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 extracellular Ca$^{2+}$. In contrast, little attention has been directed toward the early alterations in intracellular Ca$^{2+}$ ho-
meostasis caused by hepatotoxins.

Carbon tetrachloride (CC14) is mostly metabolised in the liver. It is metabolically activated by cytochrome P450 to a free radical trichloromethyl radical. The mechanism by which this free radical produces damage remain controversial (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 Ca2+ changes caused by (CC14) liver injury in rats. Furthermore, this study is a trial to declare the relation between Ca2+ homeostasis, free oxygen radical and adenosine pathways in relation to hepatotoxicity-induced experimentally with CC14.

Vol. 30, No. 1 & 2 Jan. & April, 2000

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 CCl4 in a single dose of 0.15 ml/kg orally (Melsisi et al., 1993) and served as a hepatic injured 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 CC14 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 spectrophotometrically 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 (3 weeks before CC14 & one week after CC14-induced hepatotoxicity), 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 (CCL4) (M±SE).

<table>
<thead>
<tr>
<th>Group</th>
<th>SGPT</th>
<th>SGOT</th>
<th>Malondialdehyde (MDA) n.mol/L</th>
<th>Intrahepatic calcium (mg/gm. Liver tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>11.33±1.5</td>
<td>30.3±0.7</td>
<td>1.167±0.117</td>
<td>0.071±0.005</td>
</tr>
<tr>
<td>Carbon tetra chloride induced liver injury</td>
<td>22 ± 1.2</td>
<td>50±1.4</td>
<td>2.997±0.062 P&lt;0.001</td>
<td>0.969±0.019 P&lt;0.001</td>
</tr>
<tr>
<td>0.15 ml/kg once intra gastrically.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Group</th>
<th>SGPT</th>
<th>SGOT</th>
<th>(MDA) n.mol/L</th>
<th>Intrahepatic Ca^{2+} (mg/gm. Liver tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC14 induced liver injury.15 ml/kg. single dose</td>
<td>22±1.2</td>
<td>50±1.4</td>
<td>2.997±0.062</td>
<td>0.969±0.019</td>
</tr>
<tr>
<td>Dipyridamole treated (2.25 mg/kg orally for 4 weeks)</td>
<td>11.3±1.8</td>
<td>31.1±0.75</td>
<td>0.98±0.052 P&lt;0.001</td>
<td>0.36±0.043 P&lt;0.001</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>SGPT</th>
<th>SGOT</th>
<th>(MDA) n.mol/L</th>
<th>Intrahepatic Ca^{2+} (mg/gm. Liver tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC14 induced liver injury</td>
<td>22±1.2</td>
<td>50±1.4</td>
<td>2.997±0.062</td>
<td>0.969±0.19</td>
</tr>
<tr>
<td>Nifedipine treated (for 4 weeks, 2 mg/kg, orally)</td>
<td>12±1.2</td>
<td>31.3±0.58</td>
<td>0.9±0.08</td>
<td>0.15±0.009</td>
</tr>
</tbody>
</table>

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

Vol. 30, No. 1 & 2 Jan. & April, 2000
DISCUSSION

In the present study CC14 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 CCL4 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 & Gandolfi, 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 CC14 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 (P1 & 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 P2Y1 & P2 Y2 receptors. These receptors typically have a common pathway in phospholipase (PLC). In addition, activation of purines receptors inhibit ATP-induced Ca\(^{2+}\) influx via P2 receptors (Abbracchio & Burnstock, 1994 & Abbracchio et al., 1995 a).

Administration of nifedipine in a dose of 2 (mg/kg/day) intragastrically before and after CC14-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\(^{2+}\) 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 triphosphatase, which serves as membrane bound Ca\(^{2+}\) pumps to extrude the ion & So maintain cytoplasmic Ca\(^{2+}\) at low lev-
EFFECTS OF DIPYRIDAMOLE AND NIFEDIPINE etc...

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 CC14-induced hepatotoxicity. Both of them exerts a free radical 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 CC14 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 CC14. The fourth group, received dipyridamole in a dose of (2.25mg/kg/day) intragastrically for the previous period.

It was found that administration of CC14 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 nifedipine have a hepatocytoprotective effect. This effect may be due to free radical scavenging effect and
ability to decrease intrahepatic calcium levels. Further studies of these results on hepatic patients are recommended especially in patient given nifedipine and dipyridamole for associated cardio-vascular problems.

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التأثير الواقعي المحتمل لكل من الديبيريدامول والنيفيدبين على السمية الكبدية المحدثة عملياً برابعي كلوريد الكربون

د. سوية عبد اللطيف مقبل

مدرسة الفارماكولوجيا الإكلينيكية - طب المنصورة

أجري هذا البحث لدراسة إحتمال وجود تأثير وفاثي لكل من عقاري الديبيريدامول والنيفيدبين على السمية الكبدية المحدثة في الفئران بواسطة راباعي كلوريد الكربون، وذلك من خلال تقليل الشقوق الحر، ومستوى الكالسيوم في الكبد.

إستخدم في إجراء هذا البحث عدد (40) فأراً أيضاً وقسمت إلى 4 مجموعات، كل مجموعة تتكون من 10 فئران كالالتالي:

المجموعة الأولى: لم يحدث بها سمية كبدية وأعطيت محلول ملح بنفس الكمية المستخدمة لإذابة الدواء لفترة 4 أسابيع. (مجموعة ضابطة عادية)

المجموعة الثانية: عبارت عن فئران مصابه سمية كبدية محدثه برابع كلوريد الكربون بجرعة 15 مجم/كم من طريقة المعدة. (مجموعة ضابطة مصابة سمية كبدية)

المجموعة الثالثة: أحدث بها سمية بعد 3 أسابيع من إعطاءها عقار النيفيدبين وأسبوع بعد إحداث تلك السمية. وذلك بجرعة 2 مجم/كم بواسطة أنيبوب معدية.

المجموعة الرابعة: أحدث بها سمية كبدية بعد 3 أسابيع من إعطاءها عقار الديبيريدامول ولمدة أسبوع آخر بعد إحداث تلك السمية. وذلك بجرعة 25 مجم/كم عن طريق المعدة.

وتم تقييم إحداث السمية الكبدية بقياس معدل إنزيمات الفرانز أميناز وأيضاً قياس كل من الشقوق الحررة في السيرير ومعدل الكالسيوم داخل الكبد.

وعلى ضوء هذه الدراسة يمكن استخلاص أن كل من دوائي النيفيدبين والديبيريدامول لهما تأثير

Vol. 30, No. 1 & 2 Jan. & April, 2000
محذرة على الحماية من السمية الكبدية عن طريق إنقاص الشعور الحرة في المصل وكذلك معدل الكالسيوم في الكبد، ونوصي بدراسة هذا الأمر في مرضى الكبد وخصوصاً في المرضى الذين يتناولون هذه الأدوية لأغراض أخرى مثل أمراض القلب وضغط الدم المرتفع.