# Age-Related Features of Cytoflavin Effectiveness during Experimental Myocardial Ischemia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 156, No. 12, pp. 749-752, December, 2013
Original article submitted October 18, 2012

Administration of Cytoflavin to young rats with experimental myocardial ischemia was followed by activation of mitochondrial enzymes and significant accumulation of ATP in the myocardium and blood plasma, but did not modulate the stability of cardiomyocyte membranes and signs of hypoxia. Cytoflavin increased the severity of metabolic disorders and depleted the energy reserves of cells in old rats with myocardial ischemia.

**Key Words:** *myocardial ischemia; cardioprotection; Cytoflavin; experiment*

Myocardial ischemia is a serious disease that ranks first in the morbidity and mortality rate throughout the world, including Russia [14]. Therefore, this problem requires a solution. Many authors hypothesized that administration of cardioprotective agents (trimetazidine, phosphocreatine, mildronate, *etc*.) can increase the effectiveness of standard drug therapy and interventional methods for the treatment of CHD [2,6]. However, the effectiveness of metabolic correctors significantly varies in various groups of patients [5]. Hence, the mechanisms of the effect of these agents under various conditions require further investigations.

This work was designed to study a combination drug Cytoflavin, which contains the following four components: succinic acid, nicotinamide, riboflavin, and inosine (riboxine). Cytoflavin is prescribed for cerebral ischemia. However, the cardiocytoprotective effect of this drug in combination therapy with perftoran was also evaluated in patients with acute myocardial ischemia [7]. Aging is accompanied by the development of metabolic disturbances in cells, which probably requires additional medicinal correction [13]. The age-related features of Cytoflavin effectiveness during myocardial ischemia remain unknown.

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Here we studied the effectiveness of Cytoflavin, as a potential cardiocytoprotective agent, in rats of various age groups with experimental myocardial ischemia.

## MATERIALS AND METHODS

Experiments were performed on male Wistar rats maintained in a vivarium under standard conditions. The animals ($n$=32) were divided into the following groups: 10- ($n$=6) and 24-month-old ($n$=6) intact rats; 10- and 24-month-old ($n$=5) rats with experimental IHD, and 10- ($n$=5) and 24-month-old ($n$=5) rats with IHD receiving Cytoflavin. These age groups were formed taking into account the fact that 10- and 24-month-old rats correspond to middle-age (45-50 years) and elderly people (70-75 years), respectively. The maintenance of animals and all manipulations were performed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental Purposes (Strasbourg, 1986).

Experimental CHD was induced as described elsewhere [4]. The animals received daily subcutaneous injections of epinephrine solution (0.1%, 0.1 ml) and hydrocortisone emulsion (2.5%, 1 ml) for a week. The therapeutic dose of Cytoflavin was calculated by the Yu. R. Rybolovlev formula [11]. Cytoflavin in a dose of 0.07 ml/100 g in 1.5 ml 0.9% NaCl was injected

intravenously once a day, which corresponds to the recommended dose for humans (10 ml intravenously dropwise in 200 ml 0.9% NaCl once a day).

The animals were euthanized by decapitation under light ether anesthesia. The heart was isolated and washed from the blood with cold 0.9% NaCl. Further manipulations were performed at 4°C. The myocardial homogenate was prepared. The mitochondria were isolated as described previously [10]. The activities of mitochondrial enzymes SDH, pyruvate dehydrogenase (PDH), and citrate synthase (CS) were measured spectrophotometrically [8]. The contents of ATP and ADP in blood plasma and myocardial homogenate were measured as described previously [8]. The concentrations of 2,3-diphosphoglycerate (2,3-DPG) [9], ATP, and ADP [3] in erythrocytes were measured spectrophotometrically. Lactate concentration in blood plasma and myocardial homogenate was estimated with Olvex kits. Pyruvate content in blood plasma and myocardial homogenate was measured spectrophotometrically [8]. The activity of cardiac creatine phosphokinase (CPK-MB) in blood plasma was estimated with SpectroMed kits. Type 1 LDH ($LDH_1$) activity in blood plasma was estimated with Labsystem kits. CPK activity in myocardial homogenate was estimated with Vital Diagnostiks SPb kits (St. Petersburg). Activities of hexokinase and phosphofructokinase were measured spectrophotometrically.

The results were analyzed by SPSS 11 software. The mean values were compared by Student's *t* test.

## RESULTS

The results of our study are shown in Table 1. Modeling of myocardial ischemia in 10-month-old rats was accompanied by an increase in 2,3-DPG content in erythrocytes and decrease in ATP concentration in erythrocytes, blood plasma, and myocardial homogenate. These changes illustrate the development of tissue hypoxia and energy deficiency. An increase in the activities of specific myocardial enzymes (CPK-MB and $LDH_1$) in blood plasma reflects destabilization of cardiomyocyte membranes and "loss" of enzymes from the cytoplasm. The activities of SDH, CS, and PDH decreased in mitochondria. These changes reflect the reduction of oxidative phosphorylation and decarboxylation of pyruvate, which contributes to the development of energy deficiency during ischemia. The myocardium was characterized by activation of glycolytic enzymes hexokinase and phosphofructokinase. We also revealed the activation of a substrate phosphorylation enzyme CPK, which provides cardiomyocytes with energy during oxygen deficiency.

Comparison of intact animals from various age groups (10 and 24 months) showed that old rats are characterized by a significant ($p<0.01$) decrease in the level of ATP and ADP in erythrocytes and concentration of ATP in blood plasma and myocardial homogenate. The observed changes can be related to a significant decrease in the activities of SDH, CS, and PDH, which results in the suppression of oxidation-reduction reactions. Aging was accompanied by a considerable decrease in lactate accumulation in the myocardium and instability of cardiomyocyte cytoplasmic membranes. This conclusion was derived from the appearance of organ-specific enzymes in blood plasma (CPK-MB and $LDH_1$) of old rats even under normal conditions (Table 1).

The direction of metabolic processes in 24-month-old rats after modeling of CHD did not differ from that in 10-month-old animals with experimental myocardial ischemia. These rats were characterized by the development of severe metabolic disorders, increase in the degree of tissue hypoxia, and destabilization of cardiomyocyte membranes.

Administration of Cytoflavin to young rats was followed by activation of SDH, CS, and PDH, which contributed to an increase in the concentration of ATP in the myocardial homogenate and blood plasma. These data illustrate the mechanism for a synergistic effect of Cytoflavin components. Succinic acid, as an intracellular metabolite of the Krebs cycle, is transformed into another metabolite (fumaric acid) under the influence of SDH, which stimulates aerobic glycolysis and tissue respiration. Riboflavin (vitamin $B_2$) is a flavin coenzyme of SDH, which activates SDH and other enzymes of the Krebs cycle. Nicotinamide (vitamin PP) undergoes intracellular transformation into NAD and its phosphate (NADPH), which activates nicotinamide-dependent enzyme of the Krebs cycle for cellular respiration and stimulation of ATP synthesis. Inosine (riboxine) is a precursor of ATP, which activates some enzymes of the Krebs cycle and stimulates the synthesis of key nucleotide enzymes (flavin adenine dinucleotide, FAD, and NAD) [11]. Taking into account the dynamics of 2,3-DPH, CPK-MB, and $LDH_1$, it can be concluded that the observed metabolic changes in cardiomyocytes are not followed by a decrease in the severity of tissue hypoxia and stabilization of membranes.

The effect of Cytoflavin in old rats with myocardial ischemia was opposite to that observed in young animals. We revealed a decrease in the activities of mitochondrial enzymes (SDH, CS, and PDH), ATP concentration in the plasma and erythrocytes tended to decrease. The amount of this energy substrate in the myocardium remained practically unchanged under specified conditions. These changes were accompanied by an increase in the degree of tissue hypoxia (elevation of 2,3-DPH in erythrocytes) and severe desta-

**TABLE 1.** Myocardial Metabolism in 10- and 24-Month-Old Rats under Normal Conditions, during Experimental CHD, and after Administration of Cytoflavin ($M \pm m$)

| Parameter | 10-month-old rats | | | 24-month-old rats | | |
|---|---|---|---|---|---|---|
| | intact ($n$=6) | CHD ($n$=5) | CHD+Cyto-flavin ($n$=5) | intact ($n$=6) | CHD ($n$=5) | CHD+Cyto-flavin ($n$=5) |
| Erythrocytes | | | | | | |
| ATP, μmol/liter | 664.54±14.49$^{++xx}$ | 594.44±5.75** | 582.47±5.24** | 529.03±7.36$^{++xx}$ | 474.09±8.94** | 458.91±4.66** |
| ADP, μmol/liter | 315.11±8.78 | 330.53±16.05 | 316.05±3.01 | 219.40±8.56$^{x}$ | 241.95±12.44 | 248.04±4.81* |
| 2,3-DPH, μmol/liter | 4.82±0.29$^{++xx}$ | 7.21±0.32** | 6.84±0.15** | 3.30±0.17$^{++xx}$ | 5.92±0.16** | 6.15±0.07** |
| Blood plasma | | | | | | |
| ATP, μmol/liter | 200.08±3.47$^{++xx}$ | 162.81±4.57**$^{x}$ | 177.70±2.15**$^{+}$ | 177.09±4.03$^{xx}$ | 166.99±4.46 | 158.15±3.15** |
| ADP, μmol/liter | 75.92±1.58$^{xx}$ | 79.31±1.13$^{xx}$ | 91.21±0.79**$^{++}$ | 87.31±1.43 | 87.51±2.69 | 83.50±1.93 |
| pyruvate, μmol/liter | 58.59±2.26$^{x}$ | 59.95±1.02$^{xx}$ | 51.57±0.68*$^{++}$ | 62.75±1.70$^{++x}$ | 52.06±1.43** | 69.19±1.30* |
| lactate, μmol/liter | 0.50±0.03$^{xx}$ | 0.62±0.14 | 0.67±0.02** | 0.73±0.04$^{++xx}$ | 0.99±0.03** | 1.08±0.05** |
| CPK-MB, μcat/liter | 0±0$^{++xx}$ | 0.25±0.04** | 0.21±0.02** | 0.09±0.01$^{++xx}$ | 0.33±0.02** | 0.35±0.02** |
| $LDH_1$, μcat/liter | 0.02±0.00$^{++xx}$ | 0.09±0.01** | 0.09±0.00** | 0.04±0.00$^{++xx}$ | 0.11±0.00** | 0.12±0.00** |
| Mitochondria | | | | | | |
| SDH, nmol/mg×min | 17.82±1.10$^{++xx}$ | 11.83±0.47**$^{x}$ | 13.45±0.47**$^{+}$ | 10.46±0.44$^{++xx}$ | 7.74±0.21** | 6.55±0.25** |
| CS, nmol/mg×min | 3.94±0.23$^{++x}$ | 2.38±0.21**$^{x}$ | 3.21±0.20*$^{+}$ | 2.72±0.28$^{+xx}$ | 1.92±0.05* | 1.65±0.08** |
| PDH, μmol NAD/mg×min | 31.04±0.89$^{++}$ | 21.68±0.90**$^{xx}$ | 31.34±0.73$^{++}$ | 22.92±1.09$^{+x}$ | 19.51±0.66* | 18.98±0.53* |
| Myocardial homogenate | | | | | | |
| hexokinase, μmol/mg protein×h | 27.38±1.20$^{++xx}$ | 36.22±0.54**$^{x}$ | 34.25±0.62**$^{+}$ | 27.91±0.76$^{++xx}$ | 33.33±1.59** | 22.48±0.64** |
| CPK, μcat/g protein×h | 106.13±18.71 | 138.95±1.24$^{xx}$ | 104.76±3.56$^{++}$ | 120.25±3.71$^{xx}$ | 129.21±7.62 | 96.51±0.69** |
| PFK, μmol/g protein×h | 12.97±0.54$^{++xx}$ | 16.38±0.71**$^{x}$ | 19.38±0.62**$^{+}$ | 12.01±0.67$^{++}$ | 19.47±0.40** | 10.74±0.40 |
| pyruvate, μmol/g tissue | 0.16±0.01$^{x}$ | 0.19±0.01 | 0.19±0.01* | 0.14±0.01 | 0.14±0.00 | 0.14±0.00 |
| lactate, μmol/g tissue | 3.10±0.34$^{+}$ | 2.09±0.24*$^{x}$ | 3.33±0.39$^{+}$ | 2.60±0.22$^{+x}$ | 1.66±0.20* | 1.76±0.22* |
| ATP, μmol/liter | 3.08±0.24$^{++x}$ | 1.18±0.08**$^{xx}$ | 2.09±0.16*$^{++}$ | 2.03±0.09$^{++xx}$ | 1.06±0.03** | 1.17±0.05** |

**Note.** PFK, phosphofructokinase. *$p$<0.05 and **$p$<0.01 compared to intact rats of the same age; $^{+}p$<0.05 and $^{++}p$<0.01 compared to the CHD group of the same age; $^{x}p$<0.05 and $^{xx}p$<0.01 compared to the CHD+Cytoflavin group of the same age.

bilization of cardiomyocyte membranes (increase in the concentrations of CPK-MB and $LDH_1$ in blood plasma). The observed changes can be associated with the effectiveness of Cytoflavin only under conditions of an adequate oxygen supply to cardiac cells [12]. This medicinal agent activates aerobic mechanisms of energy supply and, therefore, increases the myocardial demands for oxygen [1]. An age-related increase in the degree of ischemia and tissue hypoxia counteracts the positive effect of Cytoflavin. Stimulation of aerobic

mechanisms for energy supply in old animals with ischemia is followed by disadaptation, distress, and cardiotoxicity of the metabolic agent.

We conclude that administration of Cytoflavin has a cardioprotective effect on 10-month-old rats with experimental myocardial ischemia. This drug increases the activity of mitochondrial enzymes and, therefore, improves energy supply to the myocardium. Cytoflavin has no effect on stabilization of cardiomyocyte membranes and does not decrease the degree of tissue hypoxia in young (10 month) rats with experimental myocardial ischemia. Cytoflavin possesses cardiotoxic properties and increases the severity of metabolic disorders in old (24 months) rats with experimental myocardial ischemia, which is manifested in the depletion of energy reserves in cardiomyocytes.

This work was supported by the Belgorod State National Research University (project No. 419112011, January 17, 2012; "Clinical and Pharmacological Approaches to the Personalization of Therapy with Metabolic Drugs in Patients with Coronary Heart Disease"; performed within the framework of the State Task).

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