The Use of Leucovorin Orally in Normal and Leukemic L1210 Mice to Prevent the Toxicity and Gastrointestinal Lesions Caused by High Doses of Methotrexate

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SUMMARY

Leucovorin p.o. was as effective as parenterally in preventing weight loss and toxic deaths in normal mice from high doses of methotrexate. Histological sections of proximal jejunum demonstrate the protection afforded by delayed p.o. leucovorin from the marked disruption of villi and mucosal glands produced by methotrexate. Mice bearing leukemia L1210 treated with methotrexate plus leucovorin p.o. were able to tolerate 2- to 4-fold increases in the dose of methotrexate tolerated by mice treated with methotrexate alone. This resulted in a greater increase in survival time of leukemic mice. The therapeutic advantage of the regimen of delayed leucovorin given in conjunction with methotrexate is maintained when leucovorin is administered p.o.

INTRODUCTION

The ability of leucovorin (Lederle Laboratories, Pearl River, N. Y.: N5-formyltetrahydrofolic acid, citrovorum factor, folic acid) to reverse the toxicity and antitumor activity of folate antagonists has been demonstrated (5). When leucovorin administration is delayed 12 to 24 hr after antifolate therapy, the antineoplastic effect of the folate antagonist is retained, while host toxicity is still reduced (8).

MTX remains in wide use in the treatment of choriocarcinoma (15), leukemia (1), and carcinoma (17, 20). Leucovorin is used as an antidote for MTX overdosage (9) and to decrease systemic toxicity after local intraarterial infusions of MTX (14). Leucovorin has generally been administered parenterally, either i.m. (16) or i.v. (11). Recently, Djerassi (4) and Bertino (personal communication) have used the p.o. route to administer leucovorin.

In the present studies, the efficacy of p.o. administered leucovorin was examined in normal and leukemic mice.

MATERIALS AND METHODS

The experimental methods for the evaluation of the toxicity and the anti-L1210 effects of MTX and leucovorin have been described (6). CDFi (BALB/c X DBA/2) F1 hybrid male mice weighing from 18 to 26 g were obtained from the production colonies of the Cancer Chemotherapy National Service Center.

Stock ascitic L1210 tumor was taken from DBA/2 male mice and a uniform suspension was made in Hanks' balanced salt solution. L1210 cells, 1 X 106, in 0.2 ml solution were injected s.c. in the right flank.

In studies with normal or leukemic mice, MTX was dissolved in 2% sodium bicarbonate and given s.c. in the intrascapular region every 4th day (7) for a total of 5 treatments. Leucovorin was dissolved in distilled water and administered either p.o. or s.c. in the posterior cervical area every 4th day 24 hr after MTX administration. The appropriate concentrations of drugs were injected in a volume of 0.01 ml/g body weight.

For the histological examination of the small intestine, normal mice were treated with MTX, 160 mg/kg s.c., on Days 1 and 5. Leucovorin was administered p.o. or s.c. 24 hr following MTX therapy (Days 2 and 6). Mice were sacrificed on Day 8 and the proximal jejunum was immediately removed. The tissues were fixed in neutral buffered formalin, dehydrated, embedded in paraffin, sectioned at 5 to 6 μ, and stained with hematoxylin and eosin.

RESULTS

Table 1 shows, in terms of the percentage of toxic deaths and weight loss, the protection afforded by delayed leucovorin administration against the toxicity of high doses of MTX in normal mice. Leucovorin was as effective p.o. as parenterally in preventing toxic deaths and weight loss.

Leukemic mice receiving MTX and delayed leucovorin also demonstrated significantly less weight loss than that resulting from the same dose of MTX alone. This protection from toxicity enabled the mice bearing L1210 to tolerate 2- to 4-fold increases in the dose of MTX. The ability to use higher doses of MTX resulted in greater
Table 1
The effect of delayed leucovorin administered p.o. or s.c. on the toxicity and anti-L1210

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<th>Activity of methotrexate*</th>
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*CDF, male mice (18 to 26 g) were treated with MTX in doses indicated s.c. every 4th day for 5 treatments. Leucovorin, 200 mg/kg, was given p.o. or s.c. 24 hr after each dose of MTX.
†Mice were observed for toxicity for 90 days.
‡Weight change is the body weight difference from Day 1 to Day 7 (after 2 MTX treatments) minus controls.
§Mice inoculated s.c. in the right hind leg with 10⁶ L1210 cells from stock DBA/2 mice bearing ascitic L1210. MTX treatment started 1 day after tumor implantation.
|| ILS % is the percentage increase in median survival time compared with controls.

antitumor effectiveness as evidenced by greater increases in survival time (Table 1). MTX, 160 mg/kg every 4 days, plus delayed leucovorin, 200 mg/kg p.o., produced an 88% ILS of tumorous mice as compared with controls. MTX, 40 mg/kg every 4 days, alone yielded an ILS of 55% with all mice having large local subcutaneous tumors at the time of death. Higher doses of MTX alone were toxic. Leucovorin alone had no effect on survival time or weight change as compared with untreated controls. Delayed leucovorin p.o. was as effective as s.c. in protecting against MTX toxicity and enabling the use of higher doses of MTX.

Histological Observations on the Use of Oral Leucovorin to Prevent the Gastrointestinal Lesions of MTX. The small intestine of mice treated with MTX alone was boggy, edematous, and distended with a straw yellow fluid. The small intestines of mice receiving MTX plus delayed p.o. or s.c. leucovorin appeared normal on gross examination.

Fig. 1 illustrates the normal jejunal epithelium of untreated mice. Leucovorin, 200 mg/kg every 4 days, does not alter the histological appearance of the jejunum.

High parenteral doses of MTX alone produced marked disruption of both villi and mucosal glands in the jejunum (Fig. 2). Fig. 3 illustrates the loss of the orderly basilar arrangement of the nuclei of the jejunal epithelium of mice treated with high doses of MTX s.c. The cells are rounded, enlarged, and beginning to slough. There is cytoplasmic vacuolization, nuclear swelling, and prominent nucleoli. The brush border is not present. Lymphocytic infiltration is prominent.

Leucovorin, 200 mg/kg administered p.o. 24 hr after each dose of MTX, protected the jejunum against the g.i. toxicity of MTX. There is slight flattening of the villi, but the villi and mucosal glands are intact (Fig. 4). There is slight swelling and piling up of nuclei, but the nuclei are in the basal position and the brush border is present (Fig. 5).

Delayed leucovorin given s.c. also protected against the jejunal lesions of MTX, with only slight swelling and piling up of nuclei on histological section (Fig. 6).

DISCUSSION

In the present studies, delayed leucovorin p.o. protected mice from the weight loss and lethal toxicity produced by high doses of MTX in normal mice. MTX and delayed leucovorin p.o. resulted in greater increases in life-span of mice bearing leukemia L1210 than did MTX alone. Leucovorin administered p.o. provided as much protection from MTX toxicity in both normal and leukemic mice as did leucovorin administered s.c.

Clinically, the g.i. toxicity of MTX is well documented (3). The morphology of the g.i. lesions produced by antifolates ranges from the appearance of cytoplasmic inclusion bodies (22), flattening of the crypt epithelium (23), and epithelial metaplasia (24) to desquamation of the epi-
thelial lining (18) and hemorrhagic ulcers (21). In the present study the jejunal sections taken 72 hr after the last dose of MTX demonstrated marked disruption of both villi and mucosal glands. The epithelial cells were increased in size, as described by Jacobson (21) using rat intestine. The cells were rounded and showed cytoplasmic vacuolization, nuclear swelling, and prominent nucleoli.

These morphological lesions resulting from high dose MTX reflect the effect of an antimetabolite on a tissue with a high rate of cellular proliferation (13). The average generation time of mouse ileum crypt epithelial cells has been determined to be about 19 hr, with DNA synthesis occupying about 7.5 hr (19). MTX blocks DNA synthesis by inhibiting dihydrofolate reductase and preventing the synthesis of thymidylic acid from deoxyuridylic acid (3).

Delayed leucovorin is postulated to "rescue" normal cells which have been inhibited in the synthesis of DNA but are still viable (2). Leucovorin promptly reverses the inhibition by MTX of the incorporation of tritiated deoxyuridine into DNA by leukemic cells in vitro (10). Morphologically, this is apparently reflected in the prevention of the g.i. lesions caused by MTX with the addition of delayed leucovorin. The jejunal sections of mice receiving the g.i. lesions caused by MTX with the addition of de

References

Oral Leucovorin and Methotrexate

Fig. 1. Normal mouse jejunum of untreated controls. H & E, × 160.

Fig. 2. Jejunum of mouse treated with MTX, 160 mg/kg s.c., every 4 days (Days 1 and 5). Section was taken on Day 8. There is marked disruption of both villi and mucosal glands with infiltration of chronic inflammatory cells. H & E, × 160.

Fig. 3. Higher power of Fig. 2 showing flattened, markedly disorganized villi of jejunum of mouse treated with MTX. The epithelium has lost the orderly basilar arrangement of nuclei and brush border. The cells are rounded and beginning to slough. The cells show cytoplasmic vacuolization, nuclear swelling, and prominent nucleoli. Lymphocytic infiltration is apparent. H & E, × 400.

Fig. 4. Jejunum of mouse treated with MTX, 160 mg/kg s.c., on Days 1 and 5 and leucovorin, 200 mg/kg p.o., on Days 2 and 6 (24 hr after MTX therapy). The section was taken on Day 8. The delayed leucovorin has protected the intestine from the profound toxic effects of MTX. There is increased mitotic activity and some flattening of the villi. H & E, × 160.

Fig. 5. Higher power of Fig. 4, showing fairly normal villi of jejunum of mouse treated with MTX and protected by delayed leucovorin. Nuclei are basal and villous structure is generally intact. There is some swelling and piling up of nuclei. H & E, × 400.

Fig. 6. Slightly tangential section of jejunal villi of mouse treated with MTX, 160 mg/kg, on Days 1 and 5 and leucovorin, 200 mg/kg s.c., on Days 2 and 6. The section was taken Day 8. There is some swelling and piling up of nuclei, but nuclei are basal and villous structure is generally intact. H & E, × 400.
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