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Efficacy of Lasalocid against Murine *Pneumocystis carinii* Pneumonitis

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The efficacy of the ionophore lasalocid against *Pneumocystis carinii* pneumonitis in corticosteroid-immunosuppressed Sprague-Dawley rats was investigated. Lasalocid was effective in the prevention of the pneumonitis in a dose-dependent manner. At dosages of 0, 5, 10, and 20 mg/kg/day, *P. carinii* infection rates were 92, 60, 20, and 0%, respectively, during dexamethasone immunosuppression. Also, lasalocid compared favorably with other drugs known to have anti-*P. carinii* activity, including trimethoprim-sulfamethoxazole, atovaquone, and dapsone-trimethoprim.

Pneumocystis carinii pneumonitis (PCP) is one of the most common serious opportunistic infections in patients with AIDS. More than 70% of these patients are at risk for PCP. In the past, we and others have shown several compounds, including trimethoprim-sulfamethoxazole (5), dapsone (6), atovaquone (7), trimetrexate (1), clindamycin-primaquine (13), and aerosol pentamidine (2), to be effective against this organism in animal models. Subsequent clinical trials with humans demonstrated remarkably similar results for each of the drugs. Because of serious adverse reactions to some of these drugs and a lack of efficacy in some patients, new, safe, and effective drugs are needed.

Lasalocid, a polyether carboxylic ionophore, was isolated from *Streptomyces lasaliensis* (17). Figure 1 shows the structure of this compound. It is used as an anticoccidial drug in farm animals. Also, it is effective against *Cryptosporidium parvum* in immunosuppressed rats (14). We studied the efficacy of lasalocid against PCP and compared its effect with atovaquone, dapsone-trimethoprim, and trimethoprim-sulfamethoxazole in the rat model.

Four-month-old virus-free Sprague-Dawley female rats (Harlan Laboratories, Indianapolis, Ind.) were divided into groups of 10 rats and immunosuppressed with 2.5 mg of dexamethasone per liter supplemented with 500 mg of tetracycline hydrochloride (Sumycin; E. R. Squibb & Sons, Princeton, N.J.) per liter in the drinking water. Such rats are expected to become heavily infected with *P. carinii* by 4 weeks of dexamethasone treatment (11). Drug administration began on the day immunosuppression started and was continued for 4 weeks. Ten rats in each group received lasalocid (Sigma, St. Louis, Mo.) in daily doses of 5, 10, or 20 mg/kg of body weight administered in food (6). Another group of animals received 10 and 100 mg of atovaquone (Burroughs-Wellcome, Research Triangle Park, N.C.) per kg of body weight per day. Two groups of rats were treated with high (25 to 20 mg/kg/day) or low (2.5 to 2.0 mg/kg/day) doses of dapsone (Jacobus, Princeton, N.J.) and trimethoprim (Proloprim; Burroughs-Wellcome) in food. Two other groups of rats received high (20 to 100 mg/kg/day) or low (2 to 10 mg/kg/day) doses of trimethoprim and sulfamethoxazole in drinking water. Two groups of immunosuppressed rats were kept as controls. After 4 weeks of drug administration the animals were killed and the lungs were removed and fixed in 10% buffered formalin for histopathological studies. Lung sections were prepared with silver ammoniacal stain and examined microscopically. The lung sections were scored for severity of PCP lesions from 0 to 3+ as previously described (11).

Lasalocid was effective in the prevention of PCP in a dose-dependent manner. Results are shown in Table 1. None of the rats treated with 20 mg/kg were infected (0 of 10), whereas 92% of the controls (11 of 12) became infected. Rats receiving atovaquone (100 mg/kg/day), dapsone-trimethoprim (25/20 mg/kg/day), and trimethoprim-sulfamethoxazole (20/100 and 2/10 mg/kg/day) were also *P. carinii* free. In contrast, 50 to 60% of rats treated with low-dose atovaquone (10 mg/kg/day), lasalocid (5 mg/kg), and dapsone-trimethoprim (2.5/2.0 mg/kg) were infected with *P. carinii.*

This study shows that lasalocid has activity against murine PCP in a dose-dependent manner. Lasalocid is a polyether carboxylic acid ionophore and offers promise for a new class of drugs against *P. carinii* infection. Ionophores are lipid soluble compounds that transport polar cations such as Ca^{2+} across the cell membranes and serve as important tools in studying cell signaling (9, 10). Lasalocid is also an effective anticoccidial drug used in poultry and farm animals. In one study this compound had potent cardiovascular properties, with dilation of coronary arteries in dogs (12), and a study with horses found the 50% lethal dose to be 21.5 mg/kg/day (4). The 50% lethal dose of lasalocid for rats is 122 mg/kg and for mice is 146 mg/kg (3). More studies of the toxicity of this compound are needed. In our studies no fatalities occurred in rats given 20 mg/kg/day for 4 weeks, suggesting no obvious toxicity. Also, the dosage of 10 mg/kg/day was protective in 80% of animals. Thus, the dosage required to protect 100% of animals is between 10 and 20 mg/kg/day.

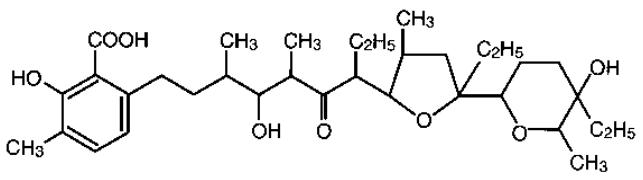

FIG. 1. Chemical structure of lasalocid.

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TABLE 1. PCP in immunosuppressed rats treated with different compounds

Drug and dosage (mg/kg/day)[a]	No. of rats					% of rats with PCP
	Total[b]	With PCP scored as[c]:				
		0	1+	2+	3+	
Lasalocid						
20	10	0	0	0	0	0
10	10	8	0	2	0	20
5	10	4	2	2	2	60
Control 1	12	1	4	4	3	92
Atovaquone						
100	10	10	0	0	0	0
10	8	4	0	3	1	50
Dapsone + TMP						
25 + 20	7	7	0	0	0	0
2.5 + 2.0	9	5	0	2	2	55
TMP + SMZ						
20 + 100	7	7	0	0	0	0
2 + 10	7	7	0	0	0	0
Control 2	8	0	0	0	8	100

[a] Control group 1 was used for lasalocid-treated groups, and control group 2 was used in a separate experiment with the other drugs. TMP, trimethoprim; SMZ, sulfamethoxazole.

[b] Excludes rats dying early from unrelated causes (bacterial or fungal infection).

[c] The extent of PCP lesions was scored from 0 to 3+ (most severe) on the basis of comparison with immunosuppressed but untreated control rats.

No studies have been reported to delineate the mechanism of antimicrobial activity of lasalocid in protozoa and fungi. Limited studies with *Streptococcus bovis* suggest that lasalocid catalyzes potassium-hydrogen exchange differences in the bacterial cell (15). Long and Jeffers found that lasalocid and other ionophorous drugs exert anticoccidial effects on the primary invasive stage and on the gametogonous stage of *Eimeria tenella* and *Eimeria necatrix*, although the mechanism is unknown (8).

In summary, lasalocid compares well with more common drugs, such as atovaquone and trimethoprim-sulfamethoxazole, in prevention of PCP.

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