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Comparative murine metabolism and disposition of class II anthracycline antibiotics*

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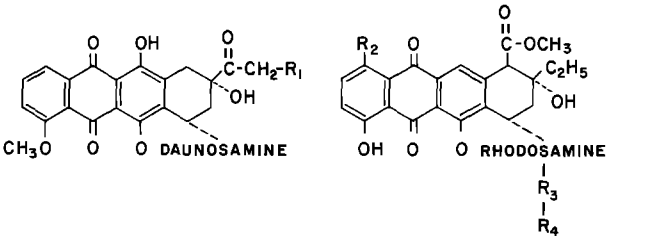
Summary. The metabolism and tissue distribution of aclacinomycin A (ACL), marcellomycin (MCM), and musettamycin (MST), three new anthracycline antibiotics, were compared after IV administration to mice. In plasma, total MCM- and ACL-derived fluorescence declined according to first-order kinetics, whereas an initial decline followed by a rebound was observed for MST. In plasma, MCM remained the predominant compound. ACL was eliminated more quickly, and was replaced by two metabolites, the reduced glycoside M_1, and an aglycone. In the case of MST, two unidentified metabolites were observed in concentrations equivalent to that of the parent drug.

The three drugs were distributed widely to organs, but only ACL achieved measurable concentrations in the brain. Initially, high concentrations of all three drugs were present in the lungs, but these decreased quickly to values similar to those present in the liver and kidneys. Intermediate concentrations of the three drugs were measured in heart and skeletal muscle. Splenic concentrations of all three drugs rose progressively, reaching a maximum at 8 h after injection in the case of ACL and MST, and at 24 h after injection in the case of MCM. Concentrations of the metabolites of MCM and MST were low in all organs except liver and kidney, where the aglycones 7-deoxypyrromycinone and bisanhydropyrromycinone were seen. The metabolism of ACL was extensive. Aglycones were dominant in the liver and kidneys, whereas reduced glycosides predominated in the spleen. These observations indicate that the murine pharmacology of these three structurally similar drugs differs markedly.

Introduction

Anthracycline antibiotics constitute a major class of antitumor agents [28]. Two anthracyclines, doxorubicin (Adriamycin, DOX) and daunorubicin (DNR), have been used extensively to treat a wide variety of solid and hematologic malignancies in humans [28]. However, their use may be complicated or limited by the occurrence of serious and sometimes life-threatening side effects, including a cumulative, dose-related cardiomyopathy associated with progressive and often irreversible heart failure [26]. As a result, there have been continuous efforts to develop new naturally occurring and semisynthetic anthracyclines with better therapeutic indices [20].

Aclacinomycin A (ACL) and marcellomycin (MCM) (Fig. 1) are new anthracycline antibiotics that have been recently introduced into clinical trials [15, 18, 22, 23, 25, 27]. The structures of MCM and ACL differ from those of DOX and DNR in the aglycone chromophore, and they have trisaccharides linked to carbon 7, in contrast to DOX and DNR, which contain only one sugar at this position. Musettamycin (MST) (Fig. 1) is a disaccharidic anthracycline similar to MCM but without the terminal deoxyfucose [21]. In addition to structural differences, the anthracycline antibiotics differ in their effects on nucleic acid biosynthesis. MCM, ACL, and MST, designated class II anthracyclines, inhibit nucleolar RNA synthesis at concentrations 100- to 1000-fold lower than those required to inhibit DNA synthesis [5, 8, 23, 24]. In contrast, class I anthracyclines, including DOX and DNR, inhibit the syntheses of



COMPOUND	R_1
Daunorubicin	H
Adriamycin	OH

COMPOUND	R_2	R_3	R_4
Aclacinomycin-A	H	Deoxyfucose	L-Cinerulose
Marcellomycin	OH	Deoxyfucose	Deoxyfucose
Musettamycin	OH	Deoxyfucose	H

Fig. 1. Structures of doxorubicin (Adriamycin), daunorubicin, aclacinomycin A, musettamycin, and marcellomycin

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DNA and nucleolar RNA at similar concentrations [5, 8, 23, 24].

ACL has already undergone phase I and II clinical testing [3, 18, 19, 23, 25, 27] and has demonstrated antitumor activity, especially in acute leukemia. In addition, this drug induces less alopecia and extravasation necrosis than do DOX and DNR. In the two phase I clinical trials conducted thus far with MCM, myelosuppression has been the major and dose-limiting toxicity [15, 22]. In addition, the myelotoxicity of MCM is erratic and unpredictable, both among individual patients and for repeated courses in a given patient. To date, no clinical trials have been performed with MST.

The human pharmacokinetics of MCM and ACL are different [6, 11, 16]. After bolus injection of ACL the parent drug quickly disappears from plasma and is replaced by two aglycones whose concentrations exceed those of parent drug [11]. This produces a decrease followed by a rebound in plasma total drug-derived fluorescence. In contrast, following treatment with MCM there is a progessive decrease of total drug-derived fluorescence, with parent drug remaining the predominant fluorescent compound [6]. No animal or clinical pharmacokinetic data are available for MST.

Our laboratory has recently published a study on the murine metabolism and disposition of MCM [7]. We demonstrated that the metabolic pathways for this drug were qualitatively similar in mouse and human, but that there were important quantitative differences. To complete the comparison between ACL and MCM in the same species and between human and mouse for each of these two drugs, we undertook to characterize the murine metabolism and disposition of ACL. In addition, we wanted to characterize the murine metabolism and disposition of MST to allow comparison of these properties for all three structurally related class II anthracyclines.

Materials and methods

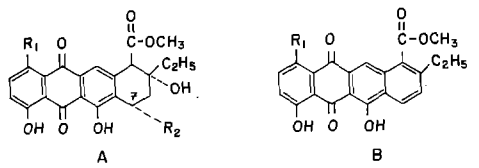
Drug supply and purity. MST was supplied by Bristol-Myers Laboratories (Syracuse, NY). ACL was provided by Sanraku-Ocean Co., Ltd (Tokyo, Japan). Drug purity was assessed on 250 μm silica gel 60 plates (E. Merck, Darmstadt, Germany), which were developed in an ascending fashion to a solvent front of 16 cm in a mixture of chloroform: methanol-glacial acetic acid: water, 80:20:14:6 by volume (system I) for MST or chloroform: methanol:water, 80:20:3 by volume (system II) for ACL. ACL proved to be more than 99% pure. MST contained only 80.9% MST, the remaining fluorescent material representing 8.0% MCM, 8.2% bisanhydropyrromycinone, and 2.9% pyrromycine (Fig. 2). Because of very limited availability of MST, further purification of MST was not feasible.

Animals. Male Swiss-Webster mice weighing 25–30 g were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass). The animals were fed a standard chow diet (NIH Rat and Mouse Ration, Ralston Purina, St. Louis, Mo) and were housed in groups of 25 per cage, in a controlled anvironment with 12-h light and dark cycles.

Treatment and tissue preparation. Mice received the drugs by IV bolus injections into a tail vein, at a dosage of 20.6 μmol/kg (i.e., equivalent to 16.9 mg/kg for ACL and 14.7 mg/kg for MST) in a volume of 0.10–0.15 ml. This dosage corresponded to that used in our previous study of MCM (20.6 μmol/kg or 17.4 mg/kg) [7]. ACL was dissolved in 0.01 *M* glucuronic acid. MST was dissolved in 0.01 *M* glucuronic acid:dimethyl sulfoxide: 0.154 *M* sodium chloride, 5:5:90 by volume. In no case did the vehicle affect the stability of the drugs. At 1, 3, 5, 10, 15, and 30 min, and 1, 2, 4, 8, 16, 24, 48, and 72 h after injection, groups of 6 mice were anesthetized with diethyl ether. Blood was collected from the retro-orbital venous plexus and placed into iced, heparinized (50 units/tube) Eppendorf microtubes. Mice were then killed by cervical dislocation, and brains, hearts, lungs, livers, kidneys, spleens, and skeletal muscles were quickly removed, weighed, frozen on dry ice, and stored at −20 °C until analysis. Blood samples were centrifuged at 12 200 *g* for 2 min and the resulting plasma supernatant was frozen on dry ice and stored at −20 °C until analysis.

Plasma analyses. Plasma analyses were performed according to a modification of the method described by Benjamin et al. [2]. For each time point, three of the six plasma samples were assayed for total drug fluorescence. To this end, 0.3 ml plasma was extracted with 1.2 ml isopropanol:2.16 *N* sulfuric acid, 75:25 by volume. The extracts were stored overnight at 4 °C and then centrifuged at 14 500 *g* for 15 min at 4 °C, after which the total fluorescence of the resulting supernatant solution was measured. Fluorescence was determined with an Aminco-Bowman spectrofluorometer (SLM Instruments, Inc., Urbana, Ill) with an excitation wavelength of 470 nm and an emission wavelength of 550 nm. Fluorescence values were corrected for endogenous, nonspecific fluorescence determined in plasma of control animals that had received vehicle only and were quantified by comparison to a simultaneously prepared standard curve in which known amounts of parent drug were added to 0.3-ml aliquots of 0.154 *M* NaCl.

The three other plasma samples obtained at each time point were pooled and assayed for individual fluorescent species. For this purpose, 0.5-ml samples of plasma were each extracted by addition of 2 ml chloroform:isopropanol, 1:1 by volume, and saturating amounts of $(NH_4)_2SO_4$. The organic phase was separated by centrifugation at 27 000 *g* for 10 min at 4 °C, collected, and dried under a nitrogen jet. The dried residue was redissolved in 100 μl chloroform:methanol, 1:1 by volume, and 40-μl portions were spotted onto TLC plates. All TLC plates were routinely spotted with standards. These consisted of ACL and authentic metabolites (M_1, N_1, bisanhydroaklavinone, 7-deoxyaklavinone, and E_1, the 7,7'-dimer of 7-deoxaklavinone), in the case ACL and of MST, and related anthracyclines (pyrromycine, pyrromycinone, 7-deoxypyrromycinone, and bisanhydropyrromycinone) in the case of MST (Fig. 2). The plates were developed for 16 cm in an ascending fashion in ethylacetate, air-dried, and then developed for 12 cm in an ascending fashion in system I (MST) or system II (ACL). Occasionally, when a better separation of the aglycones was desired, TLC plates were developed in system III, which consisted of a mixture of chloroform:methanol-Glacial acetic acid, 100:2:2.5 by volume. Fluorescent spots were identified under 254 nm light (UVS-54 Mineralight, Ultra-Violet Products, San Gabriel, Calif) and then were scraped from the plate. Fluores-



A B

COMPOUND	BASIC STRUCTURE		
Aclacinomycin A	A	H	Rhodosamine - Deoxyfucose - L Cinerulose
Marcellomycin	A	OH	Rhodosamine - Deoxyfucose - Deoxyfucose
M_2	A	OH	Rhodosamine - Rhodinose ? - Rhodinose ?
M_1	A	H	Rhodosamine - Deoxyfucose - Aminocetose
N_1	A	H	Rhodosamine - Deoxyfucose - Rhodinose
Musettamycin	A	OH	Rhodosamine - Deoxyfucose
Aklavine	A	H	Rhodosamine
Pyrromycine	A	OH	Rhodosamine
Aklavinone	A	H	OH
Pyrromycinone	A	OH	OH
7-Deoxyaklavinone	A	H	H
7-Deoxypyrromycinone	A	OH	H
Bisanhydroaklavinone	B	H	H
Bisanhydropyrromycinone	B	OH	H

[a] The 7,7' Dimer of 7-Deoxyaklavinone corresponds to 2 molecules of 7-Deoxyaklavinone linked to each other in position 7

Fig. 2. Structures of aclacinomycin A, marcellomycin, musettamycin, and some of their metabolites

cent adsorbents were eluted into 2 ml isopropanol:2.16 *N* sulfuric acid, 75:25 by volume, and assayed for fluorescence content. The concentration of each fluorescent metabolite was calculated as the product of its relative amount on TLC and the total fluorescence, and corrected for nonspecific plasma fluorescence. The assay in plasma was linear between at least 0.001 and 1 μM and the lowest limit of detection was 0.001 μM.

Organ analyses. Three of the six samples obtained at each time were assayed for total drug fluorescence. Organs were homogenized with a Polytron homogenizer (Brinkman Instruments, Westbury, NY) in 10–20 volumes of isopropanol:2.16 *N* sulfuric acid, 75:25 by volume. After centrifugation at 27 000 *g* for 10 min at 4 °C the drug equivalent fluorescence of the supernatant fluid was determined and quantified by comparison with a standard curve prepared simultaneously and in the same way as described for plasma.

The remaining organs obtained at each time were assayed for individual fluorescent species. Each liver, kidney, or lung was assayed individually. Due to their lower drug contents and smaller sizes, brains, muscles, spleens, and hearts obtained at each time point were pooled, and each type organ pool was extracted as a single sample. In addition, because of the relatively low fluorescence efficiency of ACL, the organs obtained from the animals treated with this drug were spiked with a DNR internal standard (10 nmol/g). Organs were homogenized with a Polytron homogenizer in 10–20 volumes of iced chloroform:methanol, 2:1 by volume. The resulting homogenate was clarified by filtration through glass wool and was evaporated to dryness under nitrogen. The dried extract was redissolved in 0.3–0.4 ml chloroform:methanol, 1:1 by volume, and 20 to 60 µl aliquots were spotted onto TLC plates. The plates were developed in an ascending fashion in ethylacetate for 16 cm and then for 12 cm in system I (MST or in system II (ACL). The plates were processed and concentrations were determined as described above for plasma. The lowest limit of detection in the organs was 0.1 nmol/g

Identification of metabolites. Metabolites (Fig. 2) were presumptively identified by co-chromatography with known standards on TLC and HPLC. Pyrromycin and pyrromycinone were kindly supplied by Bristol-Myers Laboratories (Syracuse, NY). Aklavin, aklavinone, 7-deoxyaklavinone, M_1, N_1, bisanhydroaklavinone, and the 7,7'-dimer of 7-deoxyaklavinone (E_1) were kindly supplied by Dr T. Oki of Sanraku-Ocean Co., Ltd (Tokyo, Japan). Bisanhydropyrromycinone and 7-deoxypyrromycinone were prepared as described previously [7].

HPLC analyses. HPLC analyses were done by a modification of the method of Andrews et al. [1] on a Spectra Physics 3500 B HPLC (Spectra Physics, Santa Clara, Calif) fitted with a µ Bondapak-phenyl column (3.9 mm × 30 cm) (Waters Associates, Milford, Mass). The mobile phase consisted of a 10-min linear gradient of 32%–72% tetrahydrofuran in 0.1% (w/v) ammonium formate buffer (pH 4.0) pumped at a flow rate of 2 ml/min. Fluorescence was detected with an Aminco Fluoromonitor (SLM Instruments, Inc., Urbana, Ill) fitted with a 470-nm excitation filter and a 500-nm cutoff emission filter.

Pharmacokinetic analysis. Computer modeling of the plasma concentrations of ACL and MST was accomplished using the MLAB program [17] (NIH, Bethesda, MD), and pharmacokinetic parameters were calculated from the modeled data. The same program was used in our previous study of MCM [7].

Statistical analysis. Student's *t*-test was used for statistical comparisons.

Results

Plasma pharmacokinetics: total drug fluorescence

In the case of ACL, plasma concentrations of total drug-derived fluorescence declined progressively (Fig. 3), with no drug related fluorescence detectable by 72 h after IV injection. A similar behavior has been described for MCM in our previous study [7]. After treatment with MST, total drug-derived fluorescence decreased to a minimum at 5–10 min after injection. This was followed by a rebound and a final elimination phase (Fig. 3). Drug fluorescence was still detectable at 72 h after treatment.

Marcellomycin. The plasma pharmacokinetics of MCM and its metabolites in mice, and the identification of these metabolites, have been described in detail in our previous study [7]. For the purpose of comparison, the most important pharmacokinetic parameters for MCM are listed in Table 1.

Aclacinomycin A. ACL was no longer detectable in plasma by 8 h after injection (Fig. 4). The pharmacokinetic parameters for ACL in plasma are listed in Table 1. ACL disappeared from plasma with a terminal half-life of 2.1 h and had a total body clearance of 0.46 l/min/m^2 (Table 1). The AUC was 2.24 $\mu M \times$ h. After treatment with ACL, two metabolites were observed (Fig. 4). They were more per-

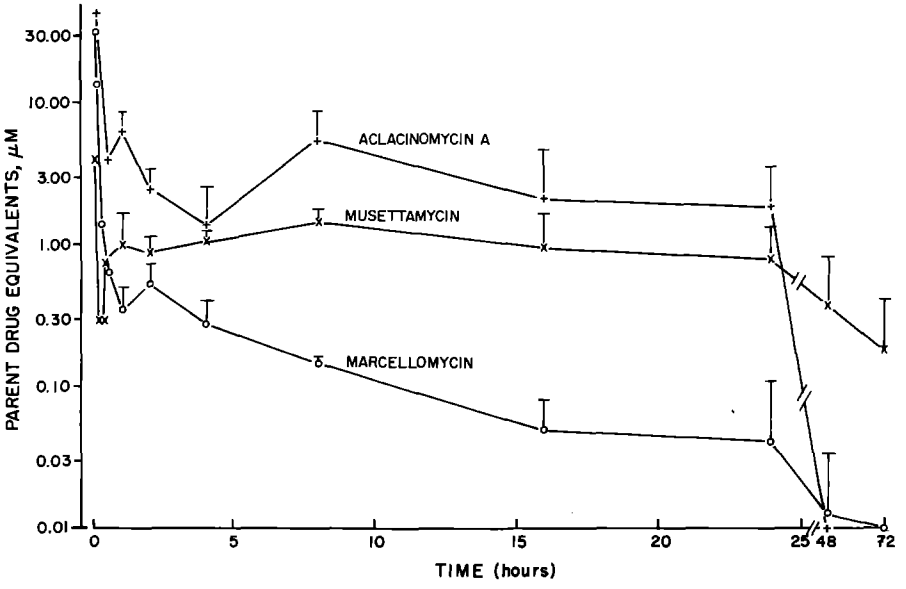


Fig. 3. Plasma total fluorescence in mice treated with aclacinomycin A, marcellomycin, or musettamycin. Aclacinomycin A, marcellomycin, or musettamycin was injected IV (20.6 μmol/kg) to mice. At specified times, plasma was obtained. Total fluorescence was determined after acid/alcohol extraction (as described in *Materials and methods*). Each *point* represents the mean of three determinations. *Bars* indicate the SD

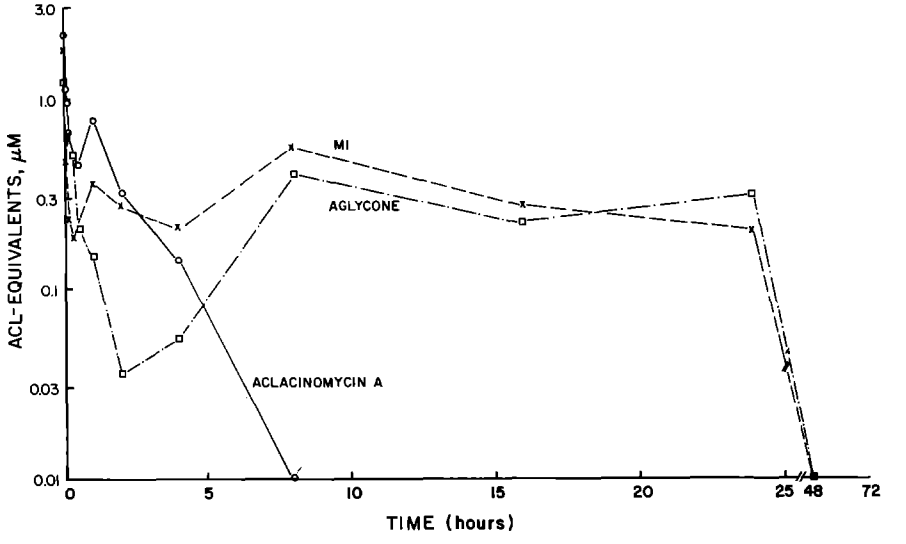


Fig. 4. Plasma concentration of parent drug and metabolites in mice injected with aclacinomycin A. Aclacinomycin A was injected IV (20.6 μmol/kg) to mice. Plasma was obtained at specified times. Parent drug and metabolites were separated by TLC and quantified by fluorescence as described in *Materials and methods*. Each *point* represents the mean of three determinations. SD bars have been omitted for clarity, but SD was usually less than 25% of the means

Table 1. Plasma pharmacokinetic parameters for marcellomycin, aclacinomycin and musettamycin

Parameter[a]	MCM	ACL	MST
Peak concentration (μM)	28.57	2.14	1.71
Time of peak (min)	1	1	1
Apparent volume of central compartment (l/m²)	1.22	20.9	0.78
Terminal half-life (h)	7.5	2.3	18.9
AUC (μMxh)	3.76	2.24	11.13
Total body clearance (l/m²/min)	0.27	0.46	0.09

[a] MCM, ACL or MST was injected IV (20.6 μmol/kg) to mice. Plasma was obtained at specified times, and parent drug and metabolites were analyzed by TLC as described in *Materials and methods*. Pharmacokinetic parameters were obtained by simulation with the MLAB program (Division of Computer Resources and Technology, NIH, Bethesda, Md)

sistent than the parent drug, and by 8 h after injection they accounted for 100% of the total drug-derived fluorescence in plasma. The first of these metabolites had the same chromatographic behavior as standard M1. The second metabolite had the mobility of an aglycone, but insufficient plasma material precluded further identification of this compound. The plasma concentration of this metabolite was maximal (1.23 μ*M*) at 1 min after treatment and the AUC for this compound was 9.74 μ*M*×h. The peak plasma concentration of M1 (1.81 μ*M*) was observed at 1 min after injection and the AUC for M1 was 10.08 μ*M*×h.

Musettamycin. After injection of MST, plasma concentrations of parent drug decreased to a minimum within 5–10 min (Fig. 5). This was followed by a rebound and a final elimination phase. The terminal half-life for MST was 18.9 h, the AUC was 11.13 μ*M*×h, and the total body clearance was 0.09 l/min/m² (Table 1). Following administration of MST, two metabolites were seen in plasma.

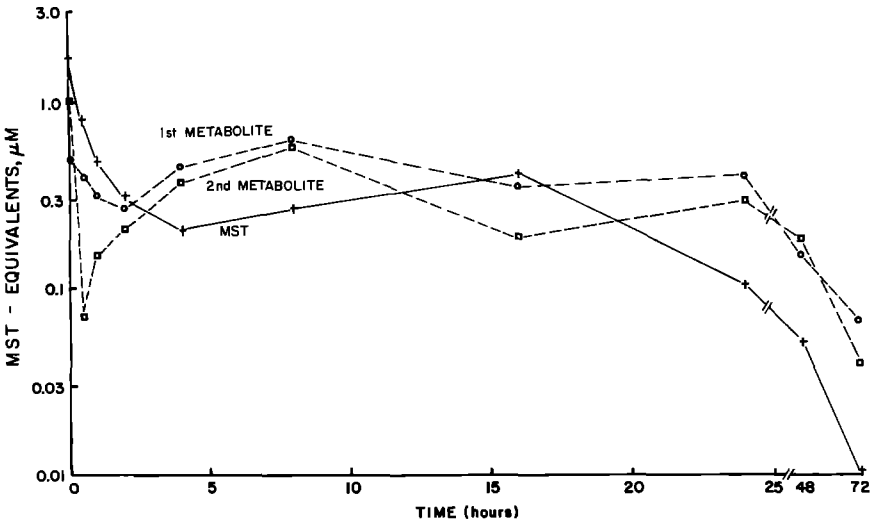


Fig. 5. Plasma concentrations of parent drug and metabolites in mice following injections of musettamycin 20.6 μmol/kg IV. Plasma was obtained at specified times. Parent drug and metabolites were separated by TLC and quantified by fluorescence as described in *Materials and methods*. Each *point* represents the mean of three determinations. SD bars have been omitted for clarity, but SD was usually less than 25% of the mean

These metabolites had TLC properties differing from any of our standards, but insufficient amounts of metabolites were available to allow further identification of these compounds. The peak plasma concentrations of these two metabolites were 0.50 μ*M* and 1.09 μ*M*. An initial rapid decline in concentrations of these two metabolites was followed by rebound phenomena similar to those described for total drug-derived fluorescence and parent drug. Both metabolites were more persistent than MST, and by 4 h after injection their concentrations exceeded those of MST. Their AUCs were 19.01 and 14.43 μ*M*× h, respectively. No metabolites having the TLC properties of bisanhydropyrromycinone or 7-deoxypyrromycinone were seen after treatment with MST.

Table 2. Metabolites of marcellomycin, aclacinomycin A, and musettamycin in murine livers, kidneys, and spleens[a]

Organ	AUC (% of AUC for total drug-derived fluorescence)		
	MCM	ACL	MST
Liver			
Parent drug	81.8 ± 3.2[b]	2.0 ± 1.0[c]	43.1 ± 3.1[d]
Aglycones	9.2 ± 2.6	90.8 ± 2.0[c]	50.9 ± 2.3[d]
Kidney			
Parent drug	92.2 ± 6.5	1.6 ± 0.3	88.6 ± 2.5
Aglycones	3.2 ± 0.1	71.8 ± 5.5[b]	9.0 ± 3.1
Spleen[e]			
Parent drug	93.6	6.7	82.1
Aglycones	0.7	17.5	12.9
M_1 and N_1	–	71.5	–

[a] MCM, ACL or MST was injected IV (20.6 μmol/kg) to mice. Organs were obtained at specified times. Parent drug and metabolites were analyzed by TLC as described in *Materials and methods*. AUCs were calculated by the trapezoidale rule
[b] Mean ± SD
[c] Statistically different from MCM and MST ($P \leq 0.001$)
[d] Statistically different from MCM ($P \leq 0.001$)
[e] Based on a single extraction of three pooled spleens at each time point

Tissue drug content

MCM. Concentrations of total drug-related fluorescence, parent drug, and metabolites have been described in detail in our previous study [7]. For the purpose of comparison, some parameters for MCM and metabolites in organs are listed in Table 2.

ACL. After administration of ACL, the highest concentration of total drug-derived fluorescence was initially observed in the lungs (Fig. 6). However, pulmonary concentrations of total fluorescence were exceeded by those in liver by 15 min after injection, and by those in the kidneys by 2 h after treatment. Hepatic and renal concentrations of total drug-derived fluorescence were relatively stable for 24 h, and then decreased to negligible values by 72 h. In heart, a peak concentration of 60.0 nmol/g for total drug-derived fluorescence was noted at 3 min after treatment. The total fluorescence then decreased steadily and became undetectable by 48 h after treatment. Skeletal muscle contained intermediate concentrations of total drug-derived fluorescence. The initial splenic drug-equivalent concentration was 13.2 nmol/g, but rose progressively to reach a maximum (88.4 nmol/g) by 8 h after injection. There was a slight but not significant decrease in the weight of this organ from 0.12 ± 0.06 g to 0.09 ± 0.02 g (mean ± SD) between 1 min and 24 h after injection. At 8 h after injection, the splenic weight was still 0.16 ± 0.06 g. Therefore, the relative increase in splenic total drug-derived fluorescence at that point cannot be explained entirely by a decrease in organ weight. Finally, the total drug-derived fluorescence observed in brain ranged from 3.7 to 42.0 nmol/g between 1 min and 24 h, but was undetectable by 48 h after injection.

Substantial amounts of metabolites were seen in organs after treatment with ACL. In liver, metabolites accounted for most of the fluorescent material (Table 2). The AUCs for the aglycones, bisanhydroaklavinone and 7-deoxyaklavinone, represented 47.7% ± 7.6% and 37.9% ± 6.1%, respectively, of the AUC of total drug-derived fluorescence. Lower amounts of the 7,7'-dimer of 7-deoxyaklavinone, M1, and N1 were also observed. In the kidneys, bisanhydroaklavinone and 7-deoxyaklavinone were predominant.

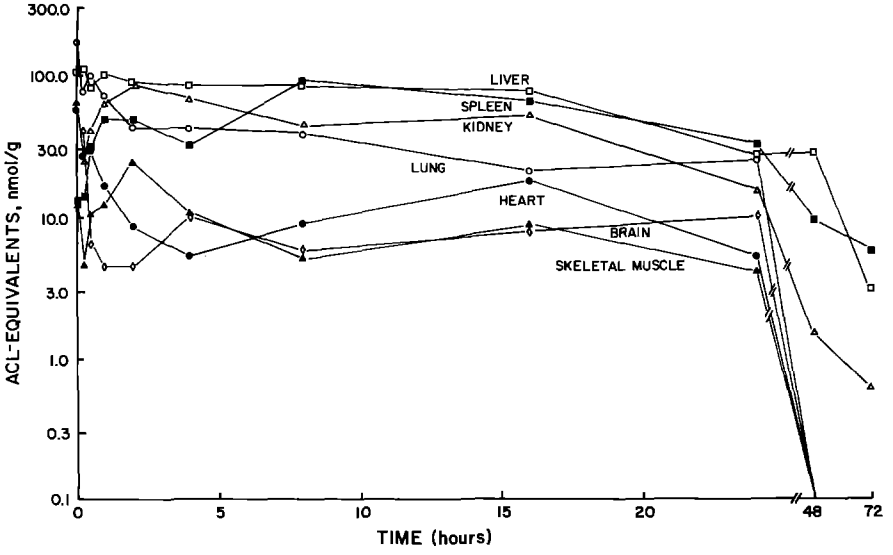


Fig. 6. Total fluorescence in organs of mice following injections of aclacinomycin A. 20.6 μmol/kg IV. At specified times organs were obtained. Total fluorescence was determined after acid/alcohol extraction as described in *Materials and methods*. Each *point* represents the mean of three determinations. SD bars have been omitted for clarity, but SD was usually less than 50% of the mean

Their AUCs represented, respectively, 37.6%±6.0% and 29.7%±4.2% of the AUC for total drug-derived fluorescence (Table 2). The hepatic and renal AUCs for the aglycones after treatment with ACL were statistically higher than the hepatic and renal AUCs for the aglycones after treatment with MCM ($P<0.001$) or MST ($P<0.001$). In spleen, M1 and N1 were the major fluorescent compounds, their AUCs representing 41.6% and 29.2% of the AUC for total drug-derived fluorescence (Table 2). Varying concentrations of parent drug and metabolites were observed in brain, heart, lungs, and skeletal muscle. Parent drug and N1 predominated in heart and lungs; brain contained mainly parent drug and M_1; and aglycones were dominant in skeletal muscle.

MST. After treatment with MST, the highest concentration of total drug-derived fluorescence was initially observed in the lungs (Fig. 7). Pulmonary concentrations of total drug-derived fluorescence decreased quickly, and by 15 min after injection the lungs, kidneys, and liver contained similar concentrations of total drug-derived fluorescence. Hepatic and renal concentrations of total drug-derived fluorescence remained fairly stable up to 48 h after injection. Skeletal muscle and heart contained intermediate amounts of total drug-related fluorescence. Negligible values were measured in brain. In spleen, the concentration of total drug-derived fluorescence increased from 33.43 nmol/g at 1 min to 119.47 nmol/g at 8 h after injection. There was a significant decrease in the splenic weight from 0.10±0.02 g at 1 min to 0.06±0.01 g at 24 h ($P<0.02$). However, at 8 h after injection the splenic weight was still 0.09±0.03 g, a value not statistically different from that of splenic weight at 1 min. Therefore, the relative increase in splenic concentration of total drug-derived fluorescence cannot be explained entirely by a decrease in splenic weight.

After treatment with MST, parent drug was the predominant compound in all organs except liver. The results for kidney, liver, and spleen are indicated in Table 2. In liver, two aglycones were seen. Their AUCs represented 39.0% and 12.1% of the AUC for total drug-derived fluorescence. They were identified tentatively as bisanhydro-

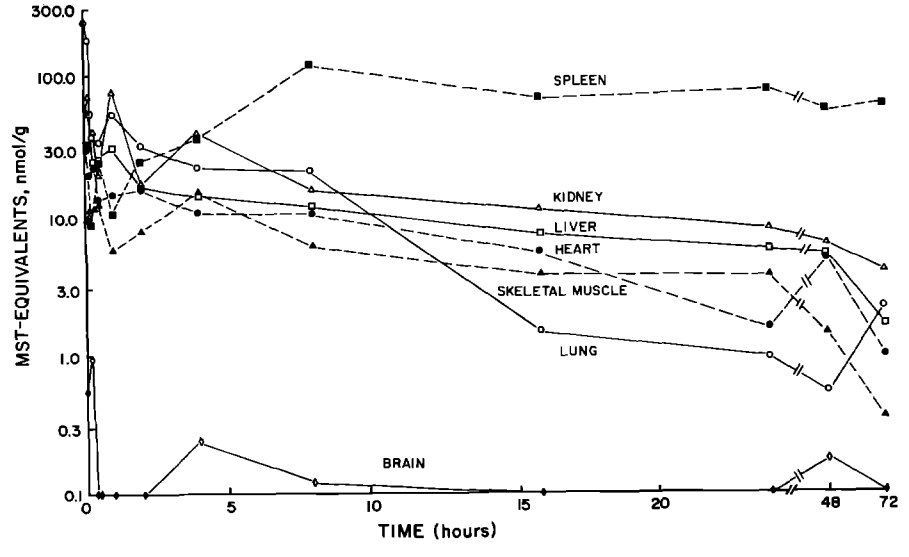


Fig. 7. Total fluorescence in organs of mice following injections of musettamycin 20.6 μmol/kg IV. At specified times organs were obtained. Total fluorescence was determined after acid/alcohol extraction as described in *Materials and methods*. Each *point* represents the mean of three determinations. SD bars have been omitted for clarity, but SD was usually less than 50% of the mean

pyrromycinone and 7-deoxypyrromycinone by co-chromatography with known standards. The hepatic AUC for aglycones after treatment with MST was significantly higher than the hepatic AUC for aglycones after treatment with MCM ($P < 0.001$). In kidney and spleen, parent compound was the predominant drug species present.

Discussion

The murine plasma pharmacokinetics of ACL mimicked, in part, the human plasma pharmacokinetics of this drug [11, 16]. Parent drug disappeared quickly in both mice and humans. Concentrations of metabolites exceeded that of the parent drug by 8 h in mice and by 1–4 h in human beings [11, 16]. Quantitatively, however, the production of these metabolites in man was sufficient to raise the total drug-derived fluorescence to values higher than those observed just after the administration of the drug. This rebound phenomenon was not seen in mice. In both mice and humans [11] one of the plasma metabolites was an aglycone, identified in humans as bisanhydroaklavinone. However, the identity of the second plasma metabolite differed between mice and humans. In mouse plasma we observed the reduced glycoside M1, whereas the second plasma metabolite in humans was tentatively identified as bisanhydroaklavinic acid [11]. However, the presence of M1 and N1 in human urines indicates that reduced glycosides are also produced by human beings [12].

We have previously reported on the similarities between the murine and human pharmacokinetics of MCM [6, 7]. In both species, plasma concentrations of parent drug decrease progressively, and the same metabolites, two aglycones and a polar conjugate, have been observed in both mice and humans.

Musettamycin (MST) is the disaccharidic anthracycline corresponding to MCM. Neither MST nor any other disaccaridic anthracyclines has yet been introduced into clinical trials, nor have the metabolism and disposition of these novel compounds been studied in animals. After IV injection of MST to mice, plasma total drug-derived fluorescence initially decreased. This was followed by a rebound that was due both to an increase in the concentration of MST and to the appearance of metabolites. The rebound in parent drug concentration is not easy to explain. It could correspond to the accumulation followed by the release of drug from a specific compartment or possibly an effect of the drug-delivery vehicle. With regard to the latter possibility, we could not demonstrate any pharmacokinetic differences when MCM was given in 5% or 10% dimethyl sulfoxide, with or without 0.01 *M* glucuronic acid (data not shown). Unfortunately, insufficient supplies of MST precluded further investigations with that drug. After administration of MST, two metabolites were seen at concentrations exceeding those of parent drug. These metabolites had chromatographic properties different from any of our standards, but insufficient quantities were available for further structural definition. They were less polar than MST and are therefore unlikely to represent conjugates of the parent drug. The structures of these metabolites remain unidentified.

In concert with their plasma pharmacokinetics, the organ distributions of MCM, ACL, and MST were profoundly different, although they shared a few common characteristics. For all three durgs, the greatest initial concentrations were measured in the lungs. This phenomenon has been described for a number of other anthracyclines, such as N,N-dimethyl-Adriamycin [9], N,N-dimethyl-daunorubicin [9], some iron–Adriamycin complexes [13], and Adriamycin octanoylhydrazone [10]. For some of these drugs, microembolic phenomena in the pulmonary capillaries, related to problems with drug solubility in plasma, have been proposed as the basis for the very high concentrations of drug found in lung tissue. Both MST and MCM are poorly soluble in aqueous media and could conceivably form particulates, obstructing small pulmonary vessels and leading to drug retention in the lungs. However, we did not observe any signs of respiratory distress after injection of either drug. In addition, ACL, which is highly soluble in aqueous media, also produced high peak concentrations in the lungs. Therefore, several factors may be involved in the pulmonary affinity of anthracyclines possessing differing aqueous solubilities.

Splenic concentrations of total drug-derived fluorescence increased progressively after IV injection of each class II anthracycline, with a maximum concentration being reached by 24 h for MCM [7] and by 8 h for ACL and MST. This increase in splenic total fluorescence has been described for a number of other anthracyclines and is usually explained by a decrease in splenic weight [7, 9, 10]. In the cases of MST and ACL, a decrease of the splenic weight was observed over the course of the experiments, but at the time when the highest splenic total drug-derived fluorescence was measured no significant change in splenic weight had yet occurred. Therefore, other factors, such as preferential uptake by reticuloendothelial cells, may contribute to this phenomenon.

Negligible amounts of drug-related fluorescence were measured in brain after treatment with MST. The same phenomenon was observed with MCM [7]. After administration of ACL, however, concentrations of total drug-derived fluorescence in the range of 3–42 nmol/g were observed. The brain's total drug-derived fluorescence was composed of approximately equal fractions of parent compound, reduced glycosides, and aglycones. Since ACM's reduced glycosidic metabolites are claimed to be active [14], cerebral tissue was significantly exposed to active compounds. To what extent this also applies to human brain remains to be established.

The metabolites observed in tissues were quite different for ACL, MCM, and MST. In the case of MCM and MST, significant concentrations of metabolites were seen only in liver. In both cases, 7-deoxypyrromycinone was present. In the case of MST significant amounts of bisanhydropyrromycinone were also observed. Since in vitro rat liver preparations convert MCM to three aglycones: 7-deoxypyrromycinone, bisanhydropyrromycine, and a third, less abundant aglycone [4], it is likely that these metabolites resulted from hepatic metabolism. However, since small quantities of bisanhydropyrromycinone contaminated the parent drug, hepatic sequestration of the exogenous aglycone, however unlikely, cannot be excluded.

The extent of murine metabolism of ACL appeared to be much greater than that of either MCM or MST. In liver, parent compound was virtually absent, and the summed AUCs for all aglycones represented 85.6% of the AUC calculated for total drug-derived fluorescence. In the kidneys, the AUC for the aglycones represented 67.3% of the AUC for total drug-derived fluorescence. In contrast, the AUC

in spleen, for the reduced glycosides, M1 and N1, amounted to 71.5% of the AUC for total drug-derived fluorescence. Other organs contained various proportions of parent drug and metabolites. The organ concentrations of the different metabolites may be related to specific enzyme localization, or to a variable capacity of these organs to concentrate different metabolites of ACL. These two possibilities cannot be distinguished at present.

In conclusion, the metabolic pathways for ACL are qualitatively similar in mouse and human. Such a qualitative similarity is also observed in the case of MCM. However, in both cases there are important quantitative differences between mouse and man. Finally, despite their structural similarity, the class II anthracyclines, MCM, ACL, and MST, have very different metabolic and pharmacokinetic behaviors.

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