Archives of Nephrology

ISSN: 2639-3573

Volume 1, Issue 2, 2018, PP: 31-35

The Diverging Effects of Erythropoietin and U-74389gon γ-Glutamyl Transferase Levels

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Abstract

***Aim:** This study calculated the effects on gamma-glutamyltransferase (γgt) levels, after treatment with either of 2 drugs: the erythropoietin (Epo) and the antioxidant lazaroid (L) drug U-74389G. The calculation was based on the results of 2 preliminary studies, each one of which estimated the certain influence, after the respective drug usage in an induced ischemia reperfusion (IR) animal experiment.*

***Materials and methods:** The 2 main experimental endpoints at which the serum γgtlevels (γgtl) were evaluated was the 60th reperfusion min (for the groups A, C and E) and the 120th reperfusion min (for the groups B, D and F). Specially, the groups A and B were processed without drugs, groups C and D after Epo administration; whereas groups E and F after the L administration.*

***Results:** The first preliminary study of Epo presented a non significant hypo transfer asemic effect by 4.62%±12.56% (p-value=0.5534). The second preliminary study of U-74389G presented a non significant hyper transfer asemic effect by 1.24%+13.20% (p-value=0.8877). These 2 studies were co-evaluated since they came from the same experimental setting. The outcome of the co-evaluation was that these 2 drugs have diverging metabolic effects on serum γgtl. (p-value=0.0000).*

***Conclusions:** The anti-oxidant capacities of U-74389G ascribe anabolic effects rather than catabolic ones Epo ascribes to serum γgtl(p-value=0.0000).*

Keywords: *ischemia; erythropoietin; U-74389G; gamma-glutamyl transferase levels; reperfusion*

Introduction

The lazaroid U-74389G (L) is famous for its hyper transfer asemic[1] capacity (p-value=0.8877). U-74389G as a novel antioxidant factor, implicates exactly only 259 published studies. The ischemia reperfusion (IR) type of experiments was noted in 18.53% of these studies. A tissue protective feature of U-74389G was obvious in these IR studies. The U-74389G chemically known as 21- [4- (2, 6-di-1-pyrrolidinyl-4-pyrimidinyl)- 1-piperazinyl]- pregna-1, 4, 9 (11)-triene-3, 20-dione maleate salt is an antioxidant complex, which prevents the lipid peroxidation either iron-dependent, or arachidonic acid-induced one. Animal kidney, liver, brain microvascular endothelial cells monolayers and heart models were protected by U-74389G after IR injury. U-74389G also attenuates the leukocytes; down-regulates the proinflammatory gene; treats the endotoxin shock; produces cytokine; enhances the mononuclear immunity; protects the endothelium and presents anti shock property.

Erythropoietin (Epo) even if is not famous for its hypotransferasemic action (p-value=0.5534), it can be used as a reference drug for comparison with U-74389G. Although Epo is met in over 30,437 published biomedical studies, only a 3.56% of them negotiate the known type of IR experiments. Nevertheless, Epo as a cytokine, it is worth of being studied about its effects on serum γ-glutamyl transferase (γgt) levels too.

This experimental work tried to compare the effects of the above drugs on a rat induced IR protocol. They were tested by calculating the serumγgt levels (γgtl) alterations.

Materials and Methods

Animal Preparation

The Vet licenses under 3693/12-11- 2010 & 14/10-1-2012 numbers, the granting company and the experiment location are mentioned in preliminary references[1,2]. The human animal care of Albino female Wistar rats, the 7 days pre-experimental *ad libitum* diet, the non-stop intra-experimental anesthesiologic techniques, the acidometry, the electrocardiogram, the oxygen supply and post-experimental euthanasia are also described in preliminary references. Rats were 16 – 18 weeks old. They were randomly assigned to six (6) groups consisted in N=10. The stage of 45 min hypoxia was common for all 6 groups. Afterwards, reperfusion of 60 min was followed in group A; reperfusion of 120 min in group B; immediate Epo intravenous (IV) administration and reperfusion of 60 min in group C; immediate Epo IV administration and reperfusion of 120 min in group D; immediate U-74389G IV administration and reperfusion of 60 min in group E; and immediate U-74389G IV administration and reperfusion of 120 min in group F. The dose height assessment for both drugs are described at preliminary studies as 10 mg/Kg body mass.

Ischemia was caused by laparotomic clamping the inferior aorta over renal arteries with forceps for 45 min. The clamp removal was restoring the inferior aorta patency and reperfusion. After exclusion of the blood flow, the protocol of IR was applied, as described above for each experimental group. The drugs were administered at the time of reperfusion; through inferior vena cava catheter. The γgtlwere determined at 60th min of reperfusion (for A, C and E groups) and at 120th min of reperfusion (for B, D and F groups). However, the predicted γgtl values were not used since a weak relation was rised with animals' mass (p-value=0.9532).

Statistical Analysis

Table 1 presents the (%)hypotransferasemic influence of Epo regarding reoxygenation time. Also, Table 2 presents the (%) hypertransferasemicinfluence of U-74389G regarding reperfusion time. Chi-square tests were applied using the ratios which produced the (%) results per endpoint. The outcomes of chi-square tests are depicted at Table 3. The statistical analysis was performed by Stata 6.0 software [Stata 6.0, StataCorp LP, Texas, USA].

Table 1. *The (%) hypotransferasemic influence of erythropoietin in connection with reperfusion time*

hypotransferasemia±SD		Reperfusion time	p-value
-19.35%	±43.54%	1h	0.2362
-12.70%	±63.87%	1.5h	0.3541
-6.06%	±77.98%	2h	0.7800
+6.35%	±57.94%	reperfusion	0.6264
-4.62%	±12.56%	interaction	0.5534

Table 2. *The (%) hypertransferasemic influence of U-74389G in connection with reperfusion time*

hypotransferasemia±SD		Reperfusion time	p-value
-19.35%	±43.54%	1h	0.2362
-6.82%	±66.01%	1.5h	0.6442
+5.71%	±68.41%	2h	0.7809
+13.64%	±72.04%	reperfusion	0.3769
+1.24%	±13.20%	interaction	0.8877

Results

The successive application of chi-square tests revealed that U-74389G recessed the γgtl the same as Epo did by 1 [0.5776868 - 1.731042] at 1h (p-value=1.0000); however, it was less catabolic by 0.5367033-fold [0.5353613 - 0.5380487] (p-value=0.0000) at 1.5h, less catabolic by 0.9428571-fold [0.3920045 - 2.267758] (p-value=0.8982) at 2h, by 2.146813-fold [2.141445 - 2.152195](p-value=0.0000) without drugs and anabolic by 0.2683513-fold [-0.2697471 - -0.2669627] than Epowhether all variables have been considered (p-value=0.0000).

Discussion

The unique available study investigating the hyper transferasemic effect of U-74389G onγgtl was the preliminary one[1]. Although the most famous activities of neuroprotection and membrane-stabilization properties, it accumulates in the cell membrane, protecting vascular endothelium from peroxidative damage but hardly penetrates the blood-brain barrier. It elicits a beneficial effect in ototoxicity and Duchenne muscular dystrophy. It increases γgt, superoxide dismutase (SOD) and glutathione (GSH) levels in oxygen-exposed cells. It treats septic states and acts as immunosuppressant in flap survival. It prevents the learning impairments, it delays the early synaptic transmission decay during hypoxia improving energetic state of neurons. It shows antiproliferative properties on brain cancer cells and is considered as a new promising anti inflammatory drug for the treatment of reperfusion syndrome in IR injuries.

The same authors confirmed[2] the short-term hypo transferasemic effect of Epo preparations in non iron deficient individuals.Woodard LE et al attempted[3] kidney-specific gene transfer following hydrodynamic tail vein injection using the kidney-specific podocin and γgtlpromoters, but found expression primarily in the liver. Injection of a transposon expressing Eporaised the haematocrit in vivo in uninephrectomised mice. Rjiba-Touati K et al explored[4] the protective effect of rhEPO against mitomycin C (MMC)-induced heart, liver, and renal dysfunction. The results showed that MMC induced a significant increase in serum γgtl. rhEPO treatment restored serum biochemical parameters and histological damage caused by MMC exposure in adult male Wistar rats. Ali BH et al investigated[5] here the effect of adenine-induced CKD on the activity of L-γgtlin serum and Epoin renal tissue. Adenine feeding induced CKD, accompanied by significant decreases (P<0.05) in the serum concentrations of Epo in rats. Coilly A et al significantly improved[6] SVR rates after protease inhibitors (PI) as peginterferon/ribavirin in HCV G1 patients. Their use to treat HCV recurrence after liver transplantation (LT) is a challenge. The most common adverse effect was anemia (n=34, 92%), treated with Epoand/or a ribavirin dose reduction. Rjiba-Touati K et al showed[7] that Cisp-induced a marked renal and liver failure characterized by a significant increase in serum γgtl. rhEPO treatments restored serum biochemical parameters changed due to Cisp exposure. van Klaveren RJ et al exposed[8] rat type II cells for 2 days to air, 60% O_2 or 85% O_2 with or without 30 μM U-74389G or 100 μM NAC At 48 h after isolation. Exposure to 60% O_2 decreasedγgtl and GSH by -47 and -34%, respectively. After 85% O_2-exposureγgtldecreased by -55%. NAC treatment decreasedγgtlactivity by -42% in the air-exposed cells. After 60% O_2, U-74389G led to significantlyγgtl(+117%). After 85% O_2 U-74389G increasedγgtl+72%. The results show that hyperoxia decreases rat type II cellγgtlactivity in vitro. Restoration of the GSH levels by NAC did not restoreγgtl. The lazaroid U-74389G with vitamin E-like properties effectively prevented the decrease inγgtland GSH, so that direct inactivation of the membrane-boundγgtlby hyperoxia is the most likely mechanism. Bonatsos V et al investigated[9] the effects of infusion of lazaroid U-74389G on cytokines and liver structure in a liver I/R rat model. γgtlwas statistically significantly reduced in treated groups (p=0.015) than control groups. Administration of U-74389G in liver I/Rinjury has potential in attenuating liver damage.

According to above, table 3 shows that U-74389G has anabolic effects by 0.2683513-fold [-0.2697471 - -0.2669627] than Epo whether all variables have been considered (p-value=0.0000); a trend accentuated along time, in Epo non-deficient rats. A meta-analysis of these ratios from the same experiment, for 18 other seric variables, provides comparable results (table 4)[10 11].

Table 3. *The U-74389G / erythropoietin efficacies ratios on serum γgt levels hyperuricemia after chi-square tests application*

Odds ratio	[95% Conf. Interval]		p-values	Endpoint
1	.5776868	1.731042	1.0000	1h
.5367033	.5353613	.5380487	0.0000	1.5h
.9428571	.3920045	2.267758	0.8982	2h
2.146813	2.141445	2.152195	0.0000	reperfusion
-.2683513	-.2697471	-.2669627	0.0000	interaction

Table 4. *A U-74389G / erythropoietin efficacies ratios meta-analysis on 18 hematologic variableç (15 variables with balancing efficacies and 3 variables with opposite efficacies)[10-11].*

Endpoint Variable	1h	p-value	1.5h	p-value	2h	p-value	Reperfusion time	p-value	interaction	p-value
WBC	0.957451	0.3782	1.396122	0.0000	1.918237	0.0000	1.71622	0.0000	1.601887	0.0000
RBC count	0.961059	0.0000	1.733395	0.0000	6.519657	0.0000	1.039524	0.0000	1.309673	0.0000
Hematocrit	38.424	0.0000	9.076658	0.0000	6.222898	0.0000	1.001356	0.2184	12.66419	0.0000
Hemoglobin	1.268689	0.0000	1.839035	0.0000	13.1658	0.0000	1.252422	0.0000	1.94889	0.0000
MCH	151.125	0.0000	4.246814	0.0000	2.709729	0.0000	1.177347	0.0000	4.362893	0.0000
MCV	150.8518	0.0000	4.236722	0.0000	2.704247	0.0000	1.180156	0.0000	4.352528	0.0000
RbcDW	3.306773	0.0000	3.023389	0.0000	2.655885	0.0000	0.2259914	0.0000	2.370353	0.0000
Platelet count	2.42839	0.0000	6.00238	0.0000	6.1333429	0.0000	3.939027	0.0000	37.62979	0.0000
MPV	145.8532	0.0000	4.053619	0.0000	2.603947	0.0000	1.2334644	0.0000	4.164431	0.0000
Platelet DW	0.6940233	0.0000	1.319118	0.0000	2.206972	0.0000	2.2484006	0.0000	2.458888	0.0000
Glucose	156.4991	0.0000	4.53659	0.0000	2.81397	0.0000	0.9073196	0.0000	4.660603	0.0000
Urea	158.4209	0.0000	4.50889	0.0000	2.850291	0.0000	0.9017775	0.0000	4.632148	0.0000
Creatinine	168.9034	0.0000	4.872332	0.0000	3.039572	0.0000	1.0262016	0.0000	5.005523	0.0000
Total proteins	155.9562	0.0000	4.421079	0.0000	2.803573	0.0000	0.8842162	0.0000	4.541934	0.0000
Albumins	0.2457507	0.0073	0.5303472	0.0000	0.6243052	0.0465	1.237477	0.0000	0.5000416	0.0000
Mean	13.8573100	0.0255	3.0278414	0.0000	3.1511336	0.0030	1.1390705	0.0144	3.5992801	0.0000

Endpoint Variable	1h	p-value	1.5h	p-value	2h	p-value	Reperfusion time	p-value	interaction	p-value
Mean corpuscular hemoglobin concentrations	-0.2774225	0.0000	-0.5504722	0.0000	-0.8522433	0.0000	+3.044774	0.0000	-0.7793243	0.0000
Plateletcrit	-0.2312044	0.0000	-0.6719365	0.0000	-1.330756	0.0886	+5.620077	0.0000	-0.9771515	0.0000
ALT	+0.5955473	0.0000	-1.157335	0.0000	+7.967324	0.0000	+0.4734427	0.0000	-0.6208232	0.0000
Mean	-0.4757810	0.0000	-0.7536578	0.0000	-0.5221354	0.0295	+2.0084217	0.0000	-0.7790213	0.0000

Conclusion

The anti-oxidant agent U-74389G was proved having diverging metabolic properties onγgtlby 0.2683513-fold [-0.2697471 - -0.2669627] than Epo (p-value=0.0000); a trend accentuated along the short term time frame of the experiment in rats. A biochemical investigation remains about how U-74389G mediates in these actions.

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Citation: **Constantinos Tsompos, Constantinos Panoulis, Konstantinos Toutouzas, et al.** *The Diverging Effects of Erythropoietin and U-74389gon γ-Glutamyl Transferase Levels. Archives of Nephrology. 2018; 1(2): 31-35.*