The Effect of Erythropoietin on Sodium During Ischemia Reperfusion Injury in Rats

Ratlarda İskemi Reperfüzyon Hasarı Sırasında Eritropoietinin Sodyum Üzerine Etkisi

İskemi Reperfüzyon Hasarı / Ischemia Reperfusion Injury

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Özet

Amaç: Bu çalışmanın amacı rat modelinde özellikle iskemi reperfüzyon protokolünde eritropoietini test etmektir. Bu molekülün biyokimyasal olarak kan sodyum düzeyine olumlu etkisi ya da etkisizliği çalışılmıştır. Gereç ve Yöntem: Ortalama kilosu 247,7 gram olan 40 rat kullanılmıştır. Sodyum düzeyi; reperfüzyon sonrası 60. dakikada (Grup A ve C), reperfüzyon sonrası 120.dakikada (grup B ve D) ölçülmüştür. Grup A ve B de eritropoietin verilmezken, grup C ve D de eritropoietin verilmiştir. Bulgular: 1) Eritropoietin uygulaması sodyumu anlamlı olmayarak 0.3 mmol/l kadar [-1.43338 mmol/l - 2.03338 mmol/l] (P= 0.7280), eşli t-testi ile de uyumlu olarak (p= 0.6992), artırmıştır. 2) Reperfüzyon zamanı sodyumu anlamlı olmayarak 1.1 mmol/l [-2.798181 mmol/l - 0.598181 mmol/l] (P= 0.1976), ve eşli t-testi ile uyumlu olarak (p= 0.1625) azaltmıştır. 3) Eritropoietin uygulaması ve reperfüzyon zamanı etkileşimi sodyum düzeylerini anlamlı olmayarak 0.1636364 mmol/l [-1.209211 mmol/l - 0.8819382 mmol/l] azaltmıştır (P= 0.7531). Tartışma: Eritropoietin uygulaması ve aynı zamanda reperfüzyon zamanı ile ilişkisinin sodyum üzerinde çeşitli anlamlı olmayan 2 saat gibi bir dar bir zamanda kısa dönem etkileri vardır. Daha uzun süreli bir çalışma daha anlamlı sonuçlar gösterebilir.

Anahtar Kelimeler

Eritropoietin; Sodyum; Reperfüzyon

Abstract

Aim: of this experiment study was the erythropoietin testing, on rat model and particularly on ischemia reperfusion protocol. The beneficial or the non effect of that molecule was studied biochemically on blood sodium. Material and Method: used were 40 rats of mean weight 247.7 gr. Sodium was measured on these time points: on 60 min after reperfusion (groups A and C), and on 120 min after reperfusion (groups B and D), A and B without but C and D with erythropoietin administration. Results: were that 1) erythropoietin administration increased non significantly the sodium by 0.3 mmol/l [-1.43338 mmol/l - 2.03338 mmol/l] (P= 0.7280), in accordance also with paired t-test (P= 0.6992), 2) reperfusion time decreased non significantly the sodium by 1.1 mmol/l [-2.798181 mmol/l - 0.598181 mmol/l] (P= 0.1976), in accordance also with paired t-test (P= 0.1625), and 3) interaction of erythropoietin administration and reperfusion time decreased non significantly the sodium levels by 0.1636364 mmol/l [-1.209211 mmol/l - 0.8819382 mmol/l] (P= 0.7531). Discussion: Erythropoietin administration as well its interaction with reperfusion time have miscellaneous non significant short – term effects on sodium on the narrow context of 2 hours. Perhaps, a longer study time may reveal clearer and more significant effects.

Keywords

Erythropoietin; Sodium; Reperfusion

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Introduction

Tissue ischemia and reperfusion (IR) remain out of main causes of permanent or transient damage with serious implications on near organs and certainly on patients' health. The use of erythropoietin (Epo) is a well established knowledge a lot of years ago. However, even if important progress has been made, satisfactory answers have not been given yet in fundamental questions, as, by what velocity this factor acts, when should it be administered, and in which dosage. The particularly satisfactory action of erythropoietin in stem blood cells recovery was noted by already performed experiments. It was realized that this certain factor has been tried in IR experiments, after international literature (PubMed - medline) careful examination. However, just few relative reports were found, not covering completely this particular object of chemical panel. Also, a lot of publications concerned trial of such particular other molecules of growth factors supprimers in which the studied molecule also belongs to. In the present study, Epo will be tried to find out whether is able to influence serum sodium.

Material and Method

Aim of present experimental study was the trial of erythropoietin in rat animal model and certainly in IR protocol. The benefit or not of that particular molecule was studied measuring serum sodium. This experimental study was approved by Scientific committee of Ippokrateion General Hospital, Athens University, and by Veterinary Address of East Attiki Prefecture and institutional and national guide for the care and use of laboratory animals was followed. Also, this study was laid out by Exprerimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki, and all of settings including of consumables, equipment and substances used, were a courtesy of that S. A. Wistar albino rats were used in accordance with accepted standards of humane animal care. They spent in laboratory 7 days before experimentation with easy access in water and food. They were randomly assigned into the following experimental groups (10 animals in each group). The experiment was acute, that is, the animal usage was completed by following experimentation times expiring as awakening and preservation did not exist.

1 - Ischemia for 45 min and afterwards reperfusion for 60 min (group A).

2 - Ischemia for 45 min and afterwards reperfusion for 120 min (group B).

3 - Ischemia for 45 min and afterwards immediate Epo intravenous (IV) administration and reperfusion for 60 min (group C).

4 - Ischemia for 45 min and afterwards immediate Epo IV administration and reperfusion for 120 min (group D).

The molecule Epo dose was 10 mg/Kg body weight of animals. The experiment was beginning by prenarcosis and general anaesthesia administration in animals. Their electrocardiogram and acidometry were continuously monitored. The vessels concerning blood supply, were prepared so as their flow to be excluded by forceps. After exclusion, the IR protocol was applied, described more in experimental groups. The molecules were administered at the time of reperfusion, through inferior vena cava (catheterization had been preceded at experiment beginning, after general anaesthesia establishment). The sodium levels measurement was performed on these time points:

1 - on 60 min of reperfusion (groups A and C),

2 - on 120 min of reperfusion (groups B and D).

Sodium is being considered a reliable index substance of metabolism being of great clinical diagnostic significance. Also, rats weight could be potentially a confusing factor, e.g. fatter rats to have greater blood sodium levels. This suspicion will be investigated. Rats were introduced into general anaesthesia by initial intramuscular (IM) administration of 0.5 cc compound, constituted by 0.25 cc xylazine, [25 cc, 20mg/cc] and 0.25 cc ketamine hydrochloride [1000, 100mg/cc, 10cc]. 0.03 cc butorphanol [10mg/cc, 10cc] anaesthesia was administered subcutaneously (S.C) before laparotomy. Continuous oxygen supply was administered during whole experiment performance. Ischemia was caused by clapping inferior aorta for 45 min after laparotomic access. Reperfusion was achieved by removing clapping and inferior aorta patency re-establishment.

40 female albino Wistar rats of mean weight 247.7 gr [Std. Dev: 34.99172 gr] were used, min weight ≥ 165 gr and max weight < 320 gr. 20 control rats mean weight 252.5 gr [Std. Dev: 39.31988 gr] suffered by ischemia for 45 min and then reperfusion.

Group A

Reperfusion which lasted 60 min concerned 10 controls rats of mean weight 243 gr [Std. Dev: 45.77724 gr], mean sodium 137.8 mmol/l [Std. Dev: 2.299758 mmol/l] (Table 1).

Group B

Reperfusion which lasted 120 min concerned 10 controls rats of mean weight 262 gr [Std. Dev: 31.10913 gr], mean sodium 137.4 mmol/l [Std. Dev: 2.75681 mmol/l] (Table 1).

20 rats of mean weight 242.9 gr [Std. Dev: 30.3105 gr] suffered by ischemia for 45 min and then reperfusion in the beginning of which 10 mg Epo/kg body weight were IV administered.

Group C

Reperfusion which lasted 60 min concerned 10 Epo rats of mean weight 242.8 gr [Std. Dev: 29.33636 gr], mean sodium 138.8 mmol/l [Std. Dev: 1.988858 mmol/l] (Table 1).

Group D

Reperfusion which lasted 120 min concerned 10 Epo rats of mean weight 243 gr [Std. Dev: 32.84644 gr], mean sodium 137 mmol/l [Std. Dev: 3.496029 mmol/l] (Table 1).

Weight comparison of each one from 4 rats groups initially was performed with other one from 3 remained groups applying statistical paired t-test. (Table 2). Some weight correlations resulted statistically significant. Any emerging significant difference among sodium levels, will be investigated whether owed

Table 1. Weight and sodium mean levels and Std. Dev. of groups

Groups	Variable	Mean	Std. Dev
A	Weight	243 gr	45.77724 gr
	Sodium	137.8 mmol/l	2.299758 mmol/l
B	Weight	262 gr	31.10913 gr
	Sodium	137.4 mmol/l	2.75681 mmol/l
C	Weight	242.8 gr	29.33636 gr
	Sodium	138.8 mmol/l	1.988858 mmol/l
D	Weight	243 gr	32.84644 gr
	Sodium	137 mmol/l	3.496029 mmol/l

Std. Dev: standard deviation

in the above mentioned significant weight correlations. Along, sodium comparison of each one from 4 rats groups initially was performed with other one from 3 remainder groups applying statistical paired t-test. (Table 2).

Table 2. Statistical significance of mean values difference for groups after statistical paired t test application.

DG	Variable	Difference	p-value
A-B	Weight	-19 gr	0.3555
	Sodium	0.4 mmol/l	0.6476
A-C	Weight	0,2 gr	0.9900
	Sodium	-1 mmol/l	0.2987
A-D	Weight	0 gr	1.0000
	Sodium	0.8 mmol/l	0.5830
B-C	Weight	19,2 gr	0.0478
	Sodium	-1.4 mmol/l	0.2177
B-D	Weight	19 gr	0.2113
	Sodium	0.4 mmol/l	0.7544
C-D	Weight	-0,2 gr	0.9883
	Sodium	1.8 mmol/l	0.1879

DG: difference for groups

Results

Applying generalised linear models (glm) with dependent variable the sodium levels and independent variables the Epo administration or no, the reperfusion time and their interaction, results in: 1) Epo administration increased non significantly the sodium by 0.3 mmol/l [-1.43338 mmol/l - 2.03338 mmol/l] (P= 0.7280), in accordance also with paired t-test (P= 0.6992), 2) reperfusion time decreased non significantly the sodium by 1.1 mmol/l [-2.798181 mmol/l - 0.598181 mmol/l] (P= 0.1976), in accordance also with paired t-test (P= 0.1625), and 3) interaction of Epo administration and reperfusion time decreased non significantly the sodium levels by 0.1636364 mmol/l [-1.209211 mmol/l - 0.8819382 mmol/l] (P= 0.7531). Reviewing the above and table 2, the table 3 sums up concerning the alteration influence of Epo in connection with reperfusion time. Inserting the rats weight as independent variable at glm, a non significant relation turns on sodium levels (p= 0.1556), so as to further investigation does not need.

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

			p-values	
Alteration	95% c. in.	Reperfusion time	t-test	glm
-1 mmol/l	-1.019995 mmol/l - 3.019995 mmol/l	1h	0.2987	0.3121
-0.3 mmol/l	-1.43338 mmol/l - 2.03338 mmol/l	1.5h	0.6992	0.7280
0.4 mmol/l	-3.357918 mmol/l - 2.557918 mmol/l	2h	0.7544	0.7796

c.in: confidence interval

Discussion

Unpleasantly, a few situations concern whether ischemia can influence the sodium levels in bibliography. Only the 2 following references show the decreasing effect of ischemia on sodium. So, Reinés A et al found that the endogenous modulator endobain E inhibited the endogenous synaptosomal membrane Na(+)K(+)-ATPase enzyme activity in rat cerebral IR cortex [1]. Olsen NV found that acute hypoxemia increases excretion of sodium and water until 6 hours, when an adaptive time-dependent course of renal functional changes in hypoxemia takes over [2]. On the contrary, there are a lot of cases reporting how the sodium levels fluctuations affect the function of various organs. Such examples will be described. Isolated sodium administration is impossible. The administration of sodium is by means of normal saline in simplest cases. Sodium is being administered associated with a drug or a factor influencing the serum sodium levels in more complex cases. The drug moiety action usually prevails on the sodium one but isolated sodium administration and study is impossible. The following 6 situations show that sodium administration aggravates circulation. Kuka J et al impaired cardiac mitochondrial energy metabolism during IR injury in Wistar rats administering high dose of sodium pivalate [3]. Rehni AK et al deteriorated the ischemic postconditioning giving before cerebral IR injury diethyl dithiocarbamic sodium in mice [4]. Hale SL et al claimed that selective inhibitors of late sodium current may decrease the late sodium-dependent intracellular calcium overload during IR in rabbits [5]. Mozaffari MS et al associated short-term high salt feeding intake with IR injury in male rats [6]. Badet L et al found that rat cold-stored kidneys preserved in Institute Georges Lopez-1 (IGL-1) solution tolerated IR injury better than those preserved in Na-UW (University of Wisconsin) solution [7]. Kadambi A et al subjected groups of rat spinotrapezius muscles to IR + 0.9% saline exhibiting significant deterioration than control group [8]. The following 5 situations show that sodium administration improves circulation. Nossaman BD et al showed that significant vasodilator activity in the hindlimb vascular bed of the cat is mediated by a cGMP- dependent mechanism [9]. Nordlander M et al increased heart rate in cardiac operated anesthetized patients and maintained renal function and splanchnic blood flow in rats by sodium nitroprusside [10]. Wang Y et al protected the heart against IR injury by Na+-H+ exchanger isoform 1 inhibition [11]. Schütte H et al maintained endothelial integrity in a subsequent rabbit lung vasculature IR maneuver by IV administration of sodium nitroprusside [12]. Piana RN et al enhanced pigs coronary blood flow immediately on reperfusion in the IR-saline group. Endothelium-independent responses to sodium nitroprusside were unaltered in arterioles and venules [13]. Schmidt TA et al measured Na,K-ATPase concentration from various parts stable during IR, of porcine and canine myocardium. A relationship between higher concentration of Na,K-ATPase and larger pressure work is suggested [14]. In one case, sodium compound administration could not influence circulation. Hoshida S et al did not affect sodium nitroprusside-induced relaxation in coronary IR Watanabe rabbits [15].

Respectively, only 2 situations show the opposite effects Epo exerts on sodium levels. Neylon M et al suggested that plasma atrial natriuretic peptide (ANP) was raised by the actions of hypoxia or Epo on the rats atrium and further proposed the diuresis and natriuresis seen during air breathing [16]. Freudenthaler S et al increased concentrations of endogenous angiotensin II and PRA and decreased GFR and RPF significantly, with no concomitant alteration of Epo regulation in humans [17]. Reversely, the majority of the following examples concern

the influence of sodium levels fluctuation on Epo. So, the following 6 situations show that sodium administration increases Epo concentration. Lalle M et al improved the efficacy of daily epoetin-α IV dose with 62.5 mg sodium ferric gluconate in cancer patients [18]. Bolaños L et al improved HD patients in maintenance rhEpo administration with continuous IV sodium ferric gluconate regimen [19]. Freudenthaler SM et al increased Epo concentration in plasma up to 290% of the baseline level treating short-term healthy male hypoxic volunteers by 0.9% sodium chloride [20]. Gargano G et al improved epoetin-α or r-HuEPO SC administration by sodium ferrous gluconate administered IV along [21],[22]. Olivieri NF et al treated patients with Cooley's anemia with oral sodium phenylbutyrate augmenting both fetal and total hemoglobin production [23]. The last 2 situations show that sodium administration declines Epo action. Krystal G et al noted loss of biological activity markedly reduced, in presence of 0.1% sodium dodecyl sulphate in preparations with specific activities of 100-300 units of Ep/mg protein [24]. Honoré PM et al found HD CKD patients being susceptible to short-term errors in sodium content of dialysate as well pathways (e.g. erythropoietin hyporesponsiveness) have come into play [25].

Conclusion

Epo administration as well its interaction with reperfusion time have miscellaneous non significant short – term effects on sodium on the narrow context of 2 hours. This comes from both bibliography and experiment. Perhaps, a longer study time may reveal clearer and more significant effects since 2 hours do not assist in safe conclusions extraction.

Competing interests

The authors declare that they have no competing interests.

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