Antiischemic Activity of Immobilized Cytochrome C

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The antiischemic activity of polyethylene glycol- and dialdehyde dextran-immobilized cytochrome C is experimentally studied on rats. Immobilization of cytochrome C on these matrices enhanced the enzyme activity assayed by restriction of the necrotic zone 4 hours after coronary occlusion.

Key Words: *immobilized cytochrome C; antiischemic activity; cytochrome C*

Cytochrome C is an effective antihypoxic and antiischemic agent [2,4,5]. At present, the development of biotechnological cytochrome C, which is identical to the enzyme of animal origin, opens up new prospects for its wide use [1].

However, as with many other endogenous agents, the use of cytochrome C is restricted by their short-term action. In light of this, it is of great interest to study the possibility of prolonging the effect of cytochrome C through immobilization on polymeric matrices.

The aim of the present study was to evaluate the antiischemic activity of cytochrome C immobilized on polyethylene glycol (PEG) and dialdehyde dextran (DAD) matrices.

MATERIALS AND METHODS

Experiments were carried out on random-bred male rats weighing 250-300 g narcotized with sodium ethaminal (40 mg/kg, i.p.). The animals were housed under standard conditions in the vivarium of the Russian Research Center for the Safety of Bioactive Compounds. The most integral parameter, the size of the necrotic zone in acute myocardial infarction, served as the measure of the antiischemic properties of cytochrome C. To this end, myocardial infarction was modeled in artificially ventilated animals by ligation of the descending branch of the left coronary artery at the level of the bottom margin of the auricle. The rats were sacrificed 4 hours after coronary occlusion and the size of the necrotic and ischemic zones was determined by the differential indicator method [3], based on differential quantitative assay of Evans blue (ischemic zone) and formazan red (necrosis).

Two modifications of PEG-immobilized biotechnological cytochrome C were studied: $CPEG_4$ (m.w. of PEG=4000), where one molecule of cytochrome C is associated with 3 ($CPEG_4$-3) and 6 ($CPEG_4$-6) PEG molecules, and $CPEG_{10}$ (m.w. of PEG=10,000), where one molecule of cytochrome C is associated with 3, 6, and 9 PEG molecules ($CPEG_{10}$-3, $CPEG_{10}$-6, and $CPEG_{10}$-9, respectively). The total molecular weight of cytochrome C-DAD complexes (CDAD) was 150,000. Biotechnological cytochrome C served as the reference preparation. All substances were injected one time intravenously immediately after coronary occlusion. The total volume of injected solution did not exceed 1 ml. The data were processed statistically using the Student *t* test.

RESULTS

Biotechnological cytochrome C in a dose of 5 mg/kg had no effect on the formation of the necrotic zone in comparison with the control (Table 1). However, when injected in doses of 10 and 20 mg/kg, cytochrome C reduced the size of the necrotic

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TABLE 1. Effect of Immobilized Biotechnological Cytochrome C on the Size of the Necrotic Zone 4 Hours after Coronary Occlusion in Rats

Experimental conditions	Dose, mg/kg	Content of cytochrome C in the complex cytochrome+sorbent, mg/kg	Ischemic zone/ total weight of myocardium, %	Necrotic zone/ total weight of myocardium, %	Necrotic zone/ ischemic zone, %
Control	-	-	34±2.6	22±2.0	68±4.3
Cytochrome C	20.0	20.0	27±2.9	9±1.4*	32±3.4*
	10.0	10.0	33±4.2	16±3.4	49±4.2*
	5.0	5.0	38±3.0	26±2.6	64±3.4
$CPEG_4$-3	24.0	12.0	34±3.3	16±2.4	49±7.9*
$CPEG_4$-6	24.0	8.0	33±1.0	14±1.1*	42±3.6*
$CPEG_{10}$-3	24.0	7.0	23±1.2	10±1.3*	37±6.0*
$CPEG_{10}$-6	24.0	4.0	33±3.3	16±2.8	46±5.5*
$CPEG_{10}$-9	24.0	3.0	32±3.1	14±3.1	41±7.1*
CDAD	3.5	1.0	23±2.6	13±0.8	59±3.7
CDAD	17.2	5.0	27±1.7	12±1.6	45±2.8*

Note. *$p<0.05$ in comparison with the control.

zone to 49±4.2 and 32±3.4% of that of the ischemic zone, respectively.

Cytochrome C immobilized on PEG_4 exhibited pronounced antiischemic properties. For instance, $CPEG_4$-3 and $CPEG_4$-6 shrank the necrotic zone to 49±7.9 and 42±3.6% of the ischemic zone and their antiischemic activity was close to that of cytochrome C in a dose of 10 mg/kg.

PEG_{10}-immobilized cytochrome C exhibited a pronounced antiischemic effect in a dose range of 3-7 mg/kg, i.e., in doses at which free cytochrome C was ineffective. Thus, $CPEG_{10}$-9 and $CPEG_{10}$-6 reliably reduced the size of the necrotic zone to 41±7.1 and 46±5.5% of the ischemic zone, while $CPEG_{10}$-3 shrank the necrotic zone to 37±6% of the nonperfused area.

Injection of DAD-immobilized cytochrome C in a dose of 5 mg/kg reduced the size of the necrotic zone in the myocardium to 45±2.8% of the ischemic zone, whereas a dose of 1 mg/kg exhibited no antiischemic effect.

Our experiments showed a dose-dependent effect of cytochrome C, which is presumably due to the high molecular weight of the enzyme and the relatively low level of its transsarcolemmal transport. However, immobilization of cytochrome C on PEG and DAD matrices, despite the considerable increase of the total molecular weight, not only did not reduce but actually enhanced the antiischemic activity of the enzyme. In light of this, the observed phenomenon may be attributed to a higher affinity of cytochrome C polymeric complexes for biological membranes, or, more likely, to a slower release of the enzyme from the matrices and, thereby, a prolonged therapeutic action. This assumption is confirmed by our pharmacokinetic studies, which demonstrated that injection of CDAD maintains a therapeutic concentration of the enzyme in the blood during 24 hours, whereas after injection of free cytochrome C its concentration drops markedly after just 2 hours.

Thus, immobilization of cytochrome C on polymeric matrices is a promising trend for optimal use of the enzyme in clinical cardiology.

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