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*Reviews*

# Aminothiadiazoles

Donald L. Hill

Southern Research Institute, Kettering-Meyer Laboratory, 2000 Ninth Avenue South, Birmingham, Alabama 35205, USA

***Summary.*** *2-Amino-1,3,4-thiadiazole (ATDA) and some of its analogs have antitumor, uricogenic, and teratogenic activity. In general, these effects are reversed by nicotinamide. Although ATDA is not extensively metabolized in animals, a portion is apparently converted to an $NAD^+$ or nucleotide analog that is a potent inhibitor of IMP dehydrogenase. Inhibition of this enzyme is probably related to the increased de novo synthesis of uric acid that has been observed in man and in chick embryos. The moderate activity of ATDA against experimental tumors has led to clinical trials in man.*

## Introduction

The unusual ring structure of 2-amino-1,3,4-thiadiazole (ATDA) and related compounds (Fig. 1) and their effect in a number of experimental tumor systems have attracted considerable interest in ATDA as a cancer chemotherapeutic agent. The objective of this review is to assemble, in one place, the pertinent biological information available on aminothiadiazoles. Such information should be useful in designing drug schedules and drug combinations involving ATDA.

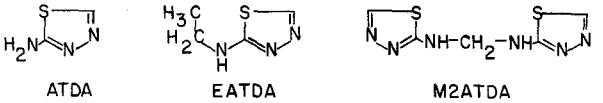


**Fig. 1.** Structures of ATDA, EATDA, and M2ATDA

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## Toxicity

In general, ATDA and related thiadiazoles have little antibacterial, antiprotozoal, or antifungal activity [6, 14, 17, 44]. ATDA has been used effectively, however, to control leaf blight caused by *Xanthomonas oryzae* in rice [47]. The activity is reversed by nicotinamide. Against *Saccharomyces carlsbergensis* and *Streptococcus faecalis,* ATDA has a moderate inhibitory effect; but 2-ethylamino-1,3,4-thiadiazole (EATDA, Fig. 1) has none [14]. EATDA is active, however, against the anaerobic microbes, *Bacteroides pseudoramosus,* and *Clostridium cylindrosporum* [6].

To two mammalian cell lines in culture, EATDA was not toxic, but ATDA was inhibitory at concentrations in the range of $10^{-5}$ to $10^{-6}$ *M* [14].

In mice, the $LD_{50}$ for ATDA is 235 mg/kg for a single IV dose and 1,910 mg/kg for an oral dose [35]. The difference indicates that the compound is poorly absorbed from the gastrointestinal tract. Pathological lesions are noted in the liver, kidneys, and spleen of mice treated with toxic doses. As a single IV injection, the highest nontoxic dose to beagle dogs was 25 mg/kg [35]. Toxic signs noted in both dogs and monkeys were weight loss, emaciation, rectal hemorrhage, salivation, and hypothermia. There were also a decrease in the number of leukocytes and biochemical and pathological evidence for hepatotoxicity and nephrotoxicity. There was no change in the concentration of uric acid in the blood, perhaps due to the presence of uricase in these animals [13].

Both ATDA and EATDA cause birth defects in rats [25]. ATDA given on days 5 and 9–10 of gestation is most toxic to embryos [2]. Resorption of fetuses occurs, and, of those remaining, up to 90% have malformations [37]. Synthesis of DNA in the embryos in inhibited by ATDA [37]. If given within

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12 h following administration of ATDA, nicotinamide and other precursors of $NAD^+$ decrease both the lethality and teratogenicity [2, 4, 37]. In these embryos, cells at the caudal end of the neural tube are killed by ATDA [3]. No teratogenicity of ATDA and EATDA is noted in the chick embryos, but their lethality to chick embryos is reversed by nicotinamide [20, 28]. The teratogenic effects of 2,2′-(methylenediimino)bis-1,3,4-thiadiazzole (M2ATDA, Fig. 1) in the hamster are reversed by nicotinamide or tryptophan, a precursor of nicotinamide [27].

In patients, limiting toxicities of EATDA were glossitis and hyperuricemia, both reversible by nicotinamide [21, 22].

## Disposition

Studies on the disposition of ATDA in experimental animals have been performed [12, 24]. An initial plasma half-life of 5 min and a terminal half-life of 10 h were observed in dogs [24]. In a more extensive investigation [12], complex pharmacokinetics were observed. In dogs and monkeys injected with 3 mg/kg, serum levels decreased linearly over a period of several hours. A possible interpretation of these results is that the drug is subject to glomerular filtration, tubular reabsorption, and secretion into the proximal tubules by an active process. Since ATDA does not bind to serum proteins, glomerular filtration would be expected. If secretion into the proximal tubules involves a process saturated at the amounts of ATDA that are present for several hours, removal from the blood would be a linear process. For a monkey given a larger dose (30 mg/kg) an exponential phase preceded the linear phase, while following a smaller dose (0.3 mg/kg) only an exponential phase was obvious. In both dogs and monkeys the serum concentration of metabolites was low and nearly constant [12]. Much of the drug was excreted unchanged in the urine. The distribution of ATDA in tissues of dogs and monkeys was, to a large extent, uniform.

For mice injected IP with 100 mg/kg, serum levels decreased logarithmically with a half-life of 2.9 h, and tissue distribution was fairly uniform [12]. Again, most of the drug was excreted without alteration.

## Biochemistry

The first notable biochemical information on aminothiadiazoles was published in 1956 [20]. During a clinical trial of EATDA prompt and marked elevations of serum uric acid and uric acid excretion were noted after each course of therapy with the agent. The level returned to normal when doses were discontinued. The parallel increases in the uric acid content of serum and urine allowed the investigators to rule out any possible renal effects on the uric acid content of serum.

The increased level of uric acid in the serum of patients given EATDA was subsequently confirmed, and increased incorporation of [$^{14}$C]glycine and [$^{14}$C]formate into uric acid was demonstrated by administering these compounds in the presence of EATDA [21, 38]. Doses of nicotinamide equal to or greater than that of EATDA completely prevented the increase in serum uric acid level, the increase in uric acid excretion, and the associated glossitis [21, 38]. Results with ATDA in patients were similar to those for EATDA [21]. 2-Acetylamino-1,3,4-thiadiazole was also uricogenic in man, but 2,5-diamino-1,3,4-thiadiazole was not. 6-Diazo-5-oxo-L-norleucine (DON) and diazoacetyl-L-serine (azaserine), glutamine antagonists known to block de novo biosynthesis of purines, prevented the uricogenic effects in man but not the oral toxicity [21].

For a patient maintained on a purine-free diet, the incorporation of [$^{14}$C]formate into uric acid was markedly increased during treatment with EATDA [21], but when EATDA and DON were given together the incorporation was similar to that seen during a control period. DON alone did not reduce the incorporation of [$^{14}$C]formate.

In another experiment, a patient was first given [$^{14}$C]adenine to label body purines [21]. At 1 day following a dose of EATDA, [$^{15}$N]ammonium chloride was given. The $^{15}$N was rapidly incorporated into uric acid, and, simultaneously, the specific activity of [$^{14}$C]uric acid in the urine fell rapidly, owing to the increased synthesis of uric acid not labeled with $^{14}$C but with $^{15}$N. Later, another dose of [$^{15}$N]ammonium chloride was administered without EATDA. In this case, the incorporation of $^{15}$N into uric acid was much less, and there was no effect on the content of urinary [$^{14}$C]uric acid. The fact that [$^{14}$C]formate and [$^{15}$N] ammonia were incorporated into uric acid faster after EATDA indicated that uric acid production de novo was increased. The increased concentrations of uric acid in the serum and urine did not come from the breakdown of nucleic acids in the tumors, for there was no evident regression of tumors and no increase in nitrogen excretion. Further, uric acid levels in serum and urine rose in a patient without apparent neoplastic disease but given EATDA [21].

The failure of DON and azaserine to inhibit normal uric acid synthesis in man [21] and their failure to reverse oral lesions caused by EATDA while inhibiting the uricogenic effect may mean that

the action of thiadiazoles is complex, involving more than one metabolic pathway or the de novo purine pathway at multiple levels. Further, DON and azaserine apparently block the increased uric acid production without affecting the related nicotinamide deficiency, leading to glossitis.

In an investigation of the effect of thiadiazoles on the purine metabolism in chick embryos, it was found that ATDA, EATDA, and *N*-acetyl-ATDA increased uric acid production [20]. 2,5-Diamino-1,3,4-thiadiazole and *N*-acetyl-ATDA-5-sulfonamide were inactive. Nicotinamide, tryptophan, and $NAD^+$ were effective in reversing the uricogenic action of EATDA. DON, azaserine, and methotrexate all decreased the uric acid content of thiadiazole-treated eggs, but the uric acid content of controls was not reduced. 6-Mercaptopurine was without effect, for it has no effect on purine synthesis in the chick embryo. In eggs injected with [$^{14}$C]formate and EATDA, there were increases in the radioactivity of hypoxanthine, inosine, xanthine, uric acid, allantoin, and serine, as compared with eggs not treated with EATDA.

6-Aminonicotinamide and 3-acetylpyridine, analogs of nicotinamide that are incorporated into $NAD^+$ by exchange, reversed the effect of EATDA in increasing the uric acid content of eggs [20]. 5-Fluoronicotinamide, however, had no activity in this regard. Insulin and sulfanilamide, compounds causing teratogenic effects in chick embryos and reversed by nicotinamide, had no activity in blocking the effects of EATDA.

ADTA strongly inhibited the incorporation of [$^{14}$C]formate and [$^{14}$C]glycine into the guanine of chick embryos [11], but incorporation of the precursors into uric acid was stimulated. The xanthine oxidase inhibitor, allopurinol (4-hydroxy-[3,4-*d*]pyrazolopyrimidine), lowered the extent of incorporation of the precursors into uric acid in the presence of ATDA. In an effect probably related to stimulation of purine synthesis, EATDA increased the content of 5-phosphoribosyl 1-pyrophosphate in mouse liver by 10- to 12-fold [23].

In mice, a high dose of EATDA inhibited, but only marginally, the increase in liver content of $NAD^+$ following a dose of nicotinamide, indicating that EATDA was not a potent, competitive antagonist of nicotinamide for the synthesis of $NAD^+$ [40]. Nevertheless, an in vitro exchange of EATDA with the nicotinamide moiety of $NAD^+$ has been demonstrated [9]. For preparation of the EATDA analog of $NAD^+$, the thiadiazole was incubated with $NAD^+$ in the presence of pig brain NADase, and the product was separated by electrophoresis. This analog has not been detected in tissues.

A brief report [15] states that ATDA at a relatively high concentration (0.5 m*M*) inhibits poly(adenosine diphosphate ribose)polymerase from baby-hamster kidney cells. This enzyme is capable of covalently binding the ADP-ribose moiety of $NAD^+$ to nuclear proteins, with the concomitant elimination of nicotinamide [18, 43], and of adding other ADP-ribose units to the first one by a $2'-1'$ glycosidic bond, yielding poly(ADP-ribose). It remains to be determined if such inhibition is important physiologically.

The effects of ADTA on ribonucleotide pools of L1210 ascites cells in vivo have been investigated [29]. The most pronounced effects were the lowering of guanine ribonucleotides and an elevation of IMP, results reversible by the simultaneous administration of nicotinamide. The changes were similar to those for mycophenolic acid, a known inhibitor of the conversion of IMP to GMP, and suggested that a primary site of action of ATDA was on IMP dehydrogenase, an enzyme important in purine nucleotide interconversion and subject to inhibition by agents of varying structure. In addition, there is evidence that this enzyme may be one of the rate-limiting enzymes in nucleic acid biosynthesis, for its absolute activity is very low, even in rapidly dividing tissues, and is correlated with rate of cell proliferation [19].

In a subsequent study, the $NAD^+$ analog containing ATDA was prepared and cleaved with pyrophosphatase to yield an ATDA-containing nucleotide for testing as an inhibitor of IMP dehydrogenase [30]. Whereas the $NAD^+$ analog was a noncompetitive inhibitor ($K_i$ = 20 μ*M*) with respect to IMP, the nucleotide analog was competitive, with a $K_i$ of 0.1 μ*M*. Both the $NAD^+$ analog and the nucleotide analog were noncompetitive with respect to $NAD^+$. Ackermann-Potter plots showed that the inhibition by the $NAD^+$ analog was pseudoirreversible but that for the nucleotide analog was reversible.

In L1210 cells in mice, a compound having the same retention time in a high-pressure liquid chromatography system as the nucleotide analog was formed from [$^{14}$C]ATDA [30]. The formation of this compound was prevented by nicotinamide. There was no evidence, however, for an $NAD^+$ analog in cell extracts, leaving the route for synthesis of the nucleotide unknown. The position of attachment of ribose to ATDA in the nucleotide is also unknown. A reasonable explanation for the absence of an intact $NAD^+$ analog containing ATDA or EATDA in treated cells is that the analogs may be better substrates for intracellular nucleotide pyrophosphatase than $NAD^+$. Further, $NAD^+$, but not the

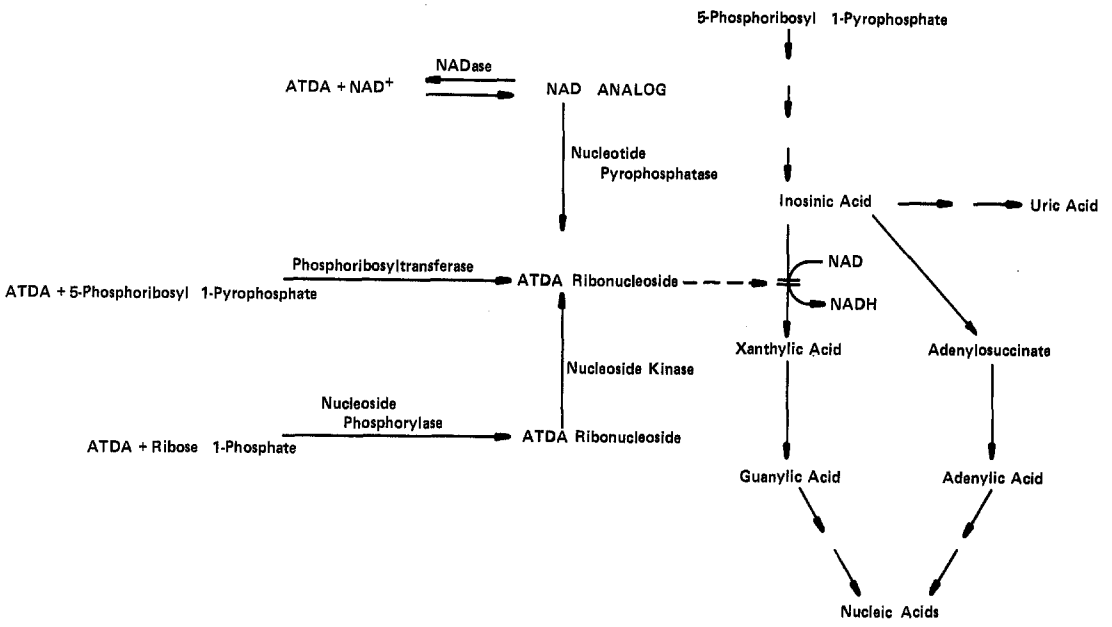


**Fig. 2.** Possible pathways for metabolism of ATDA and its site of inhibition on the pathway for purine nucleotide biosynthesis

analogs, may reach a compartment where it is protected from pyrophosphatase activity.

Possible routes of synthesis of the nucleotide analog containing ATDA (or EATDA) involve direct conversion to the nucleotide by a phosphoribosyltransferase and a sequential process involving a nucleoside phosphorylase and a nucleoside kinase (Fig. 2). Mammalian cells are known to possess nicotinamide ribosyltranferase [10], nicotinamide ribonucleoside phosphorylase [36], and nicotinamide ribonucleoside kinase [31]. However, with rat liver, which readily converted nicotinamide to the ribonucleotide, and leukemia L1210 preparations the conversion of ATDA to a nucleotide has not been detected (Bennett LL Jr, unpublished results). Further, nucleoside formation was not apparent when the same preparations were incubated with ATDA and ribose 1-phosphate. Most likely, the fraudulent nucleotides are formed following exchange of the thiadiazole with nicotinamide of the $NAD^+$ molecule and subsequent cleavage by nucleotide pyrophosphatase.

Virazole, 1-(beta-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide, is an antiviral agent metabolically converted to a monophosphate that is a potent inhibitor of IMP dehydrogenase, competitive with IMP [42]. The thiadiazoles apparently have a similar action.

Since the effects of EATDA, *N*-acetyl-ATDA, and M2ATDA are similar to ATDA and are also reversed by nicotinamide, it appears likely that their mechanisms of activation and action are the same as those for ATDA.

The mechanism of action of M2ATDA has been investigated in Japan [46]. For BALB/3T3 cells in culture, inhibition of growth was reversed by either guanosine or deoxyguanosine. Incorporation of [$^3$H]thymidine and [$^3$H]uridine into nucleic acids was strongly inhibited by M2ATDA, more quickly and up to 15 times more effectively than for ATDA. Incorporation of [$^3$H]leucine into proteins was not affected. The inhibition of nucleic acid synthesis was reversed in a competitive fashion, and evidence indicated that RNA and DNA synthesis was inhibited through a common mechanism. Incorporation of label from [$^{14}$C]hypoxanthine into acid-soluble or acid-insoluble guanine was decreased, but incorporation into adenine was not. Formaldehyde, possibly released from M2ATDA, had no effect on precursor incorporation into DNA. Because M2ATDA is more lipophilic than ATDA, it seems likely that M2ATDA more readily enters cells and then breaks down or is converted enzymatically to ATDA.

## Antitumor Activity

Against a variety of experimental tumors, ATDA was found to have modest activity. The S91 melanoma, 8110 glioblastoma, and 6C3HED lymphosarcoma all responded [34, 41]. Methyl, ethyl, alkyl, or acetyl substitution on the amino group led to less active compounds; the corresponding phenyl compound was inactive. 5-Hydroxy-ATDA was active against the melanoma and glioblastoma, but the

5-mercapto and 5-chloro analogs were not [34]. ATDA, EATDA, and *N*-acetyl-ATDA had activity against mammary adenocarcinoma, sarcoma 180, and leukemia P1534 in mice [39, 45]. The tumor inhibition was reversed by nicotinamide or nicotinic acid but not by tryptophan plus pyridoxine. The compounds were more effective in rats and mice fed a diet deficient in nicotinic acid. ATDA and EATDA also had activity against leukemia L1210 and leukemia 8174 in mice [7, 9, 16]. Nicotinic acid blocked the toxicity and antileukemic effect.

A number of analogs of nicotinamide were effective in reversing the antileukemic activity of ATDA against mouse leukemia B82 [32]. These were 5-fluoronicotinamide, 6-aminonicotinamide, *N*-hydroxymethylnicotinamide, *N,N'*methylenedinicotinamide, $NAD^+$, and 3-acetylpyridine. In contrast, isonicotinamide potentiated the effects of ATDA against this leukemia and also leukemia L1210 [33]. Having a similar potentiating effect against leukemia B82 were *N*-hydroxymethylisonicotinamide, thioisonicotinamide, 6-aminopicolinamide, 2-acetylaminoisonicotinamide, and acetylaminopicolinamide. Isonicotinic acid and 6-aminopicolinic acid were without activity in this regard. In each case of augmented activity, host toxicity was also increased. Either nicotinamide or nicotinic acid blocked the antitumor activity of the combination of ATDA plus isonicotinamide.

Activity for M2ATDA was substantial against leukemia L1210, 6C3HED lymphosarcoma, C1498 myeloid leukemia, Ehrlich ascites carcinoma, sarcoma 180, B16 melanoma, and X5563 myeloma but was marginal against two hepatomas [26]. M2ATDA also suppressed production of serum antibody in mice in response to sheep erythrocytes and the graft-versus-host response in mice [26]. It was about ten times more effective on a molar basis than ATDA for both antitumor and immunosuppressive activity. These activities for M2ATDA were prevented by simultaneous administration of an equimolar amount of nicotinic acid.

M2ATDA also inhibited the growth of bovine adenovirus type 3 and murine sarcoma virus and prevented the characteristic changes in the host cells [46].

A clinical trial of ATDA resulted in no obvious responses in a total of 17 patients treated [21], but this trial is now viewed as inadequate and phase I trials are proceeding [1].

Perhaps ATDA, given in combination with other inhibitors of the purine pathway, would provide more effective chemotherapy. Possibilities are combinations with methotrexate, which inhibits at two sites on the pathway leading to nucleotide formation [5], and 6-mercaptopurine, which has an important site of action on the first step of the pathway [8].

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