Pyrazinoic Acid Amide—An Agent Active Against Experimental Murine Tuberculosis. (19447)

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The antituberculosis efficacy of niacinamide was first described by Chorine(1). He found that doses of 500 to 1000 mg per kg administered subcutaneously produced retardation of experimental tuberculosis in guinea pigs. This effect was later rediscovered by McKenzie *et al.*(2), who reported that a concentration of 0.75% niacinamide in diet prevented the progress of tuberculosis infection in mice. Kushner *et al.*(3) then studied the activity of a series of 30 derivatives covering substitution in the acid amide group and the ring structure. A number of amide nitrogen substituted derivatives were found active but inferior to niacinamide. Isosteres of niacinamide were not mentioned in this report. Studies by Fox(4) also confirmed the *in vivo* activity of niacinamide and added additional compounds to the series of inactive niacinamide-like compounds. At the Eleventh Conference on Chemotherapy of Tuberculosis, sponsored by the Veterans Administration, St. Louis, Jan. 17, 1952, S. Kushner of Lederle, R. L. Yeager, W. G. C. Munroe and F. I. Desseau from Summit Park Hospital and W. S. Schwartz from Oteen, a facility of the Veterans Administration, announced that pyrazinoic acid amide (Aldinamide) was active against experimental tuberculosis in the mouse and against clinical infection in man (5).

Studying niacinamide isosteres, we have likewise found that pyrazinoic acid amide is more active than niacinamide or para aminosalicylic acid when administered as components in diet.

Materials and Methods. The H37Rv strain of *Mycobacterium tuberculosis*, human type, was used in these studies. It was maintained by weekly serial passage in Dubos' Tween-Albumin medium containing 0.03% Tween 80 and virulence of strain was preserved by passage through mice after five transfers in culture medium. Barckmann IS-32 white mice weighing 14 to 19 g were injected intravenously with 0.25 ml of culture suspension adjusted with sterile distilled water to permit 70% transmission of light at 620 mμ.* Treatment was begun on the day following infection. For subcutaneous administration, test compounds were dissolved in sterile distilled water at concentrations containing daily doses per mouse in 0.25 ml of solution. Compounds given by subcutaneous route were administered once daily 5 days per week. For administration in diet the compounds were ground and incorporated into a stock diet† presented *ad libitum*. Experiments were continued until at least 50% of infected untreated animals died. Mice dying during the course of the experiment as well as those surviving at the end of test period were autopsied and lungs were preserved in 10% formalin. After a minimum of 48 hours of fixation the lungs were observed for the extent of gross tuberculosis involvement. The degree of involvement was graded as 0 for no visible involvement, 1+ (to 10% of lung tissue, grossly involved), 2+ (10 to 25% gross involvement) 3+ (25 to 50% gross involvement) and 4+ (more than 50%

* On a Coleman photoelectric spectrophotometer, Model 6A.

†		%
	Yellow corn meal	31
	Whole wheat flour	30
	Casein, technical	10
	Soy bean meal	10
	Linseed oil meal	7
	Yeast dried brewers	5
	Alfalfa meal	2
	NaCl	0.5
	$CaCO_3$	0.5
	Mazola oil	3
	Cod liver oil	1
	10 mg. vit. B_2/kg.	

involvement). Extensive experience has shown that gross scores correspond closely with values obtained by histological examination. *In vitro* efficacy tests were performed both with Dubos' Tween-albumin medium and Youmans' modification of Proskauer and Beck medium. Concentrated solutions of pyrazinoic acid amide were sterilized by filtration through Seitz EK pads and added aseptically to the test medium to yield final concentrations to 1000 γ/ml. The tubes were inoculated with 0.1 ml of culture in Dubos' medium adjusted to permit 90% transmission of light at 620 mμ and inspected for growth after 14 days of incubation at 37°C. For studying development of resistance, standardized culture suspension was added to a series of tubes containing 40 to 400 γ of pyrazinoic acid amide per ml of medium. After 2 weeks incubation, culture from the tube with a turbidity of approximately 90% transmission was transferred to a fresh series of tubes containing higher concentrations of compound. This procedure was continued at 2-week intervals.

Experimental. 1) *Comparison of pyrazinoic acid amide with niacinamide.* In a typical experiment to determine efficacy in diet, the compounds were tested at 0.1, 0.5 and 1.0% concentrations using groups of 8 mice each. Pyrazinoic acid amide at 1 and 0.5% concentration gave a score of .0 and at .1% the score was 0. Niacinamide gave a score of 1.4 at 1%, 2.5 at 0.5% and 3.7 at 0.1%. Thus pyrazinoic acid amide produced complete suppression of visible tuberculosis lesions at levels where niacinamide treated mice showed minimal to moderate involvement. The compounds did not significantly influence acceptability of the diet and the weights of mice treated with effective levels of compound were similar to those of normal controls. *Pyrazinoic acid amide* was also superior to niacinamide when the compounds were administered by subcutaneous injection. The compounds were tested at doses of 20, 10 and 5 mg per day administered 5 days per week. Both compounds gave scores of less than 0.5 at 20 mg. At the 10 mg level, pyrazinoic acid amide continued to give a score of less than 0.5 but niacinamide gave a score of 1.2. At a level of 5 mg niacinamide was ineffective but pyrazinoic acid amide gave a score of 2.

TABLE I. Comparison of Pyrazinoic Acid Amide with Para-Aminosalicylic Acid, Administration by Subcutaneous Route.

Dose, mg/ mouse/day	Lung score pyrazinoic acid amide	Para-amino-salicylic acid
Trial 1. Infection of high virulence (avg survival time, 20 days)		
1	3.3	3.2
2	1.5	.9
3	1.6	1
5	.6	.4
Lung score of infected controls—3.7		
Trial 2. Infection of low virulence (avg survival time, 33 days)		
1.25	4	4
2	3.6	4
3	3.1	3.9
4	1.1	4
Lung score of infected controls—3.6		

2) Comparison of pyrazinoic acid amide with para-aminosalicylic acid. Pyrazinoic acid amide and para-aminosalicylic acid were compared for efficacy as components in diet and by parenteral administration.

A) For comparison by parenteral route 2 trials are presented in Table I. In the first, pyrazinoic acid amide and para-aminosalicylic acid were administered subcutaneously in doses of 1, 2, 3, and 5 mg per mouse per day. Groups of 8 mice each were used. Both compounds demonstrated slight efficacy at 2 and 3 mg and good efficacy at 5 mg. On actual score basis, para-aminosalicylic acid showed slightly higher activity than pyrazinoic acid amide but the difference, approximately 20%, does not appear highly significant. In a second trial using similar doses and groups of eight mice, but testing with a culture of lower virulence, pyrazinoic acid amide was superior to para-aminosalicylic acid. Para-aminosalicylic acid was ineffective at all levels up to 4 mg per mouse per day whereas pyrazinoic acid amide showed slight efficacy at doses of 3 mg and good efficacy at 4 mg. The superiority of pyrazinoic acid amide when tested against infection characterized by longer average survival time was also observed when the compounds were administered as components of diet.

TABLE II. Comparison of Pyrazinoic Acid Amide with Para-Aminosalicylic Acid, Administration of Compounds in Diet.

Compound	% in diet	Gross lung scores: Undiluted inoculum	Gross lung scores: 10^{-1} dilution inoculum
Pyrazinoic acid amide	.1	4	3.9
	.5	.1	.3
	1		.1
	2		
Para-amino-salicylic acid	.1	2.6	3.2
	.5	1.2	3.3
	1	.1	1.9
	2		.8
Infected controls		4	4

B) For comparison by diet method a trial is presented in which both drugs were tested in mice infected with standardized suspension of a highly virulent sub-culture of strain H37Rv and a 10-fold dilution of the standardized suspension. Drug diet concentrations of 0.1, 0.5, 1 and 2% were given to groups of 8 mice. The average survival time was 14 days for infected controls inoculated with the undiluted culture suspension and 33 days for infected controls inoculated with diluted culture suspension. The lung scores are shown in Table II. As was observed with administration of the drug by parenteral route, the relative activity of pyrazinoic acid amide as compared with para-aminosalicylic acid when administered in a drug diet mixture was greater in the animals subjected to the slower infection. While a superiority of pyrazinoic acid amide was evident with the undiluted suspension, this compound showed significantly higher activity against infection produced with the diluted culture suspension. Also, the relative efficacy attained with pyrazinoic acid amide increased with rising concentration of drug in the diet.

Pyrazinoic acid amide did not show a high degree of *in vitro* activity. The inhibiting concentration was 150 γ/ml in Dubos' medium and 120 γ/ml in Youmans medium. Resistance studies were performed with Dubos' Tween-Albumin medium. The inhibitory concentration rose from 150 γ/ml to more than 1000 γ/ml after 3 serial transfers.

Conclusions. 1. These data contribute to the importance of niacinamide derivatives as a new class of antituberculosis agents. One of these, pyrazinoic acid amide, is active when administered by parenteral or oral route. Under suitable experimental conditions, pyrazinoic acid amide is more active than para-aminosalicylic acid; the activity of pyrazinoic acid amide as compared with para-aminosalicylic acid increases when the average survival time for infected controls is prolonged. 2. The *in vitro* inhibitory concentration for *M. tuberculosis,* human type, strain H37Rv, tested in Dubos' Tween-Albumin medium, is 150 γ/ml. Resistance develops rapidly *in vitro;* the inhibitory concentration rises to more than 1000 γ/ml after 3 serial bi-weekly transfers.

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4. Fox, H. Herbert, Abstracts of XIIth International Congress of Pure & Applied Chemistry, 1951, 296.

5. Minutes of Eleventh Conference on Chemotherapy of Tuberculosis, Veterans Administration, St. Louis, Mo. Meeting, Jan. 17-20, 1952. To be published.

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