Research Article

Protective effects of 11-amino acid darbepoetin fragment in the experimental hypertensive neuroretinopathy

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ABSTRACT

Introduction: Hypertensive retinopathy is recorded in 66.3% of patients with hypertensive disease. As authors note, there is a lack of drugs for targeted, specific correction of retinal damage, occurring in arterial hypertension (AH). **Objective:** The objective of the study was to improve the pharmacological correction effectiveness of retinal damage with the use of 11-amino acid darbepoetin fragment PRK-002 in the experimental hypertensive neuroretinopathy. **Materials and Methods:** Hypertensive neuroretinopathy simulation was performed by daily injection of N-nitro-L-arginine methyl ester for 28 days and a single increase in intraocular pressure. The retinoprotective effects of the 11-amino acid darbepoetin fragment PRK-002 in comparison with carbamylated darbepoetin and sulodexide were estimated by the changes in the b/a coefficient. Measuring the retinal microcirculation level was carried out with the use of laser Doppler flowmetry. **Results and Discussion:** The most pronounced protective effect is demonstrated by PRK-002 in a dose of 4 μg/kg, which is expressed in reaching the target values of the b/a and the retinal microcirculation level. When pathology was corrected by carbamylated darbepoetin in a dose of 300 μg/kg, microcirculation level increased by 41.9% ($P < 0.05$) in comparison with the group without correction, but did not reach the target values; b/a increased by 30.9% ($P < 0.05$) in comparison with the group without correction and reached the target values. When pathology was corrected by sulodexide in a dose of 150 LRU/kg, the microcirculation level in retina increased by 52.5% ($P < 0.05$) in comparison with the group without correction, but did not reach the target values; b/a reached the target values. **Conclusion:** The obtained data give an experimental substantiation of the pharmacological correction possibility of retinal damage in AH by 11-amino acid darbepoetin fragment PRK-002.

KEY WORDS: 11-Amino acid darbepoetin fragment, Experimental hypertensive neuroretinopathy, PRK-002, Rats

INTRODUCTION

Hypertensive retinopathy and neuroretinopathy in patients with hypertensive disease (HD) result from pathological changes in the central retinal artery and its branches, as well as from hemodynamic changes in other vessels of the ophthalmic artery system.[1,2] Age, duration of HD, and systolic blood pressure are significant risk factors of retinopathy.[3] Local circulatory disorders in the retinal arterial system are observed in diabetic, hypertensive retinopathy and neuroretinopathy, optic nerve atrophy vascular origin, anterior ischemic optic neuropathy, etc.[4-7] The main factors in the development of retinal hypertensive angiopathy are disorders of general hemodynamics and local endothelial dysfunction of the retinal vessels.[8-10]

Hypertensive retinopathy is fraught with complications including retinal arterial occlusions, optic atrophy, and retinal vein thrombosis.[11] Acute occlusions of the retinal arteries in 91.2% of cases occur alongside cardiovascular diseases (60% – atherosclerosis and arterial hypertension).[12]

At present, the main therapy of patients with hypertensive retinal damage is aimed at the normalization of blood pressure values, as well as the correction of already formed ischemic neuropathy. In the treatment of hypertensive neuroretinopathy, drugs that improve retinal microcirculation and rheological blood properties, vasodilators, antioxidants, etc., are used.[13]

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Studying the way of how to improve retinal tissue tolerance to ischemia, in particular, developing in hypertension, is an actual problem of pharmacology and ophthalmology.[14,15] Thus, an important task is to find specific and highly effective means for correcting of hypertensive neuroretinopathy.

To study the pharmacological activity of new biologically active substances,[16] as well as study the new effects of already known drugs,[17] it is necessary to conduct experimental studies *in vitro* and *in vivo*.[18]

In our opinion, a promising renoprotective with endothelial and neuroprotective activity may be 11-amino acid darbepoetin fragment PRK-002 having a low molecular weight in comparison with carbamylated darbepoetin.[19] In view of the above, it is important to study the possibilities of pharmacological correction of hypertensive neuroretinopathy using PRK-002 in the experiment.

Objective

The objective of the study is to improve the pharmacological correction effectiveness of retinal damage with the use of 11-amino acid darbepoetin fragment PRK-002 in the experimental hypertensive neuroretinopathy.

MATERIALS AND METHODS

Experiments were carried out on 50 rats Wistar weighing 250 ± 25 g. For the study, the rats were taken with no external signs of disease, having passed the quarantine regime. Ethical principles of handling laboratory animals were observed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes, CETS No. 123.

The following groups were included in the experiment: The first group (n = 10) – a control group (with intraperitoneal [i/p] infusion of 0.9% NaCl solution in the equivalent volume for 28 days), the second (n = 10) – a group with the simulated hypertensive neuroretinopathy, the third (n = 10) – with correction of the pathology by PRK-002 in a dose of 4 µg/kg (PharmaPark Ltd., Russia), the fourth (n = 10) – with the correction of the pathology by reference drug carbamylated darbepoetin in a dose of 300 µg/kg (PharmaPark Ltd., Russia), and the fifth – with the introduction of reference drug sulodexide in a dose of 150 LRU/kg (Alfa Wassermann, Italy).

Simulation of hypertensive neuroretinopathy was performed by daily i/p injection of a non-selective inhibitor of NO synthases N-nitro-L-arginine methyl ester (L-NAME) (Sigma, Germany) in a dose of 12.5 mg/kg for 28 days and a single increase in intraocular pressure to reach 110 mmHg by applying mechanical pressure to the anterior chamber for 5 min on the 26th day of the experiment.[20]

PRK-002 was injected subcutaneously (s/c) in a dose of 4 µg/kg of rat mass to the withers area on the 1st, 4th, 7th, 10th, 13th, 16th, 19th, 22nd, 25th, and 28th days of the experiment, 30 min before administering L-NAME.

Carbamylated darbepoetin was injected s/c in a dose of 300 µg/kg of rat mass to the withers area on the 1st, 4th, 7th, 10th, 13th, 16th, 19th, 22nd, 25th, and 28th days of the experiment, 30 min before administering L-NAME.

Sulodexide in the form of capsules, 250 LRU (Vessel Due F®) was injected *per os* for 28 days in a dose of 150 LRU/kg of rat mass daily.[21]

The effectiveness of the studied pharmacological agents in the experimental hypertensive neuroretinopathy was evaluated on the 29th day of the experiment by the changes in the b/a coefficient and laser Doppler flowmetry (LDF) results.

Electroretinography (ERG) and b/a Coefficient Counting

To perform ERG, rats were kept in the dark for 30 min, then anesthetized (chloral hydrate, 300 mg/kg, i.p.). Evoked biopotentials were run at a frequency of 1–1000 Hz, amplified, averaged, and presented graphically on the screen using the MP150 data acquisition and analysis system (Biopac Systems, Inc., CA, USA).[22,23] The duration of the flashes was 0.025 s, intensity was 30 kV. ERG registration was carried out in response to a single stimulation. The ERG recording was carried out for 0.5 s on each rat in the groups. To assess the degree of retinal ischemia in the simulated hypertensive neuroretinopathy, the ratio of the amplitudes of the b- and a-waves, the b/a coefficient was evaluated. The mean was derived for each group from 10 values received and was introduced into the protocol.[24]

Measuring the retinal microcirculation level in rats was carried out with use LDF.[25] Registration was carried out by MP150 data acquisition and analysis systems and the TSD144 needle-type sensor, with AcqKnowledge 4.2 software (BIOPAC Systems, Inc., CA, USA). After animal anesthesia, assessment of microcirculation level was carried out at 10 points on the circumference of the eyeball; the recording duration of the microcirculation level readings at one point was 20 s. From the microcirculation level results at every point, the mean value was calculated, which was taken as the indicator of the microcirculation level in the retina of the experimental animal. The microcirculation value in the animal group was calculated as the mean of the values obtained from each experimental animal.[26]

Table 1: Influence of PRK-002, carbamylated darbepoetin, and sulodexide on the a- and b-waves amplitudes when correcting simulated hypertensive neuroretinopathy (M±m; *n*=10), mV

Experimental groups	The a-wave amplitudes (*n*=10)	The b-wave amplitudes (*n*=10)
Control	0.36±0.03	0.94±0.07^{y}
Simulated hypertensive neuroretinopathy	0.34±0.02	0.65±0.05*
Correction by PRK-002, 4 µg/kg	0.36±0.03	0.90±0.07^{y}
Correction by carbamylated darbepoetin, 300 µg/kg	0.35±0.03	0.88±0.06^{y}
Correction by sulodexide, 150 LRU/kg	0.35±0.03	0.81±0.05*^{y}

*P<0.05 compared to the control group; ^{y}P<0.05 compared to the group with simulated hypertensive neuroretinopathy

For all data, descriptive statistics were used, and the data were checked for normal distribution. Distribution type was determined using the criterion of Shapiro–Wilk test. In case of normal distribution, the average value (M) and standard error of the mean (m) were calculated. In cases of abnormal distribution, the median (Me) and the quartile range were calculated. Between-group differences were analyzed by parametric (t-student criterion) or non-parametric (Mann–Whitney U-test) methods, depending on the type of distribution. Differences were determined at a 0.05 significance level. Statistical analyses were performed using Statistica 10.0 software.

RESULTS

ERG and b/a Coefficient Counting

Influence of the studied pharmacological agents on the values of amplitudes a- and b-waves obtained in experimental groups is presented in Table 1.

In each group, the b/a coefficient was calculated, the values of which are presented in Table 2.

In the group with the pathology simulation, the b/a coefficient decreased by 26.5% in comparison with the control group ($P < 0.05$). Against the background of PRK-002 in a dose of 4 µg/kg and carbamylated darbepoetin in a dose of 300 µg/kg, the b/a coefficient increased by 30.9% in comparison with the group without correction ($P < 0.05$) and reached the target values. In the group with the injection of sulodexide in a dose of 150 LRU/kg, the b/a coefficient did not reach the target values, but increased by 20.9% in comparison with group without correction ($P < 0.05$).

Evaluation of Retinal Microcirculation

The LDF results are presented in Table 3.

In the group with the pathology simulation, the level of retinal blood flow decreased by 43.8% ($P < 0.05$) in comparison with the control group. When correcting pathology by PRK-002 in a dose of 4 µg/kg, blood flow level increased by 68.9% ($P < 0.05$) in comparison with the group without correction and did not differ significantly from the mean value of the control group. When correcting pathology by carbamylated darbepoetin in a dose of 300 µg/kg, the microcirculation level in retina increased by 41.9%

Table 2: Influence of PRK-002, carbamylated darbepoetin, and sulodexide on the values of the b/a coefficient when correcting simulated hypertensive neuroretinopathy (M±m; *n*=10), RU

Experimental groups	Ratio b/a (*n*=10)
Control	2.60±0.07
Simulated hypertensive neuroretinopathy	1.91±0.08*
Correction by PRK-002, 4 µg/kg	2.50±0.13^{y}
Correction by carbamylated darbepoetin, 300 µg/kg	2.51±0.06^{y}
Correction by sulodexide, 150 LRU/kg	2.31±0.10*^{y}

RU: Relative units; *P<0.05 compared to the control group; ^{y}P<0.05 compared to the group with simulated hypertensive neuroretinopathy

($P < 0.05$) in comparison with the group without correction, but did not reach the target values. When correcting pathology by sulodexide in a dose of 150 LRU/kg, the microcirculation level increased by 52.5% ($P < 0.05$) in comparison with the group without correction, but did not reach the target values.

DISCUSSION

As the previous studies showed, by the quantitative parameters of the retinal layer thickness, the neuroprotective effect is somewhat less expressed in recombinant erythropoietin than in carbamylated darbepoetin. By qualitative and quantitative morphological indices, the neuroprotective effect of carbamylated darbepoetin in a dose of 300 µg/kg[7] was revealed on the model of ischemic optic neuropathy in Wistar rats. Administration of the drug did not affect the morphological changes in the blood vessels, but the neuronal structures of the retina remained more intact than in the pathology simulating group. A similar pattern was found in the group with administration of recombinant erythropoietin in a dose of 50 IU/kg. Despite significant circulatory disturbances in the microcirculatory vessels of the retina, as well as in extraocular vessels, the neurons were relatively intact.[19]

Based on the obtained values of the b/a coefficient in the experimental groups, it follows that a positive effect on the electrophysiological state of the retina in the correction of hypertensive neuroretinopathy in descending order has PRK-002, 4 µg/kg (carbamylated darbepoetin, 300 µg/kg) > sulodexide, 150 LRU/kg.

Table 3: Influence of PRK-002, carbamylated darbepoetin, and sulodexide on the level of retinal microcirculation when correcting simulated hypertensive neuroretinopathy (M±m), perfusion units

Experimental groups	Level of microcirculation, PU (*n*=10)
Control	743.0±20.9
Simulated hypertensive neuroretinopathy	417.2±13.1*
Correction by PRK-002, 4 µg/kg	704.5±21.1^{y}
Correction by carbamylated darbepoetin, 300 µg/kg	592.4±9.6*^{y}
Correction by sulodexide, 150 LRU/kg	636.4±19.8*^{y}

PU: Perfusion units; *P<0.05 compared to the control group; ^{y}P<0.05 compared to the group with simulated hypertensive neuroretinopathy

Based on the data obtained in the evaluation of the microcirculation level in retina in the experimental groups in the simulated hypertensive neuroretinopathy, it follows that a positive effect on the state of the retinal blood flow in descending order has PRK-002, 4 µg/kg > sulodexide, 150 LRU/kg > carbamylated darbepoetin, 300 µg/kg.

It is possible to assume that PRK-002 in a dose of 4 µg/kg, carbamylated darbepoetin in a dose of 300 µg/kg, and sulodexide in a dose of 150 LRU/kg restore the endothelial nitric oxide synthase expression in retinal vessels, which was reduced by the introduction of L-NAME, which causes an improvement in retinal microcirculation and correction of electrophysiological parameters in the retina (b-wave amplitude and b/a coefficient).

CONCLUSION

Thus, when correcting hypertensive neuroretinopathy with carbamylated darbepoetin in a dose of 300 µg/kg, the microcirculation level in retina does not reach the target values, while the correcting with PRK-002 in a dose of 4 µg/kg reaches the values of the control group.

When correcting hypertensive neuroretinopathy with sulodexide in a dose of 150 LRU/kg, the mean value of the b/a coefficient in the group does not reach the target values, while the correcting with PRK-002 in a dose of 4 µg/kg reaches the values of the control group.

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