EFFECTS OF DIPYRIDAMOLE AND NIFEDIPINE ON EXPERIMENTALLY-INDUCED HEPATOTOXICITY BY CARBON-TETRACHLORIDE IN RATS

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INTRODUCTION

Dipyridamole is a potent coronary vasodilator that inhibits adenosine uptake into cells. It is frequently used as an antiplatelet drug (Brown et al., 1981). In the liver, adenosine is released into the space surrounding the hepatic arteries. Hepatic arterial dilatation can be almost tripled by adenosine and dipyridamole (Giovanni et al., 1998).

Nifedipine is one of calcium channel blockers. It was suggested that oral administration of nifedipine could prevent the incidence of halothane-induced hepatotoxicity in enzyme induced rats (Li, 1990). This may be due to prevention of the increase in cytosolic calcium concentration (Go to et al., 1990). In addition, nifedipine pretreatment exhibits a preventive effect against acetaminophen induced hepatocyte injury through lowering of intra-cellular calcium levels (Ellouk - Achard et al., 1995).

Cytosolic Calcium Ca^{2+} is an important regulator of the activity of many metabolic and structural proteins. Cells normally maintain cytoplasmic Ca^{2+} at very low levels. Ca^{2+} concentrations briefly rise several fold in response to physiological stimuli (Carafoli, 1982). Potential role of disrupted Ca^{2+} fluxes in chemically - induced liver injury was examined in many studies (Fariss et al., 1985). Speculation that Ca^{2+} could be involved in the actions of toxic substances on isolated liver cell preparations has focused primarily on extra cellular Ca^{2+}. In contrast, little attention has been directed toward the early alterations in intracellular Ca^{2+} ho-

meostasis caused by hepatotoxins.

Carbontetrachloride ($CC1_4$) is mostly metabolised in the liver. It is metabolically activated by cytochrome P_{450} to a free radical trichloromethyl radical. The mechanism by which this free radical produces damage remain controversal (Buja et al., 1988).

Molecular oxygen behaves in a biological system as an electron acceptor and produces a superoxide anion radical. It is further reduced into hydrogen peroxide and hydroxyl radical. The reactive oxygen species are highly reactive atoms or molecules that mediate oxidation of biological molecules, membranes, tissues and associated with a variety of pathological conditions (Paolisso & Giuglino, 1996).

The current study was carried out to investigate the possible effect of nifedipine and dipyridamole on intrahepatic Ca^{2+} changes caused by ($CC1_4$) liver injury in rats. Furthermore, this study is a trial to declare the relation between Ca^{2+} homeostasis, free oxygen radical and adenosine pathways in relation to hepatotoxicity-induced experimentally with $CC1_4$.

MATERIALS AND METHODS

Animals used :-

40 male albino rats, weighing 200-250gm were used throughout this study. Animals were having free access to water and food. These animals were exposed to similar environmental housing conditions.

Drugs-used :-

- Nifedipine (Epilat capsules); 10 mg is produced by Epico-Co.
- Dipyridamole (persantin tablet); 75 mg is produced by Boehringer Ingelheim Co.
- Carbon tetrachloride solvent, supplied by united Co. for Chem. & Med. Preparation.

Animal grouping :-

The animals were divided into 4 equal groups, each comprised 10 rats.

The first group; received 0.5ml saline orally/day for 4 weeks and served as a normal control group.

The second group; received CCl_4 in a single dose of 0.15 ml/kg orally (Melsisi et al., 1993) and served as a hepatic injuried control.

The third group; received nifedi-

pine in a dose of 2 mg/kg day orally (Go to et al., 1990), 3 weeks before and one week after administration of $CC1_4$ in a single dose of 0.15 ml/kg, for induction of hepatotoxicity.

The fourth group; received dipyridamole in a dose of 2.25 mg/kg/day (Paget & Barnes, 1964), orally for the previous regimen.

At the end of the study, animals were decapitated and the blood collected and the serum separated for measuring malondialdehyde (MDA), as an index of oxygen free radical and lipid peroxidation spectrophotometerically using the method of Draper & Hadley (1990) and liver functions; SGPT & SGOT. according to Reitman & Frankel (1957). The liver tissue was excised for estimation of intra hepatic Calcium levels according to Sparrow & Johnstone (1964).

Statistics

Statistical analysis of the results were carried out according to Pipkin (1984) using Student's " T " test. P was significant at <0.05.

RESULTS

Administration of Carbon tetrachloride (CC14) to rats induced a significant increase in liver enzymes (SGPT & SGOT), MDA & intrahepatic Calcium levels (Ca^{2+}), as shown in tab. (1).

Dipyridamole administration to the rats in a dose of 2.25 mg/kg/day for 4 weeks (3weeks before $CC1_4$ & one week after CC14-induced heptoxicity), produced a significant decrease in serum SGPT, SGOT, MDA & intra hepatic Ca^{2+} levels as shown in tab. (2).

Nifedipine administration to the rats in a dose of (2 mg / kg/day), orally for 4 weeks by the same regimen as above induced a significant decrease in the previously mentioned parameters, as shown in tab. (3).

Table (1) : Hepatic biochemical changes induced by carbon tetrachloride (C CL4) (M±SE).

Group n = 10	SGPT Iu/L	SGOT Iu/L	malondialdehyde (MDA) n.mol/L	Intrahepatic calcium (mg/gm. Liver tissue)
Normal control	11.33±1.5	30.3±0.7	1.167±0.117	0.071±0.005
Carbontetrachloride induced liver injury 0.15 ml/kg once intra gastrically.	22 ± 1.2	50±1.4 P<0.05	2.997±0.062 P<0.001	0.969±0.019 P<0.001

P= Significance of difference between CCL4 treated group & non-treated group .
SE= Standard error .

Table (2) : Effect of dipyridamole on Serum SGPT, SGOT, Malondialdelyde (MDA) and intrahepatic calcium Ca^{2+}. (M±SE).

Group n = 10	SGPT Iu/L	SGOT Iu/L	(MDA) n.mol/L	Intrahepatic Ca^{2+} (mg/gm. Liver tissue)
CC14 induced liver injury.15 ml/kg. single dose)	22±1.2	50±1.4	2.997±0.062	0.969±0.019
Dipyridamole treated (2.25 mg/kg orally for 4 weeks)	11.3±1.8	31.1±0.75 p<0.05	0.998±0.052 P<0.001	0.363±0.043 P<0.001

P= Significance of difference between treated group & non-treated group (control group).
SE= Standard error .

Table (3) : Effect of nifedipine on Serum SGPT, SGOT, MDA and intrahepatic Ca^{2+} levels.

Group	SGPT Iu/L	SGOT Iu/L	(MDA) n.mol/L	Intrahepatic Ca^{2+} (mg/gm. Liver tissue)
CC14 induced liver injury	22±1.2	50±1.4	2.997±0.062	0.969±0.19
Nifeddipine treated (for 4 weeks, 2 mg/kg, erally)	12±1.2 P<0.05	31.3±0.58 p<0.05	0.9±0.08 P<0.001	0.151±0.009 P<0.001

P= Significance of difference between nifedipine treated group & control group).
SE= Standard error .

DISCUSSION

In the present study $CC1_4$ administration produced a significant liver injury as indicated by increased liver enzymes, intrahepatic Ca^{2+} & MDA. These results are in accordance with Buja et al. (1988) & Reed et al. (1990) where they proposed that CCL_4 is metabolically activated by cytochrome P-450 to a free radical; trichloromethyl radical. The radicals produced peroxidation of unsaturated lipids of endoplasmic reticulum, which resulted in distortion and destruction of membranes and produced new free radicals derived from the lipids of the membranes. The free radicals also bind covalently to proteins, DNA & lipids (Sipes & Gandofli, 1982). In addition sustained increase in cytosolic-Ca^{2+} have been shown to increase phospholipase activity resulting in increased lipid peroxidation (Bellomo et al., 1983).

Intragastric administration of dipyridamole in a dose of 2.25mg/kg/day before and after $CC1_4$ administration produced a significant decrease in SGPT, SGOT, intra hepatic Ca^{2+} and MDA. It has been suggested that the mechanism of action of dipyridamole is due to inhibition of adenosine uptake into cells leading to an increase in interstitial fluid adenosine level (Hintze & Vanter, 1983). Effects of adenosine on purines receptors (P_1 & P2) were studied by Vera & Geoffrey (1998), where they had found that many cells including hepatocytes express more than one subtype of purines receptors as P_2Y_1 & P_2 Y_2 receptors. These receptors typically have a common pathway in phospholipase (PLC). In addition, activation of purines receptors inhibit ATP-induced Ca^{2+} influx via P_2 receptors (Abbracchio & Burnstock, 1994 & Abbracchio et al., 1995 a).

Administration of nifedipine in a dose of 2 (mg/kg/day) intragastrically before and after $CC1_4$-induced liver toxicity, produced a significant improvement of all parameters. These findings are in accordance with Bellomo & Orrenius, (1985), they reported that interference with Ca^{2+} homeostasis and increased levels of cytoplasmic free Ca^{2+} particpate 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 triphosphatase, which serves as membrane bound Ca^{2+} pumps to extrude the ion & So maintain cytoplasmic Ca^{2+} at low lev-

els (Bellomo et al., 1983). Furthermore, it has been shown that the microsomal Ca^{2+} sequestering system in cells such as hepatocytes are sensitive to oxidative stress (Jones et al., 1983). A sustained increase in cytosolic Ca^{2+} may mediate its adverse effects on cellular viability via activation of endonucleases, proteases and phospholipases (Siesjo, 1989), as well as enhanced production and accumulation of free radicals such as superoxide, hydrogen peroxide and hydroxyl radicals (Buja et al., 1988). This can lead to a chain of reactions involving lipid peroxides and hydroperoxide eventually resulting in membrane damage and alterations of membrane-bound protein function including the Ca^{2+} AT -pase.

From this study, it could be concluded that dipyridamole is as effective as nifedipine in protection against $CC1_4$-induced hepatotoxicity. Both of them exerts a free rdical scavenging activity and a lowering effect on intrahepatic calcium levels as a part of their cytoprotective effect.

Summary

The present work was conducted to evaluate the possible in vivo effect of dipyridamole and nifedipine on free oxygen radicals and intrahepatic calcium levels in Carbon-tetrachloride-induced hepatotoxicity.

40 male-albino rats were used and divided into 4 equal groups. The first group consisted of normal rats, received intragastric saline (0.5 ml) for 4 weeks. The second group received $CC1_4$ in a single dose (0.15ml) intragastrically and served as a hepatotoxicity control. The third group, received nifedipine in a dose of (2mg/kg/day) intragastrically, 3 weeks before and one week after administration of $CC1_4$. The fourth group, received dipyridamole in a dose of (2.25mg/kg/day) intragastrically for the previous period.

It was found that administration of $CC1_4$ to rats produced a significant hepatotoxicity as assessed by the increase of SGPT & SGOT. Furthermore these rats showed a significant increase in malondialdehyde level (MDA) and intrahepatic calcium. Administration of either nifedipine or dipyridamole produced a significant decrease in these parameters. These results suggest that dipyridamole and nidedipine have a hepatocytoprotective effect. This effect may be due to free radical scavenging effect and

ability to decrease intrahepatic calciurn levels. Further studies of these results on hepatic patients are recommended especially in patient given nifedipine and dipyridarnole for associated cardio-vascular problems.

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التأثير الوقائى المحتمل لكل من الديبير يداموك والنيفديبين على السُمية الكبدية المحدثه معملياً برباعى كلوريد الكربون

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أجرى هذا البحث لدراسه إحتمال وجود تأثير وقائى لكل من عقارى الديبيردامول والنيفيديبين على السُميه الكبديه المحدثه فى الفئران بواسط رباعى كلوريد الكربون ، وذلك من خلال تقليل الشقوق الحره ومستوى الكالسيوم فى الكبد .

إستُخدم فى إجراء هذا البحث عدد (٤٠) فأراً أبيضاً وقسمت إلى ٤ مجموعات، كل مجموعة تتكون من ١٠ فئران كالتالى :-

المجموعه الأولى : لم يحُدثْ بها سُمية كبدية وأعطيت محلول ملح بنفس الكمية المستخدمة لإذابة الدواء ولمدة ٤ أسابيع. (مجموعة ضابطة عادية)

المجموعة الثانيه : عباره عن فئران مصابه بسُميه كبديه محدثه بواسطة رباعى كلوريد الكربون بجرعة ، ١٥ر. مجم/كجم عن طريق المعدة .(مجموعة ضابطة مصابة بسُمية كبدية).

المجموعة الثالثة: أحدث بها سُمية بعد ٣ أسابيع من إعطائها عقار النيفيديبين وأسبوع بعد إحداث تلك السُمية . وذلك بجرعة ٢ مجم/كجم بواسطة أنبوبه معدية .

المجموعة الرابعة : أحدث بها سُمية كبدية بعد ٣ أسابيع من إعطائها عقار الديبيريدامول ولمدة أسبوع آخر بعد إحداث تلك السُمية . وذلك بجرعة ٢٥ر٢ مجم/كجم عن طريق المعدة .

وتم تقييم إحداث السُمية الكبدية بقياس معدل إنزيمات الترانزأميناز وأيضاً قياس كل من الشقوق الحرة فى السيرم ومعدل الكالسيوم داخل الكبد .

وعلى ضوء هذه الدراسة يمكن إستخلاص أن كل من دوائى النيفيديبين والديبيريدامول لهما تأثير

متقارب على الحماية من السُمية الكبدية عن طريق إنقاص الشقوق الحرة فى المصل وكذلك معدل الكالسيوم فى الكبد، ونوصى بدراسة هذا الأثر فى مرضى الكبد وخصوصاً فى المرضى الذين يتناولون هذه الأدوية لأغراض أخرى مثل أمراض القلب وضغط الدم المرتفع .