Novel Bioreductive Activation Mechanism of Mitomycin C Derivatives Bearing a Disulfide Substituent in Their Quinone

Qiao-Yun He, Helena Maruenda, and Maria Tomasz*

Department of Chemistry, Hunter College
City University of New York
New York, New York 10021

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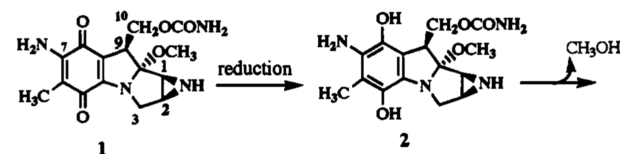
Mitomycin C (MC; **1**) is a natural antitumor antibiotic, used in anticancer chemotherapy. Upon reductive activation it alkylates and cross-links DNA.[1] The resulting DNA monoadducts and bisadducts have been structurally elucidated.[2] MC is the prototype bioreductive alkylating agent as its reactions with DNA and nucleophiles in general are absolutely dependent on reduction of its quinone under physiological conditions.[1a,3] Reduction of the quinone induces spontaneous elimination of CH_3OH from the 9/9a positions (**3**). This, in turn, activates the two "masked" alkylating functions of MC at the C1 (aziridine) and C10 (carbamate) positions, resulting in a successive incorporation of two nucleophiles (Scheme 1).[2] There is ample evidence that the cytotoxicity and antitumor activity of MC *in vivo* are also dependent on reduction.[4,5] Resistance of tumor cells to MC has been related frequently to deficient reductase activity.[5] Thus, the requirement for enzymatic reduction of MC is a potentially limiting factor in the therapeutic efficiency of the drug.

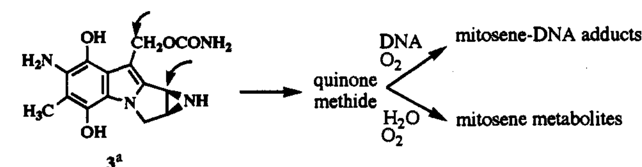
We report a novel, *nonenzymatic* bioreductive activation mechanism of a class of designed MC analogs carrying a disulfide group in their quinone side chain. Two of these (**4**; BMY-25067[6] and KW-2419[7]) (Chart 1) are superior to MC as anticancer agents; both are under clinical trials. Each was designed[6,7] on the basis of the hypothesis that their disulfide group may mediate nonenzymatic reduction of the quinone, initiated by thiols, e.g., glutathione (GSH) (Scheme 2). We tested this hypothesis experimentally as follows.

A series of disulfide analogs **4**, monosulfide **5**, and MC[8] itself were incubated with 5 mM GSH in 50 mM Tris-HCl (pH 7.5) buffer at room temperature. Reductive activation of these mitomycins was monitored by a UV assay based on the conversion of the 7-aminomitosane chromophore ($\lambda_{max} \sim 370$) to the chromophore of 7-aminomitosenes ($\lambda_{max} \sim 320$ nm) as a result of the reduction.[1a,10] As seen in Figure 1, MC was not reduced by GSH. However, all three group I disulfide analogs (**4**) were fully reduced in less than 2 h. In sharp contrast, the group II disulfide analogs (**4**) and the monosulfide **5** were unreduced even after 25 h. The kinetic course of the reductions (Figure 1d) was calculated from the UV spectral changes.

Next, it was tested whether the GSH-initiated reduction activated the mitomycin derivatives to the DNA-cross-linking species (cf. Scheme 1). The mitomycins were incubated with a mixture of 1.2 μM pBR322 DNA (linearized; ^{32}P-labeled at the 3′ ends) and 120 μM calf thymus DNA in the presence of 5 mM GSH or 0.08 mM dithiothreitol (DTT) in 20 mM Tris-HCl–1 mM EDTA (pH 7.4) buffer for 1 h at room temperature. The DNA was then isolated and assayed for cross-links by a sensitive method.[11] The results paralleled perfectly with the results of the reduction assay: group I disulfides in the presence of DTT or

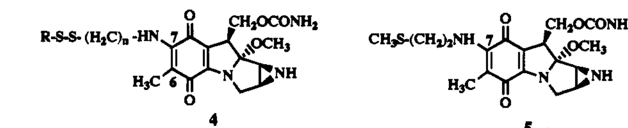
Scheme 1





[a] Arrows depict sites of nucleophilic substitutions in subsequent steps.

Chart 1



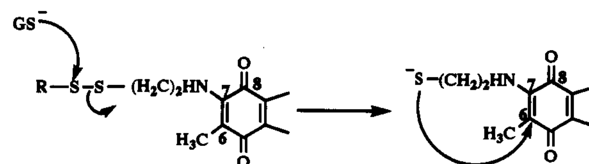
Group I:

n = 2:
- BMY-25067 (R = p - nitrophenyl)
- KW-2149 [R = $HOOC(NH_2)CH(CH_2)_2CONHCH_2CH_2$-]
- " n = 2 dimer " [R = mitomycin C - (N^7- ethyl)]

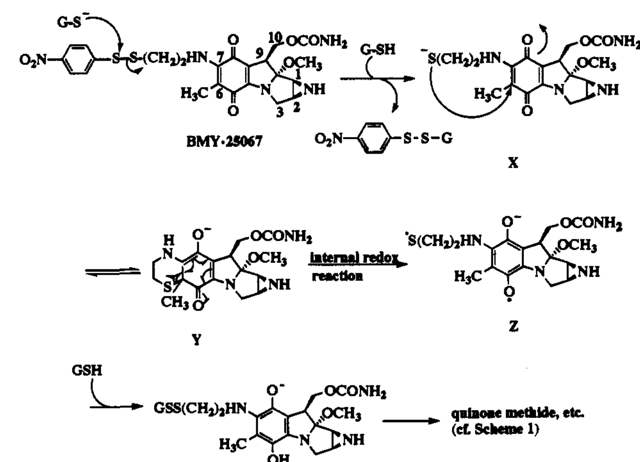
Group II:

n = 4: " n = 4 dimer " [R = mitomycin C - (N^7 - butyl)]
n = 6: " n = 6 dimer " [R = mitomycin C - (N^7 - hexyl)]

Scheme 2



Scheme 3



(1) (a) Iyer, V. N.; Szybalski, W. *Science* **1964**, *145*, 55–58. (b) Tomasz, M.; Mercado, C. M.; Olson, J.; Chatterjie, N. *Biochemistry* **1974**, *13*, 4878–4887.
(2) For a review and references, see: Tomasz, M. In *Molecular Aspects of Anticancer Drug-DNA Interactions;* Neidle, S., Waring, M., Eds.; MacMillan: London, 1994; Vol. 2, pp 312–349.
(3) Kennedy, K. A.; Rockwell, S.; Sartorelli, A. C. *Cancer Res.* **1980**, *40*, 2356–2360.
(4) Moulder, J. E.; Rockwell, S. *Cancer Metastasis Rev.* **1987**, *5*, 313–341.
(5) Wilson, K. K. V.; Long, B. H.; Chakrabarty, S.; Brattain, D. E.; Brattain, M. G. *Cancer Res.* **1985**, *45*, 5281–5286.
(6) Vyas, D. M.; Chiang, Y.; Benigni, D.; Rose, W. C.; Bradner, W. T. In *Recent Advances in Chemotherapy. Anticancer Section*; Tshigami, J., Ed.; University of Tokyo Press: Tokyo, 1985; pp 485–486.
(7) Kono, M.; Saitoh, Y.; Kasai, M.; Sato, A.; Shirahata, K.; Morimoto, M.; Ashizawa, T. *Chem. Pharm. Bull.* **1989**, *37*, 1128–1130.
(8) MC and BMY-25067 were supplied by Dr. D. M. Vyas, Bristol-Myers Squibb Research Laboratory, Wallingford, CT. KW-2419, *n* = 2 dimer, and **5** were received from Dr. M. Kasai, Kyowa Hakko Kogyo Pharmaceutical Research Laboratories, Shimotogari, Japan. The *n* = 4 and *n* = 6 dimers were synthesized by us, using a published procedure.[9]
(9) Kono, M.; Saitoh, Y.; Kasai, M.; Shirahata, K.; Morimoto, M.; Ashizawa, T. *J. Antibiot.* **1993**, *46*, 1428–1438.
(10) Tomasz, M.; Lipman, R. *Biochemistry* **1981**, *20*, 5056–5061.

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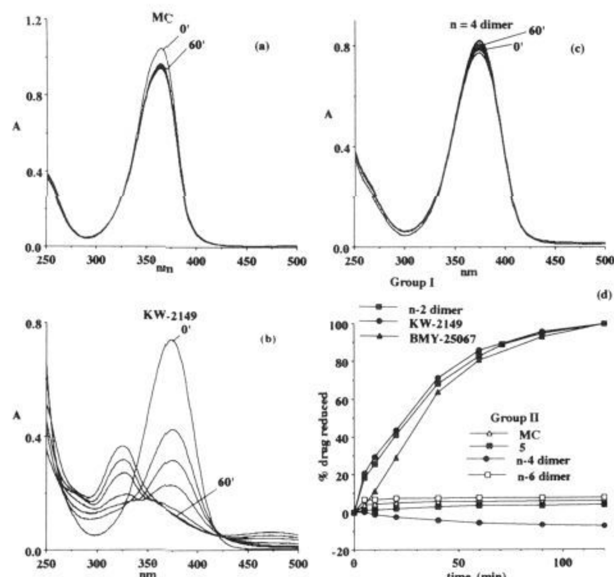

Figure 1. UV assay of reduction of mitomycins by glutathione. The reduction mixtures (see text) were incubated in UV cells (1-cm path), and spectra were determined at 0-, 5-, 10-, 20-, 40-, 60-, and 120-min and 12- and 24-h reaction times, using a Varian Cary 3 spectrophotometer. (a) MC; (b) KW-2149; (c) $n = 4$ dimer; (d) kinetic plots calculated from the spectral changes at 370 min.

GSH cross-linked the DNA (Figure 2b–f) while MC, the group II disulfides, and monosulfide **5** were completely inactive (Figure 2a,d,f).

It is concluded from these results that reductive activation of the mitomycin system by GSH and DTT is dependent upon the presence of a disulfide function, linked to the 7-amino group of MC. Furthermore, the $-(CH_2)_n-$ linker must be two carbons long ($n = 2$). This suggests a mechanism involving *intramolecular electron transfer* from the disulfide to the quinone as shown in Scheme 3 for BMY-25067. GS^--initiated disulfide exchange releases the free thiol (**X**), which then adds to the quinone,[12,13] leading to the cyclic intermediate **Y**.[14] Homolytic S–C bond cleavage gives the semiquinone anion radical **Z**, which is then reduced to the hydroquinone by excess GSH, accomplishing the reduction of the mitomycin to the activated, hydroquinone state.[15] This mechanism accounts for the $n = 2$ requirement since formation of the six-membered ring cyclic intermediate **Y**[14] is favorable, in contrast to the cases of the $n = 4$ and $n = 6$ dimers (**4**) in which unfavorable eight- or 10-membered ring formation would be required. Consistent with a favorable intramolecular cyclization mechanism, external thiols (GSH or DTT) do not reduce the quinone directly, as seen by their lack of reaction with MC itself.[16] Verification of this mechanism by characterizing stable end products and reactive intermediates is in progress.

Drug activation by thiols is an important mechanism in the action of the enediyne antibiotics[17] and other drugs.[18] However, the thiol-induced activation of the mitomycin quinone occurs by a conceptually novel mechanism, i.e., by thiol-to-quinone electron transfer, as shown here. In a recent salient structure–activity relationship study[9] the $n = 2$ dimer (**4**) was found to be hundreds-fold more cytotoxic to HeLa cells and more effective against tumors *in vivo* than the higher members ($n = 3$–12) of the dimer homolog series or MC itself. This demonstrates that the self-reductive (nonenzymatic) activation capability[19] of the disulfides, described here, plays a role in their superior antitumor activity. Linking an $RSS(CH_2)_2NH$ substituent as a "self-reducing" device to the quinone of a bioreductive alkylating agent might be useful in general in the design of new antitumor drugs.

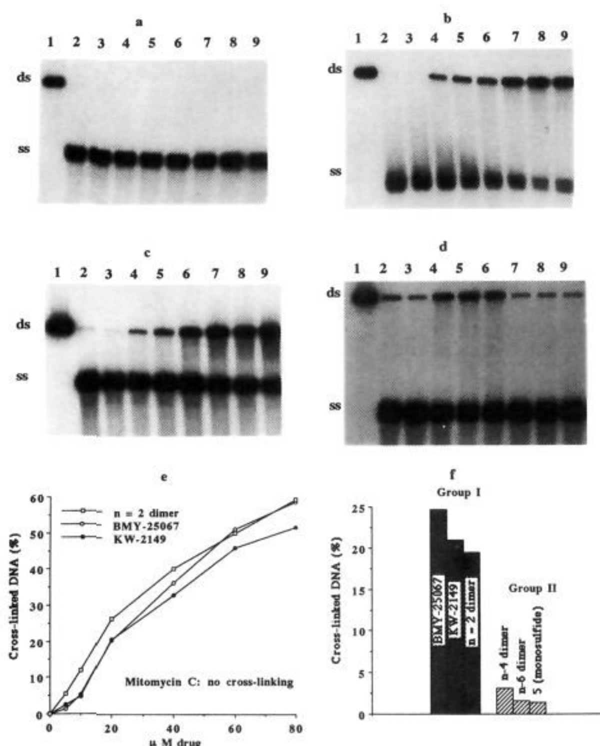

Figure 2. Gel electrophoretic assay of cross-linking of ^{32}P-labeled pBR322 DNA[11] by MC and the disulfide and monosulfide analogs upon activation by thiols. (a–c) Complete system: Incubation mixture containing drug, DNA, and thiol; see text. Lanes: 1, control linear ^{32}P-labeled pBR322 DNA; 2, same, heat-denatured; 3, complete system minus thiol; 4–9, complete system, with drug concentrations 5, 10, 20, 40, 60, and 80 μM, respectively. (a) Drug: MC. Thiol: DTT. (b) Drug: BMY-25067. Thiol: DTT. (c) Drug: KW-2149. Thiol: GSH. (d) Complete system: drug (20 μM), DNA (118 μM), GSH (5 mM); see text. Lanes 1–3: same as in a–c. Lanes 4–9: complete system; drugs are BMY-25067, KW-2149, $n = 2$ dimer, $n = 4$ dimer, $n = 6$ dimer, and monosulfide **5**, respectively. (e) Percent cross-linked DNA as a function of drug concentration, calculated from densitometric measurements of gel autoradiograms. Thiol: GSH. (f) Percent cross-linked DNA calculated from densitometric measurement of the autoradiogram in d.

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(11) Hartley, J.; Berardini, M. D.; Souhami, R. L. *Anal. Biochem.* **1991**, *193*, 131–134.

(12) Normally thiols do not reduce quinones. Rather, they react by Michael addition followed by aromatization by prototropy, to form a thioether-substituted hydroquinone.[13]

(13) Wardman, P. *Free Radical Res. Commun.* **1990**, *8*, 219–229.

(14) Two alternative cyclic intermediates, fully compatible with the mechanistic argument, are also feasible: one, resulting from sulfide attack at C8, the other, at C7.

(15) A *heterolytic* internal redox mechanism involving nucleophilic attack of a second GS^- at the sulfur of **Y** is also a possibility.

(16) See also: Sharma, M.; Tomasz, M. *Chem. Res. Toxicol.* **1994**, *7*, 390–400.

(17) Dedon, P. C.; Goldberg, I. H. *Chem. Res. Toxicol.* **1992**, *5*, 311–332.

(18) Silverman, R. B. *The Organic Chemistry of Drug Design and Drug Action*; Academic Press: San Diego, 1992; pp 387–388.

(19) This capability is *additional* to the enzymatic reductive activation mechanism common to the mitomycins (He, Q.-Y. Ph.D. Thesis, CUNY, 1994).