ACTIVITY OF DI-N-HYDROXYQUINOXALINE DERIVATIVES AND OF DEPOT SULFONAMIDES IN EXPERIMENTAL NAG VIBRIO INFECTION

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UDC 616.34-002.7.579.843.1]-092.9-085.281: [547.863.11+547.551.525.211.1]-036.8

KEY WORDS: muscles; NAG vibrio infection; dioxidine; quinoxidine; lysozyme.

Intestinal infections are characterized by an increase in the number of causative agents with multiple drug resistance [2-5, 7]; the search for new and effective chemotherapeutic remedies is thus of great practical importance [1].

The object of this investigation was to study the activity of new di-N-hydroxyquinoxaline derivatives — dioxidine and quinoxidine [6] — and of two depot sulfonamides — sulfalen and sulfamonomethoxine — in experimental NAT vibrio infection. Dioxidine and quinoxidine are new original drugs developed at the S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute; sulfalen and sulfamonomethoxine are depot sulfonamides described in the literature; in the USSR they have been synthesized by the Ordzhonikidze Institute. Data on the activity of these substances in NAG infection are not to be found in the literature.

EXPERIMENTAL METHOD

The bacteriostatic and bactericidal activity of dioxidine against 115 strains of NAG vibrios was determined in accordance with the technical instruction No. 250 of the Ministry of Health of the USSR, dated 1975. The strains were isolated in 1970-1975 from patients with intestinal infections, from vibrio carriers, and from external environmental objects. Their morphological, cultural, biochemical, and serologic properties were studied in preliminary experiments.

In mice with experimental NAG infection caused by intraperitoneal inoculation of the animals activity of dioxidine, quinoxidine, sulfalen, and sulfamonomethoxine was studied. The experiments were carried out on 830 noninbred albino mice weighing 18-20 g. Strain No. 10703 of NAG vibrio, virulent for mice and rabbits, and sensitive to dioxidine in a concentration of 4 μg/ml, was used for infection. To study the activity of the drugs on the basis of the animals' survival rate, an infecting dose of 5×10^8 bacterial cells in 0.5 ml physiological saline was used: This dose caused the death of 90-100% of untreated control mice. The sterilizing action of the drugs on NAG vibrios was studied in experiments with a smaller infecting dose, namely 1×10^8 bacterial cells, by means of which it was possible to study the ability to culture living vibrios during a long period of survival of the untreated control animals (up to 30 days). Treatment began immediately after infection, and the drugs were given internally in a single dose or over a period of 3 days.

In a special series of experiments the effect of lysozyme (given internally or subcutaneously) on the sterilizing effect of dioxidine and quinoxidine was studied. Daily throughout the period of observation on the animals (10-30 days) bacteriological specimens of liver and spleen homogenates, the contents of the stomach and intestine, and the feces were cultured in peptone water and on alkaline agar and TCBS medium. The effectiveness of the drugs was assessed on the basis of the survival rate and length of survival of the animals, the clinical picture of disease, and the results of the bacteriological tests.

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Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 5, pp. 76-78, May, 1982. Original article submitted November 27, 1981.

0007-4888/82/9305-0637$07.50

TABLE 1. Effectiveness of Dioxidine, Quinoxidine, Sulfalen, and Sulfamonomethoxine in Albino Mice with Experimental Septicemia Caused by Intraperitoneal Injection of NAG Vibrios (5×10^8 bacterial cells)

Treatment	Dose, mg/kg (once daily)	Mode of administration	Number of animals	Number which survived, %		Total length of survival of animals	Assessment of activity of dose
Control			150	11	7,3	110/1500	
Dioxidine	200	Subcutaneously	53	48	90	480/530	Highly active
		Internally	70	68	97	680/700	
	100	Subcutaneously	23	21	91	210/230	"
		Internally	23	22	96	220/230	"
	50	Subcutaneously	20	18	90	180/200	"
		Internally	30	27	90	270/300	
	25	Subcutaneously	20	16	80	160/200	"
		Internally	18	17	94	170/180	
	12,5	"	33	24	73	240/330	Active
	5	"	30	0	0		Inactive
Quinoxidine	500	Internally	74	68	92	680/740	Highly active
	250	"	25	23	92	230/250	"
	50	"	25	21	84	210/250	"
Sulfalen	500	"	25	7	28	70/250	Weakly active
Sulfamonomethoxine	500	"	30	0	0		Inactive

Legend. Numerator gives number of mouse-days in the given group of animals, denominator gives maximal possible number of mouse-days.

TABLE 2. Positive Cultures of NAG Vibrios from Mice with Experimental NAG Infection (infecting dose 1×10^8 bacterial cells, intraperitoneally)

Treatment	Dose, mg/kg	No. of animals	Positive cultures on undermentioned days*					
			2—3	4—5	6—7	11—15	16—20	21—30
Control		41	+	+	+	+	+	+
Dioxidine	200	35	+	±	—	—	—	—
Dioxidine + lysozyme	100+1	35	±	—	—	—	—	—
Quinoxidine	500	35	+	±	—	—	—	—
Quinoixdine + lysozyme	250+1	35	±	—	—	—	—	—

*Time at which vibrios were seeded from 3-4 mice.

Legend. +) Positive cultures from all objects tested, ±) some cultures positive, —) all cultures negative.

EXPERIMENTAL RESULTS

A study of 115 strains of NAG vibrios showed that in their morphological, cultural, biochemical, and serologic properties all were typical representatives of this species. Dioxidine was highly active *in vitro* against all strains of NAG vibrios. The minimal inhibitory concentrations varied from 1 to 62 μg/ml. In concentrations of 4-250 μg/ml dioxidine had a bactericidal action. As Table 1 shows dioxidine, in doses of 25-250 mg/kg daily, by internal or subcutaneous administration in a single dose or over a period of 3 days, gave a marked chemotherapeutic effect in experimental NAG infection, resulting in survival of 80-97% of the treated animals, although with virtually no effect on prolonging the life of the mice which died. In a dose of 5 mg/kg daily dioxidine was inactive. Quinoxidine also had high chemotherapeutic activity in the doses tested. Under the experimental conditions used, with an infecting dose of 5 × 10 bacterial cells, sulfalen had only weak activity despite the use of very high doses (500 mg/kg daily), and sulfamonomethoxine had no chemotherapeutic action.

Attempts to grow cultures of NAG vibrios after the use of a smaller infecting dose showed that dioxidine in a dose of 200 mg/kg and quinoxidine in a dose of 500 mg/kg considerably reduced the period during which positive cultures of vibrios could be grown: Under the influence of the drugs vibrios could be isolated from the experimental animals for 30 days.

During combined treatment of NAG infection with dioxidine (100 mg/kg) or quinoxidine (250 mg/kg daily) together with lysozymes, in a dose of 1 mg per mouse internally or subcutaneously, the animals could be completely freed from NAG vibrios during the first 3 days. The results of this investigation are given in Table 2.

Dioxidine and quinoxidine, derivatives of di-N-hydroxyquinoxaline, are thus highly active in NAG vibrios, give a chemotherapeutic effect against experimental NAG infection, and have a sterilizing action. The activity of these compounds was shown to be potentiated when given in combination with lysozyme. Clinical trials of dioxidine and quinoxidine in NAG infection in man and also the study of the activity of other quinoxiline derivatives against NAG vibrios and the further study of the action of lysozyme in conjunction with dioxidine or quinoxidine, is interesting with the aim of developing optimal programs for the clinical use of these compounds. The depot sulfonamides sulfalen and sulfamonomethoxine are of no practical interest as drugs for the treatment of NAG infection in man.

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