Clinical Experience with 6-Aminonicotinamide

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SUMMARY

The toxicity of 6-Aminonicotinamide in 60 patients with advanced malignant disease has been described. The toxicity took two clinical forms: a “deficiency” state with characteristics of a mixed B-complex vitamin deficiency, and a neurologic disturbance presumably due to a direct action on the central nervous system. Individual susceptibility was widely variable. 6-Aminonicotinamide exhibited a cumulative action. In general, toxicity could be considered a factor of total dosage. A safe daily dosage schedule has been established at 0.2 mg/kg of body weight for a 4-week period, and 0.4 mg/kg for a 2-week period. Within this tolerated dosage schedule 6-aminonicotinamide failed to exhibit any significant tumoricidal properties.

6-Aminonicotinamide (6-AN), a potent antagonist of nicotinamide (3), has shown promising activity against a number of experimental neoplasms, including the Walker carcinoma 256, mammary Adenocarcinomas 755 and C3H, and lymphosarcoma 6C5HED (2, 6, 9). A striking augmentation of the radiotherapeutic “cure” rate in the Adenocarcinoma 755 by simultaneous administration of 6-Aminonicotinamide and 6-mercaptopurine has been established (5, 6).

Although the exact mechanism of action of 6-AN has not been clearly established, it is thought to function as a true antimetabolite of nicotinamide (3), being incorporated into pyridine nucleotides (DPN and TPN) in place of nicotinamide to give the corresponding 6-AN analog. Not only does the formation of such pyridine nucleotide analog deplete the tissues of DPN and TPN, but these analogs also competitively inhibit enzymatic reactions in which pyridine nucleotides play a part as coenzymes. The presence of a 6-AN analog of DPN has been demonstrated in tissues of rabbits, rats, and mice treated with 6-AN, in yeast and bacteria grown in the presence of 6-AN, and in neoplastic tissue from rats bearing Walker carcinoma 256 (4). Recently, Dietrich1 has similarly proved the existence of a 6-AN analog in liver and tumor tissue taken from a patient treated with 6-AN.

The toxicity of 6-AN has been studied extensively in laboratory animals (1–3, 7, 8, 10–12).2-8'4 In all species investigated, the most prominent feature of intoxication was that of central nervous system damage, manifested by motor weakness, hind limb paralysis progressing to quadriplegia, coma, and death. Pathologically, specific degenerative changes in the gray matter of the anterior horns of the spinal cord and the nuclei of the brain stem have been demonstrated. In addition, splenic involution (atrophy of lymph follicles), adrenal, hepatic, and testicular focal degenerative changes, and atrophic lesions of the buccal and gastrointestinal mucosa have been observed. In some respects, these changes are similar to those seen in pellagra; the nervous system lesions, however, are quite unlike those reported in nicotinic acid-deficient animals. Hematologic depression, in the form of rapid disappearance of reticulocytes, and the reduction in the number of circulating leuko-

1 L. S. Dietrich, personal communication.
2 Research Laboratories of Frank W. Horner, Ltd., Montreal, Canada.
3 A. E. Earl, Ciba Pharmaceutical Laboratories, Summit, New Jersey.
4 J. Oleson, Lederle Laboratories, Pearl River, N.Y.
cytes (particularly lymphocytes), have been described in mice.

The LD$_{50}$ of 6-AN, when the drug is given intraperitoneally to mice, rats, and guinea pigs, has been established at 35, 11, and 10 mg/kg of body weight, respectively. Individual response, however, in each mammalian species studied, has been extremely variable. For instance, Sternberg (10), described five dogs given repeated daily doses of 4 mg/kg either intravenously or by mouth; one showed no disturbance after ten doses, two demonstrated cerebral excitation after a week and died at 8 and 18 days, and two became quadriplegic after 7 days of treatment, without signs of cerebral excitation. The cumulative effect of 6-AN has been established beyond doubt. Twenty-five per cent of rats given a daily dose of 1.0 mg/kg were dead in 12 days as a result of drug intoxication; dogs, on the other hand, survived as long as 30 days without signs of toxicity, at the same daily dosage. Complete protection against toxicity to 6-AN toxicity in rats has been provided by the simultaneous administration of nicotinamide; 50 mg/kg of nicotinamide brought about an eightfold increase in the LD$_{50}$ of 6-AN in mice (3), and in CFN male rats given 20 mg/kg of 6-AN (or about twice the LD 50) 50 per cent of the animals were fully protected by doses of 3.3 mg/kg of nicotinamide given intraperitoneally immediately prior to the antimetabolite (11). Toxic changes have likewise been reversed by large amounts of nicotinamide given immediately after the appearance of symptoms.

MATERIALS AND METHODS

During the past year, 60 patients at the Francis Delafield and Presbyterian Hospitals, all with advanced neoplastic disease, received 6-AN in varying dosages, and for varying periods of time. A wide variety of tumor types was included in this study, as shown in Table 1. The only criterion for patient selection was an anticipated survival time, in terms of the primary disease, of at least 4 weeks. Most patients, prior to and after 6-AN therapy, underwent a complete laboratory screening covering hematopoietic, renal, and hepatic systems. In addition, electroencephalography was carried out at periodic intervals in a number of individuals. When indicated, audiometric studies were done. Daily intake and output records were kept, as well as weekly weights when possible.

The drug was administered exclusively by the intramuscular route, on a unit per weight basis. The first group of patients studied received a daily dose of 0.2 mg/kg of body weight for a 2-week period. This dose was increased, in increments of 0.2 mg/kg, in successive patient groups. The highest daily dose administered was 1.5 mg/kg; toxicity at this level was such that further increase was considered unwarranted. Once a pattern of toxicity had been established for a 2-week course of treatment at the varying dosages, long-term (4-6-week) studies of drug tolerance were begun. It was apparent from the short-term (2-week) experience that any dosage higher than 0.2 mg/kg would not be tolerated by most patients over a longer period. Consequently, long-term investigation of 6-AN was restricted almost entirely to this initial level of 0.2 mg/kg.

It should be emphasized that the purpose of this study was to evaluate the toxicity of 6-aminonicotinamide, not its tumoricidal properties. As such, the results enumerated below relate primarily to drug tolerance. No specific efforts have been made to quantitate tumor changes, grossly or histologically, during and after administration of 6-AN. Inevitably, however, certain observations have been made relative to tumor regression or acceleration and to changes in the clinical status of these patients with reference to their underlying disease.

RESULTS

Considerable variability was noted in the response of patients to uniform amounts of 6-AN. In general, the more depleted the patient nutritionally, the more severe and immediate the toxic response, presumably owing either to lower initial levels of nicotinamide or of tryptophan (a nicotinamide precursor) in the body, or to impaired
detoxification of the drug within the liver. On the other hand, young individuals, without extensive weight loss or liver involvement, were able to tolerate relatively large amounts of 6-AN for considerable periods of time without evident toxicity.

In the majority of patients, however, a definite pattern of toxicity was noted, related in general to total drug dosage. At the initial dose level studied, 0.2 mg/kg, no serious toxicity was encountered during the first 4 weeks of administration, although four of the 30 individuals in this group exhibited a transient “deficiency” state from 7 to 24 days after 6-AN was begun. This took the form of a rather selective ocular disturbance: blepharitis, conjunctivitis, excessive tearing, burning, and photophobia. In only one instance were symptoms severe enough to warrant discontinuation of the drug. When 6-AN was continued at this dosage for over 1 month, more serious forms of toxicity related to the central nervous system were encountered. Of the ten patients who were given 0.2 mg/kg from 4 to 8 weeks, three developed some degree of 8th nerve damage, manifested by a gross or audiometrically detected hearing defect. In one, after 54 days of drug administration for a total dose of 691 mg., serious bilateral nerve deafness occurred which necessitated the use of a hearing aid. The other two patients, after 39 and 44 days of treatment, developed minimal hearing loss clinically; in each case, the defect was documented by audiometric studies. Despite the administration of large doses of nicotinamide (up to 1.5 gm., O.D.), reversibility of the nerve damage was not achieved in these individuals, although the follow-up period was relatively short.

At a level of 0.4 mg/kg (twelve patients) “deficiency” changes occurred earlier and with greater frequency. In addition to ocular signs, oral toxicity was noted for the first time. This took the form of “dry mouth,” progressing to true stomatitis with buccal ulceration, cheilosis, and glossitis. In two patients this dosage was continued beyond 14 days; one received 1380 mg. over a 75-day period, the other 1065 mg. over a 50-day span. Both passed through an early stage of blepharitis but began to develop, at 6 and 8 weeks, respectively, signs of central nervous system toxicity manifested by lethargy, tinnitus, and deafness. The effects of nicotinamide in reversing these changes could not be evaluated because of short survivals.

Seven patients received 6-AN in a daily dose of 0.6 mg/kg. Ocular and oral toxicity appeared rapidly in five; three patients, in addition, developed headache, vertigo, and tinnitus as early as 4 days after institution of therapy. These cerebral changes had disappeared entirely 2 weeks after cessation of drug. It is impossible to say whether this recovery was influenced by the large nicotinamide supplements provided. No long-term studies could be carried out in this group.

With a dose level of 0.8 mg/kg, and increased to 1.5 mg/kg, immediate reactions to 6-AN were noted with greater frequency. Nausea, vomiting, and headache occurred within minutes after each intramuscular injection in six of the fifteen patients in this group, necessitating discontinuation of the drug within 2-4 days. Marked disorientation occurred in three individuals after a week of therapy, and variable hearing loss was detected in an additional three patients, one of whom exhibited not only severe deafness, but tinnitus, disorientation, and ataxia as early as 6 days after the start of therapy (1.5 mg/kg). Strangely enough, two patients survived a 14-day course of 6-AN at 1.0 mg/kg without demonstrable toxicity of any kind, again demonstrating the wide variability of patient response.

No alterations in hematopoietic, hepatic, or renal function could be demonstrated in any of the patients studied.

**Electroencephalographic Studies**

In Table 2, electroencephalographic (EEG) changes in thirteen patients who received 6-AN are recorded. It is apparent that the incidence and degree of central nervous system change, as measured by the EEG, are related closely to dosage of 6-AN. At 0.2 mg/kg, despite prolonged administration, no EEG abnormality was observed. From 0.6 to 1.5 mg/kg, however, all patients demonstrated diffuse EEG abnormalities during treatment. No patient developed cerebral signs (disorientation, lethargy) without a corresponding EEG change. In two instances, on the other hand, an EEG abnormality was noted in the absence of clinical signs; this discrepancy was assumed to be due to the short duration of treatment (4-10 days).

Of interest is the fact that marked 8th nerve damage occurred selectively in two patients, there being no associated central nervous system signs or symptoms, and no EEG changes.

A typical EEG tracing is illustrated in Chart 1, showing a diffuse change throughout all leads, with reversion to a normal appearance 2 weeks after drug cessation and niacin administration. Where post-treatment tracings were obtained, this same reversibility was demonstrated.

**Audiometric Studies**

Studies on six patients with a clinical hearing deficit incurred during 6-AN administration re-
<table>
<thead>
<tr>
<th>Case no.</th>
<th>Diagnosis</th>
<th>Duration of 6-AN treatment (days)</th>
<th>Dose of 6-AN (mg/kg)</th>
<th>Hearing loss</th>
<th>Central nervous signs</th>
<th>EEG changes</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gastric</td>
<td>32</td>
<td>0.2</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>Died 1 day after treatment. Autopsy: liver moderately involved by tumor; brain not examined.</td>
</tr>
<tr>
<td>2</td>
<td>Breast</td>
<td>56</td>
<td>0.2</td>
<td>Marked</td>
<td>0</td>
<td>0</td>
<td>Developed blepharitis and stomatitis during treatment. Deafness still marked 6 mo. after treatment.</td>
</tr>
<tr>
<td>3</td>
<td>Endometrium</td>
<td>10</td>
<td>0.4</td>
<td>None</td>
<td>0</td>
<td>Grossly abnormal before 6-AN. Became less abnormal during and after treatment.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Colon</td>
<td>37</td>
<td>0.4</td>
<td>Marked</td>
<td>0</td>
<td>Mild, nonspecific</td>
<td>Died 3 weeks after 6-AN treatment. Autopsy showed massive liver metastases. Brain normal.</td>
</tr>
<tr>
<td>5</td>
<td>Cervix</td>
<td>10</td>
<td>0.4</td>
<td>None</td>
<td>Lethargic, poor coordination before treatment. No change during treatment.</td>
<td>Grossly abnormal 11 days after treatment started.</td>
<td>Died 2 months after treatment. Blepharitis and stomatitis during treatment.</td>
</tr>
<tr>
<td>6</td>
<td>Pancreas</td>
<td>74</td>
<td>0.4</td>
<td>Marked</td>
<td>Developed vertigo, lethargy, 2 mo. after treatment started.</td>
<td>Became grossly abnormal. No EEG obtained after 6-AN treatment.</td>
<td>Died 7 weeks after treatment. No clinical evidence of liver failure or CNS metastases.</td>
</tr>
<tr>
<td>7</td>
<td>Esophagus</td>
<td>15</td>
<td>0.4</td>
<td>0</td>
<td>Developed disorientation which cleared after treatment stopped.</td>
<td>Moderately abnormal to grossly abnormal during treatment. Follow-up EEG not obtained.</td>
<td>Died 3 mo. after treatment. Autopsy: no liver involvement; brain not examined.</td>
</tr>
<tr>
<td>8</td>
<td>Colon</td>
<td>5</td>
<td>0.6</td>
<td>0</td>
<td>Unsteadiness and lethargy. Cleared after treatment.</td>
<td>Became abnormal and cleared when treatment stopped.</td>
<td>Died 1 mo. after treatment. Toxic symptoms included blepharitis &amp; stomatitis. Autopsy: normal liver and brain.</td>
</tr>
<tr>
<td>9</td>
<td>Rectum</td>
<td>6</td>
<td>0.6</td>
<td>0</td>
<td>Disorientation, lethargy, and unsteadiness occurred during treatment and cleared after 6-AN stopped.</td>
<td>Became grossly abnormal and cleared when treatment stopped.</td>
<td>Died 1 mo. after treatment. Toxic symptoms included blepharitis and stomatitis.</td>
</tr>
<tr>
<td>10</td>
<td>Cervix</td>
<td>14</td>
<td>1.0</td>
<td>0</td>
<td>Disorientation and lethargy from 9th day on.</td>
<td>Diffusely abnormal</td>
<td>Terminal condition. Question as to whether CNS changes due to 6-AN or not.</td>
</tr>
</tbody>
</table>
revealed the same pattern of a mild to marked bilateral 8th nerve deafness, presumably on a toxic basis. Serial audiograms after cessation of treatment were not possible in any case, and we consequently cannot speak with any reliability concerning the reversibility of these changes. On the basis of three patients with post-treatment survival times of between 1 and 4 months, however, it was our impression that permanent 8th nerve damage had been inflicted. Improvement with massive nicotinamide therapy was minimal in two of these patients, and clinically absent in the third.

**TUMOR RESPONSE TO 6-AN**

No particular attempts were made to study tumor response to 6-AN. Gross measurements, serial biopsies, and x-ray studies were not routinely carried out to assess tissue changes, inasmuch as our principal concern was drug toxicity. The majority of these patients had widespread intra-abdominal disease which did not lend itself to accurate bedside evaluation; where tumors were palpable or visible, however, gross observations were, of course, made at frequent intervals.

A pathologically documented tumor response did not occur in any case. One patient, with a renal carcinoma metastatic to the inguinal area, showed apparent tumor regression during therapy, the large inguinal mass softening and diminishing in size. However, it was felt possible that this represented resolution of a hematoma in the necrotic tumor center. Autopsy examination of the tumor demonstrated necrosis, but it was impossible to link this definitely to 6-AN administration. A second patient, with an enormous retroperitoneal leiomyosarcoma, received a 4-week course of 6-AN at a daily dose of 0.4 mg/kg; not only did she feel vastly improved subjectively, with return of appetite and strength, but her abdominal girth measurement decreased slightly. Four months later she asked for a second course of 6-AN because of returning symptoms and abdominal enlargement, and again she enjoyed a significant symptomatic improvement as well as a measurable decrease in girth measurement. Her third course of 6-AN therapy, 3 months later, was not attended by the same success, however, and she finally died, 18 months after her tumor had been declared surgically unresectable, and 4 months after her last exposure to 6-AN. This was an isolated instance of apparent tumor response; in no other case was there even a suggestive change in tumor appearance, size, or consistency during administration of 6-AN.

Four patients with extensive metastatic hepatic involvement, who were running a persistently febrile course, owing, presumably, to tissue necrosis, showed an interesting response to 6-AN in the form of fever lysis. In each instance, the temperature elevation, which had been resistant to intensive and varied antibiotic therapy, returned rapidly to normal after 6-AN was begun. A normal temperature was maintained during drug therapy, and a return to pretreatment levels was observed after drug cessation. Whether this phenomenon was due to local tumor changes attendant upon 6-AN administration, or to a central antipyretic effect of the drug, is impossible to determine.

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**TABLE 2—Continued**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Diagnosis</th>
<th>Duration 6A-N treatment (days)</th>
<th>Dose of 6-AN (mg/kg)</th>
<th>Hearing loss</th>
<th>Central nervous signs</th>
<th>EEG changes</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Kidney</td>
<td>15</td>
<td>1.5</td>
<td>Slight</td>
<td>Disorientation, lethargy, from 7th day on.</td>
<td>Diffusely abnormal</td>
<td>Condition poor. Slight deafness noted during the 9th week of treatment along with disorientation. Blepharitis present after 6 days.</td>
</tr>
<tr>
<td>12</td>
<td>Sigmoid</td>
<td>7</td>
<td>1.5</td>
<td>Marked</td>
<td>Vertigo, disorientation, tinnitus, ataxia starting on 6th day.</td>
<td>Diffusely abnormal</td>
<td>Terminal. Blepharitis present on 6th day as well as CNS changes.</td>
</tr>
<tr>
<td>13</td>
<td>Colon</td>
<td>4</td>
<td>1.5</td>
<td>None</td>
<td>0</td>
<td>Diffusely abnormal</td>
<td>6-AN discontinued because of severe stomatitis and blepharitis.</td>
</tr>
</tbody>
</table>
DISCUSSION

It is apparent from the foregoing that the toxic response to 6-AN assumes two clinical forms, dependent on dosage and individual susceptibility. These may occur simultaneously, sequentially, or independently. The first, induced by prolonged administration of 6-AN in low dosage, emerges as the picture of a mixed B-complex vitamin deficiency. One would have reason to expect a pure nicotinic-acid deficiency, clinically resembling pellagra (although clinical pellagra is probably a mixed deficiency in itself). Such is not the case. The oral ulcerations, cheilosis, and glossitis are suggestive of pellagra, to be sure; but the ocular

signs, which are the first and most prominent features of the 6-AN-induced deficiency, resemble ariboflavinosis far more than they do pellagra. In addition, the dermatitis and diarrhea generally associated with pellagra are absent in these patients. These clinical data would support the contention that pellagra, as classically described in man, is a mixed, rather than a pure deficiency. It also suggests that 6-AN has wider biologic activity than was previously assumed. We have not attempted to prevent or reverse 6-AN toxicity by using vitamins other than nicotinic acid; the effect of riboflavin, in particular, on the 6-AN-induced ocular changes would be of considerable interest.

The second form of 6-AN toxicity, resulting from higher dosage, appears as the result of an apparently direct action on the central nervous system. Disorientation and lethargy are the most frequently encountered aberrations; however, severe headache and precipitous vomiting, of central origin, may follow closely the intramuscular administration of the drug. Of interest is the particular sensitivity of the 8th nerve to 6-AN. Bilateral nerve deafness, with or without associated tinnitus and vertigo, may be the only manifestation of central damage and, in fact, may develop in the presence of a normal electroencephalogram. At the dose levels observed during these studies, no instance of motor disability occurred, such as has been described in virtually all the acute toxicity experiments in laboratory animals. This is probably a factor of dosage rather than a true species difference.

There is convincing evidence that 6-AN exhibits a cumulative effect. The higher the daily

drug dosage, the more rapidly does toxicity manifest itself. The neurologic changes seen after a week at 1.0 mg/kg, for instance, approximate those that appear after 6 weeks of 6-AN administration at 0.2 mg/kg. In general, then, it can be said that toxicity is a factor of total drug dosage.

Although 6-AN is a highly toxic product, capable at high concentrations of severe, and probably irreversible, neurologic damage, it has a distinct clinical advantage in that it almost always gives warning, in the form of “deficiency” signs and symptoms, before dangerous levels are reached. Unfortunately, in the concentrations used in our studies and considered safe, 6-AN has failed to demonstrate appreciable tumoricidal properties. Its possible usefulness, therefore, as an isolated anticancer agent, is certainly limited by its toxicity. On the other hand, used in conjunction with other antimetabolites, 6-AN, in safe dosage, might play an important role.

CHART 1.—Electroencephalographic changes during administration of 6-aminonicotinamide. Treatment as follows:

3-6-58 PRE-TREATMENT 3-17-58 4TH POST-TREATMENT DAY 3-26-58 12TH POST-TREATMENT DAY

6-aminonicotinamide, 0.6 mg/kg, 3-8-58 through 3-13-58; niacin, 100 mg t.i.d., 3-14-58 through 3-26-58.
REFERENCES

Clinical Experience with 6-Aminonicotinamide

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