Folate and genomic stability: differential effect of methylated and oxidised folate on DNA damage and ROS production in human colon fibroblasts

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Folates are water-soluble B vitamins, which maintain DNA stability by regulating DNA biosynthesis, repair and methylation1. Low dietary folate status is associated with an increased risk of colon cancer2. Conversely, recent mandatory food fortification with oxidised folic acid has been linked with an increase in colon cancer incidence3. Moreover, high dose folic acid supplementation has been associated with an increase in premalignant adenomas or colon malignancies in some human intervention studies4. This study evaluates the ability of dietary folate and folic acid to maintain normal cellular characteristics, growth and viability, DNA stability, and endogenous reactive oxygen species (ROS) production in an in vitro human colon fibroblast cell model.

Primary human colon fibroblasts (CCD-18Co) were cultured for up to 14 days, in either 5-methyl-tetrahydrofolate (CH3THF) or folic acid at 3 plasma physiological concentrations corresponding to (1) post-supplementation (100 ng/mL), (2) nutritional sufficiency (10 ng/mL) or (3) moderate deficiency (2.5 ng/mL). CCD-18Co cells were seeded in 12-well plates at 1.6 × 10^4 cells per well and the effect of folate form and status measured on cell proliferation (cell growth using a Haemocytometer), cell viability and size (Cellometer® Cell Counter), and endogenous DNA strand breakage [Single Cell Gel Electrophoresis (SCGE)]. For ROS production, cells were seed in 25 cm^2 flasks at 1×10^5 cells per flask and analysed by Flow Cytometry.

CCD-18Co colon fibroblasts exposed to CH3THF showed significantly higher growth than those cultured in folic acid at 10 and 2.5 ng/mL (p < 0.001). While rate of cell growth was similar in all CH3THF concentrations (13.5-fold, 18.4-fold and 14.2-fold at 100, 10 and 2.5 ng/mL, respectively), proliferation at lower folic acid concentrations (10 and 2.5 ng/mL) was negligible (approx. 1.6-fold). Cell viability ranged from 69.2%−84.0% for CH3THF and 35.7–46.8% for folic acid. CH3THF supported cell viability better than folic acid at all concentrations (p < 0.02). A significant increase in cell diameter was observed in cells grown in folic acid compared with those grown in CH3THF [21.9 µm (SEM = 1.2 µm) versus 17.8 µm (SEM = 1.3 µm)] at 2.5 ng/mL (p < 0.03). Endogenous DNA strand breakage increased with decreasing folate status (p < 0.03), with fibroblasts grown in folic acid showing approx. 0.8-fold higher DNA breakage than cells cultured in CH3THF. ROS production was elevated in cells cultured in folic acid compared with those grown in CH3THF (up to 3.8-fold) (p < 0.02).

These results show that, compared with colon fibroblast cells exposed to CH3THF, folic acid at physiological concentrations, alters several biomarkers associated with colon carcinogenesis including inducing abnormal proliferation, DNA instability and increased ROS production.

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