0026-895X/91/030454-05$03.00/0

MOLECULAR PHARMACOLOGY, 40:454-458

Mechanisms for the Modulation of Alkylating Activity by the Quinone Group in Quinone Alkylating Agents

ASHER BEGLEITER, MARSHA K. LEITH, and SU-SHU PAN

Departments of Internal Medicine, (A.B., M.K.L.) and Pharmacology and Therapeutics (A.B.), University of Manitoba, and the Manitoba Institute of Cell Biology (A.B., M.K.L.), Winnipeg, Manitoba, R3E 0V9, Canada, and Division of Developmental Therapeutics, University of Maryland Cancer Center, Baltimore, Maryland 21201 (S.-S.P.)

Received January 11, 1991; Accepted June 12, 1991

SUMMARY

Previous studies have demonstrated that the quinone group may play an important role in modulating the alkylating activity of quinone alkylating agents. Introduction of a quinone moiety markedly increased the alkylating activity and cytotoxic activity of the model quinone alkylating agents benzoquinone mustard and benzoquinone dimustard. However, the cytotoxic and DNA-damaging activity of benzoquinone mustard was considerably greater than that of benzoquinone dimustard. In this study, we have investigated the role of the quinone group as a modulator of alkylating activity in these antitumor agents, using extracellular assays to eliminate differences due to cellular drug uptake and metabolism. Evidence was obtained that the alkylating activities of both benzoquinone mustard and benzoquinone dimustard were enhanced by reduction of the quinone group. In addition, when these agents were reduced, they displayed equal alkylating activity. This finding suggests that the difference in the activity of these agents in cells is not due to intrinsic differences in alkylating activities of the activated forms of these agents. Electrochemical studies revealed that benzoquinone dimustard has a lower redox potential than benzoquinone mustard and, thus, is less easily reduced. Inactivation and spectroscopic studies suggested that a major reason for the differences in activity between benzoquinone mustard and benzoquinone dimustard may be the rapid inactivation of the dimustard before its reduction. This effect may be enhanced by the lower redox potential of benzoquinone dimustard, compared with benzoquinone mustard. These findings support the hypothesis that the quinone group can modulate the alkylating activity of quinone alkylating agents; however, the mechanisms by which this modulation occurs may vary for different antitumor agents.

Quinone-containing antitumor agents are clinically important in the treatment of a wide variety of tumors. The anthracycline agents doxorubicin and daunorubicin contain the quinone moiety, and considerable evidence supports a role for free radicals generated by redox reactions of the quinone ring in the activity of these agents (1–5). Free radicals can produce their cytotoxic effects by lipid peroxidation (6) or by the induction of DNA strand breaks (7, 8). Other quinone-containing antitumor agents, such as MMC and AZQ, and the anthracycline analog 3′-(3-cyano-4-morpholinyl)-3′-deaminoadriamycin also possess alkylating activity (9–15). Although the quinone ring may contribute to the cytocidal action of these agents by formation of free radicals (1, 7, 16), the quinone moiety can also influence the alkylating activity of these agents (7, 9–12, 16–19).

We have previously described studies of the activity of the model quinone alkylating agents BM and BDM (Fig. 1) in L5178Y lymphoblasts (20–24). BM and BDM were significantly more cytotoxic to L5178Y cells compared with the structurally related nonquinone alkylating agent aniline mustard (20, 21). Both free radical-induced DNA strand breaks and DNA-DNA cross-linking appeared to contribute to the antitumor activity of BM and BDM (20–24); however, the latter mechanism appeared to be the major contributor to antitumor activity. Additionally, the cytotoxic and DNA-damaging activity of BM was considerably greater than that of BDM (21).

In the present study, we have examined the role of the quinone group as a modulator of alkylating activity and have investigated possible causes for the difference in activity of the two model quinone alkylating agents. To eliminate factors such as differences in cellular drug uptake and metabolism, we have studied the activities of BM and BDM in extracellular assays.

Experimental Procedures

Materials. BM [di(2′-chloroethyl)amino-1,4-benzoquinone], BDM [2,5-bis(di(2′-chloroethyl)amino)-1,4-benzoquinone], and HBM [di(2′-

This work was supported by the Medical Research Council of Canada (A.B.) and by Grant CH-412 from the American Cancer Society (S.-S.P).

ABBREVIATIONS: MMC, mitomycin C; AZQ, 2,5-diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone; BM, benzoquinone mustard; BDM, benzoquinone dimustard; DMSO, dimethyl sulfoxide; DTT, dithiothreitol; NBP, *p*-nitrobenzylpyridine; TEPA, tetraethylammonium perchlorate; HBM, hydrolyzed benzoquinone mustard.

hydroxyethyl)amino-1,4-benzoquinone] were prepared as described previously (20, 21) and were freshly dissolved in DMSO for use. Isolated λ phage DNA was from Boehringer Mannheim (Dorval, Quebec, Canada) and had an approximate molecular weight of 32,000,000. Ethidium bromide, DTT, NBP, and triethylamine were obtained from Sigma Chemical Co. (St. Louis, MO). TEPA was from Kodak Laboratory Chemicals (Rochester, NY).

DNA cross-linking in λ phage DNA. Isolated λ phage DNA (2.5 units/ml) was incubated *in vitro* for up to 4 hr at 37° with various concentrations of BM or BDM, in the presence or absence of the reducing agent DTT. The incubation mixture consisted of 10 μl of DNA, 10 μl of phosphate buffer (0.5 M KH_2PO_4, pH 7.2), 10 μl of H_2O or 10 μl of DTT (1 mM), 10 μl of drug in DMSO, and 60 μl of H_2O. At selected time points, 15 μl of the incubation mixture were added to 2.5 ml of ethidium bromide solution (1.25 μM), and cross-linking was measured by the ethidium bromide fluorescence assay at pH 11.8, using a Gilson Spectro/Flo fluorimeter (7). Cross-linking was expressed as percentage of cross-linking (maximum percentage of return of fluorescence after heat denaturation).

For studies of drug inactivation, BM (12.5 μM) or BDM (12.5 μM) was incubated at 37° in H_2O in the presence or absence of DTT (125 μM). The aqueous solutions had an ambient pH range of 5.7 to 4.8. At various time points, aliquots of the drug solutions were added to buffer containing λ phage DNA. The resulting mixture was incubated with DTT for up to 3 hr, and the cross-linking activity of the drug was determined as described above. The $t_{1/2}$ (preincubation time required to reduce the cross-linking activity to 50% of the activity of drug that was not preincubated) was determined from the slope of the linear regression line of a first-order kinetic analysis of the decrease in cross-linking activity.

Alkylation of NBP. Alkylating activity was determined by a modification of the procedure described by Linford (25). Various concentrations of BM or BDM, in the presence or absence of DTT (10 mM), were incubated at 37° for 1 hr with NBP, as described previously. Alkylating activity was assayed spectroscopically by measuring the alkylation product of the drug with NBP. The absorbance of the blue-colored product was measured in a Beckman DU-8 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) at 590 nm. DTT was added, immediately before the spectroscopic determination, to samples incubated in the absence of DTT, to eliminate nonspecific absorbance due to the drug quinone group.

Spectroscopic studies. BM and BDM were incubated at 37° in water, as described above, for the studies of inactivation of these agents. At various times, the UV-visible spectra of the drug solutions were measured by scanning from 250 to 650 nm using a Milton Roy 3000 spectrophotometer (Milton Roy Company, Rochester, NY).

Electrochemical studies. The reductive potential of BM and BDM was determined electrochemically by cyclic voltammetry. Cyclic voltammetric analyses were performed according to the method described earlier (26), with a CV-1A voltammetry unit (Bioanalytical Systems, West Lafayette, IN) connected to a thermostatically controlled electrochemical cell (IBM Instruments Inc., Danbury, CT). BM and BDM solutions were prepared at 1.0 mM in DMSO with 0.1 M TEPA. Potentials were measured with a glassy carbon working electrode, a platinum wire counter electrode, and an Ag/AgCl (saturated KCl) reference electrode. Voltammograms were obtained with the cell maintained at 25°. A potential range of 0.00 to −1.70 V, with scan rates of 20, 50, 100, and 250 mV/sec, was used.

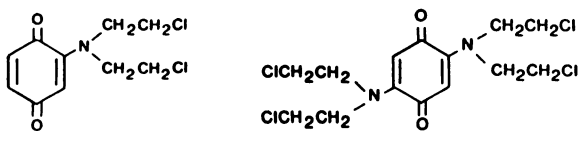

Fig. 1. Structures of the model quinone alkylating agents BM and BDM.

Results

DNA cross-linking by BM and BDM in isolated λ phage DNA. λ phage DNA was incubated at 37° with BM or BDM, in the presence or absence of DTT, and cross-linking was measured by an ethidium bromide fluorescence assay (7). BM produced significant cross-linking in both the absence and presence of DTT, although cross-linking was significantly elevated ($p < 0.05$) when the reducing agent was present (Fig. 2). In contrast, in the absence of the reducing agent BDM produced few cross-links. However, the addition of DTT resulted in a substantial increase in the level of cross-link formation by this agent, so that the cross-linking activity of BDM in the presence of the reducing agent was similar to that of BM.

Alkylation of NBP by BM and BDM. The alkylating activities of BM and BDM in the presence or absence of DTT were determined by measuring the ability of these agents to bind to the nucleophile NBP (25). BM demonstrated concentration-dependent alkylation, which was unchanged by the addition of DTT (Fig. 3). In contrast, BDM showed little alkylating activity in the absence of the reducing agent. However, the addition of DTT resulted in a substantial increase in activity, so that the alkylating activity of BDM in the presence of the reducing agent was similar to that of BM.

Inactivation of BM and BDM. In order to examine the extracellular inactivation of the model compounds, BM and BDM (12.5 μM) were incubated at 37° in water in the presence or absence of DTT. At various times, the amount of active drug remaining was determined by adding aliquots of the drug solutions to buffer containing λ phage DNA, in the presence of DTT, and measuring the cross-linking activity by ethidium bromide fluorescence assay. When BM was incubated in water in the absence of DTT, there was little inactivation, with the drug retaining 95% of its cross-linking activity after 2 hr (Fig.

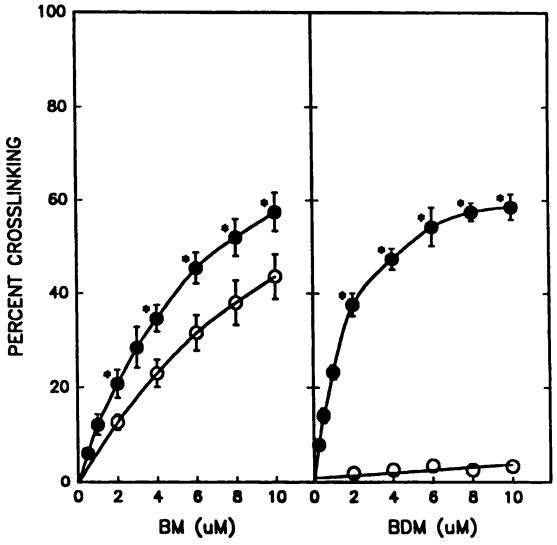

Fig. 2. DNA cross-linking by BM and BDM in isolated λ phage DNA. λ phage DNA was incubated at 37° with BM or BDM at the concentrations shown, in the presence (●) or absence (○) of 100 μM DTT. Cross-linking was determined using the ethidium bromide fluorescence assay at pH 11.8 (7) and is expressed as percentage of cross-linking (maximum percentage return of fluorescence after heat denaturation). *Points*, mean of three to seven determinations; *bars*, standard error. Cross-linking at a given drug concentration in the presence and absence of DTT was compared statistically by an unpaired *t* test comparing the significance of the difference of the means. *, $p < 0.05$.

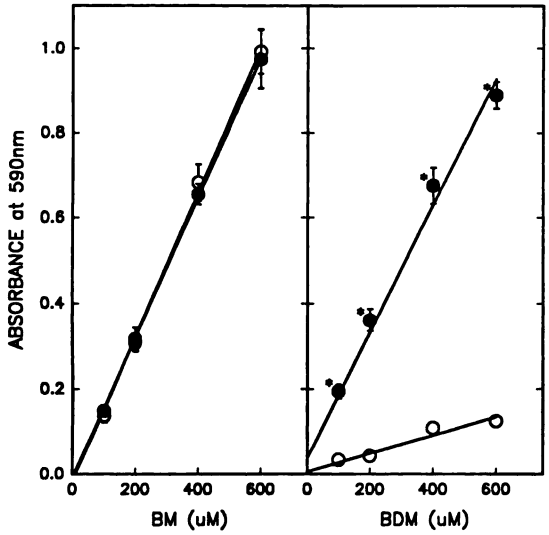

Fig. 3. Alkylation of NBP by BM and BDM. NBP was incubated at 37° for 60 min with the concentrations of drug shown, in the presence (●) or absence (○) of 10 mM DTT, and the alkylating activity was measured as described in Experimental Procedures. *Points*, mean of three to six determinations; *bars*, standard errors. Alkylation at each drug concentration in the presence and absence of DTT was compared statistically by an unpaired *t* test comparing the significance of the difference of the means. *, $p < 0.05$.

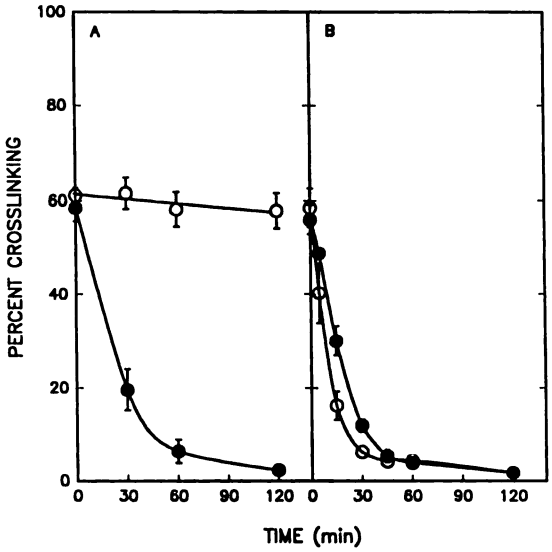

Fig. 4. Inactivation of BM and BDM. BM (12.5 μM) and BDM (12.5 μM) were incubated at 37° in water, in the presence (●) or absence (○) of 125 μM DTT. At the times shown, aliquots of the drug solutions were added to buffer containing λ phage DNA. The resulting mixture was incubated for up to 3 hr in the presence of DTT, and DNA cross-linking activity was determined using the ethidium bromide fluorescence assay, as described in Experimental Procedures. Cross-linking activity remaining after incubation in water for BM (A) and BDM (B) is expressed as percentage of cross-linking (maximum percentage return of fluorescence after heat denaturation). *Points*, mean of three to five determinations; *bars*, standard error.

4) and 91% of its activity after 4 hr. The $t_{1/2}$ (preincubation time required to reduce the cross-linking activity to 50% of the activity of drug that was not preincubated) was determined from a first-order kinetic analysis of the rate of decrease in cross-linking activity and was approximately 20 hr. However, when BM was incubated in water in the presence of DTT, drug inactivation was more rapid, with approximately 90% of the activity being lost in 1 hr. The $t_{1/2}$ for inactivation of BM in the presence of DTT was approximately 19 min.

Inactivation of BDM in the presence of DTT was similar to that of BM with DTT. Greater than 90% of the cross-linking activity of BDM was lost in 1 hr, and the $t_{1/2}$ for inactivation of this agent was approximately 17 min. However, unlike BM, there was rapid inactivation of BDM incubated in water in the absence of DTT. Under these conditions, >90% of the cross-linking activity of BDM was lost after 1 hr, and the $t_{1/2}$ for inactivation was approximately 16 min.

Spectroscopic study of inactivation of BM and BDM. To further examine the extracellular inactivation of BM and BDM, the spectroscopic changes resulting from incubation of the model compounds at 37° in water in the absence of DTT were examined. When BM was incubated in water, there was little change in the absorbance spectrum, with only a slight decrease in the peak of the parent compound at 497 nm and the appearance of a small secondary absorbance at 376 nm after 1 hr (Fig. 5A). In contrast, the spectrum of BDM incubated in water differed markedly from that of BM (Fig. 5B). There was a loss of absorbance at 370 nm during the 1-hr incubation and a gradual shift during this time to a new

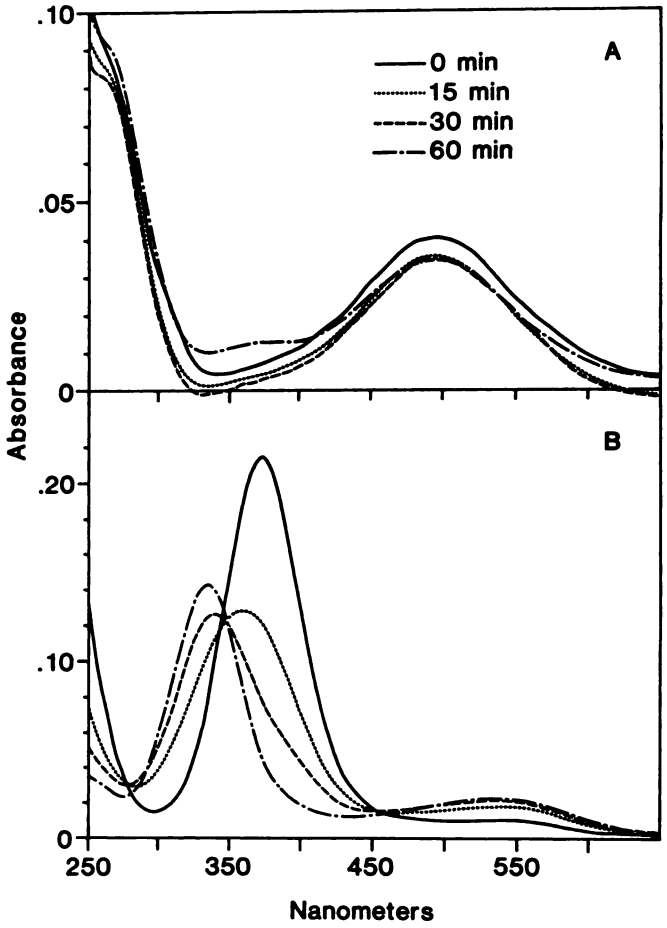

Fig. 5. Spectroscopic study of inactivation of BM and BDM. BM (12.5 μM) and BDM (12.5 μM) were incubated at 37° in water at an ambient pH ranging from 5.7 to 4.8. At the times shown, the UV-visible spectrum of each drug was scanned from 250 nm to 650 nm. The spectral scans for BM (A) and BDM (B) at various times are shown.

absorbance at 336 nm. Incubation of BM or BDM in buffer (pH 7.2) gave identical results.

Electrochemical studies of the redox reactions of BM and BDM. The reduction of the quinone group of BM and BDM was examined electrochemically using cyclic voltammetry in DMSO with 0.1 M TEPA. For both BM and BDM, the cathodic sweep gave only one wave at scan rates ranging from 20 to 250 mV/sec, and no waves were detected by the anodic sweep (Fig. 6, A and B). The reduction potentials were −0.518 and −0.723 V for BM and BDM, respectively. These results contrasted with those for a similar study with HBM. Cyclic voltammetry of this nonalkylating model quinone agent gave a cathodic sweep with two waves, which were also detected by the anodic sweep (Fig. 6C). The reduction potentials for the first and second electron reductions were −0.697 and −1.087 V, respectively.

Discussion

The precise role of the quinone moiety in the activity of quinone antitumor agents remains unclear. This functional group may influence the antitumor activity of a number of clinically important antineoplastic drugs either directly or indirectly. Reduction-oxidation reactions of the quinone group can generate oxygen free radicals and active oxygen species, which may contribute to the activity of a number of antitumor agents, including the anthracyclines (1, 3, 5, 6, 27), MMC (1, 7), AZQ (16), and streptonigrin (28). The quinone moiety can also influence the intercalating activity of DNA intercalators such as the anthracyclines, thus modifying their antitumor activity (19, 29). In addition, the quinone group can modulate the alkylating activity of quinone alkylating agents such as MMC (7, 10, 11, 18, 30), AZQ (11, 12, 16), and the model antitumor agents BM and BDM (20–24).

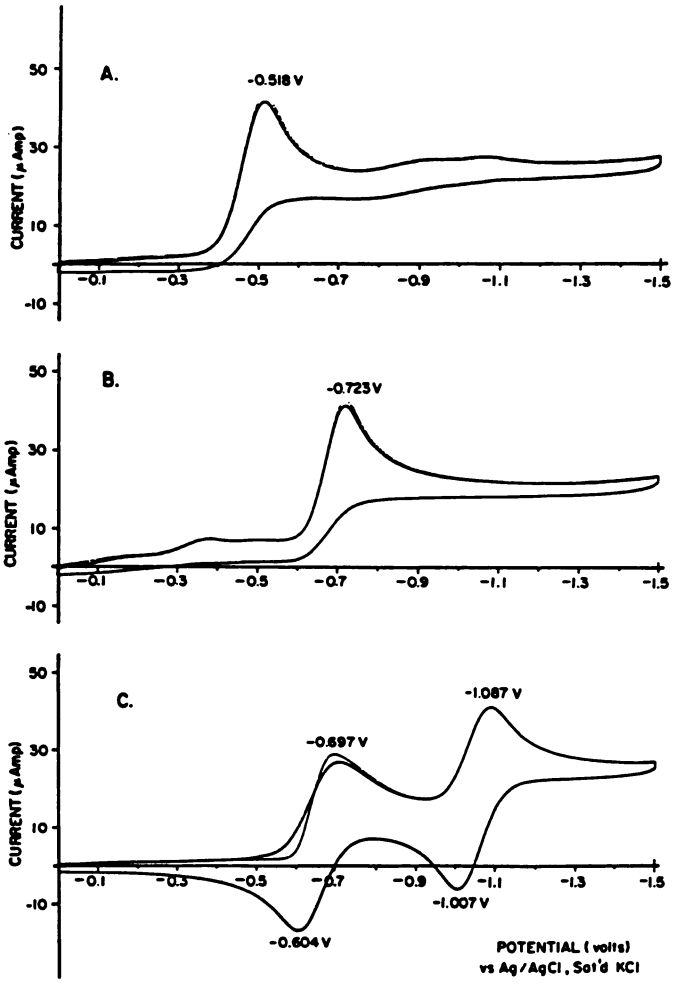

Fig. 6. Multicycle cyclic voltammograms of BM (A), BDM (B), and HBM (C). Solutions of BM, BDM, and HBM were prepared at 1.0 mM in DMSO with 0.1 M TEPA. Potentials were measured with a glassy carbon working electrode, a platinum wire counter electrode, and an Ag/AgCl (saturated KCl) reference electrode. Voltammograms were obtained at 25°, with a scan rate of 20 mV/sec, in the potential range of 0.0 to −1.70 V.

In earlier studies with the model quinone alkylating agents BM and BDM, we have shown that the presence of the quinone group enhances both the cytotoxic and cross-linking activities of these agents (20, 21). Both DNA cross-linking and oxygen radical-induced DNA strand breaks appear to contribute to the antitumor activity of these agents; however, BM is more cytotoxic and produces more DNA damage than BDM (20–23). More recently, we have found evidence that reduction of the quinone moiety by intracellular reductive enzymes can modulate the alkylating activity and cytocidal action of the agents (24). In this study, we have compared the activity of BM and BDM in extracellular systems, to investigate the mechanisms by which the quinone group modulates the alkylating activity of these agents.

The studies of cross-linking of isolated λ phage DNA and alkylation of the nucleophile NBP by BM and BDM demonstrated that reduction of the quinone moiety enhanced the cross-linking and alkylating activities of both quinone agents. This effect was most pronounced for BDM, which showed little cross-linking and alkylating activity in the absence of a reducing agent. It is not clear why DTT did not increase BM alkylation of NBP, but this finding may suggest that reduction of the quinone group of BM primarily enhances the second alkylation step of the DNA cross-linking process. Because reduced BM and BDM showed nearly identical cross-linking and alkylating activity, this study suggests that the difference in activity of these two agents in cells is not due to intrinsic differences in the alkylating properties of the activated forms of these agents.

This conclusion appears to be supported by studies of the inactivation of BM and BDM and by electrochemical studies. Whereas BM was relatively stable in its oxidized form in an aqueous environment, as was shown by the spectroscopic and cross-linking studies, oxidized BDM was rapidly converted to an inactive decomposition product. In contrast, the reduced forms of BM and BDM exhibited similar rates of inactivation of cross-linking. These findings suggest that a major reason for the differences in activity of BM and BDM in cells may be the rapid inactivation of BDM before its reduction. This effect is likely enhanced by the lower redox potential of BDM, compared with BM, making it more difficult to reduce the BDM quinone in cells and thus increasing the possibility that this agent will decompose before it can be activated. The overall activity of these agents likely represents a balance between their rate of activation and rate of decomposition. For BM, reduction may have slightly increased cross-linking but it also increased decomposition, resulting in a small net increase in cross-linking. In contrast, for BDM, reduction significantly increased alkylating activity and slightly decreased decomposition, producing

a large net increase in cross-linking. Therefore, the quinone group may act as a modulator of alkylating activity in quinone alkylating agents by influencing both the activation and the decomposition of the antitumor agents.

The absence of waves in the anodic sweeps of the electrochemical studies of BM and BDM is unexpected because, generally, quinones can undergo redox cycling, as is observed with HBM. The reasons for these findings are unknown. However, it is possible that, under the nonaqueous conditions used in the electrochemical studies, the activated forms of BM and BDM may undergo internal alkylation to form a benzomorpholino ring system, which incorporates one arm of the nitrogen mustard group and a quinone oxygen. This structure might then prevent further reduction of the semiquinone to the hydroquinone and reoxidation to the quinone moiety.

In summary, this study demonstrates that reduction of the quinone moiety activates the alkylating activity of the model quinone alkylating agents BM and BDM, that the reduced forms of these agents have equivalent alkylating activity, and that BDM is more difficult to reduce than BM. Furthermore, it suggests that a major reason for the difference in activity of BM and BDM in cells may be the rapid inactivation of BDM before its reduction. These findings further support the hypothesis that the quinone group can modulate the alkylating activity of quinone alkylating agents; however, the mechanism by which this modulation occurs may vary for different antitumor agents.

Acknowledgments

The authors thank Susan Snusher and Sophie Didych for typing the manuscript.

References

1. Doroshow, J. H. Role of hydrogen peroxide and hydroxyl radical formation in the killing of Ehrlich tumor cells by anticancer quinones. *Proc. Natl. Acad. Sci. USA* **83**:4514–4518 (1986).
2. Doroshow, J. H., G. Y. Locker, I. Ifrim, and C. E. Myers. Prevention of doxorubicin cardiac toxicity in the mouse by *N*-acetyl cysteine. *J. Clin. Invest.* **68**:1053–1064 (1981).
3. Herman, E. H., and V. J. Ferrans. Reduction of chronic doxorubicin cardiotoxicity in dogs by pretreatment with (±)-1,2-bis(3,5-dioxopiperazinyl-1-yl) propane (ICRF-187). *Cancer Res.* **41**:3436–3460 (1981).
4. Lown, J. W., H. H. Chen, J. A. Plambeck, and E. M. Acton. Further studies on the generation of reactive oxygen species from activated anthracyclines and the relationship to cytotoxic action and cardiotoxic effects. *Biochem. Pharmacol.* **31**:575–581 (1979).
5. Doroshow, J. H., G. Y. Locker, and C. E. Myers. Enzymatic defences of the mouse heart against reactive oxygen metabolites: alteration produced by doxorubicin. *J. Clin. Invest.* **65**:128–135 (1980).
6. Myers, C. E., L. Gianni, C. B. Simone, R. Klecker, and R. Greene. Oxidative destruction of erythrocyte ghost membranes by the doxorubicin-ion complex. *Biochemistry* **21**:1707–1712 (1982).
7. Lown, J. W., A. Begleiter, D. Johnston, and A. R. Morgan. Studies related to antitumor antibiotics. V. Reactions of mitomycin C with DNA examined by ethidium fluorescence assay. *Can. J. Biochem.* **54**:110–119 (1976).
8. White, H. L., and J. R. White. Lethal action and metabolic effects of streptonigrin on *Escherichia coli*. *Mol. Pharmacol.* **4**:549–565 (1968).
9. Dorr, R. T., G. T. Bowden, D. S. Alberts, and J. D. Liddil. Interaction of mitomycin C with mammalian DNA detected by alkaline elution. *Cancer Res.* **45**:3510–3516 (1985).
10. Pan, S.-S., T. Iracki, and N. R. Bachur. DNA alkylation by enzyme-activated mitomycin C. *Mol. Pharmacol.* **29**:622–628 (1986).
11. Tomasz, M., D. Chowdary, R. Lipman, S. Shinotakahara, D. Veiro, V. Walker, and G. L. Verdine. Reaction of DNA with chemically or enzymatically activated mitomycin C: isolation and structure of the major covalent adduct. *Proc. Natl. Acad. Sci. USA* **83**:6702–6706 (1986).
12. King, C. L., S.-K. Wong, and T. L. Loo. Alkylation of DNA by the new anticancer agent 3,6-diaziridinyl-2,5-bis(carboethoxyamino)-1,4-benzoquinone (AZQ). *Eur. J. Cancer Clin. Oncol.* **20**:261–264 (1984).
13. Begleiter, A., and J. B. Johnston. DNA cross-linking by 3′-(3-cyano-4-morpholinyl)-3′-deaminoadriamycin in HT-29 human colon carcinoma cells *in vitro*. *Biochem. Biophys. Res. Commun.* **131**:336–338 (1985).
14. Westendorf, J., G. Groth, G. Steinheider, and H. Marquardt. Formation of DNA-adducts and induction of DNA cross-links and chromosomal aberrations by the new potent anthracycline antitumor antibiotics: morpholinodaunomycin, cyanomorpholinodaunomycin and cyanomorpholino-adriamycin. *Cell. Biol. Toxicol.* **1**:87–101 (1985).
15. Scudder, S. A., J. M. Brown, and B. I. Sikic. DNA cross-linking and cytotoxicity of the alkylating cyanomorpholino derivative of doxorubicin in multidrug-resistant cells. *J. Natl. Cancer Inst.* **80**:1294–1298 (1988).
16. King, C. L., W. N. Hittelman, and T. L. Loo. Induction of DNA strand breaks and cross-links by 2,5-diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone in Chinese hamster ovary cells. *Cancer Res.* **44**:5634–5637 (1984).
17. Iyer, V. M., and W. Szybalski. Mitomycins and profiromycin: chemical mechanism of activation and cross-linking of DNA. *Science (Washington D. C.)* **145**:55–58 (1964).
18. Keyes, S. R., P. M. Fracasso, D. C. Heimbrook, S. Rockwell, S. G. Sligar, and A. C. Sartorelli. Role of NADPH:cytochrome *c* reductase and DT-diaphorase in the biotransformation of mitomycin C. *Cancer Res.* **44**:5638–5643 (1984).
19. Jesson, M. I., J. B. Johnston, E. Robotham, and A. Begleiter. Characterization of the DNA-DNA cross-linking activity of 3′-(3-cyano-4-morpholinyl)-3′-deaminoadriamycin. *Cancer Res.* **49**:7031–7036 (1989).
20. Begleiter, A. Cytocidal action of the quinone group and its relationship to antitumor activity. *Cancer Res.* **43**:481–484 (1983).
21. Begleiter, A. Studies on the mechanism of action of quinone antitumor agents. *Biochem. Pharmacol.* **34**:2629–2636 (1985).
22. Begleiter, A., and G. W. Blair. Quinone induced DNA damage and its relationship to antitumor activity. *Cancer Res.* **44**:78–84 (1984).
23. Begleiter, A. The contribution of alkylation to the activity of quinone antitumor agents. *Can. J. Physiol. Pharmacol.* **64**:581–585 (1986).
24. Begleiter, A., and M. K. Leith. Activity of quinone alkylating agents in quinone-resistant cells. *Cancer Res.* **50**:2872–2876 (1990).
25. Linford, J. H., 2,3,5-Tris-ethylenimino-1,4-benzoquinone (Trenimon): some chemical and biological properties. *Chem. Biol. Interact.* **6**:149–168 (1973).
26. Andrews, P. A., S. Pan, and N. R. Bachur. Electrochemical reductive activation of mitomycin C. *J. Am. Chem. Soc.* **108**:4158–4166 (1986).
27. Bachur, N. R. Anthracycline antibiotic pharmacology and metabolism. *Cancer Treat. Rep.* **63**:817–820 (1979).
28. Lown, J. W., and S.-K. Sim. Studies related to antitumor antibiotics. VIII. Cleavage of DNA by streptonigrin analogues and the relationship to antineoplastic activity. *Can. J. Biochem.* **54**:446–452 (1976).
29. Acton, E. M., G. L. Tong, C. W. Mosher, and R. L. Wolgemuth. Intensely potent morpholinyl anthracyclines. *J. Med. Chem.* **27**:638–645 (1984).
30. Begleiter, A., E. Robotham, and M. K. Leith. Increased sensitivity of quinone resistant cells to mitomycin C. *Cancer Lett.* **45**:173–176 (1989).

Send reprint requests to: Dr. A. Begleiter, Manitoba Institute of Cell Biology, 100 Olivia Street, Winnipeg, Manitoba, R3E 0V9 Canada.