ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Evaluation of carbamylated darbepoetin acute toxicity

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Article History:

Received on: 03.02.2018
Revised on: 17.06.2018
Accepted on: 19.06.2018

Keywords:

Erythropoietin,
Carbamylated darbepoetin,
Rats,
Hypoxia,
Acute toxicity

ABSTRACT

Carbamylated darbepoetin is a promising pharmacological agent with universal cytoprotective activity. To assess the acute toxicity, the drug was administered to white laboratory mice and white laboratory rats subcutaneously (from 1000 to 5000 μg / kg) and intravenously (500 to 2500 μg / kg). There was no lethality and no phenotypic changes (including from the red blood sprout) in both species of animals. The study showed that the drug carbamylated darbepoetin (Pharmapark, Russia) belongs to the IV class of low-toxic drugs.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v9i3.1554

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INTRODUCTION

The study of antihypoxic and cytoprotective activity of new compounds is one of the most urgent problems of modern pharmacology (Khadieva, T.A *et al.,* 2016; Ragulina, V.A, *et al.,* 2017; Molchanova, O.V., *et al.,* 2016; Danilenko, L.M., 2016; Peresypkina A.A *et al.,* 2017; Shakhno, E.A., *et al.,* 2016). The hypoxic condition is one of the most common causes of cell death and damage. This is due to the presence of conservative mechanisms of oxygen homeostasis regulation. One of these systems, associated with the cascade of hypoxia-inducible factor, leads to the synthesis of erythropoietin (EPO), which is a glycoprotein with a molecular weight of about 30.4 kDa (Semenza, G.L *et al.,* 2010; Stockmann, C *et al.,* 2006). This molecule is best known as a positive regulator of erythropoiesis, which is produced primarily in the kidneys in response to a decrease in oxygen concentration. However, the spectrum of physiological effects of EPO is quite wide and allows to consider it as an agent with a universal cytoprotective directivity. The cascades it initiates lead to an increase in cell resistance to damage, which has been proved in models of ischemia of almost all organs (Reznikov K.M *et al.,* 2017; Shabelnikova, A.S *et al.,* 2016; Shabelnikova, A.S *et al.,* 2016; Alehin, S.A *et al.,* 2015). With ischemic lesions of different organs, EPO causes angiogenic, antioxidant, anti-inflammatory and anti-apoptotic effects (Burger, D. *et al.,* 2009; Kertesz, N. *et al.,* 2004), which leads to a reduction in the area of damage (El Hasnaoui-Saadani R *et al.,* 2013). The realisation of the cellular effects of EPO occurs when it binds to two receptors, resulting in the formation of a homodimer. The latter leads to the activation of many intracellular factors, among which Akt plays the main role in cytoprotection (Rusai, K *et al.,* 2016). At the same time, due to the activation of a large number of secondary intermediaries, EPO is able to cause the development of such negative effects as increased endothelin production, increased tissue renin concentration, a change in the balance of prostaglandins in the vascular tissue, stimulation of angiogenesis and stimulation of

smooth muscle cells of the vessels (Fisher J.W *et al.,* 2003).

Modern pharmacology has several fundamentally different generations of drugs based on erythropoietin. By modifying carbohydrate residues, attempts have been made to reduce the effect on hematocrit and blood pressure and to improve the pharmacokinetics of the drugs. The result was the creation of several fundamentally new compounds, one of which is carbamylated darbepoetin. Harmoniously combining the best qualities of previous generations drugs, this molecule is the most promising in this group (Shukurov, R.P *et al.,* 2013; Catlin, D.H., *et al.,* 2002).

MATERIALS AND METHODS

The experiments were performed with white laboratory mice of both sexes weighing 20±2 g and white laboratory rats weighing 200±20 g. The animals were fed with full-fat mixed feeds and contained in individual ventilated cells with a temperature of 21-22° C and air humidity of 55-60%. The work was carried out in compliance with all bioethical norms according to the "European Convention for the Protection of Vertebrates used for experimentation or other scientific purposes" [Derective2014 / 63 / EU]. All experiments were approved by the local Ethics Committee (Protocol No. 7-2017 of January 14, 2017).

In the first stage of the study, the test compound was injected subcutaneously into the region of the withers or intravenously into the tail vein in laboratory doses of 1000, 2000, 3000, 4000, and 5000 μg/kg subcutaneously and 500, 1000, 1500, 2000, 2500 μg/kg intravenously. For the control group, water for injection was administered once in an equivalent volume. Doses were chosen to take into account the maximum amount of substance allowed for administration to mice subcutaneously and intravenously. For the study drug, the maximum dose for mice was 1 ml (100 μg of active substance per 0.02 kg of body weight, or 5000 μg/kg) with subcutaneous injection and 0.5 ml (50 μg of active ingredient per 0.02 kg of body weight, or 2500 μg/kg) with intravenous administration. The tested substance was administered in a finished dosage form.

Due to the lack of animals mortality during the first stage of the study, the second stage was an acute toxicity study in white laboratory rats. The rats were injected once, 3 animals of each sex in the group, subcutaneously in the withers or intravenously into the tail vein, in the maximum permissible volume for subcutaneous administration of 10 ml and 2 ml for intravenous administration, which, in terms of the active substance, was 5000 μg/kg (10 ml of 100 μg/ml = 1000 μg of active substance per 0.2 kg of body weight of the animal, or 5000 μg/kg) and 1000 μg/kg (2 ml of 100 μg/ml = 200 μg of active ingredient per 0.2 kg body weight of the animal, or 1000 μg/kg), respectively. For the control group, water for injection was administered once in an equivalent volume. The test substance was administered in a finished dosage form. During the 14 days, body weight, feed intake, and clinical signs were monitored. To assess the behavioural activity HarvardApparaturs LE 8825 (Panlab) and the original software Actitrack v2.7 were used. At day 15 of the experiment, euthanisation of animals and autopsy were performed. To assess the condition of the red blood sprout, a cytological examination of the blood and smears of the bone marrow was carried out. The survival and general condition of the animals were judged on the toxic effect of the study drug. Survival assessment was carried out for three days after seeding. Statistical analysis was performed using the Mann-Whitney test.

RESULTS

The conducted studies showed that after single subcutaneous or intravenous administration to laboratory mice and white laboratory rats of the drug Carbamylated darbepoetin (Pharmapark, Russia) in the studied doses of deterioration of the general condition and death of animals was not observed.

After a single intravenous injection of the study drug at maximal doses, both in laboratory mice and in rats, a decrease in motor activity was observed. Animals became listless, apathetic, drowsy. These changes were observed within half an hour from the moment of administration of the test substance, both in experimental and control groups, which suggests their connection with the simultaneous bolus injection of a large volume of liquid. After 30 minutes. After the administration of the test substance at the maximum dose, the behaviour of the animals did not differ from all other groups.

Characteristics of behaviour in experimental rats and mice of other groups in the first 4 hours after acute administration of the preparation, other than control animals, were not observed. No significant differences in the motor activity of the animals on the infrared IR activity monitor were established between the experimental and control groups (Tab. 1).

The intensity and nature of motor activity did not change during the experiment. Convulsions were absent in all animals that were under observation. Coordination of movements and tone of skeletal muscles in rats and mice in the experimental groups were not disturbed during the first four

Table 1: Assessment of the behavioural activity of animals in the test of actimetry (M ± m; n=10)

Criteria	Groups	
	Control	Experimental
Overall activity	14006± 1889	13586± 48
Motion stereotypes	239±62	298±41
Maximum speed	67±19	96±26

Note: * (p> 0,1) in comparison with the control.

Table 2: The dynamics of the body weight of mice after a single subcutaneous injection of carbamylated darbepoetin (Pharmapark, Russia).

	The initial mass (g)	7 days (g)	14 days (g)
Subcutaneous injection			
5000 μ/kg	20.1±2.0	20.5±1.3	21.4±0.9
4000 μ/kg	20.7±1.2	20.0±1.2	21.1±1.1
3000 μ/kg	19.4±2.1	21.0±0.8	22.3±0.7
2000 μ/kg	21.0±1.8	20.9±1.4	22.2±1.5
1000 μ/kg	20.2±1.9	21.3±1.1	22.0±1.3
Control	21.1±0.9	21.0±2.0	22.0±1.9
Intravenous injection			
2500 μ/kg	20.3±0.81	21.2±0.5	22.2±0.5
2000 μ/kg	20.2±0.7	21.2±0.6	22.2±0.7
1500 μ/kg	20.3±0.6	21.2±0.6	21.8±0.5
1000 μ/kg	20.3±0.74	21.3±0.7	22.3±0.6
500 μ/kg	20.4±0.6	21.4±0.6	21.3±0.8
Control	20.4±0.6	21.4±0.6	22.3±0,5

Table 3: Dynamics of the mass of the internal organs of mice with subcutaneous injection of the drug carbamylated darbepoetin (Pharmapark, Russia)

	Body weight at day 14						
Group	Liver	Spleen	Kidneys	Heart	Lungs	Thymus	Brain
Subcutaneous injection							
5000 μ/kg	1213±40	49.6±2.7	228.1±8.1	128.5±26.5	130.9±1.8	27.2±2.5	425.3±4.4
4000 μ/kg	1202±30	49.3±3.6	220.8±7.7	121.5±4.5	131.1±1.3	25.8±3.1	426.6±4.4
3000 μ/kg	1200±28	50.4±3.3	227.1±7.1	123.6±3.6	132.3 ±1.5	26.8±2.9	426.6±3.8
2000 μ/kg	1197±25	50.9±2.6	221.6±9.4	122.5±3.5	132.8±1.3	26.5±2.4	426.4±3.4
1000 μ/kg	1200±29	51.1±3.0	224.3±11.8	120.7±4.7	133.1±1.2	26.2±2.9	423.1±3.2
Control	1205±324	50.2±4.3	26.4±9.7	122.3±5.3	130.3±1.8	26.7±3.1	428.6±4.5
Intravenous injection							
2500 μ/kg	1198.6± 18.1	54.1±1.1	229.5±4.2	125.8±2.6	128.9±2.8	26.8±1.8	425.1±2.8
2000 μ/kg	1194.0± 8.8	53.8±1.8	227.4±3.3	126.1±1.83	130.8±2.8	26.3±1.7	427.8±2.9
1500 μ/kg	1199.3± 23.2	53.4±1.5	225.3±2.7	126.3±2.4	131.1±3.2	28.0±1.5	426.0±4.3
1000 μ/kg	1203.6± 12.1	54.2±1.5	226.9±2.9	124.0±1.2	131.3±2.9	27.3±1.7	424.8±2.7
500 μ/kg	1207.0± 9.9	54.1±1.0	228.5±3.7	125.2±3.4	128.4±2.4	25.9±2.4	427.0±3.8
Control	1197.1± 21.0	53.6±0.9	228.3±4.3	123.3±1.9	129.8±2.9	27.5±2.1	427.3±2.9

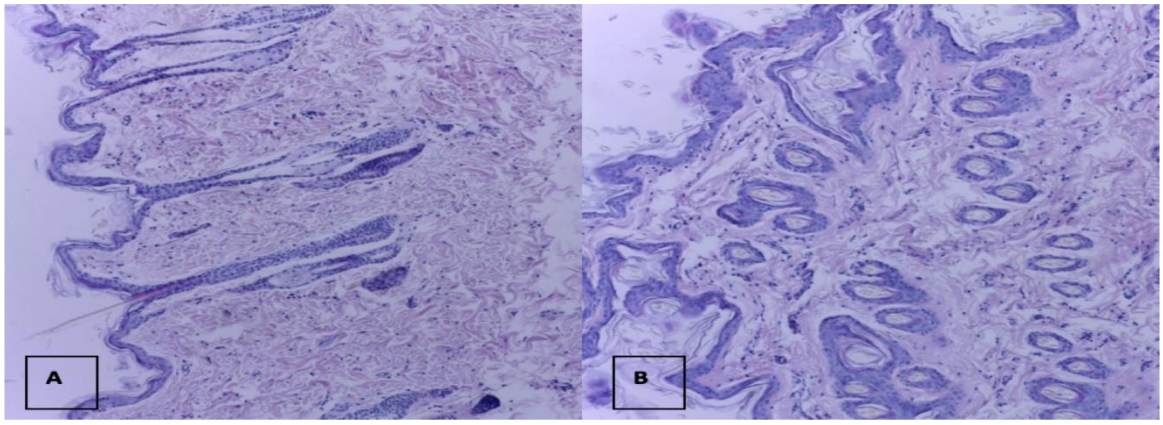

Figure 1: Histological picture of the rat's skin. A - rat skin from the control group; B - the skin of a rat receiving a drug Carbamylated darbepoetin subcutaneously at a dose of 5000 µg/kg (hematoxylin and eosin. X100).

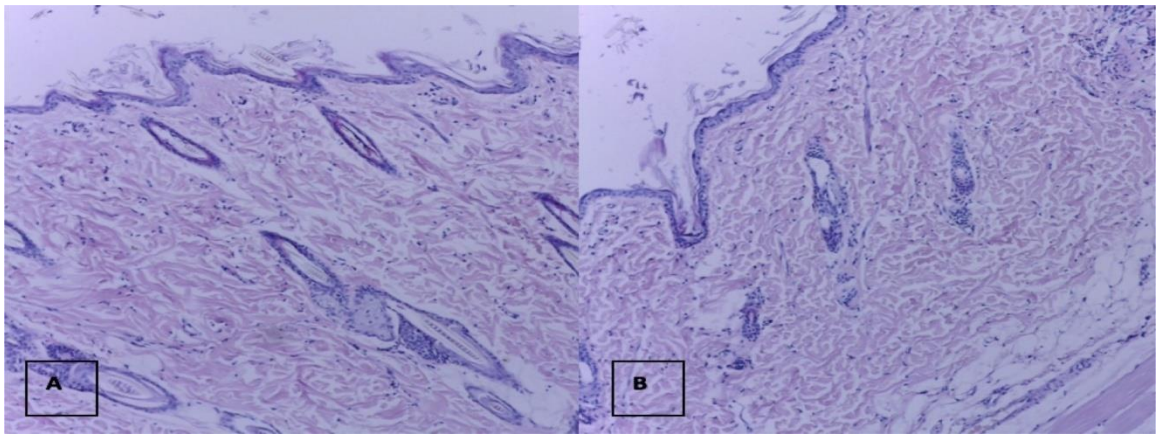

Figure 2: Histological picture of the skin of mice. A - mouse skin from the control group; B - the skin of the mouse receiving the drug Carbamylated darbepoetin subcutaneously in a dose of 5000 µg/kg (hematoxylin and eosin. X100).

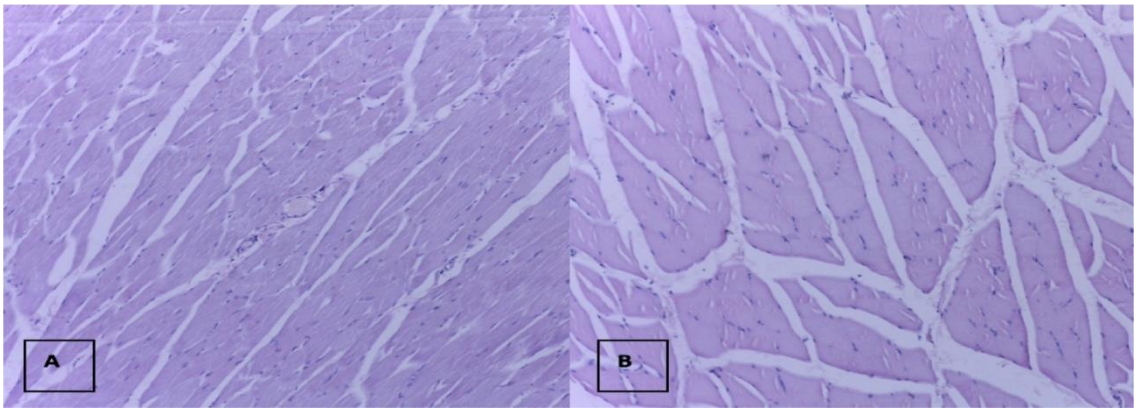

Figure 3: Histological picture of skeletal muscle in rats. A - rat muscle from the control group; B - the muscle of the rat receiving the drug Carbamylated darbepoetin subcutaneously at a dose of 5000 µg/kg (hematoxylin and eosin. X100).

hours of the experiment and during the subsequent 14-day observation period and did not differ from the corresponding parameters of the control animals. There were no deviations in the consumption of feed and water. The dynamics of body weight and average values of the mass of internal organs in the experimental groups did not have statistically significant differences from the values in the corresponding control groups (Tables 2, 3).

Macro- and microscopic examination of internal organs revealed a picture corresponding to the norm, both in the experimental and in the control group. Histological examination of the injection site in mice and rats that received the drug subcutaneously showed no signs of pathological changes and morphological differences from the control group (Figures 1-4).

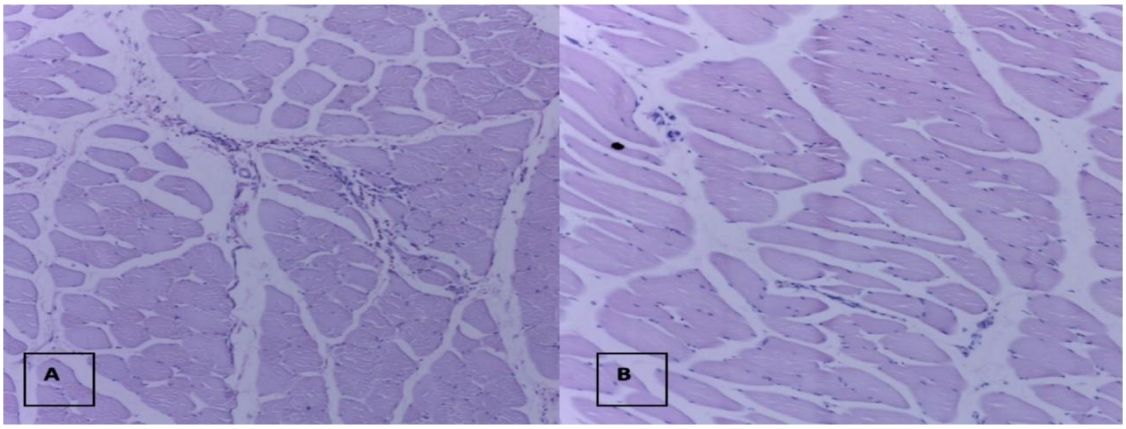

Figure 4: Histological picture of skeletal muscle in mice. A- muscle of the mouse from the control group; B - the muscle of the mouse that received the drug Carbamylated darbepoetin subcutaneously at a dose of 5000 µg/kg (hematoxylin and eosin. X100).

CONCLUSION

The study showed that the drug carbamylated darbepoetin (Pharmapark) with the subcutaneous administration at doses of 1000 µg/kg to 5000 µg/kg and intravenous administration at a dose of 500 µg/kg to 2500 µg/kg does not exhibit toxic properties and is not associated with lethality in white laboratory mice. The use of the test preparation in white laboratory rats at doses of 5000 µg subcutaneously and 1000 µg intravenously was accompanied by 100 per cent survival in the acute period (14 days) and did not lead to macro and microscopic changes in the internal organs and tissues in the injection region. The state of the red blood also did not undergo significant changes, which indicates that the drug does not affect erythropoiesis.

Based on the results of experimental studies of acute toxicity, the drug Carbamylated darbepoetin, injection solution (Pharmapark, Russia) can be classified as Class IV low-toxic drugs and should be recommended for the continuation of preclinical study (Hodge H. *et al.,* 1975).

REFERENCES

Alehin, S.A., Kolmykov, D.I., Pokrovskii, M.V., 2015. Human recombinant erythropoietin gradient dosage influence on ischemic and reperfusion liver injury. *Research result: pharmacology and clinical pharmacology,* 1(1): 9-12. doi: 10.18413/2500-235X- 2015-1- 4-9

Burger, D., Xenocostas, A., Feng, Q.P., 2009. Molecular basis of cardioprotection by erythropoietin.*Current Molecular Pharmacology,* 2(1): 56–69.

Catlin, D.H., Breitbach, A., Elliott, S., Glaspy, J., 2002. Comparison of the isoelectric focusing patterns of darbepoetin alfa, recombinant human erythropoietin, and endogenous erythropoietin from human urine. *Clinical Chemistry,* 48:2057-2059

Danilenko, L.M., Klochkova, G.N., Kizilova, I.V., Korokin, M.V., 2016 Metabolic cardioprotection: new concepts in the implementation of cardioprotective effects of meldonium. *Research result: pharmacology and clinical pharmacology*. 2(3): 95-100.

El Hasnaoui-Saadani R., Marchant, D., Pichon, A., Escoubet, B., Pezet, M., Hilfiker-Kleiner, D., Hoch, M., Pham, I., Quidu, P., Voituron, N., Journé, C., Richalet, J.P., Favret F., 2013. Epo deficiency alters cardiac adaptation to chronic hypoxia. Respiratory physiology & neurobiology, 186(2): 146–154.

Fisher J.W., 2003. Erythropoietin: physiology and pharmacology update. Experimental Biology and Medicine (Maywood), 228(1): 1-14.

Hodge H. *et al.,* Clinical Toxicology of Commercial Products. Acute Poisoning. Ed. IV, Baltimore, 1975, 427 p.

Kertesz, N., Wu J., Chen, T.H., Sucov, H.M., Wu, H., 2004. The role of erythropoietin in regulating angiogenesis.*Development biology,* 276(1): 101–110.

Khadieva, T.A., Dovgan. A.P., Pokrovka, T.G., 2016. Method of correction of endothelial dysfunction with combination of ademetionine and taurine. *Research result: pharmacology and clinical pharmacology,* 2(2):6-40.

Molchanova, O.V., Pokrovskaya, T.G., Povetkin, S.V., Reznikov, K.M., 2016. Endothelioprotective property of the combination of the thioctic acid and rosuvastatin shown in the endothelial dysfunction models. *Research result: pharmacology and clinical pharmacology,* 2(1):9-15.

Peresypkina A.A., Dolzhikov A.A., Gubareva V.O., Levkova E.A., Shabelnikova A.S., 2017. The development of hypertensive neuroretinopathy

model on Wistar rats. *Research result: pharmacology and clinical pharmacology,* 3(1):18-31.

Ragulina, V.A., Kostina, D.A., Dovgan, A.P., Burda, Y.E., Nadezhdin, S.V., 2017. Nuclear factor kappa B as a potential target for pharmacological correction endothelium-associated pathology. *Research result: pharmacology and clinical pharmacology,* 3 (1): 114-124.

Reznikov K.M., Gorbunova N.S., Kolesnichenko P.D., Tverskoy A.V., Kostina D.A., Bashkatova, D.A., Nikitina, V.A., 2017. The search for new pharmaceuticals by darbepoetin in the treatment of ischemic stroke (review of literature). *Research result: pharmacology and clinical pharmacology,* 3(1): 125-136.

Rusai, K., Prokai, A., Szebeni, B., Fekete, A., Treszl, A., Vannay, A., 2010. Role of serum and glucocorticoid-regulated kinase-1 in the protective effects of erythropoietin during renal ischemia/reperfusion injury. *Biochemical Pharmacology.* 79: 1173-1181.

Semenza, G.L. 2010. Oxygen homeostasis. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine,* 2(3): 336–361.

Shabelnikova, A.S., 2016. Correction of ischemic damage to the retina on the application of pharmacological preconditioning of recombinant erythropoietin. *Research result pharmacology and clinical pharmacology,* 2(2): 67-90 doi: 10.18413/2313-8971-2016-2-2-67-90.

Shabelnikova, A.S., Peresypkina, A.A., Gubareva, V.O., Levkova, E.A., Dolzhikov, A.A., Nikolaev, S.B., Stepchenko, A.A., 2016. Pharmacological preconditioning by recombinant erythropoietin as the possibility of increasing the stability of tissue of the retina to reperfusion ischemia in experiment. *Research result: pharmacology and clinical pharmacology,* 2(1):25-29.

Shakhno, E.A., Savitskaya, T.A., Pokrovskaya, T.G., Yakushev, V.I., Pokrovskii, M.V., Grinshpan, D.D. 2016. Use of L-arginine immobilised on activated carbon for pharmacological correction of endothelial dysfunction. *Research result: pharmacology and clinical pharmacology,* 2(1): 30-35.

Shukurov, R.P., Hamilton, R.R., Kryazhevskih, I.S., inventors; «Pharmapark LLC» assignee. Carbamylated darbepoetin 9c-depo, a method for its preparation and its use as a medicament with cytoprotective action. Russian Federation patent RF 0002575773 2013 Jul 09.

Stockmann, C., Fandrey, J., 2006. Hypoxia-induced erythropoietin production: a paradigm for oxygen-regulated gene expression. *Clinical and Experimental Pharmacology and Physiology,* 33: 968–979.