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Human tissue distribution of 4′-(9-acridinylamino)-methanesulfon-m-anisidide (NSC 141549, AMSA)

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Summary. *Concentrations of AMSA were determined by HPLC in autopsy tissue samples from five patients who had received the drug antemortem. Relative organ concentrations of AMSA varied from patient to patient; however, concentrations were generally highest in gallbladder, liver, and kidney, while low levels were generally but not invariably found in lung, testicle, muscle, fat, spleen, bladder, pancreas, colon, prostate, and brain. One patient with ventricular fibrillation and seizures had high tissue AMSA concentrations in myocardium, but low concentrations in brain. Another patient with seizures during treatment had high brain concentrations of AMSA. Relative organ concentrations were similar to those found in mice, except that mice have high AMSA concentration in their spleens whereas our patients did not, even when the spleen was infiltrated with leukemic cells. High tissue concentrations of AMSA were still present 2 weeks after treatment. AMSA concentration was lower in a renal oncocytoma (1.1 μg/g) than in surrounding kidney (2.4 μg/g).*

Introduction

The antineoplastic agent AMSA has been noted to have substantial activity against human acute leukemia [11] and there have also been sporadic reports of activity against various solid tumors, including carcinoma of the breast [9] and malignant melanoma [7, 8]. While myelosuppression is the usual dose-limiting toxicity [8, 11], hepatic dysfunction [11], seizures [10, 11], and ventricular fibrillation [6, 10, 11, 17] have also been noted.

In an attempt to determine whether these toxic manifestations could be explained on the basis of particularly high drug concentrations in liver, brain, or heart we studied autopsy tissue specimens from five patients who had been treated with AMSA antemortem.

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Abbreviation used in this paper: AMSA, 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide; HPLC, high-performance liquid chromatography; AML, acute myelogenous leukemia; LLD, lower limit of detection

Materials and methods

AMSA was supplied for phase-I and -II clinical studies by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, MD. Autopsy tissue samples were collected from four patients with acute myelogenous leukemia and one patient with metastatic malignant melanoma who died 1–14 days after receiving treatment with AMSA. One of the patients with leukemia was found at autopsy to have a clinically unsuspected renal oncocytoma.

To quantitate AMSA, 1 ml normal saline was added per gram of tissue (wet weight). The mixture was then minced and placed in a 15-ml corex tube, cooled in an ice bath, and homogenized with a model PT-10 Polytron tissue homogenizer (Brinkman Instruments, New York) at 27,000 rpm for 10–15 min. The homogenate was adjusted to pH 2.0 by adding 0.5 *N* HCl, and centrifuged at 12,000 *g* for 10 min in a Sorvall RC 2-B centrifuge. The supernatant was then thoroughly mixed with six volumes of *N*-hexane as a clean-up procedure. The organic phase was discarded and the pH of the supernatant was adjusted to 9.0 with saturated sodium borate solution. The supernatant was extracted with six volumes of ethyl acetate. The extract was then evaporated to dryness under a stream of nitrogen and reconstituted with methanol : water (9 : 1). The recovery was 75%–80% compared with known standards.

All analyses were carried out with a Waters Associates model 204 liquid chromatograph using a Bondapak μC_{18} reverse phase column (30 cm × 4.0 mm I.D.), and eluted with methanol : water : 5% sodium phosphate, pH 4.3 (450 : 50 : 3) at a flow rate of 2.0 ml/min and monitored at 254 nm. The retention time of AMSA was 10–12 min. Metabolites were not detected by this method.

A standard curve was constructed by adding known amounts of AMSA to control tissue samples. The standard curve was linear over the concentration range of interest and the lower limit of detection was 200 ng/g.

Results

Tissue concentrations of AMSA are presented in Table 1. The tissues available for assay varied from patient to patient. Where no value appears, no tissue was obtained. The highest concentration was found in the gallbladder of one patient. It is probable that bile adherent to the gallbladder wall contributed to the high values. Relative organ concentrations were different for different patients. Concentrations were generally

Table 1. Human autopsy tissue distribution of AMSA[a]

Patient	1	2	3	4	5
Sex	M	M	M	M	F
AMSA (mg/m^2/day)	75 × 7 days	30 × 2 days	40 × 3 days	90 × 1 day	90 × 5 days
Days from last treatment	1	1	5	7	13
No. of courses	1	1	2	2	2
Diagnosis	AML	AML, renal oncocytoma	Melanoma	AML	AML
Tissue AMSA (μg/g)					
Gallbladder	4.2				
Oncocytoma		1.1			
Liver	1.6	1.0	0.6	1.5	1.1
Lymph node	0.6	1.3			
Kidney	0.7	2.4	0.9	0.4	0.7
Adrenal	0.8				
Ovary					0.8
Stomach		1.0			
Thyroid	0.8				
Heart		0.7	0.3	1.0	0.6
Lung	0.8	0.4	0.5	0.4	0.5
Testicle	1.0	0.4		0.2	
Muscle	0.3	0.7			
Fat	0.5				
Spleen	0.4	0.6	< 0.2	0.4	
Bladder	0.4	0.5			
Pancreas	0.5	< 0.2			
Colon		0.4			
Prostate	0.2	0.4			
Brain	0.3		0.4	0.2	0.5

[a] An HPLC technique was used to determine AMSA concentrations in autopsy tissue obtained from patients who had received the drug within 2 weeks antemortem. Where no value appears, no tissue was obtained for assay. Patient 1 had moderate hepatic dysfunction prior to and after treatment. Patient 2 developed acute renal failure during treatment, which was due to septic shock. Patient 4 had ventricular fibrillation and patient 5 had generalized seizures due to AMSA

high in the liver. Patient 1 had moderate hepatic dysfunction at the time of AMSA administration. The liver AMSA concentration in patient 1 was comparable to those of other patients. No patient developed evidence of hepatic toxicity from AMSA. Patient 2 developed acute renal failure secondary to septic shock during treatment. This patient's renal AMSA concentrations were far higher than those of other patients. It was not felt clinically that the AMSA contributed to the renal failure.

Concentrations in myocardium were consistently less than those in liver. Of note, the highest cardiac AMSA concentration was found in patient 4. This patient had developed the acute onset of ventricular fibrillation during his last treatment with AMSA, and is the only one of the five who experienced cardiac toxicity. He was a 40-year-old male with no previous history of heart disease, but was hypokalemic at the time of treatment. His only other medications were tobramycin and ticarcillon. Autopsy revealed no abnormality of the heart other than focal myocardial congestion. No leukemic infiltrate was detected in the myocardium.

On average, the lowest concentrations were found in the brain, although in all four patients from whom brain samples were obtained concentrations in one or more other organs were as low as or lower than those in brain. Patient 4 had a grand mal seizure during his episode of ventricular fibrillation. He had the lowest brain concentrations of AMSA and it is quite possible that his seizure was due to cerebral hypoxia during his arrhythmia. Patient 5 suffered a generalized seizure during her last course of AMSA. She had had no prior neurological problems and her electrolytes were normal at the time of the seizure. She was not receiving any other medication. She had the highest cerebral concentration of AMSA among the patients for whom values were available. Autopsy revealed no evidence of leukemic infiltration of her brain, although there was evidence of disseminated intravascular coagulation involving her brain, heart, and kidneys. Her prothrombin time and fibrin split products had become elevated 5 days prior to her death, but were completely normal at the time of her seizure and had remained normal for 9 days after her seizure. It was felt that her seizure represented neurological toxicity from her AMSA.

Concentrations were generally low in lung, testicle, muscle, fat, spleen, bladder, pancreas, colon, and prostate, although for some organs only one or two samples were available for assay and for other organs single patients had moderately high AMSA concentrations in one of the organs. Leukemic infiltrates were present in the spleens of patients 1 and 2 and in the lymph nodes of patient 1. Tissue AMSA concentrations were not unusually high or low in these organs.

The patient population was too small and heterogeneous to determine whether doses or schedule of administration affected tissue distribution. In addition, the rate of clearance of AMSA from tissues could not be determined, although tissue AMSA concentrations in the patient undergoing autopsy 13 days after her last treatment did not appear to be markedly different from those in patients receiving similar doses 1 and 7 days antemortem.

Patient 2 was found at autopsy to have a clinically unsuspected renal oncocytoma. The AMSA concentration in this tumor was lower than the concentration in surrounding renal tissue, but was comparable to or higher than concentrations found in other tissues.

Discussion

AMSA is highly active against human acute myelogenous leukemia [11], and also is somewhat effective against acute lymphoblastic leukemia [11], lymphoma [1], and carcinoma of the breast [9]. Occasional responses have also been noted in malignant melanoma [7, 8], ovarian carcinoma [16], carcinoma of the lung [8, 12], and sarcomas [15]. The major dose-limiting toxicity is leukopenia [8, 16]. Life-threatening seizures [10, 11] and arrhythmias [6, 10, 11, 17] have been noted in a small number of patients. Some of these patients have been hypokalemic or hypocalcemic at the time they received AMSA.

We did not find unusually high concentrations of AMSA in either cardiac or brain tissues of our patients. However, the only patient in our series who developed cardiac toxicity had higher myocardial concentrations than did the other patients. This patient also experienced a generalized seizure that could have been due to cerebral hypoxia as a result of ventricular fibrillation. He had low AMSA concentrations in his brain. The only other patient who experienced seizures during AMSA administration had the highest observed brain concentrations of AMSA. Hence, cardiac and neurologic complications could possibly be due to excessive heart and brain AMSA concentrations rather than being due to unusual patient sensitivity to the drug. Further experience will be necessary to confirm this.

In patient 2 the AMSA was not felt to have contributed to the patient's acute renal failure, although this possibility must be considered in view of the high kidney AMSA content. No renal toxicity from AMSA has been reported previously. We feel that it is more likely that the high renal concentrations of AMSA were secondary to the renal dysfunction than vice versa. It is known that up to 40% of an administered dose of AMSA is excreted in the urine over a 3-day period [16].

Since AMSA is extensively metabolized in man, and since it has a relatively short half-life [16], it is possible that concentrations of AMSA metabolites in tissues could be considerably higher than the concentration of the parent drug. Data from studies in animals also suggest this [2]. Hence, it is possible that toxicitiy from AMSA would correlate better with tissue concentrations of metabolites than with tissue concentrations of the parent compound. Against this postulate is the fact that both cardiac and neurologic toxicity occurred acutely during AMSA infusion, at a time when relatively little metabolite would have been formed. Unfortunately, our assay method did not determine AMSA metabolite concentrations.

Generally, the relative organ concentrations of AMSA noted in our patients are similar to those noted previously in mice [2]. Any difference observed between mice and humans could be due either to species differences or to differences in timing of tissue sampling. The most marked difference noted was in splenic AMSA concentrations. Concentrations were high in mice, whereas they were low in our patients. The significance of this is unclear. However, it is notable that as with AMSA, high drug concentrations are found in the spleens of mice treated with two other agents highly active in human acute leukemia, i.e., daunorubicin [3] and cytosine arabinoside [5]. Ftorafur, another agent that attains high concentrations in mouse spleen [3], has not been extensively tested in human acute leukemia. Whether this association between a drug's splenic concentration in mice and activity against human leukemia exists by chance alone or is of true significance is unclear.

While it is not possible to define a clear time-concentration relationship for AMSA in human tissues, we can conclude that AMSA is retained for longer than 2 weeks. This would not be anticipated from its relatively short elimination half-life [16]. A third half-life has not been reported for AMSA. This may suggest that the drug remaining in tissues is quite tightly bound and is not released back into plasma. It is known that the antitumor activity of AMSA is schedule-dependent in tissue culture and that prolonged or repeated exposures are more cytotoxic than are brief exposures [4]. It is not likely that the cellular retention of AMSA in vivo would be different from that in vitro, although this has not been determined. If this assumption is correct, one might postulate that the AMSA retained in tissue is bound in such a way that it is no longer cytotoxic. On the other hand, the total amount of AMSA retained in the tissues was relatively small, and represented only a small percentage of the total dose administered. Hence, it is also very possible that late AMSA concentrations were simply too low to exhibit any cytotoxicity.

AMSA attains high concentrations in B16 melanoma in mice [14]. While the highest intracellular concentrations are found in nuclei and microsomes, substantial amounts of the drug also adhere to melanosomes. We have previously reported moderately high concentrations of AMSA in subcutaneous melanoma deposits from two of three patients studied [18], although we did not determine its subcellular localization. It has been suggested that, because of the drug's affinity for melanin, it might have substantial activity against malignant melanoma [14]. Unfortunately, it has produced only low response rates in this disease [7, 8]. It is possible that if another melanin-binding compound, such as chlorpromazine, were given after AMSA it might competitively displace the AMSA from the melanin so that it would then be available for cytotoxic interaction with the cell's DNA. This approach has not been investigated.

Moderately high concentrations of AMSA were found in a renal oncocytoma in one of our patients. Nevertheless, the concentration in the tumor was lower than the concentration in surrounding tissues.

Sensitivity of human tumors to antineoplastic agents can now be determined in vitro [13]. However, the drug concentrations studied are chosen arbitrarily on the basis of plasma pharmacokinetic parameters. It seems it would be more appropriate to choose doses that give cellular concentrations similar to those observed in human tumor biopsy samples. Hence, from our previous data in melanoma [18] and our current data in oncocytoma samples, we can surmise that it would be appropriate to use those drug doses in vitro that give a cellular concentration of AMSA of 0.4–3.4 μg/g. It is probable that as additional specimens are assayed, the observed tissue concentration range will widen somewhat.

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