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COMPARATIVE EVALUATION OF THE EFFECTIVENESS OF DIOXIDINE IN EXPERIMENTAL MENINGOENCEPHALITIS IN MICE

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UDC 616.831.9-002.022.7-085.21-036.8-092.9

The antibacterial drug dioxidine [2, 7] is of interest in the complex etiotropic therapy of purulent bacterial meningitis, when the use of other known chemotherapeutic agents is ineffective or impossible for one reason or another [3, 8]. In view of this, an experimental study of the activity of the drug on models of bacterial infections according to the data of pathomorphological investigations is substantiated.

High activity of dioxidine with respect to the indices of survival, lifetime, and health-improving effect has been established on models of generalized bacterial infections of white mice, caused by intraperitoneal infection of the animals [5]. However, on account of the absence of the morphological changes in the internal organs typical of each infection [1], it is impossible to evaluate the effectiveness of the drugs according to the results of pathomorphological investigations. In addition, it is known that the survival of the animals and the health-improving effect are far from always correlated with the absence of pathological changes in the focus of the lesion.

Most valuable from the standpoint of morphological investigations are models close in patho- and morphogenesis to the pathological processes in humans, which permit an objective evaluation of the degree of the chemotherapeutic effect in the dynamics of the infection process. Models of meningoencephalitis in mice, induced by intracerebral infection with *Ps. aeruginosa*, *Kl. pneumoniae*, and *Staph. aureus* [4, 6, 9], developed in the laboratory of chemotherapy of infectious diseases of the S. Ordzhonikidze All-Union Chemicopharmaceutical Scientific Research Institute [4, 6, 9], correspond to these requirements to a substantial degree.

In this investigation on mice we made a comparative pathological evaluation of the activity of dioxidine on a model of purulent meningoencephalitis induced by *Ps. aeruginosa* [strain 165, infecting dose (ID) $5 \cdot 10^6$ microbial cells], hemorrhagic-purulent meningoencephalitis induced by *Kl. penumoniae* (strain 444, ID 250 microbial cells), and on a model of necrotic-purulent encephalomeningitis induced by *Staph. aureus* (strain 178, polyresistant, including resistance to oxacillin, ID $4 \cdot 10^8$ and $8 \cdot 10^8$ microbial cells).

The dioxidine activity was compared with the activity of quinoxidine (the active ingredient in this drug is dioxidine), gentamycin (in experiments with *Staph. aureus* and *Ps. aeruginosa*), levomycetin and levomycetin hemisuccinate (in experiments with *Ps. aeruginosa* and *Kl. pneumoniae*) and oxacillin (in experiments with *Staph. aureus*).

S. Ordzhonikidze All-Union Chemicopharmaceutical Scientific-Research Institute. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 19, No. 10, pp. 1168-1175, October, 1985. Original article submitted December 27, 1984.

0091-150X/85/1910-0661$09.50

TABLE 1. Activity of Dioxidine and Quinoxidine in *Pyocyaneus* and *Klebsiella* Experimental Meningoencephalitis of Mice according to the Data of Pathomorphological Investigation; Single Treatment, 30 min after Infection

Pathogen of infection	Drug	Dose (mg/kg), method of administration	Average index of lesion of brain*		
			24—48 h	72 h	10—20 days
Ps. aeruginosa	Dioxidine	50, internally	3,0—3,3	3,5	—
		100, internally	0,6	1,2	0,2
		200, internally	—	0,2	0
		400, internally	0,2	0,1	0
	Quinoxidine	500, internally	0,2	—	0,4
	Control	—	4,0	All the animals died after 24—48 h	
Kl. pneumoniae	Dioxidine	500, internally	0,2	0	0
		500, subcutaneously	0	0	0
		50, internally	0,3	0,2	—
		50, subcutaneously	0,2	0,3	—
	Quinoxidine	500, internally	0,2	0	0
		200, internally	0,2	0	—
	Control	—	4,0	All the animals died after 24—48 h	

*In each of the groups examined there were 5-10 mice; the animals that survived and those that died in the indicated periods were investigated.

A total of 19 experiments were conducted on 620 white mice with pathomorphological investigation of the brain, the bones of the skull, and internal organs (liver, kidneys, heart, lungs).

The drugs were used in the most effective doses with respect to survival of the animals in the case of a single administration, including the maximum tolerable doses. Dioxidine, which exhibited the highest activity, was investigated in a wide dose range (from 12.5 to 500 mg/kg). The effectiveness of therapy was evaluated under conditions of early (30 min after infection) and delayed (after 24-48 h) beginning of therapy. Dioxidine was administered internally and subcutaneously, quinoxidine and levomycetin internally, and gentamycin, levomycetin hemisuccinate, and oxacillin subcutaneously.

For a comparison of the activity of the preparations we estimated the degree of morphological changes in the brain membranes and tissue of the animals on the basis of the criteria developed earlier [4, 6, 9] according to a four-point system, considering the peculiarities of the lesion depending on the type of pathogen. The nature of the inflammatory reaction, the degree and extent of damage to the brain membranes, the state of the blood vessels, and the nature of the changes in the brain tissue were taken into account. The maximum degree of lesion (++++ in untreated animals and in the absence of a therapeutic effect) corresponded to four points. For each group of animals we calculated the average index of lesion, taking each + as a unit. The nature of the morphological changes in the brain and internal organs was determined by the pathogen of the infection. An analysis of the results of pathomorphological investigations showed a dependence of the degree of therapeutic effect on the pathogen and peculiarities of the pathomorphological process in developing meningoencephalitis.

In the experiments with *Ps. aeruginosa* there was an acute course of the process and death of the control mice 24-48 h after infection. The animals developed diffuse infiltration of the membranes by segmentonuclear leukocytes, purulent ventriculitis and edema of the brain (Fig. 1a, b). In the most severe cases, microabscesses were detected in the brain. The average index of lesion was equal to 4.0 (Table 1). Moreover, a constant symptom of this infection was purulent pyelonephritis, kidney abscesses, and paranephritis.

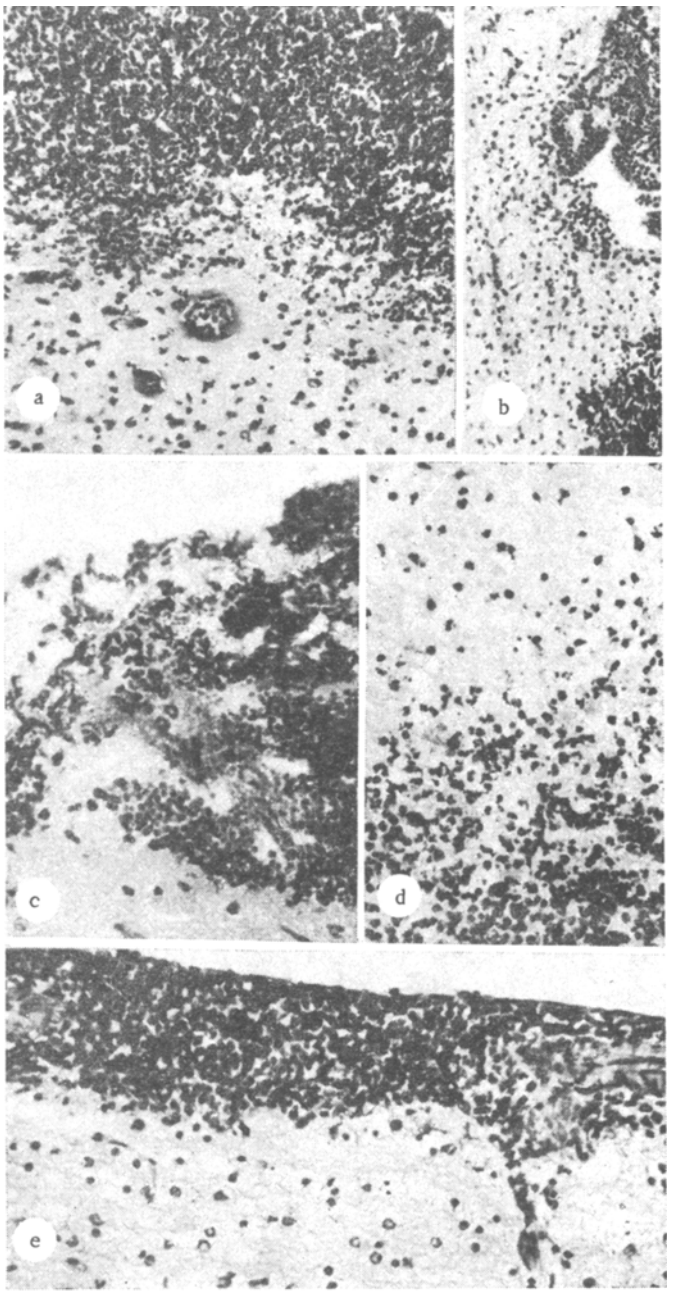

Fig. 1. Pathomorphological characteristics of experimental meningeoncephalitis in mice, induced by *Ps. aeruginosa* (a, b), *Kl. pneumoniae* (c), and *Staph. aureus* (d, e). Staining with hematoxylin and eosin. a) Purulent meningitis: the pia mater is sharply thickened, infiltrated with segmentonuclear leukocytes, penetrating into the underlying tissue, ×250; b) purulent ventriculitis: purulent exudate is in the lumen of the third ventricle of the brain and is infiltrating the vascular plexus, ×160; c) hemorrhagicpurulent meningitis: pronounced edema and penetration of the pia mater by purulent-hemorrhagic exudate with a large amount of the pathogen, ×250; d) focus of necrosis of brain tissue, infiltrated by leukocytes and surrounded by a leukocytic wall, ×260; e) focal purulent meningitis: edematous pia mater, infiltrated by segmentonuclear leukocytes, ×160.

On a model of meningoencephalitis induced by *Bacillus pyocyaneus* (*Ps. aeruginosa*), a high therapeutic effect of dioxidine and quinoxidine was established (see Table 1), as well as a dependence of

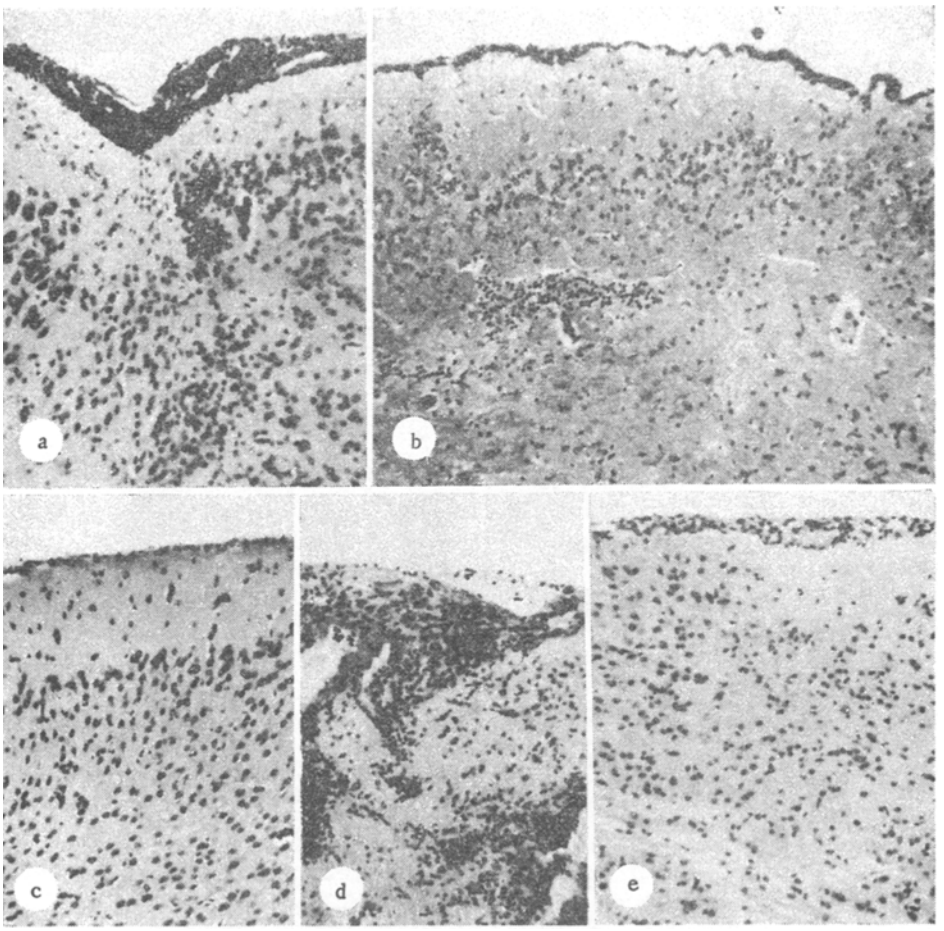

Fig. 2. Results of treatment of mice with experimental meningoencephalitis induced by *Ps. aeruginosa* (a, b), *Kl. pneumoniae* (c), and *Staph. aureus* (d, e) with derivatives of the di-N-oxide of quinoxaline and gentamycin. Staining with hematoxin and eosin. a) Treatment with gentamycin in a dose of 200 mg/kg, purulent meningitis: pia mater thickened and infiltrated by segmentonuclear leukocytes, ×160; b) treatment with quinoxidine in a dose of 400 mg/kg, focal infiltration of pia mater by lymphoid and histocytic cells, ×160; c) treatment with dioxidine in a dose of 400 mg/kg, pia mater and underlying tissue unchanged, ×160; d) treatment with gentamycin in a dose of 200 mg/kg, focus of necrosis and purulent infiltration of brain tissue, ×160; e) treatment with dioxidine in a dose of 400 mg/kg, focal infiltration of pia mater by lymphoid and histiocytic cells, ×160.

the effect on the value of the therapeutic dose. Under the influence of active doses of the drugs, the development of microabscesses in the brain and symptoms of encephalitis, purulent vetriculitis, and purulent meningitis was prevented. In part of the treated animals, the changes were manifested only in a focal thickening of the brain membranes on account of infiltration by lymphoid and histiocytic cells (Fig. 2b). The changes detected represent a therapeutically determined pathomorphosis of the infectious process. Under the influence of effective doses of the drugs, purulent pyelonephritis and abscesses in the kidneys did not develop. When doses ineffective according to morphological evaluation were used (for dioxidine 12.5-50 mg/kg), the average index of lesion approached the control, at 3.5 (see Table 1).

Gentamycin, highly active in septicemia induced by intraperitoneal injection of a culture of *Ps. aeruginosa*, proved relatively ineffective on the model of purulent meningoencephalitis, even when used in the maximum tolerable dose — 200 mg/kg (Fig. 2a). The index of lesion in this group of animals was 2.2.

When levomycitin hemisuccinate was used in a dose of 500 mg/kg, converted to levomycitin in part of the treated mice, foci of purulent inflammation in the brain tissue, as well as pur-

TABLE 2. Activity of Dioxidine and Quinoxidine in Experimental Staphylococcal Meningoencephalitis in Mice according to the Data of Pathomorphological Investigation; Single Treatment, 30 minutes after Infection*

Drug	Number of animals	Dose (mg/kg), mode of administration	Infecting dose (number of microbial cells) × 10^8	Average index of brain lesion		
				24—48 h	72 h	5—10 h
Control	38	—	1	3,0	3,3	all the animals died after 24-48 h
			4	2,0	—	
Dioxidine	15	100, subcutaneous	4	3,6—4,0	3,6	—
	12	200, subcutaneous	4	2,0—2,2	2,3	—
	25	400, internal	1	1,6	0,5	0,2—0,8
			4	—	—	0,8
	30	400, subcutaneous	1	2,4	0,5	0—0,6
			4	2,6	1,6	—
Quinoxidine	20	400, internal	1	3,0	1,3	3,0—3,5
Gentamycin	15	100, subcutaneous	4	2,6—3,3	1,6	—
	10	200, subcutaneous	4	2,1	1,5	—
Oxycillin	10	400, subcutaneous	4	3,0	1,1	—

*Animals that survived or died in the indicated periods were investigated.

ulent exudate in the ventricles and membranes of the brain (average index of lesion 2.0), were observed 24 h after intracerebral infection.

In experiments with *Kl. pneumoniae*, the mice developed hemorrhagicpurulent meningoencephalitis. Generalization of the process and death of the control animals were observed on the second to fifth days. Lesion of the vascular walls was recorded in the brain tissue, frequently with phenomena of plasma impregnation and fibrinoid necrosis, as well as pronounced microbial edema and hemorrhagic exudate (Fig. 1c).

On a model of *Klebsiella* meningoencephalitis, dioxidine in doses of 50-500 mg/kg and quinoxidine in doses of 200-500 mg/kg, administered once in early periods after infection, after 30 minutes, virtually entirely prevented the development of pathological changes in the brain tissue (see Table 1 and Fig. 2c). Under conditions of delayed treatment (beginning of administration of the drugs 24-48 h after infection), high effectiveness of dioxidine and quinoxidine ("morphological recovery") was manifested only when the drug was administered for three days. Levomycitin and levomycitin succinate were not as active as the quinoxaline derivatives. Even when the drugs were administered 30 minutes after infection, disseminated hemorrhagicpurulent meningoencephalitis was recorded in part of the mice.

In the experiments with *Staph. aureus*, the mice exhibited necrotic-purulent encephalomeningitis. A peculiarity of the model is the formation of an extensive region of necrosis at the site of introduction of the pathogen of infection, with the formation of an abscess on the second to fifth day (Fig. 1d). Osteomyelitis of the cranial bones was noted in 50% of the cases. Purulent focal (Fig. 1e) or diffuse inflammation was recorded in the brain membranes. Death of the mice occurred after 24-48 h or on the third to fifth day, depending on the value of the ID. In addition to generalization of infection, the development of extensive focal necrosis in the brain tissue is significant in pathogenesis. Metastatic purulent foci were detected in the internal organs of the animals — kidneys, lungs, myocardium, and sometimes in the liver.

On a model of staphylococcal meningoencephalitis (Table 2), dioxidine and quinoxidine were significantly less effective than in the experiments with *Ps. aeruginosa* and *Kl. pneumoniae*. Dioxidine in a dose of 100 mg/kg did not prevent the development of destructive changes in the brain tissue of the mice: after 24 h large foci of necrosis, followed by abscessing, as well as the development of purulent meningitis and osteomyelitis of the cranial bones, were detected in the region of infection. In a dose of 200 mg/kg the drug prevented the development of severe purulent lesions of the brain in two thirds of the animals; however, the average index of lesion was even higher (2.2-2.3 — see Table 2). A dose of 400 mg/kg, ensuring high survival, did not always prevent the development of destructive and in-

flammatory changes in the brain; of the 55 mice examined, which received dioxidine in this dose, foci of necrosis were detected in seven. Only in individual cases were changes limited to thickening of the brain membranes on account of lymphohistiocytic infiltration (Fig. 2e). Osteomyelitis of the cranial bones was absent; metastatic purulent lesions of the internal organs were rarely encountered (kidney abscesses and purulent pyelonephritis were detected in two out of 60 treated mice).

Quinoxidine in a dose of 400 mg/kg was less active than dioxidine: severe destructive changes in the brain were observed in 50% of the treated animals 24-48 h after injection.

Gentamycin, just as in the experiments with *Ps. aeruginosa*, according to the data of pathomorphological investigation, proved relatively ineffective on a model of staphylococcal necrotic-purulent encephalomeningitis (see Table 2, and Fig. 2d). When the maximum tolerable dose (200 mg/kg) was used, brain lesion was noted in one third of the animals; at a dose of 100 mg/kg, foci of the necrosis and abscessing of the brain tissue, as well as internal organs, were detected in all the mice. Oxacillin also proved relatively ineffective according to the data of pathomorphological investigation (see Table 2).

Thus, a comparative estimation of the effectiveness of dioxidine and quinoxidine, gentamycin, levomycitin, levomycitin hemisuccinate, and oxacillin according to the data of pathomorphological investigations conducted on three models of purulent bacterial meningoencephalitis in mice, showed that derivatives of the di-N-oxide of quinoxaline — dioxidine and quinoxidine — have a high chemotherapeutic activity. The activity of these drugs depends on the pathogen of infection. The highest effect was noted in experiments with *Ps. aeruginosa* and *Kl. pneumoniae* (despite the significant resistance of *Ps. aeruginosa* to the drugs *in vitro*: MPC $\geq$ 125 µg/ml). In experiments with *Staphylococcus*, the activity of these drugs is substantially lowered, which is evidently due primarily to the development of a severe necrotic lesion of the brain tissue under the action of staphylococcal toxin; moreover, dioxidine is somewhat more active than quinoxidine.

The high effectiveness of derivatives of the di-N-oxide in quinoxiline on certain models of meningoencephalitis, including the case of oral administration of the drugs, is apparently evidence of their good absorption and rapid penetration through the blood-brain barrier (BBB). Of interest from this standpoint is the substantial lowering of the gentamycin activity (it penetrates poorly through the BBB) on models of meningoencephalitis in comparison with its high activity on models of septicemia, unaccompanied by purulent lesion of the brain and tissue.

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