher values of $K_m$ (5·0 (SD 0·56) µM in comparison to 2·8 (SD 0·81) µM of the control group

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**Fig. 2.** (a) Uptake of 5-[\textsuperscript{14}C]-methyl-tetrahydrofuran (THF) in the colon basolateral membrane vesicles (BLMV) as a function of substrate concentration. Uptake was measured by varying 5-[\textsuperscript{14}C]-methyl-THF concentration from 0·125 to 8·0 µM in the incubation buffer (100 mM-NaCl, 80 mM-mannitol, 10 mM-HEPES, 10 mM-2-(N-morpholino)ethanesulfonic acid, pH 7·0) after incubating colon BLMV for 20 s. Control; \textbullet, ethanol fed. Mean values were significantly different from those of control: $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. (b) Lineweaver–Burk plot at pH 7·0. Control; \textbullet, ethanol fed. (c) Effect of structural analogue and inhibitors on the uptake of 5-[\textsuperscript{14}C]-methyl-THF in the colon BLMV. Uptake of 5-[\textsuperscript{14}C]-methyl-THF (0·5 µM) was measured with and without analogue (5 µM-folic acid and 5 µM-methotrexate)/inhibitor (5 mM-thymine pyrophosphate (TPP) and 25 µM-hemin) in incubation buffer of pH 7·0. Control; \textbullet, ethanol fed. (d) Uptake of 5-[\textsuperscript{14}C]-methyl-THF was determined in the presence of S–S group reacting reagent (1 mM-dithiothreitol (DTT)) and in the presence of 1 mM-ATP. None. Values are means and standard deviations represented of four separate uptake determinations, carried out in duplicate. Mean values were significantly different from those of none in control: $^*P<0.05$, $^{**}P<0.01$. Mean values significantly different from those of none in ethanol fed: $^{†}P<0.05$, $^{††}P<0.01$. 

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Fig. 3. (a) RT-PCR analysis of reduced folate carrier (RFC) and proton-coupled folate transporter (PCFT) with glyceraldehyde 3-phosphate dehydrogenase as an internal control in colon. Resolved on 1·2 % agarose gel electrophoresis, lanes 1 and 2: control; 3 and 4: ethanol fed. (b) Densitometric analysis representing relative change in PCFT and RFC mRNA expression. Values are means and standard deviations of five separate set of experiments. Mean values were significantly different from those of control: **P<0·01. (c) Western blot analysis of colon basolateral membrane (BLM) using anti RFC (58 kDa), anti-PCFT (54 kDa) antibodies, lane 1–3: control; 4–6: ethanol fed. (d) Graph represents summary data of densitometric analysis. (e and g) Association of folate transporters (PCFT and RFC) proteins with lipid rafts in colon BLM. The colon BLM were subjected to floatation on Optiprep density gradients, and fractions were collected from top of the gradients (fractions 1–4 represent detergent-resistant membrane). Fractions were separated by electrophoresis and analysed by Western blotting using (e) anti-PCFT (54 kDa) and (g) RFC (58 kDa) antibodies. The representative blot shown for PCFT and RFC expression as, upper panel lane 1–5: control; lower panel lane 1–5: ethanol fed. (f and h) Blots were scanned, and the intensity of bands was determined by densitometric analysis. Values are means and standard deviations of four separate experiments. Mean values were significantly different from those of control: *P<0·05 **P<0·01.
Transport of folate across colon basolateral membrane occurs at pH, supporting the earlier observations, which suggests that uptake was observed at pH 7.4 inside and 7.0–7.4 outside is responsible for the folate uptake, as maximum folate neither the inwardly nor the outwardly directed H⁺ be the energising force in the BLM. The results depicted that uphill folate transport, the neutral/alkaline pH was found to be the energising force in the BLM and CAM.

In order to determine the chemical driving force for the uphill folate transport, the neutral/alkaline pH was found to be the energising force in the BLM. The results depicted that neither the inwardly nor the outwardly directed H⁺ gradient is responsible for the folate uptake, as maximum folate uptake was observed at pH 7.4 inside and 7.0–7.4 outside pH, supporting the earlier observations, which suggests that folate transport across colon basolateral membrane occurs at neutral pH(11) and displayed similar characteristics as of intestinal BLM(22). Moreover, for the evaluation of the specificity of the folate transport system, structural analogues significantly inhibited the folate uptake. Less decrease in the folate transport in the presence of structural analogues methotrexate and unlabelled folic acid might be due to less affinity of the folate transport system, structural analogues significantly inhibited the folate uptake. 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Less decrease in the folate transport in the presence of structural analogues methotrexate and unlabelled folic acid might be due to less affinity of the folate transport system, structural analogues significantly inhibited the folate uptake. Less decrease in the folate transport in the presence of structural analogues methotrexate and unlabelled folic acid might be due to less affinity of the folate transport system, structural analogues significantly inhibited the folate uptake. less staining in the case of ethanol-fed rats suggests the decreased expression of PCFT and RFC.

The decreased $V_{\text{max}}$ of folate uptake process observed in ethanol-fed rats could relate to the reduced number of PCFT and RFC molecules on the colon BLM. In this context, the observed down-regulation (Fig. 3(a)–(d)) in the PCFT and the RFC mRNA corresponds to the reduced protein expression at colon BLM in ethanol-fed rats. This decrease in folate uptake in association with the down-regulation of the folate transporters in the rat colon is similar to the results of our earlier studies carried out in the small intestine(10,18) and kidneys(17,18,37,38) and might contribute to ethanol-induced folate deficiency. Moreover, the decreased folate content of colon tissue in ethanol-fed rats reflects reduced substrate availability for the transporters at the BLM under in vivo conditions and might also contribute to the colonic malabsorption in chronic alcoholism. Although we have observed a reduced folate transport across the colonic BLM in ethanol-fed rats, a substantial amount of uptake still takes place during chronic alcoholism. This might be due to the associated passive diffusion component of the transport process along with the carrier-mediated transport. An earlier study(39) carried out in L1210 leukaemia cells has shown that 20% of the total methotrexate uptake was contributed by passive diffusion; whether ethanol feeding affects this passive diffusion of 5-methyltetrahydrofolate into our experimental set is not known and is yet to be determined.

In the plane of basolateral membranes, LR could act as platforms modulating the protein activity by accumulating active pools of the transporters(51). Proteins might enter LR at the golgi level and their shuttling between the golgi and cell membranes allows the cells to exert regulatory control over the surface expression of their proteins. Utilising the Optiprep flotation, we found that folate transporter proteins – PCFT and RFC – were present in the floating fractions corresponding to lipid raft microdomains of colon basolateral membranes. Our findings demonstrated the presence of PCFT and RFC in LR of the colon membranes of rats, which might be due to alteration in the lipid composition of biological membranes by alcohol(40).

The immunohistochemical localisation of colon shows the localisation of both the folate transporters to the BBM as well as in the basolateral membrane surfaces, confirming the immunoblot experiment which demonstrated the expression of PCFT and RFC on both the BBM and BLM of colon. Moreover, less staining in the case of ethanol-fed rats suggests the decreased expression of PCFT and RFC.
Conclusions
In conclusion, these findings demonstrate that the decreased colon folate transport in chronic alcoholism may be attributed to decreased affinity and the number of folate transporter molecules across the colon BLM surfaces. Folate transport is carrier-mediated saturable, energy-independent, pH-dependent with the major involvement of RFC in folate exit across colon basolateral membrane with minimal involvement from PCFT. The decrease in uptake was associated with down-regulation of folate transporters and lipid raft associated reduction in levels of these transporters in colon BLM. The consequence of reduced folate transport across CAM results in reduced folate levels in colon.

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