Cancer Chemother Pharmacol (1992) 31: 1–5

Cancer Chemotherapy and Pharmacology

Cardiotoxicity of mitomycin A, mitomycin C, and seven N^7 analogs in vitro*

Robert T. Dorr[1], Nancy G. Shipp[1], James D. Liddil[1], Bhashyam S. Iyengar[2], Kenneth R. Kunz[2], and William A. Remers[2]

Colleges of Medicine[1] and Pharmacy[2], Department of Pharmacology and Arizona Cancer Center[1] and Department of Pharmaceutical Sciences[2], University of Arizona, Tucson, Arizona, USA

Received 18 December 1991/Accepted 14 May 1992

Summary. The alkylating antitumor agents mitomycin A (MMA), mitomycin C (MMC), and seven N^7 analogs were compared in terms of their cardiotoxic and antitumor activity in vitro. Neonatal rat-heart myocytes were sensitive to five of the compounds studied, including MMA, 7-dimethylamidinomitosane (BMY-25282), 7-(*N*-methylpiperazinyl)-mitosane (RR-194), N^7-(4-iodophenyl)-MMC (RR-208), and N^7-(4-hydroxyphenyl)-MMC (M-83) in order of descending molar potency. MMA and RR-208 possessed the greatest cytotoxic potency against 8226 human myeloma tumor cells in vitro. Two of the nine mitomycins studied, BMY-25282 and M-83, showed greater cytotoxic potency for heart cells. For these two agents, the ratio of the 50% inhibitory concentration in heart cells to that in 8226 myeloma cells was 50 and 32, respectively. For the other analogs, the tumor-cell cytotoxic potency was much higher (ranging from 200 to 7,000). For the nine mitomycin compounds, a correlation was found between heart-cell toxicity and low reduction potentials ($E_{1/2}$ values) ranging from –0.16 to –0.37 V. Thus, as the reduction potential decreased (easier reducibility), the cardiotoxic potency in vitro increased ($r = 0.81$). In contrast, mitomycins with reduction potentials of higher than –0.37 V were much less potent cardiotoxins. Thus, mitomycin C ($E_{1/2} = -0.45$ V) was noncardiotoxic even when tested at concentrations 100-fold above those pharmacologically achievable in humans. Mitomycin C also failed to enhance doxorubicin (Adriamycin) cardiotoxicity in vitro. Importantly, no correlation was found between the reduction potential and the antitumor activity of the nine analogs ($n = 0.51$), in this small series.

These results suggest that there is a relationship between the facile reduction of the quinone moiety of mitomycin antibiotics and the production of cardiotoxicity.

Introduction

The antitumor alkylating agent mitomycin C (MMC) has been associated with the production of cardiotoxicity in animals [19, 25, 28]. It has been suggested that this occurs by a cyclic one- or two-electron reduction of the quinone moiety to yield reactive oxygen free-radicals [9, 26]. In patients receiving MMC, heart damage manifested by congestive failure has been suggested when MMC is given concurrently [33] or after doxorubicin (DOX) [5, 12]. Since DOX is a well-known cardiotoxin that produces cumulative dose-dependent and irreversible damage to the myocardium [34], the relative contribution of MMC to DOX-induced heart damage remained unclear.

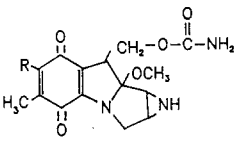
In the present study, we investigated the ability of MMC to produce cardiotoxicity in neonatal rat-heart myocytes exposed to the drug alone or in combination with DOX. In addition, MMA and seven MMC analogs exhibiting a range of quinone reduction potentials were compared in terms of their direct cardiotoxic effects on neonatal rat-heart cells.

Materials and methods

Drugs. MMC (Mutamycin) was obtained commercially as a lyophilized powder from Bristol Laboratories (Evansville, Ind.). Other analogs included MMA and the N^7 alkyl and aryl derivatives of MMC: N^7-(furfuryl)-MMC (RR-75), N^7-(4-hydroxyphenyl)-MMC (M-83), 7-(*N*-methylpiperazinyl)-mitosane (RR-194), N^7-(4-iodophenyl)-MMC (RR-208), N^7-[2-(2-furyl)ethyl]-MMC (RR-298), N^7-(4-hydroxy-3-nitrophenyl)-MMC (RR-394), and 7-dimethylamidinomitosane (BMY-25282). These compounds were selected for testing based on reduction potentials ranging from –0.16 V for BMY-25282 to –0.45 V for MMC. The analogs were synthesized and purified according to published pro-

* Supported in part by grants CA 17094 (to R.T.D.) and CA 21430 (to W.A.R.) from the Department of Health and Human Services, National Cancer Institute (Bethesda, Md.), by grant 8277-000000-1-0-YR9301 from the State of Arizona, Arizona Disease Control Research Commission (Phoenix, Ariz.), and by a Postdoctoral Fellowship Award (to K.R.K.) from the Medical Research Council of Canada

Correspondence to: R. T. Dorr, Arizona Cancer Center, 1515 N. Campbell Avenue, Tucson, Arizona 85724, USA



R	Reduction Potential $E_{1/2}$ Volts	Other Name	Chemical Synthesis (Reference)
H_2N-	-.45	mitomycin C	(Mutamycin®, Bristol Laboratories)
CH_3O-	-.21	mitomycin A	Iyengar, et al. (15)
$(CH_3)_2$-N-CH=N-	-.16	BMY 25282	Vyas, et al. (35)
HO-(C6H3)-NH-	-.37	M-83	Sami, et al. (29)
O-(C4H3O)-CH_2-NH-	-.39	RR-75	Iyengar, et al. (15)
CH_3-N(C4H6N)-N-	-.24	RR-194	Iyengar, et al. (16)
I-(C6H3)-NH-	-.33	RR-208	Sami, et al. (29)
O-(C4H3O)-CH_2-CH_2-NH-	-.42	RR-298	Iyengar, et al. (14)
HO-(C6H3)-NH- (NO_2)	-.33	RR-394	(Personal Communication; Dr. Kenn Kunz)

Fig. 1. Structures of mitomycin analogs

cedures [14–16, 29, 35], and their structures and half-wave reduction potentials ($E_{1/2}$ values) are shown in Fig. 1.

The $E_{1/2}$ values for these mitomycins were determined by differential pulse polarography on a Model 174A polarographic analyzer (E. G. and G. PARC, Princeton, N. J.). The electrolyte was 1.0 M KCl solution and the reference electrode was 0.001 M $CdCl_2$. Mitomycins were tested at 0.001 M concentration in 1.0 M KCl under the following conditions: potential scan, 0.1 V/in.; potential scan rate, 1 mV/s; voltage range, 1.5 V; initial potential, 0.1 V; modulation amplitude, 25; rate of mercury drops, 60/min. It should be noted that absolute reduction-potential values differ according to the alternate reference electrodes or incubation media used.

MMC, MMA, and BMY-25 282 were dissolved in phosphate-buffered saline (pH 7.4) and diluted with Liebovitz's M-3 medium [20] immediately prior to myocyte exposure. Because of their lipophilicity, the N^7 derivatives M-83, RR-208, RR-75, RR-194, RR-298, and RR-394 were initially dissolved in dimethylsulfoxide (DMSO, J. T. Baker, analytical grade). Subsequent serial dilutions were performed in Liebovitz M-3 medium containing 5% fetal bovine serum (Hyclone Corporation, Logan, Utah). Final concentrations of DMSO did not exceed 0.1%; DMSO was tested for cardiotoxicity at concentrations of 0.01%–10% (v/v).

Doxorubicin (Adriamycin, DOX) was purchased commercially as a sterile 10-mg lyophilized powder from Adria Laboratories (Columbus, Ohio). It was initially dissolved in phosphate-buffered saline at a concentration of 1,000 μg/ml and then frozen at –80° C for later use. The DOX aliquots were not subjected to cyclic freezing and thawing to prevent molecular self-association [7]. All subsequent DOX dilutions were performed in Liebovitz M-3 medium containing 5% fetal bovine serum.

Myocyte culture: The culture method used has been described in detail elsewhere [10]. Briefly, the hearts from 1- to 3-day-old rat pups of mixed gender (Harlan Sprague Dawley, Madison, Wis.) were minced with scalpels and exposed to seven serial 15-min digestions with 0.24% trypsin (Difco Laboratories, Detroit, Mich.) dissolved in calcium- and magnesium-free Hanks' balanced salt solution (Irvine Scientific, Santa Ana, Calif.). The digestions were pooled and washed twice in Liebovitz M-3 medium supplemented with HCO_3^- (final concentration, 0.22%) and 5% heat-inactivated fetal bovine serum [14]. Fibroblast contamination was reduced by allowing for the more rapid attachment of fibroblasts onto a 150-cm^2 plastic culture flask for 2 h with the subsequent "pouring off" of a myocyte-enriched supernatant [2]. The myocytes were then plated onto 24-well Primaria plates (Falcon Plastics, Oxnard, Calif.) at a density of approximately 4×10^5 cells/cm^2. The culture medium was Liebovitz M-3 containing 5% heat-inactivated fetal bovine serum (Hyclone Laboratories, Logan, Utah). Myocytes were again supplemented with this medium at 2 days after plating. The cells were maintained at 37° C for up to 7 days after plating.

Myocyte drug exposure. On day 3 after plating, the culture medium was exchanged with M-3 medium containing the drug(s) to be tested. For the mitomycin-analog studies, myocytes were exposed to different concentrations of each drug for 3 h at 37° C. The drug-containing medium was removed, and the cells were washed twice with fresh M-3 medium and then incubated in drug-free media for another 72 h at 37° C. The 3-day postdrug-exposure incubation period has been found to maximize the cell damage caused by known cardiotoxins such as DOX [10] and other anthracyclines [11]. In one study, (final concentration, 3.0 μM, or 1.0 μg/ml) was simultaneously added to three different DOX concentrations for evaluation of the cardiotoxic effect of MMC and DOX in combination.

Myocyte viability assays. Myocyte viability was quantitated by the measurement of intracellular adenosine triphosphate (ATP) levels normalized to total cellular protein (ATP/protein ratio) [10]. In addition, myocytes were observed visually for contraction rates just prior to cell harvesting for ATP and protein recovery. ATP was measured photometrically using the firefly luciferin-luciferase reaction [17]. Light measurements were recorded on a model 1251 Luminometer (LKB-Wallac, Finland) connected to an Apple II minicomputer for data reduction. Luciferin-luciferase reagents were obtained from a commercial source (Turner Instruments and Reagents, Mountain View, Calif.). Protein content was determined by the Bradford method [3] using a commercial Coomassie brilliant blue reagent (BioRad Laboratories, Richmond, Calif.). Each drug exposure was evaluated in sets of four observations, and cell viability was compared with the ATP/protein ratios found for simultaneously tested, untreated control myocytes.

Tumor-cell cytotoxicity assays. The MTT tetrazolium-dye assay was used to compare the relative cytotoxic activity of the analogs against one tumor cell line in vitro [23]. For these studies, the human 8226 myeloma cell line was obtained from American Type Tissue Culture (CCL 155; Rockville, Md.). This cell line grows well in suspension culture and is an IgG-lambda-secreting, β-lymphocyte-derived tumor that was originally isolated from an untreated patient with multiple myeloma [21]. The 8226 myeloma cells were grown in RPMI-1640 culture medium (Grand Island Biologicals, N.Y.) supplemented with 5% (v/v) heat-inactivated fetal bovine serum and 1% (v/v) penicillin (100 units/ml) as well as streptomycin (100 μg/ml). For the cytotoxicity studies, cells in logarithmic growth (doubling time, 27 h) were plated onto 96-well microtiter plates (Costar, Cambridge, Mass.) at a density of 4,000 cells/well. Drugs diluted in RPMI 1640 medium containing 5% fetal bovine serum were added, and the plates were then incubated at 37° C in an atmosphere containing 5% CO_2 and 95% air at 100% relative humidity for 6 days.

At the end of the 6-day exposure period, the medium was aspirated and the cells were washed twice with fresh medium (without drug). Then, 50 μl of an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide, 1 mg/ml) dye solution was added for 4 h [23]. MTT was obtained from Sigma Chemical Co. (St. Louis, Mo.). DMSO (0.1 ml/well) was used to solubilize the colored formazan dye formed by the metabolic activity of various mitochondrial reductases in viable cells [23]. Each drug concentration was tested in six wells, and viability was normalized to the amount of dye formed by untreated control cells. Dye content was measured spectrophotometrically (540-nm absorbance) on an automated microculture plate reader (Biomek 1000, Beckman Instruments, Fullerton, Calif.).

Table 1. Comparison of mitomycin-analog cytotoxicity in heart cells and tumor cells

Mitomycin	IC_{50} values (μm)[a]		
	Rat heart cells	Human 8226 myeloma cells	IC_{50} ratio (heart/tumor)
Mitomycin C	>274[b]	1.37	>200
Mitomycin A	2.1	0.009	233
BMY-25282	8.96	0.18	50
M-83	22.3	0.70	32
RR-75	>21.2[b]	0.032	>663
RR-194	12.8	0.047	272
RR-208	13.1	0.014	936
RR-298	>233[b]	0.035	>6,657
RR-394	169.6[b]	0.073	>2,323

[a] Cardiotoxic effects were not achievable at the maximal exposures listed

[b] IC_{50} values were interpolated from log-linear concentration-response curves comprising ATP/protein ratios ranging from 100% to 5% or 10% of control values

Cytotoxic specificity. The cytotoxic potency ratio was obtained by dividing the 50% inhibitory drug concentrations (IC_{50}) in the myocytes by that in the 8226 myeloma cells.

Results

Five of the nine mitomycin compounds reduced the ATP/protein ratios in the neonatal rat-heart cells in a concentration-dependent fashion (data not shown). Cardiotoxic effects were not produced by four mitosanes tested at high concentrations (Table 1). Interestingly, MMC was one of the noncardiotoxic compounds, even at concentrations of up to 300 μM (or about 100 μg/ml). Exposure to this concentration also failed to depress the synchronous rate of contraction in the myocyte cultures. In contrast, MMA was the most potent cardiotoxin of the series, followed by the N^7 derivatives BMY-25282, RR-194, RR-208, RR-75, and M-83 in order of descending potency (Table 1). The final maximal DMSO concentration used in this trial (1% v/v) reduced myocyte viability to 95% of control values. However, continuous 3-day exposure to a higher concentration of DMSO (10%, v/v) reduced myocyte viability to 55% of control levels.

For each of the cardiotoxic compounds, myocyte contraction rates were reduced from a mean value of 59 ± 15 synchronous beats/min in control cells to sporadic contractions or to a noncontractile state, depending on the drug concentration used. Although there was a rough correlation with drug concentrations, the nonsynchronous and isolated nature of the contractions made accurate quantitative comparisons of contraction rates impossible at myocyte ATP/protein ratios of $\geq 50\%$ of control values. The same trend has been reported for the anthracycline-based antitumor agents, whereby the ATP/protein ratios provided a more dynamic index of toxic injury [10].

Against the 8226 myeloma cells, MMA and RR-208 were the most potent agents (Table 1). The N^7 MMC derivatives RR-75, RR-298, RR-194, and RR-394 were roughly equicytotoxic against the tumor cells, with the IC_{50} values ranging from 0.032 to 0.073 μM, respectively, in descending order of potency. MMC displayed high cytotoxic potency in this series, whereas M-83 and BMY-25282 showed intermediate antitumor potency.

The third column in Table 1 compares the relative cytotoxic potency for tumor cells and heart cells. The results show that two of the analogs, BMY-25282 and M-83, had relatively low cytotoxic potency in the tumor cells as compared with the heart cells. In contrast, the other mitomycins, including MMA and MMC, exhibited potency ratios of over 200, indicating greater cytotoxic potency for the 8226 myeloma cells as compared with the heart cells. For some of the drugs, discrete ratios could not be obtained because IC_{50} concentrations were unachievable in the heart cells; this was due to the limited aqueous solubility of RR-75 and RR-298 in low, nontoxic DMSO concentrations.

Least-squares linear regression analysis revealed a positive correlation between a low reduction potential and cardiotoxic potency for mitomycins in the rat heart cells ($r = 0.81$). A similar relationship for the reduction potential was not evident for antitumor cytotoxic potency ($r = 0.51$). For interpretation of these correlations, it should be borne in mind that a compound with a high reduction potential such as MMC ($E_{1/2} = -0.45$ V) is more difficult to reduce than a compound with a low reduction potential such as MMA ($E_{1/2} = -0.21$ V).

MMC was also evaluated for potential additive cardiotoxicity with the anthracycline antitumor agent DOX. The effect of MMC in combination with DOX is shown in Table 2. As anticipated, DOX produced concentration-dependent suppression of ATP in the myocytes. The results also show that there was no further loss in myocyte viability following the addition of a fixed concentration of 3.0 μM MMC to cytotoxic concentrations of DOX. For reference, the MMC concentration used in combination with DOX roughly approximated the peak plasma levels obtained in patients following the administration of a single maximally tolerated MMC dose of 15–20 mg/m^2 [31]. The IC_{50} value for DOX in these assays was determined to be approximately 1.3 μM (interpolated from Table 2), which is similar to the results previously reported for this assay system [10].

Table 2. Effect of mitomycin C on DOX cardiotoxicity in vitro

Agent(s)	Viability (μg ATP/mg protein) at		
	0 DOX	0.9 μM DOX	1.8 μM DOX
Doxorubicin (% control)	7.39 ± 0.78 (100%)	5.18 ± 0.28 (70%)	2.65 ± 0.24 (36%)
Doxorubicin + 3.0 μM mitomycin C (% control)	7.12 ± 0.50 (96%)	4.70 ± 0.17 (64%)	2.87 ± 0.20 (39%)

Data represent mean values ± SD for 4 determinations at each drug concentration

Discussion

MMC is an alkylating agent that is active in a variety of solid tumors, particularly adenocarcinomas of the gastrointestinal tract [8, 24]. In addition to myelosuppression, the drug has also been associated with rare toxic effects in the lungs [6] and kidneys [13]. Cardiac toxicity has been implicated only when the drug has been given in combination with [33] or after doxorubicin therapy [5, 7, 32]. On one of these trials, 14/91 (15%) heavily pretreated breast cancer patients developed congestive heart failure (CHF) following MMC treatment [5]. A much lower CHF incidence of only 3.4% was described in patients treated with non-mitomycin-containing chemotherapy [5]. Villani et al. [33] have observed cardiopulmonary insufficiency associated with elevated systolic intervals in 5/36 (14%) breast cancer patients receiving DOX and MMC simultaneously. However, prospective studies have not confirmed a cardiotoxic interaction between DOX and MMC [27].

In dogs, lethal doses of MMC have produced hemorrhages of the pericardium and endocardium [25], whereas in Wistar rats, a mixed myocarditis with cardiac muscle lysis was noted [19]. In contrast, Monti et al. [22] could not demonstrate any evidence of cardiotoxicity for MMC in guinea pigs in vivo or in isolated atrial muscle preparations; in addition, MMC did not enhance the toxicity of DOX in the same guinea-pig test systems. This observation is consistent with the current findings.

The present results show that MMC is noncardiotoxic in vitro at concentrations 100 times greater than those achieved following clinical doses of 20 mg/m^2 [31]. Mitomycin C also failed to enhance DOX cardiotoxicity in the in vitro test system. In contrast, MMA and four of the N^7 analogs of MMC produced concentration-dependent cardiotoxic effects in vitro. The results obtained using MMA are intriguing in that this agent had the lowest IC_{50} values in both the rapidly dividing tumor cells and the nondividing heart cells. Nonetheless, MMA yielded a high myocyte/tumor cell ratio of >200 : 1. This suggests that MMA is a potent and relatively specific antitumor agent in vitro.

Other studies have documented that mitomycins with low reduction potentials (easy reducibility) produce less myelotoxicity than do agents with higher $E_{1/2}$ values such as MMC [9]. More recently, Kunz et al. [18] have reported that among several mitomycins, bioreductive activation ($E_{1/2}$ values) constitutes a highly significant independent variable for the prediction of cytotoxic potency in human tumor cells. The correlation coefficient between cytotoxic potency and low reduction potential was $r = 0.73$ for 18 mitomycin C derivatives with $E_{1/2}$ values ranging from –0.26 to –0.45 V [18]. Similar results have been reported by Pan and Gonzalez [24]. It should be borne in mind that the half-wave potentials reported in the current study cannot be directly compared with potentials measured against alternate reference electrodes [24] or values listed as $E_{1/7}$ instead of $E_{1/2}$ [30].

Since only one tumor cell line was investigated in the current study, any broad statement about the antitumor efficacy of the compounds studied would obviously be limited. Moreover, the in vitro methodology used, largely bypasses the toxicologic effects of detoxification, liver biotransformation, and hepatic injury. In addition, a single cardiotoxicity parameter, ATP content, was relied upon; this might have obviated the detection of cardiotoxins that did not ultimately interrupt the electron-transport chain. Nonetheless, several N^7 mitomycin derivatives were potent cardiotoxins in this system, including M-83 and BMY-25282. It is noteworthy that BMY-25282 has produced cardiotoxicity in animals that precluded testing in the clinic [4]. In bovine cardiac mitochondria, BMY-25282 strongly stimulates oxygen free-radical formation, whereas MMC does not [4]. These findings are consistent with the current results and suggest that as reported for DOX [27], oxygen free-radical formation can result from redox cycling of easily reduced mitomycins [26], and this may be responsible for the production of cardiotoxicity. The present findings suggest that it may be possible to preclinically select mitomycins with high tumor specificity and low toxicity. Further experiments based on this model are warranted, especially those testing MMA and some of the other relatively noncardiotoxic N^7 MMC analogs studied in the current series.

Acknowledgement. The technical assistance of Dr. S. Sami was invaluable in the performance of this study.

References

1. Bligh HF, Bartoszek A, Robson CN, Hickson ID, Kasper CB, Beggs JD, Wolf CR (1990) Activation of mitomycin C by NADPH: cytochrome P-450 reductase. Cancer Res 50: 7789
2. Blondel B, Roijen I, Cheneval JP (1970) Heart cells in culture: a simple method for increasing the proportion of myoblasts. Experientia 27: 3
3. 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
4. Bradner WT, Bregman CL, Buroker RA, Pritsos CA, Sartorelli AC (1988) Cardiotoxicity of mitomycin derivatives. Proc Am Assoc Cancer Res 29: 267
5. Buzdar AU, Legha SS, Tashima CK, Hortobagyi GN, Yap HY, Krutchik AM, Luna MA, Blumenschein GR (1978) Adriamycin and mitomycin C: possible synergistic cardiotoxicity. Cancer Treat Rep 62: 1005
6. Buzdar AU, Legha SS, Luna MA, Tashima CK (1980) Pulmonary toxicity of mitomycin. Cancer 45: 236
7. Dalmark M, Johansen P (1982) Molecular association between doxorubicin (Adriamycin) and DNA-derived bases, nucleosides, nucleotides, other aromatic compounds, and proteins in aqueous solution. Mol Pharmacol 22: 158
8. Doll DC, Weiss RB, Issell BF (1985) Mitomycin: ten years after approval for marketing. J Clin Oncol 3: 276
9. Doroshow JH (1981) Mitomycin C-enhanced superoxide and hydrogen peroxide formation in rat heart. J Pharmacol Exp Ther 218: 206
10. Dorr RT, Bozak KA, Shipp NG, Hendrix M, Alberts DS, Ahmann FR (1988) In vitro rat myocyte cardiotoxicity model for antitumor antibiotics using adenosine triphosphate/protein ratios. Cancer Res 48: 5222
11. Dorr RT, Shipp NG, Lee KM (1991) Comparison of cytotoxicity in heart cells and tumor cells exposed to DNA intercalating agents in vitro. Anti-Cancer Drugs 2: 27
12. Ganz PA, Lurie K (1983) Angina pectoris after doxorubicin and mitomycin therapy. Cancer Treat Rep 67: 98
13. Hanna WT, Krauss S, Regester RF, Murphy WM (1981) Renal disease after mitomycin C therapy. Cancer 48: 2583

14. Iyengar BS, Lin H-J, Cheng L, Remers WA (1981) Development of new mitomycin C and porfiromycin analogues. J Med Chem 24: 975
15. Iyengar BS, Sami SM, Tarnow SE, Remers WA (1983) Mitomycin C analogs with secondary amines at position 7. J Med Chem 26: 1453
16. Iyengar B, Sami S, Takahashi T, Sikorski E, Remers WA, Bradner WT (1986) Mitomycin C analogues with increased metal complexing ability. J Med Chem 29: 1760
17. Kimmich GA, Randles J, Brand JS (1975) Assay of picomole amounts of ATP, ADP and AMP using the luciferase enzyme system. Anal Biochem 69: 187
18. Kunz KR, Iyengar BS, Dorr RT, Alberts DS, Remers WA (1991) Structure-activity relationships for mitomycin C and mitomycin A analogues. J Med Chem 34: 2281
19. Levillain R, Cluzan R (1973) Cardiac toxicity of antimitotic drugs. Proc Eur Assoc Cancer Res 3: 100
20. Liebovitz A (1986) Development of tumor cell lines. Cancer Genet Cytogenet 19: 11
21. Matsuoka Y, Moore GE, Yagi Y, Pressman D (1967) Production of free light chains of immunoglobulin by a hematopoietic cell line derived from a patient with multiple myeloma. Proc Soc Exp Biol Med 125: 1246
22. Monti E, Bossa R, Galatulas I, Favalli L, Villani F, Piccinini F (1983) Interaction between doxorubicin and mitomycin C on mortality and myocardial contractility in guinea pig. Tumori 69: 113
23. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55
24. Pan SS, Gonzalez H (1990) Mitomycin antibiotic reductive potential and related pharmacological activities. Mol Pharmacol 37: 966
25. Phillips FS, Schwartz HS, Sternberg SS (1960) Pharmacology of mitomycin C: I. Toxicology and pathological effects. Cancer Res 20: 1354
26. Pritsos CA, Sartorelli AC (1986) Generation of reactive oxygen radicals through bioactivation of mitomycin antibiotics. Cancer Res 46: 3528
27. Rajagopalan S, Politi PM, Sinha BK, Myers CE (1988) Adriamycin-induced free radical formation in the perfused rat heart: implications for cardiotoxicity. Cancer Res 48: 4766
28. Ravry MJR (1979) Cardiotoxicity of mitomycin C in man and animals. Cancer Treat Rep 63: 555
29. Sami SM, Iyengar BS, Tarnow SE, Remers WA, Bradner WT, Schurig JE (1984) Mitomycin C analogues with aryl substituents on the 7-amino group. J Med Chem 27: 701
30. Svingen BA, Powis G (1981) Pulse radiolysis studies of antitumor quinones: radical lifetimes, reactivity with oxygen, and one-electron reduction potentials. Arch Biochem Biophys 209: 119
31. Van Hazel GA, Scott M, Rubin J, Moertel CG, Eagen RT, O'Connell MT, Kovach JS (1983) Pharmacokinetics of mitomycin C in patients receiving the drug alone or in combination. Cancer Treat Rep 67: 805
32. Verweij J, Funke-Kupper AJ, Teule GJJ, Pinedo HM (1988) A prospective study on the dose dependency of cardiotoxicity induced by mitomycin C. Med Oncol Tumor Pharmacother 5: 159
33. Villani F, Comazzi R, Lacaita G, Guindani A, Genitoni V, Volonterio A, Brambilla MC (1985) Possible enhancement of the cardiotoxicity of doxorubicin when combined with mitomycin C. Med Oncol Tumor Pharmacother 2: 93
34. Von Hoff DD, Layard W, Basa P, Davis HL Jr, Von Hoff AL, Rozencweig M, Muggia FM (1979) Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 91: 710
35. Vyas DM, Chang Y, Benigni D, Doyle TW (1987) A practical synthesis of mitomycin A and its analogues. J Org Chem 52: 5601