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Mutation Research 578 (2005) 317–326
Fundamental and Molecular Mechanisms of Mutagenesis
www.elsevier.com/locate/molmut
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Folic acid deficiency increases chromosomal instability, chromosome 21 aneuploidy and sensitivity to radiation-induced micronuclei

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Received 14 February 2005; received in revised form 27 May 2005; accepted 31 May 2005
Available online 11 July 2005

Abstract

Folic acid deficiency can lead to uracil incorporation into DNA, hypomethylation of DNA, inefficient DNA repair and increase chromosome malsegregation and breakage. Because ionising radiation increases demand for efficient DNA repair and also causes chromosome breaks we hypothesised that folic acid deficiency may increase sensitivity to radiation-induced chromosome breakage. We tested this hypothesis by using the cytokinesis-block micronucleus assay in 10 day WIL2-NS cell cultures at four different folic acid concentrations (0.2, 2, 20, and 200 nM) that span the "normal" physiological range in humans. The study showed a significant dose-dependent increase in frequency of binucleated cells with micronuclei and/or nucleoplasmic bridges with decreasing folic acid concentration ($P < 0.0001$, $P = 0.028$, respectively). These biomarkers of chromosomal instability were also increased in cells irradiated (1.5 Gy γ-rays) on day 9 relative to un-irradiated controls ($P < 0.05$). Folic acid deficiency and γ-irradiation were shown to have a significant interactive effect on frequency of cells containing micronuclei (two-way ANOVA, interaction $P = 0.0039$) such that the frequency of radiation-induced micronucleated cells (i.e. after subtracting baseline frequency of un-irradiated controls) increased with decreasing folic acid concentration (P-trend < 0.0001). Aneuploidy of chromosome 21, apoptosis and necrosis were increased by folic acid deficiency but not by ionising radiation. The results of this study show that folate status has an important impact on chromosomal stability and is an important modifying factor of cellular sensitivity to radiation-induced genome damage.

1. Introduction

Folate is an essential B-vitamin that occurs naturally in a wide variety of foods, such as broccoli, cabbage, cauliflower, fruit and nuts. Its synthetic oxidised form, folic acid is used in fortified foods and vitamin supplements because it is more stable than the natural reduced glutamated form. Mammals are unable to synthesize

Abbreviations: BNC, binucleated cell; BNCs, binucleated cells; MN, micronucleus; MNi, micronuclei; NPB, nucleoplasmic bridge; NDI, nuclear division index; MNed BNC, binucleated cell with one or more micronuclei; BNC with NPB, binucleated cell with one or more nucleoplasmic bridge

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0027-5107/$ – see front matter.
doi:10.1016/j.mrfmmm.2005.05.012

folate de novo and are, therefore, bound to obtain it from dietary sources. Inadequate folate intake is associated with increased risk of neural tube defects, Down's syndrome, cardiovascular disease, Alzheimer disease and various cancers [1–5].

Folate is required for the synthesis of dTMP from dUMP, is an essential factor for the production of methionine and ultimately *S*-adenosylmethionine (SAM), the primary methyl donor for DNA methylation which determines gene expression and prevents chromosomal fragility in specific regions, such as the centromere and fragile sites [6,7]. Folate deficiency reduces SAM and dTMP synthesis, causing CpG hypomethylation and excessive uracil incorporation in DNA, respectively. Excessive uracil incorporation may generate point mutations and can lead to chromosome breakage and associated micronucleus formation [5,8–11]. Additionally, reduced dTMP synthesis has been associated with folate-sensitive fragile sites expression [6], which is postulated to have a function in the development of malignancies [12]. Hypomethylation of centromeric DNA results in chromosome loss, as well as breakage of specific chromosomes, such as chromosomes 1, 9 and 16 [13]. More recently it has been shown that folic acid or 5-methyltetrahdrofolate deficiency can increase the rate of aneuploidy of chromosomes 17 and 21 in cultured human lymphocytes [14].

Significant doses of ionising radiation (5–20 cGy) and folate deficiency induce chromosomal instability of similar type and extent [15–17], i.e. increased malsegregation of whole chromosomes or segments of whole chromosomes and modified gene dosage due to the formation of asymmetric dicentric chromosomal rearrangements, nucleoplasmic bridges and the generation of amplified DNA via the breakage-fusion-bridge cycle [18,19]. Experiments with CHO cells suggested a synergistic effect between folic acid deficiency and gamma-radiation with respect to induction of DNA strand breaks and gene mutation [15]. This finding is supported by Blount et al. [5] who postulated that an interaction between folic acid deficiency and oxidative stress on genome damage could be expected because simultaneous excision of an oxidised base and uracil on opposite DNA strands within 12 bases of each other could result in the formation of double-strand breaks.

A better understanding of the dietary causes of inter-individual variation in radiation-sensitivity is needed because it is becoming increasingly evident that radiation sensitivity phenotype as detected by the micronucleus assay appears to be a good predictor of breast cancer risk and abnormal response to radiotherapy in prostate cancer patients [20,21].

Considering the known independent and similar effects of radiation or folic acid deficiency on genome stability, we hypothesized that folic acid deficiency increases the sensitivity of cells to DNA damage induced by ionising radiation. The model used to test the hypothesis consisted of 10 day cultures of the WIL2-NS lymphoblastoid cell line, derived from the spleen of a Caucasian male (American Type Culture Collection, 1992) with hereditary spherocytosis [22,23], and the comprehensive cytokinesis-block micronucleus (CBMN) assay [18,24]. The CBMN assay is a technique which allows a rapid and accurate assessment of chromosome breakage, chromosome rearrangements and chromosome loss [24–27]. The frequency of binucleated cells (BNCs) containing micronuclei (MNed BNC) or nucleoplasmic bridges (BNC with NPB), cell death parameters such as the frequency of necrotic and apoptotic cells and cytostatic effects, by measuring the nuclear division index, were determined to asses the independent and combined toxic effects of folic acid deficiency and ionising radiation. By using DNA probes to the centromeric regions of chromosomes it is possible to use the CBMN assay to determine the rate of aneuploidy in nuclei of binucleated cells and mononucleated cells and determine malsegregation pattern in these cells [14,28,29]. Furthermore, the hypothesis was tested that exposure to folic acid deficiency and/or ionising radiation may increase the risk of chromosome 21 aneuploidy.

2. Materials and methods

The experimental design is summarised in Fig. 1. To allow the effect of folic acid deficiency on chromosomal instability to become evident, cells were cultured for 10 days before harvesting on slides. WIL2-NS cultures were set up, in duplicate, at 0.3×10^6 cells/mL in 5 mL RPMI-1640 cell culture medium containing 5% dialysed fetal bovine serum (FBS) (Trace Scientific, Melbourne, Australia), 2 mM L-glutamine (Sigma, Sydney, Australia),