Impact of an angiotensin II receptor antagonist and antioxidant on count of stem cells and on morphofunctional parameters in animals

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Introduction

Now pathology of cardiovascular system is the major cause of cardiac morbidity and mortality in many countries. It's necessary to develop new methods of diagnosis and treatment of cardiovascular diseases. Studying new properties of drugs and substances to treat pathology of cardiovascular system is very important. Candesartan cilexetil is an angiotensin II receptor antagonist. This drug has a number of side effects. Resveratrol is the natural antioxidant, which has pleiotropic health beneficial effects. Effect of new combination of candesartan cilexetil with resveratrol on mobilization of stem cells was studied in the experiment. Goal. To investigate the effect of candesartan cilexetil and resveratrol at different doses on the number of stem cells, morphofunctional parameters in animals. Methods. Male Balb/C mice were used for the experiment. Animals were received intragastrically candesartan cilexetil and resveratrol at different doses for 7 weeks after physical strain. Evaluation of the effects of substances on mobilization of CD117+ stem cells from bone marrow to blood, morphofunctional parameters were detected by flow cytometry. Results. It was found that candesartan cilexetil at 3 mg/kg dose stimulated mobilization of CD117+ stem cells but was cytotoxic. Candesartan cilexetil at 1.5 mg/kg dose did not increased the number of CD117+ stem cells in bone marrow. The combination of candesartan cilexetil and resveratrol increased the amount of CD117+ stem cells in bone marrow and blood and was safe for cells. Conclusions. New combination of candesartan cilexetil and resveratrol was effective in increasing of the number of CD117+ stem cells and was safer than candesartan cilexetil alone.

Keywords: Candesartan cilexetil; resveratrol; CD117+ stem cells; apoptotic cells; micronuclei; cell cycle parameters.

Materials and methods

Male Balb/C mice were used in the experiment. Animals were divided into 8 groups. Mice of 6 groups were exposed to...
to physical strain (swimming with 2% freight of body weight) for 2 months. Then animals received intragastrically daily candesartan cilexetil and resveratrol for 7 weeks to stimulate mobilization of stem cells and angiogenesis after damaging effect of physical stress: group 1 of animals were given candesartan cilexetil at 3 mg/kg dose (maximum therapeutic dose for treatment of hypertension according to the instruction); group 2 of animals received candesartan cilexetil at 1.5 mg/kg dose (average therapeutic dose for treatment of hypertension according to the instruction), group 3 were given candesartan cilexetil at 1.5 mg/kg dose and resveratrol at 10 mg/kg dose; mice of group 4 were given candesartan cilexetil at 1.5 mg/kg dose and resveratrol at 30 mg/kg dose, group 5 of mice was administrated candesartan cilexetil at 1.5 mg/kg dose and resveratrol at 50 mg/kg dose. Parameters of bone marrow and blood of the control basic were studied before the experiment. Control group 1 of mice were intact (no physical exertion) during all time of the experiment. Animals of this group were given intragastrally daily 1% starch solution. Control 2 of animals (subjected only to physical exertion) were given intragastrally daily 1% starch solution.

The administered doses of the studied substances were calculated using the interspecific conversion formula: [8]:

$$A = \frac{B \times \kappa(B)}{\kappa(A)}$$

Where:
- $A$ – required dose;
- $B$ – known dose;
- $\kappa(B)$ – conversion factor for weight $B$;
- $\kappa(A)$ – conversion factor for weight $A$.

Flow cytometry method was used to study the number of CD117+ stem cells (endothelial progenitor cells), apoptotic and micronucleated cells, cell distribution at stages of cell cycle in bone marrow and blood of Balb/C mice.

The results are presented as means±SEM. We used Student’s t-test to compare 2 samples and one-way ANOVA for multiple comparisons followed by pair-wise comparison.

**Results and discussion**

At first the count of stem cells in bone marrow and blood of Balb/C mice was investigated. Cell surface marker CD117 (c-kit) was used to study the number of stem cells. It was found that long-term physical activity didn’t change the number of CD117+ stem cells in bone marrow and blood of Balb/C mice. Candesartan cilexetil at 3 mg/kg dose increased the number of CD117+ stem cells in bone marrow and blood of animals in comparison with the control groups (p<0.05). It was shown that candesartan cilexetil at 1.5 mg/kg dose after physical activity didn’t alter the amount of CD117+ stem cells in bone marrow. Candesartan cilexetil at 1.5 mg/kg dose increased the number of CD117+ stem cells in blood of experimental animals (p<0.05) (Figure 1 and 2).

Combination of candesartan cilexetil at 1.5 mg/kg dose and resveratrol at 10 mg/kg, 30 mg/kg and 50 mg/kg doses after long-term physical exposure significant increased the number of CD117+ stem cells in bone marrow and blood of mice in comparison with the control groups. The highest increase of the number of CD117+ stem cells was recorded in the group of animals treated with candesartan cilexetil at 1.5 mg/kg dose and resveratrol at 50 mg/kg dose (Figure 1 and 2).

![Fig 1. The number of CD117+ stem cells in bone marrow of Balb/C mice under using candesartan](image)

* – in comparison with the control basic (p<0.05); ** – in comparison with the control 1 (p<0.05); # – in comparison with the control 2 (p<0.05).
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Fig 2. The number of CD117+ stem cells in blood of Balb/C mice under using candesartan cilexetil and resveratrol

* – in comparison with the control basic (p<0.05); ** – in comparison with the control 1 (p<0.05); # – in comparison with the control 2 (p<0.05).

The effect of using of candesartan cilexetil, resveratrol and their combination on cytogenetics and cytokinetic parameters of bone marrow and blood was analyzed. The obtained data are presented in Figures 3 – 8. It was found that long-term physical overload stimulated generation of apoptotic and micronucleated cells in bone marrow and blood of Balb/C mice (Figures 3, 4, 6 and 7). Candesartan cilexetil at 3 mg/kg dose increased the number of cells with damage of genetic material in comparison with the control basic and control 1, and decreased this parameter as compared to the control 2 (p<0.05). Distribution of cells at phases of cell cycle in bone marrow of Balb/C mice under using candesartan cilexetil at high dose didn’t change. Using candesartan cilexetil at 3 mg/kg after physical overload stimulated proliferation of cells in blood in comparison with the control 2 (p<0.05) (Figures 5 and 8).

Fig 3. The number of apoptotic cells in bone marrow of Balb/C mice under using candesartan cilexetil and resveratrol

* – in comparison with the control basic (p<0.05); ** – in comparison with the control 1 (p<0.05); # – in comparison with the control 2 (p<0.05); ## – in comparison with group 1 (p<0.05).
It was shown that candesartan cilexetil at 1.5 mg/kg dose significantly increased the ratio of apoptotic cells in bone marrow and blood of Balb/C mice (Figures 3, 4, 6, and 7). Candesartan cilexetil at 1.5 mg/kg decreased the number of cells at G0/G1 stages of cell cycle in blood in comparison with the control 2 (p<0.05), so it stimulated cell proliferation and decreased negative effect of long-term physical overload (Figure 8).

Using combination of two substances stimulated generation of apoptotic cells and significantly decreased the amount of cells with micronuclei in bone marrow (Figures 3 and 4). New combination of candesartan cilexetil at 1.5 mg/kg dose with resveratrol at 10 mg/kg, 30 mg/kg and 50 mg/kg doses after long-term physical training decreased the amount of cells at G0/G1 stages of cell cycle in blood (p<0.05) (Figure 8).
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Fig 6. The number of apoptotic cells in blood of Balb/C mice under using candesartan cilexetil and resveratrol

* – in comparison with the control basic (p<0.05); ** – in comparison with the control 1 (p<0.05); # – in comparison with the control 2 (p<0.05); ## – in comparison with group 1 (p<0.05); @ – in comparison with group 2 (p<0.05).

Fig 7. The number of micronucleated cells in blood of Balb/C mice under using candesartan cilexetil and resveratrol

* – in comparison with the control basic (p<0.05); ** – in comparison with the control 1 (p<0.05); # – in comparison with the control 2 (p<0.05); ## – in comparison with group 1 (p<0.05); @ – in comparison with group 2 (p<0.05); + – in comparison with group 3 (p<0.05).

Fig 8. Distribution of cells at phases of cell cycle in blood of Balb/C mice under using candesartan cilexetil and resveratrol

* – in comparison with the control basic (p<0.05); ** – in comparison with the control 1 (p<0.05); # – in comparison with the control 2 (p<0.05); ## – in comparison with group 3 (p<0.05).
Some authors found that angiotensin II receptor antagonist candesartan cilexetil has a number of side effects. High doses of candesartan cilexetil influence the formation of separate subpopulations of cells of bone marrow [9]. G. Mantzios et al showed that candesartan cilexetil was cause of autoimmune haemolytic anaemia [10].

Conclusions

It was shown that candesartan cilexetil at high dose stimulated mobilization of CD117+ stem cells in bone marrow and blood of Balb/C mice, but was cytotoxic. Low dose of candesartan cilexetil didn’t increased the number of CD117+ stem cells in bone marrow and increased it in blood, but the effect was less in comparison with high dose of substance. This is the first evidence that use of the combination of candesartan cilexetil and resveratrol after long-term physical training stimulated generation of stem cells in bone marrow and blood of Balb/C mice. New combination of candesartan cilexetil and resveratrol was not cytotoxic and stimulated cells proliferation. These results might be used to develop new complex drug for treatment of cardiovascular diseases capable to mobilize stem cells and facilitate reparative processes in cardiovascular system.

References