Effect of folate-binding protein on intestinal transport of folic acid and 5-methyltetrahydrofolate across Caco-2 cells

Summary

Background Milk products are a potential matrix for fortification with synthetic folic acid or natural 5-methyltetrahydrofolate (5-CH₃-H₄folate) to enhance the daily folate intake. In milk, folate occurs bound to folate-binding proteins (FBP). Our previous studies with an in vitro gastrointestinal model showed that 70% of the initial FBP content of the milk product was retained in the duodenal lumen. While folic acid remained bound to FBP after gastric passage, 5-CH₃-H₄folate was mainly present as free folate in the duodenal lumen. Aim of the study To investigate the effect of FBP on the absorption of folic acid and 5-CH₃-H₄folate from the intestinal lumen. Methods The transport of [³H]-folic acid and [¹⁴C]-5-CH₃-H₄folate across enterocytes was studied in the presence or absence of bovine FBP using monolayers of Caco-2 cells grown on semi-permeable inserts in a two-compartment model. The apparent permeability coefficients (Papp) of folic acid and 5-CH₃-H₄folate were determined and compared with the permeability of reference compounds for low (mannitol) and high (caffeine) permeability. Results The transport from the apical to the basolateral side of the Caco-2 cells was higher (P < 0.05) for folic acid (Papp = 1.7*10⁻⁶ cm/s) than for 5-CH₃-H₄folate (Papp = 1.4*10⁻⁶ cm/s) after 2 h incubation to 1 µM folic acid or 5-CH₃-H₄folate test solutions (pH 7). The permeability of folic acid and 5-CH₃-H₄folate across Caco-2 monolayers appeared to be higher (P < 0.05) than that of mannitol (Papp = 0.5*10⁻⁶ cm/s) but lower (P < 0.05) than that of caffeine (Papp = 34*10⁻⁶ cm/s). The addition of FBP to the medium led to a lower (P < 0.05) intestinal transport and cellular accumulation of folic acid and 5-CH₃-H₄folate. Conclusions Compared to the reference compounds, folic acid and 5-CH₃-H₄folate showed a moderate permeability across Caco-2 cells, which indicates that folate absorption from the intestinal lumen is not likely to be complete. The intestinal transport of folic acid and 5-CH₃-H₄folate was found to be dependent on the extent of binding to FBP at the luminal side of the cells.

Key words folate – intestinal transport – folate-binding protein – Caco-2 – bioavailability

Introduction

An adequate folate intake is preventive against megaloblastic anemia [1] and reduces the risk for neural tube defects [2, 3], colon cancer [4] and cardiovascular diseases [5, 6]. In many countries mean folate intake was found to be lower than recommended or desired [7, 8] and supplementation or food fortification could be used to complement the folate intake from the natural diet. The main folate compound in non-fortified food products is 5-methyltetrahydrofolate (5-CH₃-H₄folate), while in supplements and fortified products mostly folic acid is used. Milk can be considered as a potential matrix for folate fortification because it is widely consumed and might enhance the folate bioavailability from the diet [9]. In unprocessed milk, folate is essentially bound to folate-binding proteins (FBP) [10, 11]. At saturation, FBP...
bind approximately 1 mol folate/mol protein at pH 7.2 [12] with a somewhat higher affinity for folic acid than for 5-CH₃-H₄folate [13]. The physiological role of FBP is unclear.

In recent studies, the bioaccessibility of folate from folic acid- or 5-CH₃-H₄folate-fortified milk products was investigated using a dynamic in vitro gastrointestinal model [14, 15]. Approximately 60–80% of the supplemental folate was released from the milk matrix during gastrointestinal passage and, therefore, available for absorption (bioaccessible). Addition of FBP reduced the bioaccessible fraction of folic acid to a higher extent than that of 5-CH₃-H₄folate from the fortified milk products [14, 15]. In additional studies with the gastrointestinal model it was found that approximately 80% of folic acid and 5-CH₃-H₄folate occurred bound to FBP in fortified whey suspensions with equimolar amounts of folate and FBP [16]. FBP appeared to be highly stable during gastric passage in the gastrointestinal model as 70% of the initial FBP content could be retrieved in the duodenal lumen [16]. While folic acid remained bound to FBP, the FBP-bound fraction of 5-CH₃-H₄folate gradually decreased from 79% to 5% during gastric passage. These studies show that the FBP binding characteristics are different for folic acid and 5-CH₃-H₄folate after gastric passage which could affect the absorption of both folate compounds from the intestinal lumen.

The intestinal absorption of folate has been characterized based upon in vitro and in vivo studies (mainly rat). The transport of folate across the intestinal cell membrane within physiological concentrations (<10 µM) was found, at least partly, to occur by a pH-dependent, active, carrier-mediated system [17–20]. However, contradictory results have been reported about the influence of FBP on folate uptake [21, 22] and transport [23, 24] both in vivo in rats [24] as well as in in vitro studies using isolated rat mucosal cells [21], goat brush-border membrane vesicles [22] and everted sacs of rat intestine [23].

The present study was performed to investigate the effect of luminal FBP binding to folic acid and 5-CH₃-H₄folate on the transport of both folate compounds across human epithelial cells. For this purpose, monolayers of polarized human Caco-2 cells grown on semipermeable inserts were used. Caco-2 cells cultured in a two-compartment system are widely used as an in vitro model for human intestinal absorption as they display after differentiation both biochemical and morphological characteristics of small intestinal enterocytes [25–27]. Also the permeability characteristics of compounds across Caco-2 monolayers were found to correlate well with human oral absorption in vivo [28–32]. In the present study the permeability of folic acid and 5-CH₃-H₄folate across Caco-2 cells was compared with the permeability of reference compounds.

### Materials and methods

#### Chemicals

Radiolabelled folate compounds, [³H]-folic acid (888 GBq/mmol; 37 MBq/ml) and [¹⁴C]-(RS)-5-CH₃-H₄folate (2.11 GBq/mmol; 3.7 MBq/ml), were obtained from Amersham Pharmacia (Buckinghamshire, UK). Folic acid was studied as a mixture of radiolabelled and non-radiolabelled compounds. The non-radiolabelled standard solution of folic acid (Schirck’s Laboratories, Jona, Switzerland) was controlled on purity according to Van den Berg et al. [33]. The reference compounds, [³H]-mannitol and [¹⁴C]-caffeine, were obtained from ICN Biomedicals (Irvine, CA, USA) and Perkin-Elmer life sciences (Boston, MA, USA), respectively. Sephadex G75 Superfine powder, scintillation liquid (High Ionic Fluor and Ultima Gold) and the low molecular weight gel filtration calibration kit [17-0442-01] were obtained from Amersham Pharmacia. The FBP-rich whey fraction (821 nmol FBP/g) was kindly provided from DMV International (Veghel, The Netherlands). The elution solution used for gel filtration was 0.1 mol/L phosphate buffer with 0.15 mol/L NaCl (pH 7.2) containing 13.4 g/L Na₂HPO₄, 3.5 g/L NaH₂PO₄, 8.3 g/L NaCl, 0.02 g/L Na-azide (all from Sigma, St.Louis, MO, USA).

#### Cell culture

The human colon carcinoma cell line, Caco-2, was obtained from the American Type Culture Collection (Rockville, MD, USA). Cells grown in 75 cm² flasks (Corning-Costar, Cambridge, MA, USA) were passaged weekly at a split ratio of 1:10 using 0.05 % trypsin in PBS with 0.022 % EDTA. Caco-2 cells were used at passages 35-42. The Caco-2 cells were maintained at 37 °C in an atmosphere of 5 % CO₂ in culture medium, Hepes-buffered Dulbecco’s Modified Eagle Medium (DMEM) containing 4.5 g/L glucose, supplemented with 0.15 mol/L NaCl (pH 7.2) containing 13.4 g/L Na₂HPO₄, 3.5 g/L NaH₂PO₄, 8.3 g/L NaCl, 0.02 g/L Na-azide (all from Sigma, St.Louis, MO, USA).