Combination of Captopril and Darbepoetin Alfa Attenuate Radiation-Induced-Endothelial Dysfunction

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Abstract

Background: Captopril and darbepoetin are drugs with high radio protective potential.

Materials and Methods: The experiments were performed on male 55 Wistar rats. Rats were divided into 5 equal groups: 1) Intact; 2) Control; 3) Captopril (15 mg/kg); 4) Darbepoetin alfa (5000 U/kg); 5) Captopril (15 mg/kg) + Darbepoetin alfa (5000 U/kg). To simulate the RIED animals were subjected to ionizing-radiation injury at 500 roentgens with an exposure of 20 seconds. To evaluate the endothelial dysfunction (ED) level all rats were subjected to coefficient of ED (CED) calculation and determination of blood pressure (BP) and eNOS expression level, as well as diameter of cardiomycytes and carotid intima/media ratio.

Results and Discussion: According to the degree of therapeutic effect, the used treatment schemes can be arranged in the following sequence (to reduce the effect): Captopril + Darbepoetin alfa > Captopril > Darbepoetin alfa. In this case, the combination of captopril 15 mg/kg i.p. + darbepoetin-alpha in a dose of 5000 U/kg i.p. brought the values of functional and laboratory tests to a group of intact animals.

Conclusion: The combination of captopril + darbepoetin alfa has a high radio- and endothelioprotective potential and can be recommended for further preclinical and clinical studies.

Keywords: Captopril, Darbepoetin Alfa, Dysfunction.

Background

The impact of radiation on biological systems continues to be one of the priority tasks of researches in modern biomedicine. Workers in nuclear power plants, nuclear submarines, cosmonauts, and many other professions face the risk of exposure to ionizing radiation. Patients at cancer clinics who have undergone radiation therapy are at high-increased risk for radiation-associated pathologies [1]. An important event of the last decades in radiobiology is the discovery of the contribution of radiation to the formation of cardiovascular pathology. In particular, a number of experimental and clinical observations demonstrate the contribution of radiation to the occurrence of endothelial dysfunction, arterial hypertension, dyslipidemia, and vascular wall remodeling and blood coagulation disorders [1, 2]. In spite of this, there is a certain gulf between the existing arsenal of pharmacological compounds, theoretically capable of reducing radiation-induced endothelial dysfunction (RIED) and the number of targeted preclinical trials. For example, angiotensin-converting enzyme (ACE) inhibitors [3], erythropoietin preparations [4-7, 28-30], arginase inhibitors [8, 9], antioxidants [10, 11], NO donators [10, 11], statins [12-16, 24, 25] and some other drugs [19-21, 26, 27] are shown as agents capable of blocking important for RIED pathogenetic cascades.

Materials and Methods

Bioethics

The experiments were carried out in compliance with the requirements of the federal law of the Russian Federation *On Protection of Animals against Cruel Treatment* dated June 24, 1998, the rules of laboratory practice in preclinical studies in the Russian Federation (GOST 3 51000.3-96 and GOST R 53434-2009), European Community directives (86/609 EU), the rules and the International Recommendations of the European Convention for the Protection of Vertebrate Animals used in experimental studies (1997) and the Laboratory Practice Rules adopted in the Russian Federation (order of the Ministry of Healthcare of the Russian Federation No. 708 of August 29, 2010).

Animals and Groups

The experiments were performed on male 55 Wistar rats (245-275 g). The animals were kept in a standard conventional vivarium in individually ventilated cages (Techniplast, USA). Each animal was

subject to handling procedure 3 days before the experiment.12 hours before the experiment, animals were deprived of access to feed.

Rats were divided into 5 equal groups:

- Intact – Vehicle (NaCl 0,9%) 1 ml/kg intraperitoneally 1 time per day for 7 days
- RIED – Radiation + L-NAME+ Vehicle (NaCl 0,9%) 1 ml/kg intraperitoneally 1 time per day for 7 days
- Captropril – Radiation + L-NAME+ Captopril 12.5 mg/kg intraperitoneally 1 time per day for 7 days
- Darbepoetin alfa – Radiation + L-NAME + Darbepoetin alfa 5000 U/kg intraperitoneally 1 time per 2 day for 6 days (3 times)
- Captropril + Darbepoetin alfa - Radiation + L-NAME + Captopril 12.5 mg/kg intraperitoneally 1 time per day for 7 days + Darbepoetin alfa 5000 U/kg intraperitoneally 1 time per 2 day for 6 days (3 times)

Pathology Modeling

To simulate the RIED animals were subjected to ionizing-radiation injury at 500 roentgens with an exposure of 20 seconds LD=50/30 once (ROKUS-M, Russia). To gain the level of ED due enhancement of nitric oxide deficiency and vascular wall injury we also injected L-NAME daily for 7 days once a day, intraperitoneally, at a dose of 15 mg/kg.

Endothelial dysfunction evaluation

All animals were subject to coefficient of endothelial dysfunction (CED) evaluation as described previously [22]. In brief, the common carotid artery was cathetized and connected to a pressure transducer (Biopac Systems, USA). Then a solution of acetylcholine at a dose of 40 μg/kg (endothelium-dependent vasodilation) and a solution of sodium nitroprusside at a dose of 30 μg/kg (endothelium-dependent vasodilatation) were injected into the femoral vein. The area under curve (AUC) of the pressure drop in response to nitroprusside divided by the AUC of pressure drop in response to acetylcholine was taken as CED.

Histology

After CED evaluating, the animals were euthanized by cardiac puncture under anesthesia. The heart, carotid arteries, abdominal aorta, and renal arteries were excised, fixed in 10% buffered formalin, stained in hematoxylin-eosin the intima/media for morphometrically evaluation of intima/media ratio and cardiomyocytes diameter.

Western-blot

The level of eNOS expression in abdominal aorta was determined in a cell lysate as previously described [23]. After the incubation, cell were washed three times with 5 mM phosphate buffer. The cells from one well were collected in 100 μl of lysis buffer, centrifuged for 10 min at 1000 g and subjected to polyacrylamide gel electrophoresis. Each plate was applied a mixture of pre-stained protein markers with a molecular weight range (Mm) 7000-200000 Yes (Bio-Rad Kaleidoscope Prestained Standards, USA). At the end of electrophoresis, the proteins were transferred from the gel to a nitrocellulose membrane — Western blot (60 V, 1 hour). After washing the membrane three times with 5_mM phosphate buffer pH 7.4, containing 150 mM NaCl and 0.05% Tween-200, it was treated overnight with a solution of polyclonal rabbit antibodies against human eNOS (BD Transduction Labs, USA) diluted (ratio=1:500). Then the membrane was washed and incubated for an hour with diluted at a ratio of 1:300 secondary antibodies conjugated with horseradish peroxidase (goat anti-rabbit IgG antibodies, Sigma, USA). The strip corresponding to eNOS was detected according to its molecular weight, as compared with the tap proteins. The film was dried in air, the strips were scanned and the area under the curve was calculated using the Total Lab program.

Statistical assay

The data was checked for normal distribution. The type of distribution was determined by the criterion of Shapiro-Wilk. In the case of a normal distribution, the mean value (M) and standard error of the mean (m) were calculated. In cases of non-parametric distribution, the median (Me) and quartile range (QR) were calculated. Intergroup differences were analyzed by MANOVA with post-hoc analysis or Mann-Whitney with Bonferroni correction test, depending on the type of distribution. Statistical analysis was performed using Statistic a 10.0 software.

Results and Discussion

Immediately after irradiation and for the next 7 days, the animals showed no significant changes in behavior and phenotype, except for a slight anxiety. Irradiation followed by the introduction of L-NAME led to the development of persistent hypertension, accompanied by an increase in systolic blood pressure to 181.2 ± 2.5 mm Hg. Art. (in contrast with 115.3±1.9 in intact group). When studying the functional and laboratory signs of endothelial dysfunction, an increase in CCED was observed from 1.15±0.09 to 5.75±0.10 c.u. (Fig 1.A), intima / media ratio from 0.22±0.01 to 0.31±0.02 c.u. (Fig 1.B) and a decrease in eNOS expression from 6.11±0.07 to 2.12±0.08% (Fig 1.C). At the same time, there was an increase in the diameter of myocardiocytes from 8.7±0.1 to 13.5±0.2 μm (Fig 1.D), which can be

explained as a result of a deficit of nitric oxide and an increase in the afterload on the heart.

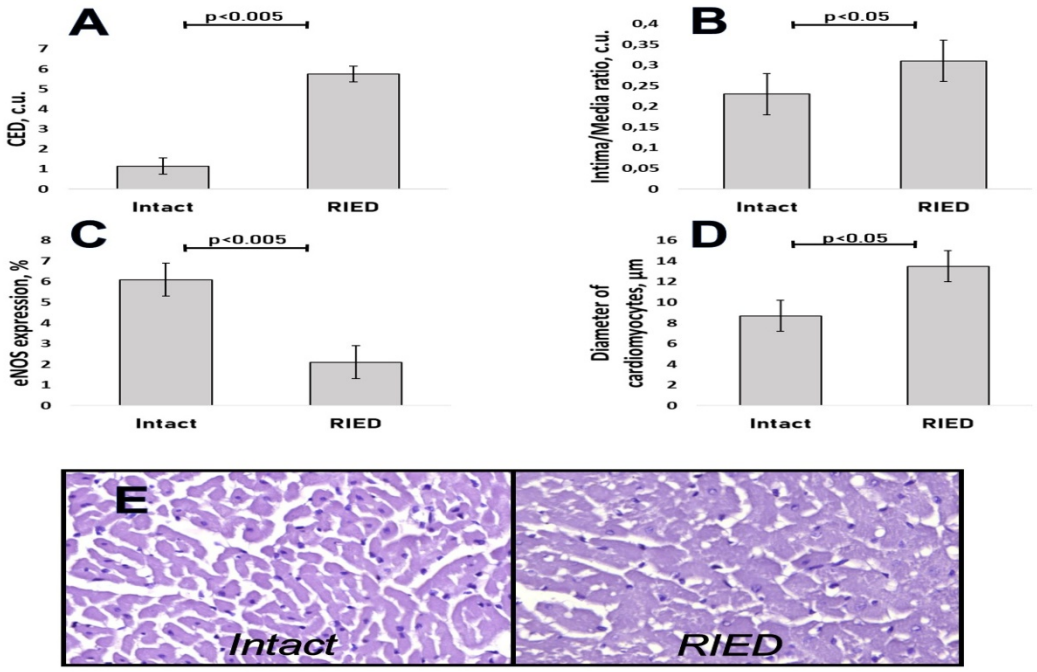

Figure -1: Impact of RIED modeling on the CED (A), Intima/Media ratio (B), eNOS expression (C) and diameter of cardiomyocytes (D, E).

Captopril darbepoetin and their combination improved functional and laboratory parameters, reducing CED (Table 1), the intima/media ratio and the diameter of cardiomyocytes, as well as increasing the expression of eNOS in comparison with the non-treated group. At the same time, according to the degree of therapeutic effect, used treatment schemes can be arranged in the following sequence (to reduce the effect): Captopril + Darbepoetin alfa > Captopril > Darbepoetin alfa.

Table -1: Impact of captopril, darbepoetin and combined therapy on the functional and laboratory parameters of rats with RIED.

Group	Systolic BP, mmHg	CED, c.u.	Intima/Media ratio, c.u.	eNOS expression, %	Diameter of cardiomyocytes, µm
Intact	115.3±1.9	1.15±0.08	0.22±0.01	6.11±0.07	8.7±0.1
RIED	181.2±2.5	5.75±0.10	0.31±0.02	2.12±0.08	13.5±0.2
Captopril	129.1±1.5*	2.69±0.09*	0.27±0.01*	3.11±0.08*	10.5±0.2*
Darbepoetin alfa	179.9±2.8	4.65±0.10*	0.30±0.03	2.49±0.09*	13.4±0.1
Captopril + Darbepoetin alfa	121.1±1.5*	1.58±0.09* ^{y}	0.24±0.01*	5.99±0.08* ^{y}	9.0±0.2* ^{y}

Note: BP – Blood pressure**;** * - p<0,05 in comparison with **RIED** group; ^{y} - p<0,05 in comparison with **Captopril** group

As can be seen from table 1, captopril significantly improved all measurable indicators, while darbepoetin alpha monotherapy did not affect the intima/media ratio, systolic pressure and diameter of cardiomyocytes.

The combination of captopril and darbepoetin showed the most pronounced effect, significantly reducing CED, eNOS expression and diameter of cardiomyocytes in comparison with captopril monotherapy (Table 1).

It is possible to associate the obtained effect with the fact that the therapeutic contribution of captopril is realized through a combination of its own radioprotective effect and hypotensive action. Perhaps darbepoetin alpha has a high endothelioprotective activity on this model, which was shown to be a significant decrease in CED, but did not show a sufficient therapeutic effect due to the fact that it is not able to reduce blood pressure.

In the case of a combination of two drugs, the radioprotective and antihypertensive effects of captopril add to the anti-apoptotic effect of

darbepoetin and protect the endothelium to a very high degree.

Conclusions

1. Simulation of RIED by irradiation of male rats 500 R results in the development of severe hypertension and endothelial dysfunction, confirmed by functional and laboratory tests.

2. Captopril at a dose of 15 mg/kg i.p. significantly improves the functional and morphological state of the vascular wall, and also prevents the development of cardiomyocyte hypertrophy.

3. Darbepoetin-alpha monotherapy at a dose of 5000 U/kg has a weak and selective therapeutic effect on the RIED expert model.

4. The combination of captopril 15 mg/kg i.p. + darbepoetin-alpha in a dose of 5000 U/kg i.p. It has a strong radio and endothelioprotective effect, bringing the values of functional and laboratory tests to a group of intact animals.

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