Anti-Ischemic Effect of a New Oxynicotinic Acid Derivative

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The effect of a new derivative of oxynicotinic acid (KONA) on experimental cerebral ischemia is examined in rats. It is demonstrated that a single dose of the preparation (30 mg/kg) significantly decreases the severity of ischemic damage and increases the survival of the animals after bilateral ligation of the common carotid arteries. Comparison with xanthinol-nicotinate shows the advantages of the new preparation. Although KONA does not inhibit free-radical oxidation *in vitro*, it does lower the content of free-radical oxidation products in rat blood plasma to the normal level.

Key Words: *cerebral ischemia; nicotinic acid derivatives; lipid peroxidation*

Being analogs of vitamins PP and B_6, the oxyderivatives of pyridine carbonic acids can be used as pharmacological preparations with a broad spectrum of biological activity. 5-Oxynicotinic acid is one of these derivatives [2]. It is thought that the hydroxyl group in position 5 endows the compound with antioxidant activity. Antioxidants and nicotinic acid are known to possess anti-ischemic activity [3,9]. On the basis of 5-oxynicotinic acid Dr. L. G. Stolyarova (Institute of Chemical Physics, Russian Academy of Sciences) synthesized a new preparation (KONA) that has a better solubility and higher stability than the parent compound.

Our aim was to use a rat model of cerebral ischemia to study the effect of KONA, which is believed to combine antioxidant activity with the properties of nicotinic acid.

MATERIALS AND METHODS

A model of severe ischemia was employed in this study. In the model, cerebral ischemia is achieved by bilateral ligation of the common carotid arteries, which results in 80-100% mortality. The severity of local and total cerebral neurological symptoms after ligation was assessed by the method of McGrow with our modifications. This method allows for calculation of the stroke-index, reflecting the severity of experimental insult.

The effect of KONA on the sequelae of acute disorders of the brain circulation was evaluated by mortality and by the severity of clinical symptoms in experimental and control animals. The effect of KONA was compared with that of xanthinol-nicotinate (X-nc), a preparation often used in clinical practice [1]. Both agents were injected intraperitoneally in a single dose of 30 mg/kg 30 min before induction of ischemia.

The plasma content of lipid peroxidation (LPO) products was determined in the blood taken from the jugular vein [7]. Lipids were isolated by the method of Folch *et al.* [8] with our modifications. Fluorescence was measured at λ_{exc}=365 nm and λ_{fl}=440 nm in a Hitachi-850 spectrofluorimeter with cutoff filters at a slit width of 5 nm. The fluorescence intensity was expressed in arbitrary units. Inorganic phosphates were measured as described [11].

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TABLE 1. Mortality Rate in Severe Experimental Ischemia of the Brain in the Control Group and after Administration of KONA and X-nc

Compared groups	Mean value in ischemic group	Mean value in ischemia+preparation group	Z value	Bilateral probability of equality or exceeding of values
Ischemia and ischemia+KONA	14.75 (18)	22 (18)	2.47745	0.013
Ischemia and ischemia+X-nc	15.86 (18)	21 (18)	1.8765	0.061

Note. Here and in Table 4 the number of animals is indicated in parentheses.

TABLE 2. Degree of Cerebral Damage in the Group with Severe Experimental Ischemia and the Influence of KONA and X-nc Calculated with the Aid of Tables of Conjugated Parameters

Compared groups	χ^2	Degrees of freedom	Significance of differences
Ischemia and ischemia+KONA	9.360	2	0.009
Ischemia and ischemia+X-nc	4.497	2	0.1
KONA and X-nc	1.800	2	0.41

The ability of KONA to inhibit LPO was studied in model inhibition of oxidation of rat liver microsomes. Oxidation was induced with 4,4'-azo-bis(4-cyanovaleric acid) (10 mM) in 100 mM phosphate buffer at pH 7.0. The kinetics of the process was assessed by oxygen absorption in an oxygenmeter (Yellow Springs Instr. Co.) using a Clarke electrode. The oxidation lag time and oxidation rate were measured within the linear segment of the oxygen absorption curve [5]. Microsomes were isolated by centrifugation as described [4]. The significance of differences was evaluated by the bilateral nonparametric Mann-Whitney test. The severity of clinical symptoms in the experimental and control groups was analyzed using the χ^2 test.

Reagents (chemical grade) were obtained from Reakhim, ascorbic acid was from Sigma, and 4,4'-azo-bis(4-cyanovaleric acid) was from Fluka. All solvents were distilled before use.

RESULTS

A protective effect of KONA against severe experimental ischemia of the brain was revealed. The number of animals surviving the ischemic insult differed considerably in the control and experimental groups. In the control group occlusion of the carotid arteries proved to be compatible with life in 11% of cases, whereas 50 and 39% of rats survived after administration of KONA and X-nc, respectively. It should be mentioned that the significance of the effect of KONA (0.013) was significantly higher than that of X-nc (0.061) (Table 1).

The number of cases with severe neurological symptoms (>7 points) in the experimental group decreased, while the number of cases with a mild course of experimental ischemic insult increased. Analysis of tables of conjugated parameters with the aid of the χ^2 test shows (Table 2) that KONA significantly reduces the degree of cerebral injury. The protective effect of X-nc has a significance level of 10%; however the difference between the effects of these preparations is not reliable.

It can be seen from Table 3 that acute ischemia considerably increases the content of Schiff bases (the end product of lipid peroxidation). It is seeen that ischemia induces no increase in the number of Schiff bases after the administration of KONA.

Analysis of the intergroup significance of differences shows that ischemic injury induces a statistically significant increase in the blood content of Schiff bases (Table 4). The effect of KONA manifests itself in a significant decrease (down to the control level) in the amount of LPO end

TABLE 3. Changes in the Plasma Schiff Bases SB Content in Rats Subjected to Cerebral Ischemia and Treated with KONA

Group	Fluorescence per mg phosphorus ($M \pm m$)	n	Coefficient of variation
Control	0.185±0.079	5	0.006
Control+KONA	0.173±0.069	4	0.004
Ischemia	1.873±2.234	4	4.991
Ischemia+KONA	0.111±0.057	2	0.003

TABLE 4. Significance of Intergroup Differences in the Plasma Schiff Bases Content

Compared groups	Mean value in control group	Mean value in experimental group	Z value	Bilateral probability of equality or exceeding of values
Control and KONA	5.2 (5)	4.75 (4)	−0.123	0.901
Control and ischemia	3.4 (5)	7 (4)	1.837	0.066
Control and ischemia + KONA	4.6 (5)	2.5 (2)	−0.968	0.333
Ischemia and ischemia + KONA	4.5 (4)	1.5 (2)	−1.620	0.1

products. However, the 10% level of reliability is due to the small number of animals included in the experiment.

Our results suggest that in the chosen model KONA does exhibit antioxidant activity. However, investigation of its ability to inhibit LPO in a model system showed that this agent affects neither the lag time of oxidation nor the oxidation rate when applied in concentrations as high as 10^{-3} M.

Free-radical damage to cell structures is an important component in the molecular mechanisms of ischemia-induced injury [6]. Our findings suggest that in the case of KONA the mechanism of action is not directly associated with inhibition of this stage of ischemia-induced injury. This does not exclude an indirect effect of KONA on free-radical oxidation. It is likely that the preparation acts at the stages preceding LPO intensification. It can be speculated that the anti-ischemic effect of KONA is due to the vascular effect of this preparation, since it is known that nicotinic acid acts as a vasodilator by altering the synthesis of PGD^2 [10].

The more pronounced anti-ischemic effect of KONA compared with that of X-nc indicates that synthetic preparations based on 5-oxynicotinic acid hold promise.

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