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The effect of erythropoietin on endosalpingeal karyorrhexis during ischemia reperfusion injury in rats

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Abstract

Introduction. *The aim of this experiment was to study the effects of the antioxidant drug "U-74389G" on rat model, particularly in ischemia reperfusion protocol. The beneficial or other effects of that molecule were studied estimating the mean endosalpingeal karyorrhexis (EK) lesions.*

Material and methods. *40 rats were used of mean weight 247.7 g. EK was evaluated 60 min after reperfusion for groups A and C and 120 min after reperfusion for groups B and D. Groups A and B without the drug but C and D with erythropoietin administration.*

Results. *Results were that erythropoietin administration kept non-significantly increased the EK scores by 0.1 (–0.0393284–0.2393284) (p = 0.1544). This finding was in accordance with the results of Wilcoxon signed-rank test (p = 0.1573). Reperfusion time non-significantly decreased the EK scores by 0.1 (–0.2393284–0.0393284) (p = 0.1544), approximately in accordance with the increased EK score by 0.1 (–0.2440518–0.0440518) of Wilcoxon signed-rank test (p = 0.1573). However, Epo administration and reperfusion time together kept non-significantly increased the EK scores by 0.0181818 (–0.0679319––0.1042955) (p = 0.6715).*

Conclusions. *Results of this study indicate that Epo administration kept non-significantly increased the EK scores. Perhaps, a longer study time than 2 hours may provide more significant effects.*

Key words: erythropoietin, endosalpingeal karyorrhexis, reperfusion

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Introduction

Tissue ischemia and reperfusion (IR) remain one of the main causes of permanent or transient damage with serious implications on adjacent organs and certainly on patients' health. The use of erythropoietin (Epo) is a well-established knowledge for many years. However, despite important progress has been made, satisfactory answers have not been obtained yet to the fundamental questions, such as, by what velocity this factor acts, when it should be administered, and in what dosage. The particularly satisfactory action of the erythropoietin in stem blood cells recovery has been

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noted in several performed experiments. Since a careful literature search (PubMed — Medline) was conducted, it was realized that this certain factor has been tried in experiments. However, just few relative reports were found, not covering completely this particular object of action velocity. A lot of publications are present on trials of other similar molecules of growth factors to which the studied molecule also belongs to.

The aim of this experimental study was to examine the effect of the drug erythropoietin on rat model and particularly in an oviducts IR protocol. The beneficial effect or non-effectiveness of that molecule were studied by evaluating endosalpingeal karyorrhexis (EK).

Material and methods

Animal preparation

This experimental study was approved by Scientific Committee of Ippokrateion General Hospital, Athens University and by Veterinary Address of East Attiki Prefecture under 3693/12–11–2010 & 14/10–1–2012 decisions. Institutional and national guide for the care and use of laboratory animals was followed. This experimental study was carried out at the Exprerimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. All settings needed for the study including of consumables, equipment and substances used, were provided by them. Albino female Wistar rats were used in accordance with accepted standards of humane animal care. They spent 7 days in laboratory before experimentation with easy access to water and food. The experiment was acute, that is, the animal usage was completed by following experimental set of times without awakening and preservation of the rodents. They were randomly assigned to four experimental groups (10 animals in each group):

— group A: ischemia for 45 min followed by reperfusion for 60 min;
— group B: ischemia for 45 min followed by reperfusion for 120 min;
— group C: ischemia for 45 min followed immediately by Epo intravenous (IV) administration and reperfusion for 60 min;
— group D: ischemia for 45 min followed immediately by Epo IV administration and reperfusion for 120 min.

The molecule erythropoietin was administered in a dose of: 10 mg/kg body weight of the animal. The experiment was beginning by prenarcosis and general anesthesia administration to the animals. Their electrocardiogram and acidometry were continuously monitored. The inferior aorta was prepared so as its blood flow could be excluded by forceps. After exclusion, the protocol of IR was applied, exactly as is described in experimental groups. The molecules were administered at the time of reperfusion, through inferior vena cava catheterization, which had been carried out after general anesthesia. The EK evaluation was performed at 60 min after reperfusion for groups A and C and at 120 min after reperfusion for groups B and D.

Protocol of the experiment

The experimental rats were given general anesthesia by initial intramuscular (IM) administration of 0.5 cc of a compound, constituting 0.25 cc xylazine, (25 cc, 20 mg/cc) and 0.25 cc ketamine hydrochloride (1000, 100 mg/cc, 10cc). 0.03 cc butorphanol (10 mg/cc, 10cc) anesthetic agent was administered subcutaneously (SC) before laparotomy. Continuous oxygen supply was administered during the whole experiment. Ischemia was caused by clamping inferior aorta over renal arteries for 45 min after laparotomic access. Reperfusion was achieved by removing the clamp and inferior aorta patency re-establishment. Forty (40) female Wistar albino rats were used of mean weight 247.7 g (SD = 34.99172 g), with min weight ≥ 165 g and max weight ≤ 320 g. Rats' weight could be potentially a confusing factor, for example fatter rats to have greater EK counts. This suspicion will be investigated. Also, detailed histopathological study [1] (pathology) and grading of EK findings was performed by scores, this is: 0 when lesions were not found, 1 when mild lesions were found, 2 when moderate lesions were found and 3 when serious lesions were found. The previous grading is transformed as follows: (0–0.499) without lesions, (0.5–1.499) the mild lesions (1.5–2.499), the moderate lesions and (2.5–3) the serious lesions damage, because the study concerns score ranges rather than point scores.

Model of ischemia-reperfusion injury

Control groups

20 control rats of mean weight 252.5 g (SD = = 39.31988 g) were subjected to ischemia for 45 min followed by reperfusion.

Group A

10 controls rats of mean weight 243 g (SD = = 45.77724 g), mean without lesions EK score 0 (SD = 0) were subjected to 60 min reperfusion (tab. 1).

Table 1. Weight and endosalpingeal karyorrhexis score mean levels and standard deviation of groups

Groups	Variable	Mean	SD
A	Weight	243 g	45.77724 g
	EK	Without lesions 0	0
B	Weight	262 g	31.10913 g
	EK	Without lesions 0	0
C	Weight	242.8 g	29.33636 g
	EK	Without lesions 0.2	0.421637
D	Weight	243 g	32.84644 g
	EK	Without lesions 0	0

SD — standart deviation; EK — endosalpingeal karyorrhexis

Table 2. Statistical significance of mean values difference for groups after statistical paired *t*-test application for weight and Wilcoxon signed-rank test for scores

DG	Variable	Difference	p-value
A-B	Weight	–19 g	0.2423
	EK	Without lesions 0	1.0000
A-C	Weight	0.2 g	0.9900
	EK	Without lesions –0.2	0.1573
A-D	Weight	0 g	1.0000
	EK	Without lesions 0	1.00001
B-C	Weight	19.2 g	0.2598
	EK	Without lesions –0.2	0.1573
B-D	Weight	19 g	0.1011
	EK	Without lesions 0	1.0000
C-D	Weight	–0.2 g	0.9883
	EK	Without lesions 0.2	0.1573

DG — difference for groups; EK — endosalpingeal karyorrhexis

Group B

10 controls rats of mean weight 262 g (SD = = 31.10913 g) mean without lesions EK score 0 (SD = 0) were subjected to 120 min reperfusion (tab. 1).

Erythropoietin group

20 rats of mean weight 242.9 g (SD = 30.3105 g) were subjected to ischemia for 45 min followed by reperfusion in the beginning of which 10 mg Epo/kg body weight were IV administered.

Group C

10 Epo rats of mean weight 242.8 g (SD = = 29.33636 g), mean mild EK score 0.2 (SD = 0.421637) were subjected to 60 min reperfusion (tab. 2).

Table 3. The increasing influence of erythropoietin in connection with reperfusion time

Increase	95% c. in.	Reperfusion time	p-values	
			Wilcoxon	glm
Without lesions –0.2	–0.0801229– –0.4801229	1 h	0.1573	0.1510
Without lesions –0.1	–0.0393284– –0.2393284	1.5 h	0.1573	0.1544
Without lesions 0	Undefined	2 h	1.0000	1.0000

Group D

10 Epo rats of mean weight 243 g (SD = 32.84644 g), mean without lesions EK score 0 (SD = 0) were subjected to 120 min reperfusion (tab. 3).

Results

Every rats' weight group initially was compared with each one from 3 remained groups applying statistical paired *t*-test. (tab. 2). Any emerging significant difference among EK scores will be investigated whether owed in the above mentioned significant weight correlations. Every EK scores rats group initially was compared with other one from 3 remained groups applying Wilcoxon signed-rank test (tab. 2). Applying generalized linear models (glm) with dependant variable the EK scores and independent variables the Epo administration or no, the reperfusion time and their interaction, resulted in: Epo administration kept non-significantly increased the EK scores by 0.1 (–0.0393284 – 0.2393284) (p = 0.1544). This finding was in accordance with the results of Wilcoxon signed-rank test (p = 0.1573). Reperfusion time non-significantly decreased the EK scores by 0.1 (–0.2393284 – 0.0393284) (p = 0.1544), approximately in accordance with the increased EK score by 0.1 (–0.2440518 – 0.0440518) of Wilcoxon signed-rank test (p = 0.1573). However, Epo administration and reperfusion time together kept non-significantly increased the EK scores by 0.0181818 (–0.0679319 – 0.1042955) (p = 0.6715). Reviewing the above and table 2, table 3 sums up concerning the decreasing influence of Epo in connection with reperfusion time. Inserting the rats weight as independent variable at glm, a non significant relation turns on (p = 0.5477), so as to further investigation is not needed.

Discussion

The following clinical situations show the association between ischemia and EK. Isik et al. [2] found lower karyorrhexis in local treatment with antithrombin in hepatic ischemia-reperfusion injury group. Taki-

zawa et al. [3] considered as major cause of neonatal hypoxic-ischemic (HI) encephalopathy the oxidative stress, which induces DNA peroxidation, apoptotic neuronal death and karyorrhexis significantly increased after 24–72 h of HI insult. Sun et al. [4] found various types of eosinophilic neurons (ENs) in post-ischemic gerbils brain. ENs with minimally abnormal nuclei and swollen cell bodies appeared at 3 h in the ischemic core and at 12 h in the periphery. In the ischemic periphery, ENs had slightly atrophic cytoplasm and sequentially developed pyknosis, karyorrhexis and karyolysis over 1 week. Folkerth et al. [5] observed nuclear karyorrhexis and/or pyknosis with cytoplasmic hypereosinophilia in neurons in the arcuate nucleus in consecutive stillbirth brains from 22 to 41 gestational weeks, considering in part on hypoxic-ischemic lesions such as white matter and brainstem gliosis for the unexplained stillbirth is based. Takizawa et al. [6] considered pontosubicular neuron necrosis as one of perinatal hypoxic-ischemic brain injury and its pathological peculiarity is neuronal apoptosis closely associated with presence of karyorrhexis. Hargitai et al. [7] associated preterm birth with hypoxic-ischemic encephalopathy, including neuronal karyorrhexis mostly at the diencephalon and brain stem. Hallak et al. [8] caused an increase in the proportion of fetal rats that had brain injury, including shrinkage of cells and karyorrhexis in the hippocampus and thalamus (from 0% to ~ 38.5%; $p < 0.05$) by hypoxia and decreased maternal oxygen tension and pH. Tan et al. [9] investigated in utero free radical production and injury following hypoxia-ischemia to premature fetal brain utilizing a rabbit model of acute placental insufficiency. Hypoxia resulted in a significant increase in nitrogen oxides, lipid peroxidation, and protein oxidation, with a concomitant decrease in total antioxidant capacity, compared with controls. Fetuses delivered 24 h post-ischemia had increased hippocampal nuclear karyorrhexis on histology compared with controls. Meng et al. [10] manifested neuronal karyorrhexis more predominant in preterm infants perinatal hypoxic-ischemic basal ganglia necrosis. Fortuna et al. [11] developed a model of mildly ischemic-hypoxic brain injury consisted of neuronal degeneration and necrosis with nuclear pyknosis and karyorrhexis. Khera [12] proposed a tentative 3 phase sequence of pathogenesis for the embryotoxic action of valproic acid (800 mg/kg) administered orally to rats on day 13 of pregnancy. In the third or embryonic phase, a pleiotropic karyorrhexis in the embryo, appeared aggravated, presumably by the preceding labyrinthine degeneration of the placental phase. Squier et al. [13] examined infant brains who were stillborn or died due to cerebral palsy in the early neonatal period. Criteria for white matter ischemia were reactive astrocytosis, macrophage infiltration, karyorrhexis and endothelial swelling or reduplication. Kalimo et al. [14] classified caudate nucleus and putamen to selectively vulnerable brain regions which incur neuronal damage induced by hypoglycemia and ischemia. By day 2–3 of recovery the great majority of the medium-sized neurons had undergone karyorrhexis and cytorrhexis, their remnants being subsequently removed by macrophages. Burton et al. [15] showed no evidence of hydatidiform mole (circumferential trophoblastic proliferation, hydrops, scalloped villi, and stromal karyorrhexis) in 85% of 25 women with a rare suspected tubal ectopic hydatidiform mole. Three cases of molar pregnancy (15%) were identified. Each of these cases had the histological features of an early complete hydatidiform mole. Their results show that tubal ectopic hydatidiform mole is a rare entity and demonstrate that it is over-diagnosed. Samaila et al. [16] showed hydatidiform mole characterized by circumferential trophoblastic proliferation, hydropic degeneration and stromal karyorrhexis in 5 females with ectopic ruptured tubal hydatidiform mole gestations.

Also, there is an association between Epo and oviducts. Lappin [17] supposed that the beneficial effects of hEpo are likely to extend to organs such as ovaries, oviducts, uterus which have Epo receptors. Sasaki et al. [18] claimed that Epo is both estrogen inducible and produced in oviducts. Masuda et al. [19] found E_2-induced stimulation of Epo mRNA in oviductal ampulla and isthmus regions, being transient, rapidly down-regulated and hypoxia inducible. The E_2 action is probably mediated through the E_2 receptor, and de novo protein synthesis is not required for E_2 induction of Epo mRNA. Ochiai et al. [20] attained the synthesis of hEpo protein attempting localized in vivo plasmid DNA gene transfer in laying chicken oviducts.

Conclusion

Results of this study indicate that Epo administration kept non-significantly increased the EK scores. Perhaps, a longer study time than 2 hours may provide more significant effects.

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