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METABOLIC ACTIVATION OF MITOMYCIN C BY LIVER MICROSOMES AND NUCLEI*

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Abstract—Bioreductive alkylating agents require reductive activation prior to exerting their cytotoxic actions. This property results in preferential toxicity to hypoxic cells. Previous data have demonstrated that mitomycin C is activated by hypoxic tumor cells and is selectively cytotoxic to these oxygen-deficient cells. The biotransformation of mitomycin C was studied in liver microsomes and nuclei and in a reconstituted, partially purified cytochrome P-450 drug-metabolizing system to provide information on these reductive processes. Both the metabolism of mitomycin C, measured by disappearance of the quinone portion of the substrate, and the formation of an alkylating metabolite(s), determined by employing 4-(*p*-nitrobenzyl)pyridine as a trapping agent, required anaerobic conditions and an NADPH-generating system, and were inhibited by O_2 and CO in both microsomes and nuclei. A reconstituted enzyme system consisting of NADPH, NADPH–cytochrome P-450 reductase, phospholipid and cytochrome P-450 converted mitomycin C to a reactive metabolite(s) under hypoxic conditions. Omission of N_2 or any component of the system decreased the metabolic activation of mitomycin C. These findings support the concept that the cytochrome P-450 system is capable of activating mitomycin C under hypoxic conditions to the alkylating metabolite(s) that is responsible for antineoplastic activity.

Mitomycin C (MC) is the prototype of a class of antitumor drugs, the bioreductive alkylating agents, which require metabolic reduction to form a species capable of alkylating critical cellular macromolecules [1]. Because MC, the only clinically available drug demonstrated to be a bioreductive alkylating agent, requires metabolic activation to form a reactive metabolite capable of producing DNA crosslinks [2–4], elucidation of possible metabolic pathways and conditions for MC activation is important to comprehend the mechanism of action of this antibiotic.

In 1962, Schwartz [5] reported that MC was metabolized by rat liver preparations under anaerobic conditions and that the majority of the MC-metabolizing activity was found to reside in the microsomal fraction and required an NADPH-generating system. Because the conditions under which MC was found to be metabolized are similar to those hypothesized to produce an alkylating species, we have measured both the metabolic disappearance of MC and the production of a reactive metabolite(s) by mouse liver microsomes. In addition, these studies have been extended to include measurement of MC activation by liver nuclei and by a reconstituted cytochrome P-450 enzyme system. The results described in this report demonstrate that NADPH, NADPH–cytochrome P-450 reductase, phospholipid, cytochrome P-450 and anaerobiasis are all required for the optimal activation of MC to an alkylating species which is considered to be the active, tumor inhibitory form of the drug.

MATERIALS AND METHODS

MC was the gift of Dr. Maxwell Gordon of the Bristol-Myers Co. (Syracuse, NY). $NADP^+$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were obtained from the Sigma Chemical Co. (St. Louis, MO). All other reagents were obtained from standard chemical sources unless otherwise specified.

Female CD-1 mice (Charles River Farms, Inc., Portage, MI) of 25–30 g body weight and male Sprague–Dawley rats (Charles River Breeder, Inc.) of 200–300 g body weight were housed under 12-hr cycles of light/dark and were maintained on Purina Lab Blox (Ralston, Purina Co., St. Louis, MO) and water *ad lib*. Animals were not deprived of food prior to being killed.

Microsomes were freshly prepared by the Ca^{2+} precipitation method of Schenkman and Cinti [6]. Preliminary experiments with microsomes prepared by ultracentrifugation showed no differences in MC metabolism when compared with microsomes prepared by the Ca^{2+} precipitation method. After the final wash, the microsomal pellets were suspended in 0.14 M KCl–0.01 M phosphate buffer (pH 7.4). Nuclei were prepared by the method of Bresnick *et*

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al. [7]. Examination of the washed nuclei by phase contrast and electron microscopy showed neither evidence of microsomal contamination nor the presence of microsomal components attached to the nuclei.

NADPH–cytochrome P-450 reductase and cytochrome P-450 were purified as described by Gibson and Schenkman [8]. Gel electrophoresis revealed multiple protein bands in the partially purified fractions; however, no reductase was found in the cytochrome P-450 fraction nor was cytochrome P-450 found in the reductase fraction. Cytochrome P-450 and cytochrome P-450 reductase activities were assayed as described previously [8]. Cytochrome P-420 content was nil.

Microsomes or nuclei were added to 0.1 M Tris–HCl (pH 7.4) containing 1.8 μmoles $NADP^+$, 12.5 μmoles glucose-6-phosphate, 12.5 μmoles $MgCl_2$ and 3 units of glucose-6-phosphate dehydrogenase in a total volume of 2.5 ml. Preliminary experiments showed that the disappearance of MC from the incubation medium and the generation of a reactive species were both linear with protein concentration up to 5.0 mg/ml for nuclei and 0.5 mg/ml for microsomes, and with time up to 20 and 12.5 min for nuclei and microsomes, respectively. Therefore, all experiments with microsomes were performed for 10 min at 0.4 mg protein/ml and with nuclei for 20 min at 2.0 mg protein/ml.

Anaerobic conditions were achieved by stoppering flasks and pregassing reaction mixtures for 10 min with prepurified nitrogen which contained less than 10 ppm oxygen (Matheson Gas, Rutherford, NJ). Except as noted, all reactions were performed under a continuous flow of nitrogen. The incubation flasks were then warmed for 2 min to generate NADPH, and the reaction was initiated by the addition of MC (0.30 mM final concentration) dissolved in acetone. The incubations were stopped by the addition of 1 ml of saturated $Ba(OH)_2$ solution and 1 ml of 15% $ZnSO_4$ to precipitate protein. MC concentrations in the supernatant fractions were determined as described below.

Reactive metabolites of MC were estimated by a modification of the method described by Wheeler *et al.* [9]. Incubation mixtures used to estimate the relative amounts of biotransformation products with alkylating capabilities were the same as those described for the metabolism experiments, except that 25 μl of a 10% acetone solution of 4-(*p*-nitrobenzyl)pyridine (Aldrich Chemical Co., Milwaukee, WI) was added to the samples prior to incubation. After incubation, each reaction was terminated by the addition of 2 ml of acetone and 1 ml of 1 M NaOH and immediately extracted with 4 ml of ethyl acetate. The organic and aqueous phases were separated by centrifugation for 2 min at 1000 *g*, and the absorbance of the organic layer at 540 nm was determined. The incubations and subsequent extractions used for estimating the presence of alkylated products were carried out under subdued light.

Metabolism of MC and production of alkylating species were also estimated in a reconstituted enzyme system described by Gibson and Schenkman [8]. This system when fully reconstituted with phospholipid, NADPH–cytochrome P-450 reductase and cytochrome P-450 was capable of metabolizing MC under anaerobic conditions in the presence of NADPH. To study the importance of each of the component parts of the system, additions or deletions of components as indicated in the text were carried out under conditions described above. The completely reconstituted enzyme system contained, in 2.5 ml total volume, 1.0 nmole cytochrome P-450, 0.01 unit of NADPH–cytochrome P-450 reductase (where 1 unit = 1000 μmoles cytochrome *c* reduced/min), 1.4 mg phospholipid (1:1 by weight of dipalmitoyl and dimyristoyl phosphatidylcholine), 12.5 μmoles glucose-6-phosphate, 12.5 μmoles $MgCl_2$, 1.8 μmoles $NADP^+$, 3 units glucose-6-phosphate dehydrogenase and, in those cases where the production of reactive species from MC was determined, 25 μl of a 10% solution of 4-(*p*-nitrobenzyl)pyridine in acetone was added and alkylated product was measured as described above.

Protein was determined by the method of Lowry *et al.* [10]. MC concentrations were estimated by determining the absorbance of the quinone form of the drug at 363 nm, and the measured absorbance was then related to that obtained using known amounts of drug. Any contribution from NADPH to the absorbance of MC at 363 nm was eliminated by determining drug-related absorbances against appropriate samples containing only the NADPH-regenerating system. The limit of sensitivity for the method was 0.1 μg/ml.

RESULTS

Both liver microsomes and isolated nuclei were capable of metabolizing MC under anaerobic conditions as measured by substrate disappearance (Table 1). The metabolism of MC required a source of reduced pyridine nucleotide, was inhibited by a CO- or O_2-containing atmosphere, and was destroyed when the enzyme source was boiled. The cytochrome P-450 content of CD-1 mouse liver microsomes was 1.02 ± 0.07 nmoles/mg protein. The enzymatic activity in microsomes based on the content of cytochrome P-450 was 17.95 nmoles MC consumed · min^{-1} · (nmole cytochrome P-450)$^{-1}$. The amount of cytochrome P-450 present in nuclei was not determined.

Because radiolabeled MC was unavailable, it was impossible to determine directly whether this agent was activated to species which could covalently bind to macromolecules. Therefore, to estimate the rates of formation of reactive metabolites, an adaptation of the method of Wheeler *et al.* [9] was employed to trap the activated drug produced during incubation as an alkylated complex of 4-(*p*-nitrobenzyl)pyridine. The rate of formation of the alkylated 4-(*p*-nitrobenzyl)pyridine product was linear with time and with protein concentration in a manner analogous to that observed in metabolism experiments. Furthermore, the presence of 4-(*p*-nitrobenzyl)pyridine in the reaction mixture did not affect the rate of MC disappearance. Figure 1 shows the results obtained for both the disappearance (metabolism) of MC and the formation of an alkylated product of 4-(*p*-nitrobenzyl)pyridine. The results demonstrated that conditions such as an

Table 1. Metabolism of mitomycin C by liver microsomes and nuclei

Conditions	Mitomycin C consumed [nmoles · min^{-1} · (mg protein)$^{-1}$]	
	Microsomes	Nuclei
Complete incubation system*	18.33 ± 1.33	1.71 ± 0.10
O_2 atmosphere†	1.43 ± 0.32	0.28 ± 0.12
CO atmosphere†	4.62 ± 1.65	0
Boiled enzyme	0	0.02 ± 0.02
Minus enzyme	0.57 ± 0.52	0.11 ± 0.11
Minus NADPH-generating system	1.85 ± 1.30	0.32 ± 0.16

* The complete system contained 0.4 mg/ml CD-1 mouse liver microsomal or 2.0 mg/ml CD-1 mouse liver nuclear protein and an NADPH-generating system; incubation was performed under continuously flowing N_2 at 37° for 10 min as described in Materials and Methods. Each value is the mean ± S.E. for at least three separate preparations.

† Either atmospheric air or CO replaced N_2 for these incubations.

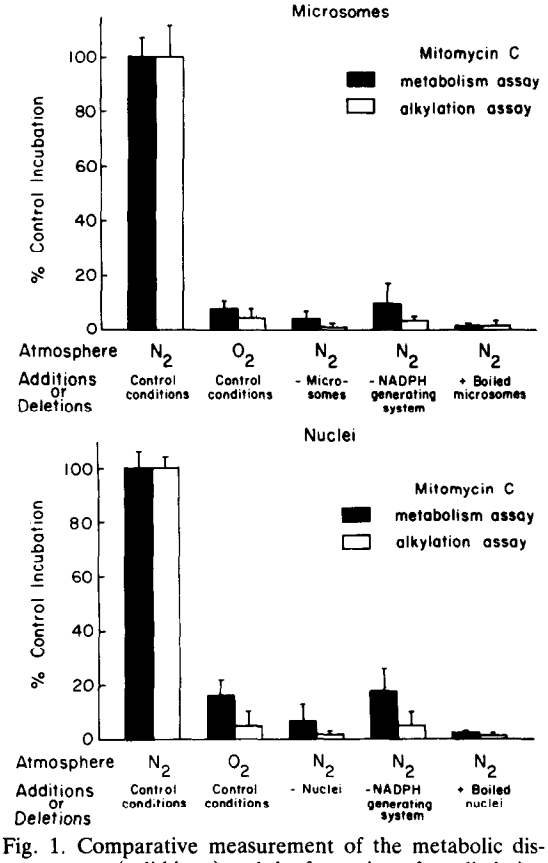

Fig. 1. Comparative measurement of the metabolic disappearance (solid bars) and the formation of an alkylating species (open bars) of MC. Studies were conducted with both CD-1 mouse liver microsomes (upper panel) and nuclei (lower panel) as described in Materials and Methods. The mean ± S.E. for three to six determinations is shown. Control conditions consisted of the addition of the NADPH-generating system, enzyme source and the trapping agent in the case of the alkylation assay, and the incubation was performed under a continuous flow of N_2. Control values for metabolic disappearance were 18.33 ± 1.33 and 1.71 ± 0.10 nmoles · min^{-1} · (mg protein)$^{-1}$ for microsomal and nuclear preparations, respectively. Control values for formation of alkylating species were 14.52 ± 1.60 and 1.63 ± 0.10 ΔA_{540} · min^{-1} · (mg protein)$^{-1}$ for microsomal and nuclear preparations, respectively.

O_2-containing atmosphere, a lack of or a denatured enzyme source, or the omission of a source of reduced pyridine nucleotide inhibit both the metabolism of MC and the formation of a reactive form of the drug.

To characterize further the mechanism involved in the metabolic activation of MC by mouse liver microsomes, the effects of the addition of sodium azide, sodium nitrite, and potassium cyanide to the incubation mixture were determined. The addition of potassium cyanide at 0.5 mM final concentration resulted in a 35–45% stimulation of both MC metabolism and generation of a reactive species, whereas 1.0 mM sodium nitrite or sodium azide had little effect on the metabolism of MC as compared to control but did inhibit the formation of the 4-(*p*-nitrobenzyl)pyridine alkylated product by 15–20%.

Both the rate of metabolic disappearance and the rate of formation of reactive MC metabolites catalyzed by microsomes increased in concert in a linear fashion as the concentration of drug in the incubation medium was elevated (Fig. 2). As reported previously [5], it was not possible to saturate the enzyme with MC when either metabolism or production of activated drug was measured due to the limited solubility of the drug in aqueous media.

The metabolism and activation of MC to a reactive species by a reconstituted cytochrome P-450 drug-metabolizing enzyme system were evaluated to determine the involvement of individual components in the measured phenomena (Table 2). Neither NADPH–cytochrome P-450 reductase alone nor the reductase in combination with phospholipid was capable of activating or metabolizing the drug optimally. Furthermore, the substitution of cytochrome *c* for cytochrome P-450 did not result in efficient metabolism or conversion of drug to species capable of alkylation. Both the activation of MC to a reactive species and the metabolic disappearance of the antibiotic catalyzed by the reconstituted enzyme system were inhibited by an O_2-containing environment. Similarly, omission of phospholipid or the source of NADPH, or the replacement of NADPH with NADH, markedly decreased or prevented both activation and metabolism of drug. The fully reconstituted system metabolized MC at a rate of

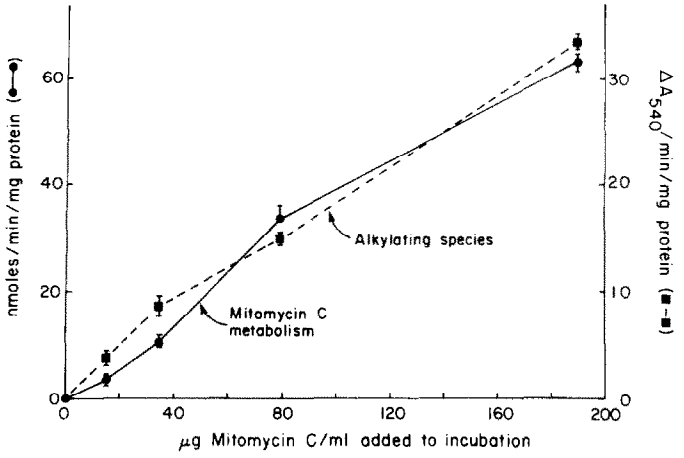

Fig. 2. Relationship between the metabolism of MC (●) and the generation of an alkylated product (■) by CD-1 mouse liver microsomes. Incubations were conducted with different concentrations of antibiotic as described in Materials and Methods. Each point is the mean ± S.E. for three determinations.

15.68 nmoles MC · min^{-1} · (nmole cytochrome P-450)$^{-1}$.

DISCUSSION

MC is the prototype of a class of cancer chemotherapeutic drugs, the bioreductive alkylating agents, which are selectively toxic to hypoxic tumor cells. We have evidence in support of the rationale that the differential cytotoxicity of bioreductive alkylating agents to oxygenated and hypoxic tumor cells is the result of their mode of enzymatic activation [11–15]. Hypoxic tumor cells exist in poorly vascularized regions of solid neoplasms and therefore are oxygen deficient. This oxygen poor environment appears (a) to be permissive for oxygen-sensitive reductive metabolism of agents like MC and misonidazole, and (b) to result in enhanced activation of these agents to reactive species under hypoxic conditions [11, 12]. Previous reports from this laboratory employing Sarcoma 180 and EMT6 tumor cells have demonstrated that these cells activate MC under hypoxic conditions and that MC is selectively toxic to hypoxic tumor cells *in vitro* [13–15]. However, due to the limited amount of enzymatic material which could be conveniently obtained from cultured cells, another source of enzyme was employed for further studies on the pathways by which this antibiotic is metabolically activated under conditions of O_2 deficiency.

Table 2. Metabolism and activation of mitomycin C by a reconstituted rat liver enzyme system

	Percent of control	
	Metabolism*	Alkylation†
fpt + PL + P-450 + NADPH + N_2‡	100	100
fpt + NADPH + N_2	0§	16
fpt + PL + NADPH + N_2	18	11
fpt + P-450 + NADPH + N_2	0	17
fpt + PL + P-450 + N_2	14	15
fpt + PL + P-450 + NADH + N_2	16	10
fpt + PL + P-450 + NADPH + O_2	20	4
fpt + PL + cytc + NADPH + N_2	37	39
fpt + PL + cytc + P-450 + NADPH + N_2	101	90

* Metabolism refers to the rate of disappearance of MC from the incubation medium as measured spectrophotometrically.

† Alkylation refers to the measurement of the rate of formation of an alkylated product of 4-(*p*-nitrobenzyl)pyridine.

‡ The control incubation represents the metabolism of MC and the formation of a reactive species of MC by a fully reconstituted enzyme system isolated from rat liver consisting of NADPH–cytochrome P-450 reductase (fpt), phospholipid (PL), cytochrome P-450 (P-450) and NADPH, incubated under a nitrogen atmosphere (N_2) as described in Materials and Methods. Abbreviation: cytc, cytochrome c.

§ Each number refers to the mean of duplicate experiments on two separate purifications of cytochrome P-450. The ranges of these means for these experiments were ± 2% of control for duplicate experiments.

Early reports [2, 5] provided evidence that MC was metabolized in bacteria and mammalian liver. Subcellular fractionation of liver showed that MC-metabolizing activity was present mainly in the microsomal fraction and required anaerobic conditions [5]. Because the studies of Iyer and Szybalski [2] in bacteria and our work [14] in tumor cells suggested that substrate disappearance and the activation of the antibiotic to a reactive species were related, we have conducted studies with both liver microsomes and nuclei to determine whether these phenomena were linked. The results of these investigations show that the enzymatic system(s) present in nuclei and in microsomes is similar to that which we have reported for tumor cells [14]. In both the liver subcellular fractions and tumor cell preparations, oxygen almost completely abolished any detectable activation of MC to a 4-(*p*-nitrobenzyl)pyridine trappable metabolite under the conditions employed. In addition, a source of reduced pyridine nucleotide was also required for both metabolism of MC and activation of the drug to reactive species in liver microsomes and in tumor cell homogenates.

Lack of inhibition of the microsomal metabolism or activation of MC by sodium azide, sodium nitrite and potassium cyanide suggests that microsomal azoreductase [16] was not important for MC activation in this system and that the microsomes were not contaminated by mitochondrial enzymes [17].

Handa and Sato [18], Bachur *et al.* [19–21], Sinha [22] and Komiyama *et al.* [23] have shown that MC stimulates the microsomal oxidation of NADPH under aerobic conditions. This result is attributed to interaction of MC with NADPH–cytochrome P-450 reductase to produce a one-electron reduction of MC, which in the presence of O_2 transfers the electron to O_2 to form superoxide. To understand more fully the enzymatic components involved in the metabolic activation of MC to a 4-(*p*-nitrobenzyl)pyridine trappable metabolite(s), an isolated reconstituted cytochrome P-450-metabolizing system was employed. This enzyme preparation, isolated from uninduced rats, has been shown to metabolize benzphetamine, aminopyrine, ethylmorphine and *p*-chloro-*N*-methylaniline [8]. The present report demonstrates that this system can also mediate the metabolism and activation of MC. However, the system functioned optimally only when all of the necessary component parts were present simultaneously; these included phospholipid, the reductase, reduced pyridine nucleotide, cytochrome P-450 and anaerobic conditions. Specifically, the NADPH–cytochrome P-450 reductase, which can apparently catalyze the formation of semiquinone [20, 21], does not efficiently activate MC, even in the presence of phospholipid, to reactive species which can be trapped using 4-(*p*-nitrobenzyl)pyridine. Similarly, NADPH–cytochrome P-450 reductase did not appreciably catalyze the metabolic disappearance of MC. Efficient activation of MC in these experiments appeared to require cytochrome P-450 as the electron acceptor for the reductase, because the substitution of cytochrome *c* for cytochrome P-450 in the enzymatic system led to a decrease in both the rate of activation and metabolism of the drug. This is in contrast to the findings of Komiyama *et al.* [23] who reported that, under relatively long incubation times of 30–60 min, MC disappeared from incubation media containing xanthine oxidase or NADPH–cytochrome P-450 reductase or microsomes under anaerobic conditions. In our experience, the rates of metabolic disappearance and formation of a reactive species from MC were linear for a maximum of 12.5 min in both microsomes and the reconstituted enzyme system. Therefore, all incubations were performed for 10 min. The catalytic activity of NADPH–cytochrome P-450 reductase alone was only 10–18% of the activity exhibited by the fully reconstituted enzyme system. In comparison, the metabolizing activity of the reconstituted enzyme system was approximately 88%, on a per nmole cytochrome P-450 basis, of the activity present in native microsomes. The inhibition of MC metabolism by CO was photoreversible with a wavelength maximum of 450 nm (unpublished observations). Thus, cytochrome P-450 is necessary for optimal metabolism and formation of a reactive species of MC. Similar results for the metabolism of another quinone, benzo[*a*]pyrene-3,6-quinone, by the cytochrome P-450 system have been reported [24].

Because the cytochrome P-450 system is also present in liver nuclei [7, 25–29], the ability of isolated mouse liver nuclei to activate MC was also evaluated using nuclei isolated by the method of Bresnick *et al.* [7]. The purity of these preparations was assessed by light and electron microscopy. No evidence of cytoplasmic contamination was observed; this finding was of importance since the specific activity of the nuclear enzyme system was approximately 10% of that found in the microsomes. Nuclei have been shown to metabolize several substances which are activated to alkylating species by cytochrome P-450 systems, including benzo[*a*]pyrene [7, 25, 26, 28, 29], 2-acetylaminofluorene [27], 2-aminofluorene [27], and 7,12-dimethylbenz[*a*]anthracene [30]. It is possible that the activation of MC to short-lived reactive species near a critical target molecule, DNA, by nuclear enzymes may play an important role in the cytotoxic properties of MC.

The data presented in this report are concerned with the anaerobic metabolism and activation of MC, but others have demonstrated that MC also undergoes futile reduction–oxidation cycles to produce superoxide radicals and other toxic oxygen species [18–23]. Thus, MC is cytotoxic towards both normally aerated and hypoxic tumor cells, with a given concentration of antibiotic being more toxic for hypoxic cells [14, 15]. It is possible that two distinct mechanisms for activation of MC occur. The mechanism in hypoxic cells would require full reduction of the quinone moiety to the hydroquinone followed by loss of methanol and intramolecular rearrangement to form a reactive quinone methide species capable of crosslinking DNA. The reactive quinone methide hypothetically could be produced efficiently near its target, DNA, by the enzyme systems described here and elsewhere [11–15] and result in cytotoxicity by virtue of lesions generated at the level of the genome. In oxygenated cells, one-electron reduction of MC to the semiquinone with subsequent reoxidation to the quinone and production of super-

oxide anion and associated metal catalyzed oxygen byproducts may result in cytotoxicity if toxic species are produced in concentrations sufficient to overwhelm the natural defenses of cells against such damage. Anthracyclines have been hypothesized to undergo such activation to a semiquinone form and have been shown to undergo futile reduction–oxidation cycles with superoxide production [18–20]. The futile redox cycling of the anthracyclines to produce superoxide anions, and not the production of adriamycin free radical species, has been shown to result in DNA strand breaks [31]. Administration of vitamin E or *N*-acetylcysteine, however, has been shown not to affect the antineoplastic properties of the anthracyclines [32, 33], suggesting that superoxide production is not the major mechanism by which these agents induce tumor cell kill. However, vitamin E or *N*-acetylcysteine administration has been reported to ameliorate the cardiotoxic properties of the anthracyclines which may well be due to the generation of toxic O_2 species during futile oxidation–reduction cycles [33, 34]. Similar studies in tumor-bearing animals or in cell culture have not been performed with MC. Liver is a relatively well-oxygenated tissue [35], and thus one would not expect the conversion of MC to a reactive quinone methide to occur under normal physiological conditions. In agreement with this conclusion, gross liver damage or functional liver changes have not been reported to occur as a result of treatment with this antibiotic.

Appreciation of the metabolic routes of activation of MC has led to the suggestion that this bioreductive alkylating agent may have primary utility as a hypoxic cell directed agent in the therapy of solid tumors [11–15]. Since the O_2-dependent toxicity of MC towards normal cells, which are for the most part relatively well-oxygenated, occurs at drug concentrations higher than those required for comparable toxicity to hypoxic cells, this antibiotic might well be optimally employed in the treatment of solid tumors at a low dosage level in combination with other agents (e.g. radiation, streptonigrin, and bleomycin) directed specifically towards the well-oxygenated cellular components of tumors.

REFERENCES

1. A. J. Lin, L. A. Cosby and A. C. Sartorelli, in *Cancer Chemotherapy* (Ed. A. C. Sartorelli), p. 71. American Chemical Society, Washington (1976).
2. V. N. Iyer and W. Szybalski, *Science* **145**, 55 (1964).
3. W. Szybalski and V. G. Arneson, *Molec. Pharmac.* **1**, 202 (1965).
4. H. S. Schwartz, J. E. Sodergren and F. S. Philips, *Science* **142**, 118 (1963).
5. H. S. Schwartz, *J. Pharmac. exp. Ther.* **136**, 250 (1962).
6. J. B. Schenkman and D. Cinti, *Life Sci.* **11**, 247 (1972).
7. E. Bresnick, J. B. Vaught, A. H. L. Chuang, T. A. Stoming, D. Bockman and H. Mukhtar, *Archs Biochem. Biophys.* **181**, 257 (1977).
8. G. G. Gibson and J. B. Schenkman, *J. biol. Chem.* **253**, 5957 (1978).
9. G. P. Wheeler, B. J. Bowdon, J. A. Gremsley and H. H. Lloyd, *Cancer Res.* **34**, 194 (1974).
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
11. K. A. Kennedy, B. A. Teicher, S. Rockwell and A. C. Sartorelli, *Biochem. Pharmac.* **29**, 1 (1980).
12. K. A. Kennedy, B. A. Teicher, S. Rockwell and A. C. Sartorelli, in *Molecular Actions and Targets for Cancer Chemotherapeutic Agents* (Eds. A. C. Sartorelli, J. R. Bertino and J. S. Lazo), Vol. 24, p. 85. Academic Press, New York (1981).
13. S. Rockwell and K. A. Kennedy, *Int. J. Radiat. Oncol. Biol. Phys.* **5**, 1673 (1979).
14. K. A. Kennedy, S. Rockwell and A. C. Sartorelli, *Cancer Res.* **40**, 2356 (1980).
15. B. A. Teicher, J. S. Lazo and A. C. Sartorelli, *Cancer Res.* **41**, 73 (1981).
16. R. Kahl, U. Wulff and K. J. Arther, *Xenobiotica* **8**, 359 (1978).
17. G. Powis and L. Wincentsen, *Biochem. Pharmac.* **29**, 347 (1980).
18. K. Handa and S. Sato, *Gann* **66**, 43 (1975).
19. N. R. Bachur, S. L. Gordon and M. V. Gee, *Molec. Pharmac.* **13**, 90 (1977).
20. N. R. Bachur, S. L. Gordon and M. V. Gee, *Cancer Res.* **38**, 1745 (1978).
21. N. R. Bachur, S. L. Gordon, M. V. Gee and H. Kon, *Proc. natn. Acad. Sci. U.S.A.* **76**, 954 (1979).
22. B. K. Sinha, *Chem. Biol. Interact.* **30**, 67 (1980).
23. T. Komiyama, T. Oki and T. Inui, *J. Pharmacobio-Dynamics* **2**, 407 (1979).
24. J. Capdevila, R. W. Estabrook and R. A. Prough, *Biochem. biophys. Res. Commun.* **83**, 1291 (1978).
25. J. B. Vaught and E. Bresnick, *Biochem. biophys. Res. Commun.* **69**, 587 (1976).
26. A. S. Khandwala and C. B. Kasper, *Biochem. biophys. Res. Commun.* **54**, 1241 (1973).
27. S. Sakai, C. E. Reinhold, P. J. Wirth and S. S. Thorgeirsson, *Cancer Res.* **38**, 2058 (1978).
28. W. E. Fahl, C. R. Jefcoate and C. B. Kasper, *J. biol. Chem.* **253**, 3106 (1978).
29. J. M. Pezzuto, M. A. Lea and C. S. Yang, *Cancer Res.* **37**, 3427 (1977).
30. E. G. Rogan, P. Mailander and E. Cavalieri, *Proc. natn. Acad. Sci. U.S.A.* **73**, 457 (1976).
31. V. Berlin and W. A. Haseltine, *J. biol. Chem.* **256**, 4747 (1981).
32. R. W. Freeman, J. S. MacDonald, R. D. Olson, R. C. Boerth, J. A. Oates and R. D. Harbison, *Toxic. appl. Pharmac.* **54**, 168 (1980).
33. C. E. Myers, W. P. McGuire, R. H. Liss, I. Ifrim, K. Grotzinger and R. C. Young, *Science* **197**, 165 (1977).
34. R. D. Olson, J. S. MacDonald, C. J. Van Boxtel, R. C. Boerth, R. D. Harbison, A. E. Slonim, R. W. Freeman and J. A. Oates, *J. Pharmac. exp. Ther.* **215**, 450 (1980).
35. W. R. Jondorf, in *Concepts in Drug Metabolism*, Part B (Eds. P. J. Jenner and B. Testa), p. 307. Marcel-Dekker, New York (1981).