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Effects of erythropoetine and oxymetolone coadministration on serum level of malonyldehaldyde, sorbitol dehydrogenase, gluthathione reductase, serum creatinine and BUN after kidney ischemic reperfusion in dog

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ABSTRACT

The present study was conducted to evaluate the effects of erythropoetine (Epo) and oxymetolone (Oxy) coadministration on serum level of malonyldehaldyde (MDA) , sorbitol dehydrogenase, gluthathion reductase, serum creatinin and BUN after kidney ischemic reperfusion(I/R) in dog. 24 dogs were randomly divided to 4 groups: 1) Control 2) Oxymetolon (3 mg/kg) 3) Erythropoetine (500 IU/kg) 4) Oxymetolone/erythropoetine (3 mg/kg / 500 IU/kg). Blood samples were taken 5 times: Zero day (Before Ischemia), 45 minutes after Ischemia, 24 hour after repefusion, 7 days after Ischemia, 14 days after Ischemia. Serum samples were sent to Labrotary for malonyldehaldyde (MDA), sorbitol dehydrogenase (SDH), gluthathion reductase (GR), serum creatinin (SCr) and BUN Analysis. Administration of oxymetolone and erythropetene alone and in combination decreased serum MDA level significantly in Epo and Oxy/Epo group ($p<0.05$). Serum SDH leve deeacreased in all groups compare the zero time. But administration of Epo and Epo/Oxy deacreased serum SDH level significantly between different times ($p<0.05$) and difference in Oxy and Control group was not significant. Additionaly, serum GR level decreased significantly in Epo, Oxy and Epo/Oxy groups in all times compare the zero time ($P<0.05$). But decrease in control group compare the zero time was not significant. BUN and SCr level did not differ significantly in all groups compare the zero time.

Keywords: erythropoetine, oxymetolone, antioxidant status, kidney, dog

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INTRODUCTION

Acute renal injury (ARI) in critically ill patients is highly associated with poor prognosis, and despite the increasing efforts to alleviate fatal consequences of ARI, the mortality rate among these patients remains a severe problem [1,2,3]. Tubular necrosis and interstitial infiltration of inflammatory cells are characteristic pathologic changes in the kidney with I/R. The inflammatory responses following I/R include oxidative stress, increased production of inflammatory cytokines, and infiltration of neutrophils and macrophages [4].

Erythropoietin (EPO) is a 30.4 kDa glycoprotein hormone and hypoxia-inducible hematopoietic growth factor, produced primarily in the kidney [5, 6]. The essential function of the erythropoietin is regulation of red blood cell production [7]. In an ischemic condition, the EPO receptor will be up-regulated [8,9,10,11]. After that, EPO can

activate multiple intracellular signaling, including mitogenactivated protein kinase (MAPK), c-Jun N-terminal Kinase (JNK), and phosphatidylinositol 3-kinase signaling cascades [12,13,14], and induces the subsequent transcription of anti-apoptotic [15] and anti-oxidative genes [16]. Based on these properties, EPO has emerged as an efficient renoprotective agent against renal dysfunction and injury caused by hypoxia, oxidative stress, and hemorrhagic shock [17].Oxymetholone (17β-hydroxy-2-[hydroxymethylene]-17-methyl-5α-androstan-3-one) is a 17α-alkylated anabolic-androgenic steroid and a synthetic derivative of testosterone. It has been approved by the US Food and Drug Administration for the treatment of anemias caused by deficient red cell production. [18]

The objective of the current study was to compare the effects of Erythropoetine (EPO) and Oxymetolone (OXY) coadministration or alone on serum level of Malonyldehaldyde (MDA), Sorbitol Dehydrogenase (SDH), Gluthathione Reductase (GR), Serum Creatinine (SCr) and BUN after kidney I/R in dog.

MATERIALS AND METHODS

Animals

This study was performed on 24 male dogs weighting 20 to 25 kg. Male dogs were obtained from the central animal laboratory of Tabriz medical university and housed in pathogen-free cages. Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the United States guidelines (United States National Institutes for Health publication no. 85-23, revised in 1985) and our ethical committee on animal care approved the protocol. All dogs received a standard diet and had free access to water; also they were housed in a 12-h light/dark cycle and 23±2°C. The animals were randomly divided into four experimental groups (n=6): 1- I/R group: in dogs subjected to renal ischemia for 45 min; 2- I/R-OXY group: in dogs administered OXY (3 mg/kg, intramuscularly) 20 min prior to I/R; 3- I/R-EPO group: in dogs administered EPO (500 U/kg, intramuscularly) 20 min prior to I/R; and 4- I/R-OXY-EPO group, in which dogs administered both of OXY and EPO (3 mg/kg and 500 U/kg respectively, intramuscularly).

Surgical Procedure:

Animals were anesthetized with Ketamine hydrochloride (Ketamine 10%, Alfasan, Woerden- Holland, 50 mg/kg) and Xylazine (Xylazin 2%,Alfasan, Worden-Holland, 5mg/kg) intramusculary. A midline celiotomy was made in each dog and the left kidney became available. Ischemia-reperfusion injury was induced by applying a noncrushing microvascular clamp on the left renal artery for 45 minutes. After 45 minutes of ischemia, the clamp was removed and the tissue was closed in layers. Then, the animals were returned to their cages, reperfusion period was 24 hour after surgery.

Biochemical measurements

Blood samples were taken from Saphenous vein at 5 different times: 1) Zero time: before induction of Ischemia 2) 45 minutes after Ischemia; 3) 24 hours after reperfusion 4) 7 days after reperfusion 5) 14 days after reperfusion. After each blood sampling, for obtaining serums, samples were centrifuged at 5000 rpm for 10 minutes. Finally, serums sent Tabriz branch, Islamic Azad University laboratory for determining concentrations of MDA, SDH, GR ,serum Creatinine and blood urea nitrogen.

Statistical Analysis

Statistical analysis were conducted with the Statistical Package for Social Science (SPSS) to determine significancy between groups. Results are expressed as means ± SEM. MDA, SDH, GR, SCr and BUN were compared between different times and also between groups by one-way ANOVA test. Probability values of less than 0.05 ($P<0.05$) were considered significant and Probability values of less than 0.01 ($P<0.01$) were considered as very significant.

RESULTS

Serum MDA level differed significantly ($p<0.05$) at 45 minutes after ischemia, 24 hours, 7 days and 14th days after reperfusion compared with zero time in IR and IR/OXY groups. However, in IR/EPO group differences in serum MDA level was significant ($p<0.05$) only at 45 minutes after ischemia and 24 hours after reperfusion. But, in IR/OXY/EPO group serum MDA level showed a significant ($p<0.05$) difference just at 45 minutes after ischemia compare to zero time. Furthermore, after 14 days of reperfusion IR/EPO and IR/OXY/EPO had a very significant ($p<0.01$) decrease in serum level of MDA compared with IR and IR/OXY groups.

Table 1: Serum concentrations of MDA at different times in all groups[1]

	Zero Time	45m after Ischemia	P Value	24h after Reperfusion	P Value	7d after Reperfusion	P Value	14d after Reperfusion	P Value
IR	16.10 ±0.007	26.10 ± 0.03	0.021	28.00 ± 0.003	0.017	24.00 ± 0.001	0.024	22.00 ± 0.05	0.03
IR/OXY	16.50 ± 0.31	24.02 ± 0.001**	0.025	26.00 ± 0.01**	0.015	21.00 ± 0.05	0.045	20.00 ± 0.01	0.04
IR/EPO	16.20 ± 0.05	20.02 ± 0.001*	0.040	22.01 ± 0.007*	0.031	18.05 ± 0.007*	0.06	17.09 ± 0.03*	0.07
IR/EPO/OXY	16.35 ±0.006	20.00 ± 0.54*	0.040	19.00 ± 0.05*	0.051	17.00 ± 0.47*	0.07	17.00 ± 0.007*	0.07

[1] The results are reported as Mean ±SEM
** The mean difference is very significant at the <0.01 level in column.*
*** The mean difference is significant at the <0.05 level in column.*

Serum SDH level differed significantly ($p<0.05$) at 45 minutes after ischemia, 24 hours, 7 days and 14th days after reperfusion compared with zero time in all groups. In addition, after 14 days of reperfusion IR/EPO and IR/OXY/EPO had a significant ($p<0.05$) increase in serum level of SDH compared with IR and IR/OXY groups.

Table 2: Serum concentrations of SDH at different times in all groups[1]

	Zero Time	45m after Ischemia	P Value	24h after Reperfusion	P Value	7d after Reperfusion	P Value	14d after Reperfusion	P Value
IR	6.60 ± 0.05	3.20 ± 0.002	0.017	3.50 ± 0.001	0.01	4.00 ± 0.05	0.035	4.00 ± 0.06	0.035
IR/OXY	7.60 ± 0.07	3.50 ± 0.002	0.01	4.00 ± 0.002	0.02	5.50 ± 0.007	0.03	5.50 ± 0.007	0.03
IR/EPO	7.10 ± 0.05	5.50 ± 0.002**	0.03	5.00 ± 0.01**	0.03	6.00 ± 0.05**	0.05	6.00 ± 0.03**	0.05
IR/EPO/OXY	7.60 ± 0.007	5.50 ± 0.003**	0.03	5.50 ± 0.07**	0.03	6.00 ± 0.83**	0.045	6.50 ± 0.003**	0.05

[1] The results are reported as Mean ±SEM
** The mean difference is very significant at the <0.01 level in column.*
*** The mean difference is significant at the <0.05 level in column.*

Serum GR level differed significantly ($p<0.05$) at 45 minutes after ischemia, 24 hours, 7 days and 14th days after reperfusion compared with zero time in IR and IR/OXY groups. But, in IR/EPO group differences in serum GR level was significant ($p<0.05$) only at 45 minutes after ischemia and 24 hours after reperfusion. But, in IR/OXY/EPO group serum GR level showed a significant ($p<0.05$) difference just at 45 minutes after ischemia compare to zero time. Furthermore, after 14 days of reperfusion IR/OXY, IR/EPO and IR/OXY/EPO had a significant ($p<0.05$) increase in serum level of GR compared with IR. But the difference between IR/EPO/OXY and IR group was very significant ($p<0.01$).

Table 3: Serum concentrations of GR at different times in all groups [1]

	Zero Time	45m after Ischemia	P Value	24h after Reperfusion	P Value	7d after Reperfusion	P Value	14d after Reperfusion	P Value
IR	20.12 ± 0.01	13.70 ± 0.03	0.01	13.00 ± 0.004	0.01	14.00 ± 0.002	0.021	15.50 ± 0.002	0.02
IR/OXY	20.19 ± 0.02	15.30 ± 0.003**	0.027	15.20 ± 0.007**	0.027	17.01 ± 0.00**	0.04	17.50 ± 0.05**	0.02
IR/EPO	20.20 ± 0.01	16.01 ± 0.00*	0.03	16.50 ± 0.003*	0.03	17.90 ± 0.008*	0.05	18.02 ± 0.001**	0.05
IR/EPO/OXY	20.25 ±0.002	16.09 ± 0.007*	0.03	18.02 ± 0.005*	0.058	18.90 ± 0.05*	0.06	18.94 ± 0.08*	0.06

[1] The results are reported as Mean ±SEM
** The mean difference is very significant at the <0.01 level in column.*
*** The mean difference is significant at the <0.05 level in column.*
BUN levels did not show any significant (p>0.05) difference at different times in all groups except in IR group at 24 hours after reperfusion, that it was significant (p<0.05) compared with zero time.

Table 4: BUN at different times between all groups [1]

	Zero Time	45m after Ischemia	P Value	24h after Reperfusion	P Value	7d after Reperfusion	P Value	14d after Reperfusion	P Value
IR	18.02 ± 0.01	26.00 ± 0.003	0.008	22.00 ± 0.003	0.02	20.05 ± 0.001	0.06	20.04 ± 0.00	0.06
IR/OXY	20.02 ± 0.01	22.00 ± 0.05*	0.06	22.00 ± 0.01	0.06	20.05 ± 0.001	NS	20.02 ± 0.006	NS
IR/EPO	19.00 ±0.001	21.00 ± 0.05*	0.065	20.00 ± 0.01	0.06	19.90 ± 0.001	NS	19.02 ± 0.002	NS
IR/EPO/OXY	21.00 ± 0.01	22.05 ± 0.001*	0.07	22.00 ± 0.007	0.07	21.07 ± 0.003	NS	21.05 ± 0.003	NS

[1] The results are reported as Mean ±SEM
** The mean difference is very significant at the <0.01 level in column.*
*** The mean difference is significant at the <0.05 level in column.*

Serum concentrations of Creatinine showed a significant increase at 45 minutes after ischemia and 24 hours after reperfusion in all groups compare to zero time. But 14 days after reperfusion, differences in SCr level was not significant (p>0.05) compare to zero time. Also there was not significant (p>0.05) differences at 14 days of reperfusion between experimental groups.

Table 5: Serum concentrations of Creatinine at different times between all groups [1]

	Zero Time	45m after Ischemia	P Value	24h after Reperfusion	P Value	7d after Reperfusion	P Value	14d after Reperfusion	P Value
IR	1.20 ±0.002	2.20 ± 0.001	0.03	2.80 ± 0.007	0.01	1.90 ± 0.002	0.04	1.70 ± 0.05	0.05
IR/OXY	1.50 ± 0.02	2.20 ± 0.002	0.02	2.60 ± 0.005	0.01	1.60 ± 0.05	0.06	1.50 ± 0.037	NS
IR/EPO	1.10 ± 0.05	2.02 ± 0.003	0.03	2.01 ± 0.009	0.03	1.80 ± 0.05	0.05	1.80 ± 0.47	0.05
IR/EPO/OXY	1.10 ± 0.04	2.10 ± 0.003	0.04	2.20 ± 0.01	0.03	1.50 ± 0.05	0.05	1.50 ± 0.001	0.05

[1] The results are reported as Mean ±SEM
** The mean difference is very significant at the <0.01 level in column.*
*** The mean difference is significant at the <0.05 level in column.*

DISCUSSION

The results of this study indicate that erythropoetine (EPO) have more protective effects than oxymetholone (OXY) on renal injury that induced by renal artery Ischemic/Reperfusion in dogs. Also, co-administration of EPO and OXY had more protective effects than individual administration. These results are in agreement with earlier studies [19, 20, 21]. EPO can be used as a reno-protective substance in diabetic patients that suffering renal failure with vessel origin [22].

There are several mechanisms that Erythropoietin exerts its protective effects through them. One of this mechanisms is ATP-dependent potassium channels. ATP-dependent potassium channels have very important role in the occurrence of apoptic injuries. So that, blocking this channels increase caspase-3 and TNF-alpha levels in kidney tissue and induces harmful effects. But EPO applies its protective effects with a direct impact on ATP-dependent potassium channels. [20] Another mechanism that EPO exerts its cytoprotective effects in renal tissue includes bcl-2 and bone morphogenic protein-7 gene induction, that builds an anti-apoptotic molecule and an anti-fibrotic and proregenerative factor respectively. [22] Another protective effect of EPO that have been mentioned by Yang et al. (2001) in renal transplantation was enhancing apoptosis in inflammated cells and also reduction of inflammation in interstitial cells of kidney and remodeling of kidney through the cytokine interleukin (IL-1β) and Caspase-3 activation. [23]

However, EPO take an important role in controlling Anemia due to renal disease, chemotherapy and other disease that affecting kidney. EPO applies its cytoprotective effects by reacting with its receptors in non-hematopoietic tissues including mytogenesis, angiogenesis, inhibition of apoptosis and promoting vascular healing. The results of this study showed that administration of EPO individually and without any other therapeutic adjuvants such as nanderolone and oxymetholone can improve renal performance in dogs suffering acute renal failure (ARF) and

finally decrease the mortality rate.

CONCLUSION

The results of this study showed that administration of erythropoetine had a beneficial effect on antioxidant status in compare of administration of Oxymetolone. Also, coadministration of erythropoetine and Oxymetolone had more beneficial effects than individually administration of erythropoetine and Oxymetolone.

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