Cancer Chemother. Pharmacol. 3, 135–141 (1979)

Cancer Chemotherapy and Pharmacology

General Review

m-AMSA and PALA: Two New Agents in Cancer Chemotherapy*

M. Rozencweig[1], D. D. Von Hoff, R. L. Cysyk, and F. M. Muggia

Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland, USA

Summary. *4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA) and N-(phosphonacetyl)-L-aspartate (PALA) are two new anticancer agents that have been recently introduced into clinical investigation. This review summarizes the preclinical information that has accumulated with these compounds as well as the very preliminary data presently available from early clinical trials. This information indicates the promising potential of* m*-AMSA and PALA in the treatment of cancer.*

Introduction

The therapeutic success achieved with anticancer chemotherapy in a variety of tumor types, such as Hodgkin's disease [14] and testicular cancer [18], provides strong support for the development of strategies aimed at broadening the spectrum of malignancies curable with chemotherapy. In this respect, the identification of new anticancer agents has traditionally been one of the main objectives of the Division of Cancer Treatment of the National Cancer Institute, involving complex and difficult preclinical and clinical work.

Most encouraging results have been obtained with combination chemotherapy [13]. Prime reasons for integrating agents into such regimens are the proven antitumor activity of the individual agents and the feasibility of their combined administration at full or nearly full doses. These requirements should be of major concern when new agents are selected for clinical testing. The need for agents endowed with little or no myelosuppressive activity must be particularly stressed.

The development of analogs of established agents represents a major facet in new drug programs [26]. The results of efforts in this direction have been generally inconclusive, leading to the question of specific methods of evaluation [5, 32, 34] and further emphasizing the need for new classes of chemotherapeutic agents, as illustrated by the increasingly explored potential of *cis*-diamminedichloroplatinum (II) [28, 33].

The antitumor activity of new agents is first detected or confirmed in animal screening systems before their introduction into clinical trials. These systems have recently been revised at the Division of Cancer Treatment of the National Cancer Institute [16]. The new procedure shows a disease-specific orientation and consists of a panel of spontaneous and allografted murine tumors as well as human tumor xenografts. The practical implications of this new screening remain to be determined. Proper clinical feedback is necessary for correct assessment of these systems and to promote continuous attempts at improving their relevance. This will require a great deal of coordination to obtain adequate clinical data [4, 27] and to avoid incomplete and meaningless investigation [40] with agents selected for clinical investigation on the basis of these experimental findings.

4′-(9-Acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA; NSC-249992) and *N*-(phosphonacetyl)-L-aspartate (PALA; NSC-224131) represent new types of anticancer agents that have been evaluated in the new screening panel. This review summarizes the preclinical information that has accumulated with these compounds and the very preliminary data recently obtained in early clinical trials. This information points to the great potential of these new agents in the treatment of cancer.

m-AMSA

The antitumor activity of acriflavin, an acridine dye, was reported by Mellanby in 1933 [25]. Since then, nu-

Reprint requests should be addressed to: M. Rozencweig

1 *Present address:* Investigational Drug Section, Institut Jules Bordet, 1, rue Héger-Bordet, B-1000 Brussels, Belgium

* Presented in part at the USA-Japan Agreement Meeting, Tokyo, June 1978

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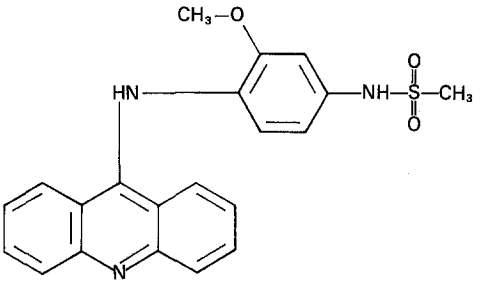

Fig. 1. Chemical structure of *m*-AMSA

Table 1. Activity of *m*-AMSA and PALA in tumor panel systems [16]

	AMSA	PALA
L1210 Leukemia	+	−
P388 Leukemia	+	±
B16 Melanoma	+	+
Lewis lung	+	+
Colon 26	+	+
Colon 38	+	±
Colon xenograft CX-1	NT	−
Colon xenograft CX-2	±	−
Mammary xenograft MX-1	−	+
CD8 F1 Mammary	+	+
Lung xenograft LX-1	NT	−

merous acridine derivatives have been produced in a search for compounds with an enhanced therapeutic index. *m*-AMSA is one of the various acridine derivatives synthesized by Cain and Atwell [3] (Fig. 1).

m-AMSA is active against a wide spectrum of murine tumors (Table 1) [3, 9, 16, 30]. These include the ascitic form of the L1210 and the P388 leukemias, the Lewis lung carcinoma, the spontaneous C3H mammary adenocarcinoma, and the mammary tumor in $CD8F_1$ mice. No schedule dependency of this experimental antitumor activity has been detected. It is of particular interest that the drug is curative in the commonly resistant B16 melanoma. This has been related to an enhanced localization of *m*-AMSA in melanoma cells, followed by a tight binding to melanoma granules [36]. No antitumor activity was detected in the intracerebrally inoculated L1210, the transplantable colon tumors 36 and 38, the colon xenografts in mice, or the MX1 breast xenograft. In addition to its antitumor effects, *m*-AMSA also exhibits antiviral [2] and immunosuppressive activities [1].

Since the acridines, as a class, interact with DNA, attempts to define the mechanism of cytotoxicity of *m*-AMSA have so far been directed at studying DNA effects [43]. However, *m*-AMSA is structurally dissimilar from simple acridines in that the 9-methanesulfonanilide moiety is orthogonal to the acridine ring system [3] and thus might be expected to interfere with intercalation [42]. Gormley et al. [17] studied the interaction of *m*-AMSA with calf thymus DNA and various chemically defined synthetic copolymers. They found that *m*-AMSA does intercalate into DNA base pairs, that it interacts with both AT and GC base pairs, and that the drug alters the template properties of DNA as determined by inhibition of nucleic acid polymerizing enzymes. A study by Furlong et al. [15] on the effects of *m*-AMSA on macromolecular synthesis in L1210 cells in vivo and in vitro demonstrated that DNA synthesis is substantially inhibited at *m*-AMSA concentrations that do not interfere with RNA synthesis. In addition, *m*-AMSA was found to produce DNA fragmentation in L1210 cells (exposed in vivo or in vitro), as shown by centrifugation of cell lysates on alkaline sucrose gradients. In vivo experiments revealed cross-resistance to *m*-AMSA of a subline of P-388 leukemic cells resistant to a variety of DNA intercalating agents [21].

The kinetic response to *m*-AMSA was investigated in cycling and noncycling cells [39]. In these experiments, CHO cells were exposed to the drug for 2 h, and the cell-cycle distributions were determined 30 h later. Cycling cells were shown to accumulate in G2 and this phenomenon was dose-dependent. CHO cells arrested in G1 were also treated prior to return to cycle. At moderate concentrations, these noncycling cells were two to four times more resistant to the drug than cycling cells. At higher doses the cell kill effects were similar in cycling and noncycling cells, but the progression through the cell cycle was not markedly affected in noncycling cells. Overall, the kinetic response to *m*-AMSA resembled that observed with ADM.

m-AMSA was also found to induce cell cycle-specific chromosome damage in CHO cells [12]. In these experiments, synchronized cells were exposed to *m*-AMSA at various phases of the cell cycle. Severe damage was seen, especially after treatment during the early G2 phase, with more than 20 breaks per cell. This is in contrast to ADM, which induces more chromosome damage in the S phase than in G2.

A preclinical pharmacology study [10] in rodents, with ^{14}C-*m*-AMSA labeled in the acridine nucleus, showed that radioactivity was well distributed to all tissues except the brain. The pharmacologic disposition of *m*-AMSA appeared to be related to the susceptibility of the 9-carbon atom of the acridine ring to nucleophilic attack by endogenous thiols, which results in the release of the 9-methanesulfanilide moiety and the formation of the corresponding 9-alkylthioether of acridine. Biliary excretion was the primary route of excretion, with more than 50% of the administered dose excreted in the bile within 2 h after IV administration. Less than 2% of the biliary radioactivity was unchanged *m*-AMSA. The predominating biliary metabolite was the glutathione-9-thioether of acridine as determined by field desorption

mass spectroscopy. This metabolite is actively excreted into the bile by a transport mechanism that is saturable with therapeutic doses in rats.

Multiphasic plasma decay curves are observed, with an early rapid component followed by a more gradual decline. This pattern of disappearance is believed to be due to an interaction of *m*-AMSA with thiol groups of protein, forming stable protein-acridine complexes and thus causing prolonged retention of the acridine moiety in plasma and certain tissues. Eleven percent of the administered radioactivity can be recovered in the urine by 24 h, approximately 22% of it being unchanged *m*-AMSA. Although thiolysis can occur nonenzymatically, *m*-AMSA is an excellent substrate for glutathione-S-transferase purified from rat liver. Plasma levels and biliary excretion of *m*-AMSA and its metabolite can be influenced by the in vivo manipulation of glutathione-S-transferase activity and levels of glutathione (GSH). Enhancement of in vivo transferase activity by phenobarbital pretreatment produces a decrease in the plasma half-life of unchanged *m*-AMSA and a corresponding increase in biliary excretion. Depletion of GSH levels results in a prolonged plasma half-life of unchanged *m*-AMSA and a corresponding decrease in biliary excretion (Shoemaker and Cysyk, unpublished results).

After oral administration of ^{14}C-*m*-AMSA to rats (100 mg/kg), 60%—90% of the radioactivity can be recovered in the bile (Rozencweig and Cysyk, unpublished data). At oral doses giving similar levels of radioactivity in the liver, considerably lower concentrations of *m*-AMSA, particularly in its unchanged form, are seen in most other organs than after IV administration [11].

m-AMSA is only modestly effective against L1210 implanted IP when given orally. This is probably a consequence of its rapid uptake by the liver. The following experiment was carried out by Cysyk and co-workers [11] to determine whether the high levels of drug seen in the liver might result in an antitumor effect in liver disease. *m*-AMSA was given orally at various dose levels 5 days after IV L1210 inoculation to groups of ten mice. The liver was removed 24 h thereafter and bioassayed for the content of tumor cells. After the highest doses, no L1210 cells were detected in the liver in most animals. These findings point to the potential of *m*-AMSA for the treatment of liver metastases, particularly by the oral route.

Toxicology studies of *m*-AMSA in beagle dogs and rhesus monkeys showed that the dog was the more sensitive of the two animal species (M. C. Henry et al., Report II TRI-TOX 249992-76-2, NCI, Oct. 20, 1976). The toxic dose low for the beagle dog on a single-dose schedule was 31.2 mg/m^2. In dogs and monkeys, the most frequently observed toxic effects were related to the liver and the gastrointestinal, lymphatic, and hematopoietic systems. The liver abnormalities seen at higher doses included elevated alkaline phosphatase, SGOT, SGPT, and bilirubin levels. Histologic alterations included cytoplasmic vacuolization, congestion, hemosiderosis, and hemorrhage. Gastrointestinal toxicity was manifested in anorexia, emesis, and bloody diarrhea. There was congestion, hemorrhage, degeneration, and necrosis of intestinal mucous membranes. Lymphatic tissues showed hemorrhage, congestion, and necrosis.

Anemia and leukopenia were noted in peripheral blood and were dose-related. However, thrombocytopenia was not a prominent manifestation of toxicity in dogs and monkeys. A consistent effect was noted in the animals' bone marrow, with observed hypoplasia being dose-related and reversible. Other side effects included local tissue reactions at injection sites. In the dog, reversible congestion and hemorrhage in the kidneys were noted, with transient increases in BUN and serum creatinine levels and with hematuria and proteinuria.

For clinical IV use, *m*-AMSA is formulated as two sterile liquids that are aseptically combined prior to use. Each 2-ml flint ampule contains 1.5 ml solution of *m*-AMSA in anhydrous *N,N*-dimethylacetamide (50 mg *m*-AMSA/ml). Each 20-ml amber vial contains 13.5 ml 0.0353 *M* L-lactic acid. The flint ampule (1.5 ml) containing 75 mg *m*-AMSA is added to the vial of L-lactic acid solution and the contents are mixed. The resulting orange-red solution contains 5 mg *m*-AMSA/ml. Because of phlebitis occurring at the dose of > 70 mg/m^2, it has been recommended that the drug be diluted in 500 ml 5% dextrose in water and infused over 1 h [41].

Phase I clinical trials with a variety of schedules of oral and IV *m*-AMSA have been completed. Published results are available for only two of these trials. Von Hoff et al. [41] reported their experience at the National Cancer Institute with single IV doses repeated every 28 days, while Legha et al. [24], at the MD Anderson Hospital, investigated a schedule involving IV administration daily for 3 days every 3 weeks.

Leukopenia was the major dose-limiting toxic effect of *m*-AMSA in both trials. Leukopenia was dose-related (Table 2) and reproducible at the recommended dose schedule for Phase II trials in solid tumors: 120 mg/m^2 or 40 mg/m^2 three times every 3—4 weeks. Mild thrombocytopenia also occurs. No evidence of cumulative myelosuppression was noted in patients receiving repeated courses of *m*-AMSA.

Moderate to severe phlebitis has been described at doses of 70 mg/m^2 or over per course. This can be avoided with sufficient dilution of the drug. Alopecia, nausea, and vomiting may also be encountered occasionally.

A generalized pruritic macular skin rash was noted in one patient 24 h after the administration of 50 mg *m*-AMSA/m^2 [41]. The rash resolved spontaneously within

2 days. At the 160 mg/m² dose level, two patients developed apparently drug-induced mild and transient elevations in serum alkaline phosphatase [41].

Pharmacokinetic studies have been reported in one patient, *m*-AMSA containing ^{14}C-label in the 9-carbon atom of the acridine ring being used [41]. Plasma levels of unchanged free *m*-AMSA declined in a biphasic manner with a half-life of 12 min for the distribution phase and 2.5 h for the excretion phase. The corresponding figures for total plasma radioactivity were 11 min and 4.5 h. No unchanged free drug could be detected in the plasma after 23 h.

Phase I clinical trials do not purport to determine the antitumor activity of a new drug. Nevertheless, objective tumor regressions and beneficial responses have been noted in these early trials in a number of tumor types, including ovary and lung adenocarcinoma, melanoma, and acute myeloblastic leukemia.

Table 2. Myelosuppression induced by *m*-AMSA at increasing dose levels repeated every 28 days [41]

Dose (mg/m²)	No. of patients/ no. of courses	Median WBC nadir × 10^3/mm³ (range)	Median platelet nadir × 10^3/mm³ (range)
10	3/3	5.4 (–)	211 (–)
20	3/4	4.4 (3.1–6.1)	210 (200–290)
33	3/3	3.3 (–)	175 (–)
50	3/4	4.9 (4.8–6.0)	260 (260–390)*
70	3/6	2.9 (2.2–4.5)	220 (190–250)
90	4/5	2.7 (1.8–4.3)	140 (120–390)
120	8/11	3.0 (0.5–4.9)	210 (15–325)
160	2/3	1.4 (1.2–1.6)	155 (90–200)

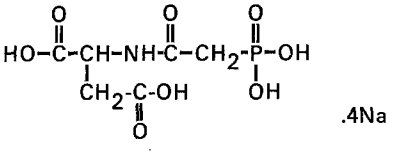

Fig. 2. Chemical structure of PALA

PALA

PALA (Fig. 2) is a potent inhibitor of aspartate transcarbamylase, a key enzyme in the de novo biosynthesis of pyrimidine nucleotides [7]. This inhibition results in cytotoxic effects, which have been shown to be reversed in vivo by uridine and carbamyl aspartate [20]. Cells with natural or acquired resistance to PALA are generally characterized by a high activity of aspartate transcarbamylase [22, 23]. This enzymatic activity correlates directly with the rate of cell proliferation [38, 45]. The degree of cell resistance to PALA might also be related to inefficient cell uptake mechanisms or neutralizing pyrimidine salvage pathways [19]. PALA has been shown to enhance the entry of 5-fluorouracil into nuclear RNA [29].

The spectrum of activity of PALA in experimental tumors appears to be unique (Table 1) [9, 16, 30, 35]. PALA is curative without clear schedule dependency in the Lewis lung carcinoma, which is refractory to most cytotoxic agents with a few exceptions such as cyclophosphamide or nitrosoureas [35]. On the other hand, L1210 and P388 murine leukemias are relatively or completely resistant to PALA, whereas these tumor models are extremely sensitive to most agents. Although inhibition of the spleen aspartate transcarbamylase has been described after oral administration in the mouse [44], no antitumor activity has been observed with oral PALA in experimental murine tumors.

The correlation between tumor growth inhibition, activity of aspartate transcarbamylase, and doubling time has been studied by Johnson et al. [22] (Table 3). L1210, L5178Y, and P388 cell lines are resistant in vivo to PALA. In vitro, large concentrations are needed to inhibit the tumor growth of these cell lines, and enzyme activity is relatively high whereas doubling time is relatively short. In contrast, Lewis lung, B16 melanoma, and glioma 26 are highly sensitive to PALA in vivo. In vitro, a 50% growth rate inhibition of these solid tumors is obtained at concentrations 20 times lower than in the leukemias. These solid tumor cells showed enzyme activity twice as low and doubling times twice as long as leukemic cells.

Table 3. PALA sensitivity, aspartate transcarbamylase levels, and doubling time of murine tumors in vitro [22]

Cell line	μ*M* PALA for 50% growth rate inhibition	ATCase activity (nmol/mg protein/min)	Doubling time (h)
L1210 leukemia	45 ± 1	55 ± 8	17 ± 3
L5178Y leukemia	91 ± 4	64 ± 6	13 ± 1
P388 leukemia	39 ± 5	52 ± 8	16 ± 1
Lewis lung carcinoma	3.9 ± 1.0	19 ± 1	24 ± 3
B16 Melanoma	2.0 ± 0.5	29 ± 5	27 ± 4
Glioma 26	3.4 ± 0.3	29 ± 5	35 ± 8

Although the L1210 saystem has been widely used as a prime screening system, drugs that are inactive in the L1210 system appear to be most beneficial for combination chemotherapy in man. A striking correlation has been suggested between myelosuppression in man and the increase in lifespan (ILS) in L1210 [31]. An ILS of 25% is the accepted limit of activity in this system. Only 50% of nonmyelosuppressive drugs exceed this limit, whereas it is exceeded with most drugs inducing dose-limiting myelosuppression in man (Fig. 3).

The effect of PALA on cell-mediated immune response was measured by the rejection rate of a tumor allograft in mice [22]. Almost all controls rejected an inoculum of L1210 cells, and only 2 of 24 animals died of leukemia. PALA at various dose levels did not alter the rejection of the tumor. In contrast, the cell-mediated immune response was markedly inhibited in mice receiving CTX.

Following the administration of PALA at 400 mg/kg daily for 5 days in mice, all animals died by day 22. Even at this dose, a total lack of myelosuppression was found throughout the experimental period. Histologic evaluation of various organs from mice treated with PALA at this lethal dose did not reveal any pathologic changes except in the liver, where PALA produced a dose-dependent and dose-limiting toxicity [22].

Toxicologic studies in large animals confirmed the absence of drug-induced bone marrow impairment (E. P. Denine et al., Report SORI-KM-77-663-3890-1, National Cancer Institute, December 2, 1977) (Table 4). In these studies, the dog proved more sensitive to PALA than the monkey. The toxic dose low in the dog was 2.4 g/m^2 for the single-dose study and between 0.3 and 0.6 g/m^2/day for the schedule of daily administration for 5 days. Dose-limiting toxicity resulted from toxic effects on the gastrointestinal tract and the central nervous system (CNS). Gastrointestinal toxicity was dose-related and reversible at sublethal doses. It was manifested in emesis, diarrhea, hematemesis, and hematochezia. Histologic examination revealed hemorrhagic and suppurative atrophy of the intestinal mucosa. CNS effects at sublethal doses were essentially apparent with the single-dose schedule and included seizures, loss of corneal reflex, and ataxia. No histologic lesions could be identified to account for these CNS manifestations. Finally, sporadic and transient shifts in serum enzymes and electrolytes were also encountered but could not be clearly related to specific target organ impairment.

Various assays have been determined for quantitating PALA [6, 8, 37], but few pharmacokinetic data are available at this point. The physiological disposition of the drug is being studied in mice, rats, dogs, and monkeys [6]. Preliminary data indicate that PALA is rapidly eliminated in the urine, where up to 80% of the administered dose could be recovered within the first 12 h. Chromatographic analysis of serum and urine indicated no detectable PALA metabolism. After IV administration of ^{14}C-PALA (120 mg/m^2) to mice, the drug was not taken up to any large extent by the individual normal tissues except kidney and bone. PALA was cleared rapidly from all tissues except bone, where it was retained at high concentrations for at least 24 h. No difference in uptake could be detected between solid L1210 leukemia and Lewis lung carcinoma. Pharmacokinetic studies with the oral route (120 mg/m^2) have also been undertaken in animals. Based on urinary recovery of radioactivity, these studies led to the conclusion that absorption was poor, which is consistent with the lack of experimental antitumor activity noted with oral administration. A better understanding of the pharmacologic disposition of PALA must await the publication of additional data.

Several Phase I clinical trials have been completed with a variety of schedules (Table 5), and results of these

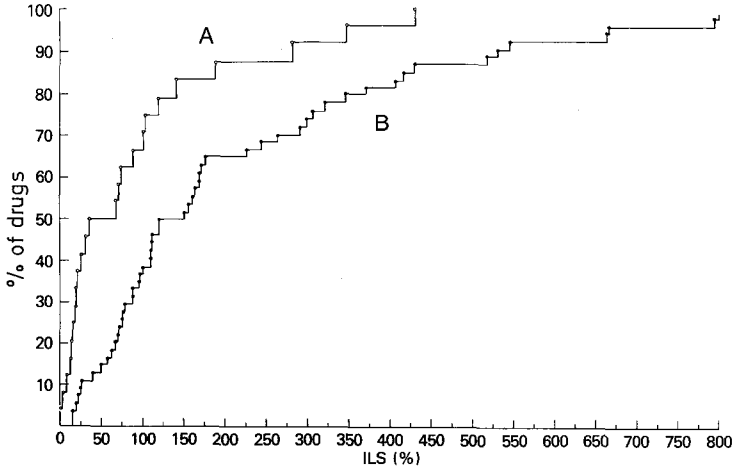

Fig. 3. Distribution of 54 drugs inducing dose-limiting myelosuppression in man (**B**) and 24 drugs not inducing dose-limiting myelosuppression in man (**A**) as a function of the increase in lifespan observed in L1210 murine leukemia relative to untreated controls (ILS%) [31]

Table 4. Preclinical toxicology of PALA in dogs

Dose schedule (mg/m² × days)	Lethality	Toxicity			
		GI	CNS	Hepatic	Hemato
9.6 × 1	2/2	+	+	+	−
4.8 × 1	0/2	+	+	−	−
2.4 × 1	0/2	+	+	−	−
1.2 × 5	2/2	+	±	+	−
0.6 × 5	0/2	+	−	+	−
0.3 × 5	0/2	−	−	−	−

Table 5. Phase-I studies with PALA

Institution	IV Schedule
MD Anderson	Every 2 weeks
	Daily for 3 days every 2 weeks
National Cancer Institute	Daily for 5 days every 3 weeks
Mayo Clinic	Daily for 5 days every 6 weeks
Memorial	Weekly or twice weekly
Mt. Sinai	Continuous infusion (24 h or 5 days)
Sidney Farber	Continuous infusion (5 days)

trials will be published soon. It appears that mucocutaneous toxicity is the major dose-limiting factor of the drug with all the schedules that have been investigated. Phase II studies with PALA are presently being initiated in a rapidly increasing number of tumor types.

Discussion

The drug development program of the Division of Cancer Treatment of the National Cancer Institute has recently reorganized its screening program towards a human disease-specific orientation. The two new anticancer agents presented in this paper are both products of this new screening system. Complete clinical trials with these agents should help validate or invalidate the new screening methodology.

It will take extensive clinical trials to incorporate both *m*-AMSA and PALA into our therapeutic armementarium. However this preclinical and clinical background constitutes a solid foundation on which to build subsequent clinical experience.

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Received March 7, 1979/Accepted April 23, 1979