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Mitomycin Antibiotic Reductive Potential and Related Pharmacological Activities*

SU-SHU PAN and HECTOR GONZALEZ

Division of Developmental Therapeutics, University of Maryland Cancer Center, Baltimore, Maryland 21201

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SUMMARY

Relationships of reductive potential, kinetics of enzymatic reduction, augmented oxygen consumption, and cytotoxicity were determined for seven clinically relevant mitomycin antibiotics. Potentials for one-electron reduction were obtained by cyclic voltammetry analysis in dimethyl sulfoxide with 0.1 M tetraethylammonium perchlorate. These potentials were −0.55 V for N^7-acetylmitomycin C, −0.61 V for mitomycin A, −0.75 V for N^7-(*p*-hydroxyphenyl)mitomycin C, −0.79 V for N^7-(dimethylaminomethylene)mitomycin C, −0.81 V for N^7-(2-(4-nitrophenyldithio)-ethyl)mitomycin C, −0.87 V for mitomycin C, and −0.89 V for porfiromycin. All seven antibiotics were reduced by xanthine oxidase and NADPH-cytochrome P450 reductase, but the rate of reduction varied for each antibiotic and each enzyme. The less negative the reductive potential of an antibiotic, the more easily that antibiotic was reduced enzymatically. These seven mitomycin antibiotics also augmented oxygen consumption by rat liver microsomes. As with their reduction by xanthine oxidase and NADPH-cytochrome P450 reductase, the less negative the reductive potential of an antibiotic, the more it augmented oxygen consumption. Cytotoxicity of each antibiotic was assessed by defining the IC_{50} against HCT 116 human colon carcinoma cells. A relationship between the reductive potential of these antibiotics and their cytotoxicity against HCT 116 cells was also observed.

MC is an antitumor antibiotic that is active against a variety of animal and human tumors (1). Its cumulative myelosuppressive effect has prevented it from gaining wider clinical acceptance (1). In recent years, several promising mitomycin analogues were reported to be less myelosuppressive than MC in animal models. These analogs included Ac-MC (2), 7-cysteaminomitosane (3), DMAM-MC (4), and M-83 (5). However, Ac-MC and DMAM-MC were subsequently found to be cardiotoxic (6, 7). Other side effects such as liver toxicity were uncovered during autologous bone marrow transplant studies that suggested additional limitations to the use of MC (8, 9). Future efforts are needed to develop new analogues that have the ability to circumvent these side effects yet maintain the efficacy of MC. At present, it is known that enzymatic activation or acidic hydrolysis is required for the action of MC (10–13), and its stimulation of oxygen free radical formation is believed to be related to cardiotoxicity (6, 7, 14). All of these mechanistic properties involve a key reaction, i.e., reduction of quinone moiety of these antibiotics. We feel that the reductive potential of mitomycin analogues may play a key role as an indicator for their activation and their subsequent activities and may be utilized as a preliminary criterion for the analysis of analogues. This paper will present the relationships of reductive potential, kinetics of enzymatic reduction, induction of oxygen consumption, and cytotoxicity for seven mitomycin antibiotics with different substituent groups at the C-7 position of mitomycin (Fig. 1).

Materials and Methods

Reagents. MC, PFM, and MA were kindly supplied by the Natural Products Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). Ac-MC (BMY-26605), DMAM-MC (BMY-25282), and NPSSE-MC (BMY-25067) were kindly provided by Dr. Stephen Carter, Bristol Laboratories (Wallingford, CT). M-83 was provided by Dr. Makoto Morimoto, Kyowa Hakko Kofyo Co. (Nagaizui, Sunto, Shizuoka, Japan). NADPH-cytochrome P450 reductase and rat liver microsomes were isolated by the methods described by Yasukochi and Masters (15). Bovine milk xanthine oxidase was isolated by the method of Nelson and Handler (16). The maximum absorbance wavelength of each antibiotic was obtained by scanning in 50 mM Tris·HCl at pH 7.5, with a Cary 118 spectrophotometer (Varian, Palo Alto, CA). These wavelengths were 365 nm for MC, PFM, and NPSSE-MC, 320 nm for MA, 328 nm for Ac-MC, 382 nm for M-83, and 392 nm for DMAM-MC.

Cyclic voltammetry. Cyclic voltammetric analyses were performed according to the method described earlier (17), with a CV-1A voltam-

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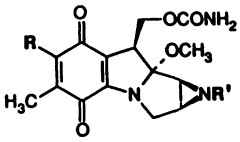
ABBREVIATIONS: MC, mitomycin C; PFM, porfiromycin; Ac-MC, N^7-acetylmitomycin C; MA, mitomycin A; M-83, N^7-(*p*-hydroxyphenyl)mitomycin C; DMAM-MC, N^7-(dimethylaminomethylene)mitomycin C; NPSSE-MC, N^7-(2-(4-nitrophenyldithio)-ethyl)mitomycin C; PBS, phosphate-buffered saline; HPLC, high pressure liquid chromatography; DMSO, dimethyl sulfoxide.

metry unit (Bioanalytical Systems, West Lafayette, IN) connected to a thermostatically controlled electrochemical cell (IBM Instruments Inc., Danbury, CT). All antibiotic solutions were prepared at 0.8 mM in DMSO with 0.1 M tetraethylammonium perchlorate. Potentials were measured with a glassy carbon working electrode, a platinum wire counter electrode, and an Ag/AgCl (saturated KCl) reference electrode. A potential range of 0.00 to −1.70 V with scan rates of 20, 50, 100, 200, and 500 mV/sec, was used for all seven compounds.

Enzymatic reduction of antibiotics. The reduction of each antibiotic by xanthine oxidase and NADPH-cytochrome P450 reductase was performed in assays described earlier, with some modification (13). The 250-μl reaction mixtures contained 50 mM Tris·HCl at pH 7.5, 0.5 mM NADH, 32 μg of xanthine oxidase (or 0.5 mM NADPH and 2 μg of NADPH-cytochrome P450 reductase), and one antibiotic at its appropriate concentration. Reactions were carried out anaerobically for 1 to 15 min at 37° with shaking. After termination of the reaction, aliquots of the solution were injected onto the HPLC column for analysis (13).

Oxygen consumption. Oxygen consumption by rat liver microsomes was measured with a Clark-type electrode in a Yellow Springs Instrument model 53 oxygen analyzer (Yellow Springs, OH), as described by Bachur *et al.* (18). The reaction mixture contained 0.1 M phosphate buffer at pH 7.5, 0.5 mM NADPH, 1 mM diethylenetriaminepentaacetic acid, and 0.5 mg of microsomes in a total of 1.5 ml. Baseline oxygen consumption was first measured without any antibiotics. Then antibiotics, dissolved at designated concentrations in DMSO, were added to the reaction mixture and drug-enhanced oxygen consumption was measured. When the antibiotic was added, a final concentration of 0.4% DMSO was established in the reaction chamber. Control experiments with an equivalent amount of DMSO showed no effect on microsomal activity.

HPLC analyses. A Hewlett-Packard 1090M HPLC with a diode array detector (Palo Alto, CA) was used for all analyses of antibiotics and their products. A Brownlee 5-μm RP-18 (220 × 4.6 mm) column and a 7-μm RP-18 (15 × 3.2 mm) guard column were used. The mobile phase consisted of a 15-min linear gradient of 0 to 50% methanol in 10 mM potassium phosphate buffer at pH 7.0. The flow rate was 1.0 ml/min, and the column eluate was monitored at the appropriate maximum absorbance wavelength of each compound. Antibiotic concentrations in reaction mixtures were estimated by integration of the peak area and comparison with those of concomitantly analyzed external standards.



ANTIBIOTICS	R	R'
Ac-MC (BMY-26605)	$CH_3-\overset{O}{\overset{\|}{C}}-NH-$	H
MA	CH_3-O-	H
M-83 (KW-2083)	HO–C$_6$H$_4$–NH–	H
DMAM-MC (BMY-25282)	$(H_3C)_2N-CH=N-$	H
NPSSE-MC (BMY-25067)	O_2N–C$_6$H$_4$–S–S–CH_2CH_2NH-	H
MC	NH_2-	H
PFM	NH_2-	CH_3

Fig. 1. Structural formulae of mitomycin antibiotics.

Cell culture and cell survival. HCT 116 human colon carcinoma cells were maintained in McCoy's medium 5A, as described previously by Wilson *et al.* (19). Cell survival after drug treatment was assessed by colony formation as follows. Confluent HCT 116 cells were seeded at 300, 600, and 900 cells/35-mm culture plate and incubated overnight at 37° to allow cells to adhere. Cells were exposed to each antibiotic at six different concentrations for 1 hr at 37°, washed twice with PBS, and returned to fresh growth medium. Incubation was resumed at 37° for up to 14 days, until visible colonies developed. Medium was removed and plates were rinsed with saline. Cells were fixed and stained for 20 min with 0.25% crystal violet in methanol/formaldehyde (90:10). After thorough rinsing with water, colonies containing more than 50 cells were counted as survivors, and a survival curve for each antibiotic was plotted.

Partition coefficient. The partition coefficient between octanol and PBS was determined for each antibiotic. This was accomplished by vigorously mixing 1 ml of a solution of the antibiotic in PBS with an equal volume of octanol, centrifuging the mixture at 1000 × *g* for 10 min, and measuring the concentrations of the antibiotic in the organic and aqueous layers.

Results

Cyclic voltammetry. Cyclic voltammetric analyses for the seven mitomycin antibiotics were conducted at a potential range between 0.00 and −1.70 V and at five different scan rates. The best resolution of peaks was obtained at a scan rate of 20 mV/sec, and these results are presented (Fig. 2, Table 1). The data in Table 1 are arranged in order of increasingly negative reductive potential of the first peak of each antibiotic. In DMSO, electrochemical reduction occurred in two one-electron-transferring steps. The first cathodic wave corresponds to the transfer of an electron to the mitomycin quinone, to form an anion radical. The second cathodic wave corresponds to the transfer of an electron to the mitomycin radical anion, to generate the dianion (17). The first waves are quasireversible charge-transfer processes where the separation of cathodic and anodic peak potential ($E_p^c - E_p^a$) varied between 102 and 159 mV (Table 1). The second cathodic wave was seen for all seven antibiotics, but on the anodic sweep a poorly defined anodic peak was observed with each compound except NPSSE-MC (Table 1). This probably reflects rapid reoxidation of the dianion of these antibiotics. Repetitive scans for each antibiotic revealed consistent cathodic and anodic waves.

Kinetics of antibiotic reduction. All seven antibiotics were reductively metabolized by xanthine oxidase and NADPH-cytochrome P450 reductase (Table 2, Fig. 3). Disappearance of antibiotics, as measured at their maximum absorbance wavelength during analysis, was dependent on substrate and enzyme concentration. Solubility limits precluded achieving concentrations of any antibiotic that would saturate either enzyme. Therefore, Michaelis-Menten kinetic parameters could not be obtained. The rate of reduction of a fixed initial concentration of each antibiotic was calculated from a time course. Ac-MC, MA, M-83, and DMAM-MC were used at 0.5 mM. MC and PFM were used at 1.0 mM and NPSSE-MC was used at 0.1 mM. The enzyme concentration in each mixture was adjusted accordingly. Incubation times were between 0 and 15 min, during which there existed a linear relationship between time and drug metabolism (with a correlation coefficient of >0.98 for each antibiotic) (Table 2). Under these reaction conditions, Ac-MC was reduced by xanthine oxidase most rapidly, whereas MA, M-83, DMAM-MC, NPSSE-MC, MC,

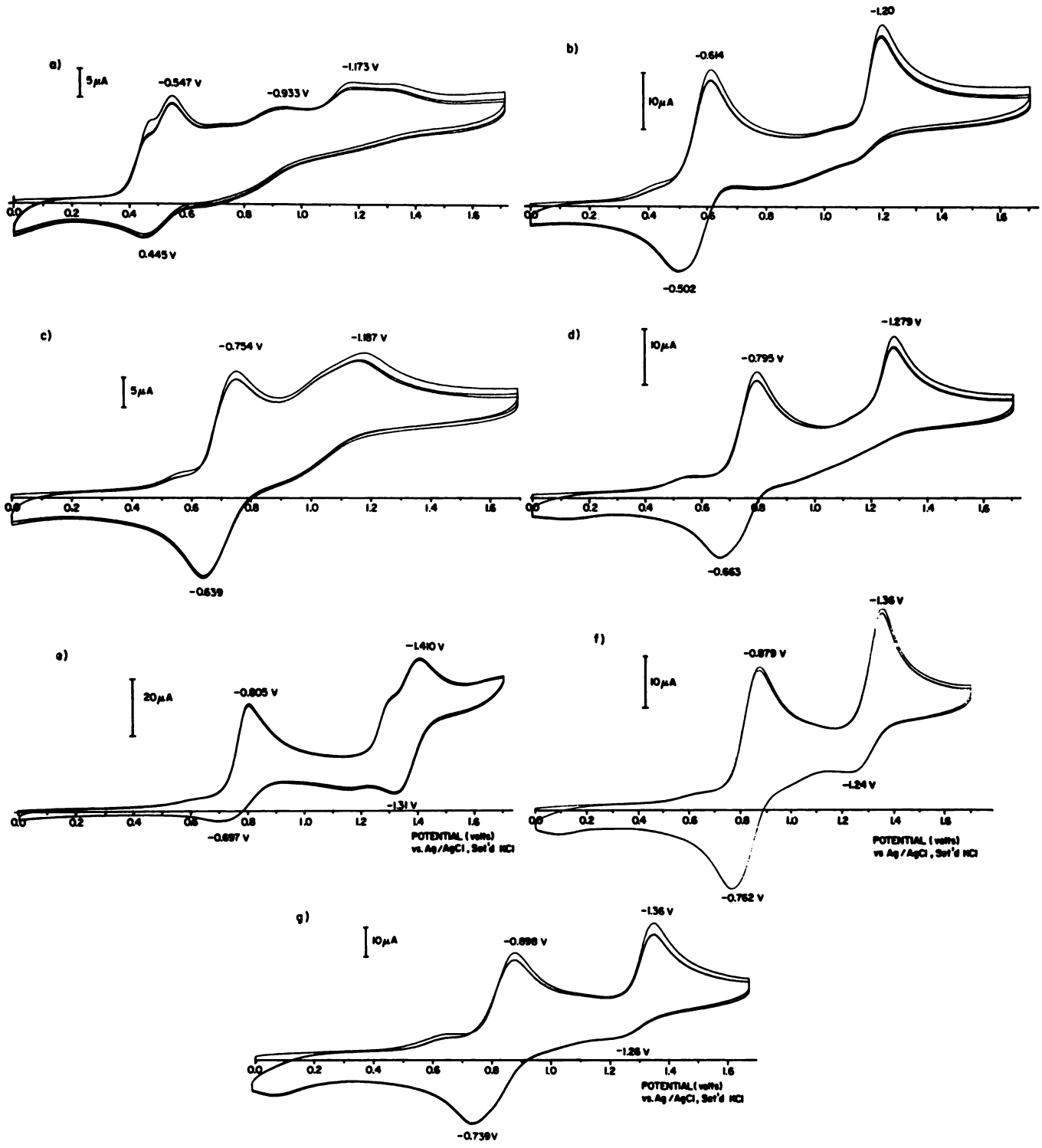


Fig. 2. Cyclic voltammographs of mitomycin antibiotics. a, Ac-MC; b, MA; c, M-83; d, DMAM-MC; e, NPSSE-MC; f, PFM; g, MC. Details of the method used are described in Materials and Methods. Each tracing was made with a scan rate of 20 mV/sec.

and PFM were metabolized at progressively slower rates (Table 2). Similar results were obtained with NADPH-cytochrome P450 reductase (data not shown). In addition, the primary metabolites of each of these antibiotics were further metabolized, as indicated by their disappearance during longer incubation times. A linear relationship was seen between the reductive potential of the first electron transfer and the rate of enzymatic reduction of these compounds (Fig. 3). The less negative the potential of the antibiotic, the more rapidly it was reduced.

Oxygen consumption. The ability of antibiotics to augment oxygen consumption was measured at five concentrations of each antibiotic. Due to the high activity of some of the antibiotics under the conditions of study, drugs were studied at different concentrations. Ac-MC, MA, M-83, DMAM-MC, and NPSSE-MC were studied at concentrations between 2.0 and

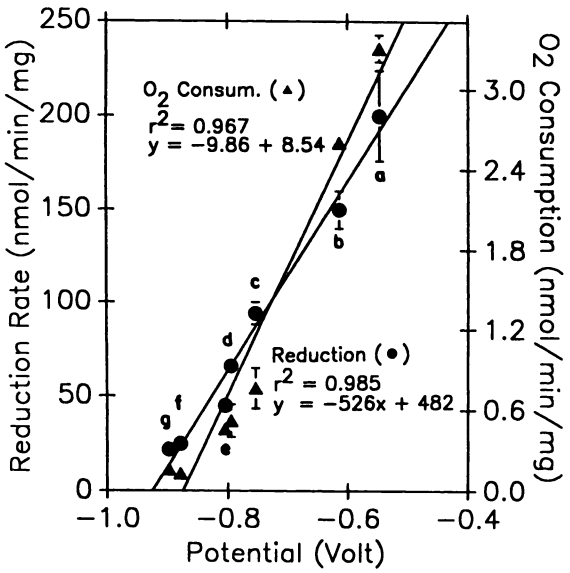


Fig. 3. Relationships of first electron-reductive potential of mitomycin antibiotics to 1) the rate of reduction by xanthine oxidase (●) and 2) the amount of augmentation of oxygen consumption by rat liver microsomes (▲). a, Ac-MC; b, MA; c, M-83; d, DMAM-MC; e, NPSSE-MC; f, PFM; g, MC.

TABLE 1

Cyclic voltammetric peak potential (V) for mitomycin C and analogues

The potentials were measured against an Ag/AgCl (saturated KCl) reference electrode. Details of the method used are described in Materials and Methods.

Mitomycin analogues	Wave I[a]			Wave II		
	E_p^c	E_p^a	ΔE_p	E_p^c	E_p^a	ΔE_p
	V					
Ac-MC	−0.547	−0.445	0.102	−1.173	PD[b]	
MA	−0.614	−0.502	0.112	−1.200	PD	
M-83	−0.754	−0.639	0.115	−1.187	PD	
DMAM-MC	−0.795	−0.663	0.132	−1.279	PD	
NPSSE-MC	−0.805	−0.697	0.108	−1.410	1.315	0.095
PFM	−0.879	−0.762	0.117	−1.360	−1.24	0.120
MC	−0.898	−0.739	0.159	−1.360	−1.26	0.100

[a] E_p^c, potential of cathodic peak; E_p^a, potential of anodic peak; $\Delta E_p = E_p^c - E_p^a$.
[b] PD, poorly defined.

25.0 μM, whereas MC and PFM were studied at concentrations between 50.0 and 500.0 μM. All seven antibiotics augmented oxygen consumption by rat liver microsomes (Table 2, Fig. 3). Oxygen consumption was dependent on both the concentration of microsomes and the concentration of antibiotics. For each antibiotic, there existed a linear relationship between the rate of oxygen consumption and the drug concentration (data not shown). From these relationships, the rate of augmented oxygen consumption for each antibiotic was calculated (Table 2, Fig. 3). There were large differences among the antibiotics in terms of their ability to augment oxygen consumption. There was a linear relationship between the rate of augmentation by these antibiotics and the first electron-reduction potentials (Fig. 3). The less negative the reductive potential of a drug, the more it augmented oxygen consumption by rat liver microsomes.

Cytotoxicity. The IC_{50} for each antibiotic was calculated from cell survival curves, which were obtained by exposing HCT 116 cells to six concentrations of each antibiotic (Table 2). With the exception of Ac-MC, the degree of cytotoxicity of these antibiotics followed the same trend as did the other three characteristics of these compounds. The trend, however, was not linear. NPSSE-MC, a compound with a relatively high reductive potential close to that of MC, was dramatically more active against HCT 116 cells than was MC. Among the six antibiotics tested, MA was most and M-83 was second most toxic against HCT 116 cells, while neither antibiotic had the least negative potential, whereas Ac-MC had the least negative potential yet it was less toxic than MA.

Partition coefficient. The partition coefficients between octanol and PBS were 0.31 for Ac-MC, 2.56 for MA, 2.69 for M-83, 1.12 for DMAM-MC, 10.2 for NPSSE-MC, 0.36 for MC, and 0.98 for PFM. NPSSE-MC appeared to be the most lipophilic.

Discussion

Previously we have shown, by electrochemical and enzymatic methods, that one-electron reduction of MC is sufficient to convert it to active intermediates (13, 17). This radical-forming action is a prerequisite initial step before subsequent metabolic actions that generate metabolites and alkylated products (13). The present study of seven mitomycin antibiotics showed that the reductive potential of the first electron transfer is affected by the substituent group at the C-7 position. The more electronegative the substituent at C-7, the less negative is the reductive potential for the compound. For example, substitution of a methoxy group for an amino group results in a large potential shift from −0.872 V to −0.614 V (Table 1). Electronegative substituents on the quinone appear to have a stabilizing effect on the semiquinone radical anion, by helping to disperse electron density in the ring.

Our data demonstrate linear relationships between the first electron-reductive potential of seven mitomycin antibiotics, their rates of enzymatic reduction, and their ability to augment oxygen consumption by rat liver microsomes. We feel that it is reasonable to utilize the rate of enzymatic reduction as an index for the generation of active intermediates that can interact with macromolecules and to utilize the amount of augmented oxygen consumption as an index for the generation of oxygen radicals that may cause cardiotoxicity and cytotoxicity. Compounds with low first electron-reductive potentials (less negative) may suffer from two unfavorable consequences, 1) inducing too much oxygen consumption, which may lead to active oxygen radical formation, and 2) being too reactive, which may render them highly cytotoxic. Four of the antibiotics shown in this paper to have low reductive potentials fall into these categories; Ac-MC and DMAM-MC are cardiotoxic (6, 7); MA has proved to be too toxic for clinical use; and M-83, an initially promising analogue, also proved unsuitably toxic (5). Only NPSSE-MC, with a reductive potential lower than those of MC and PFM, has not been found to be cardiotoxic (7).

Correlation between reductive potential and cytotoxicity of antibiotics against HCT 116 cells was less clear. NPSSE-MC is an analogue with a reductive potential close to that of MC and lower than that of Ac-MC, yet its cytotoxicity against HCT 116 cells was 10 times greater than that of MC and 2 times greater than that of Ac-MC. It seems that the cytotoxicity of these MC analogues does not depend totally on the two aforementioned reactions that are related to reductive potential, i.e., the rate of generating active intermediates and oxygen consumption. The reasons for this are yet to be determined. One possible cause of differences in cellular cytotoxicity could be

TABLE 2

Relationships of reductive potential and pharmacological activities of mitomycin C and analogues

Mitomycin analogues	E_p^c of wave I[a]	Rate of reduction by xanthine oxidase[b]	O_2 consumption by microsomes[c]	IC_{50}[d]
	V	*nmol/min/mg*	*nmol/min/mg*	*μM*
Ac-MC (BYM-26605)	−0.547	200 ± 30	3.29 ± 0.09	0.8 ± 0.2
MA	−0.614	150 ± 10	2.58 ± 0.08	0.02 ± 0.03
M-83 (KW-2083)	−0.754	94 ± 6	0.76 ± 0.15	0.2 ± 0.02
DMAM-MC (BYM-25282)	−0.795	66 ± 4	0.52 ± 0.12	ND[e]
NPSSE-MC (BYM-25067)	−0.805	45 ± 3	0.46 ± 0.06	0.4 ± 0.05
PFM	−0.879	25 ± 1	0.13 ± 0.018	5 ± 1.0
MC	−0.872	22 ± 2	0.16 ± 0.018	5 ± 1.0

[a] E_p^c, potential of cathodic peak.
[b] Determined by time courses between 0 and 15 min. Values represent means ± standard deviations of three experiments each performed in triplicate.
[c] Each antibiotic was measured at five drug concentrations. Values represent means ± standard deviations of three experiments, each performed in triplicate.
[d] Each IC_{50} was calculated from cell survival curves obtained by exposing HCT 116 cells to six concentrations of each antibiotic. Values represent the means ± standard deviations obtained from three experiments with triplicate plates in each.
[e] ND, not determined.

differential uptake of the mitomycin analogues. We reported earlier that PFM enters HCT 116 cells by diffusion (20). NPSSE-MC is much more lipophilic than the other compounds tested. High lipophilicity would facilitate its diffusion across the cell membrane. However, at present, uptake cannot be determined for the other compounds, because no analytical measurement is sufficiently sensitive and no radioactive forms of the analogues are available. In the case of Ac-MC, deacetylation following cellular uptake, converting Ac-MC to MC, may play a role. Furthermore, the specificity of cellular alkylation by each antibiotic could also vary due to the substitution.

There is a continuing search for analogues of MC with improved therapeutic indices. Emphasis has been directed toward reducing myelosuppression and maintaining antitumor efficacy. Several new MC analogues with low reductive potential and high reactivity have proven to be less myelosuppressive (2–5) and are able to circumvent MC resistance (19), but these analogues also produced new toxicities not previously associated with MC (6, 7). Therefore, analogues possessing reductive potentials closer to those of MC and PFM, but with substituent groups on the C-7 or C-10 position that facilitate binding or alkylation, may have better therapeutic indices. New analogues with such properties may be a better direction for investigation.

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References

1. Crooke, S. T. Mitoymcin C: an overview, in *Mitomycin C: Current Status and New Developments*. (S. K. Carter and S. T. Crook, eds.). Academic Press, Inc., New York, 1–4 (1979).
2. Schlein, A., J. E. Schurig, W. C. Rose, A. R. Farwell, A. P. Florczyk, T. Kaneko, H. Wong, and W. T. Bradner. Evaluation of BMY-26605 as a potentially less myelosuppressive mitomycin analog. *Proc. Am. Assoc. Cancer Res.* **26**:210 (1985).
3. Bradner, W. T., W. C. Rose, J. E. Schurig, A. Schlein, and J. B. Huftalen. Antitumor activity and toxicity in animals of RR-150 (7-cysteaminomitosane), a new mitomycin derivative. *Cancer Res.* **44**:5619–5623 (1984).
4. Bradner, W. T., W. C. Rose, J. E. Schurig, A. P. Florezyk, J. B. Huftalen, and J. J. Catino. Antitumor activity and toxicity in animals of BMY-25282, a new mitomycin derivative. *Cancer Res.* **45**:6475–6481 (1985).
5. Yoshio, S. M-93: a new derivative of mitomycin. *Cancer Treat. Symp.* **1**:29–35 (1983).
6. Pritsos, C. A., and A. C. Sartorelli. Generation of reactive oxygen radicals through bioactivation of mitomycin antibiotics. *Cancer Res.* **46**:3528–3582 (1986).
7. Bradner, W. T., C. L. Bregman, R. A. Buroker, C. A. Pritsos, and A. C. Sartorelli. Cardiotoxicity of mitomycin derivatives. *Proc. Am. Assoc. Cancer Res.* **26**:267 (1988).
8. Lazarus, H. M., M. R. Gottfried, R. H. Herzig, G. L. Phillips, R. S. Weiner, G. P. Sama, J. Fay, S. N. Wolff, O. Sudilovsky, R. P. Gale, and G. P. Herzig. Veno-occlusive disease of the liver after high-dose mitomycin C therapy and autologous bone marrow transplantation. *Cancer (Phila.)* 49:1789–1795 (1982).
9. Schlicher, R. B., J. D. Young, V. Ratanathorathorn, and L. H. Baker. High dose mitomycin C (MMC) and autologous bone marrow transplantation: clinical results and pharmacokinetics. *Blut* **45**:83–184 (1982).
10. Schwartz, H. S. Pharmacology of mitomycin C. III. *In vitro* metabolism by rat liver. *J. Pharmacol. Exp. Ther.* **136**:250–258 (1962).
11. Tomasz, M., and R. Lipman. Alkylation reactions of mitomycin C and pH. *J. Am. Chem. Soc.* **101**:6063–6067 (1979).
12. Tomasz, M., and R. Lipman. Reductive metabolism and alkylating activity of mitomycin C induced by rat liver microsomes. *Biochemistry* **20**:5056–5061 (1981).
13. Pan, S., P. Andrews, C. Glover, and N. R. Bachur. Reductive activation of mitomycin C and mitomycin C metabolites catalyzed by NADPH cytochrome P-450 reductase and xanthine oxidase. *J. Biol. Chem.* **259**:959–966 (1984).
14. Doroshow, J. H. Mitomycin C-enhanced superoxide and hydrogen peroxide formation in rat heart. *J. Pharmacol. Exp. Ther.* **218**:206–211 (1981).
15. Yasukochi, Y., and B. S. S. Masters. Some properties of a detergent-solubilized NADPH-cytochrome *c* (cytochrome P450) reductase purified by biospecific affinity chromatography. *J. Biol. Chem.* **251**:5337–5344 (1976).
16. Nelson, C. A., and P. Handler. Preparation of bovine xanthine oxidase and the subunit structures of some iron flavoproteins. *J. Biol. Chem.* **234**:5368–5373 (1968).
17. Andrews, P. A., S. Pan, and N. R. Bachur. Electrochemical reductive activation of mitomycin C. *J. Am. Chem. Soc.* **108**:4158–4166 (1986).
18. Bachur, N. R., S. L. Gordon, and M. V. Gee. Anthracycline antibiotics augmentation of microsomal electron transport and free radical formation. *Mol. Pharmacol.* **13**:901–910 (1977).
19. Willson, J. K. V., B. H. Long, S. Chakabarty, D. D. Brattain, and M. G. Brattain. Effects of BMY 25202, a mitomycin C analogue, in mitomycin C-resistant human colon cancer cells. *Cancer Res.* **45**:5281–5286 (1985).
20. Pan, S., R. Johnson, H. Gonzalez, and V. Thohan. Mechanisms of transport and intracellular binding of porfiromycin in HCT 116 human colon carcinoma cells. *Cancer Res.* **49**:5048–5053 (1989).

Send reprint requests to: Su-shu Pan, Division of Developmental Therapeutics, University of Maryland Cancer Center, 655 W. Baltimore Street, Baltimore, MD 21201.