PHARMACOLOGY AND TOXICOLOGY

Correction with Cordarone of Changes in Cathepsin D Activity Induced by Modeled Circulation Insufficiency and Toxic Action of Strophanthin K

P. A. Galenko-Yaroshevskii, S. M. Lemkina, and Ya. V. Kostin

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Some biochemical mechanisms of the protective effect of cordarone against cardiotoxic activity of strophanthin K are studied in a rat model of circulation insufficiency. The potassium channel blocker and the cardiac glycoside are found to have opposite effects on the lysosome apparatus of the heart.

Key Words: *strophanthin K; cathepsin D; circulation insufficiency*

Intoxication with cardiac glycosides is a specific problem in the therapy of circulation insufficiency (CI) [1] which prompts the search for rational pharmacological correction of hypersensitivity to the toxic action of cardiac glycosides and a comprehensive study of its pathogenic mechanisms. Previous studies have proved that low tolerance to the cardiotoxicity of strophanthin K (SK) can be successfully corrected with the potassium channel blocker cordarone [2]. However, the mechanism of its protective effect remains unclear. Since the development of various pathological processes involves lysosomes [4], it seems important to investigate their role both in the realization of SK toxicity and in the protective effect of cordarone under conditions of CI.

The aim of the present study was to assess the activity of cathepsin D (CD, EC 3.4.23.5), a lysosomal enzyme involved in protein catabolism, and the functional state of lysosomal membranes against the background of a toxic dose of SK and its combination with cordarone in a model of CI.

Department of Pharmacology, Kuban Medical Academy, Krasnodar

MATERIALS AND METHODS

Experiments were performed on 68 male albino rats weighing 160-200 g narcotized with sodium thiopental (40 mg/kg, i.p.). Circulation insufficiency was modeled by repetitive administration of large doses of the β-adrenomimetic isadrine. The development of CI was judged from hemodynamic disturbances, anatomic and histological changes in internal organs, and altered tolerance to arrhythmogenic and toxic action of SK [1,2]. An arrhythmogenic dose of the glycoside inducing the appearance of group extrasystoles or bigeminy in preliminary tests on intact rats [1,2] was used as the toxic dose. The choice of the therapeutic dose of cordarone was based on published data on its specific pharmacological activity [2].

Free and total CD activity was determined spectrophotometrically by the concentration of final products of the CD-catalyzed reaction [5], using lyophilized human hemoglobin (RENAM, Russia) as the substrate. Free CD activity was measured in a fresh myocardium homogenate. The total CD activity was determined in the same homogenate treated

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TABLE 1. Cathepsin D Activity in the Heart of Intact Rats and Animals with Experimental CI After Single Intravenous Administration of Strophanthin K, Cordarone, and Their Combination

Experimental conditions	Activity, μmol/min×g protein		Free/total activity, %
	free	total	
Intact rats			
Control	0.0460±0.0016	0.0745±0.0019	62.0±2.1
15 min after injection of:			
SK	0.0109±0.0011*	0.0319±0.0007*	34.0±3.3*
cordarone	0.0117±0.0009*	0.0413±0.0010*	28.3±2.1*
SK+cordarone	0.0102±0.0004*	0.0319±0.0006*	32.0±0.9*
30 min after injection of:			
SK	0.1029±0.0023*	0.1122±0.0030*	91.8±1.2*
cordarone	0.0101±0.0011*	0.0392±0.0023*	25.6±1.2*
SK+cordarone	0.0463±0.021	0.0784±0.0007	59.1±2.1
Experimental CI			
Control	0.0722±0.0012*	0.0801±0.0012*	90.1±0.8*
15 min after injection of:			
SK	0.0650±0.0012*	0.0765±0.0007*	84.9±1.1*
cordarone	0.0217±0.0008*	0.0719±0.0016*	30.2±0.7*
SK+cordarone	0.0474±0.0064*	0.0697±0.0024*	67.4±6.6*
30 min after injection of:			
SK	0.1161±0.0023*	0.1194±0.0018*	97.3±0.4*
cordarone	0.0120±0.0005*	0.0601±0.0012*	20.0±1.2*
SK+cordarone	0.0413±0.0017*	0.0662±0.0017*	62.3±1.6*

Note. Mean values of 4-9 experiments are presented. *Differences are significant in comparison with the corresponding controls.

with the nonionogenic detergent Triton X-100. The stability of lysosomal membranes was assessed from the percent ratio of free to total activities.

The data were processed statistically using standard ANOVA tests [3].

RESULTS

Table 1 shows that modeled CI resulted in a marked activation of the acid proteinase CD, the free/total ratio being markedly increased (by 45.3%) due to a more pronounced increase in the free activity. This indicates labilization of lysosomal membranes and CD release into the cytosol. A single intravenous administration of the toxic dose of SK induced biphasic changes in CD activity. In intact animals, free and total CD activities dropped 15 min postinjection, amounting for 23.7 and 42.8% of the basal activity, respectively. However, 30 min postinjection both parameters rose and attained 223.7 and 150.6%, respectively. As evidenced by the free/total CD percent ratio, the enzyme-substrate interaction 15 min after injection of the minimal arrhythmogenic dose of SK was weaker than that 30 min postinjection. Administration of the toxic dose of SK to animals with experimental CI led to a significant decrease in free and total CD activity: to 90 and 95.5%, respectively, in comparison with basal activities in these animals, the free/total ratio being also decreased. At the same time, 15 min after SK injection the share of free activity and the free/total ratio attained 141.3 and 136.9%, respectively, compared with that in intact controls. This implies an increased lability of lysosomal membranes in animals with CI. Thirty min postinjection, both free and total CD increased considerably, attaining values of 160.8 and 149.1%, respectively, in comparison with the corresponding activities in animals with CI. A significant increase was recorded in the free/total CD activity ratio, indicating a further increase in the lysosomal membrane lability, which is typical of CI and is probably responsible for reduced SK tolerance.

The potassium channel blocker cordarone, a potent protector against SK cardiotoxicity [2], inhibited free and total CD activity and stabilized lysosomal membranes both in intact and CI rats, as evidenced by a considerable decrease in the mem-

brane permeability (Table 1). Combination of cordarone with SK (toxic dose) not only prevented SK-induced activation of hydrolytic enzymes in intact rats, but also normalized the functional state of lysosomal membranes. This combination also restored the initial free/total CD activity ratio in rats with CI, which proved the stabilization of lysosomal membranes compared with the control. This was due to opposite effects of cordarone and toxic dose of SK on free and total CD activity. The inhibitory effect of cordarone on CD activity arises from the ability of this α, β, χ-blocker to inhibit lysosomal β-adrenoreceptors [4], which are thought to participate in the regulation of the functional state of lysosomes and the activity of hydrolytic enzymes [4].

Thus, changes in CD activity after administration of the toxic dose of SK in combination with cordarone point to a possible biochemical mechanism of the protective effect of cordarone against glycoside intoxication. This mechanism consists in reducing labilization of lysosomal membranes occurring in CI. Our findings indicate that primary effects of SK can be corrected by directed modulation of the heart lysosomal system, which makes it possible to optimize clinical application of SK.

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Antiedematous Effect of the Preparation Polyosm in Brain Ischemia

M. B. Plotnikov, V. I. Smol'yakova, and G. A. Chernysheva

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The effect of the preparation Polyosm (polyethylene oxide 400) on cerebral edema (impedance measurements) and cerebral circulation is studied in brain ischemia caused by ligation of the left common carotid artery and reduction of blood flow through the right common carotid artery to 25% of the original level. The preparation markedly reduces cerebral edema and induces transient improvements in cerebral circulation.

Key Words: *cerebral edema; brain ischemia; polyethylene oxide*

The preparation "solution of polyethylene oxide 400 30%" is an osmotically active compound used in the therapy of glaucoma [6]. This preparation increases osmotic activity of the blood and decreases intraocular pressure. There is evidence that intravenous administration of polyethylene oxide 400 (PEO 400) is more efficient [6]. It was demonstrated that intravenous administration of PEO 400 in a dose of 6 g/kg markedly decreases the water content in the brain [1]. Based on these findings, we have developed the preparation Polyosm, which is a solution of PEO 400 for intravenous administration. The aim of the present study was to examine the effect of Polyosm on brain ischemia in conscious rats.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 350-380 g. Cerebral ischemia in conscious

Institute of Pharmacology, Scientific Center of the Russian Academy of Medical Sciences, Tomsk

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