Effect of Active Fraction of Cerebral on Expression of Caspase-3 and β-Amyloid Precursor Protein during Therapy of Hemorrhagic Stroke in the Acute and Delayed Periods

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Active anti-stroke fraction of Cerebral preparation (extract of water-soluble molecules from brain tissue of animals with hemorrhagic stroke) decreased caspase-3 expression and improved survival of experimental animals in the acute period after hemorrhagic stroke.

Key Words: *hemorrhagic stroke; caspase-3; β-amyloid precursor protein; active fraction of preparation Cerebral*

Hemorrhagic stroke (HS) constitutes 15% of cerebral circulatory disorders [12]. The search for preparations for the therapy of this condition is an urgent problem.

Cerebral is an extract of water-soluble molecules from the brain cortex of animals survived HS [1]. After intranasal administration this preparation is transported to brain cells over the olfactory tract bypassing the blood-brain barrier, which determines its rapid therapeutic effect in patients with HS and advantages over traditional drugs [1,2].

A considerable number of nerve cells undergo apoptosis during HS. Caspase-3 is the main effector protease in apoptosis. This cysteine protease cleaves various structural proteins maintaining architectonics and integrity of the cytoplasm and nucleus [6]. β-Amyloid precursor protein is one of the substrates for caspase-3 [5]. This protein in physiological concentration stimulates cell proliferation and adhesion [11], provides a relation between the cytoskeleton and extracellular space, and regulates or directs axonal growth [10]. Overexpression of β-amyloid precursor protein and accumulation of degradation products (*e.g.*, neurotoxic β-amyloid) are the main marker of apoptosis.

Here we studied the effects of Cerebral on the expression of caspase-3 and β-amyloid precursor protein mRNA in the brain of rats during the acute (6 and 14 days) and delayed period after HS (6 months).

MATERIALS AND METHODS

Experiments were performed on 100 male outbred albino rats weighing 280-350 g.

The rats were divided into 4 groups: control; HS; HS and administration of Cerebral; and HS and administration of Cerebral in combination with verapamil.

The animas were narcotized with ether and fixed in a stereotaxis. Brain tissue (area of the internal capsule) was destructed with a mandrel knife. Autoblood (100 μl) was administered into the site of damage after 2-3 min.

Active fraction of Cerebral was obtained by preparative separation of components from a lyophilized preparation by reversed-phase chromatography on a Milikhrom-4 chromatograph. A 10×250-mm column

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was packed with Lichrosorb C18 sorbent. The active fraction of Cerebral was subjected to repeated chromatography to obtain the homogenous peptide product with a molecular weight of 350-500 Da.

The active fraction of Cerebral was administered intranasally once a day over 3 days after stroke (2 drops into each nostril). Verapamil (2.5 mg/kg) was injected intramuscularly once a day for 4 days after the end of Cerebral treatment.

Total RNA was isolated by the method of phenol extraction [9].

Expression of caspase-3 and β-amyloid mRNA was studied in survivors 6 and 14 days and 6 months after stroke. The study was performed by DNA-RNA hybridization on nitrocellulose filters [9,10] using Litekh probes (caspase-3, 5'-ctcaaattccgtgccaccttccggt taacacgagtgagg-3'; β-amyloid, 5'-gcatcgcttacaaactcacc aactaggcaccggtaaggaa-3').

The probes were labeled with [γ-^{32}P]ATP (>3000 Ci/mM, Amersham Pharmacia Biotech) [9].

Radioactivity of the filter was measured on a Beta-1 scintillation counter.

The results were analyzed by nonparametric Mann—Whitney test using Statistica 6.0 software.

RESULTS

The active fraction of Cerebral produced a therapeutic effect in albino rats during the acute period of HS (severe destructive process).

The mortality rate in experimental animals was maximum over the first 2 weeks (Fig. 1). Mortality was lower in treated rats. In the control group only 4 of 12 animals with HS survived after 2.5 weeks (*vs.* 9 of 13 animals in the experimental group). However, only 5 rats of the Cerebral group survived by the end of observations.

During the acute period of HS (6-14 days) expression of caspase-3 increased and surpassed that in control animals (Fig. 2). Expression of caspase-3 mRNA in rats of the Cerebral group was higher than in control animals, but lower compared to rats not receiving drug therapy ($p<0.05$).

The content of effector protease mRNA decreased in the follow-up period. These changes were not observed in rats receiving the active fraction of Cerebral.

The content of β-amyloid precursor protein mRNA practically does not change in the acute period of stroke. Therapy with the active fraction of Cerebral slightly decreased β-amyloid expression after 6 and 14 days (Fig. 3). However, β-amyloid expression increased in the delayed period after stroke. It was probably associated with trophic influences after HS. Published data show that the decrease in the concentration of trophic factors (*e.g.*, NGF) increases expression of

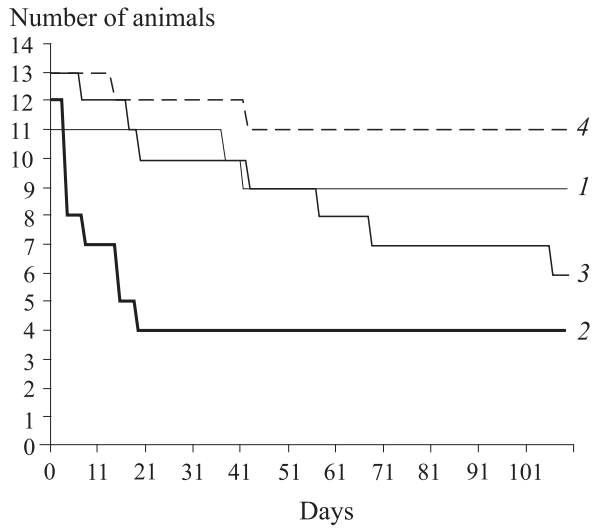

Fig. 1. Survival of animals after experimental hemorrhagic stroke (HS) and Cerebral therapy: control (*1*); HS (*2*); HS+Cerebral (*3*); HS+Cerebral+verapamil (*4*).

β-amyloid RNA, which is followed by activation of caspase-3 [4,6]. These changes probably took place in our experiments.

Biomolecular study showed that during the acute and delayed period after HS, expression of caspase-3 and β-amyloid precursor protein in the brain tissue of rats receiving Cerebral and verapamil is much higher than in animals receiving Cerebral alone ($p<0.01$).These data indicate that blockade of calcium entry into nerve cells reduces the proapoptotic effect of cerebral. Previous studies showed that verapamil and nifedipin block potential-dependent calcium channels and prevent hydroxycholesterol-induced apoptosis in cultured striated muscle cells [4]. However, chelation of extra-

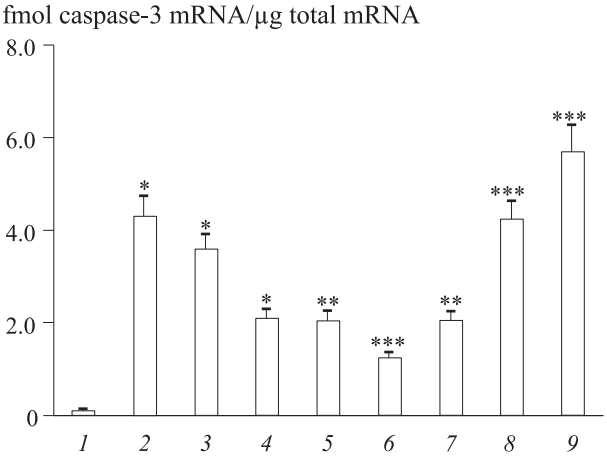

Fig. 2. Effect of Cerebral on caspase-3 expression in the brain of rats receiving therapy during the acute and delayed period after HS. Here and in Fig. 3: control (*1*); HS, 6 days (*2*); HS, 14 days (*3*); HS, 6 months (*4*); HS+Cerebral, 6 days (*5*); HS+Cerebral, 14 days (*6*); HS+Cerebral, 6 months (*7*); HS+Cerebral+verapamil, 14 days (*8*); HS+Cerebral+verapamil, 6 months (*9*). *$p<0.05$, **$p<0.025$, and ***$p<0.01$ compared to the control.

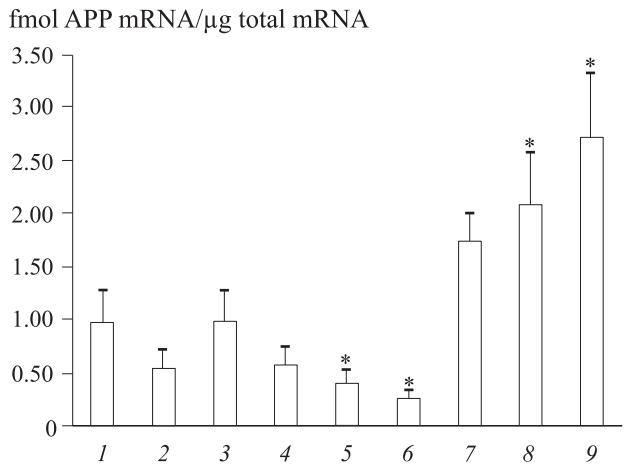

Fig. 3. Effect of Cerebral on β-amyloid precursor protein (APP) expression in the brain of rats receiving therapy during the acute and delayed period after HS.

cellular calcium leads to activation of caspase-3 and initiation of programmed cell death [7]. Our results and published data suggest that either the effect of the active fraction from Cerebral is associated with the entry of exogenous calcium and activity of verapamil-blocked channels, or verapamil produced an opposite effect in animals with HS. Under these conditions, the proposed scheme of treatment with active fraction of Cerebral in a specified dose does not produce the anti-apoptotic effect.

Our results indicate that treatment with active fraction of Cerebral alone or in combination with verapamil produces an antiapoptotic effect during the acute period after stroke. It decreases expression of caspase-3 (marker protease of apoptosis) and increases survival of animals. Administration of a calcium channel blocker has the delayed consequences after stroke, which is associated with apoptosis in brain cells (for example, vascular dementia).

Long-term observations showed that administration of the test preparation for 3 days produces only a transitory therapeutic effect in animals. Probably, this effect will be more pronounced and prolonged after long-term treatment with the preparation.

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