Effects of Drotaverine Hydrochloride on Viability of Rat Cultured Cerebellar Granulocytes

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The neurocytotoxic effect of drotaverine hydrochloride was studied in culture of rat cerebellar granulocytes. Incubation of cells with 100 and 250 μM drotaverine reduced neuronal survival to 60 and 4%, respectively.

Key Words: *drotaverine; cerebellar granulocyte culture; neurocytotoxicity; stroke*

Treatment of brain stroke is one of the priorities of modern neurology. Increasing incidence of brain stroke and high mortality from this condition, severe disability of patients with rather limited prospects of recovery of their functions and working capacity determine the medical and social consequences of the disease and necessitate the development of effective complex system for rehabilitation of patients after stroke.

An important problem of drug correction of changes in metabolism and cerebral hemodynamics in patients with acute disorders of cerebral circulation is to attain the balance between the severity of these disorders and energy requirements of the brain tissue. This balance can be attained by using a complex of drugs of several pharmacological groups: vasodilators, nootropic drugs, drugs improving the metabolic processes in the brain, *etc.* According to some data, vasodilators, for example, drotaverine hydrochloride (nicospan, no-spa) promote normalization of cerebral circulation and oxygen and glucose supply to the brain [1].

It is noteworthy, however, that vasoactive drugs, particularly in high doses, in addition to dilatation of the cerebral vessels can significantly reduce their total peripheral resistance and systemic BP, which can lead to the progress of cerebral ischemia under conditions of chronic cerebral circulation insufficiency. These effects have been described, for example, for papaverine and its hydroderivatives [7,8]. It is therefore important to study the neurotoxic effects of drotaverine, a structural and functional analog of papaverine.

We studied the effects of drotaverine hydrochloride (drotaverine) on survival of cultured cerebellar granulocytes.

MATERIALS AND METHODS

Cerebellar cells of 7-day-old rats were cultured as described previously [2]. On day 7 of culturing, drotaverine (Biomed) dissolved in bidistilled water was added to nutrient medium in a final concentrations of 100 and 250 μM. Similar volumes of bidistilled water were added into control cultures. After 4 h, the cultures were fixed and stained with cresyl violet after Nissl.

The preparations were photographed in the Olympus microscope (Olympus Optical Corp.) with a CC-12 digital camera at ×40. The percentage of dead neurons in each visual field was evaluated in 5-15 microphotographs for each culture.

The results were statistically processed using Student's *t* test.

RESULTS

The neurocytotoxic effect of drotaverine was clearly concentration-dependent. High concentration (250 μM) caused virtually complete death of nerve cells (Figs. 1, 2).

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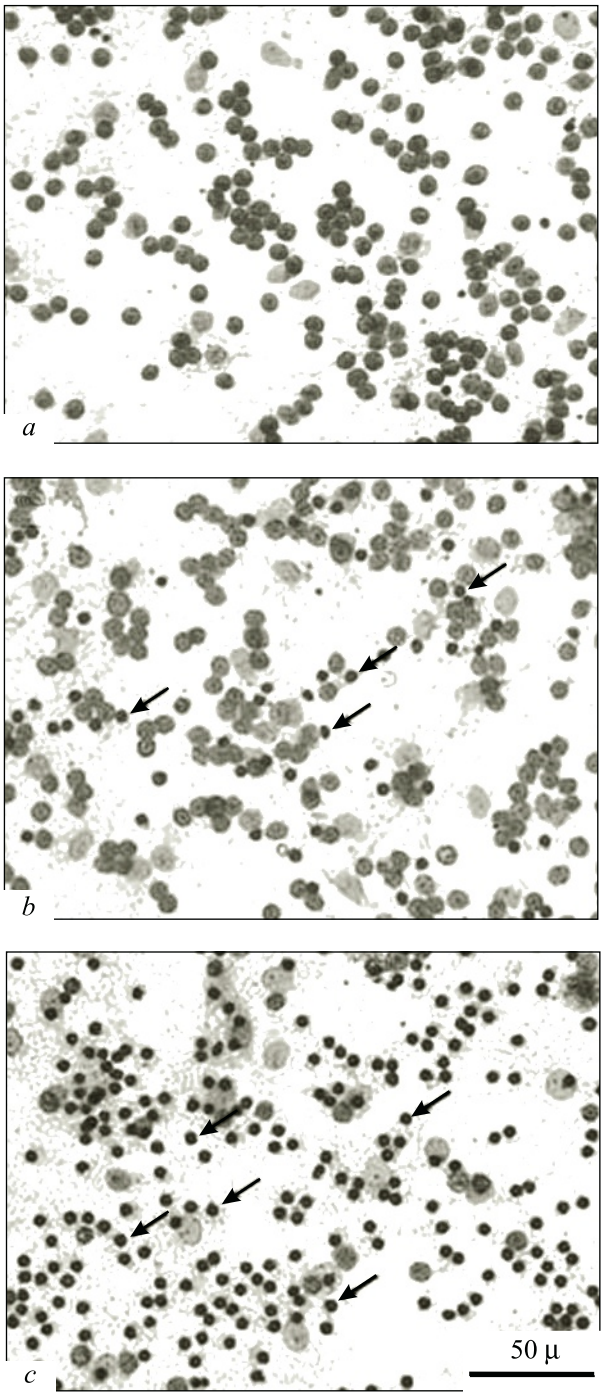

Fig. 1. Drotaverine neurotoxicity in cerebellar cell culture. *a*) control; *b*, *c*) drotaverine, 100 and 250 μM, respectively. *a*) numerous intact cerebellar round granulocytes without signs of destruction; *b*, *c*) pyknotic nuclei of dead neurons (arrows) among intact cells. Nissl's cresyl violet staining.

Despite the absence of data on drotaverine penetration into CNS *in vivo*, its use in high doses leading to hyperdilatation of the cerebral blood vessels and to increase of their permeability can lead to opening of access for drotaverine directly to nerve cells. Therefore, drotaverine overdosage is fraught with a much more serious side effect than that mentioned in pharmacological descriptions of this drug.

The mechanisms of neurocytotoxic effect of drotaverine can be due to its structure. It is a tetrahydroisoquinoline (Fig. 3, *a*). Compounds of this type are synthesized in animal organisms. Some of them, for example 1-benzyl-1,2,3,4-tetrahydroisoquinoline (Fig. 3, *b*) and salsolinol (1-methyl-1,2,3,4-tetrahydroisoquinoline; Fig. 3, *c*) are neurotoxic [3,6,10] and their endogenous formation is assumed to be one of the main causes of Parkinson's disease [4].

In the body, drotaverine can be reduced to 1-benzyl-1,2,3,4-tetrahydroisoquinoline, which can explain its neurotoxicity, but as there are no data on drotaverine metabolites, it is just a hypothesis.

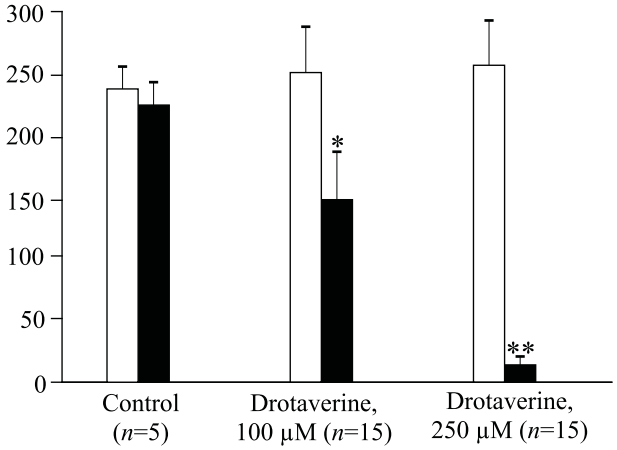

Fig. 2. Number of intact granulocytes per field of view. *$p<0.05$, **$p<0.01$ in comparison with the control. Open bars: before therapy; dark bars: after therapy.

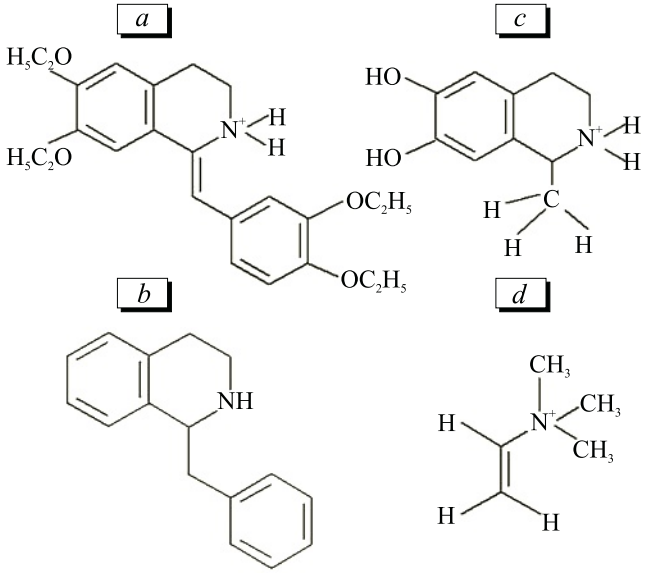

Fig. 3. Chemical formulae of substances. *a*) drotaverine; *b*) 1-benzyl-1,2,3,4-tetrahydroisoquinoline; *c*) salsolinol (1-methyl-1,2,3,4-tetrahydroisoquinoline); *d*) neurine.

Presumably, the mechanism of drotaverine neurotoxicity is identical to the mechanism of toxic effect of neurine (vinyl trimethyl ammonium hydroxide; Fig. 3, *d*) – product of acetylcholine endogenous transformation [9]. Drotaverine and neurine have a double bond in a similar position in relation to the ammonium group and during oxidation can be transformed into highly toxic epoxyderivative compounds [5].

Whatever is the mechanism of the neurotoxicity found in our study, these data should be taken into consideration in drotaverine therapy.

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