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The effect of 2-substituted thiazolidine-4(*R*)-carboxylic acids on non-protein sulphydryl levels and sulphurtransferase activities in mouse liver and brain

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Abstract—2-Substituted thiazolidine-4(*R*)-carboxylic acids (TD) were found to increase the concentration of non-protein sulphydryls (NPSH) and the activity of rhodanese (thiosulphate sulphurtransferase, EC 2.8.1.1) and 3-mercaptopyruvate sulphurtransferase (EC 2.8.1.2) in mouse liver. These properties suggest TDs are potentially hepatoprotective compounds. However TDs also cause depletion of NPSH in the mouse brain and this may be the reason for their toxic side effects on the central nervous system.

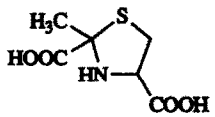
L-Cysteine (L-cys*) in mammalian cells is of a dietary origin and is also produced by transsulphuration of methionine. L-cys is the rate limiting amino acid in glutathione (GSH) biosynthesis [1]. GSH is believed to be a form of L-cys storage [2] and practically accounts for the whole non-protein sulphydryls (NPSH) of the cell [3]. An increase of GSH is useful in protecting the cells against the toxic effects of drugs, other foreign compounds and oxygen [3]. GSH cannot be used as a drug because it does not enter the cell [4]. Utilization of free L-cys for therapy is limited by its toxicity as it causes damage to the CNS [5].

Thiazolidine derivatives the cyclic products of a non-enzymatic reaction of L-cys with carbonyl compounds (Scheme 1) are potentially capable of releasing L-cys *in vivo*, either by non-enzymatic solvolysis [6] or by metabolic action [7]. The work reported compares the effect of different thiazolidine derivatives (TD) on the NPSH levels in mouse liver and brain and on the activity of enzymes participating in "sulphane sulphur" metabolism, i.e. rhodanese (EC 2.8.1.1) and 3-mercaptopyruvate sulphurtransferase (MPST) (EC 2.8.1.2).

Materials and Methods

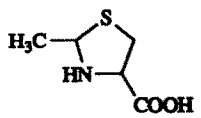
Chemicals. The following thiazolidine derivatives used were the condensation products of L-cys with (1) pyruvate, (2) acetaldehyde, (3) formaldehyde, (4) and (5) D- and L-arabinose, (6) D-ribose, (7) and (8) D- and L-fucose, (9) D-mannose, (10) D-galactose, (11) D-glucose, (12) L-xylose and (13) D-lyxose.

(1) CP



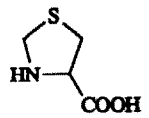
2-methyl-thiazolidine-2,4-dicarboxylic acid

(2) CA



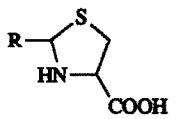
2-methyl-thiazolidine-4-carboxylic acid

(3) CF



thiazolidine-4-carboxylic acid

The subsequent 2-polyhydroxyalkyl-thiazolidine 4-carboxylic acids (products of condensation of L-cys with sugars):



(4) CAr(D) R = 2(*S*)-D-arabino-tetrahydroxybutyl
(5) CAr(L) R = 2(*R*)-L-arabino-tetrahydroxybutyl
(6) CR(D) R = 2(*S*)-D-ribo-tetrahydroxybutyl
(7) CFuc(D) R = 2(*R*)-D-fuco-tetrahydroxypentyl
(8) CFuc(L) R = 2(*S*)-L-fuco-tetrahydroxypentyl
(9) CM(D) R = 2(*R*)-D-manno-pentahydroxypentyl
(10) CGal(D) R = 2(*R*)-D-galacto-pentahydroxypentyl
(11) CGlu(D) R = 2(*S*)-D-gluco-pentahydroxypentyl
(12) CX(L) R = 2(*S*)-L-xylo-tetrahydroxybutyl
(13) CL(D) R = 2(*S*)-D-lyxo-tetrahydroxybutyl

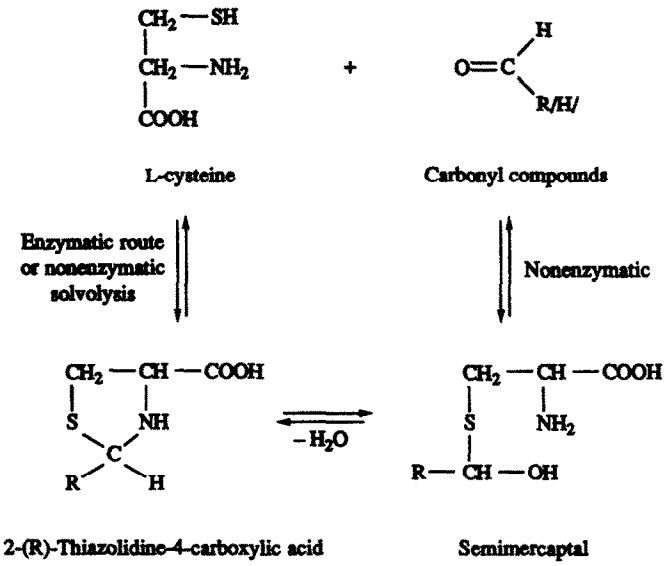
Synthesis of compounds 4–10 and 13 has been described elsewhere [8] (method A). Compounds 11 and 12 were obtained by a modified method of Weitzel *et al.* [9] in dry methanol. Stereochemistry of the products was determined by combined polarimetric and ^{1}H-NMR methods as described previously [8].

CP and CF were synthesized according to Schubert [10], CA according to Nagasawa *et al.* [6] and 3-mercaptopyruvate ammonium salt according to Sprinson and Chargraff [11] in the Laboratory of Organic Synthesis, Jagiellonian University, Cracow, Poland. The purity was checked by TLC. GSH and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were from the Sigma Chemical Co. (St Louis, MO, U.S.A.).

Animals. Female Swiss mice, 2–3 months old, weighing approximately 20 g, kept on a standard diet for mice were used. The animals had free access to food and water and were not starved before being killed by decapitation.

Preparation of material for investigations. The animals were given i.p. injections of TD (12 μmol/g), once a day for 3 days. The control animals were injected with the same volumes (0.5 mL) of isotonic saline. On the fourth

* Abbreviations: Cys-Acid, cysteic acid; CS-Acid, cysteine sulphinic acid; GSH, reduced glutathione; L-cys, L-cysteine; MPST, 3-mercaptopyruvate sulphurtransferase; TD, thiazolidine derivatives; NPSH, non-protein sulphydryls; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid).



Scheme 1. The synthesis of thiazolidine-4(*R*)-carboxylic acids. Transitionally formed thiosemiacetal undergoes cyclization, when the amino group is in close proximity.

day, the mice were killed and the livers and brains were dissected, washed with cold saline, weighed and frozen at −30° until needed. After thawing, the tissues were homogenized with 0.1 M phosphate buffer, pH 7.4 (liver 1 g:5 mL, brain 1 g:2 mL) and centrifuged at 650 *g* for 15 min. The supernatants were added to the incubation medium.

Methods. MPST activity was determined using the method of Kun and Fanshier [12] with the modification of Kasperczyk *et al.* [13]. Rhodanese activity was assessed according to the method of Sörbo [14].

NPSH was measured by reaction with DTNB to give a compound that absorbs at 412 nm (Elman's method) [15], with the modification of Moron *et al.* [16]. The protein content was measured according to the method of Lowry *et al.* [17].

The data were analysed using Student's *t*-test.

Results and Discussion

Table 1 shows that the tested TD elevated the NPSH in mouse liver. The most active compound was CP, a product of non-enzymatic condensation of L-cys and pyruvate, formed in the course of L-cys metabolism both *in vitro* [18] and *in vivo* [19]. In the liver, TD also triggers an increase in the activity of enzymes related to anaerobic L-cys sulphur metabolism (Table 1), which in turn leads to the formation and biodegradation of the pools of "sulphane sulphur"

Table 1. Levels of NPSH and the activities of rhodanese and MPST in mouse liver

	NPSH nmol/mg protein		MPST μmol pyruvate/mg protein		Rhodanese μmol SCN^-/mg protein	
TD	X	SD	X	SD	X	SD
Control	65.4	±7.1	20.6	±1.4	1.79	±0.46
CP	106.5‡	±11.2	27.9‡	±3.0	2.83‡	±0.39
CA	89.3‡	±6.7	24.3‡	±1.7	2.81‡	±0.30
CF	78.0‡	±9.3	20.9	±2.2	1.45*	±0.22
CAr(D)	89.8‡	±10.4	33.3‡	±1.7	3.07‡	±0.13
CAr(L)	66.7	±7.1	24.4‡	±2.1	3.34‡	±0.20
CR(D)	70.4*	±6.3	26.0‡	±2.4	2.55‡	±0.14
CFuc(D)	71.3*	±10.2	26.5‡	±1.1	3.37‡	±0.22
CFuc(L)	61.8	±11.3	21.3	±1.9	2.58‡	±0.15
CM(D)	71.9*	±6.2	26.1‡	±1.3	2.04	±0.33
CGal(D)	97.0‡	±10.1	22.8‡	±1.1	2.18†	±0.13
CGlu(D)	69.7	±3.8	23.1‡	±1.5	2.53‡	±0.10
CX(L)	68.4	±7.4	23.0‡	±2.9	3.28‡	±0.18
CL(D)	72.3†	±8.6	22.6†	±2.9	3.17‡	±0.40

NPSH was assayed by the DTNB method and was expressed as nmol/mg protein. The specific activity of rhodanese was expressed as μmoles of SCN^-/5 min incubation per 1 mg protein and that of MPST as μmoles of pyruvate/15 min incubation per 1 mg protein.

X, mean and SD of 15–20 determinations.

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

Table 2. Levels of NPSH and the activities of rhodanese and MPST in mouse brain

TD	NPSH nmol/mg protein		MPST μmol pyruvate/mg protein		Rhodanese μmol SCN^-/mg protein	
	X	SD	X	SD	X	SD
Control	22.2	±3.1	11.9	±0.7	0.247	±0.020
CP	17.8*	±2.3	10.5	±1.9	0.407‡	±0.048
CA	18.6	±1.9	10.3†	±0.2	0.250	±0.020
CF	20.2	±2.4	12.3	±0.1	0.402‡	±0.040
CAr(D)	17.5*	±1.9	10.1‡	±0.3	0.131‡	±0.020
CAr(L)	16.7*	±2.3	11.7	±0.2	0.324‡	±0.016
CR(D)	15.6†	±1.0	11.1*	±0.1	0.244	±0.018
CFuc(D)	10.4‡	±2.0	7.7‡	±0.3	0.170‡	±0.020
CFuc(L)	16.5†	±1.2	11.3	±0.3	0.188‡	±0.032
CM(D)	15.6†	±2.1	12.7	±0.1	0.104‡	±0.010
CGal(D)	15.5†	±1.3	10.2*	±2.2	0.204†	±0.027
CGlu(D)	17.4*	±1.4	8.7‡	±0.2	0.196‡	±0.014
CX(L)	15.2†	±1.8	7.4‡	±0.2	0.251	±0.086
CL(D)	17.6*	±2.7	8.2‡	±1.1	0.116‡	±0.040

The determination conditions were the same as in Table 1.
* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$.

which have been considered as natural regulatory agents [20].

On the other hand, a decrease of NPSH concentration was observed in the brain following the administration of TD (Table 2). It should be emphasized that an increase of NPSH levels in the liver and a decrease in the brain is produced by adducts L-cys with toxic formaldehyde (CF) and acetaldehyde (CA) as well as with naturally occurring aldoses or pyruvate. Therefore, the observed effects of TD must be connected with the cys, and not with the aldehyde moiety in the thiazolidine.

The decrease of GSH concentration in the brain (Table 2) suggests that not only free cys [21], but also a variety of TDs which can be a potential source of this amino acid *in vivo* may be toxic for the CNS. This is possibly due to the low rate of GSH synthesis in the brain [22] which can lead to accumulation of free cysteine. The cytotoxicity of cysteine and the GSH depletion [21, 23] are related to formation of H_2O_2, and radicals accompanying the spontaneous, very rapid oxidation to cystine. The cys may also react with intracellular GSH to yield the mixed disulphide (CYSSG) [24]. Olney *et al.* [25] suggest that a possible mechanism of cys-induced toxicity could also be related to oxidation to cysteic acid (Cys-Acid) and cystine sulphinic acid (CS-Acid) in the CNS cells. The neurological disturbances were observed following the administration of CF as hepatoprotective agent (Heparegen) [26]. The metabolic route of CF in liver is through *N*-formylcysteine [7] which may, probably, be also oxidated respectively to *N*-formylcysteic acid and *N*-formylcysteine sulphinic acid, although this has not been proved.

CA in contrast to CF easily undergoes spontaneous solvolysis (CA has a methyl radical at the second position of the thiazolidine ring) and does not require metabolism to furnish cys *in vivo* [6]. As it has been demonstrated by Nagasawa *et al.* [6], CA is less toxic than CF and also offers more efficient protection against the toxicity of acetaminophen. This is confirmed by these studies which show that CA in the liver increases NPSH concentration much more than CF (Table 1).

The fact that the studied TD compounds to varying degrees increase the GSH concentration in the liver and decrease its values in the brain (Tables 1 and 2) can be the result of the different chemical character of the R group at the second position in the thiazolidine ring.

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