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Hepatoprotective effects of diethylcarbamazine in acute liver damage induced by carbon tetrachloride in rats

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ABSTRACT This research was carried out to determine a potential role of leukotrienes in the acute hepatotoxicity induced by CCl_4 in rats. An inhibitor of leukotrienes biosynthesis, diethylcarbamazine (DEC, 25 and 50 mg $\cdot kg^{-1}$ ip) exerted hepatoprotective effects, decreasing the activity of alanine aminotransferase in serum and the concentration of liver triglycerides. DEC reduced histological damage of liver evidenced by electron microscopy. The hepatoprotective effects of DEC were dose-dependent. The results favor the role of leukotrienes in CCl_4 hepatotoxicity.

KEY WORDS diethylcarbamazine; liver; carbon tetrachloride poisoning; alanine aminotransferase; triglycerides

Leukotrienes belong to a group of compounds termed "eicosanoids"[1] and play a particularly important role in the induction and pathophysiology of liver injury[2,3]. Diethylcarbamazine (DEC) is a potent antifilarial drug in man[4] and inhibits leukotrienes biosynthesis, preventing the conversion of 5-HPETE to leukotriene A_4[5]. Therefore, we decided to test whether DEC is able to prevent acute liver damage induced by carbon tetrachloride (CCl_4) in rats in order to study the role of leukotrienes in the CCl_4 - induced hepatotoxicity.

MATERIALS AND METHODS

CCl_4-induced acute liver injury Sprague-Dawley rats, ♂ (235±*s* 15 g) were used in these experiments. Food was withdrawn 12 h before administration of CCl_4, but water was supplied *ad lib*. DEC (10, 25, and 50 mg $\cdot kg^{-1}$) was dissolved in water and injected ip 1 ml $\cdot kg^{-1}$, 30 min before ip CCl_4 1 ml $\cdot kg^{-1}$ of a suspension (20 % v/v) in olive oil[6]. The blood was obtained from the tail for enzymic determinations. Rats were decapitated and the livers were taken for determination of triglycerides and for ultrastructural studies.

Alanine aminotransferase (ALT) in serum A colorimetric test was used[7] with Kits of Hoffmann-La Roche (Switzerland).

Triglycerides (TG) concentration in liver Liver triglycerides were extracted in methanol-chloroform and determined by a colorimetric procedure[8] at 439 nm. Tripalmitine was used as standard.

Electron microscopy Liver was fixed in 3.2 % glutaraldehyde prepared in phosphate buffer 0.1 mol $\cdot L^{-1}$. After 1 h, the tissue was postfixed in 2 % osmium tetroxide for 1 h and finally embedded in a polymer resin (Spurr). Ultrathin sections were prepared on copper 400 mesh grids, stained with saturated uranyl acetate and Reynold's lead citrate[9], and examined using a JEM 100 S electron microscope.

Statistical analysis Groups were compared using a one-way ANOVA with completely randomized design and a Duncan's multiple comparison test.

Drugs and chemicals Diethylcarbamazine citrate was obtained from Wellcome (UK). All other reagents were of AR.

RESULTS

DEC 25 and 50 mg $\cdot kg^{-1}$ ip reduced ALT activity in rat serum and liver TG in rats poisoned with CCl_4 (Tab 1).

Liver showed swelling of hepatocyte membranes system (plasma membrane,

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Tab 1. Effects of diethylcarbamazine (DEC) on ALT activity in rat serum and liver triglycerides. $n=6$, $\bar{x}\pm s$. $^{a}P>0.05$, $^{b}P<0.05$, $^{c}P<0.01$ *vs* CCl_4 group.

CCl_4/ ml·kg^{-1}	DEC/ mg·kg^{-1}	ALT activity/ U·L^{-1}	Triglycerides/ mg·g^{-1}
0	0	12.1±1.54	6.2±0.5
1	0	102.4±0.58	25.2±0.42
1	10	94.0±3.84[a]	22.1±1.60[a]
1	25	70.1±6.24[b]	12.3±0.5[c]
1	50	62.0±5.39[b]	9.8±1.61[c]
0	50	11.5±1.80	8.3±1.81

endoplasmic reticulum and nuclear envelope) in rats poisoned with CCl_4. Dilation of endoplasmic reticulum and nuclear envelope are observed. Hepatoprotective effect of DEC (25 and 50 mg·kg^{-1} ip) was found in CCl_4-induced liver damage. The hepatocyte organelles, mainly endoplasmic reticulum, mitochondria, Golgi apparatus, and hepatocyte membrane system appeared to be well preserved. Hepatocyte membrane system is well preserved. Normal mitochondria are also observed (Fig 1, Plate 4).

DISCUSSION

DEC exerts hepatoprotective effects on CCl_4-induced liver damage in rats. There is fragmentary evidence for a possible role of arachidonic acid (AA) products during the development of liver injury. Formation of $P_GF_{2\alpha}$ and PGE_2 by liver microsomes was increased from CCl_4-cirrhotic rats[10]. Prostacyclins ip reduced the acute liver damage induced by CCl_4[11]. Leukotrienes are also products of AA peroxidation. BW755C, a dual cyclooxygenase and lipoxygenase inhibitor, reduces apparent indicators of liver cirrhosis in rats induced by CCl_4[12], and was more effective than indometacin in decreasing liver damage. These results gave evidence of leukotrienes role in CCl_4-induced liver injury[12].

However, BW755C inhibits lipid peroxidation as an antioxidant agent[13]. Antioxidant compounds such as alpha tocopherol, silymarin, cianidanol, diethyldithiocarbamate and many others are potent inhibitors of CCl_4-induced liver damage, which is mainly dependent of lipid peroxidation[14].

The use of BW755C to evaluate the role of leukotrienes in models of inflammation is misleading due to antioxidative properties (eg, scavenging effect against oxygen radicals) and should be discontinued[13].

Using chemiluminescence methods we have found that DEC is lacking of antioxidative effects[15]. It provides evidence of the role of leukotrienes in CCl_4 - induced liver damage in rats.

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nociceptive effect of intracerebroventricular injection tetrapeptide Asn-Ala-Gly-Ala in rats

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RACT The antinociceptive effect of intracerebroventricular injection (icv) of Asn-ly-Ala (NAGA), a partial sequence of ropin, was studied in rats. The potasontophoresis-induced tail flick was used isure the pain threshold. The antinoci- effect of NAGA, which was dosedent (icv, 0.03 — 0.24 μmol/rat) and sting (90 min), was reversed by ne (icv, 0.26 mg·kg^{-1}) and inhibited i-MEK serum (titre: 1:5000, 5 μl) -LEK serum (titre: 1:5000, 5 μl). -induced antinociception was scarcely d by anti-β-EP serum (titre: 1:30 000, or anti - Dyn A_{1-13} serum (titre: 0, 5 μl). It was suggested that the iceptive effect of NAGA may be associated with the release of met-enkephalin and leu-enkephalin in rat brain.

KEY WORDS analgesia; naloxone; endorphins; immune sera; neuropeptides

The tetrapeptide Asn-Ala-Gly-Ala (NAGA), a sequence of human β-lipotropin$_{14-17}$[1], was isolated from human brain[2]. Intracisternal injection of NAGA to mice produced dose-dependent, long-lasting, and naloxone-reversible antinociceptive effect as evaluted by the hot plate and tail flick methods[3], and the tail-pressure and phenylbenzoquinone-induced writhing tests[4] in mice. In the present study, we report the correlation between NAGA antinociceptive effect and endogenous opioid peptides in rats.

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