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Mitomycin C is not metabolized by but is an inhibitor of human kidney NAD(P)H:(quinone-acceptor)oxidoreductase

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Summary. It has been suggested that quinone reductase [NAD(P)H:(quinone-acceptor)oxidoreductase], also known as DT-diaphorase, protects hypoxic cells against mitomycin C cytotoxicity by metabolizing mitomycin C to less toxic metabolites. This hypothesis is based on an increase in mitomycin C's cytotoxicity in the presence of the potent quinone reductase inhibitor dicumarol. It has been suggested that under aerobic conditions the metabolism of mitomycin C by quinone reductase leads to the formation of cytotoxic metabolites. In the present study, mitomycin C was found not to be a substrate for partially purified quinone reductase from human kidney. Mitomycin C did not cause the oxidation of NADPH by quinone reductase and there was no utilization of mitomycin C and no appearance of its metabolites. Quinone reductase did not catalyze the formation of alkylating metabolites from mitomycin C, determined by the lack of formation of 4-(*p*-nitrobenzyl)pyridine conjugates. However, mitomycin C was a weak competitive inhibitor of quinone reductase with dichloroindophenol as the substrate, with K_i = 0.32 m*M*. Therefore, the alteration of mitomycin C's cytotoxicity by dicumarol in tumor cell lines appears to involve a mechanism other than the direct inhibition of mitomycin C reduction by quinone reductase.

Introduction

Mitomycin C is a quinone-containing antibiotic with antitumor activity in a number of experimental animal tumors [9]. It is used clinically in the treatment of breast,and head, and neck tumors [12]. More recently, it has been used by intravesicular administration for bladder cancer and infused in the hepatic artery for metastatic colon cancer [30].

The critical cytotoxic event produced by mitomycin C is thought to be the cross-linking of complementary strands of DNA [16], although the monofunctional alkylation of DNA or other nucleophilic sites [41] and formation of toxic oxygen radicals may also be important for its antitumor activity [3, 39]. The biological activity of mitomycin C requires its reduction. Mitomycin C is known to undergo one-electron reduction by flavoenzymes, including NADPH cytochrome P-450 reductase and xanthine oxidase, to the short-lived mitomycin C semiquinone radical [18, 31]. One-electron reduction has been proposed by some workers to be responsible for the biological activity of mitomycin C [28, 31, 36]. Other workers have suggested that the two-electron reduction of mitomycin C to the hydroquinone is responsible for biological activity [17, 26, 32]. A major enzyme catalyzing the two-electron reduction of quinones is quinone reductase [NAD(P)H:(quinone-acceptor)oxidoreductase, E.C. 1.6.99.2], formerly known as DT-diaphorase [13, 15]. The potent inhibitor of quinone reductase, dicumarol, has been reported to increase the formation of alkylating species from mitomycin C incubated with NADPH and sonicates of EMT6 mouse mammary carcinoma or of V79 Chinese hamster lung fibroblasts [20]. Dicumarol also increases the cytotoxicity of mitomycin C to hypoxic EMT6 carcinoma cells, although it decreases mitomycin C's cytotoxicity to euoxic cells [20, 22]. These findings have led to the suggestion that quinone reductase may protect cells from mitomycin C's cytotoxicity under hypoxic conditions by reducing the intracellular concentration of the drug [20]. This is similar to the protection proposed for quinone reductase against the toxicity of simple quinones [25, 27]. It has been suggested that under aerobic conditions the metabolism of mitomycin C by quinone reductase leads to the formation of cytotoxic products [23].

Despite these suggestions, there have been no studies to determine whether mitomycin C is indeed a substrate for quinone reductase. We now report evidence showing that mitomycin C is not a substrate for metabolism by human kidney quinone reductase but an inhibitor of the enzyme.

Materials and methods

Mitomycin C, dicumarol, flavin adenine dinucleotide (FAD), and bovine serum albumin (Fraction V) were purchased from Sigma Chemical Co. (St. Louis, Mo). 2,6-Dichloroindophenol and 4-(*p*-nitrobenzyl)pyridine were purchased from Aldrich Chemical Company (Milwaukee, Wis). NADPH was purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind).

Quinone reductase was prepared from the cytosol of normal human kidneys obtained as surgical waste material. The kidneys were placed in chilled Roswell Park Memorial Institute media 1640 within 16 min of their arrival in the pathology laboratory, transported to the laboratory,

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and homogenized in 2 volumes of 0.25 *M* sucrose. The method for preparation of quinone reductase is a modification of that previously described [35]. Briefly, the cytosol from approximately 25 g human kidney was applied to a 1 × 15 cm column of Blue Sepharose CL-6B (Pharmacia Inc., Piscataway, NJ) and the column washed, first with 200 ml 10 m*M* sodium phosphate buffer (pH 7.4), and then with 100 ml 2 *M* NaCl in 10 m*M* sodium phosphate buffer (pH 7.4). Quinone reductase still bound to the column was eluted with 100 ml 2 *M* NaCl containing 4 *M* urea, the eluate containing activity concentrated to 8 ml by ultrafiltration using an Amicon PM10 membrane (Millipore, Bedford, Mass) and applied to a 2.5 × 45 cm column of Sephadex G-25. The fractions containing activity were then applied to a 0.5 × 10 cm column of hydroxylapatite (Bio-Rad Laboratories, Richmond, Calif) and eluted with a 100 ml linear gradient of 10–300 m*M* sodium phosphate buffer (pH 6.8). A small amount of quinone reductase was eluted at this step. The major portion of quinone reductase activity was eluted from the column with 30 ml 300 m*M* sodium phosphate buffer (pH 6.8). FAD (1 μ*M*) was added to each fraction to stabilize the enzyme. All steps were carried out at 4°C and the purified enzyme was stored at −80°C. The recovery of the major quinone reductase fraction was 10% of the total activity in the cytosol, and it had a specific activity measured by NADPH oxidation, with dichloroindophenol as the substrate, at 21°C of 576 nmol/min mg^{-1}, which represented a 448-fold purification from cytosol. The activity was completely inhibited by 30 μ*M* dicumarol. Human kidney microsomes were prepared from fresh kidney homogenate by centrifuging at 10,000 *g* for 15 min, then at 110,000 *g* for 60 min, and finally washed by resuspending twice in 0.25 *M* sucrose. Rat liver microsomes were prepared in the same way and stored at −80°C until use. Protein was measured by the dye-binding method of Bradford [6], using a commercial test kit (Bio-Rad Laboratories, Richmond, Calif) with crystalline bovine serum albumin as a standard.

Quinone reductase activity was measured by the oxidation of NADPH at 340 nm, or by the reduction of dichloroindophenol at 600 m, at 21°C with an incubation mixture containing 50 m*M* TRIS-HCl buffer (pH 7.8), 0.7 mg/ml bovine serum albumin, and 0.11 μg/ml quinone reductase at an incubation volume of 1 ml. Dichloroindophenol or mitomycin C dissolved in 10–100 μl water was added immediately before 0.1 μmol NADPH dissolved in 10 μl TRIS-HCl buffer. There was no oxidation of NADPH by dichloroindophenol or mitomycin C in the absence of enzyme. All measurements were made in triplicate. For kinetic studies, the concentration of dichloroindophenol was varied between 15 and 75 μ*M* and the concentration of NADPH, between 10 and 40 μ*M*. Kinetic analysis of the data was carried out with the Enzfitter computer program (Elsevier-Biosoft, Cambridge, UK).

Studies of product formation from mitomycin C by quinone reductase were made using a similar incubation system but with 3 m*M* NADPH, 1 m*M* mitomycin C, and 0.7 mg/ml rat liver microsomes or 1 μg/ml quinone reductase at a volume of 2 ml and a temperature of 37°C under both aerobic and anaerobic conditions. Samples of the incubation medium were taken at 20 min intervals and protein was precipitated with half volumes each of acetonitrile and 10% zinc sulfate. The mixture was centrifuged at 10,000 *g* for 5 min, and mitomycin C and metabolites in 50 μl supernatant fluid were assayed by high-performance liquid chromatography (HPLC) [31].

The formation of alkylating metabolites from mitomycin C by quinone reductase or human kidney microsomes was measured by reaction with 4-(*p*-nitrobenzyl)pyridine using a modification of the method of Kennedy et al. [19]. The incubation mixture contained, at a volume of 2 ml, 50 m*M* TRIS-HCl (pH 7.8), 0.7 mg/ml bovine serum albumin, 1 μg/ml quinone reductase or 0.27 mg/ml human kidney microsomal protein, 1 m*M* mitomycin C, and 9 m*M* 4-(*p*-nitrobenzyl)pyridine. The reaction was started by the addition of NADPH to a concentration of 3 m*M*. Incubations were conducted under anaerobic or aerobic conditions for 20 min at 37°C. The reaction was terminated by adding 2 ml acetone and 1 ml 1 *M* NaOH. The mixture was extracted immediately with 4 ml ethyl acetate, centrifuged at 2,000 *g* for 5 min, and the absorbance of the organic layer measured at 540 nm.

Results

Human kidney quinone reductase

Human kidney is a rich source of quinone reductase in comparison to other human tissues [33]. The majority of human kidney quinone reductase activity is in the cell supernatant, with the microsomal fraction containing less than 1% and the mitochondrial fraction containing 7% of the total quinone reductase activity. The partially purified enzyme used was the major quinone reductase in human kidney and represented 75% of the activity that could be eluted from the hydroxylapatate column. Some studies were conducted with a minor form of quinone reductase that eluted from the hydroxylapatate column prior to the major form and was approximately 17% of the total activity.

Mitomycin C as a substrate for quinone reductase

The effect of mitomycin C on the oxidation of NADPH by quinone reductase measured spectrophotometrically is shown in Fig. 1. Mitomycin C itself had a significant absorbance at 340 nm, although this did not interfere with the measurement of NADPH oxidation. There was no evidence for stimulation of NADPH oxidation by mitomycin C even in the presence of a 20-fold excess of quinone reductase necessary to produce measurable NADPH oxidation with dichloroindophenol as a substrate. Mitomycin C also caused no oxidation of NADPH using the minor form of quinone reductase from human kidney cytosol (results not shown). Attempts to study NADPH oxidation by a fluorometric technique were unsuccessful because of the extensive quenching of NADPH fluorescence by both dichloroindophenol and mitomycin C.

Mitomycin C as an inhibitor of quinone reductase

Mitomycin C was an inhibitor of dichloroindophenol reduction by quinone reductase, measured by the change in absorbance at 600 nm (Fig. 2). Inhibition by mitomycin C was competitive with dichloroindophenol with a K_i of 319 μ*M*. Interaction with NADPH showed partially uncompetitive inhibition [11] at low concentrations of NADPH and activation at high concentrations of NADPH. Separate studies showed that mitomycin C did not cause the reoxidation of reduced dichloroindophenol.

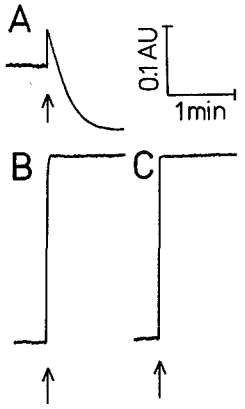

Fig. 1. The effect of dichloroindophenol and mitomycin C on the oxidation of NADPH by quinone reductase. Incubations were conducted at 21°C and contained in 50 m*M* TRIS-HCl (pH 7.8), 0.7 mg/ml bovine serum albumin, 0.3 m*M* NADPH and *A*, 0.11 μg/ml quinone reductase and 25 μ*M* dichloroindophenol; *B*, 0.11 μg/ml quinone reductase and 25 μ*M* mitomycin C; and *C*, 2.2 μg/ml quinone reductase and 25 μ*M* mitomycin C. Recordings are absorbance at 340 nm. Dichloroindophenol and mitomycin C were added at the arrows

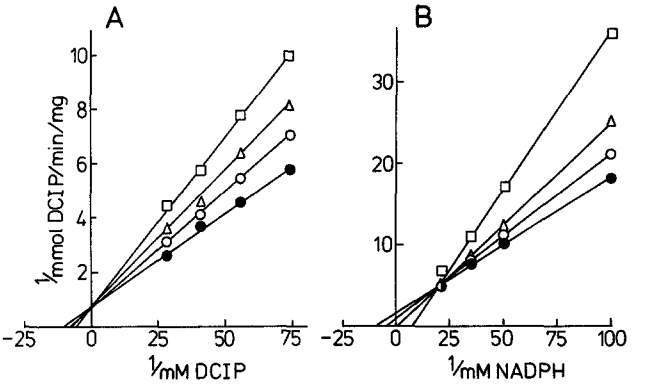

Fig. 2. Lineweaver-Burk plots showing inhibition of quinone reductase by mitomycin C, measured by reduction of dichloroindophenol at 600 nm. Assay conditions are described in the text. (●) No mitomycin C; (○) 50 μ*M* mitomycin C; (△) 100 μ*M* mitomycin C; (□) 200 μ*M* mitomycin C. *A*, varying concentrations of dichloroindophenol (DCIP); *B*, varying concentrations of NADPH

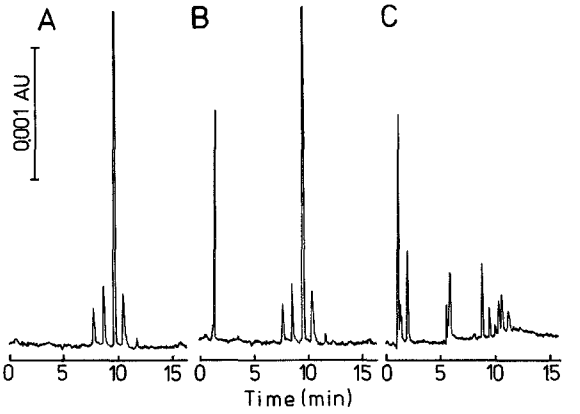

Fig. 3. HPLC chromatograms of mitomycin C and metabolites. Absorbance was measured at 313 nm. Assay conditions are described in the text and were at 37°C under N_2. *A*, Mitomycin C incubated with quinone reductase for 20 min. The major peak is mitomycin C, the minor peaks are its spontaneous degradation products. *B*, Mitomycin C incubated with quinone reductase and NADPH for 20 min. The peak at 1 min is derived from NADPH. *C*, Mitomycin C incubated with rat liver microsomes and NADPH for 20 min

Table 1. Formation of alkylating metabolite(s) from mitomycin C

	Quinone reductase OD 540 nm	Kidney microsomes OD 540 nm
Air, NADPH	0.029	0.037
N_2, NADPH	0.038	0.100
N_2, NADPH, dicumarol	0.034	0.103
N_2, no NADPH	0.031	0.011
N_2, NADPH, no enzyme	0.024	0.014

Alkylating metabolites generated from mitomycin C were measured at 540 nm as the colored product formed by reaction with 4-(*p*-nitrobenzyl)pyridine. Mitomycin C (1 m*M*) was incubated with 1.1 μg/ml quinone reductase or 270 μg/ml human kidney microsomes, 3 m*M* NADPH, with and without 9 μ*M* dicumarol, under N_2 or in air, in a dark environment at 37°C for 20 min. Values are means of three separate determinations

HPLC analysis of mitomycin C metabolites

There was no utilization of mitomycin C or formation of metabolites from mitomycin C incubated with quinone reductase and NADPH under anaerobic conditions, compared with incubations in which NADPH was omitted (Fig. 3). There was also no formation of metabolites from mitomycin C incubated with the minor form of human kidney quinone reductase (results not shown). When mitomycin C was incubated with rat liver microsomes and NADPH under anaerobic conditions as a positive control, several metabolite peaks were formed, as previously reported [31].

Formation of alkylating metabolites

No formation of alkylating metabolites from mitomycin C was measured by conjugation with 4-(*p*-nitrobenzyl)pyridine in the presence of quinone reductase and NADPH (Table 1). Mitomycin C incubated with human kidney microsomes and NADPH under anaerobic conditions formed alkylating metabolites. Formation of the alkylating metabolites by kidney microsomes was partially inhibited in air and unaffected by the addition of dicumarol.

Discussion

Quinone reductase has been reported to catalyze the reduction of a number of simple quinones to hydroquinones [13, 15], and will also catalyze the reduction of quinoneimines [34], azo compounds [14], and a transition metal [10]. Although some hydroquinones can reportedly autooxidize to form reactive oxygen species [7, 29], the formation of hydroquinones by quinone reductase has been thought to provide a cellular protection mechanism against quinone toxicity [2, 4, 8, 24, 25, 27, 38]. The hydroquinones of most simple quinones are conjugated with glucuronide and excreted from the cell [24]. Some halogenated dimethyl-

naphthoquinones, however, are activated by quinone reductase [5, 37]. The most compelling evidence for a role of quinone reductase in modulating cellular quinone toxicity are reports of changes in the toxicity of quinones produced by dicumarol, a potent inhibitor of quinone reductase [5, 27, 35, 38].

The suggested role for quinone reductase in the cellular metabolism of mitomycin C is based upon the findings that dicumarol increases both the formation of alkylating species from mitomycin C by cell sonicates and NADPH [19, 20] and the cytotoxicity of mitomycin C to hypoxic EMT6 cells that contain quinone reductase [21–23]. Under aerobic conditions there is a decrease in the cytotoxicity of mitomycin C to EMT6 cells in the presence of dicumarol. Studies with L1210 cells that lack measurable quinone reductase activity have shown an increase in mitomycin C cytotoxicity by dicumarol under hypoxic conditions but no protective effect under aerobic conditions [23]. The findings have been interpreted to suggest that quinone reductase metabolizes mitomycin C to toxic species in air, but under hypoxic conditions the metabolism of mitomycin C by quinone reductase in cells containing the enzyme is a protective mechanism [20, 23].

In the present study we could not demonstrate that mitomycin C is a substrate for metabolism by a partially purified preparation of human kidney cytosolic quinone reductase. There was no measurable increase in NADPH oxidation by the enzyme in the presence of mitomycin C and no loss of mitomycin C or formation of reduced products of mitomycin C detected by HPLC. Chemical studies have suggested that when mitomycin C is reduced to mitomycin C hydroquinone, the most likely product of metabolism by quinone reductase, it rapidly breaks down to form alkylating species [17, 32]. No formation of alkylating metabolites was detected by conjugation with 4-(*p*-nitrobenzyl)pyridine when mitomycin C was incubated with quinone reductase and NADPH. This is further evidence against the metabolism of mitomycin C by quinone reductase. In the same system, human kidney microsomes, which contain little quinone reductase activity but have NADPH-cytochrome P-450 reductase activity, formed alkylating metabolites from mitomycin C. Although mitomycin C was not a substrate for quinone reductase, it was a competitive inhibitor of the enzyme, using dichloroindophenol as the substrate with a K_i of 319 μM.

The lack of metabolism of mitomycin C by quinone reductase observed in this study may apply to other complex antitumor quinones since doxorubicin has also been reported not to be a substrate for metabolism by rat liver quinone reductase [40].

If mitomycin C is not a substrate for metabolism by quinone reductase, the alteration in mitomycin C's cytotoxicity by dicumarol must involve a mechanism(s) other than inhibition of quinone reductase. Keyes et al. [23] came to a similar conclusion for the action of dicumarol in increasing mitomycin C's toxicity in hypoxic cells lacking quinone reductase activity. Akman et al. [1] have reported the potentiation of the cytotoxicity of the simple quinone menadione to L1210 cells by dicumarol and proposed a mechanism that did not involve quinone reductase.

In summary, we could not demonstrate that mitomycin C is a substrate for metabolism by quinone reductase purified from human kidney cytosol. Mitomycin C is, however, a weak inhibitor of the quinone reductase.

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References

1. Akman SA, Dietrich M, Chlebowski R, Doroshow J, Block JB (1985) Menadione (K3) and dicumarol (D) synergy vs leukemia L1210. Proc Am Assoc Cancer Res 26: 325
2. Ames BN (1983) Dietary carcinogens and anticarcinogens. Oxygen radials and degenerative diseases. Science 221: 1256
3. Bachur NR, Gordon SL, Gee MV (1978) A general mechanism for microsomal activation of quinone anticancer agents to free radicals. Cancer Res 36: 1745
4. Benson AM, Hunkeler MJ, Talalay P (1980) Increase of NAD(P)H:quinone reductase by dietary antioxidants: possible role in protection against carcinogenesis and toxicity. Proc Natl Acad Sci USA 77: 5216
5. Berger MS, Talcott RE, Rosenblum ML, Silva M, AliOsman F, Smith MT (1985) Use of quinones as brain-tumor therapy: Preliminary results of preclinical laboratory investigations. J Toxicol Enviror Health 16: 713
6. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72: 248
7. Cadenas E, Boveris A, Ragan CI, Stoppani AOM (1977) Production of superoxide radicals and hydrogen peroxide by NADH-ubiquinone reductase and ubiquinol-cytochrome C reductase from beef heart mitochondria. Arch Biochem Biophys 180: 248
8. Chesis PL, Levin DE, Smith MT, Ernster L, Ames BN (1984) Mutagenicity of quinones: pathways of metabolic activation and detoxification. Proc Natl Acad Sci USA 81: 1696
9. Crooke ST, Bradner WT (1976) Mitomycin C: a review. Cancer Treat Rev 3: 121
10. De Flora S, Morelli A, Basso C, Romano M, Serra D, De Flora A (1985) Prominent role of DT-diaphorase as a cellular mechanism reducing chromium VI and reverting its mutagenicity. Cancer Res 45: 3188
11. Dixon M, Webb EC (1979) Enzyme inhibition and activation: In: Dixon M (ed) Enzymes. Academic Press, New York, pp 343
12. Dorr RT, Fritz WL (1980) Cancer chemotherapy handbook. Elsevier, New York, pp 548
13. Ernster L (1967) DT-diaphorase. Methods Enzymol 10: 309
14. Huang MT, Miwa GT, Lu AYH (1979) Rat liver cytosolic azoreductase. Purification and characterization. J Biol Chem 254: 3930
15. Iyanagi T, Yamazaki I (1970) One-electron-transfer reductions in biochemical systems: V. Difference in the mechanism of quinone reduction by the NADH dehydrogenase and the NAD(P)H dehydrogenase (DT-diaphorase). Biochim Biophys Acta 216: 282
16. Iyer VN, Szybalski W (1963) A molecular mechanism of mitomycin action: linking of complementary DNA strands. Proc Natl Acad Sci USA 50: 355
17. Iyer VN, Szybalski W (1964) Mitomycins and porfiromycin: Chemical mechanism of activation and crosslinking of DNA. Science 145: 55
18. Kalyanaraman B, Perez-Reyes E, Mason RP (1980) Spin-trapping and direct electron spin resonance investigations of the redox metabolism of quinone anticancer drugs. Biochim Biophys Acta 630: 119
19. Kennedy KA, Rockwell S, Sartorelli AC (1980) Preferential activation of mitomycin C to cytotoxic metabolites by hypoxic tumor cells. Cancer Res 40: 2356
20. Keyes SR, Fracasso PM, Heimbrook DC, Rockwell S, Sligar SG, Sartorelli AC (1984) Role of NADPH cytochrome C reductase and DT-diaphorase in the biotransformation of mitomycin C. Cancer Res 44: 5638

21. Keyes SR, Rockwell S, Sartorelli AC (1985) Enhancement of mitomycin C cytotoxicity to hypoxic cells by dicumarol in vivo and in vitro. Cancer Res 45: 213
22. Keyes SR, Rockwell S, Sartorelli AC (1986) Mechanistic studies on dicumarol enhanced toxicity of mitomycin C and porfiromycin to hypoxic EMT6 tumor cells. Proc Am Assoc Cancer Res 27: 233
23. Keyes SR, Rockwell S, Sartorelli AC (1987) Studies on the modulation of mitomycin C cytotoxicity by dicumarol. Proc Am Assoc Cancer Res 28: 411
24. Lind C, Vadi H, Ernster L (1978) Metabolism of benzo(a)pyrene-3,6-quinone and 3-hydroxybenzo(a)pyrene in liver microsomes from 3-methylcholanthrene-treated rats. Possible role of DT-diaphorase in the formation of glucuronyl conjugates. Arch Biochem Biophys 190: 97
25. Lind C, Hochstein P, Ernster L (1982) DT-Diaphorase as a quinone reductase: a cellular control device against semiquinone and superoxide radical formation. Arch Biochem Biophys 216: 178
26. Moore HW (1977) Bioactivation as a model for drug design bioreductive alkylation. Science 197: 527
27. Morrison H, Jernstrom B, Nordenskjold M, Thor H, Orrenius S (1984) Induction of DNA damage by menadione (2-methyl-14-naphthoquinone) in primary cultures of rat hepatocytes. Biochem Pharmacol 33: 1763
28. Nagata C, Matsuyama A (1970) On the mechanism of action of the carcinostatic antibiotic mitomycin C. Progress in antibiotics and anticancer chemotherapy. Proceedings, 6th International Congress of Chemotherapy, vol II. University Park Press, Baltimore, MD, p 423
29. Nickerson WJ, Falcone G, Strauss G (1963) Studies on quinone thioethers: I. Mechanism of formation and properties of thiodone. Biochemistry 2: 537
30. Ogawa M, Rozenczweig M, Staquet MJ (eds) (1982) Mitomycin C: Current impact on cancer chemotherapy. Excerpta Medica, Amsterdam
31. Pan SS, Andrews PA, Glover CJ, Bachur NR (1984) Reductive activation of mitomycin C and mitomycin C metabolites catalyzed by NADPH-cytochrome P-450 reductase and xanthine oxidase. J Biol Chem 259: 959
32. Peterson DW, Fisher J (1986) Autocatalytic quinone methide formation from mitomycin C. Biochemistry 25: 4077
33. Powis G (1987) NAD(P)H:(Quinone-acceptor)oxidoreductase (QAO) activity in human normal and tumor tissues. Proceedings, 10th International Congress of Pharmacology, Sydney, Australia, August 23–28, 1987, p 35
34. Powis G, Hodnett EM, Santone KS, Lee See K, Melder DC (1987 a) The role of metabolism and redox cycling in the cytotoxicity of antitumor quinoneimines and quinonediimines. Cancer Res 47: 2363
35. Powis G, Lee See K, Santone KS, Melder D, Hodnett EM (1987 b) Quinoneimines as substrates for quinone reductase (NAD(P)H:(quinone-acceptor)oxidoreductase) and the effect of dicumarol on their cytotoxicity. Biochem Pharmacol 36: 2473
36. Sartorelli AC (1986) The role of mitomycin antibiotics in the chemotherapy of solid tumors. Biochem Pharmacol 35: 67
37. Talcott RE, Rosenblum M, Levin VA (1983) Possible role of DT-diaphorase in the bioactivation of antitumor quinones. Biochem Biophys Res Commun 111: 346
38. Thor H, Smith MT, Hartzell P, Bellomo G, Jewell SA, Orrenius S (1982) The metabolism of menadione (2-methyl-1,4-naphthoquinone) by isolated hepatocytes. A study of the implication of oxidative stress in intact cells. J Biol Chem 257: 12419
39. Tomasz M (1976) H_2O_2 generation during the redox cycle of mitomycin C and DNA-bound mitomycin C. Chem Biol Interact 13: 89
40. Wallin R (1986) Adriamycin and DT-diaphorase. Cancer Lett 30: 97
41. Weissbach A, Lisio A (1965) Alkylation of nucleic acids by mitomycin C and porfiromycin. Biochemistry 4: 196

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