CARDIOPROTECTIVE EFFECTS OF ERYTHROPOIETIN HORMONE: A STUDY ON A POSSIBLE ROLE IN HEART FAILURE AND MYOCARDIAL INFARCTION: ELUCIDATION OF UNDERLYING MECHANISMS.

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دراسة فى الدور الوقائى المحتمل لهرمون الاريثروبيوتين علي عضلة القلب فى حالات ضعف عضلة القلب واحتشاء القلب مع توضيح الآليات المؤدية لذلك.

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ABBREVIATIONS

ACE	Angiotensin-converting enzyme
ACF	AortoCaval fistula.
Apaf-1	Apoptotic protease activating factor 1
ARC	Apoptosis repressor with caspase recruitment domain.
AT_1R	Angiotensin II type-1 receptor.
BK	Bradykinin.
BNP	B-type (Brain) natriuritic peptide.
CED-4	Cell death protein
CK	Creatine Kinase
COX	Cyclooxygenase enzyme.
Cyt c	cytochrome *c*
DAB	2,3-Diaminobenzidine tetrachloride
DCM	Dilated Cardiomyopathy.
DOCA	deoxycorticosterone.
EDV	End Diastolic Volume.
EMCV	M-variant of encephalomyocarditis virus
EPOR	Erythropoietin receptor.
FADD	Fas-Associated protein with Death Domain
FLIP	FADD-like inhibitory proteins
GLUT	Glucose transporter.
GPRKs	G protein-coupled receptor kinases
ICM	Ischemic Cardiomyopathy
iNOS	Inducible Nitric oxide sythase
L-NAME	NG-nitro-L-arginine methyl ester
MCP-1	Monocyte chemoattractant protein.
NO	Nitric Oxide.
PARP	Poly (ADP-ribose) polymerase.
PGC-1α	proliferator activator receptor γ coactivator-1 α
PPAR	Peroxisome proliferator-activated receptor
RAAS	Renin-Angiotesin-aldosterone-system.
SP-SHRs	sterone-prone spontaneously hypertensive rats
SWOP	Second window of protection.
TCAs	Tricyclic antidepressants.

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Erythropoietin (Epo), a hematopoietic cytokine that is basically released by the adult kidney in response to hypoxia,and is a potent stimulator of bone marrow red blood cell production**.** To fulfill its principal function, i.e. the regulation of red cell production, EPO binds to a surface receptor (EPO receptor) on the erythroid progenitor cells. After activation of the receptor and subsequent intracellular signaling cascades, survival of these cells is promoted by anti-apoptotic mechanisms.

Besides its primary role in promoting erythrocyte survival and differentiation, it has been recently discovered that this cytokine has several properties that can extend beyond its capacity to produce RBCs.

Recombinant human EPO (rhEPO) is widely used for the treatment of anemia occurring in the context of surgery, cancer, HIV, kidney failure . Its safety and toxicity profiles have been well established. Epo has been recently shown to enhance exercise capacity in patients with chronic heart failure as well as direct actions on platelets, vascular endothelium, smooth muscle, and myocytes of the heart.

A direct effect of EPO hormone in improving cardiac performance in pressure overload heart failure has also been recently observed. Studies on EPO $R^{-/-}$ null rescued mice suggested a potential protective role for EPO . However, despite a strong link between EPO receptor activation and cardiac improvement, the underlying mechanisms are still unclear. A possible induction of angiogenesis and angiogenic factors could explain such an effect (***Westenbrink et al , 2007).***

Furthermore, a strong correlation between plasma levels of Epo and left ventricular functions in cardiomyopathy was also reported ***(Longhu et al , 2006)***.

In the context of ischemia , a cytoprotective effect for Epo hormone has been recently verified by data obtained from experimental studies showing that erythropoietin can have a dramatic neuroprotective effect in animal models of cerebral ischemia. This protective effect is attributed to an antiapoptic effect on neuronal cells**,** that is similar to its principal action on erythroid progenitor cells. This raised the possibility that erythropoietin can have an antiapoptotic effect on cardimyocyte as well ***(Moon et al , 2003).***

Indeed some recent studies have demonstrated that in in-vitro isolated perfused hearts exposed to Epo are protected against subsequent hypoxia ***(Cai et al, 2003).*** These protective actions have also been directly verified on cardiac cells as well. On isolated cultured H9c2 cardiac myoblasts (embryonic rat heart cells) preconditioned with EPO showed a better response to hypoxia ***(Parsa et al , 2003).***This suggests that the direct actions of Epo to prevent myocyte death independently of its effects on red blood cell number or cells other than cardiac myocytes .This emphasizes the potential role of Epo in the treatment of hearts particularly in limiting infarct expansion and attenuating the post-infarct deterioration in hemodynamic function. Furthermore, evidences for the existence of delayed and immediate actions of Epo suggest that mechanisms proposed involve a genomic action as well as a rapid non genomic action probably referred to its ability to scavange free radicals particularly H_2O_2. Despite such observations the signal transduction pathways activated by Epo to protect the heart have not been clearly identified. However, the erythropoietin receptor EPOR is widely distributed in the cardiovascular system, including endothelial cells, smooth muscle cells and cardiomyocytes and despite a wide overlap in the signaling pathways activated by EPO-R, an anti-apoptotic activity is strongly suggested in the cardiovascular system.In myocardial infarction

apoptosis has been reported at acute stages of evolution in the ischemic area as well as in remote zones. In the ischemic area it might be a determinant of the final size of the infarct and it seems to depend on the presence of post-ischemic reperfusion. It seems likely that the preconditioning with Epo with other agents can confer myocardial protection against ischemia-reperfusion injury, in terms of reduction in cellular apoptosis and necrosis ***(Daisuke et al , 2006).***

According to the for mentioned information, we hypothesized a potential therapeutic role for EPO in the failing heart both in improving cardiac performance and increasing resistance in the face of ischemic challenges. We also hypothesized that such protective function for EPO hormone can be partially based on it antiapoptotic actions.

AIM OF THE WORK:

The aim of this study therefore, is to:

1- Evaluate the direct effect of in vivo Epo administration on improving the myocardial contractility in a rat model of heart failure.

2- Clarify the role of Epo preconditioning in cardioprotection against ischemic injury using an in vitro model of global ischemia.

3- Unravel the possible underlying mechanisms for EPO protective effects in heart failure and associated infarction.

HEART FAILURE : PATHOPHYSIOLOGY AND CONSEQUENCES

Heart failure is the pathophysiologic state in which the heart fails to pump blood at a rate sufficient for the requirements of the metabolizing tissues and/or pumps only from an abnormally elevated diastolic filling pressure. This impairment of pump performance leads to increased pulmonary and systemic venous pressures, reduced cardiac output and molecular abnormalities that cause progressive deterioration of the heart and premature myocardial cell death.

It is estimated that there are more than 15 million new cases of heart failure each year worldwide. There are about 500,000 new cases of heart failure diagnosed each year in the USA, and ten times that number of Americans currently in heart failure. The numbers are rapidly increasing because of the aging population. Heart failure is the leading cause of hospitalization of patients over 65 years in age. Despite many new advances in drug therapy and cardiac assist devices, the prognosis for chronic heart failure remains very poor. One year mortality figures are 50-60% for patients diagnosed with severe failure, 15-30% in mild to moderate failure, and about 10% in mild or asymptomatic failure ***(Towbin JA and Bowles NE, 2002).***

The primary abnormality in heart failure is the hemodynamic disorders including: impaired pump performance leading to increased pulmonary and systemic venous pressures, fluid retention, and low cardiac

output. The fall in cardiac output leads to activation of several neurohormonal compensatory mechanisms aimed at improving the mechanical functions of the heart such as norepinephrine, vasopressin, and atrial natriuretic peptide.

Norepinephrine increases afterload by systemic vasoconstriction and increases chronotropy and inotropy by direct stimulation of cardiac myocytes. Plasma concentrations of various other neuroendocrine markers correlate with both the severity of heart failure and the long term prognosis. For example, raised plasma concentrations of N-terminal and C-terminal atrial natriuretic peptide and of brain natriuretic peptide are independent predictors of mortality in patients with chronic heart failure. Patients with congestive heart failure and raised plasma noradrenaline concentrations also have a worse prognosis ***(Reynolds M. Delgado and James T. Willerson, 1999).***

As heart failure advances and/or becomes progressively decompensated, there is a relative decline in the counterregulatory effects of endogenous vasodilators, including nitric oxide (NO), prostaglandins (PGs), bradykinin (BK), atrial natriuretic peptide (ANP), and B-type natriuretic peptide (BNP). This occurs simultaneously with the increase in vasoconstrictor substances from the RAAS and adrenergic systems. This fosters further increases in vasoconstriction and thus preload and afterload, leading to cardiac fibroblast proliferation, adverse myocardial remodeling, and antinatriuresis with total body fluid excess and worsening of CHF symptoms ***(Gary S., 2001).***

It was also reported that there is an increase in plasma level of Epo in proportion to the degree of cardiac dysfunction. Patients with CHF by