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# Potentiation of antimetabolite antitumor activity in vivo by dipyridamole and amphotericin B

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**Summary.** Previous studies have shown that dipyridamole (DP), a potent nucleoside transport inhibitor blocking the rescue effect of exogenous nucleosides, markedly potentiates the cytotoxicity of antimetabolites. However, no enhancement of the chemotherapeutic effect of antimetabolites by DP in vivo has yet been reported. This study provided evidence that the combination of DP and amphotericin B (AmB) significantly potentiated the inhibitory effect of 5-fluorouracil (FU) or methotrexate (MTX) against a panel of transplantable tumors including sarcoma 180, cervical carcinoma U14, and Lewis lung carcinoma in mice. No significant increase in toxicity was induced by this combination in treated mice. Our results indicate that the combination of DP and AmB with antimetabolites is potentially useful in cancer chemotherapy.

## Introduction

Antimetabolites currently used in cancer chemotherapy are inhibitors of the de novo pathways of nucleotide biosynthesis, but they cannot inhibit the salvage pathways. The cytotoxicity of antimetabolites to mammalian cell can be reversed by exogenous nucleosides. In vivo, the circulating nucleosides might protect tumor cells from antimetabolite action. The reasons why antimetabolites fail to cure human neoplasms [3, 11] are attributed, at least in part, to the operation of purine and pyrimidine salvage [24].

In recent years many studies have demonstrated that a combination of inhibitors of key enzymes in de novo biosynthesis and salvage nucleoside transport provide synergistic cytotoxic action. Dipyridamole (DP), an inhibitor of nucleoside transport, has been effective in blocking the rescue by nucleosides against acivicin cytotoxicity in rat hepatoma 3924A and human colon cancer cells (VAC05) [9, 24, 27]. It has enhanced the cytotoxicity of methotrexate (MTX) in a variety of cell lines [1, 2, 16], potentiated the cytotoxicity of 5-fluorouracil (FU) to human colon cancer cells [10], and shown a synergistic effect with *N*-phosphonacetyl-l-aspartate (PALA) against ovarian cancer and other tumor cells [4, 5]. DP has also potentiated the effect of acivicin or PALA on nucleotide levels in tumor cells in vivo [4, 25]. However, i.v. infusion of this drug has failed to block thymidine incorporation into DNA in Walker carcinosarcoma cells in rats [17]. The chemotherapeutic effect of MTX was not markedly improved when its combination with DP was tested against Ridgway osteogenic sarcoma or L1210 leukemia in mice [16].

Recent studies have demonstrated that cultured rat hepatoma 3924A and human colon cancer HT-29 cells in the lag and log phases were highly sensitive to DP, whereas cells in the stationary phase were insensitive. Amphotericin B (AmB) has effectively restored the inhibitory effect of DP on stationary cancer cells [28, 29]. The cultured monolayer cells in the stationary phase are known to be more similar to cells in solid tumors than are the more frequently used log-phase cells. Therefore, one of the reasons why the antitumor activities of antimetabolites plus DP in vivo are not dramatically improved might be attributed to the insensitivity of tumor cells to DP. This suggests that the enhancement by AmB of the inhibitory action of DP in stationary-phase cells should be relevant in the treatment of solid tumors whose growth fractions are low.

This paper reports that the combination of DP and AmB enhanced the inhibitory effects of antimetabolites (FU, MTX) against a panel of transplantable tumors including sarcoma 180, cervical carcinoma U14, and Lewis lung carcinoma in mice without a significant increase in toxicity.

## Materials and methods

*Drugs.* AmB was purchased from E. R. Squibb & Sons, Inc. (Princeton, NJ, USA). DP was purchased from Shanghai No. 10 pharmaceutical works (Shanghai, China). MTX and FU were purchased from Shanghai No. 12 and No. 13 pharmaceutical works (Shanghai, China), respectively.

*Animal experiments.* Male or female Kunming mice or C57/BL mice weighing 18–22 g were obtained from the Institute of Medical Laboratory Animals, Chinese Academy of Medical Sciences. Transplantable tumors, including sarcoma 180 and cervical carcinoma U14 in Kuming mice and Lewis lung carcinoma in C57/BL mice, were used. Tumor cell suspensions were prepared with a tissue grinder by the addition of 1 part tumor tissue to 5 parts normal saline (w/v). For each mouse, 0.2 ml suspension was injected s.c. in the axilla region. Treatments by i.p. injection

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**Table 1.** Inhibitory effect of MTX, DP, and AmB on the growth of sarcoma 180 in mice[a]

| Experiment number[b] | Treatment | Mice ($n$) | | Body weight change (g) | Tumor weight | |
|---|---|---|---|---|---|---|
| | | Begin | End | | Mean (g) ± SD | % of control[c] |
| I | Control | 10 | 10 | +6.1 | 3.30±0.35 | |
| | MTX | 10 | 9 | +1.0 | 2.26±0.27 | 67 |
| | DP | 10 | 10 | +4.2 | 3.40±0.35 | 103 |
| | AmB | 10 | 10 | +2.4 | 2.81±0.51 | 83 |
| | MTX + DP | 10 | 10 | +2.4 | 2.82±0.71 | 84 (1.2) |
| | MTX + AmB | 10 | 9 | −0.5 | 2.20±0.42 | 65 (1.2) |
| | MTX + DP + AmB | 10 | 10 | +1.4 | 1.46±0.26 | 43* (0.7) |
| II | Control | 10 | 10 | −0.6 | 3.82±0.32 | |
| | MTX | 10 | 9 | −4.5 | 2.37±0.21 | 62 |
| | DP | 10 | 10 | −0.3 | 3.52±0.29 | 92 |
| | AmB | 10 | 10 | −2.0 | 3.31±0.22 | 82 |
| | MTX + DP | 10 | 10 | −2.0 | 2.49±0.19 | 65 (1.1) |
| | MTX + AmB | 10 | 10 | −3.6 | 2.20±0.22 | 58 (1.1) |
| | MTX + DP + AmB | 10 | 9 | −1.9 | 1.64±0.25 | 43* (0.9) |

[a] Dose of drugs: MTX, 1 mg/kg; DP, 10 mg/kg; AmB, 5 mg/kg; given i.p. every other day × 5
[b] Experiment I, treatment started 24 h after tumor inoculation; Experiment II, treatment started on day 7 after tumor inoculation
[c] Numbers in parentheses indicate the coefficient of drug interaction (see *Materials and methods*)
* Significantly different from any other group; $P < 0.01$

started 24 h or 7 days after s.c. tumor inoculation; drugs were given sequentially, as follows: AmB, DP, and FU (or MTX), with a 30-min interval between drugs. Controls were injected with normal saline. Mice were sacrificed on day 11 after the initiation of treatment and the tumors were weighed. Data were expressed as percentage of tumor weight, and Student's $t$-test was used for statistical analysis. The evaluation of two-drug (antimetabolite + DP or AmB) and three-drug (antimetabolite + DP + AmB) combinations was done following a previously described method [7, 21], with modifications. The coefficient of drug interaction was defined as the ratio between the percentage of tumor weight for a drug combination and the product of the percentage of tumor weight for the individual drugs. Coefficient values of $<1$ were considered to show synergism, those of $>1$, antagonism, and those of $\sim 1$, additive effect.

*Histological examination of tumors.* Mice were sacrificed 24 h after the last injection of drugs. Tumor tissue blocks were rapidly removed and fixed in Bouin's solution. Sections were stained with hematoxylin and eosin and observed under a microscope with a rectangular net-like micrometer. A number of optical fields (0.01 $mm^2$ each) were examined along the peripheral area of the tumor, and the number of tumor cells and mitotic figures were recorded.

*Examination of bone marrow cellularity.* Femurs were removed and the bone marrow cavity was washed out with 5 ml white-blood-cell diluting solution. After a single cell suspension was made by pushing and drawing the syringe, the nucleated cells were counted under a microscope.

*Examination of the intestinal mucosa.* A small fragment of the small intestine (3–5 mm from the pylorus) was removed from each mouse and sections were prepared as described above. Mitotic figures and degenerated cells in 20 optical fields of the intestinal mucosa and crypt cells in 10 longitudinally sectioned crypts of Lieberkühn were counted as previously described by Cheng et al. [6]. A 100-μm segment of each crypt was examined.

*Histopathologic examination of organs.* The heart, lung, liver, spleen, pancreas, kidney, and adrenal gland were examined under a microscope for pathologic changes.

## Results

### *Effects of the DP, AmB, and MTX combination on tumor growth*

DP plus AmB significantly enhanced the inhibitory effect of MTX on sarcoma 180. Replicate experiments showed that MTX + DP + AmB exhibited greater inhibition than any other combination against sarcoma 180 when treatment started at 24 h or on day 7 after tumor inoculation. The difference in inhibition between the group given MTX + DP + AmB and all other groups was significant ($P < 0.01$); the 0.7 coefficient of drug interaction showed synergism for the three-drug combination. However, the inhibitory effect in the groups given MTX + DP or MTX + AmB was not significantly different from that in the group given MTX alone (Table 1).

### *Effects of the DP, AmB, and FU combination on tumor growth*

As shown in Table 2, DP plus AmB also markedly potentiated the inhibitory effect of FU on sarcoma 180. The inhibitory effect of FU + DP + AmB was much greater than that seen in other groups; the percentage of tumor weight decreased to 28%, which was significantly different ($P < 0.01$) from that of FU, FU + DP, or FU + AmB. The coefficient of drug interaction (0.7) showed a synergistic effect for the three-drug combination.

When DP and AmB were combined with FU, the growth of Lewis lung carcinoma was much slower than that observed for either FU alone or the combinations of

**Table 2.** Inhibitory effect of FU, DP, and AmB on the growth of sarcoma 180 in mice[a]

| Treatment | Mice ($n$) | | Body weight change (g) | Tumor weight | |
|---|---|---|---|---|---|
| | Begin | End | | Mean (g) ± SD | % of control[b] |
| Control | 10 | 10 | +3.4 | 3.41 ± 0.48 | |
| FU | 10 | 9 | +1.7 | 2.08 ± 0.40 | 55 |
| DP | 10 | 10 | +3.4 | 3.32 ± 0.70 | 97 |
| AmB | 10 | 10 | +0.4 | 2.62 ± 0.51 | 74 |
| FU + DP | 10 | 10 | +1.9 | 1.70 ± 0.41 | 49 (0.9) |
| FU + AmB | 10 | 9 | +0.9 | 1.52 ± 0.48 | 45 (1.1) |
| FU + DP + AmB | 10 | 9 | +1.1 | 1.00 ± 0.25 | 28* (0.7) |

[a] Dose of drugs: FU, 20 mg/kg; DP, 10 mg/kg; AmB, 2.5 mg/kg; given i.p. daily for 10 days
[b] Numbers in parentheses indicate the coefficient of drug interaction (see *Materials and methods*)
* Significantly different from any other group; $P < 0.01$

**Table 3.** Inhibitory effect of FU, DP, and AmB on the growth of Lewis lung carcinoma in mice[a]

| Experiment number[b] | Treatment | Mice ($n$) | | Body weight change (g) | Tumor weight | |
|---|---|---|---|---|---|---|
| | | Begin | End | | Mean (g) ± SD | % of control[c] |
| I | Control | 5 | 5 | +2.4 | 3.76 ± 1.03 | |
| | FU | 5 | 5 | +0.4 | 2.62 ± 0.82 | 70 |
| | DP | 5 | 5 | +2.2 | 3.27 ± 0.84 | 87 |
| | AmB | 5 | 5 | +1.0 | 3.66 ± 0.52 | 97 |
| | FU + DP | 5 | 5 | +0.4 | 3.58 ± 0.98 | 95 (1.6) |
| | FU + AmB | 5 | 5 | −0.1 | 2.40 ± 0.62 | 64 (0.9) |
| | FU + DP + AmB | 5 | 5 | −0.4 | 1.51 ± 0.84 | 40* (0.7) |
| II | Control | 10 | 10 | +0.7 | 5.71 ± 0.48 | |
| | FU | 10 | 9 | +0.2 | 4.78 ± 0.85 | 83 |
| | DP | 10 | 9 | +0.2 | 5.65 ± 0.76 | 95 |
| | AmB | 10 | 9 | +0.8 | 5.31 ± 0.81 | 93 |
| | FU + DP | 10 | 10 | +0.6 | 4.21 ± 0.60 | 74 (0.9) |
| | FU + AmB | 10 | 9 | −0.2 | 4.27 ± 0.70 | 75 (1.0) |
| | FU + DP + AmB | 10 | 9 | −0.4 | 2.76 ± 0.68 | 48* (0.7) |

[a] Dose of drugs: FU, 15 mg/kg; DP, 10 mg/kg; AmB, 2.5 mg/kg; given i.p. daily for 10 days
[b] Experiment I, treatment started 24 h after tumor inoculation; Experiment II, treatment started on day 7 after tumor inoculation
[c] Numbers in parentheses indicate the coefficient of drug interaction (see *Materials and methods*)
* Significantly different from any other group; $P < 0.01$

**Table 4.** Inhibitory effect of FU, DP, and AmB on the growth of cervical carcinoma U14 in mice[a]

| Treatment | Mice ($n$) | | Body weight change (g) | Tumor weight | |
|---|---|---|---|---|---|
| | Begin | End | | Mean (g) ± SD | % of control[b] |
| Control | 10 | 10 | +4.3 | 4.85 ± 0.60 | |
| FU | 10 | 10 | +2.4 | 2.18 ± 0.41 | 45 |
| DP | 10 | 10 | +2.8 | 3.88 ± 0.47 | 80 |
| AmB | 10 | 10 | +2.8 | 3.58 ± 0.46 | 73 |
| FU + DP | 10 | 10 | +1.2 | 2.01 ± 0.42 | 42 (1.2) |
| FU + AmB | 10 | 10 | +0.8 | 1.96 ± 0.35 | 41 (1.2) |
| FU + DP + AmB | 10 | 10 | +1.1 | 1.40 ± 0.29 | 28* (1.0) |

[a] Dose of drugs: FU, 15 mg/kg; DP, 10 mg/kg; AmB, 2.5 mg/kg; given i.p. daily for 10 days
[b] Numbers in parentheses indicate the coefficient of drug interaction (see *Materials and methods*)
* Significantly different from any other group; $P < 0.01$

FU + DP and FU + AmB. The therapeutic effect of FU was markedly enhanced by DP + AmB. The difference in inhibitory effect between the group given FU + DP + AmB and all other groups was highly significant ($P < 0.01$). The coefficient of drug interaction in two experiments (0.7 in both) showed a synergistic effect for the three-drug combinations; however, that of the groups given FU + DP or FU + AmB showed no synergism (Table 3). In cervical carcinoma U14, the three-drug combination showed an additive effect (Table 4).

**Table 5.** Changes in tumor cell density and mitosis in sarcoma 180 or Lewis lung carcinoma after treatment[a]

| Tumor | Treatment | Tumor cell density[b] | | Mitotic figures[b] | |
|---|---|---|---|---|---|
| | | Mean ± SD | % | Mean ± SD | % |
| S180 | Control | 831 ± 26 | 100 | 17 ± 2.6 | 100 |
| | MTX | 583 ± 9 | 70 | 12 ± 1.2 | 71 |
| | DP | 742 ± 32 | 89 | 12 ± 1.0 | 71 |
| | AmB | 687 ± 6 | 83 | 13 ± 2.1 | 77 |
| | MTX + DP | 608 ± 10 | 73 | 12 ± 1.5 | 71 |
| | MTX + AmB | 554 ± 27 | 67 | 12 ± 2.3 | 71 |
| | MTX + DP + AmB | 362 ± 11 | 44* | 7 ± 2.7 | 41* |
| Lewis | Control | 1041 ± 58 | 100 | 25 ± 4.0 | 100 |
| | FU | 756 ± 18 | 73 | 24 ± 3.6 | 96 |
| | DP | 969 ± 48 | 93 | 25 ± 2.7 | 100 |
| | AmB | 974 ± 39 | 94 | 23 ± 2.8 | 93 |
| | FU + DP | 769 ± 16 | 74 | 24 ± 2.8 | 96 |
| | FU + AmB | 766 ± 12 | 74 | 13 ± 1.2 | 52 |
| | FU + DP + AmB | 410 ± 24 | 39* | 10 ± 2.4 | 40* |

[a] Dose of drugs for sarcoma 180-bearing mice: MTX, 1 mg/kg; DP, 10 mg/kg; AmB, 5 mg/kg; given i.p. every other day × 5. Drug doses for Lewis lung carcinoma-bearing mice: FU, 15 mg/kg; DP, 10 mg/kg; AmB, 2.5 mg/kg; given i.p. daily for 10 days

[b] Number of cells and mitotic figures in an area of $10 \times 0.01$ mm$^2$ viewed under a microscope

* Significantly different from any other group; $P < 0.01$

**Table 6.** Changes in nucleated cells of bone marrow and crypt cells of the small intestine in treated mice[a]

| Experiment number[b] | Treatment | Nucleated cells per femur ($\times 10^6$) | Crypt cells[c] | Mitotic figures[d] |
|---|---|---|---|---|
| I | Control | 9.39 ± 0.54 | 310 ± 4.2 | 59.4 ± 5.9 |
| | MTX | 9.00 ± 0.94 | 311 ± 4.8 | 51.1 ± 4.2 |
| | DP | 9.49 ± 0.72 | 314 ± 4.8 | 55.9 ± 2.1 |
| | AmB | 9.52 ± 0.49 | 311 ± 2.2 | 56.8 ± 3.5 |
| | MTX + DP | 9.10 ± 0.63 | 311 ± 2.9 | 52.5 ± 2.8 |
| | MTX + AmB | 9.60 ± 0.94 | 314 ± 3.4 | 51.3 ± 1.7 |
| | MTX + DP + AmB | 8.93 ± 0.32 | 305 ± 4.9 | 52.2 ± 2.5 |
| II | Control | 10.43 ± 0.54 | 313 ± 4.1 | 62.2 ± 5.7 |
| | FU | 9.22 ± 3.06 | 289 ± 6.4 | 53.6 ± 6.0 |
| | DP | 10.48 ± 0.89 | 307 ± 6.0 | 58.7 ± 3.6 |
| | AmB | 10.16 ± 0.51 | 309 ± 5.2 | 60.4 ± 3.4 |
| | FU + DP | 8.39 ± 0.79 | 296 ± 9.0 | 59.6 ± 3.3 |
| | FU + AmB | 8.40 ± 0.72 | 302 ± 5.7 | 54.2 ± 5.2 |
| | FU + DP + AmB | 8.38 ± 1.39 | 300 ± 6.9 | 56.6 ± 5.4 |

[a] Mean ± SD for bone marrow assay ($n = 5$) and small intestine assay ($n = 10$)

[b] Experiment I, dose of drugs: MTX, 1 mg/kg; DP, 10 mg/kg; AmB, 5 mg/kg; given i.p. every other day × 5. Assays were done on day 11 after the initial injection. Experiment II, dose of drugs: FU, 15 mg/kg; DP, 10 mg/kg; AmB, 2.5 mg/kg; given i.p. daily for 7 days. Assays were done on day 8 after the initial injection

[c] Number of crypt cells in 10 longitudinally sectioned crypts of Lieberkühn from the small intestine. A 100 μm segment of each crypt was examined

[d] Number of mitotic figures in 20 optical fields of the intestinal mucosa, equivalent to an area of $20 \times 0.01$ mm$^2$. Magnification, $10 \times 100$

*Changes in tumor cell density and mitosis*

Examination of the sections showed that sarcoma 180 and Lewis lung carcinoma cells grew vigorously with considerable mitosis in control groups. There were many necrotic areas in tumors in groups given the three-drug combinations (MTX + DP + AmB or FU + DP + AmB). The nuclei of the tumor cells were swollen and the cell density was decreased. The number of cells and mitotic figures in the group given MTX + DP + AmB were reduced by 56% and 59%, respectively; those in the group given FU + DP + AmB decreased by 61% and 60%, respectively. Values for the groups given the three-drug combinations were significantly different ($P < 0.01$) from those for any other group (Table 5).

*Toxicity*

Relative to nontreated controls, mice treated with FU or MTX alone or related combinations showed slight weight loss or slower weight gain. However, no significant bodyweight differences were observed between mice treated with single antimetabolites and those given related combinations (Tables 1–4). No inhibition on bone marrow cellularity was found in sarcoma 180-bearing mice at therapeutic doses of MTX. There was no significant difference in nucleated cell counts between the group given MTX + DP + AmB and that given MTX alone. In Lewis lung carcinoma-bearing mice, FU caused a slight decrease in bone marrow cellularity. FU + DP + AmB also induced a slight decrease in bone marrow cells, but it was not significantly different from that caused by FU alone (Table 6).

In sarcoma 180-bearing mice, the combination of DP, AmB, and MTX did not cause marked changes in crypt cells from the small intestine at therapeutic doses. No significant difference was found in the number of crypt cells and mitoses between the group given MTX + DP + AmB and that given MTX. In Lewis lung carcinoma-bearing mice, FU caused a slight decrease in the number of crypt cells and mitoses at therapeutic doses; however, the number of crypt cells and mitoses in the group given FU + DP + AmB did not differ significantly from those in the group given FU. The number of degenerated cells remained at approximately the same level in treated and control groups (Table 6). At therapeutic doses, no pathologic changes were found in the heart, lung, liver, spleen, pancreas, kidney, or adrenal gland of treated mice.

## Discussion

Previous studies have demonstrated that the combination of DP with various antimetabolites displays synergistic cytotoxicity to cancer cells in vitro [1, 2, 4, 9, 10, 16, 26, 27]; however, DP fails to potentiate the antitumor activity of antimetabolites in vivo. No augmentation of the antitumor effect of antimetabolites by DP has yet been reported. Pharmacokinetic studies [12, 17] have found that DP binds extensively to rat and human serum proteins. DP-serum-protein binding might be one of the possible factors that reduce the efficacy of DP-antimetabolite combinations in vivo.

Another possible factor might involve the insensitivity of tumor cells to DP. A recent investigation has shown that in the same cell line, sensitivity to DP displays marked changes during the course of growth in culture. Cultured

hepatoma and colon cancer cells in the lag and log phases are highly sensitive to DP, whereas cells in the stationary phase are comparatively insensitive to the drug. Notably, AmB can render stationary-phase cells sensitive to DP [28, 29], a phenomenon that is relevant to the efficacy of the latter, particularly in solid tumors with highly compacted cells and a smaller growth fraction. Therefore, it is possible to enhance the antitumor effect of antimetabolites in vivo by using a combination of DP and AmB. In the present study we demonstrated that the combination of DP and AmB indeed augmented the antitumor effect of antimetabolites; however, the use of DP without AmB failed to accomplish this augmentation. To our knowledge, this is the first report that the combination of DP and AmB significantly enhances the antitumor effect of antimetabolites in vivo.

AmB is an antifungal antibiotic that increases membrane permeability. AmB alone exhibits no established antitumor activity; however, it has been shown to potentiate the effect of various chemotherapeutic agents [13, 18, 19, 23] and has been used in combination with antitumor drugs in clinical trials [20]. In addition, it has been shown to induce sensitivity in drug-resistant cells [14]. There are at least two mechanisms underlying the effect of AmB. One involves a stimulation of the immune system of the host when AmB is combined with antitumor agents that have few, if any, immunosuppressive effects [22]. The other involves a potentiation of the cytotoxicity of anticancer agents, which likely results from the increased uptake of the agents by tumor cells due to the enhancement of membrane permeability by AmB [13]. The combination of AmB with FU or MTX did not potentiate antitumor activity at doses used in our experiments. The augmentation of the antitumor activity of antimetabolites probably cannot be attributed to the stimulation of the host immune system or the increased uptake of the drug caused by AmB. However, AmB might increase the sensitivity of tumor cells to DP, which blocks the nucleoside rescue and potentiates the antitumor activity of antimetabolites.

DP has been reported to enhance the toxicity of MTX or PALA in mice, with more deaths in combination than in single-drug treatments [5, 16]. However, it is important to know whether the combination of DP and AmB increases the toxicity of FU or MTX at therapeutic doses. At sublethal doses, FU and MTX are known to cause the depletion of hemopoietic cells in bone marrow and damage to intestinal mucosa, especially the crypts of Lieberkühn. The enlargement of nuclei and nucleoli, pycnosis, and necrosis of crypt cells have occurred after administration of these drugs [8, 15]. For evaluation of the toxic effects of antimetabolites and other anticancer drugs, quantitative histologic methods have been developed in which crypt cells and their mitotic figures were counted on the sections, showing that the decrease in the number of crypt cells and mitotic figures were related to the dose of drug [6].

In the present study, the changes in nucleated bone marrow cells and intestinal crypt cells in treated mice were investigated. Our data showed that the combination of DP and AmB with FU or MTX did not markedly affect nucleated bone marrow cells or crypt cells from the small intestine in mice. No significant differences were observed between mice treated with the three-drug combinations and those given FU or MTX alone. In addition, no pathologic changes in the heart, lung, liver, spleen, pancreas, kidney, or adrenal gland were found in mice treated with the three-drug combinations. At therapeutic doses, the combination of DP and AmB evidently does not increase FU or MTX toxicity in mice.

Our results show that the combination of DP and AmB with antimetabolites (FU, MTX) enhanced the chemotherapeutic effect of the latter without significantly increasing their toxicity in vivo. Both DP and AmB are licensed drugs; therefore, this combination is potentially useful in antimetabolite chemotherapy of neoplastic diseases.

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