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# Clinical and clinical pharmacologic studies of 2-amino-1,3,4-thiadiazole (A-TDA : NSC 4728)*

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**Summary.** A clinical phase I–II evaluation of 2-amino-1,3,4-thiadiazole (A-TDA) administered daily, twice a week, or weekly was undertaken, in which 71 patients were treated with a range of doses from 2 mg/m$^2$ to 200 mg/m$^2$. Pharmacokinetic studies employing high-performance liquid chromatography (HPLC) demonstrated a terminal *(β)* serum half-life of 2.19 h.

Stomatitis, dermatitis, nausea, vomiting, and lethargy were observed. No significant leukopenia or thrombocytopenia, however, was noted. A-TDA administration led to hyperuricemia, which was adequately controlled with concurrent administration of allopurinol.

Antitumor responses included one partial response in a patient with large cell carcinoma of the lung and three objective responses (2 non-small cell lung and 1 squamous cell carcinoma of the esophagus). Two patients with adenocarcinoma of the lung had a marked improvement of psoriasis during A-TDA therapy. Further phase II studies in patients with cancer and trials in patients with psoriasis are recommended.

## Introduction

The antitumor and uricogenic properties of 2-substituted thiadiazoles have been recognized for nearly 30 years [7]. The antitumor effect of 2-ethylamino-1,3,4-thiadiazole (EA-TDA) in murine systems and the inhibition of this effect by nicotinamide were first reported in 1955 [11]. Clinical trials of another member of this class of compounds, 2-amino-1,3,4-thiadiazole (A-TDA), were also limited by marked hyperuricemia as well as painful stomatitis [6]. Nicotinamide was shown to prevent both of these clinically limiting toxic effects, but also abolished the antitumor effect in experimental systems.

Since those early studies, concomitant administration of the xanthine oxidase inhibitor, allopurinol, has prevented the limiting toxic effect of hyperuricemia [5]. Nelson et al [9, 10] have presented data which support the concept that both the antitumor and the uricogenic effects of A-TDA are related to an inhibition of inosine monophosphate dehydrogenase activity. With a better understanding of the mechanism of action of this drug and the availability of pharmacologic methods to control hyperuricemia, clinical interst in A-TDA and related thiadiazole nucleoside compounds has received renewed attention.

## Materials and methods

A total of 71 patients were treated with A-TDA at the Vermont Regional Cancer Center of the University of Vermont, 42 patients receiving the drug in a phase I trial and 29 being treated in a subsequent phase II trial directed at non-small cell lung cancer. Patient characteristics and tumor types for the two studies are shown in Table 1.

To be eligible for the phase I portion of the study, patients had to have histologically proven cancer that had failed to respond to standard therapy or for which there was no therapy with a reasonable expectation of benefit. Informed consent consistent with the standards of the University of Vermont was obtained from all patients. It was necessary for patients to have a serum bilirubin of 2 mg% or less, serum creatinine of 2.5 mg% or less, a platelet count of at least 100000/mm$^3$, and a white blood cell count of at least 3500/mm$^3$.

A-TDA was supplied by the Investigational Drug Branch of the National Cancer Institute as a lyophilized powder. The drug was reconstituted with sterile water to a final concentration of 10 mg/ml and administered IV over 5–10 min.

In the initial, dose-finding (phase I) portion of this clinical trial, A-TDA was given in a dose of 12 mg/m$^2$ daily. This was based on one-third of the Toxic Dose Low observed in preclinical toxicologic tests performed in rhesus monkeys [13]. Administration was projected for 5 consecutive days. However, in the first patients treated, stomatitis occurred after 2 days in one patient and 4 days in another. This stomatitis was severe and limiting. Therefore, the frequency of drug administration was decreased. As shown in Table 2, limiting stomatitis occurred in approximately 50% of patients given A-TDA twice weekly at all doses from 12 to 125 mg/m$^2$. On a weekly schedule, it was possible to give 125 mg/m$^2$ with less frequent and less severe stomatitis. That dose and schedule were used in nine patients and

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**Table 1.** Patient characteristics

| | *Phase I* | *Phase II* |
|---|---|---|
| Number of patients in study | 42 | 29 |
| Male | 27 | 20 |
| Female | 15 | 9 |
| Median age (range) | 61 (33–77) | 59 (41–79) |
| Median Karnofsky performance status (range) | 60 (30–90) | 70 (50–90) |
| Prior treatment | | |
| Radiation and chemotherapy | 14 | 2 |
| Radiation alone | 14 | 13 |
| Chemotherapy alone | 11 | 0 |
| None | 3 | 14 |
| Tumor types | | |
| Non-small cell carcinoma of the lung | 19 | 29 |
| Breast carcinoma | 4 | |
| Ovarian carcinoma | 3 | |
| Adenocarcinoma of the rectum | 3 | |
| Osteosarcoma | 2 | |
| Gastric adenocarcinoma | 2 | |
| Renal cell carcinoma | 2 | |
| Adenocarcinoma – unknown primary | 1 | |
| Bladder carcinoma | 1 | |
| Synovial sarcoma | 1 | |
| Prostate carcinoma | 1 | |
| Endometrial carcinoma | 1 | |
| Melanoma | 1 | |
| Esophageal squamous cell carcinoma | 1 | |

**Table 2.** Phase I courses and toxicity

| Dose ($mg/m^2$) | No. of patients | Total no.[a] of doses | No. of patients with stomatitis | No. of patients with dermatitis |
|---|---|---|---|---|
| 2 qd × 5 | 1 | 5 | – | – |
| 5 qd × 5 | 1 | 5 | – | – |
| 12 qd × 2 | 2 | 4 | 1 | – |
| 12 qd × 4 | 1 | 4 | 1 | – |
| 12 qd × 5 | 2 | 10 | – | 1 |
| 12 2/week | 2 | 8 | 1 | 2 |
| 18 2/week | 5 | 16 | 1 | 1 |
| 27 2/week | 9 | 42 | 5 | – |
| 36 7/week | 7 | 27 | 3 | – |
| 50 2/week | 12 | 35 | 3 | 1 |
| 75 2/week | 12 | 40 | 6 | 5 |
| 125 2/week | 7 | 21 | 5 | 2 |
| 100 weekly | 1 | 3 | – | – |
| 125 weekly | 9 | 44 | 3 | – |
| 150 weekly | 3 | 5 | – | – |
| 175 weekly | 1 | 5 | – | – |
| 200 weekly | 2 | 2 | 2 | – |

[a] Some patients were treated with more than one dose level. The number of doses per patient was variable, depending on toxicity, dose escalation, and behavior of the underlying malignancy

recommended for further phase II studies, although slightly higher doses (150 and 175 $mg/m^2$) were tolerated in four patients. When 200 $mg/m^2$ was given in a single dose it produced severe, unacceptable toxicity in two patients.

Initial treatment was with A-TDA only. Serum uric acid measurements were obtained each day prior to therapy. Allopurinol was given only if the serum uric acid was elevated to greater than 10 mg%. Hyperuricemia uniformly occurred even at the lowest administered doses, and subsequently, all patients received allopurinol (100 mg t.i.d.) prior to and throughout A-TDA therapy. For those patients who experienced severe stomatitis, nicotinamide (100 mg PO t.i.d.) was given. Patients were cautioned to avoid multivitamin preparations containing niacin or nicotinamide.

*Assay of A-TDA.* Blood samples were obtained at specified time points after the injection of A-TDA. Samples were

centrifuged at 600 *g* for 10 min, and the serum was stored for up to 1 week at −20 °C prior to assay of drug levels. The concentration of A-TDA in serum was determined by a combined thin-layer and high-performance liquid chromatographic (HPLC) method. The assay and the quantitation of A-TDA in human serum have been described elsewhere [1]. This method was specifically designed for the analysis of A-TDA and permits assay of A-TDA in fluids containing as little as 400 ng A-TDA/ml as well as separation and quantitative analysis of nicotinamide and allopurinol.

Human serum samples were prepared for TLC by addition of 9 volumes of acetonitrile. After centrifugation (600 *g*, 10 min at 5 °C), the supernatant was collected and brought to dryness under vacuum with air purging at 37 °C using a Fisher IMD sample concentrator (Fisher Scientific, Pittsburgh, Pa). The residue was reconstituted with 0.4 ml HPLC-grade water. The samples (50 µl aliquots) were manually spotted onto Avicel-F (250 µm particle size; Analtech, Newark, Del) TLC plates along with the appropriate A-TDA, nicotinamide, allopurinol and uric acid standards.

TLC plates were developed at ambient room temperature in a closed chamber over a 5-h period using a solvent system of 2-propanol-water (70:30, v/v). This procedure resulted in $R_f$ values of 0.42 for uric acid, 0.78 for allopurinol, 0.80 for A-TDA, and 0.92 for nicotinamide. UV (254 nm) absorbing spots, which corresponded to uric acid or nicotinamide standards, were scraped separately from the plate, eluted in 0.4 water, sonicated and analyzed individually with the HPLC system. The single spot containing both A-TDA and allopurinol was likewise scraped from the plate, eluted in 0.4 ml water with sonication, and injected onto the HPLC apparatus.

HPLC conditions included a mobile phase consisting of an isocratic system of water-methanol (99:1, v/v), and the paired-ion reagent 1-octanesulfonic acid (buffered to a pH of 3.0) at a final concentration of 5 m*M* of each of the four compounds. The concentration was measured by UV absorption at 254 nm, which was considered optimal for A-TDA. Quantitative analysis was based on peak areas which were computed using a preset integration program in the software data system of Spectra Physics Model 8000 microprocessor-controlled high-performance liquid chromatograph equipped with a data system. The HPLC was equipped with a Schoeffel Model 770 variable-wavelength UV detector set at 254 nm. The column (30 cm × 3.9 mm I. D.) was a 10-µm particle size reversed-phase $C_{18}$ µ Bondapak (Waters Assoc.). A guard column (7 cm × 0.2 mm I. D.), packed with Co:Pell ODS, particle size 30–38 µm (Whatman, Clifton, NJ) was installed to protect the main column. Samples were injected onto the column through a 100-µl loop using a manual injector.

*A-TDA pharmacokinetic parameters.* Aminothiadiazole serum disappearance was essentially biphasic, and calculations of basic pharmacokinetic parameters were based upon an open 2-compartment model according to the formula: $C = C_0^A e^{-\alpha t} + C_0^B e^{-\beta t}$ where C is the serum concentration at any time (t), $C^A$ and $\alpha$ are constants for the function representing drug elimination [1]. The volume of distribution ($V_d$) was calculated using the formula: $V_d = D/\beta\ (C_0^A/\alpha + C_0^B/\beta)$, where D is the dose of administered drug.

## Results

### *Toxicity*

The incidence of stomatitis and skin rash is shown with dose levels used in Tables 2 and 3. Stomatitis was the most frequent and severe side effect, resulting in dose and schedule limitation in the phase I portion of the study. One presentation was that of a diffuse erythematous glossitis with ulcerations along the lateral margins of the tongue. This was noted in patients with good dentition as well as those with carious teeth or no teeth at all. Other patients experienced an angular cheilitis with secondary infection in the most severe cases. Several patients also had generalized erythema of the buccal mucosa with minimal involvement of the pharynx and palate.

Oral toxicity most commonly occurred within the first 24–36 h after administration of A-TDA and was most severe between 48 and 72 h. There was usually rapid resolution of mouth soreness after 3 days, but occasional patients required up to 10 days for complete healing. All forms of oral toxicity were painful, and there was often generalized mouth soreness without visible lesions.

Retreatment with A-TDA prior to complete resolution of mouth soreness caused more severe oral toxicity. There

**Table 3.** Phase II courses and toxicity

| Dose (mg/m²) | No. of patients | No. of patients with stomatitis | No. of patients with dermatitis |
|---|---|---|---|
| 125 weekly × 1 | 4 | 1 | 1 |
| × 2 | 9 | 2 | 1 |
| × 3 | 3 | – | – |
| × 4 | 4[b] | – | – |
| × 5 | 2 | – | – |
| × 6 | 3[b] | – | – |
| × 7 | 2[a] | 1 | 1 |
| × 9 | 1[a] | – | 1 |
| × 10 | 1[b] | – | – |

[a] Transient toxicity with each dose precluding escalation
[b] Escalation to higher dose in one patient

did not appear to be a simple relationship, however, between the degree of oral toxicity and the dose of A-TDA. Three patients with significant mouth pain were given nicotinamide without benefit. The use of allopurinol did not appear to influence the occurrence or severity of the oral toxicity. Oral toxicity was quite variable from patient to patient, and even with once-weekly doses some patients developed mouth soreness requiring treatment delay.

Six patients in the phase I trial and four in the phase II study experienced dermatitis, which occurred in two clinical patterns. Dermatitis was only seen in the phase I trial in those patients who received daily treatment, as was given in the initial patients, or at doses of 75 mg/m$^2$ or greater given twice weekly. Four patients had lesions which were 3–10 mm in size, well marginated, crusted, and superficially eroded. These occurred primarily on the face, but also on the trunk and scalp. Three patients developed seborrheic dermatitis with greasy scales on an erythematous base that was most severe in the eyebrows and nasolabial fold areas, but was also seen on the scalp, eyelids, and behind the ears. Both types of skin reaction appeared within 3 days after initiation of A-TDA administration and usually resolved within a week. Neither type of skin reaction was pruritic or painful.

Prior studies with A-TDA and related thiadiazoles had shown that hyperuricemia could be expected in humans treated with the drug. Elevation of serum uric acid occurred in most patients not treated with allopurinol in this series. As expected, no significant elevation of serum uric acid was observed in patients treated concomitantly with allopurinol and A-TDA.

Other toxicities were mild and infrequent. Seven patients experienced mild nausea and vomiting occurring 6–12 h after treatment and resolving after 24 h. This occurred at a dose range of 75–125 mg/m$^2$. Six patients treated with 125 mg/m$^2$ weekly complained of malaise and sleepiness occurring 2–3 days after therapy. There was no evidence of A-TDA-induced myelosuppression. Two patients experienced minimal scalp alopecia at 75 and 150 mg/m$^2$. Although A-TDA is excreted principally in the urine, minor patient-to-patient variation of renal function did not appear to result in a quantitative change in toxicity.

*Pharmacokinetic studies*

The basic pharmacokinetic parameters determined after administration of a single IV dose of A-TDA at 50 mg/m$^2$ are shown in Table 4. In seven patients with normal renal and liver function, the average drug distribution half-life ($t_{1/2}\alpha$) was 4.96 min (range, 2.32–9.06 min) and the average drug elimination half-life ($t_{1/2}\beta$) was 2.19 h (range, 1.19–3.80 h). The volume of distribution of A-TDA in these patients averaged 23.09 l/m$^2$, which is close to total body water content.

Three patients who received 75 mg/m$^2$ A-TDA every 3 days had serial serum drug levels determined after the first and second doses. No serum A-TDA was detectable 72 h after the second drug dose, suggesting that serum drug accumulation is not likely with this dose and schedule of administration.

*Clinical response*

Forty-two patients were treated in a traditional phase I trial. In the phase I study, a patient with squamous cell carcinoma of the lung exhibited a less than partial response by measurement of chest X-ray lesions after a total dose of 550 mg/m$^2$ given as doses of 50, 75, then 125 mg/m$^2$ twice a week. The tumor progressed after 2 months despite continued therapy. Another patient with unresectable squamous cell carcinoma of the cervical esophagus experienced improvement in symptoms, with lessened dysphagia and minor improvement as measured by barium swallow after a total of 575 mg/m$^2$ of A-TDA given as 125, 75, or 100 mg/m$^2$ per dose. The disease progressed after 2 months despite continued treatment. It was determined that 125 mg/m$^2$ A-TDA given once a week was the maximum tolerated dose based on skin and mucosal toxicity and was, therefore, recommended as the starting dose for a phase II trial. Therefore, 29 patients with non-small cell carcinoma of the lung were then treated according to the phase II schedule, with dose regulation dictated by the severity of stomatitis.

In the phase II portion of the study, two patients with large cell carcinoma of the lung demonstrated responses to A-TDA. One patient had a less than partial response after receiving weekly doses of drug to a total of 1200 mg/m$^2$ over 9 weeks. In this patient, six doses of 125 mg/m$^2$ were administered without toxicity and the dose was increased to 150 mg/m$^2$ for three doses. The disease progressed despite continued treatment after 3 months from the time of maximal response. Another patient demonstrated a partial response by measurement of CXR lesions after two doses of 125 mg/m$^2$. The tumor progressed after 1 month of continued weekly treatment. Two patients with coincident psoriasis had improvement of their skin lesions while receiving A-TDA therapy.

**Table 4.** A-TDA Pharmacokinetic parameters

| Patient | Dose | | $t_{1/2}\alpha$ (min) | $t_{1/2}\beta$ [h] | Vd[a] (liters/m$^2$) |
|---|---|---|---|---|---|
| | mg/m$^2$ | mg | | | |
| 1 | 50 | 82 | 3.42 | 2.31 | 30.56 |
| 2 | 50 | 82 | 2.32 | 1.09 | 15.96 |
| 3 | 50 | 89 | 9.06 | 2.39 | 27.31 |
| 4 | 50 | 81 | 4.73 | 3.80 | 18.23 |
| 5 | 50 | 77 | 6.78 | 1.25 | 27.78 |
| 6 | 50 | 95 | 4.47 | 1.18 | 19.09 |
| 7 | 50 | 85 | 3.92 | 3.34 | 22.73 |
| | Mean | | 4.96 | 2.19 | 23.09 |
| | SE | | 0.856 | 0.411 | 2.10 |

[a] $V_d$ = Volume of distribution

## Discussion

The uricogenic effect of 2-amino-1,3,4-thiadiazole and related thiadiazole and thiazole compounds is no longer a limiting factor for this class of drugs in clinical cancer trials. Doses of A-TDA higher than those used prior to the availability of allopurinol have resulted in a dose- and schedule-limiting toxicity of oral mucositis but also in the attainment of objective clinical antitumor effect.

The human serum A-TDA half-life of 2.19 h determined in the present study is similar to that observed in mice in our laboratory and that reported by others [3]. This is in marked contrast, however, to the much longer A-

TDA half-life values of 6 h in dogs given 60 mg/m$^2$ [3] and 10 h in dogs given 500 mg/m$^2$ reported by Lu and Loo [8]. The pharmacokinetics of A-TDA are complex however, and appear to be dose-dependent. As reported by El Dareer [3], administration of a small dose of A-TDA (3.6 mg/m$^2$) to monkeys results in serum drug levels that decrease in an exponential fasion, with a half-life under 4 h, while for monkeys given a larger dose (36 mg/m$^2$), serum levels decreased linearly with a half-life of 13 h. Finally, monkeys given 360 mg/m$^2$ A-TDA exhibited both exponential and linear phases of drug elimination. As discussed by El Dareer [3], this type of dose-dependent kinetics suggests saturation of metabolic or, more likely, excretion mechanisms. Whether similar dose-dependent kinetics exist for humans remains to be determined.

2-β-D-Ribofuranosylthiazole-4-carboxamide (tiazofurin) has been shown by Jayaram et al [4] and Cooney et al [2] to form an analogue of NAD in which tiazofurin is substituted for nicotinamide. That analogue is a potent inhibitor of IMP dehydrogenase, as is the comparable analogue of A-TDA. In clinical studies at the Vermont Regional Cancer Center [12] tiazofurin has been shown to produce a uricogenic effect similar to that caused by A-TDA and it, too, is inhibited by allopurinol.

Preclinical studies of tiazofurin suggest that it may be a more effective antitumor agent than A-TDA. Therefore, it may be anticipated that tiazufurin and other compounds with the same mechanism of action may prove to have useful clinical activity in patients with cancer.

Although limited therapeutic antitumor activity was observed in the study reported here, the fact that some responses were seen in lung cancer has encouraged further phase II studies of A-TDA and has provided further impetus to the investigation of a series of related compounds.

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