Folic Acid Supplementation and Cell Kinetics of Rectal Mucosa in Patients with Ulcerative Colitis

Guido Biasco,1 Ursula Zannoni, Gian Maria Paganelli, Renato Santucci, Paolo Giovannetti, Giovanni Rivolta, Rita Miniero, Loris Pironi, Carlo Calabrese, Giulio Di Febo, and Mario Miglioli


Abstract

It has been suggested that colon cancer risk in ulcerative colitis (UC) is correlated to a reduced bioavailability of folate. We studied the effects of folic acid supplementation on the pattern of rectal cell proliferation in patients affected by long-standing UC. In the rectal mucosa of these patients, an expansion of proliferating cells to the crypt surface is found frequently. This abnormality is considered an intermediate biomarker in chemoprevention trials. Twenty-four patients (13 males; age, 26–70 years; UC duration, 7–34 years) with UC in remission for 1 month at least were assigned randomly to one of the following treatments: (a) folic acid (15 mg/day) or (b) placebo. Cell proliferation was analyzed through immunohistochemistry on sections of rectal biopsies incubated for 1 hour in a culture medium containing bromodeoxyuridine. Fragments were taken at admission to the study and after 3 months of treatment. As compared to the baseline values, after 3 months of therapy in patients treated with folic acid, a significant reduction of the frequency of occurrence of labeled cells in the upper 40% of the crypts (φh value) was observed (0.1836 ± 0.0278 versus 0.1023 ± 0.0255; P < 0.01). On the contrary, no significant proliferative changes were observed in the placebo group. These results suggest that folate supplementation contributes to regulating rectal cell proliferation in patients with long-standing UC. These findings may be significant for chemoprevention of colon cancer in these patients.

Introduction

UC is a disease that predisposes one to the development of colorectal cancer (1–5). Patients affected by this disease are usually treated long-term with sulfapyridine (SASP), a drug that impairs the absorption of folates (6). A retrospective case-control study suggests that the administration of folic acid can reverse or slow the progression of precancer in the colorectal mucosa of UC patients (7). To verify the possible chemoprevention action of folic acid on colorectal cancer in UC we carried out a prospective study on patients affected by this disease for more than 7 years. The intermediate endpoint of the study has been the regression of cell proliferation abnormalities usually observed in rectal mucosa affected by UC (8).

Materials and Methods

Study Design. The study was double-blind double-dummy study comparing the effects of folic acid versus placebo in patients affected by long-standing UC for more than 7 years. Before admission to the study, patients underwent proctoscopy and biopsies to evaluate histology and cell proliferation of the rectal mucosa (see below). After endoscopy, they were randomly assigned to one of the following treatments: (a) folic acid (as calcium folate: Citofolin, Bracco S.p.A, Milano, Italy), 15 mg once a day p.o. and (b) undistinguishable placebo once a day p.o. The duration of the treatment was 3 months. Patients were invited not to change their lifestyle and dietary habits during this period.

Patients. Thirty patients affected by long-standing (more than 7 years) disease were invited to take part in the study. Informed consent was obtained from all of the patients. Exclusion criteria were previous or in-course treatment with folate acid, detection of precancer in colorectal mucosa, pregnancy, and relapse of the disease. All eligible patients were in remission for more than 1 month. During the study, six patients had an acute relapse of the disease; they were excluded and not replaced. Table 1 shows the clinical features of the 24 patients who concluded the study. Thirteen patients were randomly assigned to the active arm of the folic acid of the treatment and 11 to the placebo treatment.

Endoscopy and Biopsies. Proctoscopy was carried out without any bowel-cleaning preparation. During endoscopy, six biopsies were taken from flat mucosa 10–15 cm from the anal verge using biopsy minforces. Two biopsies were immediately fixed in 10% buffered formalin for routine histological examination. The histological activity of ulcerative colitis was evaluated according to the classification of Truelove and Richards (9).

Cell Kinetics. The remaining four specimens were cut in 1 mm-thick fragments and oriented mucosal side up on filter paper in small Petri dishes. The culture medium was Eagle’s basic solution supplemented by 10% FCS plus (BrdUrd) in a concentration of 320 μM/l. The dishes were placed in a rolling chamber at 37°C for 1 h with an atmosphere of 95% O2 and 5% CO2. After the incubation, the specimens were fixed in ethanol 70%, dehydrated, and embedded in paraffin according to routine histologic procedures. Nonconsecutive 3-μm sections were cut and mounted on the slides to obtain samples of different crypts. The slides were processed with the avidin-biotin peroxidase complex technique (Vectastain ABC kit; Vector Laboratories)

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2 To whom requests for reprints should be addressed.
3 The abbreviations used are: UC, ulcerative colitis; SASP, salicylazosulfapyridine; BrdUrd, 5’-bromodeoxyuridine; LI, labeling index.
using an anti-BrdUrd monoclonal antibody (Becton-Dickinson) at a 1:100 dilution in PBS. Denaturation of DNA was done prior to immunohistochemical procedure with 2N HCl for 20 min at 37°C. Finally, slides were stained with diaminobenzidine solution (Sigma Chemical Co.) and weakly counterstained with hematoxylin.

For cell kinetics evaluation, each crypt was divided into five compartments of equal size. The compartments were referred to by ordinal numbers from 1 (bottom) to 5 (surface). For each case, the BrdUrd Labeling Index (LI), both overall and for each compartment, and the BrdUrd-labeled cell frequency in each compartment, and the BrdUrd Labeling Index (LI), both overall and for each compartment, were calculated by the equation:

\[
\text{Labeling index} = \frac{\text{Number of BrdUrd-labeled cells}}{\text{Total number of cells}} \times \frac{100}{\text{Ht}}
\]

where TF = total blood folate concentration, PF = plasma folate concentrations, Ht = hematocrit, and 21 is the dilution factor. Normal values were 3–17 ng/ml for plasma folate and 150–600 ng/ml for RBC folate.

**Folate Assay.** A fasting venous blood sample was drawn in the morning at the admission and at the end of the study. Plasma and RBC folate concentration were measured according to a previously described method (11). Briefly, blood samples were collected into tubes containing EDTA. Plasma was separated by centrifugation. For RBC folate evaluation, 100 μl of EDTA whole blood was mixed with 2.0 ml of freshly prepared 1% ascorbic acid solution to lyse the cells. Both plasma and hemolysate were protected from light by a black paper and immediately stored at -20°C until assays were performed using the radioimmunoassay method (Solid Phase No Boil Folic acid kit, Diagnostic Products Corp., Los Angeles, CA). RBC folate acid concentration was calculated by the equation:

\[
\text{RBC} = 21 \times \frac{\text{TF}}{\text{PF} \times \frac{100 - \text{Ht}}{100}} \times \frac{100}{\text{Ht}}
\]
Discussion

Some evidence in the literature supports the hypothesis of a protective role of folate toward cancer. Experimental studies show that the supplementation of folic acid stabilizes the DNA in rats treated with methylazoxymethanol (12), whereas folate deficiency facilitates the development of cancer after exposure to chemical carcinogens (13). Epidemiological reports indicate an inverse relationship between dietary folate intake and colorectal cancer (14, 15). In humans, oral supplementation of folic acid, 10 mg once a day for 3 months, can reverse cervical dysplasia (16). Moreover, bronchial squamous metaplasia improves in smokers treated with folate (17), and the development of colorectal adenomas seems to be inhibited by folate assumption (18).

In a retrospective study, Lashner et al. indicate that UC patients with colorectal precancer or cancer showed lower serum folate levels than UC patients without malignancy (7). These observations suggest that folate acid supplementation may be useful to control the risk of colorectal cancer in the disease.

Our results are consistent with this hypothesis. The statistically significant decrease in the frequency of occurrence of proliferating cells in the upper 40% of the crypt (φh) suggests that folate supplementation is effective in reducing cell proliferation abnormalities in the rectal mucosa of patients with UC. An expansion of the proliferative compartment is one of the earliest changes occurring during induction of colonic adenocarcinomas in mice by 1,2-dimethylhydrazine (19). This abnormality is also observed in flat mucosa of patients with colorectal neoplasias, suggesting that it is a step on a common pathway that leads to increasing cellular atypia and malignant transformation (20). Thus, this abnormality has been regarded as an intermediate biomarker for determination of the effectiveness of nutritional intervention therapy (21).

Other substances, such as calcium, vitamins, and ω-3 fatty acids are all able to normalize the cell proliferation pattern in the rectal mucosa of patients at high risk of colorectal cancer (22–24). These substances are considered to have a "protective" action, and they are proposed for Phase III chemoprevention trials.

Folic acid is an important coenzyme in purine and pyrimidine synthesis as well as in aminoacid metabolism, methylation of biogenic amines and the initiation of protein synthesis (25, 26). These properties are related to DNA synthesis and may, at least in part, explain our results.

SASP administration can affect the absorption of folate (6). Some suggest that this may be pathogenetic pathway contributing to colorectal carcinogenesis in UC (7). In our patients, the duration of assumption of SASP was extremely variable. Moreover, some patients ceased the assumption of SASP several years before admission to the study, so the possible implications of SASP intake on the our results is questionable.

We believe that this observation does not change the relevance of the obtained data. Intrinsinc properties of folic acid may control cell proliferation. We believe that our results, derived from a prospective study, could be useful for the development of strategies for the control of colorectal cancer in UC.

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References


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