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The acute effect of erythropoietin on red blood cell distribution width levels during hypoxia reoxygenation injury in rats

Abstract

Objective: This experimental study examined the effect of erythropoietin on rat model and particularly in an hypoxia-reoxygenation (HR) protocol. The effect of that molecule was studied biochemically using blood mean red blood cells distribution width (RDW) levels.

Materials and methods: 40 rats of mean weight 247.7 g were used in the study. RDW levels were measured at 60 min (groups A and C) and at 120 min (groups B and D) of reoxygenation. Erythropoietin was administered only in groups C and D.

Results: Epo administration non-significantly decreased the RDW levels by 1.64% +2.53% (p=0.5159). Reperfusion time non-significantly decreased the RDW levels by 1.19% +2.52% (p=0.5405). However, erythropoietin administration and reperfusion time together produced a non significant combined effect in decreasing the RWD levels by 1.06% +1.43% (p= 0.4733).

Conclusion: Erythropoietin administration, reoxygenation time and their interaction have non-significant decreasing effect on RDW levels. A longer study time or a higher Epo dose is required for future investigation of this variable.

Keywords: hypoxia, erythropoietin, red blood cell distribution width, reoxygenation

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Introduction

Erythropoietin (Epo) is generally one of the more well studied growth factors. Epo implicates over 28,373 known biomedical studies at present. 8.63% at least of these studies concern tissue hypoxia and reoxygenation (HR) experiments. Certainly, important progress has been made concerning the Epo usage in reversing the HR kind of transient or permanent injuries including adjacent organs and certainly patients' health. Nevertheless, satisfactory answers have not been provided yet to basic questions, as, its action velocity, the administration timing and the dosage. The concept is to forward the knowledge away from the original action of Epo in stem blood cells recovery. However, just few related reports were found, not covering completely more specific matters. A numeric evaluation of the Epo efficacy was yielded by a meta-analysis of 19 published seric variables, based on the same experimental setting, at the same endpoints (Table 1). The special aim of this experimental work was to study the effect of Epo on a rat model and mainly in an HR protocol. The effect of Epo molecule was tested by measuring the blood mean red blood cells distribution width (RDW) levels.

Materials and methods

Animal preparation

Prefectural veterinary Address of East Attiki licensed this experiment under 3693/12-11-2010 & 14/10-1-2012 decisions. Every substance, equipment and consumable needed for the study was a courtesy of ELPEN Pharmaceuticals Co Inc. S.A. at Pikermi, Attiki. Formal humane animal care was adopted for female albino Wistar rats. That care included normal 7 days pre-experimental housing in laboratory with ad libitum diet. Furthermore, it used preceded prenarcotic and general anesthesiologic techniques,[1-4] nonstop electrocardiogram, acidometry and oxygen supply. Finally it did not permit post-experimental preservation of the rodents.

The rodents were randomly delivered to four experimental groups; each one consisted by 10 animals. The 4 groups had common the stage of preceded hypoxia of 45 min induced by laparotomic forceps clamping inferior aorta over renal arteries. Afterwards, reoxygenation was restored by removing the clamp and reestablishment of inferior aorta patency. Reoxygenation of 60 min was followed for group A. Reoxygenation of 120 min was followed for group B. Immediate Epo intravenous (IV) administration and reoxygenation of 60 min was followed for group C. Immediate Epo IV administration and reoxygenation of 120 min was followed for group D. The dosage for molecule Epo was 10 mg/kg body mass per animal. Epo administration was performed at the time of reoxygenation, through catheterized inferior vena cava. The RDW levels evaluations were performed at 60 min of reoxygenation for A and C groups and at 120 min of reoxygenation for B and D groups. The mean mass of the forty (40) female Wistar albino rats used was 247.7 g [Standard Deviation (SD): 34.99172 g], min weight 165 g and max weight 320 g. Rats' mass could be probably a confusing factor, e.g. the more obese rats to have higher RDW levels. This suspicion was also investigated.

Model of induced hypoxia-reoxygenation injury

Control groups: Hypoxia lasted 45 min in 20 control rats of mean mass 252.5 g [SD: 39.31988 g], followed by reoxygenation.

I. **A group:** Reoxygenation lasted 60 min in 10 controls rats of mean mass 243 g [SD: 45.77724 g], mean RDW levels 11.42 % [SD: 1.081974 %] (Table 2).

II. **B group:** Reoxygenation lasted 120 min in 10 controls rats of mean mass 262 g [SD: 31.10913 g], mean RDW levels 11.26 % [SD: 0.7515908 %] (Table 2).

Erythropoietin group: Hypoxia lasted 45 min in 20 control rats of mean mass 242.9 g [SD: 30.3105 g], followed by reoxygenation along with 10 mg Epo /kg body mass were IV administered.

I. C group: Reoxygenation lasted 60 min in 10 Epo rats of mean mass 242.8 g [SD: 29.33636 g], mean RDW levels 11.21 % [SD: 0.9146342 %] (Table 2).

II. D group: Reoxygenation lasted 120 min in 10 Epo rats of mean mass 243 g [SD: 32.84644 g], mean RDW levels 11.1 % [SD: 0.8055365 %] (Table 2).

Table 2 Weight and RDW levels and SD of groups

Groups	Variable	Mean	SD
A	Weight	243 g	45.77724 g
	RDW	11.42%	1.08%
B	Weight	262 g	31.10913 g
	RDW	11.26%	0.75%
C	Weight	242.8 g	29.33636 g
	RDW	11.21%	0.91%
D	Weight	243 g	32.84644 g
	RDW	11.10%	0.81%

Statistical analysis

Every weight and RDW level group was compared with each other from 3 remained groups applying respective statistical standard t-tests (Table 3). If any probable significant difference among RDW levels was raised, it would be investigated whether owed in any respective probable significant mass correlation (Table 3). Then, the application of generalized linear models (glm) was followed. It included as dependant variable the RDW levels. The 3 independent variables were the Epo administration or no, the reoxygenation time and their interaction. Inserting the rats' mass as independent variable at glm, a non-significant correlation appeared with RDW levels (p=0.2561), so as to further investigation was interrupted.

Results

The glm resulted in: Epo administration non-significantly decreased the RDW levels by 0.185 % [-0.7459541 % -0.3759541 %] (p=0.5084). This finding was accordant with the results of standard t-test (p=0.5234). Reoxygenation time non-significantly decreased the RDW levels by 0.135 % [-0.69749 % - 0.42749 %] (p=0.6299) also accordant with standard t-test (p=0.4512). However, the interaction of Epo administration and reoxygenation time none significantly decreased the RWD levels by 0.12 % [-0.4588305 % - 0.2170123 %] (p= 0.4733). Reviewing the above and (Table 3-5) present, concerning the declining influence of Epo versus reoxygenation time.

Table 3 Statistical significance of mean values difference for groups (DG) after statistical standard t test application

DG	Variable	Difference	p-value
A-B	Weight	-19 g	0.2423
	RDW	0.16%	0.6299
A-C	Weight	0,2 g	0.99
	RDW	0.21%	0.6957
A-D	Weight	0 g	1
	RDW	0.32%	0.4405
B-C	Weight	19,2 g	0.0478
	RDW	0.05%	0.8918
B-D	Weight	19 g	0.2113
	RDW	0.16%	0.564
C-D	Weight	-0,2 g	0.9883
	RDW	0.11%	0.5199

Discussion

Certainly, RDW levels are influenced by hypoxia. Shrestha et al.,[5] noticed neutrophil gelatinase-associated lipocalin (NGAL), an iron-regulatory glycoprotein, to be upregulated systemically in response to ischemia.[5] Plasma NGAL levels were inversely correlated with indices of anemia including RDW levels (P=.007) independent on underlying oxidant stress and estimated myeloperoxidase levels (P=.045). Isik et al.,[6] have shown RDW levels as an independent correlate predictor index of adverse outcomes also associated with both presence and severity of isolated ischemia than baseline RDW levels measured at ischemic patients and controls.[6]

Table 4 The decreasing influence of erythropoietin in connection with reperfusion time

p-Values				
Decrease	**95% c. in.**	**Reperfusion Time**	**t-Test**	**g/m**
0.21%	-1.151256 % - 0.7312558 %	1h	0.6957	0.6449
0.19%	-0.7459541 % - 0.3759541 %	1.5h	0.5234	0.5084
0.16%	-0.8919467 % - 0.5719469 %	2h	0.564	0.6516
0.14%	-0.69749 % - 0.42749 %	reperfusion time	0.4512	0.6299
0.12%	-0.4588305 % - 0.2170123 %	interaction		0.4733

Table 5 The (%) decreasing influence of erythropoietin in connection with reperfusion time

Decrease	+SD	Reperfusion Time	p-values
1.85%	4.24%	1h	0.6703
1.64%	2.53%	1.5h	0.5159
1.43%	3.34%	2h	0.6078
1.19%	2.52%	reperfusion time	0.5405
1.06%	1.43%	interaction	0.4733

Afsar et al.,[7] related RDW levels with Epo resistance in iron replete hemodialysis patients (p=0.023).[7] Blain et al.,[8] did not noticed modification at RDW levels by aging.[8] Also, Epo levels were not influenced in subjects aged until 65 years old but a decrease in Epo production was marked by further aging. Brill et al.,[9] guided anemia evaluation by basic diagnostic studies including RDW values.[9] Treatment should be directed anemia correction by use of recombinant human Epo. Ribeiro et al.,[10] studied the impact of α-actinin-3 (ACTN3 R577X) and Epo (Epo T→G) polymorphisms on serum lipid peroxidation and hemogram.[10] Both types of polymorphism had effect on the runners' response to pequi oil: Epo TT and TG genotypes caused significant responses in RDW values, emphasizing the importance of nutrigenomic effects.

Conclusion

Epo administration, reoxygenation time and their interaction have non-significant declining effect on RDW levels. A longer study time or a higher Epo dose is required for future investigation of this variable.

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Conflicts of interest

Authors declare that there is no conflict of interest.

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