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ARTICLE

Modification of Acute and Chronic Liver Damage by Thiazolidine Compounds

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As high sulfhydril levels were shown to reduce the action of agents causing tissue-injury, increasing glutathion concentrations may have cytoprotective potential. In this study the hepatoprotective effects of several derivatives of 4-carboxy-5,5-dimethyl thiazolidine, a modulator of glutathion metabolism were studied in rat liver damaged with CCl_4. It was found that 4(S) carboxy 5,5-dimethyl-2 (5'-nitro-2-furyl) thiazolidine (dimethyl-thiazolidine-nitrofuran: DTNF) had the most significant hepatoprotective action; therefore it was subjected to detailed investigation in various models for acute and chronic liver injury. This compound was shown to ameliorate allyl-alcohol induced liver injury in rats, galactosamine induced hepatitis of mice and CCl_4 induced chronic liver damage in rats. Our study on protein synthesis in primary hepatocyte suspension culture showed that cell injury induced by CCl_4 could be reduced in the presence of this thiazolidine compound. (Pathology Oncology Research Vol 1, No1, 60–63, 1995)

Key words: thiazolidine, liver damage, cirrhosis, hepatoprotection

Introduction

Thiazolidine compounds represent one group of hepatoprotective agents which act by elevating liver glutathion concentration or by interacting with electrophilic groups.[1] Thus the direct or indirect neutralization of endogenous or exogenous toxic substances is implicated in the hepatoprotection. Previous studies on the reaction between mercapto amino acids and aldoses resulted in obtaining (4R)-2-polyhydroxyalkyl-thiazolidine-4-carboxyl acid.[2,3] Investigation on the pharmacological properties of thiazolidine-4-carboxylic acids, and especially 2-polyhydroxyalkyl-4-thiazolidine carboxylic acids, showed that these compounds can protect against the hepatotoxicity elicited by high doses of acetaminophen in mice.[8,9]

The purpose of the present study was to decide whether these new thiazolidine compounds have hepatoprotective action in acute and chronic liver damage in rats.

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Materials and Methods

Chemicals

The thiazolidine compounds used in the present studies were prepared from D-penicillamine with diverse aldehyds in aqueous solution at room temperature as reported previously.[2,3] The 2-substituted 5-5'-dimethyl-thiazolidine-4(S) carboxilic acids are 2(R,S)-epimeric mixtures with reproducible melting points and of near constant optical rotation. The chemical analysis of these compounds were performed according to the methods described by one of the (Z.Gy.) authors.[5,6] The main characteristics of the compounds synthesized for the present hepatoprotective studies are shown in *Table 1.*

Animal experiments

CFY male rats were purchased from LATI Gödöllő Hungary and fed on a standard LATI chow. In each experimental group there were 6-8 rats with 180-200 g body weight. Liver damage was induced by intragastric administration of CCl_4 (diluted with corn oil 1:3) in a dose of 1.5 ml/kg. Thiazolidine compounds were given in the indi-

Table 1. **The chemical characteristics of thiazolidine compounds**

No.	*Substituent*	*Melting point (°C)*	*Yield (%)*	*Specific rotation [α]D*
1.	5'-nitro-2-furyl	147–148	72	-159 (0.829)
2.	4'-pyridyl	149–151	54.5	+ 42.7 (1.320)
3.	1'-methyl-pyrrol-2'-yl	126–128	68	+87.5 (0.471)
4.	4'-methylthiophenyl	150–152	97	+174.4 (0.516)
5.	4'-fluorophenyl	126–127	90	+86.9 (0.473)
6.	5'-indol-2-yl	140–142	57	+18 (0.551)
7.	2'-thienyl	129–131	62	+56 (0.549)
8.	2'-benzofuryl	96	68.6	+215 (0.511)
9.	3'-nitrophenyl (DTNF)	139–140	84.6	-26.4 (0.588)

cated doses per orally 3h before CCl_4 poisoning. The animals were sacrificed 48 or 72h later in ether narcosis by exsanguination via aorta descendens, and the blood was collected. The liver was removed, immediately frozen on dry ice, except the middle lobe, which was fixed in formaline for histological sections.

Liver injury was also studied in rats receiving 20 ml/kg allylalcohol (1:100 dilution stock i.p.). Treatments with the thiazolidine compound were performed 1h before poisoning. Evaluation of the experiment took place 24h after allylalcohol treatment.

Galactosamine induced hepatitis was studied in male CBA mice, by applying a dose of 750 mg/kg i.p. Treatments with the test compounds were performed 6h after poisoning. For the histological study the animals were killed 24h later.

Table 2. **Relationship between chemical structure and hepatoprotection of 4(S)-carboxy-5-dimethyl thiazolidine derivatives[a]**

Thiazolidine Substituents (R)[b]	*Protective effect on SGOT elevation[c]*	*Extent of necrotic lesions[d]*
4-pyridyl-	9%	++
3-pyridyl-	14%	++
1'-methyl-2pyrrolyl	14%	++
4'-methylthio phenyl	20%	++
4'-fluorophenyl	23%[e]	++
5-indolyl-	20%	++
2-thienyl-	20%	++
2-benzofuryl	24%	++
5'-nitro-2-furyl	43%	+

a: CFY rats (n=6) were treated with the test compounds (100 mg/kg i.p. doses), then 3 hours later 1.5 ml/kg CCl_4 was administered per orally. Animals were killed 48 hours after CCl_4 treatments.
b: thiazolidine 2-H substituted by R-
c: P=C-T / Cx100 where C is the change in control liver upon toxin treatment, T is the change upon treatment with the test compound in the damaged liver.
d: Severity of the lesions in the liver was assessed by the extent of necrotic changes in liver lobulae in histological sections. The sections from liver treated with CCl_4 alone showed a score of +++.
e: Statistically significant difference between the values in only CCl_4 treated and in CCl_4 plus test compound treated groups ($p< 0.05$).

Chronic liver damage was induced by administering CCl_4 perorally to rats in a dose of 0.15 ml/kg, twice a week for 1 month (fibrosis model) or in a dose of 0.3 ml/kg for 15 weeks (cirrhosis model). At the third week of the experiment DTNF was administered 5 hours after beginning of CCl_4 treatments. For the study of the effects on the already developed liver cirrhosis, rats were treated with the thiazolidine compound from the 15th week for 4 weeks after cancelling hepatotoxin administration. Animals in control groups received 5% Tween 80 as vehicle i.p.

Methods for evaluations of liver injury

For the estimation of quantitative changes in acute liver injury, glutamate-oxalacetate transaminase activity of the sera (SGOT) and glycogen and triglyceride content of liver were measured according to established methods.[1,4,7,10] The amount of collagen was determined according to the quantity of hydroxyproline associated to protein.[12] Histological evaluations were performed in HE stained liver sections. The severity of necrotic changes, fatty degeneration and connective tissue accumulation was scored surveying 50 lobulae per section and are given in arbitrary scale units.

Table 3. **Hepatoprotective action of 2-(5'-nitro-2-furyl)-4-(S)carboxy-5,5-dimethyl-thiazolidine (DTNF) in CCl_4 induced liver injury[a]**

Treatment	*Triglyceride mg/g w.w.*	*%*	*Glycogen mg/g w.w.*	*%*
Control	6.9±3.0	100	46.3±6.4	100
CCl_4 alone 48 hrs	39.0±4.4	562	3.9±1.3	8
CCl_4+DTNF 48 hrs	14.2±2.8[+]	205	32.5±2.1[+]	70
CCl_4 alone 72 hrs	20.9±6.8	302	16.7±4.9[+]	36
CCl_4+DTNF 72 hrs	17.1±6.2	247	48.8±4.6[+]	105
DTNF alone 48 hrs	5.5±4.0	79	46.1±5.0	99
DTNF alone 72 hrs	6.5±1.8	94	44.5±3.9	96

a: CFY rats were treated with DTNF in 100 mg/kg dose i.p., then 3 hours later 1.5 ml/kg CCl_4 was administered p.o. Animals were sacrified at 48 or 72 hours of the experiment as indicated and serum GOT, liver triglyceride and glycogen content were measured; there were 6 animals in each of the treatment groups.
+: Significantly different from the value for the CCl_4 treated group.

Statistics

The significance of differences between means were assessed using Student t test; $P>0.05$ was regarded as significant.

Results

To compare the hepatoprotective actions of the 4(S)-carboxy-5,5-dimethyl thiazolidine compounds, doses of 100 mg/kg were administered in rats 3 hours prior CCl_4 indu-

ced liver injury. *Table 2* shows hepatoprotective activity in term of reducing the SGOT elevation. The most effective derivatives offered 24-43 percent protection if the elevation of SGOT was measured, but in certain cases the necrotic lesions were also ameliorated. One of these compounds, 2-(5'-nitro-2'-furyl)-4(S)-carboxy-5,5-dimethyl thiazolidine (DTNF), was selected as the most potent because it was able to modify favorably the accumulated triglyceride and the reduced glycogen content induced by CCl_4 *(Table 3)*. In addition, as *Table 4* indicates, allylalcohol induced acute liver injury was also reduced by DTNF. The hepatoprotective effect of DTNF was further studied in galactosamine induced liver injury i.e. in an acute experiment, modeling "hepatitis" in mice. Cyanidanol-3 was used in a dose of 250 mg/kg as a reference compound *(Table 5)*. Morphological evaluations of fatty liver and hepatocellular necrosis were carried out and demonstrated a pronounced amelioration of these lesions upon treatment with DTNF in a dose of 100 mg/kg.

Table 4. **Actions of DTNF on allylalcohol induced liver injury**

Treatment	*SGOT (mmol/ml × hour)*
Control	1.80±0.43
Allylalcohol 20 ml/kg	4.16±1.60
Allylalcohol 20 ml/kg +DTNF 100 mg/kg	2.84±0.90

a: CFY rats were treated with DTNF (100 mg/kg i.p.) and 1 hour later allylalcohol was administered i.p. in a dose of 20 ml/kg. SGOT activity was measured 24 hours after poisoning (n=5).

Table 6 summarizes the results of the experiments designed for the evaluation of the effects of DTNF in chronically induced liver injury. It was found that applying 100 mg/kg DTNF 5 hours after each administration of CCl_4 significantly prevented the development of chronic liver injury. This conclusion is based on biochemical measurements of collagen levels and evaluation of histological sections (data not shown).

Table 5. **Modification of galactosamine induced hepatitis by DTNF in mice**

Treatment[a]	*Morphological alterations*[b]	
	Fatty liver (score 0–3)	*Necrosis (score 0–3)*
Control	0	0
Galactosamine alone	3.2	2.7
Galactosamine + DTNF	2.0	1.2
Galactosamine + Cyanidanol-3	0.8	2.4

a: 750 mg/kg galactosamine was given to mice i.p. and 6 hours later, treatments with the test compounds were carried out i.p. in doses of 100 mg/kg (n=5). Animals were killed 48 hrs after poisoning.
b: The severity of the necrotic lesions and fatty liver were evaluated in a semi-quantitative manner in liver sections after HE staining.

Discussion

Studies on the derivatives of 4-carboxy-5,5-dimethyl-thiazolidines revealed that some of these compounds have hepatoprotective activity against liver injury *(Table 2)*. Structures containing 4'-pyridyl, 3'-pyridyl or 1'-methyl-2-pyrrolyl residues as substituents of 2-hydrogen in the thiazolidine ring were ineffective in contrast to those containing phenyl groups with either fluorine and methylthio groups or condensed heterocycles e.g. 5-indolyl or 2-benzofuryl derivatives. The best results were obtained, however, with compounds containing heterocyclic substituents of thiazolidine (2'-thienyl and 5'-nitro-2'-furyl derivatives). Hepatoprotective actions of these compounds were evaluated on the basis of both biochemical (serum GOT activity, lipid, glycogen content of the liver) and histological (assessment of the necrotic lesions) methods (data not shown). The compound 2-(5'-nitro-2-2-furyl)-4-(S)carboxy-5,5-dimethyl thiazolidine (DTNF) was studied further in both the CCl_4 and other models of liver injury.

Table 6. **Effect of 2-(5'-nitro-2-furyl)-4 carboxy 5',5-dimethyl-thiazolidine (DTNF) on the induction of rat liver cirrhosis**

Treatment		*Collagen*	*Histology*			
CCl_4[a] *p.os 15 weeks*	*DTNF*[b] *i.p. 3–15 weeks*	*OH-proline µg/mg DNA*	*Fibrosis*		*Cirrhosis*	
			Mod	*Ext*	*Mod*	*Ext*
–	–	122±11	0/10	0/10	0/10	0/10
0.3 ml/kg	–	366±51	1/7	–	–	6/7
0.3 ml/kg	10 mg/kg	305±64	6/9	1/9	2/9	–
0.3 ml/kg	100 mg/kg	276±38[c]	3/5	1/5	1/5	–

Mod = moderate; Ext = extensive
a: Twice weekly
b: Treatment 5 hours after CCl_4
c: Difference from CCl_4 injured rats is significant at $p = 0.05$

Our results indicate that the hepatoprotective activity of DTNF is not restricted to CCl_4 induced liver injury because the allylalcohol induced liver damage and galactosamine induced acute hepatitis could both be ameliorated by treatments with this compound. It was remarkable in our experiment that using the galactosamine model, the effect of DTNF against fatty liver and the necrotic lesions surpassed that of cyanidanol-3. In addition the development of chronic liver damage was also favourably modified by simultaneous treatments with DTNF. However, these effects were present only in animals treated with the high i.p. dose (100 mg/kg) of DTNF simultaneously with CCl_4. No significant effect was observed either by histological or biochemical evaluations when DTNF was administered after the development of cirrhosis.

The effectivity of DTNF on the acute CCl_4 induced liver injury may be attributed at least in part to the cytoprotection

offered by high intracellular sulfhydril level. Since it was reported that the protective mechanism of other 2-oxo-4 carboxythiazolidine compounds against liver toxicity could be related to the generation of cysteine[9,10] it is a possibility that DTNF also acts this way. To test this possibility is the aim of forthcoming experiment.

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