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PROTECTION OF CCL_4-INDUCED LIVER DAMAGE IN RATS BY SOME CALCIUM CHANNEL BLOCKERS

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Abstract: Liver necrosis was produced in rats by administering 3 doses o a mixture o carbon tetrachloide + olive oil, 2 ml/kg, ip. The liver damage was evienced by the elevated levels serun aspartate aminotrans ferase (AST), alanine aminotransferase (AIT) and gamma glutamyl transpeptidase (γ-GT) and by histopathological observations of liver sections. Nitrendipine, nimodipine and nisoldipine (1 mg/100 g of rat, ip) significantly reduced these elevated levels of AST, AlT and γ-GT. Carbon tetrachloride induced liver necrosis was also found to be significantly reduced in nitrendipine, nimodipine and nisoldipine pre-treated animals as observed macroscopically and histologically.

Key words: nitrendipine nimodipine nisoldipine liver necrosis carbon tetrachloride

INTRODUCTION

Carbon tetrachloride ($CC1_4$) induces fatty liver and liver cell necrosis (1). Though the precise mechanism is not known, several effects of $CC1_4$ seem to play a role such as inhibition of triacylglycerol release from the liver (1) and increase lipoperoxidation in membranes, whose structural integrity is necessary for lipoprotein release, finally resulting in liver triacylglycerol accumulation and destruction of liver cells. In addition Long (2) demonstrated that cytosolic Ca^{2+} is elevated upto 100 folds in rat hepatocytes exposed to $CC1_4$ which is capable of initiating irreversible liver cell injury. Nitrendipine (NT), nimodipine (NM) and nisoldipine (NS) new dihydropyridine type Ca^{2+} entry blockers exhibit vasodilator, antihypertensive, tissue protective and antiperoxidative properties (3, 4, 5, 6). Zotz and co-workers (7) reported that NT reduces nephrotoxic and hepatotoxic effects of cyclosporin-A in patients after kidney transplantation. However, no study has so far been reported describing effects of NT, NM and NS in $CC1_4$ induced liver damage in rats. Therefore, the present study was carried out in rats to explore if NT, NM and NS would reduce the biochemical and histological changes associated with $CC1_4$ induced liver damage in rats.

METHODS

Male albino rats of Wistar strain (HAU, Hisar, 150-180 g) with free access to standard diet and tap water were used. Animals were divided into 5 groups of 10 each. Group I served as control (which received 3 injections of olive oil, 2 ml/kg, ip as vehicle). In group II, fresh mixture of CCl_4+olive oil (1:1) was given on 1st, 4th and 7th day in doses of 2 ml/kg, ip. The group IInd animals were also administered placebo solvent ip (polyethylene glycol + glycerin + water for injection) for 10 consecutive days. In groups III, IV and V, NT, NM and NS (1 mg/100 g of rat) respectively were administered ip. In addition to $CC1_4$. A 0.1% stock solution of NT, NM and NS was prepared using the placebo solvent of the following composition, 969 g Polyethylene glycol 400 + 60 g glycerin + 100 g water for injection. These drugs were protected from light during weighing and handling because

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they are light sensitive. NT, NM and NS were given once daily, beginning one day prior to the experiments and continued for 10 consecutive days. On 10th day blood was withdrawn directly from the heart without using anaesthesia and serum was separated by centrifugation for biochemical studies. Whole livers were removed after sacrificing the animals and preserved in 10% formal saline. By the standard technique serial sections (5 mm) were cut and stained with haemotoxyline and eosine. Aspartate amino transferase (AST; EC 2.6.1.2) and alanine amino transferase (ALT; EC 2.6.1.2.) levels in serum of different groups were assayed (8). Gamma glutamyl transpeptidase (γ-GT; EC: 2.3.2.2) activity in serum was determined (9).

RESULTS

Gross examination of rat liver from control group showed normal appearance, red colour, smooth and regular under surface without any evidence of haemorrhage and necrosis, while CCl_4 treated livers showed multiple area of necrosis without massive haemorrhagic patches. Large part of liver was covered with white slough and there were multiple white patches indicating necrotic areas. Liver from CCl_4 treated rats showed characteristic nut meg appearance. The under surfaces of 80% of the livers were irregular/nodular. Livers from NT, NM and NS treated groups were almost normal in appearance regarding colour and under surfaces except slight congestion. Livers from NS treated groups showed minimum congested areas as compared to NT and NS treated groups.

Histology of liver from control group showed portal triad, rows of hepatocytes or normal arrangements of hepatocytes with nuclei, while CCl_4 treated liver sections showed intense centrilobular necrosis, sinusoidal haemorrhagic congestion and extensive fatty changes. Hepatocytes in centrilobular zone were enlarged and contained lipids. Hepatocytes in periportal zone were also enlarged and the normal architectural pattern was destroyed with severe vacuolization of surviving periportal hepatocytes (Fig. 1a). Histology of liver sections of NT or NM + CCl_4 treated rats revealed few areas of congestion, spoty necrosis with minimum fatty changes (Fig. 1b and 1c). In NS + CCl_4 treated group the congestion was much less in liver sections as compared to NT or NM + CCl_4 treated groups (Fig. 1d).

Administration of CCl_4 to rats produced a significant elevation of serum AST, ALT and γ-GT levels as compared to control (Table I). There was a significant ($P<0.01$) reduction in serum AST, ALT and r-GT levels in rats treated with NT+CCl_4, NM + CCl_4 and NS + CCl_4 as compared to animals treated with CCL_4 alone.

TABLE 1 : Effect of nitrendipine (NT), nimodipine (NM) and nisoldipine (NS) 1 mg/100 g of rat, i.p. on serum aminotransferase and gamma glutamyl transpeptidase (γ-GT) levels in rats with CCl_4 induced liver damage. (Value are mean ± SE of 10 animals in each group).

Group	*Liver weight (gm) (% of body weight)*	*AST IU/L*	*ALT IU/L*	*γ-GT U/ml*
Control	4.29 ± 0.11	18.10 ± 0.17	15.70 ± 0.81	33.65 ± 0.46
CCL_4	3.68 ± 0.13	49.60 ± 0.10	56.00 ± 0.91	64.75 ± 0.30
CCL_4 + NT	4.47 ± 0.14*	27.20 ± 0.28*	26.30 ± 0.20*	42.45 ± 0.53*
CCL_4 + NM	4.45 ± 0.04*	26.70 ± 0.39*	25.15 ± 0.22*	43.05 ± 0.35*
CCL_4 + NS	4.33 ± 0.06*	24.10 ± 0.31*	22.30 ± 0.15*	37.83 ± 0.23*

*$P < 0.01$ when compared with CCl_4 treated group (unpaired "t" test).

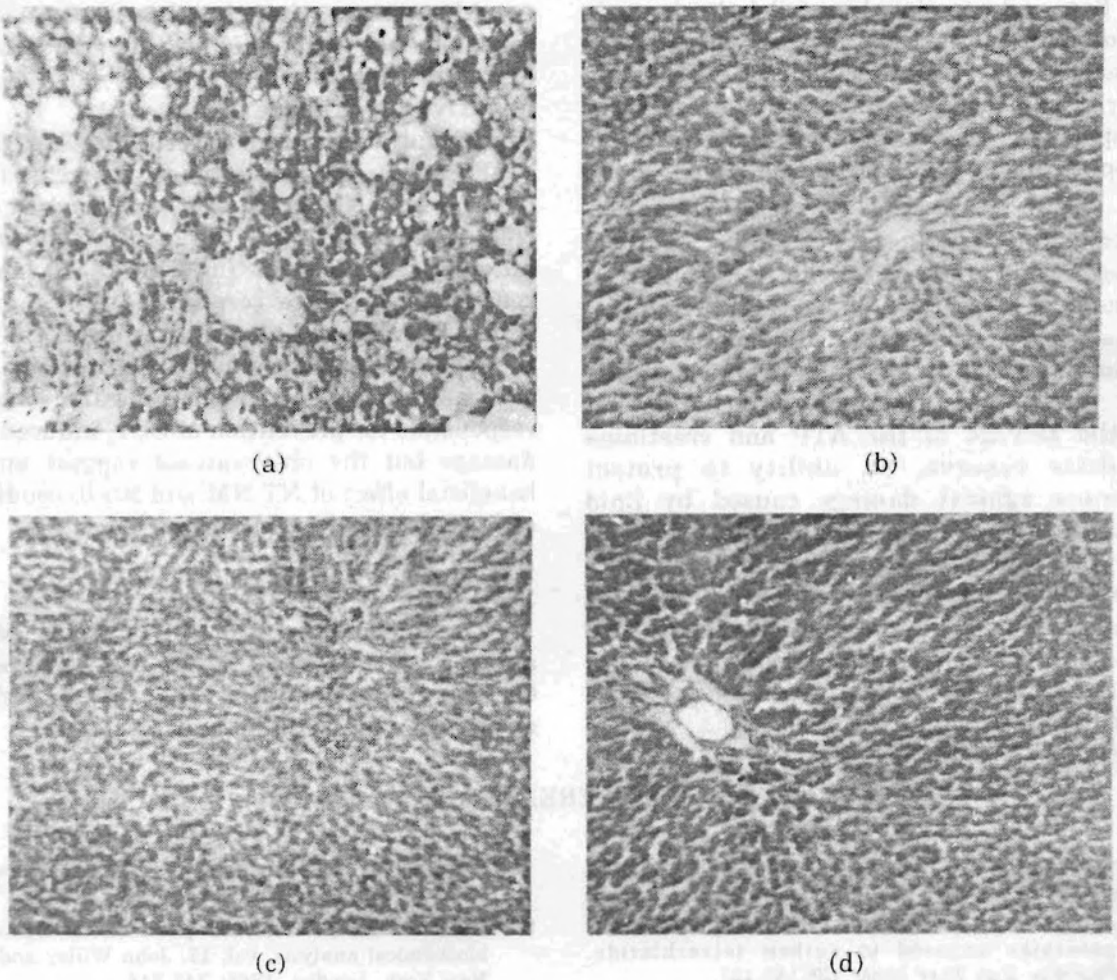

Fig. 1a : Liver section of CCl_4 treated rat showing intense centrilobular necrosis, haemorrhage (↑) and extensive fatty change (low power: 10 X 10).
Fig. 1b : Liver section from nitrendipine + CCl_4 treated rat showing spotty necrosis with fatty changes.
Fig. 1c : Liver section from nimodipine + CCl_4 treated rat showing slight fatty changes.
Fig. 1d : Liver section from nisoldipine + CCl_4 treated rat showing minimum fatty change.

DISCUSSION

The histological and biochemical changes indicate that NT, NM and NS protects liver from CCl_4 induced damage. This may be attributed to alteration in extracellular and intracellular Ca^{2+} concentration, general vasodilator action or to antilipoperoxidative properties of calcium channel blockers. NT, NM and NS are well known selective Ca^{2+} influx blockers in the myocardium, vascular smooth muscles and various parts of the brain (3, 10). It is possible that NT, NM & NS inhibit Ca^{2+} influx and modulate intracellular calcium which helps help in preventing ca^{2+} accumulation in liver cells, since it was demonstrated that cytosolic Ca^{2+} is elevated 100 folds in rat hepatocytes exposed to CCl_4 which is capable in initiating irreversible liver cell injury (2).

General vasodilator effect of these calcium antagonists improve hepatic blood flow which may be useful in preventing CCl_4 induced centrilobular hypoxia, since reduced hepatic

blood flow and associated centrilobular hypoxia account for the centrilobular necrosis in CCl_4 poisoning. Zotz (7) showed that NT reduces the nephrotoxic and hepatotoxic effects in cyclosporin-A treated patients after kidney transplantation by increasing liver blood flow.

Mak and Weglick (11) have reported antiperoxidant effect of calcium antagonists in sarcolemmal membrane. Nayler and Britnell (4) have suggested that Ca^{2+} antagonists provide cellular protection by an unknown mechanism. Many factors may be involved including not only the salvage of the ATP and creatinine phosphate reserve, an ability to protect membrane against damage caused by lipid peroxidation may be major contributory factor. If the antilipoperoxidative effect of Ca^{2+} blockers occurs in liver cells also, it may be beneficial for preventing CCl_4 induced liver damage, since increased lipoperoxidation in membrane is also decisive pathogenic factor in etiology of CCl_4 induced liver damage.

Alterations in the levels of serum tansaminases AST, ALT r-GT may has the highest sensitivity as well as positive and negative predictive values (12, 13, 14). Significantly low levels of serum AST, ALT and γ-GT levels in rats simultaneously treated with CCl_4 + NT, NM or NS as compared to CCl_4 treated alone indicates the involvement of Ca^{2+} antagonists in some important biochemical reactions which are responsible for protection from CCl_4 induced liver damage in rats. From the results of present study it is difficult to infer the exact molecular and biochemical mechanism responsible for prevention of CCl_4 induced liver damage but the observations suggest marked beneficial effect of NT NM and NS in conditions of liver damage produced by CCl_4.

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