EFFECT OF SOME CALCIUM CHANNEL BLOCKERS (NIFEDIPINE AND AMLODIPINE) ON THE INJURY ASSOCIATED TO HEPATIC ISCHEMIA-REPERFUSION IN ALBINO RATS

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ABSTRACT

Hepatic ischemia is an important factor in the development of hepatic degeneration and necrosis in different hepatic pathological conditions.

This study was conducted to declare the effect of pretreatment of albino rats with dihydropyridine L-type calcium channel blockers (nifedipine and amlodipine) on hepatic ischemia reperfusion (I/R). Furthermore this study investigated the effect of I/R on plasma levels of endothelin-1 (ET-1) and nitric oxide (NO) and their pathophysiologic links to maintenance of hepatic function.

This study was carried on 36 male albino rats. The animals were divided into 6 equal groups, each consisted of 6 rats. Group (1): sham operated (rats subjected to anesthesia and laparotomy). Group (II): control I/R rats, pre-treated with saline. Group (III): treated with nifedipine (2mg/kg/day) intra-gastric for 6 weeks before exposure to sham operation. Group (IV) : I/R treated with nifedipine with the same previously mentioned dose and for the same duration before induction of I/R. Group (V): amlodipine pretreated (0.5mg/kg/day) for 6 weeks, intragastrically and then exposed to sham operation. Group (VI): received amlodipine 0.5mg/kg/day for 6 weeks before being subjected to I/R. Transient hepatic ischemia for 90 minutes was done under anesthesia by hepatic vascular pedicle clamping

followed by 30 minutes reperfusion. Hepatic cell function was tested by measuring plasma alanine transferase (ALT). This is in addition to measurements of hepatic tissue calcium content, plasma endothelin-1 and plasma nitrites. It was found that I/R produced a significant increase in plasma ALT, ET-1, nitrites and hepatic tissue calcium. Both nifedipine and amlodipine induced hepato-protective effect confirmed by prevention of the elevation in plasma levels of ALT and hepatic tissue calcium load. Each of nifedipine and amlodipine had equally prevented the hepatic accumulation of calcium. In spite of this equi-effective protection any of the drugs exerted significant changes in plasma ET-1 levels, while plasma nitrites levels remained high. Further studies of these results on hepatic patients are recommended especially in patients given nifedipine or amlodipine for associated cardiovascular problems.

INTRODUCTION

Hepatic ischemia occurs in hemodynamic and cardiogenic shock and interruption of hepatic blood flow can be necessary during extensive liver resection for trauma or tumors and in liver transplantation, during which resections are frequently performed under complete or partial vascular occlusion to reduce blood loss and it has been shown that intermittent rather continuous pedicle occlusion leads to lower post-operative morbidity and mortality rates [1,2]

Endothelin family (endothelin1, 2, 3) consists of 21 amino acid [3]. Endothelin-1 (ET-1) is produced mainly by endothelial cells ,vascular smooth muscle cells and to a lesser extent by hepatocytes. ET-2 is mainly produced within the kidney and intestine, whereas the highest levels of ET-3 are found in the brain [4]. Endothelin receptors (ET_A & ET_B) are present in many internal organs, e.g. heart, adrenals, kidneys, lung tissue and central nervous system [5].

Liver has been considered to be a major organ that greatly alters its functions through mechanisms involving ET-1, one of the most potent vasoconstrictors produced by vascular endothelial cells. ETA receptor is predominantly expressed on vascular smooth muscle cells and executes vasoconstriction [7,8] on the other

hand, ETB receptor occurs in endothelial cells, Kupffer cells, and vascular smooth muscle cells and its stimulation in endothelial cell induces nitric oxide (NO) mediated vaso-relaxation through activation of constitutive NO synthase [9,10]. The factors stimulate endothelin production by endothelial cells include mechanical stimulations of the endothelium, thrombin, calcium ions, epinephrine, angiotensin II, vasopressin, dopamine, erythropoietin, cytokines as IL-1 , insulin-like growth transforming factor beta, endotoxins and lipids (low density lipoproteins and high density lipoproteins). The substances inhibiting endothelin synthesis are NO, cyclic guanosine monophosphate (cGMP), atrial natriuretic peptide (ANP), prostacyclin (PGI_2) and bradykinins [4]. Although circulating endothelin levels may not fully reflect local vascular production because more than two thirds of endothelin is released abluminally [11], elevated plasma endothelin levels may indicate overproduction of the peptide. Elevated endothelin plasma levels have been described in atherosclerosis [12,13] and other cardiovascular diseases such as stroke, pulmonary hypertension, cardiogenic shock and congestive heart failure [14,15,16]. Importantly plasma endothelin levels after acute myocardial infarction are elevated and correlate with the severity of the disease [13,14]. In addition to vasoconstriction, it might also promote vascular cell proliferation in atherosclerosis with consecutive narrowing of the vessels, especially in situations with enhanced endothelin release and thus further deteriorate organ perfusion and oxygen supply.

Calcium channel entry blockers (CCEBs) are drugs extensively used in cardiology practice. CCEBs include a broad spectrum of various drugs with different actions on the heart and the vessels [17]. Three groups of routinely used CCEB, the high voltage (L-type) calcium channel have been accepted phenylalkylamines (e.g. verapamil), dihydropyridines (nifedipine, nimodipine ,amlodipine) and benzothiazepines (e.g. diltiazem). Clevidipine is another IV group of 1, 4 dihydropyridine with ultrashort activity, being rapidly hydrolyzed by esterases [18, 19]. The first generation dihydropyridine L-type CCBs, such as nifedipine, were developed as selective and

powerful vasodilator [20, 21]. To increase the selectivity of nifedipine, a series of 2nd generation CCEB e.g nimodipine was developed by altering nifedipine's physicochemical and pharmacodynamic properties [22]. A third generation series of L-type CCBs has also been developed and at least one of these, amlodipine [23]. It is very likely that part of the difference between amlodipine and nifedipine stems from the unexpected property of amlodipine to release NO [24].

The aim of the present work is to study the effect of pre- treatment with dihydropyridine L-type calcium channel blockers (nifedipine and amlodipine) on hepatic ischemia-reperfusion (I/R) induced injury in albino rats. In addition the purpose of this study was to examine the effects of I/R on plasma levels of ET-1 and its pathophysiologic links to maintenance of hepatic functions.

MATERIALS & METHODS

Animals used :

This experiment was carried on 36 male albino rats, weighing 220-260 grams/rat. Animals were having free access to water & food. They were exposed to similar housing conditions of heat & humidity.

Drugs used :

- Nifedipine (Epilat capsules): 10 mg, supplied by Epico-Co.
- Amlodipine (Norvasc tablets): 5 mg, supplied by Pfizer-Co.

Animals grouping :

The animals were divided into 6 equal groups, each consisted of 6 rats.

Group (1) : Sham operated (anesthesia + laparotomy) but without hilum clamping of the hepatic vascular pedicle. This group pretreated with intragastric saline, 0.5 ml/kg/day for 6 weeks.

Group (2) : Control group (anesthesia + ischemia reperfusion; I/R). These animals pretreated with saline in the previous amount and duration and subjected to 90 minutes hepatic ischemia induced by hilum clamping of hepatic vascular pedicle, followed by 30 minutes of reperfusion.

Group (3) : Pretreated with intragas-

tric nifedipine in a dose of (2 mg/kg/day) for 6 weeks and then exposed to sham operation[25].

Group (4) : I/R pretreated with nifedipine in a dose of 2 mg/kg/day, intragastrically for 6 weeks [25].

Group (5) : Pretreated with amlodipine 0.5 mg/kg/day intragastrically for 6 successive weeks and exposed to sham operation[26].

Group (6) : Rats with induced I/R and pretreated with amlodipine (0.5 mg/kg /day), intragastrically for 6 weeks [26].

Surgical procedures :

Liver ischemia was induced according to the method of Nauta et al. and Peralta et al. (6, 27). Hepatic ischemia was performed under general anesthesia one hour after the last drug dose. Sodium thiopental (the used anesthetic agent) was given in a dose of 30 mg/kg body weight intraperitoneally (IP) for induction and repeated when required in a dose of 10 mg/kg, IP for maintenance (27). After section of the ligaments of the liver, hepatic ischemia was induced for 90 minutes by hilum clamping of hepatic vascular pedicle using bulldog clamp. During the period of ischemia, 0.5 ml of saline was given IP every 30 minutes to maintain hemodynamic stability. Reperfusion was established by removal of the clamp. After 30 minutes of reperfusion, the animals were killed by knifing and their livers were immediately removed for assay of hepatic calcium content. In addition, blood was collected for estimation of plasma alanine amino transaminase (ALT), endothelin and nitric oxide (nitrites).

Estimation of ALT :

ALT. activity was determined by enzymatic technique [28] using Biotic laboratories kits. The activity was expressed as units/liter.

MEASUREMENT OF NITRIC OXIDE

This is done by measuring nitrite (NO_2) which is one of two primary stable and non-volatile breakdown products of NO This method is in accord to Griess et al : [29] and Bredt and Snyder [30]. Nitrites, a stable oxidation product of NO are measured by a spectrophotometer assay using the Griess reagents, sulfanilamide,

HCl and N- (1-napthyl) ethylenediamine (NED).

Measurement of plasma immunoreactive endothelin -1 (irET-1) :

It was determined according to Rubanyi et al & Gian et al. (31& 32). Plasma samples were drawn into chilled EDTA tubes (1mg/ml blood) containing aprotonin (500 KIU/ ml of blood). Centrifuge the blood at 1.600 xg for 15 minutes at 0°C. Stored at -70°C until the time of assay.

Measurements of hepatic calcium tissue :

At the end of the 30 minutes of reperfusion, the hepatic tissue calcium load was measured using Perkin- Elmer 22380 atomic absorption spectro-photometer with air acetylene flame. Its value was expressed in mg/ gm liver tissue [33].

Statistics :

Student's t-test according to Pipkins [34] was used to compare statistically significant changes between control groups and test groups. P value = or< 0.05 was considered to be significant.

RESULTS

A significant increase in liver enzyme (ALT) and hepatic tissue calcium contents (from 12.1±0.3 & 0.09 ± 0.002 to 24 ± 0.4 & 0.99 ± 0.002, respectively) were observed in the group subjected to ischemia reperfusion as compared to the control group (Tab. 1). In addition I/R induced a significant increase in both plasma levels of ET-1 and nitrites (from 5.1 ± .1 and 21.7+1.1 to 9.3 ± 0.2 and 29.8 +0.9) respectively in comparison to sham operated group (tab2 & Fig 1). When ischemia was preceded by nifedipine administration to the rats in a dose of 2 mg/kg/day intragastrically for 6 weeks, the elevation in plasma ALT and hepatic calcium levels were prevented (table 1) . Also when I/R was preceded by administration of amlodipine to the rats in a dose of 0.5 mg/kg/day intragastrically for 6 weeks, the increase in plasma ALT & hepatic tissue calcium levels were prevented (Tab.1)

In addition nifedipine pretreatment induced a non significant changes in plasma immunoreactive endotheliun-1 (irET-1) levels accompanied by increase in plasma nitrites in comparison to I/R control groups (from 9.3

±0.2 to 8.9 ±0.7 for ir ET-1 and from 29.8 ± 0.9 to 37.2 ±3.3 for nitrites, Tab. 2 & Fig. 1)

Also, amlodipine pretreatment induced non-significant changes in plasma ir ET-1 levels (from 9.3 ± 0.2 to 9.1± 0.8) accompanied by increase in plasma nitrites (from 29.8 ± 0.9 to 50.8 ± 2.1) as showed in (tab. 2 & Fig.1).

Table (1): Effect of intragastric pretreatment by either nifedipine (2 mg/kg/day) or amlodipine (0.5 mg/kg/day) for 6 weeks on plasma ALT and on hepatic tissue calcium content in hepatic ischemia reperfusion (I/R) injury in rats (Mean ± SE).

Groups N = 6	Group (1) Sham-operated (Anesthesia + laparotomy)	Group (2) Control IR	Group (3) Sham + nifedipine	Group (4) IR + nifedipine	Group (5) Sham + amlodipine	Group (6) IR + amlodipine
Plasma ALT (IU/L)	12.1 ± 0.3	24 ± 0.4•	12.1 ± 0.3	14.5 ± 1.1*	12.2 ± 0.2	13.5 ± 0.9*
Hepatic tissue calcium (mg/gm liver tissue)	0.09 ± 0.002	0.99 ± 0.002•	0.099 ± 0.003	0.09 ± 0.004*	0.089 ± 0.003	0.08 ± 0.005*

SE = standard error.

ALT = alanine transaminase

• = Significant difference between control I/R and sham operated group.

*= Significant difference between I/R drug pre- treated (amlodipine or nifedipine) and I/R control group.

Table (2): Effect of intragastric pretreatment by either nifedipine (2 mg/kg/day) or amlodipine (0.5 mg/kg/day) for 6 weeks on plasma levels of endothelin-1 like immunoreactivity (ir ET-1) and nitrites in rats with induced hepatic I/R (mean ± SE).

Groups N = 6	Group (1) Sham-operated (Anesthesia + laparotomy)	Group (2) Control IR	Group (3) Sham + nifedipine	Group (4) IR + nifedipine	Group (5) Sham + amlodipine	Group (6) IR + amlodipine
Plasma irET-1 pg/ml	5.1 ± 0.1	9.3 ± 0.2•	5.2 ± 0.3	8.9 ± 0.7	4.9 ± 0.2	9.1 ± 0.8
Plasma nitrites (µmol/L)	21.7± 1.1	29.8 ± 0.9•	21.9 ± 0.3	37.2 ± 3.3+	22.1 ± 0.8	50.8 ± 2.1+

SE = standard error.

ALT = alanine transaminase

• = Significant difference between control I/R & sham operated group.

+ = Significant difference between I/R and drug pre- treated (amlodipine or nifedipine) and I/R control group.

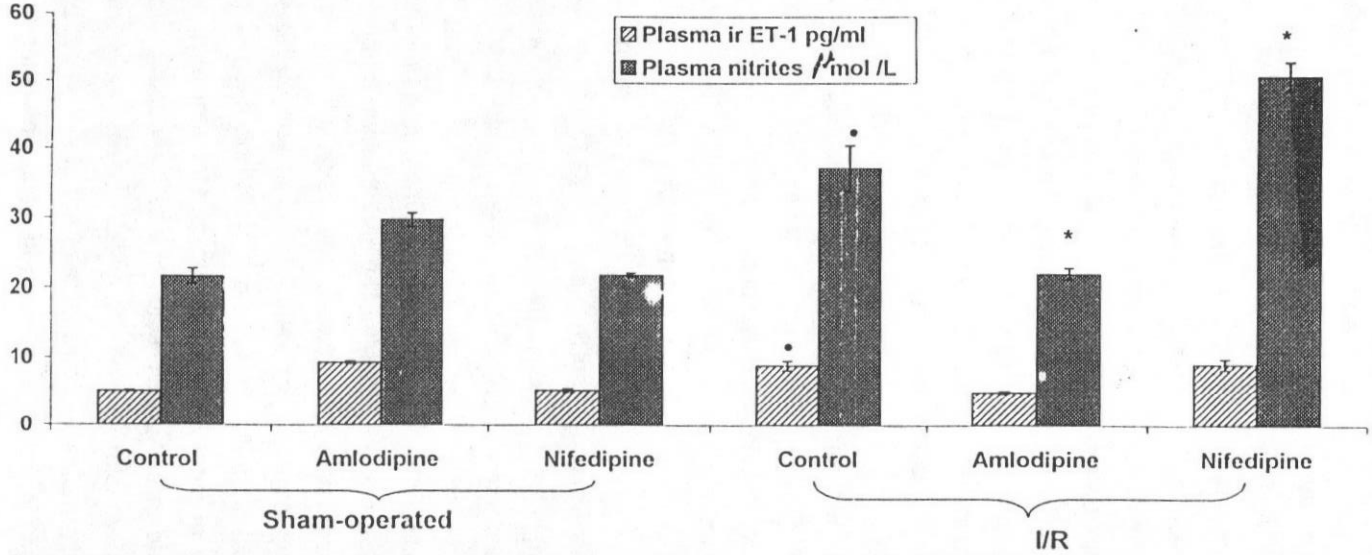

Fig (1): Effect of intra-gastric pretreatment by either amlodipine (0.5 mg/kg/day) for 6 weeks or nifedipine (2 mg/kg/day) for 6 weeks on plasma levels of endothelin-1 like immuno reactivity (ir ET-1) and nitrites in rats with induced hepatic I/R (mean ± SE).

SE = standard error.
ALT = alanine transaminase .
• = Significant difference between control I/R & sham operated groups .
* = significant difference between I/R drug pre- treated (amlodipine or nifedipine) and I/R control groups .

DISCUSSION

Liver cell injury is a common event in clinical practice. Ischemia reperfusion injury considered of the most important causes of liver cell injuries. A number of surgical maneuvers require a period of liver ischemia, on reperfusion hepatic injury results from failure of the microcirculation and excessive inflammatory responses [35]. Furthermore, anoxic liver injury occurs during organ preservation for liver transplantation as a treatment of end stage of liver disease [36]. Therefore, in the present work model of I/R was chosen due to its important clinical relevance [35, 36].

In the present study, rat groups subjected to I/R showed significant hepatic function alternations demonstrated by increase in plasma levels of ALT. There were also significant increases in hepatic tissue calcium levels. These findings are in accord with previous studies [37, 38]. They reported that interference with Ca+2 homeostasis and increased levels of cytoplasmic free ca +2 participate in cell injury through disruption of cellular thiol homeostasis .Certain proteins are highly sensitive to changes in the thiol status, including Ca^{2+} - dependent adenosine triphosphate which serves as membrane bound Ca^{2+} pumps to the ion that maintain cytoplasmic calcium at low levels [39,40] . A sustained elevation in cytoplasmic Ca^{2+} may mediate adverse effects on cellular viability via activation of endonucleases and phospholipases as well as enhanced production and accumulation of free radicals [41, 42]. This can lead to a chain of reactions involving lipid peroxidation eventually resulting in membrane damage and alternations of membrane bound protein function including the Ca^{2+} -AT pase. Furthermore, I/R provoked a significant increase in plasma endothelin-1 and nitrites. These results supported by the study of Taniani et al [43], as they found that during anoxia re-oxygenation , plasma ET-1 levels were elevated and blockade of its effect by endothelin antagonists attenuated reperfusion injury, suggesting deteriorating effects of this vasoconstrictor peptide on hepato- portal hemodynamics. Mechanism by which ET-1 aggravates the hepatic reperfusion injury has been thought to involve impairment of sinusoidal patency through its vasoconstrictor actions

(44, 45). This notation was supported by previous studies showed that trans- portal administration of ET-1 induced a marked vasoconstriction at portal venules and a reduction of bile flow (46). The increase in plasma nitrites levels associated with I/R could by explained on the light of Taniani et al study (43), where they reported that ET-1 is released during the early period of re-oxygenation and stimulates ETB receptors -mediated signaling to trigger NO-dependent and independent mechanisms.

Despite markedly different chemical structure, all compounds of the three main classes of Ca^{2+} antagonists (dihydropyridines, phenylalkylamines and benzothiazepines) inhibit the inward flow of Ca^{2+} ions through (L- type) calcium channels (44). However, because of binding at different receptor sites, different pharmacokinetic properties, and different effects at the levels of the cardiovascular (coronary and peripheral arteries, cardiac conduction system and myocardium) and extracardiovascular systems, each of these compounds has its own advantages and disadvantages (44). For these reasons two members of calcium channel antagonists were used in the present study to evaluate any possible differences in their effects on I/R.

Pretreatment of rats with nifedipine before induction of ischemia - reperfusion produced a significant decrease in hepatic calcium contents , plasma ALT and plasma ET-1 with significant increase in plasma nitrites level as compared to I/R control group. These finding are in agreement with Goto et al, (25) and Sogni et al.(45). They reported that nifedipine could prevent the incidence of halothane induced hepatotoxicity by preventing the increase in cytosolic calcium concentrations. Moreover, in vitro study revealed that nifedipine pretreatment exhibited a preventive effect against acetaminophen - induced hepatocytes injury by keeping intracellular calcium levels within the normal control values (46,47). Using the isolated rat hepatocyte exposed to ethanol, Cobreros et al. (48) observed a significant decrease in ALT and lipid peroxidation after nifedipine treatment. The present study extends these findings to in vivo model. Although several NO synthase isoforms

have been isolated all are homologous and divided into two categories with different regulations and activities. The constitutive isoforms in neuronal and endothelial cells are always present [49]. These NO synthase isoforms are inactive until intracellular Ca^{2+} levels increase, the Ca^{2+} binding level increase, the Ca^{2+} -binding protein calmodulin binds to Ca^{2+} and Ca^{2+} calmodulin complex binds to and activates NO synthase (49). The constitutive NO synthase isoforms then synthesize small amount of NO until intracellular Ca^{2+} levels decrease. In contrast, the inducible NO synthase isoform is normally not expressed in macrophages and hepatocytes when activated by specific cytokines these cells produce inducible NO synthase enzyme that synthesize large amounts of NO. Although the inducible synthase is Ca^{2+} independent, the dihydropyridine Ca^{2+} - channel antagonist, nifedipine reduced bacterial lipopolysaccharide induced NO production in cultured macrophages [49]. Thus, in certain preparations acute treatment with Ca^{2+} antagonists may directly stimulate NO release as well as facilitate the effects of NO at the level of vascular smooth muscle cells. It seems that at least part of the increased NO release by nifedipine is due to a protection from reactive oxygen species (ROS), which deactivates NO [50]. However, chronic nifedipine therapy does not improve endothelial NO release in normotensive animals [51]. The evidence of slow onset and long duration of action, suggested that amlodipine had pharmacological properties distinct from other calcium channel antagonists particularly nifedipine [52]. In addition a number of studies have suggested that amlodipine inhibits platelet aggregation [53]. Changes in shear stress are thought to be important in the regulation of NO production, whenever blood flow changes, so do shear and NO release [54]. Therefore all vasoactive drugs have the potential to release NO because of the changes in physical forces that distort the endothelial cells. Amlodipine prompts the production of NO through the activation of eNOS. The effect on eNOS is apparently transduced via activation of angiotensin receptor followed by the generation of kinins & stimulation of the B_2- kinin receptor [52]. Amlodipine, unlike nifedipine or diltiazem, caused a dose

dependent release of nitrite, the hydration product of NO. The ability of amlodipine to release NO was unexpected, because: NOS is a calcium dependent enzyme and amlodipine should reduced intra-endothelial cell calcium and there are no L-type calcium channels on endothelial cells for amlodipine to block [52]. Several studies have suggested that small vessels are more dependent on the influx of extra-cellular Ca^{2+} than larger vessel [49]. Furthermore, it has been shown that nifedipine strongly reduced contraction due to ET-1 in porcine ciliary arteries [55]. This may also explain why, in the human microcirculation of normal subjects, intra-arterial administration of verapamil or nifedipine fully prevents ET- 1 induced contractions [56]. Interestingly calcium antagonists can reverse endothelin induced contraction in both the porcine coronary as well as the human internal mammary artery [57, 58].

The probable reason for this is that, in contracting cells, ET lowers the membrane potential of vascular smooth muscle cells [59] which led to opening voltage operated Ca^{2+} channels. Therefore, once contractions have developed, Ca^{2+} antagonists are able to exert an inhibitory effect. Although ET-1 was originally considered to be an endogenous activator of voltage- operated Ca^{2+} channel [49], it has been shown that ET-1 interacts with specific receptors on vascular smooth muscle (ETA & ETB receptors) mediating vasoconstriction and proliferation. On endothelial cells, ET interacts with ETB- receptors, stimulating the formation of NO and PGI2 [49]. ETA receptors bind endothelin-1 and to a lesser degree, endothelin 3; ETB receptors bind endothelin -1 and endothelin 3 with similar affinity [60, 61]

Administration of amlodipine in a dose of 0.5 mg/kg/day for 6 weeks to rats before induction of I/R produced a non -significant changes in plasma ET-1. In contrast to this study, Inigo et al. [61] found that ET-1 concentrations were significantly higher during amlodipine compared with losartan treatment in crossover trial in renal transplant recipients. Furthermore, in vitro study reported that amlodipine at high dose (20 mg/kg/day) increased preproendothelin-1 expression in rat

ventricles and aorta of normotensive rats because this dose can activate the sympathetic nervous systems and the rennin angiotensin system. Both nor-adrenaline and angiotensin II stimulate preproendothelin-1 gene expression [62]. However, amlodipine attenuates the ischemia and reperfusion- induced increase in cardiac ET-1 binding sites in hearts from rats pretreated with amlodipine (0.25-0.5 mg/kg) [63]. In addition in a human study, twenty and forty minutes ischemia caused a time dependent increase in ET-1 binding density. Amlodipine pretreatment attenuated this increase in a time and dose dependent manner. Amlodipine (0.25-0.5 mg/kg) also suppressed the ischemia induced increase in ET-1 binding site density [64].

In conclusion both nifedipine and amlodipine exerted a protective effect against the injury associated to hepatic ischemia reperfusion in rats. Six weeks of therapy with nifedipine or amlodipine improves liver function by increasing the bioavailability of nitric oxide (this increase of NO levels was higher in groups treated with amlodipine than in that treated with nifedipine) and by preventing the increase of hepatic calcium content that occurred with I/R injury.

REFERENCES

1- Belghiti J, Noun R, Malafosse R, Jagotp, Sauvanet A, Pierangeli F, Marty J & Farges O (1999) : Continuous versus intermittent portal triad clamping for liver resection. Ann surg; 299:369.

2- Grenesse D, Laurens M, Gugenhem J, Heurteaux C , Curio R, Benard R & Schmid. Alliana A (2001) : Intermittent ischemia reduces warm hypoxia -Reoxygenation induced -JNK1/SAK1 activation & apoptosis in rat hepatocytes. Hepatology; 34:972.

3- Goraca A, (2002) : New views on the role of Endothelin.. Endocrine regulations; 36: 161.

4- Inoue A, Yana Gisawa M, Kimure S, Kasuya Y, Miyauchi T, Goto K & Masaki T (1989):

The human endothelin family: three structurally & pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci (USA); 86: 2 863.

5- **Yanagi Sawa M (1994) :** The endothelin system. A new target for therapeutic intervention . Circulation; 89: 1320.

6- **Nauta RJ, Uribe M, Walsh DB, Miller D & Butteer Field A (1989) :** Description of a chronic in vivo model for the study of warm hepatic ischemia reperfusion injury. Surg Res. Commun.;6 :241.

7- **Okumura S, Takei Y, Kawano S, Nagank, Maseroda E, Goto M & Tsuji S (1994) :** Vasoactive effect of endotholin-1. Hepatology; 19:55.

8- **Rockey Dc & Chung JJ (1995) :** Inducible nitric oxide synthase in rat hepatic lipocytes & the effect of nitric oxide on lipocyte contractility. J Clin Invest.; 95:1199.

9- **Housset C, Rockey, DC & Bissell DM (1993) :** Endothelin receptors in rat liver lipocytes as a contractile target for endothelin -1 .Pro Natl Aca Sci US; 90:9266.

10- **Hirata Y, Emori T, Eguchi S, Kannok, Imau T, Ohta K & Marumo F (1993) :** Endotheiin receptor subtype B mediates synthesis of nitric oxide by cultured bovine endothelial cells. J Clin Invest; 91:1367.

11- **Wagner of, Christ G, Wogta J, Vierhapper H, Parzer S, Nowotny OJ, Schneider B, Waldhaust W & Binder BR (1992) :** Polar secretion of endothelin-1 by cultured endothelial cells. J Biol Chem.; 267: 16066.

12- **Lerman A, Edwards BS, Hallett JW, Heulblein OM, Sandberg SM & Burnett JJ (1991) :** Circulating and tissue endothelin immuno-

reactivity in advanced atherosclerosis. N Engl J Med; 325:997.

13- **Stewart DJ, Cernacek O, Costelio KB, Roulcau JL (1992)** : Elevated endothelin-1 in heart failure & loss of normal response to postural change. Circulation; 85:510.

14- **Gernacek P & Stewart D J (1989)** : Immunoreactive endothelin in human plasma: marked elevations in patients in cardiogenic shock. Biochem Biophys Res Commun; 161:562.

15- **Lechleitner P, Gen ser N, Mair J, Artner DE, Dienstt F & Puschendrof B (1992)** : Endothelin-1 in patient with complicated and uncomplicated myocardial infraction. J Clin Invest; 70:1070.

16- **Stewart D J, Kubac G, Gostello KB, Cernacek P (1991)** : Increased plasma endothelin-1 in the early hours of acute myocardial infarction. Am Coll Cardiol; 18:38.

17- **Apastolakos M & Varon M (1996)** : Anti-arrythmic and anti-ischemic properties of calcium channel antagonists . New Horiz; 4:45-57.

18- **Huraux C, Makita T, Szalnn F, Nordlander M & Levy J (1997)** : The vasodilator effects of clevidipine on human internal mammary artery. Anesth Analg; 85: 1000.

19- **Ericsson H, Fakt C, Jolin Mellgard A, Nardlander M, Sohtell L & Sunzel L (1999)** : Clinical and pharmacokinetic results with a new ultrashort acting calcium antagonist, clevidipine; following gradually increasing intravenous doses to healthy volunteers. Br J Clin Pharmacol; 47:531.

20- **Vatner SF & Hintze TH (1982)** : Effects of a calcium channel antagonist on large and

small coronary arteries in conscious dogs. Circulation; 66:579.

21- **Hintze TH & Vatner SF (1983) :** Comparison of effects of nifedipine and nitroglycerin on large and small coronary arteries and cardiac function in conscious dogs. Circ Res J; 52 (suppl)139.

22- **Elkayam U (1998) :** Calcium channel blockers in heart failure. Cardiology; 89 (suppl) : 38.

23- **Deleeuw PW & Birkenhager WH (2002) :** The effects of calcium channel blockers on cardiovascular outcomes; a review of randomized controlled trial. Blood Press; 11:71.

24- **Zhang X and Hintze T (1998) :** Amlodipine release nitric oxide from canine coronary micro vessels. An unexpected mechanism of action of a calcium channel blocking agent .Circulation; 97:576.

25- **Goto T, Ohwan K, Matsumoto N, Miyazaki T, Murokami I, Ohhata M and Shiata K (1990) :** Protective effect of calcium channel blockers on the liver against halothane hepatitis in rats. Masui; 39 (2) 204.

26- **Kin S, Zhan Y, I zumic Y and Iwao H (2003) :** Cardiovascular effect of combination of perindopril ,condesratan and amlodipine in hypertensive rats. Hypert; 35:769.

27- **Peralta C, Hotter G, Closa D, Gelpi E, Bulbena G and Catafau JR (1997) :** Protective effect of preconditioning on the injury associated to hepatic ischemia reperfusion in the rat : role of nitric oxide and adenosine. Hepatology; 25:934.

28- **Schmidt E & Schmidt F. (1973) :** Colorimetric determination of GPT. Enzyme Biol Clin, 3:1.

29- **Griess P (1897) :** Bemerkungen

Zu derabhand Lung der H.H. Weselsky and Benedikt "Ueber einige azoverbindungen" Chem. Ber.; 12,426 Quoted from analytical biochemistry; 126:131-138 (1982).

30- **Bredt DS and Synder SH (1999)** : Nitric oxide a physiological messenger molecule. Ann Rev Biochem; 63:175.

31- **Rubanyi GM & Polokof F (1994)** : Human Endothelin-1 enzyme immunometric assay. Pharm. Rev; 46:326.

32- **Gian PR, Teresa MS, Giovanna A & A chille CP (2000)** : Measurement of endothelin: clinical and research use. Ann Clin Biochem; 37; 6081.

33- **Sparrow MP & Johnstone BM (1964)** : A rapid micromethod for extra action of Ca++ and Mg ++ from tissue. Biochem Biophys Acta; 90:425.

34- **Pipkins FB (1984)** : Statistics analysis of the obtained data by descriptive and comparative analysis in: "Medical statistics Made Ease" Churchill Livingstone Publication. London, Mellbourne , New York.

35- **Inglott F and Mathie R (2000)** : Nitric oxide and hepatic ischemia reperfusion injury. Hepatogastroenterology; 47 (36): 1722.

36- **Sileri P, Schena S, Morini S, Rastellinic pharm S and Benedetti C (2001)** : Pyruvate inhibits hepatic ischemia reperfusion injury in rats. Transplantation, 72 (1):27.

37- **Bellomo, Gefand Orrenius S (1985)** : Altered thiol and calcium homeostasis in oxidative hepatocellular injury. Hepatology Baltimore; 5 : 876.

38- **Banbieri A, Zontaf Saracino ML, Dondi O, Frattini P,**

Lucchelli A, Santagosting M, Daastanna G, Lawshri MB and Zonata A (1996) : Evolution of reperfusion syndrome after liver ischemia in the rat. J surg Res; 62 (2) : 153.

39- Belloma GF, Mirabelli P, Richelini I and Orrenius SO (1983) : Critical role of sulphhydryl group (s) in ATP-dependent Ca2+ sequestration by the plasma membrane fraction from rat liver. FEBs Lett.; 163:139.

40- Taddei S and Salvetti A (2002) : Endothelial dysfunction in essential hypertension: clinical implications. J Hypertension; 20:1671.

41- Buja LM, Hogler HK and Willenson JT (1988) : Altered calcium homeostasis oxidative hepatocellular injury. Hepatology Baltimore; 5 : 876.

42- Ghiadonil, Magagna A, Versani D, kardasz I, Huang Y, Taddeis and Salvetti A (2003) : Different effect of anti-hypertensive drugs in conduit artery endothelial function. Hypertension; 41 : 1281.

43- Taniani H, Suematsu M, Suzuki T, Norimizu S, Hor R, Ischimura Y and Nimura Y (2001) : Endothelin-B receptor mediated protection against anoxia reoxygenation injury in perfused rat liver, nitric oxide dependent and independent mechanisms. Hepatology; 33 (4): 894.

44- Mitsuoka H, Suzuki S, Sakaguchi T, Baba S Miwa M, KonnoH and Nakamura S (1999) : Contribution of endothelin-1 microcirculatory impairment in total hepatic ischemia and reperfusion injury. Transplantation; 67 : 514.

45- Sogni P, Moreau R, Gromola A, Gadano A, Cailmail S Calmus Y, Clozel M (1998) :

Benificial hemodynamic effects of bosentan, a mixed ETA and ETB receptor antagonist, in portal hypertensive rats. Hepatology; 28:65.

46- **Bauer M, Zhang JX., Bauer I and Ckmens MG (1994) :** ET- induced alternations of hepatic sinusoids. Am J Physiol; 266: G 624.

47- **Taddei S, Virdis A, Ghiadoni L and Salvetti A (2002) :** Effects of antihypertensive drugs on endothelial dysfunction: clinical implications. Drugs; 62:265.

48- **Cobreros A, Sainz L, Lasheras B and Cenarru E (1997) :** Hepatotoxicity of ethanol: protective effect of calcium channel blockers in isolated hepatocytes .liver; April 17 (2)L 76.

49- **Ruschitzka FT, Luscher TF and Noll G (1999) :** Calcium antagonists and endothelial function. J Clin Basic Cardiol; 2:175.

50- **Berkels R, Egink G, Marsen TA, Bartels H, Roesen R and Klaus w (2001) :** Nifedipine increases endothelial nitric oxide bioavailability by antioxidative mechanisms. Hypertension; 37:240.

51- **Tschudi mR, Griscione L, Novoscl D, Pfeiffer K and Luscher TF (1994) :** Antihypertensive therapy augments endothelium dependent relaxations in coronary arteries of spontaneously hypertensive rats. Circulation; 89:2212.

52- **Mason RP, Marche T and Hintze H (2003) :** Novel vascular biology of third generation L-type calcium channel antagonists. Ancillary action of amlodipine. Arterioscler Thrombovasc Biol; 22:215.

53- **Chou TC, Li CY, Yen MH and Ding YA (1999) :** Antiplatelet effect of amlodipine

: a possible mechanism through a nitric oxide mediated process. Biochem Pharmacol; 58:1657.

54- **Booy C, H wang T, Sykes M, Mitchell BJ, Kemp BE, Lum H and JOH (2002) :** Shear stress stimulates phosphorylation of eNOS at ser 635 by a protein kinase A-dependent mechanism .Am J Physiol Heart Circ Physiol; 283:H1819.

55- **Meyer P, Lang MG, Flammer J and Luscher TF (1995) :** Effects of calcium channel blockers on the response to endothelin-1 bradykinin and sodium nitroprusside in procine ciliary arteries. Exp Eye Res; 60:505.

56- **Kiowski W, Luscher TF, Linder L and Buhler FR (1991) :** Endothelin-1 induced vasoconstriction in humans. Reversal by calcium channel blockade but not by nitrovasodilators or endothelium derived relaxing factor. Circulation; 83:469.

57- **Kung CF, Tschudi MR, Noll G, Clozei JP and Luscher TF (1995) :** Differential effects of calcium antagonist miberfradil in epicardial intramyocardial coronary arteries. J Cardiovasc Pharmacol; 26:312.

58- **Yang Z, Bauer E, Von Segesser T, Stulz P, Turina M and Luscher TF (1990) :** Different mobilization of calcium in endothelin-1 induced contractions in human arteries and veins: Effects of calcium antagonists. J Cardiovasc Pharmacol; 16:654.

59- **Miller VM, Komori K, Burnett JJ and Vanhoute PM (1989) :** Different sensitivity to endothelin in canine arteries and veins. Am J Physiol; 257:H1127.

60- **Wenzel R, Duthiers N, Noll G, Bucher J, Kaufmann U and Luscher T (1996) :** Endothelin and calcium antag-

onist in the skin microcirculation of patients with coronary artery disease. Circulation; 94: 316.

61- Inigo P, Campristal J, Laro S, Piera C, Campos B, Bescos M, Oppenheimer F and Rivera F (2001) : Effects of losartan and amlodipine on intra renal hemodynamics and TGF-B1 plasma levels in a crossover trial in renal transplant recipients. JAm Soc Nephrol; 12:822.

62- Peter K, Nicole M, Han K and Maurice W (2004) : Amlodipine at high dose increase preproendothelin-1 expression in the ventricles and aorta of normotensive rats. J Hypertension; 22 (4):827.

63- Nayler WG, OU RC, Gu XH and Casley DJ (1992) : Effect of amlodipine pretreatment on ischemia reperfusion induced increase in cardiac endothelin-1 binding site density. J Cardiovasc Pharmacol.; 20 (3): 416.

64- Wigeysunder HC, Hansen MS, Stanton E, Gropp AS, Hall C, Dhalla S and Ghali J (2003) : Neurohormons and oxidative stress in non-ischemic cardiomyopathy: relationship to survival and the effect of treatment with amlodipine. Am Heart J; 146 (2):291.

الملخص العربى

تأثير بعض قافلات قنوات الكالسيوم (النيفيديبين والأمليوديبين) على الإصابة التى تحدث فى كبد الفئران البيضاء نتيجة تعرضها لقصور فى الدورة الدموية بالكبد ثم أعادة ضخ الدم.

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يعتبر القصور فى الدورة الدموية للكبد عاملا مهما فى حدوث وتطور الضمور وكذلك الموت للخلايا الكبددية فى مختلف حالات أمراض الكبد .

أجرى هذا البحث لدراسة احتمال وجود تأثير وقائى لكل من عقارى النبفيديبين والأمليوديبين على الاصابة التى تحدث فى الكبد نتيجة لقصور فى الدورة الدموية به ثم استعادة وصول الدم بعد فترة.

تم اجراء هذا البحث على ٣٦ فأراً أبيضاً من الذكور السليمة صحياً بوزن يتراوح من ٢٢٠ إلى ٢٦٠ جرام، قسمت الفئران البيضاء الى ٦ مجموعات متساوية .

المجموعة الأولى : قياسية (لم تعرض إلى قصور فى الدورة الدموية الكبدية).

المجموعة الثانية : قياسية (عرضت لقصور فى الدورة الدموية الكبدية).

المجموعة الثالثة : معالجة بدواء النيفيديبين بجرعة ٢ مجم/كجم يومياً لمدة ٦ أسابيع متتالية ولم تعرض لقصور فى الدورة الدموية الكبدية).

المجموعة الرابعة : معالجة بالنيفيديبين بنفس الجرعة ولنفس المدة السابق ذكرها ولكنها عرضت إلى قصور فى الدورة الدموية الكبدية بعد العلاج .

المجموعة الخامسة : معالجة بدواء الأملوديبين بجرعة ٠,٥ مجم/كجم يومياً لمدة ٦ أسابيع متتالية بينما لم يتم تعريضها إلى قصور فى الدورة الكبدية).

المجموعة السادسة : مثل السابقة ولكنها عرضت إلى قصور فى الدورة الدموية لمدة ٩٠ دقيقة ثم إعادة ضخ الدم لمدة ٣٠ دقيقة.

أحدث القصور الدموى الكبدى فى الفئران المحضرة بغلق الحزمه الوعائية الدموية الداخلة للكبد لمدة ٩٠ دقيقة متبوعة بفترة ٣٠ دقيقة اعادة للدموية وذلك برفع الغلق عن الحزمة.

اختبرت وظيفة الخلايا الكبدية بقياس مستوى انزيم الألنين أمينوتر انسفيراز فى البلازما وقياس درجة تراكم الكالسيوم بالنسيج الكبدى وقياس كل من مستوى الاندوثيلين - ١ وكذلك مستوى النيتريدات فى البلازما.

فى هذا النموذج التجريبى أشارت النتائج إلى زيادة مستوى أنزيم الألنين أمينوتر انسفيراز فى البلازما وكذلك زيادة كل من كمية الكالسيوم فى الأنسجة الكبدية ومستوى الاندوثيلين - ١ وأيضاً مستوى النيتريدات فى البلازما. كما وجد أنه لكل من دوائى النيفيديبين والاملوديبين تأثير متساوى فى حماية الكبد من الإصابة بقصور الدورة الدموية الكبدية واتضح ذلك بانخفاض مستوى انزيم الألنين أمينوتر انسفيراز فى البلازما وكذلك كمية الكالسيوم فى الأنسجة الكبدية. وبالرغم من هذا التأثير الإيجابى المماثل فلم يحدث أى من تغير ذو دلالة احصائية فى مستوى الاندوثيلين- ١ فى البلازما، بينما ظل مستوى النيتريدات فى البلازما مرتفعاً وكان أكثر ارتفاعاً مع دواء الأملوديبين. وعلى ضوء هذا البحث نوصى بدراسة هذه الآثار فى مرضى الكبد وخصوصاً فى المرضى الذين يتناولون هذه الأدوية لأغراض أخرى مثل مشكلات قصور وظائف الجهاز الدورى والقلب.