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ORIGINAL ARTICLE

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Preclinical pharmacology of 2-methoxyantimycin A compounds as novel antitumor agents

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Abstract *Purpose*: The present study was designed to determine pharmacological and biochemical properties of 2-methoxyantimycin A analogs (OMe-A1, OMe-A2, OMe-A3, and OMe-A5), which are novel antitumor compounds, and provide a basis for future pharmaceutical development, preclinical evaluation, and clinical trials. *Methods*: A high-performance liquid chromatography (HPLC) method was established and employed to assess the biostability of these analogs and to determine their pharmacokinetic properties in mice and rats. *Results*: In vitro biostability of the 2-methoxyantimycin analogs was esterase-dependent, compound-dependent, and species-dependent. In the absence of esterase inhibitors, all of the analogs were relatively unstable. Stability was greater, however, in human and dog plasma than in rat and mouse plasma. In the presence of esterase inhibitors, OMe-A1 was stable at 37°C for 60 min in mouse and rat plasma, moderately stable in human plasma, and unstable in dog plasma. OMe-A2 was generally stable in all types of plasma. OMe-A3 was stable in dog and rat plasma, but not in human or mouse plasma. OMe-A5 was stable in human and dog plasma, but not in mouse or rat plasma. Each of these analogs was highly bound to plasma proteins. Of S9 fractions from four species, human S9 was least efficient in metabolizing OMe-A3. Following an intravenous dose of OMe-A1 in mice, plasma levels decreased rapidly, with an initial half-life of 2.7 min and a terminal half life of 34 min. Following an intraperitoneal dose in mice, plasma levels decreased less rapidly with a terminal half-life of 215 min. Following an intravenous dose of OMe-A1 or OMe-A3 in rats, plasma levels decreased more rapidly with initial half-lives of about 1.0 min. At an equivalent dose, OMe-A3 had a faster clearance than OMe-A1. *Conclusions*: For 2-methoxyantimycin A analogs, species differences in biostability, metabolism, and pharmacokinetics may be pertinent in assessing their pharmacological and toxicological profiles and antitumor activity in humans.

Keywords 2-Methoxyantimycin A · HPLC · Metabolism · Protein binding · Pharmacokinetics

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Introduction

One of the characteristics of malignant cells is loss of apoptosis, resulting in uncontrolled cell growth. Proteins of the Bcl2 family are involved in the response of cells to apoptotic stimuli. In general, Bcl2 proteins can be classified into two groups: proapoptotic (such as Bax and Bid) and antiapoptotic (such as Bcl2 and Bcl-x_L). Bcl-x_L, a pore-forming oncoprotein, is a component of mitochondrial membranes [1]. When cancer cells are exposed to radiation or chemotherapeutic drugs or are deprived of growth factors, those overexpressing Bcl-x_L demonstrate increased cell survival and multidrug resistance [2–4]. In human cancers, Bcl-x_L is often overexpressed [5]; this is the case in nearly all advanced prostate cancers [6]. For breast cancer, Bcl-x_L overexpression is associated with high tumor grade and nodal metastases [7]. Therefore, inhibitors of anti-apoptotic proteins such as Bcl2 and Bcl-x_L would be expected to be potent antitumor agents.

In fact, by binding to the hydrophobic groove on the surface of Bcl-x_L, proapoptotic proteins such as Bax inhibit the function of the oncoprotein [5]. There is an

increasing interest in developing small molecules that mimic the Bcl-x_L-binding effects of Bax-like proapoptotic proteins as cancer therapeutic agents [5, 8, 9]. Antimycin A3, an antibiotic isolated from *Streptomyces* sp. [10], and its analogs exhibit inhibitory effects on Bcl2 and Bcl-x_L [8, 9]. Antimycin A3 is, however, also a potent inhibitor of mitochondrial electron transport [8]. Given to mice as a single dose intravenously, this compound is highly toxic, with an LD_{50} of 1 mg/kg (information provided by the National Cancer Institute). Therefore, the clinical utility of this compound as an anticancer agent may be limited. More recently, efforts have been devoted to the development of novel antimycin analogs as antitumor agents. Several representative analogs are illustrated in Fig. 1.

Biochemical studies have indicated that one of the derivatives of antimycin A3, 2-methoxyantimycin A3 (OMe-A3), binds to the hydrophobic groove of Bcl-x_L and inhibits Bcl-x_L-induced pore formation but does not interfere with mitochondrial respiration [8]. OMe-A3 induces apoptosis in cell lines derived from human multiple myelomas that overexpress Bcl-x_L. Human myeloma cells RPMI-8226, transplanted into mice as xenografts, respond to OMe-A3 given intravenously as repeated doses of about 1.5 mg/kg (information provided by the National Cancer Institute). In OMe-A3 treated mice, host toxicity is limited, suggesting that it is a candidate for further clinical development.

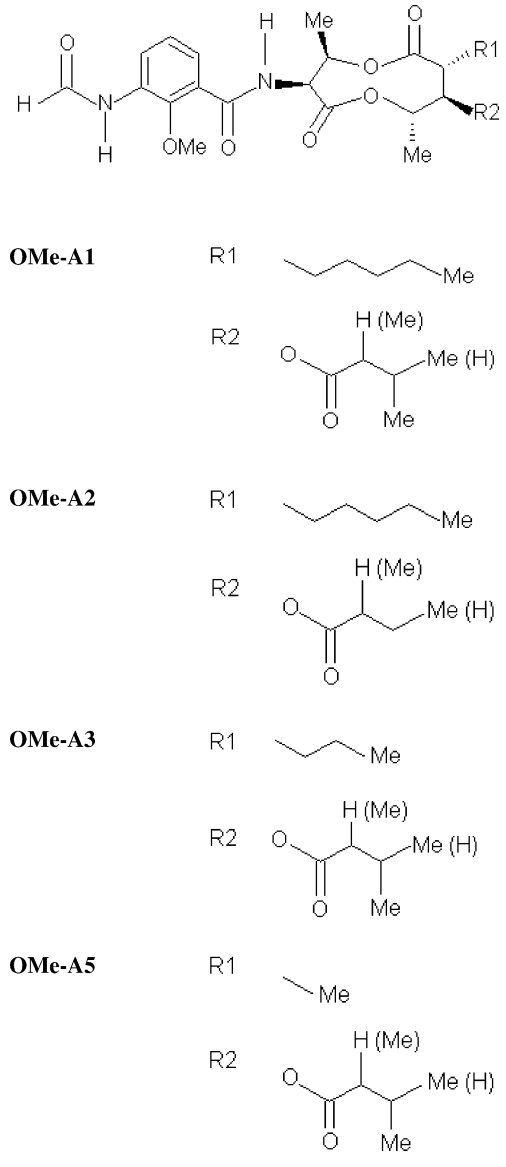

Fig. 1 Structure of 2-methoxyantimycin A (OMe-A) analogs

The purpose of this investigation was to determine pharmacological and biochemical properties of various 2-methoxyantimycin A analogs (OMe-A1, OMe-A2, OMe-A3, and OMe-A5) in various in vitro and in vivo models. Emphasis was on species differences in drug metabolism and disposition. The results from the present study may be useful in deciding if these compounds are appropriate for clinical evaluation as anticancer agents and, if so, to provide information useful for future pharmaceutical development, preclinical evaluation and clinical trials.

Materials and methods

Test compounds, chemicals, reagents, and animals

All chemicals and solvents used for sample preparation and HPLC analysis were analytical/HPLC grade. Acetonitrile, methanol, and phosphoric acid were purchased from Fisher Scientific (Atlanta, Ga.). Commercially available plasma samples of mouse, rat, dog, and humans were purchased from Lampire Biological Laboratories (Pipersville, Pa.). These plasma samples, obtained from pathogen-free animals or normal human healthy volunteers, were rapidly frozen, kept at −80°C, shipped to our laboratory in dry ice, and then kept at −80°C until use. Hepatic S9 fractions from male CD2F1 mice, male Sprague-Dawley rats, male dogs, and male humans were purchased from In Vitro Technologies (Boston, Mass.). The Developmental Therapeutics Program, National Cancer Institute, provided the test compounds, HL60 and RPMI-8226 cells, and male CD2F1 mice. Male Fischer 344 rats with implanted jugular cannulas were obtained from Charles River Laboratories (Cambridge, Mass.). All animal use and care procedures were approved by the Institutional Committee on Animal Use and Care at the University of Alabama at Birmingham.

Analytical method

The HPLC equipment consisted of a Hewlett Packard 1050 ChemStation with a fluorescence detector (Agilent 100 series). Quantification of the test compounds was achieved with a Zorbax SB-C18 (5 μm, 150 × 4.6 mm) analytical column protected by a LiChroCART 100

RP-18 guard column. The mobile phase was methanol: ammonium acetate buffer (87:13, v/v; 0.2 *M*; pH 5.8, adjusted with HCl). The flow rate was 1 ml/min. The column elute was monitored fluorometrically at an excitation wavelength of 232 nm and an emission wavelength of 418 nm.

For analysis of samples, two volumes of cold methanol/acetonitrile (50:50, v/v) were added to portions of plasma, culture medium, or cell extract. The preparations were mixed and then centrifuged to obtain clear supernatants, which were dried at room temperature by a stream of compressed air. With this method, no effects on test drug stability were observed. The residues were dissolved in 200 µl of the mobile phase described above, and a portion (80 µl) was injected onto the HPLC column. For each of the test compounds, the amounts present in samples were quantified using standard curves established from the peak areas. In the investigated concentration range of 10–500 ng/ml, the calibration curves were linear, with mean correlation coefficients (r^2) of >0.999. The final concentration of each sample was calculated from the standard curves and adjusted by the corresponding recovery rate. The mean recovery rates, in the presence of NaF/EDTA, were determined to be $70 \pm 5\%$. Intraday (triplicate samples) and interday (3 days) variations, assayed with three different concentrations (50, 100, and 500 ng/ml), were acceptable (<5% for each of the plasma samples from mice, rats, dogs, and humans, and cell culture medium). The retention time and limit of quantitation (LOQ) of each compound are shown in Table 1.

Stability in plasma

Plasma samples from mouse, rat, dog, and humans were incubated at 37°C with or without addition of NaF (2.5 mg/ml) and EDTA (1 mg/ml). NaF and EDTA were used as esterase inhibitors to prevent in vitro degradation by esterase activity. To these preparations were added OMe-A1, A2, A3, or A5 dissolved in methanol to result in a final concentration of 500 ng/ml in each plasma sample. Portions of plasma, taken at designated times after an addition of analog, were extracted and analyzed as described above. Additionally, the stability of OMe-A1, OMe-A2, OMe-A3, and OMe-A5 in the plasma from mice and humans was also evaluated at −80°C. From these preparations, portions were analyzed immediately; other portions were quickly frozen and then stored at −80°C for 1, 2, and 4 weeks before analysis.

Table 1 HPLC analysis of OMe-A analogs

Test compound	Retention time (min)	LOQ (ng/ml)	Standard curve (ng/ml)
OMe-A1	6.0	4.6	10–500
OMe-A2	7.3	14.3	10–500
OMe-A3	8.3	7.3	10–500
OMe-A5	5.6	17.2	10–500

Stability in cell culture medium

HL60 and RPMI-8226 cells were maintained in cell culture medium containing (or not containing) 20% fetal bovine serum (FBS). OMe-A1 (500 ng/ml) was added to a culture containing 200,000 cells/ml, and to control cell-free medium. Portions of culture medium were removed at various times and, as appropriate, centrifuged to collect cells. Samples of cell-free medium, to which NaF/EDTA was added, were extracted using the aforementioned procedure for the treatment of plasma samples. Collected cells were suspended in phosphate-buffered saline containing NaF/EDTA, subjected to sonication, and then extracted and analyzed as described above for plasma samples.

Binding to plasma proteins

Protein binding properties of the OMe-A analogs were analyzed using a previously described protocol involving the use of a microultrafiltration system [11]. Solutions of the OMe-A analogs were diluted with plasma (from mouse, rat, dog, or humans) containing NaF/EDTA to a final concentration of 1000 ng/ml. Controls (to measure non-specific binding to membranes) were diluted with water (containing NaF/EDTA) to the same final concentration. These preparations were incubated at 37°C for 20 min, at which time portions were taken and placed into the sample reservoirs of Amicon Centrifree ultrafiltration systems (Millipore Corporation, Bedford, Mass.). The filter systems were centrifuged at 37°C until the reservoirs were dry. From each filtrate, portions were taken for analysis by HPLC. The amounts present in the filtrates were designated as "free drug" (F). The concentrations of the unfiltered solutions were also determined and expressed as the "total drug" concentration (T). The percentage of test analogs bound to plasma proteins was calculated from the following equation:

$$\text{Percent of bound} = [(T - F)/T] \times 100.$$

Similar experiments were performed for OMe-A3 in dog or human plasma in the absence of NaF/EDTA.

S9 metabolism

To evaluate metabolism of the test compounds by S9 fractions from various species, reaction systems containing OMe-A3 (500 or 2000 ng/ml) and S9 (1 mg protein/ml) in 100 m *M* Tris buffer (pH 7.4) were maintained at 37°C. At designated times, i.e., 0, 5, 10, 20, 30, 45, and 60 min, triplicate portions of the mixtures were removed, processed, and analyzed by a procedure similar to that described above for plasma samples.

Pharmacokinetic studies with mice and rats

To determine the pharmacokinetics of OMe-A1 in male CD2F1 mice, groups of three were dosed either intravenously (tail vein) or intraperitoneally with OMe-A1 (20 mg/kg) in a vehicle of Cremophor (5%) and ethanol (5%) in 0.9% saline. For both dosing routes, the volume administered was 5 ml/kg body weight. At various times, animals were anesthetized by inhalation of ether, and blood samples were collected by cardiac puncture into tubes containing NaF/EDTA (dry powder, 4.375 mg NaF and 1.75 mg EDTA in each tube). Plasma was prepared and immediately frozen at −80°C. The protocols for the pharmacokinetic studies were essentially the same as previously described [12–14].

Male Fischer 344 rats with implanted jugular cannulas (five per group) were dosed via a lateral tail vein with either OMe-A1 or OMe-A3 (0.25 mg/kg). The dosing vehicle was the same as that used for mice; the dosing volume was 1.3 ml/kg body weight. Blood samples were collected via the implanted jugular catheter, and plasma was prepared and analyzed. Further, samples of plasma, urine, liver, and kidney were collected from rats kept in metabolism cages for 24 h [15]. NaF and EDTA were added to tubes (4.375 mg NaF and 1.75 mg EDTA) for collection of blood and urine samples and to the homogenizing buffer (NaF 2.5 mg/ml and EDTA 1 mg/ml). Pharmacokinetic parameters were estimated using WinNonlin (Mountain View, Calif.) as reported previously [14]. They included the area under the curve (AUC, ng h/ml), initial half-life ($T_{1/2\alpha}$, min), terminal half-life ($T_{1/2\beta}$, min), maximum concentration (C_{max}, ng/ml), clearance (CL, ml/kg/h), mean residence time (MRT, min), and volume of distribution (V_{ss}, ml/kg). A two-compartment model was used to fit the concentration-time curves.

Results

Stability in plasma

There were remarkable species differences in in vitro stability in plasma among the OMe-A analogs when the esterase inhibitors NaF/EDTA were not added into the assay system (Fig. 2). For all four compounds, stability was generally greater in dog and human plasma than in rat and mouse plasma. After 60 min, 61% of the added OMe-A1 and 53% of the added OMe-A2 remained in dog plasma. For the other compounds and other types of plasma, however, $<33\%$ of the amounts added were present at this time.

In the presence of NaF/EDTA, the stability of all four compounds was substantially increased, with greater than 60% of the amounts added being present after a 60-min incubation. This suggests that esterase-associated metabolism is the major degradation pathway for OMe-A compounds. As shown in Fig. 2a, OMe-A1 was substantially stable in mouse, rat, and human plasma. In the presence of NaF/EDTA, the stability of OMe-A1 in dog plasma was increased for early time points (up to 10 min), but no changes were observed afterwards (Fig. 2a), indicating that esterase activity is responsible only for initial degradation of this compound. As shown in Fig. 2b, OMe-A2 was generally stable in all types of plasma containing NaF/EDTA: greater than 80% of the amounts added were present after a 60-min incubation and there were no statistically

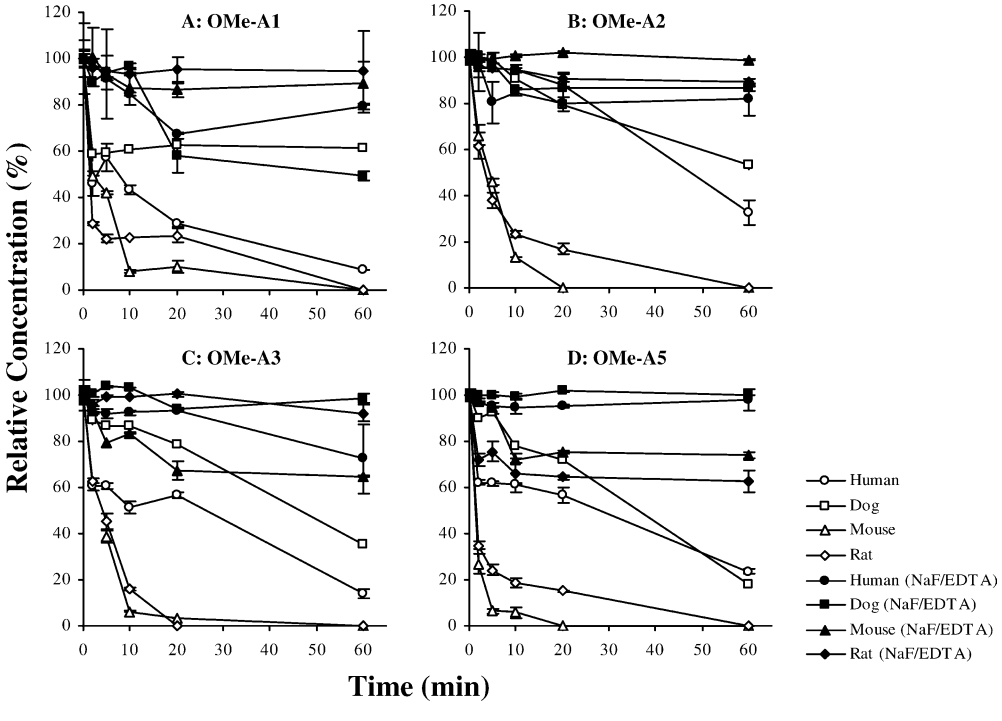

Fig. 2 Stability of OMe-A1 (**a**), OMe-A2 (**b**), OMe-A3 (**c**), and OMe-A4 (**d**) in mouse, rat, dog, and human plasma in the presence or absence of esterase inhibitors NaF and EDTA

significant differences among plasma samples from the four different species. Under the same assay conditions, OMe-A3 was largely stable in dog and rat plasma, with greater than 92% of the amounts added being present at 60 min. There were notable differences in stability of OMe-A3 for the other two species, with 72% and 65% of the amounts added being present at 60 min in human and mouse plasma, respectively (Fig. 2c). OMe-A5 was stable in human and dog plasma, with greater than 98% of the amounts added being present at 60 min. There were substantial decreases in concentration for mouse and rat plasma, with 74% and 63% of the amounts added being present at 60 min, respectively (Fig. 2d).

We further determined the long-term stability of these analogs under frozen storage conditions that may be used in future clinical studies. In the absence of NaF/EDTA at −80°C, none of the compounds was completely stable over 4 weeks in human or mouse plasma (Table 2). Under the storage conditions without NaF/EDTA, OMe-A1 was the most stable, with 86% and 78% of the amounts added being present after 4 weeks for human and mouse plasma, respectively (Table 2). Under these conditions, all analogs were more stable in human plasma than in mouse plasma. In mouse plasma, OMe-A5 was the most unstable analog: only 27% of the amount added was present at that time (Table 2). As seen in the aforementioned in vitro assays, NaF/EDTA increased the stability of all four OMe-A analogs in both human and mouse plasma (Table 2). In human plasma, OMe-A2 was less stable than the other three analogs, with a decrease of 18% of the amount added. In mouse plasma, OMe-A1 and OMe-A3 were more stable than the other two compounds, with OMe-A5 being the least stable under these storage conditions (Table 2).

Stability in cell culture medium

The relative instability of some OMe-A analogs in vitro in the absence of esterase inhibitors led us to investigate further the stability of these compounds under cell culture conditions, which may have implications for the extrapolation in vitro results to in vivo settings. We tested cellular uptake and stability of OMe-A1 in cell culture with FBS-containing or FBS-free medium. As shown in Fig. 3a, OMe-A1 was taken up rapidly and accumulated markedly in HL60 cells cultured with the FBS-free medium (Fig. 3a). In contrast, more OMe-A1 was detected in FBS-containing medium than in FBS-free medium (Fig. 3b). Greater stability of OMe-A1 in the presence of FBS may be associated with protein binding of this compound. With RPMI-8226 cells and medium, there was no appreciable difference from the results obtained with HL-60 cells (not shown).

Protein binding

Considering the in vitro stability of the test compounds, protein binding assays were accomplished in the presence of NaF/EDTA and with a relatively short but sufficient incubation period (20 min). Duplicate samples were run for each assay. As shown in Table 3, extensive plasma protein binding (>99%) was observed for all test compounds in all plasma samples from four species. In the absence of NaF/EDTA, similar results were obtained for OMe-A3 in dog and human plasma (not shown).

Table 2 Stability of OMe-A analogs stored at −80°C for 4 weeks. The data presented are the mean ± SD percentages of the amounts added (500 ng/ml) present after 4 weeks of storage

Test compound	Human plasma		Mouse plasma	
	Without NaF/EDTA	With NaF/EDTA	Without NaF/EDTA	With NaF/EDTA
OMe-A1	85.8 ± 0.4	93.4 ± 0.2	78.2 ± 0.6	95.8 ± 0.2
OMe-A2	70.3 ± 0.4	82.6 ± 0.3	62.7 ± 0.2	77.2 ± 0.2
OMe-A3	72.8 ± 0.6	95.0 ± 0.3	45.4 ± 0.0	89.9 ± 0.5
OMe-A5	79.2 ± 0.3	92.3 ± 0.6	27.4 ± 0.1	63.8 ± 0.1

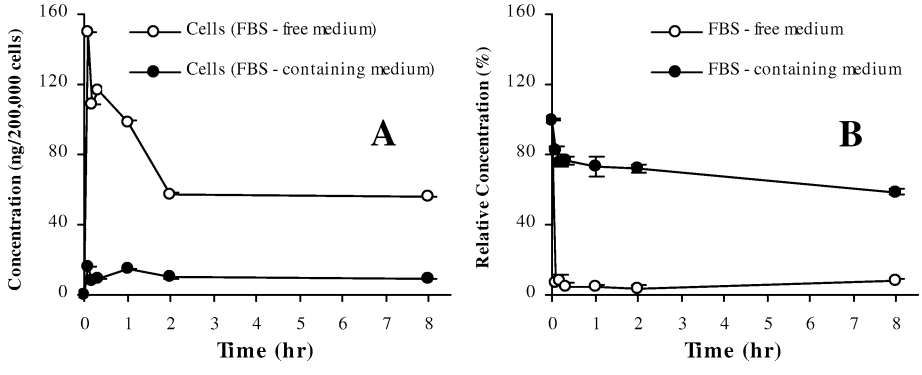

Fig. 3 Stability and accumulation of OMe-A1 in HL-60 cells grown in FBS-containing or FBS-free medium. The contents of OMe-A1 in the cells (**a**) and medium without cells (**b**) were analyzed by HPLC

S9 metabolism

In experiments to determine the effect of S9 proteins on the stability of OMe-A3 (500 or 2000 ng/ml), the initial levels of the compound dropped rapidly, even in the absence of S9 proteins (Fig. 4). Thus, OMe-A3 was not stable in solution at pH 7.4. Under these conditions, degradation of OMe-A3 was observed after incubation with mouse, rat, and dog S9 components. As shown in Fig. 4a, at a concentration of 500 ng/ml, the half-lives of OMe-A3 in the presence of mouse, rat, or dog S9 were approximately 2 min. However, in the presence of human S9 components, a prolonged half-life (10 min) was observed, indicating a relatively weak S9 metabolic capability in humans compared with other species. At a concentration of 2000 ng/ml, the half-lives of OMe-A3 in the presence of mouse, rat, or dog S9 components were shorter than 5 min (Fig. 4b). In the presence of human S9 components, however, the concentration curve was not significantly different from that of control (no S9 added), indicating again no appreciable S9 activity towards OMe-A3 metabolism.

Pharmacokinetics

Following an intravenous dose of OMe-A1 (20 mg/kg) in mice, plasma levels dropped rapidly, with the concentration at 30 min after dosing (1040 ng/ml) being about 15% of that at 5 min after dosing (Fig. 5). Pharmacokinetic modeling indicated that a two-compartment model best fit the concentration-time curve (Table 4). The terminal half life ($T_{1/2\beta}$) was 34 min. Nevertheless, the drug was still detectable (15 ng/ml) at 24 h after dosing (Fig. 5). With an intraperitoneal dose of 20 mg/kg, plasma levels increased to a maximum at 30 min after dosing, then decreased with a $T_{1/2\beta}$ value of 215 min. The drug was still detectable (18 ng/ml) at 24 h after dosing. Relative to dosing intraperitoneally, the intravenous dose gave higher AUC and C_{max} values and a lower $T_{1/2}$ value (Table 4). Bioavailability by the intraperitoneal route was approximately 62%.

For rats dosed intravenously with OMe-A1 (0.25 mg/kg) (Fig. 6), plasma levels dropped quickly, with a distribution half-life ($T_{1/2\alpha}$) of 1.0 min and terminal half-life ($T_{1/2\beta}$) of 35.8 min (Table 5). For rats dosed intravenously with OMe-A3 (0.25 mg/kg), plasma levels dropped even more quickly, with a $T_{1/2\alpha}$ of 0.9 min (Table 5). At 2 h after dosing, the concentration of OMe-A1 was 10 ng/ml; OMe-A3 was undetectable at 10 min after dosing. Compared with OMe-A1, OMe-A3 had smaller values of AUC and V_{ss}, shorter MRT, and lower C_{max}, and its clearance was faster (Table 5). It should be noted that larger standard errors in modeling pharmacokinetic parameters for OMe-3 data were observed, presumably due to the limitation of available data points. For these rats, little or no detectable amounts of OMe-A1 or OMe-A3 were present in the urine, collected over a period of 24 h, or in livers or kidneys taken at 24 h after dosing.

Table 3 Protein binding (%) of OMe-A1, OMe-A2, OMe-A3, and OMe-A5 in mouse, rat, dog, and human plasma (1000 ng/ml, initial concentration)

Test compound	Mouse	Rat	Dog	Human
OMe-A1	99.0 ± 0.40	99.9 ± 0.03	99.8 ± 0.31	99.5 ± 0.73
OMe-A2	99.7 ± 0.06	99.9 ± 0.0	99.9 ± 0.09	99.9 ± 0.02
OMe-A3	99.1 ± 0.31	99.8 ± 0.12	99.3 ± 0.60	99.9 ± 0.07
OMe-A5	99.7 ± 0.09	99.9 ± 0.0	99.1 ± 0.64	99.6 ± 0.54

Discussion

The OMe-A compounds are promising, novel antitumor agents that are under extensive preclinical evaluation. Results from the present investigation allow several conclusions. First, in vitro studies with plasma from various species demonstrated that stability of these compounds depends not only on the presence of NaF/EDTA (for esterase inhibition) but also on the structure of the compound, the temperature, and the type of plasma present. The major degradation pathway appeared to be associated with esterase activity in plasma, since, for most of the analogs, addition of NaF/EDTA retarded in vitro degradation. In future clinical trials, species differences in degradation of these compounds should be considered, especially for monitoring of drug levels and host toxicity.

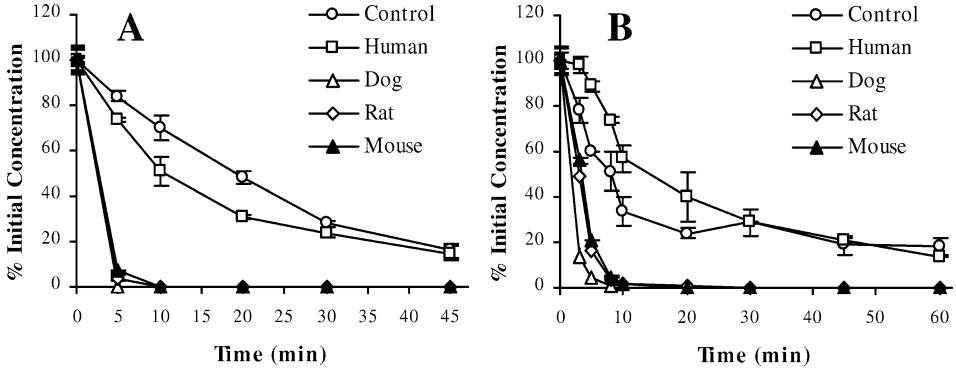

Fig. 4 Mouse, rat, dog, and human S9 metabolism of OMe-A3 at an initial concentration of 500 ng/ml (**a**) or 2000 ng/ml (**b**)

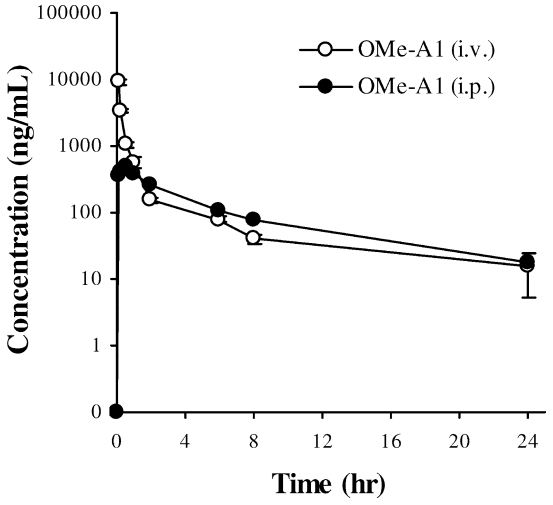

Fig. 5 Pharmacokinetics of OMe-A1 in CD2F1 mice dosed intravenously or intraperitoneally with 20 mg/kg

Table 4 Pharmacokinetic parameters for OMe-A1 following intravenous or intraperitoneal injection into male CD2F1 mice

Parameter	Intravenous dose	Intraperitoneal dose
Dose (mg/kg)	20	20
AUC (ng h/ml)	3209 ± 76	2000 ± 411
$T_{1/2\alpha}$ (min)	2.7 ± 0.1	33.6 ± 41.4
$T_{1/2\beta}$ (min)	34.1 ± 0.1	214.8 ± 69.4
C_{max} (ng/ml)	26,939 ± 864	528.9 ± 465.7
CL (ml/kg/h)	6232 ± 149	10,001 ± 2062
MRT (min)	25.8 ± 2.8	222.1 ± 50.1
V_{ss} (ml/kg)	2685 ± 249	32,160 ± 1882
F (bioavailability)	–	0.62

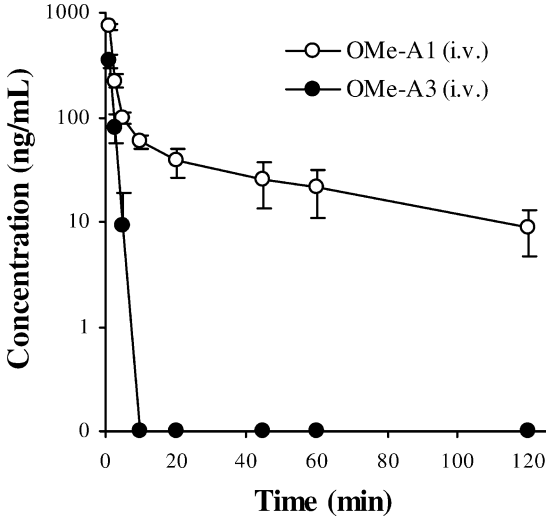

Fig. 6 Pharmacokinetics of OMe-A1 and OMe-A3 in F344 rats dosed intravenously with 0.25 mg/kg

Table 5 Pharmacokinetic parameters of OMe-A1 and OMe-A3 following intravenous injection into Fischer 344 rats

Parameter	OMe-A1	OMe-A3
Dose (mg/kg)	0.25	0.25
AUC (ng h/ml)	87.2 ± 5.3	16.4 ± 5.0
$T_{1/2\alpha}$ (min)	1.0 ± 0.02	0.9 ± 0.05
$T_{1/2\beta}$ (min)	35.8 ± 4.8	8.9
C_{max} (ng/ml)	1442 ± 21	744 ± 38
CL (ml/kg/h)	2866 ± 174	15,233 ± 1929
MRT (min)	33.1 ± 5.3	1.32 ± 4.3
V_{ss} (ml/kg)	1581 ± 167	336 ± 1055

Second, the S9 components from mice, rats, and dogs, showed appreciable capacity for metabolizing OMe-A3 in the in vitro assay. In contrast, human S9 fractions had limited capacity to metabolize these compounds. It is likely that in humans metabolism of OMe-A compounds occurs primarily in plasma. Because of the lack of microsomal metabolism, there would probably be a slower clearance in humans than in other species. The exact metabolic pathways are not known and metabolites were not identified in the present study. Therefore, it remains to be determined whether these apparent species differences would have an impact on the drug availability to tumor tissue versus host tissues, therapeutic effectiveness, dosing and schedule, and toxicity to hosts.

Third, regardless of structure or species, binding of OMe-A analogs to plasma proteins was >99%. This may explain why, in some cases, these compounds have prolonged elimination half lives. One may anticipate that these compounds will interact with other drugs binding to plasma proteins and that possible competition for binding sites and disposition of these bound drugs will be different for individual patients with varying levels of plasma proteins. It is generally accepted that the biological activity of drugs is largely associated with free (unbound) drug levels in the blood circulation, and not with total drug levels. Therefore, in clinical trials, patients with low levels of total plasma proteins and those being concurrently treated with other drugs should be carefully monitored for toxic effects [11].

Fourth, pharmacokinetic studies in mice dosed intravenously indicated that, relative to intraperitoneal dosing, OMe-A1 has a short plasma half-life and a relatively large AUC value. The sixfold longer terminal half-life for intraperitoneal dosing may reflect continuing absorption from the peritoneal cavity. A pharmacokinetic study in rats involving a lower dose administered intravenously showed that OMe-A3 is eliminated from plasma more readily than OMe-A1. Thus, pharmacokinetic values vary depending upon the route of administration and on the structure of the analog.

Since we used NaF/EDTA in the collection of biological samples, in vitro degradation of the test compounds after sampling was minimized. Therefore, the short plasma half lives in rodents may be related to instability of these compounds in plasma as seen with in

vitro assays. The results of pharmacokinetic studies in rodents may be useful in designing future pharmacokinetic and efficacy studies in humans. For example, a short plasma half-life may indicate that there is a need for closer monitoring of drug levels in a pharmacokinetic study and continuous infusion of the drugs in an efficacy study.

In summary, these results relating to the biostability and pharmacokinetics of antimycin analogs are important to the future development and evaluation of this class of compounds, not only for assessment of their antitumor activity but also for evaluation of their potential toxicity. Since, in terms of degradation of these compounds, human plasma is similar to dog plasma, it may be useful, in future studies, to use the dog as a preclinical model for evaluation of toxic effects. A toxicokinetic study in dogs may be valuable to future human clinical trials.

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References

1. Halestrap AP, Doran E, Gillespie JP, O'Toole A (2000) Mitochondria and cell death. Biochem Soc Trans 28:170–177
2. Minn AJ, Rudin CM, Boise LH, Thompson CB (1995) Expression of bcl-xL can confer a multidrug resistance phenotype. Blood 86:1903–1910
3. Decaudin D, Geley S, Hirsch T, Castedo M, Marchetti P, Macho A, Kofler R, Kroemer G (1997) Bcl-2 and Bcl-XL antagonize the mitochondrial dysfunction preceding nuclear apoptosis induced by chemotherapeutic agents. Cancer Res 57:62–67
4. Huang DC, Cory S, Strasser A (1997) Bcl-2, Bcl-XL and adenovirus protein E1B19kD are functionally equivalent in their ability to inhibit cell death. Oncogene 14:405–414
5. Wang S, Yang D, Lippman ME (2003) Targeting Bcl-2 and Bcl-XL with nonpeptidic small-molecule antagonists. Semin Oncol 30 [Suppl 16]:133–142
6. Krajewska M, Krajewski S, Epstein JI, Shabaik A, Sauvageot J, Song K, Kitada S, Reed JC (1996) Immunohistochemical analysis of bcl-2, bax, bcl-X, and mcl-1 expression in prostate cancers. Am J Pathol 148:1567–1576
7. Olopade OI, Adeyanju MO, Safa AR, Hagos F, Mick R, Thompson CB, Recant WM (1997) Recant overexpression of BCL-x protein in primary breast cancer is associated with high tumor grade and nodal metastases. Cancer J Sci Am 3:230–237
8. Tzung SP, Kim KM, Basañez G, Giedt CD, Simon J, Zimmerberg J, Zhang KY, Hockenbery DM (2001) Antimycin A mimics a cell-death-inducing Bcl-2 homology domain 3. Nat Cell Biol 3:183–191
9. Kim KM, Giedt CD, Basañez G, O'Neill JW, Hill JJ, Han YH, Tzung SP, Zimmerberg J, Hockenbery DM, Zhang KY (2001) Biophysical characterization of recombinant human Bcl-2 and its interactions with an inhibitory ligand, antimycin A. Biochemistry 40:4911–4922
10. Dunshee BR, Leben C, Keitt GW, Strong FM (1949) The isolation and properties of antimycin A. J Am Chem Soc 71:2436–2437
11. Agrawal S, Zhang X, Cai Q, Zhao H, Tan W, Yu D, Zhang R (1998) Effect of aspirin on pharmacokinetics of antisense oligonucleotides in rats. J Drug Target 5:303–312
12. Zhang R, Li Y, Cai Q, Liu T, Sun H, Chambless B (1998) Preclinical pharmacology of the natural product anticancer agent 10-hydroxycamptothecin, an inhibitor of topoisomerase I. Cancer Chemother Pharmacol 41:257–267
13. Wang H, Cai Q, Zeng X, Yu D, Agrawal S, Zhang R (1999) Anti-tumor activity and pharmacokinetics of a mixed-backbone antisense oligonucleotide targeted to RIα subunit of protein kinase A after oral administration. Proc Natl Acad Sci U S A 96:13989–13994
14. Wang H, Li M, Rinehart JJ, Zhang R (2004) Pretreatment with dexamethasone increases anti-tumor activity of carboplatin and gemcitabine in mice bearing human cancer xenografts: in vivo activity, pharmacokinetics and clinical implications for cancer chemotherapy. Clin Cancer Res 10:1633–1644
15. Zhang R, Lu Z, Zhao H, Zhang X, Diasio RB, Habus I, Jiang Z, Iyer RP, Yu D, Agrawal S (1995) In vivo stability, disposition and metabolism of a "hybrid" oligonucleotide phosphorothioate in rats. Biochem Pharmacol 50:545–556