Biochemical Pharmacology, Vol. 34, No. 3, pp. 415–416, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

INFLUENCE OF CIMETIDINE ON CCl_4-INDUCED LIVER INJURY AND SURVIVAL IN RATS

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Liver injury produced by CCl_4 depends on a toxic agent which has to be metabolized by the liver NADPH-cytochrome P_{450} enzyme system to a highly reactive intermediate. It has been suggested that this toxic intermediate is the trichlormethyl radical ($CCl_3^{\bullet}$) producing maximum damage to the liver. This radical can covalently bind to neighbouring components in the endoplasmic reticulum and may also initiate lipid peroxidation (1,2). The histamine H_2-receptor antagonist cimetidine, widely used in the therapy of peptic ulcer disease, has been reported to impair cytochrome P_{450} dependent drug metabolism(3). Our investigations were done to examine whether cimetidine may reduce liver damage produced by carbon tetrachloride and improve survival in CCl_4 treated rats.

MATERIAL AND METHODS

Four groups of fed Sprague-Dawley rats weighing 230-250g(Thomae,Biberach/Riß,F.R.G.) recieved an intraperitoneal injection of 40mg/kg cimetidine(commercially available ampules from Smith-Kline-Dauelsberg,3400 Göttingen,F.R.G.): 150min. before injection of 2,5ml CCl_4/kg(group A), simultaneously with injection(group B), 150min after injection(group C) and as controls,2,5ml olive oil was used(group D). CCl_4 and olive oil were given through a stomach tube. The animals of group E received only 2,5ml CCl_4/kg and those of group F only an aliquot of olive oil. All animals were killed 24 hours later in ether anesthesia and blood samples were taken by cardiac puncture. In serum, enzymatic activity was measured photometrically using a standardized kit (Boehringer,6800 Mannheim,F.R.G.). Histological examination of liver tissue were performed(HE and PAS staining) on about 50% of randomized animals. Student's t-test was used for statistical evaluation, means given as $\bar{x} \pm$ s.d. In a second series, survival of rats was investigated, when controls received 5ml CCl_4/kg and the two other groups were treated additionally with 40mg/kg or 120mg/kg cimetidine simultaneously.

RESULTS AND DISCUSSION

When aspartate aminotransferase is measured in serum after application of 2,5ml CCl_4/kg 24 hours later, the enzymatic activity has risen to high values(1894 ± 1610 U/L). An additional injection of 40mg/kg cimetidine reduces significantly the release of this enzyme into serum(Table 1).

TABLE 1: Effect of a single dose of CCl_4 and/or cimetidine(CIM) on serum activities of aspartate aminotransferase(ASAT)

group	treatment	number of animals	ASAT (U/L)	stat.eval.
A	CIM + 150min later CCl_4	21	445 ± 354	$p < 0.0005$
B	CCl_4 + CIM simultaneously	9	338 ± 116	$p < 0.005$
C	CCl_4 + 150min later CIM	7	357 ± 211	$p < 0,01$
D	CIM + olive oil	11	106 ± 24	$p < 0,005$
E	CCl_4	18	1894 ±1610	
F	olive oil	11	84 ± 18	$p < 0,0005$

The animals received 2,5ml CCl_4/kg by stomach tube or an aliquot of olive oil. CIM was injected intraperitoneally in a dose of 40mg/kg. Statistical evaluation(stat. eval.) was performed against group E.

Light microscopy of CCl_4 injured livers shows the typical changes(numerous pericentral necroses, single cell necroses, ballooned hepatozytes and liver cells with the

signs of fatty degeneration). The additional application of 40mg/kg cimetidine histologically reveals a disappearance of cohesive focal necroses, few scattered single cell necroses, but obviously more ballooned cells and cells with fatty degeneration(Table 2).

TABLE 2: Semiquantitative determination of liver damage due to CCL_4 modified by administration of cimetidine(CIM).

treatment		CCL_4	CIM 150min before CCL_4	CIM + CCL_4 simultaneous	CCL_4 150min before CIM
irreversible damage (necrobiosis)	Ø		2		
	(+)		5	2	1
	+	4	7	3	3
	++	6	2		1
reversible damages: fatty changes ballonization loss of glycogen etc.	Ø		1		
	(+)	2	4		
	+	7	4	1	
	++	1	7	4	5
number of animals		10	16	5	5

In regard to irreversible changes,the sign"Ø" means absence of any necroses, the sign"(+)" means scarcely single cell necroses, the sign"+" a moderate and the sign "++" a high amount of single cell necroses and/or cohesive focal necroses of the parenchyme. In regard to reversible changes, the sign "Ø" means absence of any sign of cellular alteration, the sign"(+)" an alteration up to 10%, the sign"+" an alteration of 10 to 40% and the sign"++" an alteration of more than 40% of the parenchyme

When the dosage of CCL_4 was augmented to 5ml/kg, all animals died. A simultaneous injection of 40mg/kg cimetidine had no effect on survival, whereas 5 of 9 rats,given 120mg/kg,survived(Table 3).

TABLE 3: Survival of rats, treated with 5ml CCL_4/kg and various doses of cimetidine (CIM) simultaneously

treatment	number of animals	survivors
CCL_4	9	0
CCL_4 + 40mg/kg CIM	9	0
CCL_4 + 120mg/kg CIM	9	5

We conclude that cimetidine significantly reduces the hepatotoxic effects of CCL_4 and markedly improves the survival of rats in high dosages. These effects may be explained by the reversible inhibition of cytochrome P_{450}(4,5). Recently it has been demonstrated that cimetidine may exert inhibitory effects on the reductive pathway of halothane, another polyhalogenated hydrocarbon(6). Perhaps cimetidine might be useful in the therapy of acute CCL_4 poisoning in humans.

REFERENCES

1. R.O.Recknagel, Pharmacol.Rev. 19,145 (1967)
2. T.F.Slater, in Biochemical Mechanisms of Liver Injury(Ed.T.F.Slater),Academic Press,London,1 (1978)
3. J.Puurunen,O.Pelkonen,Eur.J.Pharmacol. 55,335 (1979)
4. S.Rendić,V.Sumjić,R.Toso,F.Kajfez,H.H.Ruf, Xenobiotica9,555 (1979)
5. R.G.Knodell,J.L.Holtzman,D.L.Crankshaw,N.M.Steele,L.N.Stanley, Gastroenterology 82,84 (1982)
6. J.L.Plummer,S.Wanwimolruk,M.A.Jenner,P.de la M.Hall,M.J.Cousins, Drug Metabol. Dispos. 12,106 (1983)