Antibacterial Properties of Orinase in Infected Mice. (23081)

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The search for an orally active agent effective in treatment of diabetes mellitus has been carried on for many years. Recently, a group of synthetic sulfonamides has been reported to be capable, when administered orally, of reducing blood sugar concentrations in clinical diabetes(1-5) as well as in experimental animals(5-7).

While continuous administration of any physiologically active drug presents the obvious problem of toxicity, the constant use of a sulfonamide, even though not a sulfanilamide-like compound,* presents another aspect of toxicity not commonly encountered, *viz.*, the effect of the compound on bacterial flora of the host. This question is particularly pertinent in the diabetic individual in view of his increased susceptibility to infection. It therefore appeared advisable to investigate the antibacterial properties of some of the newly discovered antidiabetic agents. In this paper the antibacterial properties of 2 compounds having hypoglycemic activity, 1 - (p-methylphenylsulfonyl)-3 - butylurea or Orinase† and 1-(p-aminophenylsulfonyl)-3-butylurea, are compared with a commonly used triple sulfa mixture.

Methods. Male white mice (CF-1), 18-20 g, were selected at random from animal pools. Technics for establishing mouse infections, maintenance of cultures, etc., have been described(8,9). Freshly prepared stock suspensions in aqueous 0.25% Methocel‡ were prepared at beginning of each experiment and diluted so that the desired dose was contained in 0.2 ml when administered subcutaneously, or in 0.5 ml when administered by oral intubation. The triple sulfa mixture was prepared by thoroughly mixing in 1:1:1 ratio sulfadiazine, sulfamerazine and sulfamethazine. After blending, the mixture was considered an entity and used as the other two compounds. Preliminary work in non-infected animals demonstrated that subcutaneous doses as high as 400 mg/kg/day and oral doses as high as 800-1000 mg/kg/day of the sulfonamide compounds could be safely given to mice at daily intervals over a period of 6 days without gross signs of toxicity. The infecting dose and the drug were administered so that the drug was given immediately following the infecting dose and continued at daily intervals for 6 days. On the seventh day the surviving animals were sacrificed. Evaluation of activity was based on median protective dose (CD_{50}) as calculated by the method of Reed and Muench(10), and the standard error calculated as described by Miller and Tainter (11).

Results. Table II summarizes our data of the comparison of 1-(p-methylphenylsulfonyl)-3-butylurea with 1-(p-aminophenylsulfonyl)-3-butylurea and the triple sulfa mixture in infected mice. The failure of 1-(p-methylphenylsulfonyl) - 3 - butylurea to protect mice infected with lethal challenges of 10 different organisms is quite clearly demonstrated. None of the infected groups survived in statistically significant numbers in those instances where 1-(p-methylphenylsulfonyl)-3-butylurea was given via either oral or subcutaneous routes. This is in contrast to 1-(p-aminophenylsulfonyl)-3-butylurea which possesses a high degree of antibacterial activity in all infections studied with the exception of *Pseudomonas aeruginosa* (Table I). The latter compound is inferior to the triple sulfa mixture.

It is felt that the failure of 1-(p-methylphenylsulfonyl)-3-butylurea to protect infected mice was due to a true lack of antibacterial activity and that the animals did not die of cumulative toxicity produced by infection superimposed upon a near lethal hypoglycemia caused by a large dose of 1-(p-methylphenylsulfonyl)-3-butylurea. If death

* Unpublished.

† Orinase is the Upjohn trade name for 1-(p-methylphenylsulfonyl)-3-butylurea.

‡ Dow Chemical Co., Midland, Mich.

TABLE I. Comparison of *In Vivo* Antibacterial Activities* of Orinase, 1-(p-Methylphenylsulfonyl)-3-Butylurea, 1-(p-Aminophenylsulfonyl)-3-Butylurea, and a Triple Sulfa Mixture.

Organism	1-(p-aminophenylsulfonyl)-3-butylurea		Orinase		Triple sulfa	
	Subcut.	Oral	Subcut.	Oral	Subcut.	Oral
Streptococcus hemolyticus	66.1	54.3	>400	>600	37	10
Diplococcus pneumoniae I	353	236	"	"	114	121
Micrococcus aureus	100	50	"	"	18	10
Pasteurella multocida	55.1	46.8	"	"	14	10
Klebsiella pneumoniae	327	200	"	"	45	80
Proteus vulgaris	31.2	20.2	"	"	2.7	2.1
Pseudomonas aeruginosa	>500	395	"	"	31.4	28.3
Salmonella typhi	12.6	12.6	"	"	13	10
Salmonella paratyphi B	70.7	64	"	"	2.5	4.2
Escherichia coli	100	83	"	"	13.5	9.3

* Expressed as median protective dose in mg/kg/day.

TABLE II. Effect of Glucose* on Antibacterial Activities of 1-(p-Aminophenylsulfonyl)-3-Butylurea and Orinase, 1-(p-Methylphenylsulfonyl)-3-Butylurea in *S. hemolyticus* Infected Mice.

Log dil'n of challenge dose	Orinase†		1-(p-aminophenylsulfonyl)-3-butylurea		Control‡	
	Mort. rate		Mort. rate		Mort. rate	
	With glucose	Without glucose	With glucose	Without glucose	With glucose	Without glucose
4	20/20	20/20	0/20	0/20	10/10	10/10
5	"	19/20	"	"	"	"
6	16/20	17/20	"	"	5/10	8/10
7	5/20	11/20	"	"	"	1/10
8	0/20	0/20	"	"	1/10	0/10
Avg LD_{50}§	6.5	6.9	>4.0	>4.0	6.6	6.4

* Given as 5% in the drinking water. † Admin. orally by direct intubation at 500 mg/kg/day. ‡ Infected but untreated. § Expressed as log of median lethal dose.

had been due to increased susceptibility of the animal, due to a low blood sugar concentration or other forms of toxicity plus the infection, it could be expected that fewer bacterial cells in the challenge, as demonstrated by a higher LD_{50}, would cause death of the animals. Further, if the toxic effect of low blood sugar were countered with glucose administered to the animal, a therapeutic pattern resembling that of 1-(p-aminophenylsulfonyl)-3-butylurea should be observed. In an effort to demonstrate this, the following experiment was designed and carried out.

Two groups of 200 mice were treated with either 1 - (p-methylphenylsulfonyl)-3 - butylurea or 1-(p-aminophenylsulfonyl)-3-butylurea. The 2 groups were further divided into 5 groups of 40 mice, and each group was challenged with *Streptococcus hemolyticus* serially diluted in 10-fold increments. One-half of the animals treated with 1-(p-methylphenylsulfonyl)-3-butylurea was given glucose in drinking water, while the remaining half was given plain water. Appropriate untreated infected controls were used for comparative purposes. The median lethal dose of *S. hemolyticus* for each group was used as a measure of altered susceptibility to the infection.

It can be noted from the data (Table II) that addition of glucose (5%) to the infected animals did not alter susceptibility of the different groups to the *S. hemolyticus* infections. The groups receiving 1-(p-aminophenylsulfonyl)-3-butylurea was protected whether glucose was administered to mice or not, and the susceptibility of mice receiving 1-(p-methylphenylsulfonyl)-3-butylurea was unchanged in the untreated starved controls.

The influence of one bacteriologically active agent upon activity of a second antibacterial agent has been studied in detail by numerous workers. Many types of different ac-

TABLE III. Effect of Orinase, 1-(p-Methylphenylsulfonyl)-3-Butylurea on the Activity of Tetracycline in *S. hemolyticus* Infected Mice, 20 Mice per Series.

Drug level*				Drug level*			
Orinase	Tetracycline	Mort. ratio†	CD_{50}‡	Orinase	Tetracycline	Mort. ratio†	CD_{50}‡
250	.3	19/20	.73 (.53-.93)	None	.3	17/20	.72 (.60-.84)
"	.6	14/20		"	.6	12/20	
"	1.2	1/20		"	1.2	5/20	
"	2.4	0/20		"	2.4	1/20	
"	4.8	"		"	4.8	0/20	

* Expressed in mg/kg/day. † No. dead/total No. ‡ Median protective dose (95% confidence limits).

tions, including synergism and antagonism, have been demonstrated depending upon the antibiotics and upon the proportions. To establish that 1-(p-methylphenylsulfonyl)-3-butylurea does not interfere with antibacterial action of an antibiotic, the compound was administered orally to a group of infected mice being treated subcutaneously with tetracycline§ while a second group of infected mice was treated with the antibiotic alone. The median protective dose of tetracycline was determined both alone and in the presence of 250 mg/kg/day of 1-(p-methylphenylsulfonyl)-3-butylurea and used as the basis of evaluation.

From the results presented in Table III, it is obvious that 1-(p-methylphenylsulfonyl)-3-butylurea does not interfere with the protective action of tetracycline in *S. hemolyticus* infected mice, and that tetracycline therapy could be carried out in the presence of relatively large daily doses of 1-(p-methylphenylsulfonyl)-3-butylurea.

Summary. 1. The chemotherapeutic properties of 2 newly discovered sulfonamide antidiabetic agents have been studied in infected mice and compared to a commonly used triple sulfa mixture. Data demonstrating that 1-(p-methylphenylsulfonyl)-3-butylurea is devoid of any antibacterial activity and that 1-(p-aminophenylsulfonyl)-3-butylurea is active as an antibacterial agent have been presented. Lack of antibacterial activity of 1-(p-methylphenylsulfonyl)-3-butylurea can be attributed to absence of the para-amino group. This para-amino group is present in each of the ingredients in the triple sulfa mixture as well as other sulfanilamide-like compounds. 2. It has been demonstrated that failure of 1-(p-methylphenylsulfonyl)-3-butylurea to protect infected mice was due to a true lack of antibacterial activity, and death of test animals was not attributed to toxicity. 3. Large daily doses of 1-(p-methylphenylsulfonyl)-3-butylurea have been shown not to interfere with therapeutic activity of tetracycline in infected mice.

§ Upjohn Tetracycline (Panmycin) was used.

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