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# Influence of Scheduling on Therapeutic and Toxic Effect of AMSA in Lewis Lung Carcinoma*

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***Summary.*** *The antitumor activity and toxic effect of AMSA were studied in Lewis lung carcinoma (3LL) at various stages of growth. The total dose of drug injected IP was 15 mg/kg, which is equivalent to the $LD_{10}$. Different administration schedules were tested, these being single-injection schedules (day 1, 7, or 10 after tumor implantation) and repeated low-dose-injection regimens (days 1, 4, and 7 and days 1–7 after tumor implantation).*

*Tumor weight inhibition, retardation of growth, reduction in the number of metastases, and median survival time of treated mice over controls were analyzed as end-points to evaluate the antitumor activity of AMSA. Early deaths and changes in white blood cell count were considered as parameters of toxicity. Our findings can be summarized as follows: (1) AMSA is only minimally effective against primary 3LL tumor at all the growth stages examined and no schedule-dependency is detected; (2) a greater reduction in metastases (70%–77%) is found when the drug is administered fractionally than when it is given in a single dose (39%–60%); (3) irreversible leukopenia is induced by the single-dose schedule of AMSA administration while after repeated low doses the white blood cell counts are in the same range of those of the control groups.*

*Therefore, because of the schedule-dependency of toxicity and reduction in metastases, fractionated administration of AMSA at this dose level would be suitable for adjuvant chemotherapy.*

## Introduction

Major progress in cancer chemotherapy has resulted both from the discovery of new antineoplastic drugs and from proper therapeutic designs for clinically established agents. Clinical reports indicate that lung cancer represents an increasingly relevant problem because of its rising frequency, high mortality rate and, with few exceptions [4], limited responsiveness to chemotherapeutic agents [5, 7] whose activity is more favorably assessed in a variety of other human malignancies.

Conflicting data exist in the literature about the antitumor activity of 4'-(9-acridinylamino)methanesulfon-m-anisidide (AMSA), a synthetic acridine derivative [2], in lung cancer. In fact, in spite of encouraging data from phase I clinical trials [14], phase II evaluation of AMSA indicates only a marginal activity of this drugs, if any, in lung cancer patients [15, 19]. In addition, marked hematopoietic toxicity of this drug has been observed, so that myelosuppression, primarily leukopenia, is the major dose-limiting factor [3, 17, 19].

On the basis of these premises we wanted to verify the antitumor activity and toxic effect elicited by AMSA in Lewis lung carcinoma (3LL), which is so far one of the most suitable models for human lung cancer [20].

Experimental studies on the therapeutic effects of AMSA on 3LL have already been reported by other authors, but undetailed and often contrasting results have been obtained [9, 10, 17]. Moreover, because no clear dependency of the effect of the administration schedule was demonstrated consistently in the mouse tumor system for this drug, we investigated the influence of different schedules of AMSA on both antitumor activity and toxic effect.

## Materials and Methods

AMSA (acridinyl anisidide, Bristol-Myers Co. International Division) as supplied for clinical use was reconstituted in a solution containing acridinyl anisidide 5 mg/ml in 10% v/v *N,N*-dimethylacetamide and 0.0318 *M* L-lactid acid, just before use. This solution was further diluted 20-fold with 5% dextrose in distilled water. The AMSA dose used was 15 mg/kg body weight, corresponding in our experimental conditions to the $LD_{10}$. Adult female C57BL/6 mice about 2 months old and weighing 20–22 g were used throughout this work. Each recipient mouse was inoculated IM with a suspension of 3LL containing $2.5 \times 10^5$ viable cells, prepared as previously described [11]. 3LL-bearing mice were assigned randomly to the following treatment groups, each containing 10–25 animals.

*Schedule A:* controls, receiving 5% dextrose in single injections; *Schedule $A_1$:* AMSA 15 mg/kg given on day 1 after tumor implantation; *Schedule $A_2$:* AMSA 15 mg/kg given on day 7 after tumor implantation; *Schedule $A_3$:* AMSA 15 mg/kg given on day 10 after tumor implantation; *Schedule B:* controls, receiving 5% dextrose in repeated injections; *Schedule $B_1$:* AMSA 5 mg/kg/dose given on days 1, 4, and 7 after tumor implantation; *Schedule $B_2$:* AMSA 2.14 mg/kg/dose given from day 1 to day 7 after tumor implantation.

Median survival times (MST) of treated and control animals, tumor volumes, and estimated reduction in the

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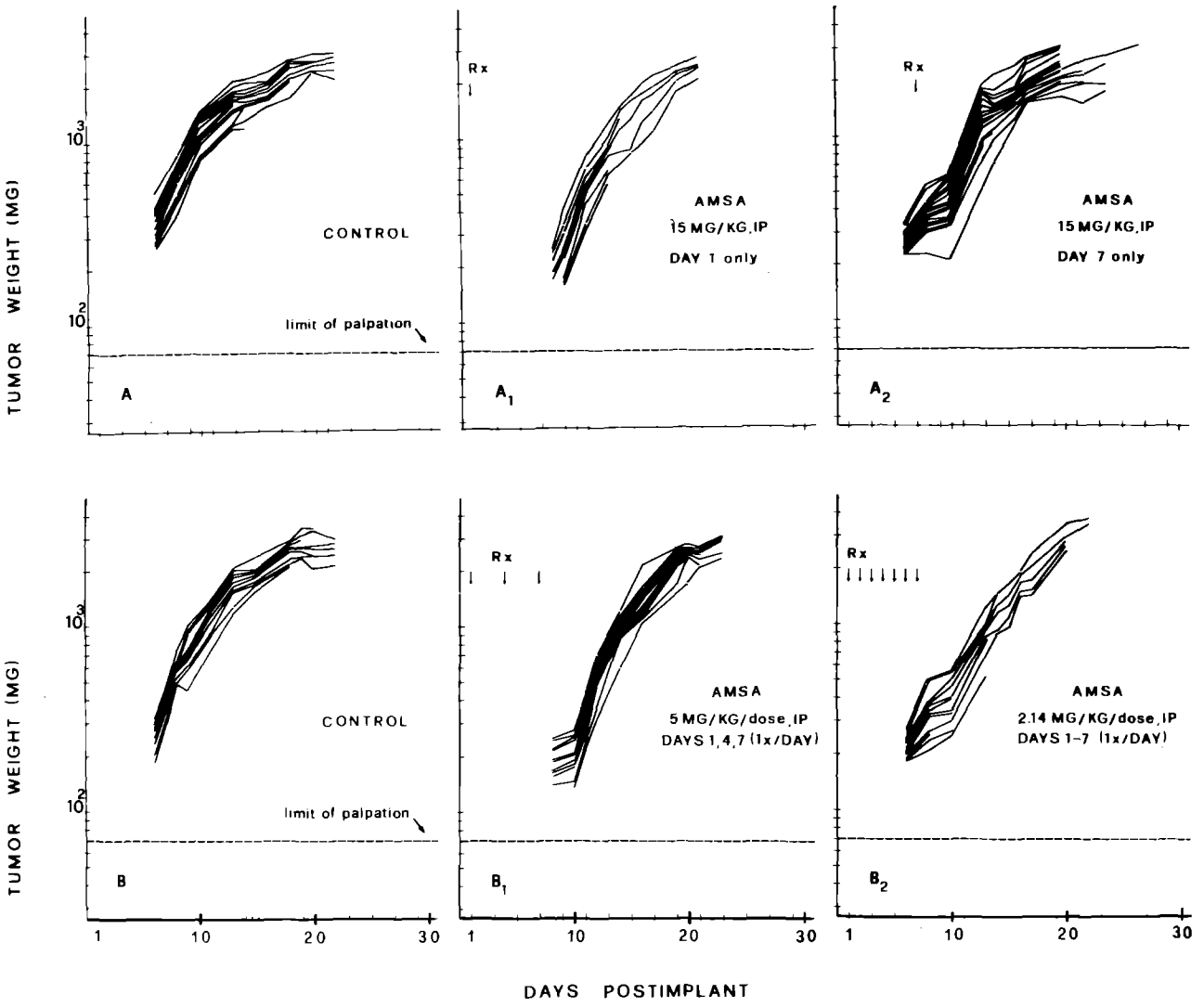


**Fig. 1.** Effect of AMSA 15 mg/kg given according to different schedules on primary 3LL tumor. *Panel A* Control, 5% dextrose, single injection; *A1* AMSA 15 mg/kg on day 1 only; *A2* AMSA 15 mg/kg on day 7 only. *Panel B* Control, 5% dextrose, repeated injections; *B1* AMSA 5 mg/kg/dose on days 1, 4, and 7; *B2* AMSA 2.14 mg/kg/dose on days 1–7. 3LL-bearing mice were inoculated with $2.5 \times 10^5$ tumor cells IM on day 0. Each group consisted of 12–15 mice

**Table 1.** Effect of AMSA on Lewis lung carcinoma, as function of different schedules of administration

| | Therapeutic effect | | | | | | No. of early deaths/[e] No. of treated mice |
|---|---|---|---|---|---|---|---|
| | Average tumor weight (mg) ± SE[a] | | T–C[b] (days) | No. of metastases[c] median (range) | | T/C[d] (%) | |
| | T | C | | T | C | | |
| A1: AMSA 15 mg/kg, day 1 only | 560 ± 67* | 1,237 ± 53 | 4.0 | 43 (13–95) ∧ | 70 (31–131) | 91 | 5/15 |
| A2: AMSA 15 mg/kg, day 7 only | 483 ± 24* | 1,076 ± 50 | 3.0 | 28 (6–76)* | 70 (31–131) | 91 | 15/25 |
| A3: AMSA 15 mg/kg, day 10 only | 875 ± 77* | 1,699 ± 78 | 5.0 | n.e. | n.e. | 92 | 8/10 |
| B1: AMSA 5 mg/kg/dose, days 1, 4, 7 | 549 ± 32* | 1,121 ± 64 | 4.5 | 26 (3–50)** | 87 (31–122) | 99 | 0/25 |
| B2: AMSA 2.14 mg/kg/dose, days 1–7 | 516 ± 84* | 1,130 ± 80 | 4.5 | 20 (13–35)§§ | 87 (31–122) | 86 | 0/15 |

[a] Tumor weight on 12th day of growth [8]. * $P < 0.01$ vs control (Student's *t*-test)

[b] T–C, tumor growth delay. The time taken to reach 1,000 mg was used

[c] Lung metastases were counted on the 21st day after tumor implantation. ∧ not significantly different from the control; * $P < 0.01$ vs control; ** $P < 0.05$ vs control; §§ $P = 0.01$ vs control (Mann–Whitney *U*-test)

[d] Median survival time of treated mice/median survival time of control mice, × 100. MST of controls was 23 days (16–32)

[e] No early death was observed in the control groups

number of metastases were calculated according to the method of Geran et al. [8]. Early deaths (i.e., deaths occurring within 7 days after the end of treatment) presumed to be attributable to acute toxicity [22] were disregarded in calculation of MST.

Fifteen mice from treated groups (*(schedules $A_2$* and *$B_1$)* and control groups were chosen for hematologic evaluation. Individual blood samples were aspirated from the orbital sinus in the morning, because of the known diurnal variations in total leukocytes [18] and heparinized with Liquemin (Roche). The samples were collected every day for the *schedule $A_2$* animals and every other day for control and *schedule $B_1$* mice, starting from the day of treatment. The total WBC count was performed in a Thoma hemocytometer and for the differential leukocyte count (minimum 200 cells) smears stained with May-Grunwald-Giemsa were prepared.

For histologic examination of target organs, such as liver, kidney, lung, heart, and brain, five mice treated according to the three schedules cited above were designed and sacrificed 3 days after the end of treatment (i.e., on day 10). The organs were preserved in a Bouin solution, embedded in paraffin wax, sectioned at 4–6 μm, and stained with hematoxylin & eosin.

## Results

### *Therapeutic Effect of AMSA*

In Fig. 1 the response of individual 3LL-bearing mice to different schedules of AMSA is plotted, and in Table 1 the main activity end-points are listed. The curves shown in Fig. 1 (panels A1 and A2) represent the pattern of 3LL growth after AMSA 15 mg/kg given in a single dose on day 1 or 7 after tumor implantation.

As can be seen, in both cases the drug is only moderately effective against this tumor, which is usually refractory to chemotherapy. In fact, treated groups show neither a relevant regression of the tumor mass (55%) nor a significant retardation of tumor growth in comparison with the control group (panel A). A very similar response is still elicited by the primary tumor site if treatment with the same total dose of AMSA (15 mg/kg) is performed at a later stage of growth (i.e., on day 10 after tumor implantation; *schedule A3,* Table 1).

As regards the metastases response, reductions of 39% and 60% in the number of lung nodules are observed when AMSA is given on day 1 or 7, respectively. No increase in lifespan is induced by the single-dose treatment at any of the growth stages examined (Table 1).

When AMSA is administered on three occasions over a 1-week period (5 mg/kg/dose on days 1, 4, and 7 after tumor implantation; *schedule B1*) the same modest effect on primary tumor control is observed (tumor regression 51%), while a significantly more pronounced reduction in the number of metastases is achieved (70%) (see panel B1 of Fig. 1, and Table 1). Finally, a further fractionation of the same total dose of AMSA every 24 h over the first week of tumor growth *(schedule B2)* fails to produce any additional improvement of the therapeutic response compared with *schedule B1* (see panel B2 of Fig. 1 and Table 1).

### *Toxic Effects of AMSA*

As regards the toxic effects induced by different AMSA schedules Table 1 shows that after a single dose of 15 mg/kg a high percentage of early deaths occurs: 33% by day 1, 60% by day 7, and 80% by day 10 of treatment. It is interesting that, as demonstrated for other antineoplastic drugs, the increase in toxic deaths is related to the tumor growth and is probably due to hematopoietic toxicity [6].

In contrast the same total dose of AMSA, if fractionally administered (*schedule B1* and *B2*), does not cause early deaths. In addition, acute treatment leads to a weight loss of up to 15%, while after fractionated treatment the maximum weight loss observed is only 2% (data not shown).

To evaluate the schedule-dependency of such toxic effects at both hematologic and histologic levels, total peripheral WBC count and morphologic examination of target organs were carried out.

Figure 2 shows the changes in total WBC value occurring during tumor growth both in two selected treatment groups (*schedules A2* and *B1*) and in untreated control animals. As can be seen, mice receiving a single dose of AMSA show a

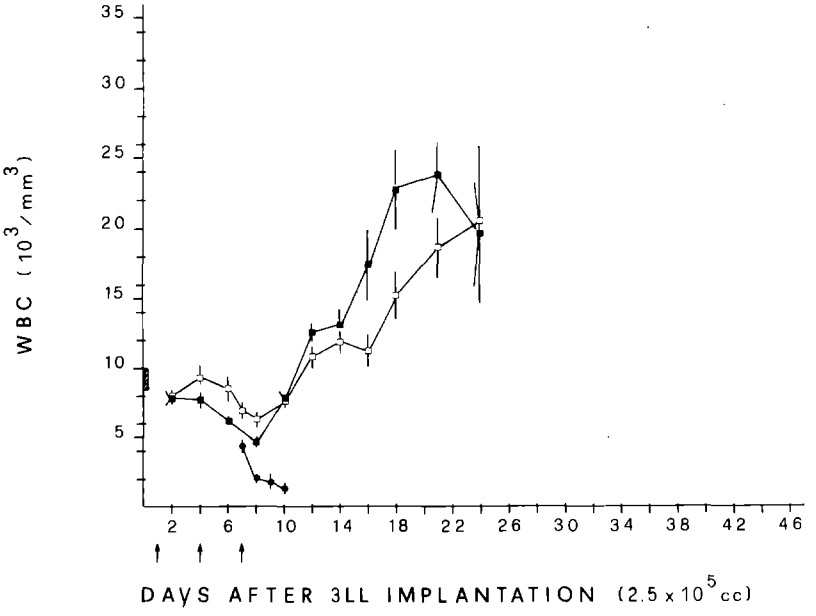


**Fig. 2.** Myelotoxic effect of AMSA 15 mg/kg given in a single dose (● day 7 only) or in divided doses (■ mg/kg/dose on days 1, 4, and 7). 3LL-bearing mice were inoculated with $2.5 \times 10^5$ tumor cells IM on day 0. Each group consisted of 15 mice. (□) control: 5% dextrose; *black area,* normal range of peripheral WBC; arrows, time of drug administration

rapid drop in WBC 24 h after treatment. Such leukopenia persists until death of the animals, with no recovery. In contrast, the same total dose of the drug given according to a fractionation regimen induces only a gradual leukocyte reduction during treatment, reaching its nadir on day 8. After this a full recovery of the WBC count occurs, the same level as in the untreated controls being reached on day 10 (i.e., 3 days after the end of treatment).

After that, a marked leukocytosis is found in both treated and untreated groups. Relatively higher WBC values are observed in the treated group, although such differences are not statistically significant by the Student's *t*-test. The WBC values observed after AMSA given in a single dose, in contrast, are statistically different ($p < 0.01$) from those of both untreated and treated groups.

The differential leukocyte count revealed no alterations in the fractions of white cell types until 3 days after treatment in any group, and the relative percentages of lymphocytes and granulocytes were the same as in the healthy mice (85.5% and 14.5%, respectively). In the late stages of tumor growth granulocyte values rose to about 60% in both treated and control groups, which was probably related to an increase of inflammatory reaction due to the progressive tumor necrosis.

Histologic examination of target organs did not reveal any notable microscopic changes that were drug schedule-dependent; the slight vacuolization of hepatocytes found in both the treated groups (*schedules A2* and *B1*) was totally unspecific and similar to that observed in control group livers.

## Discussion

One of the purposes of the present work was to evaluate the therapeutic index of AMSA in an experimental lung cancer. The 3LL model was chosen because, despite the lack of direct correlation in drug sensitivity of transplantable animal tumors and spontaneously occurring tumors in man, experimental systems still play an important rôle in cancer chemotherapy studies. The results presented in this paper are consistent with the clinical findings of limited effectiveness of AMSA when used as single-agent therapy against pulmonary cancer, so confirming the reliability of 3LL as a good model for mimicking lung tumors in man [20].

Moreover, on the basis of our previous experience with other antineoplastic drugs [12] we wanted to check whether fractionated treatment could improve the antitumor activity of AMSA against 3LL. In contrast to results recorded with other experimental tumors [2], our findings indicate that the therapeutic effect of AMSA on 3LL does not vary significantly with the modality of treatment. In fact, repeated low doses of the drug display antitumoral activity similar to that of a single 15 mg/kg dose in terms of primary tumor burden reduction and of retardation of tumor growth. Only at the metastatic level was there an improvement fewer lung nodules as were observed when the fractionation schedules were used.

In contrast, comparison of the toxic deaths in the various groups clearly reveals the superiority of the repeated-low-dose treatment over the acute schedules. These results are consistent with data reported by other authors for adriamycin given by different schedules [1, 16], confirming some similarity between these two drugs [21, 23].

Differences in toxicity among various treatment modalities are even more evident when total leukocyte counts are compared. This parameter was chosen because it reflects myelopoiesis and, as previously mentioned, leukopenia is the major complication of AMSA therapy. In particular, the single dose of AMSA induces higher toxicity than does fractionated treatment. Such severe toxicity exceeds the therapeutic activity of the drug and results in a rapid irreversible leukopenia leading to death. The severity of alterations found at the hematologic level is not supported by corresponding histologic lesions. We have not found marked alterations in target organs, especially liver and kidney, as otherwise demonstrated in preclinical toxicologic studies by other authors [13] using AMSA 30–34 mg/kg. This fact, in our opinion, could be related to the following factors: the lower dose of AMSA we used, the IP route of administration, and the known rapid metabolic clearance of this drug.

In conclusion, the data presented in this paper show that AMSA given according to a multiple-dose schedule in the 3LL system, although eliciting only modest primary tumor control, is not myelotoxic and induces an appreciable reduction in the number of lung nodules. Therefore, taking into account the advantages obtained by fractionated treatment, we intend to pursue our studies with AMSA, using it as an adjuvant agent after surgery, with a view to improving the therapeutic effectiveness of this clinically important drug especially in terms of increasing host lifespan.

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