Biochemical Pharmacology, Vol. 27, pp. 2095–2098

0006-2952/78/0901-2095$02.00/0

INHIBITION OF δ-AMINOLEVULINIC ACID SYNTHETASE BY THIOACETAMIDE AND THIOACETAMIDE-*S*-OXIDE*

TAKEMI YOSHIDA and ROBERT A. NEAL†
Center in Environmental Toxicology, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232, U.S.A.

(*Received* 23 *September* 1977; *accepted* 10 *January* 1978)

Abstract—Thiocetamide and one of its metabolites, thioacetamide-*S*-oxide, were shown to inhibit δ-aminolevulinic acid (ALA) synthetase when administered *in vivo* to adult male mice. Thioacetamide and thioacetamide-*S*-oxide also inhibited the 3,5-dicarboethoxy-1,4-dihydrocollidine (DDC)-mediated induction of ALA synthetase when given either 1 hr prior to or 3 hr after the administration of DDC. The results of these studies also indicate that thioacetamide-*S*-oxide is generally a more potent inhibitor of ALA synthetase than thioacetamide.

The hepatotoxin and hepatocarcinogen thioacetamide has been shown to decrease the transfer of messenger RNA from the nucleus [1] and cause the enlargement of nucleoli associated with an increased synthesis of RNA in the nucleus [2, 3]. In addition, the *in vivo* administration of thioacetamide has been shown to inhibit hepatic mixed-function oxidase enzyme activity and decrease the concentration of hepatic cytochrome P450 [4].

It has been postulated that the toxic effect of certain hepatotoxins and carcinogens may be due to the formation of chemically reactive metabolic products. With regard to thioacetamide, the metabolite thioacetamide-*S*-oxide [5, 6] appears to be an intermediate in the pathway to the formation of the ultimate toxic metabolite. Thus, Hunter *et al.*[7] have recently shown that thioacetamide-*S*-oxide is more effective than thioacetamide in causing inhibition of hepatic mixed-function oxidase enzyme activity and hepatic necrosis. However, these studies also suggested that thioacetamide-*S*-oxide is further metabolized to the ultimate toxic metabolite [7].

The mechanism of inhibition of hepatic mixed-function oxidase activity by thioacetamide has not yet been determined. The hemoprotein cytochrome P450 plays a key role in the activity of these hepatic mixed-function oxidase enzyme systems. In turn, the key enzyme in the heme biosynthetic pathway is δ-aminolevulinic acid (ALA) synthetase. A relationship between ALA synthetase and the activity of the cytochrome P450 containing mixed-function oxidase enzymes has been suggested by several investigators [8–10].

In this study we have examined the effects of thioacetamide and its metabolite, thioacetamide-*S*-oxide, on ALA synthetase. We have found that both compounds inhibit this enzyme, with thioacetamide-*S*-oxide being generally more effective than thioacetamide. These results suggest that the decrease in hepatic mixed-function oxidase activity seen on *in vivo* administration of thioacetamide is, perhaps, the result of inhibition of ALA synthetase.

MATERIALS AND METHODS

Thioacetamide was obtained from the Fisher Scientific Co., Atlanta, Ga. Thioacetamide-*S*-oxide was synthesized by the method of Walter [11] and purified by recrystallization from hot methanol. 3,5-Dicarboethoxy-1,4-dihydrocollidine (DDC) was purchased from ICN Pharmaceuticals. Hydrocortisone was from the Sigma Chemical Co.

Male ICR Swiss mice, weighing 22–28 g, were used in these experiments. They were fed a commercial diet *ad lib.* However, they were fasted 20 hr prior to administration of thioacetamide or theoacetamide-*S*-oxide. The mice were injected interaperitoneally with different dose levels of thioacetamide and of thioacetamide-*S*-oxide dissolved in 0.9% NaCl and sacrificed at the times indicated in the text. In some experiments, mice were i.p. injected with DDC (300 mg/kg), a well known inducer of ALA synthetase, prior to or subsequent to the administration of thioacetamide or thioacetamide-*S*-oxide. Control mice were injected with the vehicle only. The livers were perfused with cold 0.9% NaCl solution *in situ*. After their removal the livers were homogenized in 3 vol. of 10 mM Tris–HCl buffer (pH 7.4) containing 0.9% NaCl and 0.5 mM EDTA using a Potter–Elvehjem homogenizer with a Teflon pestle. ALA synthetase activity was assayed by the method of Marver *et al.* [12] using a total liver homogenate as the enzyme source. The reaction mixture contained 200 μmoles pyridoxal phosphate, 150 μmoles Tris–HCl (pH 7.2), and 0.5 ml homogenate in a final volume of 2 ml. The reaction was carried out for 60 min at 37° and stopped by the addition of 0.5 ml of 25% trichloroacetic acid solution. The ALA produced was estimated colorimetrically after condensation with acetylacetone and isolation of the pyrrole compound formed on a Dowex-1-acetate column [13]. Hepatic tyrosine amino transferase

* This work was supported by U.S. Public Health Service Grants ES00075 and ES00267.

† To whom reprint requests should be sent.

Table 1. Effect of thioacetamide and thioacetamide-*S*-oxide on hepatic ALA synthetase activity in fasted mice*

Treatment	ALA synthetase activity (nmoles/g liver/hr)	
	2-Hr post-administration	4-Hr post-administration
Control	51.0 ± 4.5	48.8 ± 2.8
Thioacetamide		
25 mg/kg		22.2 ± 1.7
50 mg/kg	24.0 ± 0.5	16.1 ± 4.4
Thioacetamide-*S*-oxide		
12.5 mg/kg		27.8 ± 2.3
25 mg/kg	24.2 ± 0.4	22.1 ± 1.1

* Mice were injected intraperitoneally with thioacetamide and thioacetamide-*S*-oxide and sacrificed at the times indicated. Values are the mean ± S. E. of three to five mice.

activity was determined by the method of Rosen *et al.* [14]. Protein concentration was estimated by the biuret method using bovine serum albumin as the standard.

RESULTS

The effect of thioacetamide and thioacetamide-*S*-oxide on the basal activity of ALA synthetase is shown in Table 1. As can be seen, both thioacetamide and thioacetamide-*S*-oxide decreased hepatic ALA synthetase activity when measured at 2 and 4 hr after their administration. The effects of both compounds on the induction of ALA synthetase by DDC were also examined. Tables 2 and 3 show the effects of thioacetamide and thioacetamide-*S*-oxide on the DDC-mediated induction of ALA synthetase. As shown in Tables 2 and 3, the pretreatment of mice with thioacetamide and thioacetamide-*S*-oxide strongly inhibited the induction of ALA synthetase after DDC administration. The inhibition of ALA synthetase induction by thioacetamide and by thioacetamide-*S*-oxide appeared to be dose dependent. Almost complete inhibition of the DDC-mediated induction of ALA synthetase was observed by doses of 50 mg/kg of thioacetamide and 25 mg/kg of thioacetamide-*S*-oxide respectively.

Thioacetamide and thioacetamide-*S*-oxide are also able to inhibit DDC-mediated induction of ALA synthetase when given 3 hr after the administration of DDC (Table 4). The inhibition of ALA synthetase induction by DDC was about 38 per cent at 25 mg/kg and 76 per cent at 50 mg/kg of thioacetamide and 65 per cent at 25 mg/kg of thioacetamide-*S*-oxide. Thus, thioacetamide-*S*-oxide appeared to be more effective in inhibiting ALA synthetase induction than did an equivalent dose of thioacetamide.

In contrast to the almost complete inhibition of DDC-mediated induction of ALA synthetase produced by prior administration of thioacetamide and thioacetamide-*S*-oxide (Tables 2 and 3), the effects of these compounds on tyrosine amino transferase (TAT) induction by hydrocortisone were less dramatic. As shown in Table 5, pretreatment of mice with thioacetamide or thioacetamide-*S*-oxide resulted in a 44–46 per cent inhibition of the hydrocortisone-mediated induction of TAT. Neither thioacetamide nor thioacetamide-*S*-oxide caused any significant decrease in the basal activity of TAT when measured 4 hr after their administration.

Table 2. Effect of pretreatment of mice with thioacetamide on the induction of ALA synthetase by DDC*

Treatment	ALA synthetase activity (nmoles/g liver/hr)
None	50.5 ± 1.0
DDC	209.4 ± 29.1
DDC + thioacetamide (12.5 mg/kg)	112.5 ± 13.3
DDC + thioacetamide (25.0 mg/kg)	60.4 ± 12.1
DDC + thioacetamide (50.0 mg/kg)	37.7 ± 6.5

* Mice were given thioacetamide 1 hr prior to administration of DDC and sacrificed 3 hr after DDC administration. Each value is the mean ± S. E. of three to five mice.

Table 3. Effect of pretreatment of mice with thoacetamide-*S*-oxide on the induction of ALA synthetase by DDC*

Treatment	ALA synthetase activity (nmoles/g liver/hr)
None	57.7 ± 2.4
DDC	264.9 ± 26.4
DDC + thioacetamide-*S*-oxide (6.25 mg/kg)	155.0 ± 12.1
DDC + thioacetamide-*S*-oxide (12.5 mg/kg)	116.7 ± 19.8
DDC + thioacetamide-*S*-oxide (25.0 mg/kg)	71.2 ± 2.5

* Mice were given thioacetamide-*S*-oxide 1 hr prior to administration of DDC and sacrificed 3 hr after DDC administration. Each value is the mean ± S. E. of three to five mice.

DISCUSSION

The ability of thioacetamide and thioacetamide-*S*-oxide, administered *in vivo*, to inhibit ALA

Table 4. Effect of thioacetamide and thioacetamide-*S*-oxide, administered *in vivo* on the levels of ALA synthetase induced by DDC*

Treatment	ALA synthetase activity (nmoles/g liver/hr)
None	55.3 ± 6.1
DDC, 3 hr	278.5 ± 20.7
DDC, 6 hr	726.7 ± 4.2
DDC + thioacetamide (25 mg/kg)	470.2 ± 32.9
DDC + thioacetamide (50 mg/kg)	218.5 ± 60.9
DDC + thioacetamide-*S*-oxide (25 mg/kg)	290.1 ± 30.6

* Mice were injected with DDC (300 mg/kg, i.p.) at time 0. Three hr later mice were injected with thioacetamide and thioacetamide-*S*-oxide and sacrificed at 3 hr after treatment with these compounds. Each value is the mean ± S. E. of three to five mice.

Table 5. Effect of thioacetamide and thioacetamide-*S*-oxide on the induction of tyrosine amino transferase by hydrocortisone*

Treatment	Tyrosine amino transferase activity (μmoles/mg protein/hr)
None	1.12 ± 0.03
Hydrocortisone	4.21 ± 0.47
Thioacetamide + hydrocortisone	2.78 ± 0.18
Thioacetamide-*S*-oxide + hydrocortisone	2.85 ± 0.05

* Mice were injected with thioacetamide (50 mg/kg, i.p.) and thioacetamide-*S*-oxide (25 mg/kg, i.p.) 1 hr prior to hydrocortisone (100 mg/kg, i.p.), and sacrificed 3 hr after hydrocortisone administration. Values are the mean ± S. E. of three to five mice.

synthetase and to block induction of this enzyme by DDC is of interest in relation to the ability of these compounds to bring about a decrease in both hepatic mixed-function oxidase enzyme activities and cytochrome P450 levels. ALA synthetase is a regulatory enzyme in the heme biosynthetic pathway. Thus, the inhibition of this enzyme by thioacetamide and thioacetamide-*S*-oxide could result in a decreased availability of heme for the synthesis of cytochrome P450. Cytochrome P450 levels are not significantly decreased until 24 hr after administration of thioacetamide or thioacetamide-*S*-oxide [7]. Aminopyrine demethylase and aniline hydroxylase follow a similar pattern of decrease in activity after administration of thioacetamide. Considering the half lives of the various species of cytochrome P450 (7–8 hr or longer) [15], it is reasonable that inhibition of ALA synthetase by thioacetamide and thioacetamide-*S*-oxide could account for the decrease seen in both the levels of cytochrome P450 and the activity of the hepatic mixed-function oxidase enzyme systems. A decrease in cytochrome P450 and the activity of hepatic mixed-function oxidase enzyme systems as a result of interference with heme synthesis or stimulation of heme breakdown has also been noted with 3-amino-1,2,4-triazole [16], hemin [17] and cobaltous chloride [18]. It is possible that thioacetamide and thioacetamide-*S*-oxide may be inhibiting the synthesis of apo-cytochrome P450 as well as heme. However, previous work has shown that apo-cytochrome P450 can still be induced under conditions where the synthesis of heme is inhibited by cobaltous chloride [19]. Thus, inhibition of heme synthesis alone could account for the effect of thioacetamide and thioacetamide-*S*-oxide on hepatic cytochrome P450 levels and mixed-function oxidase activity.

Thioacetamide-*S*-oxide appears to be a more effective inhibitor of ALA synthetase than thioacetamide. Thioacetamide-*S*-oxide is also a more potent necrogenic agent in rat liver than thioacetamide [7]. An equivalent decrease in hepatic mixed-function oxidase activity is also obtained at lower doses of thioacetamide-*S*-oxide as compared to thioacetamide [7]. However, thioacetamide-*S*-oxide does not appear to be the metabolite directly responsible for liver necrosis, since the extent of necrosis produced by both thioacetamide and thioacetamide-*S*-oxide is enhanced by pretreatment of rats with phenobarbital, an inducer of the hepatic mixed-function oxidase enzyme systems, and reduced by pretreatment with cobaltous chloride, pyrazole and SKF-525-A (Smith, Kline & French), compounds which decrease the activity of hepatic mixed-function oxidase enzyme systems [7]. It is proposed that the ultimate toxic metabolite of thioacetamide is, perhaps, thioacetamide-*S*-dioxide(CH_3–CSO_2–NH_2)produced from thioacetamide-*S*-oxide in a mixed-function oxidase catalyzed reaction [7]. The present experiments were not specifically designed to show evidence for the involvement of thioacetamide-*S*-dioxide in the inhibition of ALA synthetase. However, since thioacetamide-*S*-oxide appears to be more effective than thioacetamide in inhibiting ALA synthetase *in vivo*, it appears possible that thioacetamide-*S*-oxide may be acting as an inhibitor by way of its metabolism to a more reactive intermediate such as thioacetamide-*S*-oxide.

The mechanism by which thioacetamide and thioacetamide-*S*-oxide inhibit ALA synthetase is unclear. Thioacetamide causes changes in nuclear function and morphology [1–3]. It also has been reported to inhibit protein synthesis in rat liver [20]. It is possible that thioacetamide and thioacetamide-*S*-oxide may block the DDC-mediated induction of ALA synthetase by inhibition of protein synthesis. This hypothesis is supported by the finding that the effects of thioacetamide and thioacetamide-*S*-oxide on DDC-mediated ALA synthetase induction are similar to those seen on administration of hemin or cycloheximide (data not shown). However, the difference in the degree of inhibition by thioacetamide and thioacetamide-*S*-oxide or the induction of ALA synthetase and TAT suggests that a mechanism more specific than a generalized inhibition of protein synthesis may be responsible for the inhibition of ALA synthetase activity by these compounds.

REFERENCES

1. E. Stöcker and T. Yokoyama, *Beitr. Path. Anat.* **134,** 1 (1966).
2. H. R. Adams and H. Busch, *Cancer Res.* **23,** 576 (1963).
3. D. E. Kizer, B. C. Shirley, B. Cox and B. A. Howell, *Cancer Res.* **25,** 596 (1965).
4. E. A. Barker and E. A. Smuckler, *Molec. Pharmac.* **8,** 318 (1972).
5. R. Ammon, H. Berninger, H. J. Haas and I. Landsberg, *Arzneimittel-Forsch.* **17,** 521 (1967).
6. V. Becker and W. Walter, *Acta hepato-splenol.* **12,** 129 (1965).
7. A. L. Hunter, M. A. Holscher and R. A. Neal, *J. Pharmac. exp. Ther.* **200,** 439 (1977).
8. O. Wada, Y. Yano, G. Urata and K. Nakao, *Biochem. Pharmac.* **17,** 595 (1968).
9. J. Baron and T. R. Tephly, *Archs Biochem. Biophys.* **139,** 410 (1970).
10. T. Yoshida, Y. Suzuki and M. Uchiyama, *Biochem. Pharmac.* **25,** 2418 (1976).
11. W. Walter, *Justus Liebigs Annln Chem.* **633,** 35 (1960).
12. H. S. Marver, D. P. Tschudy, M. G. Perlroth and A. Collins, *J. biol. Chem.* **241,** 2803 (1966).
13. G. Urata and S. Granick, *J. biol. Chem.* **238,** 811 (1963).

14. F. Rosen, H. R. Harding, R. J. Milholland and C. A. Nichol, *J. biol. Chem.* **238,** 3725 (1963).
15. W. Levin, D. Ryan, R. Kuntzman and A. H. Conney, *Molec. Pharmac.* **11,** 190 (1975).
16. J. Baron and T. R. Tephly, *Molec. Pharmac.* **5,** 10 (1969).
17. K. W. Bock, W. Fröhling and H. Remmer, *Biochem. Pharmac.* **22,** 1557 (1973).
18. T. R. Tephly, C. Webb, P. Trussler, F. Kniffen, E. Hasegawa and W. Piper, *Drug Metab. Dispos.* **1,** 259 (1973).
19. M. A. Correia and V. A. Meyer, *Proc. natn. Acad. Sci. U.S.A.* **72,** 400 (1975).
20. E. A. Barker, E. A. Smuckler and E. P. Benditt, *Lab. Invest.* **12,** 955 (1963).