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Efficacy of tetroxoprim/sulphadiazine in the treatment of *Pneumocystis carinii* pneumonitis in rats

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Pneumocystis carinii pneumonitis was induced using dexamethasone in male Sprague-Dawley rats. After the first death due to *Pn. carinii* occurred, nine rats received 50 mg/kg/day tetroxoprim and 120 mg/kg/day sulphadiazine. Four additional rats were given no therapy and served as a positive control. All the surviving rats including five negative controls were sacrificed four weeks after the last positive control rat died. All four rats in the positive control group and two of nine in the treated group developed *Pn. carinii* pneumonitis, whereas none of the five negative controls had evidence of *Pn. carinii* infection. The difference between the treated and untreated rats was significant ($P = 0{\cdot}05$). These results suggest that combination therapy with tetroxoprim/sulphadiazine is effective in the treatment of *Pn. carinii* pneumonitis in this animal model.

Introduction

Tetroxoprim, a diaminopyrimidine, is a selective inhibitor of dihydrofolate reductase. Tetroxoprim in combination with sulphadiazine has been shown to be effective against bacterial strains *in vitro* (Bywater, Holt & Reeves, 1979). In clinical trials the drugs were effective and well tolerated (Reeves *et al.*, 1979). Different aminopyrimidines, however, demonstrate varying affinity to the bacterial and non-bacterial reductases (Burchall, 1979), and therefore, we have investigated the efficacy of tetroxoprim/sulphadiazine in the treatment of *Pneumocystis carinii* pneumonia in a rat model. *Pn. carinii* organisms have been shown to exist as saprophytes in the lung tissue of rats, man and other animals (Ruskin, 1976). Though it is not possible to produce *Pn. carinii* pneumonia in rats by exogenous inoculation of the organism, *Pn. carinii* infection does develop by activation of latent infection provided the animals are immunosuppressed with a corticosteroid and are protected from bacterial infections by use of an antibiotic such as tetracycline (Frenkel, Good & Schulz, 1966).

Materials and methods

Male Sprague-Dawley rats weighing approximately 200 g were used. Five rats were housed in each cage. Rats were assigned to three groups: group I (negative control) consisted of five rats that received no drugs; group II (positive control) consisted of five rats

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that received dexamethasone and tetracycline; and group III (treatment group) consisted of ten rats that not only received dexamethasone and tetracycline but also tetroxoprim and sulphadiazine intragastrically after the first rat died of *Pn. carinii* infection.

Drugs

Dexamethasone sodium phosphate (Merck Sharpe & Dhome, West Point, Pa., U.S.A.) and tetracycline hydrochloride were added to the drinking water. The concentration of the drugs was 0·1 mg/100 ml and 50 mg/100 ml respectively.

The dosage for tetroxoprim and sulphadiazine (Heumann Arzneimittel, Nürnberg, W. Germany) was 50 mg/kg/day and 120 mg/kg/day respectively, administered to each rat on the basis of the average weight of the group III rats on the first day of treatment. No changes in the dosage were made thereafter. For administration, tetroxoprim and sulphadiazine were homogenized (Polytron homogenizer PT10/35) in tylose solution (1% tylose in 0·9% of NaCl solution). The concentration of tetroxoprim and sulphadiazine was adjusted in such a way that 0·5 ml of suspension contained the daily dose for each rat.

All the solutions and suspensions were prepared daily. The rats had access to water and food *ad libitum*.

Laboratory Diagnosis of Pn. carinii *pneumonia*

Autopsies were done at the time of death. Smears were made from freshly cut, unfixed specimens and histologic examination was performed on formalin fixed material as described elsewhere (Smith & Bartlett, 1982). The histological specimens were examined by one of us (I.D.C.) without prior knowledge of the treatment.

Results

Group I. All the rats survived and were killed four weeks after the last death in group II. There was no evidence of *Pn. carinii* pneumonia in any of these specimens.

Group II. One rat died of overwhelming fungal infection within the first two weeks of the study and was excluded from the statistical analysis. The other rats died of *Pn. carinii* pneumonia on the 41st, 60th, 63rd and 70th day of the experiment.

Group III. The first rat died on the 41st day of the experiment but was unfortunately cannibalized. Treatment with tetroxoprim and sulphadiazine was started on the 42nd day. The two rats that died on the 43rd and 59th day had evidence of *Pn. carinii* pneumonia. No further deaths occurred in this group. The remaining seven rats were killed four weeks after the last death in group II, namely the 98th day of the experiment. None of these had evidence of *Pn. carinii* pneumonia.

In summary, 4/4 of the positive control group and 2/9 of the treated group developed *Pn. carinii* and the difference was significant ($P = 0{\cdot}05$).

Discussion

Pn. carinii is one of the common life threatening infections in immunocompromised hosts with primary immunodeficiencies, malignant disease, organ transplants or the newly recognized acquired immunodeficiency syndrome (AIDS) (Walzer *et al.*, 1974; Masur *et al.*, 1981). In man, it is believed, though not proven, that pneumonia due to

this agent occurs as an opportunistic infection resulting from reactivation of latent organisms in immunocompromised individuals.

Pentamidine isothionate and co-trimoxazole are the only drugs shown to be effective in the treatment of *Pn. carinii* pneumonitis. In a randomised trial, co-trimoxazole and pentamidine were equally effective with respective recovery rates of 77% and 75%. In this study, co-trimoxazole was less toxic than pentamidine (Hughes *et al.*, 1978). Co-trimoxazole has also been shown to be an effective prophylactic agent against *Pn. carinii* pneumonia (Hughes *et al.*, 1977).

In this trial, all the rats in group II (positive control) that survived beyond the first week of the experiment developed *Pn. carinii* pneumonitis, indicating that immunosuppression and reactivation of latent *Pn. carinii* infection was successful in the rats receiving dexamethesone (group II and III). It also demonstrates the efficacy of tetroxoprim and sulphadiazine in combination therapy for *Pn. infection* in rats. The difference in the number of rats that developed *Pn. carinii* pneumonia in the treated and untreated rats was significant if we exclude one rat in group II from the analysis ($P=0{\cdot}05$). We believe the exclusion is acceptable as similar studies have shown that four or more weeks of immunosuppression is required before death due to *Pn. carinii* occurs (Hughes *et al.*, 1974). Furthermore, that particular rat was not considered to be the index case and the treatment was not started until the 42nd day of the experiment. Since one rat in group III received only a single dose of tetroxoprim/sulphadiazine before death it too could be excluded from the statistical analysis. If so, the difference in the rate of *Pn. carinii* pneumonia in treated versus untreated groups (4/4 vs. 1/8 respectively) is even more significant ($P=0{\cdot}01$).

As the epidemiology, pathogenesis, treatment and prophylaxis in the rat model has a strong clinical correlation (Walzer & Young, 1984), it is probable that tetroxoprim/sulphadiazine would prove to be effective in human *Pn. carinii* pneumonia. However, this remains to be seen. Comparative studies of tetroxoprim/sulphadiazine and co-trimoxazole are also needed. It is also not clear whether tetroxoprim/sulphadiazine will be better tolerated than co-trimoxazole by patients with AIDS.

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