Effects of Nicotinamide and Related Compounds on the Antileukemic Activity of 2-Amino-1,3,4-thiadiazole*

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SUMMARY

The effect of nicotinamide and a variety of nicotinamide analogs and related compounds on the activity of 2-amino-1,3,4-thiadiazole against transplanted mouse leukemia B82 has been tested. 5-Fluoronicotinamide, 6-aminonicotinamide, N-hydroxybis-methylnicotinamide, N-N'-methylene-bis-nicotinamide, DPN, and 3-acetylpyridine were as potent as nicotinamide and nicotinic acid in reversing the thiadiazole effect. Other nicotinamide analogs, the analogs of nicotinic acid, precursors and catabolic products of nicotinamide have not shown such activity. The results of this study have been compared with results obtained by another author in a different system. It has been concluded that the term “niacin antagonist” represents several different metabolic activities, all reversible by niacin.

2-Amino-1,3,4-thiadiazole (ATDA) and its derivatives have been shown to produce interesting biological effects. Oleson and his associates (16) demonstrated activity against melanoma S-91 of the DBA-line 1 mouse, glioblastoma 8110 of the A mouse, and C3HED lymphosarcoma of the C3H mouse. Burchenal and Dagg (2) reported 2-ethylamino-1,3,4-thiadiazole (EATDA) to be active against leukemia 8174 of the C58F1 mouse. Troy et al. (19) found ATDA to inhibit a glioblastoma, a melanoma, a lymphosarcoma, a mammary adenocarcinoma, Sarcoma 180, and leukemia P1534 in mice. Activity of EATDA against Adenocarcinoma 755 in C57BL mice was shown by Shapiro et al. (18), and Goldin et al. (6, 7) observed moderate inhibition of leukemia L1210 in the BDF1 mouse. A different effect was discovered by Krakoff, who demonstrated an increase in de novo synthesis of uric acid in man (11) as well as in the developing chick embryo (13). Since both the anti-neoplastic and the uricogenic effects could be reversed by nicotinic acid and nicotinamide, ATDA and EATDA are considered as niacin antagonists (18, 19).

We were surprised to find, however, that 6-aminonicotinamide, another niacin antagonist with antineoplastic activity (8, 17), reversed the tumor-inhibitory effect of ATDA. Recently, Krakoff et al. (12) reported that, in the chick embryo, 6-aminonicotinamide, 3-acetylpyridine, L-tryptophan, and di phosphopyridine nucleotide (DPN), as well as nicotinamide, counteract the uricogenic effect of EATDA. In addition, he found that 3,3-dimethyl-1-phenyltriazene potentiated the uricogenic effect of EATDA significantly (18). Subsequently, we have studied the influence of a variety of nicotinamide analogs and related compounds on the activity of ATDA against transplanted mouse leukemia B82.

MATERIALS AND METHODS

The evaluation of the chemotherapeutic activity of a given drug or drug combination was done in the following way. F1 hybrids of the BALB × C58 cross were given inoculations subcutaneously of one million cells of Leukemia B82 (4), suspended in 0.1 ml. of 0.9 per cent saline. Twenty-four hours later the mice were divided into groups of ten mice each. At the same time treatment of these groups was started. There was one untreated control
group for ten treated groups. The compounds were dissolved in 0.9 per cent saline or in 0.5 per cent carboxymethylcellulose in saline. The dilution was such that the volume to be given daily via intraperitoneal injection was 0.01 ml/gm mouse. After ten injections treatment was discontinued. Twelve to 14 days after inoculation the mice were sacrificed and autopsied and the subcutaneous tumors dissected out and weighed.

RESULTS

As shown in Chart 1, ATDA at 0.3 mM (30 mg/kg/day) produced 100 per cent tumor inhibition, which was completely reversed by nicotinamide or nicotinic acid at 0.2 mM (24 or 23 mg/kg/day, respectively). With one-tenth of these doses there was incomplete reversal of the thia diazole effect. It appears that N-hydroxymethylnicotinamide, substituted at the amino group, reversed the ATDA effect at dose levels comparable to those of nicotinamide on a molecular weight basis (Chart 1). N-N'-methylene-bis-nicotinamide appeared to be fully effective even at lower dose levels. This suggests that the compound is split,
than are the other derivatives. The effect of ATDA was reversed by 0.02 mmoles (2.8 mg/kg/day). Double or half this dose, however, had no reversing effect. The higher dose was toxic, producing tumor inhibition with pronounced weight loss, and the lower dose was too low to produce reversal. The reversing effect was rapidly lost with a slight decrease of the dose of 6-aminonicotinamide below the optimum of 0.02 mmoles/mg (Chart 2). With

5-fluoronicotinamide, a similar situation was found. There was clear reversal of the activity of ATDA with 0.2 mm (28 mg/kg/day). Lower as well as higher doses were less effective. In this case, the higher dose did not produce toxicity but was just in the lowest range to show slight antileukemic activity of its own (Chart 2). The optimum dose is 250 mg/kg/day for antileukemic activity of this compound (3).

In Table 1 the results of these experiments are summarized. The dose of nicotinamide producing a 50 per cent reversal of the ATDA effect is listed as one. The doses of the analogs or related compounds having the same effect or the highest doses tested which failed to have any effect are compared with this dose on a molecular weight basis. These figures are based on approximate dose levels and are intended only to give an impression of the order of magnitude.

Of the analogs, it appears that 5-fluoro-, 6-amino-, N-hydroxymethyl-, N-N'-methylene-bis-nicotinamide, and 3-acetylpyridine were equally as potent as nicotinamide. With 5-amino- and 5-methylnicotinamide, a tenfold dose was required to produce the same effect. The remaining compounds were ineffective even at this level. In contrast to the nicotinamide derivatives, none of the nicotinic acid analogs appeared to be as effective as nicotinic acid at the same, the tenfold, or sometimes even higher dose levels.

Of the compounds known to be involved in nicotinamide metabolism, only DPN appears to have an effect equal to that of nicotinamide. Of the precursors, L- and DL-tryptophan were ineffective up to a 200-fold dose, and 3-hydroxyanthranilic acid showed only slight activity at a 100-fold dose level. N-methylnicotinamide, N-methyl-6-pyridone-3-carboxamide, and nicotinuric acid, catabolic products of nicotinamide, failed to reverse the thiadiazole effect up to a 10- to 50-fold dose equivalent of nicotinamide.
DISCUSSION

A synopsis of these results and those of Krakoff reveals the following situation:

1. The antileukemic activity of ATDA is reversed by nicotinamide and nicotinic acid as well as by several nicotinamide analogs, some of which have other distinct biological effects.

2. 3-Acetylpyridine in combination with thiadiazole retains its own teratogenic and DPN-depressing effect but reverses the uricogenic as well as the antileukemic effect of thiadiazole. It does not possess antileukemic activity of its own.

3. 6-Aminonicotinamide in combination with thiadiazole retains its own teratogenic and DPN-depressing effect and reverses the uricogenic as well as the antileukemic effect of thiadiazole. It does have antineoplastic activity of its own.

4. 5-Fluoronicotinamide produces a deformity in the chick embryo without depression of DPN-levels and does not block the uricogenic thiadiazole effect in this system. At doses lower than those producing tumor inhibition when given alone, however, it reverses the antileukemic effect of thiadiazole.

5. 6-Aminonicotinamide reverses the antileukemic activity of thiadiazole but not that of 5-fluoronicotinamide.3

6. 3,3-Dimethyl-1-phenyl-triazene also has a teratogenic effect in the chick embryo reversible by nicotinamide. It markedly potentiates the uricogenic effect of thiadiazole (18). No such potentiation of the antileukemic effect occurs, however.

7. DL-Tryptophan reverses the uricogenic but not the antileukemic effect of thiadiazole.

8. DPN reverses the uricogenic as well as the antileukemic effect of thiadiazole.

It would appear that the relationships between these compounds are very complex. The term "nicotinamide antagonist" seems to represent an increasing variety of biological activities. Whether some of these effects are due to the formation of a fraudulent DPN, as suggested by Ciotti et al. (5), may be considered. It would appear most likely, however, that several metabolic lesions are involved.

REFERENCES


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