ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1986, p. 327–329
0066-4804/86/020327-03$02.00/0

Vol. 29, No. 2

Effect of Folinic Acid on the Capacity of Trimethoprim-Sulfamethoxazole to Prevent and Treat *Pneumocystis carinii* Pneumonia in Rats

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Received 28 May 1985/Accepted 1 November 1985

Daily administration of 1 mg of folinic acid to immunosuppressed rats with incipient or established *Pneumocystis carinii* pneumonia did not impair the capacity of trimethoprim-sulfamethoxazole to either prevent or treat this disease. These observations constitute the first experimental support for the use of folinic acid to prevent or control cytopenias that occur in patients with *Pneumocystis carinii* pneumonia who are under trimethoprim-sulfamethoxazole treatment.

The fixed combination of trimethoprim-sulfamethoxazole (T/S) has become the standard for the treatment of *Pneumocystis carinii* pneumonia (PCP). This combination is also used to prevent such infections in neutropenic or renal transplant patients. Prophylaxis resulting from T/S treatment has been associated with pancytopenia or leukopenia in renal (1, 8) and bone marrow (10) transplant patients. In addition, neutropenia has been produced or aggravated in patients with the acquired immunodeficiency syndrome (AIDS) who are under treatment with T/S (5). Others have confirmed this observation (C. B. Small, C. A. Harris, R. S. Klein, and G. H. Friedland, Program Abstr. 23rd Intersci. Conf. Antimicrob. Agents Chemother., Abstr. no. 630, 1983).

It has been suggested that because of interference with folate metabolism, T/S is responsible for hematologic abnormalities in some patients (1, 2, 10). Observations that the suppressive effect of T/S on bone marrow cultures can be reversed by the addition of folinic acid (1, 12) and that in some patients the hematologic abnormality caused by trimethoprim can be corrected by administering folinic acid (9) are consistent with this suggestion. In other patients (especially in AIDS patients) in whom neutropenia may be mediated by the peripheral destruction of leukocytes (E. Outwater and J. A. McCutchan, Abstr. Int. Conf. AIDS, abstr. no. W-22, p. 76, 1985), the administration of folinic acid may not be useful.

Although it is unclear which group of patients with T/S-induced cytopenia will benefit from the administration of folinic acid (B. J. Kinzie and J. W. Taylor, Letter, Ann. Intern. Med. **101**:565, 1984; C. Stock, Letter, Ann. Intern. Med. **102**:277, 1985), the routine addition of folinic acid to T/S therapy has been recommended (2, 4, 12). Others have warned against this procedure because of the concern that folinic acid may reduce the efficacy of T/S in the treatment of PCP (11, 13; T. Butler, Letter, Ann. Intern. Med. **102**:277, 1985; C. Stock, Letter, Ann. Intern. Med. **102**:277, 1985).

Although there are no prospective animal or human studies on the effect of dosage with folinic acid on the activity of T/S against PCP, there is one anecdotal report involving two patients in whom PCP was unresponsive to T/S until the concomitant administration of folinic acid was stopped (7). For these reasons we evaluated the effect of folinic acid on the prophylactic and therapeutic activity of T/S in experimentally induced PCP.

Male Sprague-Dawley rats (Harlan, Sprague-Dawley, Inc., Houston, Tex.) weighing approximately 200 g were housed in one room with five animals per wire cage. They had access to food and water ad libitum. Their water consumption was measured daily (6, 7), and their weights were measured weekly.

Dexamethasone sodium phosphate (Merck Sharp & Dohme, West Point, Pa.) at a concentration of 2 mg/liter of distilled drinking water was used to immunosuppress the animals. Tetracycline hydrochloride (E. R. Squibb & Sons, Princeton, N.J.) was added to the drinking water at a concentration of 500 mg/liter to suppress bacterial infection. In groups receiving T/S (Bactrim IV infusion; Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.), 355 mg of trimethoprim and 1,775 mg of sulfamethoxazole were added to each liter of the tetracycline-dexamethasone base solution. This solution provided approximately 45 and 225 mg of trimethoprim and sulfamethoxazole, respectively, per kg of body weight per rat per day. Each animal regularly drank between 20 and 30 ml of solution per day. The medicated drinking water was prepared every 4 to 5 days. Folinic acid (leucovorin calcium for injection; Lederle Laboratories, Pearl River, N.Y.) was given subcutaneously at a dosage of 1 mg/day.

A total of 106 rats were used in this study. Ten, serving as untreated controls, were fed and housed with the treatment groups but did not receive drugs. The remaining 96 rats, divided into four groups (distributed as follows) were started on drugs on the same day: group 1, 10 animals, was given tetracycline and dexamethasone in drinking water; group 2, 15 animals, was given tetracycline, dexamethasone, and T/S in drinking water; group 3, 15 animals, was given tetracycline, dexamethasone, and T/S in drinking water and 1 mg of folinic acid subcutaneously daily; and group 4, 56 animals, was given tetracycline and dexamethasone in drinking water until the first animal died with histologic evidence of PCP. On the day of the first death, the survivors in group 4 were

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TABLE 1. Effect of folinic acid therapy on PCP treatment

Group	Scheduled days of administration of[a]:			Mean wt (g)		No. of rats in original group	No. of fatalities (days after start of expt)	No. (%) of rats with PCP at:	
	TCN-Dex	T/S	FA	Initial	At death or sacrifice			Death	Sacrifice
Control	NA[b]	NA	NA	245	440	10	0 (NA)	NA	All clinically healthy
1	1–56	NA	NA	253	170	10	0 (NA)	0	5 (50)
2	1–56	1–56	NA	252	182	15	1[c] (55)		0
3	1–56	1–56	1–56	253	170	15	0 (NA)	0	0
4 A	1–84	NA	NA	250	156	7	2 (81 and 82)	1	5 (71)
4 B	1–84	57–84	NA	251	152	24	4 (63, 76, 79, and 80)	0	0
4 C	1–84	57–84	57–84	254	148	24	6 (63, 79, 79, 79, 80, and 80)	0	2 (8)

[a] TCN-Dex, Tetracycline hydrochloride and dexamethasone sodium phosphate; FA, folinic acid.
[b] NA, Not applicable.
[c] This rat was cannibalized; no specimen was obtainable for examination.

randomly divided into three subgroups: A, with 7 animals, continued to receive tetracycline and dexamethasone in drinking water; B, with 24 animals, received tetracycline, dexamethasone, and T/S; and C, with 24 animals, received tetracycline, dexamethasone, and T/S plus 1 mg of folinic acid daily. At the end of 8 weeks, the survivors in groups 1 through 3 were killed by carbon dioxide inhalation. Survivors in subgroups A, B, and C were killed 4 weeks later (a total of 12 weeks of immunosuppression).

The lungs were removed immediately after euthanasia or spontaneous death, perfused with 10% neutral buffered Formalin, and submerged in the same fixative for 18 to 24 h. Two blocks of lung tissue, one of the single left lobe and the other of the three major right lobes (azygos not included), were embedded in paraffin and sectioned at a thickness of 4 to 5 μm. Sections were stained with hematoxylin and eosin and Gomori methenamine-silver. Stained sections from each block were examined. Rats were considered free of PCP if no cysts were seen on sections stained by Gomori methenamine-silver. Rats were considered infected if characteristic intraalveolar cysts were present in association with a granular, foamy, eosinophilic intraalveolar transudate.

Control rats gained weight steadily during the study period, but all steroid-treated rats lost weight progressively (Table 1). There were 13 spontaneous deaths: none in group 1 or 3, 1 in group 2 (a rat that unfortunately was cannibalized), 2 in group 4 A (at 3 months of immunosuppression), 4 in group 4 B (during months 2 and 3 of immunosuppression), and 6 in group 4 C (at 3 months of immunosuppression). All deaths in groups 4 B and C occurred after 12 or more days of therapeutic intervention. Nine rats had evidence of pulmonary mycoses at death.

Five (50%) of the immunosuppressed rats in group 1 developed PCP after 8 weeks. None of the immunosuppressed animals that received T/S prophylactically either with (group 3) or without (group 2) folinic acid had PCP at the time of sacrifice or spontaneous death (Table 1).

The first fatality in group 4 with evidence of PCP occurred after 2 months of immunosuppression. At the end of the study, six of the seven untreated rats (subgroup A) had PCP. This high incidence of PCP was not observed in the treated groups. Instead, animals treated with T/S had no evidence of PCP at death (subgroup B). Although two animals (8%) treated with T/S and folinic acid (subgroup C) had evidence of PCP, there was no statistically significant difference between the proportions afflicted in subgroups B and C which would suggest that folinic acid had any effect on the efficacy of T/S ($P = 0.24$; Fisher's exact test). There was no difference in the severity of PCP observed in the groups.

At the high dose administered in this study, folinic acid had no effect on the capacity of T/S to prevent or treat experimentally induced PCP. This observation is supported by similar findings in the treatment of toxoplasmosis and malaria (3; C. Stock, Letter, Ann. Intern. Med. **102**:277, 1985), in which folic or folinic acid provides full protection against the hematotoxicity of pyrimethamine, a potent antifolic agent.

Although we are not prepared to advocate the routine use of folinic acid for patients undergoing treatment with T/S, the results of this study suggest that it can be safely added to the T/S regimens of patients who have become cytopenic during therapy. Further studies are needed to identify those subgroups of AIDS and non-AIDS patients who become cytopenic under T/S therapy and who consequently will benefit from the administration of folinic acid.

Technical, histologic, and veterinary support provided by Anna Chulian, Christine Bendele, and Wayne Stanley is gratefully appreciated.

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