The Protective Effect of Diltiazem Against Carbon Tetrachloride and Thioacetamide Induced Liver Injury

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Özet: *DİLTİAZEM'İN KARBONTETRAKLORİD VE THIOACETAMİDE İLE KARACİĞERDE OLUŞTURULAN HASARA KARŞI KORUYUCU ETKİSİ*

Bir kalsiyum kanal blokeri olan diltiozemin karbontetraklorid (CCl_4) ve thioacetamide ile oluşturulan karaciğer hasarına karşı koruyucu etkisi araştırıldı. CCl_4, 1 ml/kg İ.P. verildi. 24 saat sonunda ciddi karaciğer hasarı oluşturuldu. SGOT, SGPT ve ALP düzeyleri sırası ile 16, 40 ve 8 kat arttı. Thioacetamide, 8,6 m mol/kg İ.P. verildi. SGOT, SGPT ve alp düzeyleri 6, 8 ve 5 kat arttı. Diltiazem'in (30 mgr/kg İ.P.) CCl_4 ve thioacetamide'den 1 saat önce ve 7 saat sonra verilmesi ile SGOT, SGPT ve ALP düzeylerinde anlamlı düşüş görüldü. Serum enzimlerindeki düşüşler, histopatolojik olarak diltiazem tedavisiyle hepatosellüler nekrozdaki azalmanın gösterilmesi ile desteklendi.

Anahtar kelimeler: Karbon tetraklorid, diltiazem, karaciğer hasarı, thiouasetamid

Summary: *The protective effect of diltiazem, a calcium channel blocker, against carbon tetrachloride and thioacetamide- induced liver injury in rats was investigated. Carbon tetrachloride given at 1 ml/kg, i.P. resulted in severe liver injury at 24 hr in rats, as judged by the up to sixteen, forty and eight-fold elevations in liver enzymes such as SGOT, SGPT and ALP respectively. Thiocet amide given at 8.6 mmoles/kg, i.P., 24 hr later again elevated liver enzymes SGOT, SGPT and ALP six, eight and five-fold respectively, in plasma, Diltiazem (30 mg/kg,i.p.) administered l hr blefore and 7 hr after carbon tetrachlordide or thiocetamide significantly lowered serum transaminase and alkaline phosphatase levels. A reduction in serum enzymes was supported by histological findings of reduced hepatocellular necrosis in diltiazem treated rats.*

Key words: Carbon tetrachloride, diltiazem, liver injury, thioacetamide

Calcium channel blockers are now established agents employed in a number of disorders including angina in its several forms, hypertension, peripheral vascular disorders and some types of cardiac arrhytmias. However there are many other applications for which these agents have been used (1). Much is known about the beneficial effects of these drugs in preventing the cellular damage associated with calcium overload.

Many cellular processes such as DNA transcription and replication, cytoskeletal regulation, alteration in phospholipid and protein turnover and regulation of enzyme-dependent pathways are regulated by changes in free cytosolic calcium (2). Recent investigations have examined the potential role of disrupted Ca^{++} fluxes in chemically induced liver injury and it has been demonstrated that cell viability depends on extracellular calcium levels (3,4). Disturbances in intracellular homeostasis of calcium might lead to hepatocyte injury and death (5,6,7,8).

Raised intracellular calsium levels have been shown to potentiate cell damage by oxygen-free radicals (8,9), decrease mitochondrial ATP synthesis, activate Ca^{++}- ATP ase and as a result of depletion in energy reserves the cell dies (2). Administration of CCl_4 or thioacetamide to the rat is associated with large increases in the calcium content of the rat liver (10). The rise in liver cell calcium content may contribute to the cell injury associated with toxic agents. CCl_4

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Short title: Diltiazem-carbon tetrachloride and thioacetamide induced liver injury.

Table I: Treatment Groups.

Group No	8:00	Hours 9:00	16:00
I	Vehicle	Vehicle	Vehicle
II	Vehicle	CCl_4 (1 ml/kg, i.p.)	Vehicle
III	Vehicle	Thioacetamide (8.6 mmoles/kg,i.p.)	Vehicle
IV	Diltiazem (30 mg/kg, i.p.)	Vehicle	Diltiazem (30 mg/kg, i.p.)
V	Diltiazem (30 mg/kg, i.p.)	CCl_4 (1 ml/kg,i.p.)	Diltiazem (30 mg/kg, i.p.)
VI	Diltiazem (30 mg/kg, i.p.)	Thioacetamide (8.6 mmoles/kg, i.p.)	Diltiazem (30 mg/kg, i.p.)

and many other hepatotoxic agents are metabolised by the liver endoplasmic reticulum to reactive intermediates that interact with components of the liver cell and initiate a train of events leading to cell injury and centrilobular necrosis (5,10,11). Calcium channel blocking drugs have been shown to be cytoprotective in liver (12). They may protect against liver injury with a reduction in calcium concentration within the liver.

The aim of this study is to investigate the role of diltiazem in protection against CCl_4 and thioacetamide- induced hepatocellular necrosis in rats and thus to find a future role of Ca channel blockers in the management of hepatocellular injury.

MATERIALS AND METHODS

Male Wistar rats, weighing 250-350 g fasted for 16 hr before experiments, with free access to water were equally distributed within 6 groups (n=6 per group). CCl_4 , 100 % (Merck) or thioacetamide (Sigma) was administered i.p. in a single hepatotoxic dose of 1 ml/kg and 8.6 mmoles/kg. Diltiazem (Mustafa-Nevzat) was given as an i.p. injection at 30 mg/kg rat weight, 1 hr prior to and 7 hr after the toxic agent (Table I).

24 hr after administration fo the hepatotoxic agents, intracardiac blood was taken from all of the rats under light ether anaesthesia and serum transaminase (SGOT,SGPT) and alkaline phosphatase (ALP) levels were measured.

For histologic estimates of cell damage caused by toxic agents, sections of liver were fixed in 10 % formalin, sectioned and stained with haematoxylin and eosin.

Student's t-test was used in evaluating serum enzymes.

RESULTS

CCl_4 given at 1 ml/kg, i.p. resulted in severe liver injury at 24 hr in rats as judged by the up to sixteen, forty and eight-fold elevations in liver enzymes SGOT, SGPT and ALP respectively. We have also given thioacetamide at 8.6

Table II: Serum transaminase and alkaline phosphatase levels. (Values are means ± SE, n= 6).

Groups	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
I Control	155 ± 43	63 ± 8	115 ± 21
II CCl_4	2452 ± 217	2524 ± 82	970 ± 45
III Thioacetamide	903 ± 167	521 ± 86	604 ± 85
IV Diltiazem	128 ± 7	62 ± 16	124 ± 56
V CCl_4 + Diltiazem	1377 ± 40	1414 ± 162	551 ± 47
VI Thioacetamide + Diltiazem	457 ± 128	286 ± 146	348 ± 37

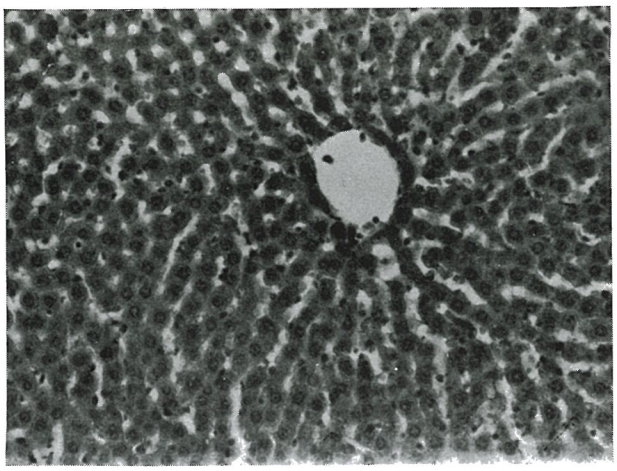

Figure 1: Normal liver tissue from rats treated with either vehicle or diltiazem (X 82, H-E).

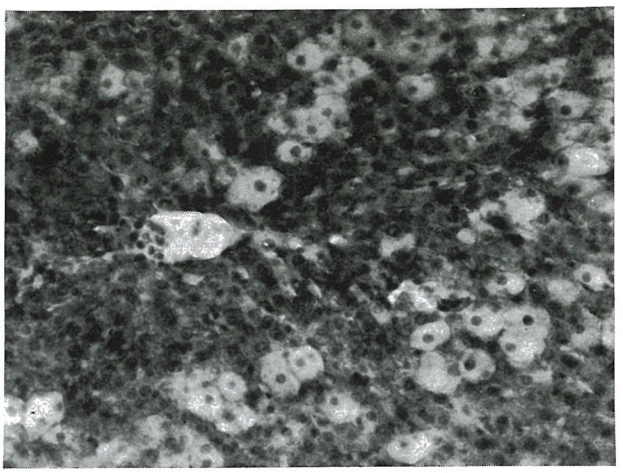

Figure 2: The development of centrilobular necrosis following a single acute dose of carbon tetrachloride. Fatty degeneration, centrizonal necrosis, pignotic changes, vacuoles and "balooning degeneration" is seen (X 82,H-E).

mmoles/kg,i.p. and found 24 hr later liver enzymes SGOT, SGPT and ALP were elevated six,eight and five-fold respectively, in plasma (Table II). These findings point to the presence of acute, toxic hepatic damage in these rats. Diltiazem (30 mg/kg,i.p.) administered prior to and 7 hr after CCl_4 and thioacetamide significantly lowered serum transaminase and alkaline phosphatase leves.

No significant difference was found between the serum transaminase and alkaline phosphatase levels of the control and diltiazem treated group ($p>0.05$) whereas significant differences was found between the control and other groups : (I and II $p< 0.001$; I and III $p< 0.001$; I and V $p< 0.001$; I and VI $p< 0.001$; II and V $p< 0.001$; III and VI $p< 0.001$, $p< 0.05$, $p<0.001$ for SGOT,SGPT and ALP respectively).

Histopathologic structures of the liver were also studied in each group using light microscopy. On microscopic evaluation of these groups, normal liver tissue samples were found in control and diltiazem treated groups (Figure I). The development of centrilobular necrosis following a single acute dose of hepatotoxic agent is presented in Figure 2 and 3. Centrizonal necrosis, fatty changes, pignotic changes, inflammatory cell infiltration, claudy swelling, "balooning degeneration" was determined in CCl_4 and thioacetamide treated groups.

Slight hepatocellular degeneration was observed in the diltiazem + CCl_4 and diltiazem + thioacetamide treated group (Figure 4,5).

DISCUSSION

Calcium channel blockers have been shown to be active on especially L-type voltage-operated calcium channels and thus reduce calcium influx. They exert their cytoprotective effects through several mechanisms such as blockade of the L-type voltage-operated calcium channels (1,13), Reduction of oxidative stress (9), antagonism at inflammatory mediator receptor sites (14,15) and inter action at other intracellular sites.

Uncontrolled cellular calcium influx is a critical factor in the pathogenesis of hepatic ischemic injury (8). Studies relating to the liver suggest that calcium channel blockers may limit hepatocellular damage especially those arising from toxic agents (2).

It has been shown that CCl_4 rapidly and severly inhibits hepatic endoplasmic reticulum calcium sequestration in rats exposed to this hepatotoxin (2,11,16). Moreover a diversity of toxic insults, including thioacetamide, galactosamine, dimethylnitrosamine, heavy metals and ischemia are known to alter tissue Ca^{++} content sig-

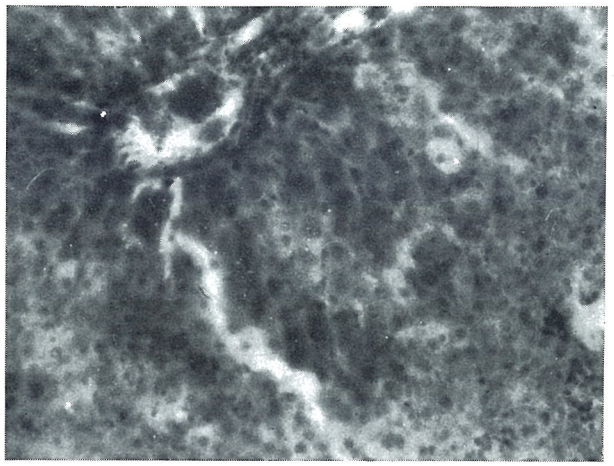

Figure 3: The development of centrilobular necrosis following a single acute dose of thioacetamide. Focel necrosis, sinuzoidal dilatation and mononuclear cell infiltration is seen (X 82, H-E).

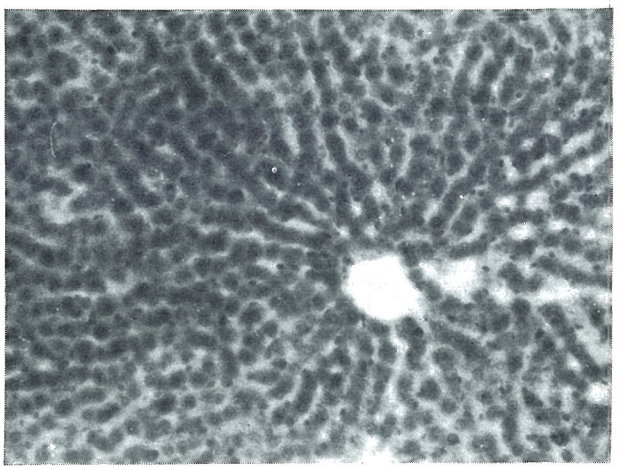

Figure 4: Liver tissue from rats treated with either diltiazem + carbontetrachloride or diltiazem + thioacetamide. Slight hepatocellular degnerative changes are seen (X 82, H-E).

nificantly (17,18,19). As a result cytosolic Ca^{++} concentrations become elevated in liver cells.In studies of isolated cells, calcium was found to be essential for CCl_4 (3), thioacetamide and other toxic agents (4) induced cell death in cultured hepatocytes.

The protective effects of nifedipine and verapamil on CCl_4 , chloroform,dimethylnitrosamine, thioacetamide and paracetamol-induced hepatocellular injury in rats have been studied (10). Nifedipine (25 mg/kg, i.p.) provided almost total protection against dimethylnitrosamine and partial protection against chloroform toxicity whereas almost complete protection against dimethylnitrosamine and some protection against CCl_4 toxicity was provided by verapamil (25 mg/kg,i.p.). In our study diltiazem (30 mg/kg,i.p.) when administered prior to and 7 hr after CCl_4 or thioacetamide, provided partial protection against these toxic agents.

Elevation in liver enzymes point to the presence of acute, toxic hepatic damage (20). Calcium channel blockers reduced significantly liver enzyme levels in rats with acute hepatic damage induced by galactosamine and CCl_4 . Moreover calcium channel blockers can interact with many other intracellular structures such as Na^+ -K+ ATP ase, cAMP phosphodiestrease, protein kinase C and calmodulin (21,22). Diltiazem (30 mg/kg, i.p.) administered prior to and 7 hr after CCl_4 or thioacetamide significantly diminished biochemical markers of liver injury and this was supported by the histological tindings of reduced hepatocellular necrosis.

Since biochemical observations were supported-by histological findings of reduced hepatocellular necrosis in diltiazem treated rats, we suggest that the benzothiapine calcium channel blocker diltiazem might have an utility in the treatment of toxic liver injury.

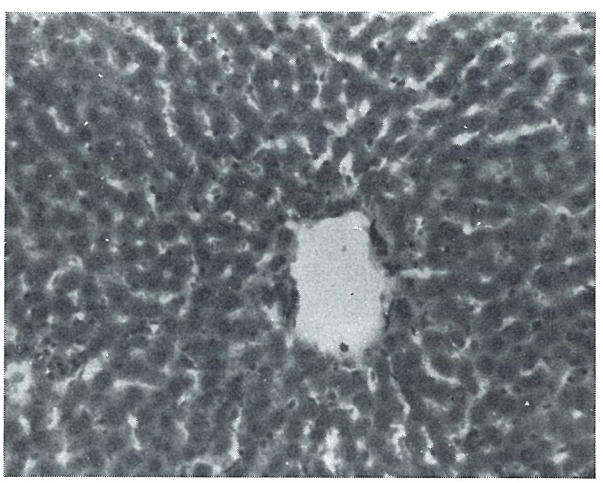

Figure 5: Liver tissue from rats treated with either diltiazem + carbontetrachloride or diltiazem + thioacetamide. Slight hepatocellular degnerative changes are seen (X 82, H-E).

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