Use of Zafirlucast, Cysteine-Containing Leukotriene Antagonist, in Epinephrine-Induced Heart Injury

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Combination of preventive and repeated (after 12 h) injections of zafirlucast in a daily dose of 40 mg/kg to animals with epinephrine-induced heart injury essentially decreased the content of leukotriene C_4, but did not prevent the increase of leukotriene B_4 content in the blood, though decreased its content in comparison with the level observed 24 h after epinephrine injection by 11%. Two injections of zafirlucast had a favorable impact on serotonin content in the myocardium (this parameter decreased by 19%), promoted a decrease in epinephrine content and normalization of the norepinephrine/epinephrine ratio, did not change prostaglandin content and ratio, and slightly decreased histamine content in the myocardium. The effect of zafirlucast can be explained by a decrease of oxidative stress. Zafirlucast decreased blood content of myofibrillar fraction of creatine phosphokinase (by 22%) and slightly improved elastic characteristics of erythrocytes. These results suggest that zafirlucast can be added to combined therapy of necrotic, stress-induced, hypoxic, and ischemic injuries of the myocardium.

Key Words: *stress; leukotriene antagonists; myocardium; blood; serotonin; prostaglandins*

The search new drugs regulating vascular effects of leukotrienes, specifically their natural and synthetic antagonists, is very important for the therapy of systemic and circulatory disorders, disturbances of cardiac hemodynamics and inflammatory involvement of the myocardium. Here we investigated changes in the content of leukotrienes, markers of necrosis and inflammation, in epinephrine-induced cardiac involvement treated by zafirlucast.

MATERIALS AND METHODS

The study was carried out on 20 Chinchilla rabbits (2-4 kg). Six animals were controls and in 14 cardiac injury was induced by epinephrine: 7 were left without treatment and 7 rabbits received 20 mg/kg zafirlucast (leukotrienes C_4, D_4, and E_4 antagonist, Zeneka) dissolved in water (injections into the ear vein) 15 min before and 12 h after epinephrine. The animals were sacrificed 24 h after epinephrine injection. All animals were kept in a vivarium under similar conditions.

Epinephrine-induced cardiac injury, measurements of prostaglandins, catecholamines, serotonin and histamine, leukotrienes and myofibrillar fraction of creatine phosphokinase (CPK-MB), and methods of statistical processing of the results were described previously [1].

RESULTS

Cysteine leukotrienes C_4, D_4, and E_4 and dihydroxyl derivative leukotriene B_4 are products of arachidonic acid oxidation catalyzed by 5-lipoxygenase [3]. The first to form is an unstable product leukotriene A_4, which is transformed in erythrocytes into leukotriene B_4 under the effect of hydrolase [4,6] and then in endothelial cells into leukotriene C_4 (reaction catalyzed by specific synthase) [2,5]. Combination of preventive

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and repeated (after 12 h) injections of zafirlucast in a dose of 20 mg/kg for 24 h to animals with epinephrine-induced cardiac injury notably (by 42%) reduced the content of leukotriene C_4, but its blood level remained high (45.6% above the normal). The effect of zafirlucast in a daily dose of 40 mg on the content of leukotriene B_4 was less expressed. The content of leukotriene B_4, although showed a trend to a decrease (being 11% lower than 24 h after epinephrine injection), was still 58% higher than in the control (in epinephrine-induced injury it by 76% surpassed the normal, Table 1).

However, no synergism in the effects of zafirlucast as leukotriene C_4, D_4, and E_4 antagonist and inhibitor of leukotriene B_4 formation was observed, at least in a dose of 40 mg/kg, despite the fact that leukotrienes B_4 and C_4 are synthesized from the same precursor A_4 in interrelated neutrophil-erythrocyte processes [4] or in endothelial cell [6].

Interestingly, zafirlucast promoted the increase in glutathione reductase activity, which did not differ from the control 24 h after epinephrine injection [2]. Previously similar effect was observed after injection of leukotriene synthase inhibitor in ischemia-reperfusion myocardial damage [2].

Two injections of zafirlucast (leukotriene antagonist) had a favorable impact on serotonin content, which decreased by 19% in the myocardium in comparison with the controls, and promoted the decrease in epinephrine content and normalization of the norepinephrine/epinephrine ratio. This effect of zafirlucast, which no doubt deserves further investigation, can be due to a decrease in oxidative stress [3,7,8] and hence, due to decreased production of free radicals (adrenochrome and nitroxyl anion).

Importantly, zafirlucast inhibited the release of CPK-MB (blood content of CPK-MB is 22% lower than in the control), which substantiates addition of this drug to combined therapy for necrotic, stress-induced, hypoxic, and ischemic myocardial injuries.

It is noteworthy that the content and ratio of prostaglandins E and $F_{2\alpha}$ and histamine did not appre-

TABLE 1. Effects of Zafirlucast on the Content of Epinephrine, Norepinephrine, Serotonin, Histamine, Prostaglandins, and Leukotrienes in the Myocardium and Blood in Epinephrine-Induced Heart Injury

Parameter	Normal	Epinephrine-induced injury	
		control	treatment
Myocardial ventricles			
Epinephrine, μg/g	0.07±0.01	0.5±0.1*	0.28±0.10*
Norepinephrine, μg/g	1.25±0.4	0.8±0.1	0.85±0.09
Norepinephrine/epinephrine	17.9±1.8	1.63±0.16*	3.05±0.14*$^{+}$
Serotonin, μg/g	6.3±0.5	10.3±1.3*	8.9±1.0***$^{++}$
Histamine, μg/g	3.4±0.5	7.8±0.6*	6.5±0.5*$^{++}$
Prostaglandin E, ng/g	3.0±0.8	2.2±0.9**	2.1±0.5**
Prostaglandin $F_{2\alpha}$, ng/g	3.4±0.6	7.4±0.9*	6.4±0.6*
Prostaglandin E/$F_{2\alpha}$	0.88±0.07	0.30±0.10*	0.33±0.05*
Blood			
Epinephrine, pg/g	4.5±0.8	5.9±0.8***	4.5±0.5***$^{++}$
Norepinephrine, μg/g	2.3±0.6	2.1±0.2	2.0±0.2
Norepinephrine/epinephrine	0.51±0.08	0.36±0.09***	0.44±0.04***
CPK-MB, nmol/ml	2.3±0.8	12.5±1.8***	9.8±1.3***$^{++}$
Serotonin, nmol/ml	39±5	56±3***	47±3***
Histamine, nmol/ml	0.24±0.05	2.85±0.15*	2.58±0.14*
Prostaglandin E, ng/g	65±3	102±15**	92±11**
Prostaglandin $F_{2\alpha}$, ng/g	105±10	402±21*	389±13*
Prostaglandin E/$F_{2\alpha}$	0.62±0.06	0.25±0.04*	0.24±0.03*
Leukotriene C_4, nmol/ml	5.7±0.8	14.3±1.5*	8.3±1.4
Leukotriene B_4, nmol/ml	5.8±0.8	10.2±2.1**	9.1±1.1**
Deformability, arb. units	12.5±3.4	89±9*	68±8*

Note. *p<0.001, **p<0.01, ***p<0.05 compared to normal; ^{+}p<0.001, ^{++}p<0.05 compared to the control.

ciably change under the effect of zafirlucast in a dose of 40 mg/kg (Table 1).

In addition, zafirlucast slightly improved elastic characteristics and stability of erythrocyte membranes (Table 1).

These results provide the basis for extending indications for the use of zafirlucast in stress-induced microfocal heart injuries with pronounced secondary inflammatory allergic reaction and tissue edema.

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