Thus, administration of CG normalizes LPO activated by CCl_4 both in the liver and in the myocardium.

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Study of Cardioprotective Effect of α-Tocopherol and Panthenol Using an Experimental Model of Ischemized-Reperfused Isolated Heart

A. O. Kumerova, A. P. Skesters, and I. Y. Skestere

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Using an experimental ischemia-reperfusion model it is found that combined treatment with α-tocopherol and panthenol markedly increases the content of endogenous antioxidant tocopherol during total ischemia and reperfusion, i.e., it improves the antioxidant state of the postischemized myocardium, thus preventing possible damage caused by stepped-up production of active oxygen forms during reoxygenation.

Key Words: *antioxidants; tocopherol; panthenol; ischemia; heart; perfusion*

The ischemized myocardium is characterized by a reduced content of antioxidants and high-energy compounds and a lowered activity of antioxidant enzymes together with an increased content of prooxidant metabolites [2]. The fate of the ischemized myocardium during reperfusion is determined not only by the magnitude and reversibility of the changes induced by ischemia itself, but also by the reperfusion period. During this period intensification of lipid peroxidation (LPO) in the vascular endothelium and in cardiomyocyte membranes, which may be caused by the elevated content of active

Latvian Medical Academy, Riga. (Presented by P. V. Sergeev, Member of the Russian Academy of Medical Sciences)

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TABLE 1. Content of Endogenous TP, MDA, Lactate, ATP, and CP and SOD Activity in Intact Rat Myocardium (Control, 12 Animals)

Endogenous TP, nmol/g protein	SOD, U/g protein	MDA, nmol/mg protein	Lactate, μmol/g protein	ATP, μmol/g protein	CP, μmol/g protein
50.3±3.47	3.15±0.13	0.144±0.015	2.48±0.21	4.17±0.15	3.18±0.34

oxygen forms during reoxygenation, should be strictly prevented [8,9]. Previously we have proven a cardioprotective effect of pantothenic acid derivatives, among them panthenol (PT), added to the perfusate [10]. PT was shown to increase the content of endogenous α-tocopherol (TP) in postischemic cardiomyocytes, which apparently underlies its antioxidant effect.

In the present study we investigated the cardioprotective effect of TP (*in vivo*) applied before ischemia and of PT (*in vitro*) added to the perfusate and cardioplegic solutions.

MATERIALS AND METHODS

The study was carried out on male Wistar rats weighing 280-320 g under thiopental narcosis. The excised heart was arrested and washed in ice-cold perfusate (without PT). A perfusion cannula was inserted into the aorta and fixed with a ligature. A closed perfusion chamber allowed for controlling the temperature and humidity of the internal environment. The heart was perfused after Langendorff with Krebs-Henseleit solution containing (in mM): NaCl 128.2, KCl 4.7, $CaCl_2$ 1.3, $MgCl_2$ 1.1, $NaHCO_3$ 20.0, and glucose 10.0. The perfusate was oxygenated with extrapure carbogen (95% O_2 and 5% CO_2). The hydrostatic pressure in the cannula was 80 cm H_2O, volume 24 ml/min, pH 7.4, temperature 37±0.1°C. The experiment was performed according to the following scheme: stabilization perfusion - 15 min; cardioplegia - 1 min at 0°C; total ischemia (TI) was accomplished via a 30-min occlusion of the aorta at 37°C; reperfusion - 30 min. After the perfusion was restarted the function of the heart was restored without mechanical or electrical stimulation. The following parameters were recorded during perfusion: coronary volume, pH of outflowing perfusate, and ECG. After completion of the protocol the heart was promptly frozen using Wollenberger's forceps and stored in liquid nitrogen before biochemical examinations. α-TP (Fluka) dissolved in sterile peroxide-free olive oil was injected intramuscularly in a dose of 30 mg/kg 24 hours prior to ischemia. PT (Serva) was added to the perfusate in a concentration of 12 kM/liter. The animals of the first group were injected with TP, the animals of the second group were injected with TP and perfused with PT-containing solution, and in the third group all preparations were omitted. The content of lactate [7], ATP [4], creatine phosphate (CP) [11], malonic dialdehyde (MDA) [14], and TP [13] and the activity of superoxide dismutase (SOD) [12] were determined.

RESULTS

After 30-min TI the concentration of lactate in the myocardium was increased in comparison with the norm (intact animals). At the same time the concentration of high-energy phosphates decreased to 41.7% and 47.7% of the normal value for ATP and CP, respectively (Tables 1 and 2). The content of endogenous TP in cardiomyocytes decreased during ischemia. SOD activity remained unchanged, whereas the concentration of MDA was considerably elevated (from 0.144 to 0.487 nmol/g, Table 3). The sharp rise of MDA (3.4-fold) suggests the intensification of LPO during TI. These results confirms the data of other investigators and substantiate the advisability of using antioxidants for the prevention of heart damage caused by intensified LPO processes [6,8].

Injection of TP did not affect the accumulation of lactate or the content of CP in the ischemized myocardium, but reliably increased the content of ATP and endogenous TP in comparison with group 3 (by 62.6 and 34.4%, respectively), while the content of MDA dropped by 36.2% (Table 3).

TABLE 2. Effect of α-TP and PT on Content of Lactate, ATP, and CP in the Myocardium (12 Animals)

Group	Ischemia			Reperfusion		
	lactate, μmol/g protein	ATP, μmol/g protein	CP, μmol/g protein	lactate, μmol/g protein	ATP, μmol/g protein	CP, μmol/g protein
Control	17.05±0.65*	1.74±0.10*	1.20±0.07*	7.49±0.28*	1.57±0.12*	1.79±0.19*
α-TP	16.33±0.70*	2.83±0.015*,**	0.95±0.11*	2.99±0.36**	3.12±0.17*,**	0.99±0.14*,**
α-TP+PT	16.67±0.28*	2.00±0.15*	0.73±0.11*,**	6.80±0.34*	2.07±0.22*	0.96±0.04*,**

Note. One and two asterisks denote $p<0.05$ vs. the control and untreated animals, respectively.

TABLE 3. Effect of α−TP and PT on SOD Activity and Content of Endogenous TP and MDA in the Myocardium

Group	Ischemia			Reperfusion		
	endogenous TP, nmol/g protein	SOD, U/g protein	MDA, nmol/mg protein	endogenous TP, nmol/g protein	SOD, U/g protein	MDA, nmol/mg protein
Control	45.0±1.98 (8)	3.35±0.29 (8)	0.487±0.03* (7)	30.2±1.25* (8)	3.67±0.18 (8)	0.346±0.013* (6)
α−TP	60.5±1.67*,** (8)	2.03±0.16*,** (8)	0.318±0.018*,** (7)	51.5±3.04*,** (8)	4.27±0.40* (8)	0.265±0.020* (6)
α−TP+PT	64.7±2.69*,** (8)	3.94±0.32 (8)	0.311±0.009*,** (8)	119.2±26.2*,** (8)	2.81±0.18** (8)	0.215±0.007*,** (7)

Note. The number of animals is shown in parentheses.

Addition of PT to the perfusate still further elevated the content of endogenous TP and SOD activity in the myocardium during ischemia and led to a drop of MDA.

Thirty-minute postischemic perfusion sharply decreased the concentration of lactate (from 17.1 to 7.5 μmol/g), although its level remained higher than that in the intact myocardium. The content of high-energy compounds was little affected, the reserves of endogenous TP were considerably depleted and its content dropped by 40.1% (from 50.3 to 20.2 nmol/g). The concentration of MDA was lower than during ischemia but still surpassed the normal value by 140.3%. As was expected, injection of TP elevated the content of endogenous TP in the myocardium during the 30-min reperfusion virtually to the normal value. SOD activity was increased to 4.27 U/g (vs. 3.15 U/g in the intact myocardium). This was probably due to the increased content of active oxygen forms, leading to the accumulation of superoxide anion radicals during reoxygenation (J. M. McCord).

Combined administration of TP and PT yielded a considerable increase in endogenous TP in the postischemic period which attained 294.7 and 136.9% in comparison with the ischemic and intact group, respectively, implying thus implies an antioxidant effect of PT.

The structure of PT is close to natural compounds (R-2,4-dihydroxy-3,3-dimethylbutyric 3-hydroxypropylamide); it is practically nontoxic, soluble in water and in perfusate. PT not only increases the concentration of TP in the postischemized myocardium but also exhibits synergism with exogenous TP, thus substantially improving the antioxidant protection of the myocardium in the postischemic and reperfusion periods. There are some published data on a beneficial cardioprotective effect of combined administration of TP and pantothenic acid derivatives [1,3,12] in hypothermia and other pathological states. This is confirmed by the low SOD activity (2.81 U/g) and low concentration of MDA (0.215 nmol/g) after a 30-min reperfusion (Table 3). Previously we have shown that pantothenic acid derivatives are precursors of CoA. The CoA pool changes due to enhanced endogenous synthesis, thus improving the adaptive capacity of the myocardium [5]. The cardioprotective effect of combined application of PT and TP is also confirmed by the accelerated recovery of heart function during reperfusion. The heart rhythm stabilizes more quickly and extrasystole is practically absent.

Thus, using experimental ischemia-reperfusion of the isolated heart model we demonstrated that combined application of TP and PT during TI and reperfusion of the heart markedly increases the content of endogenous antioxidant, i.e., it improves the antioxidant status of the postischemized heart, preventing possible damage induced by stepped-up production of active oxygen forms during reoxygenation.

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