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Review paper

Mitomycin C: mechanism of action, usefulness and limitations

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The mitomycins are antitumor antibiotics that are under investigation now for more than 30 years. Mitomycin C (MMC) is the best investigated subtype. It serves as a prototype for drugs with bioreductive alkylation, which is a unique feature of this class. MMC is mainly active under anaerobic circumstances. The pharmacokinetics are linear in a two-compartment model. The main toxicities of MMC are thrombocytopenia and leucocytopenia. Rare but severe side effects are a hemolytic uremic syndrome, pneumonitis and cardiac failure. MMC has a wide clinical antitumor spectrum with efficacy in various tumor types such as gastric cancer, pancreatic cancer, breast cancer, non-small cell lung cancer, cervical cancer, prostate cancer and bladder cancer. Still, the above mentioned side effects prevent a more widespread use. The most important features of the drug will be reviewed.

Key words: Mitomycin C, bioreductive alkylation, clinical pharmacology, hemolytic uremic syndrome.

Introduction

The mitomycins constitute a class of antitumor antibiotics isolated from *Streptomyces caespitosus*. Mitomycin C (MMC), isolated in 1958,[1] has received the greatest attention of all mitomycins, both in preclinical and clinical investigations. The drug has a wide spectrum of antitumor activity, but clinically also exerts infrequent but sometimes quite severe side effects that prevent a more widespread use.

Chemistry and mechanism of action

MMC was isolated from fermentation filtrates of *Streptomyces caespitosus* as blue violet crystals. The crystal structure and the absolute stereochemical configuration of MMC have been determined.[2] MMC has a molecular weight of 334 daltons and is soluble in water and organic solvents. Its structure is shown in Figure 1.

The structure, in which quinone, aziridine and carbamate functions are arranged around a pyrrolo[1,2-*a*] indole nucleus, is quite unique. Under acidic conditions protonation of the C_{9a}-methoxy group triggers cleavage[3] resulting in a C_9–C_{9a} double bond and opening of the aziridine ring[4] (Figure 2), while additional replacement of the C_7-amino function by a hydroxyl group and cleavage of the C_{10}-carbamate function occur at more pronounced acid conditions.[5] Under alkaline conditions the latter two changes are seen[6,7] without concomitant effects on the C_{9a}-methoxy group or the aziridine ring.

In principle, MMC inhibits DNA synthesis. Oxidized MMC is inactive: the drug requires chemical or enzymatic reductive activation to either the corresponding semiquinone or the hydroquinone form, to bind DNA by mono- or bifunctional

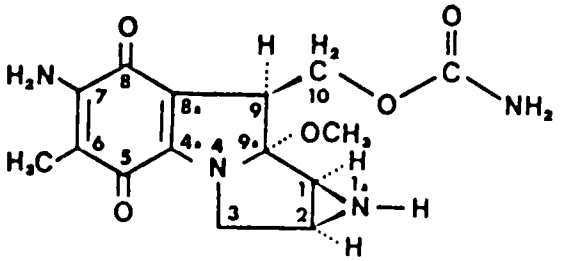

Figure 1. Structure of mitomycin C.

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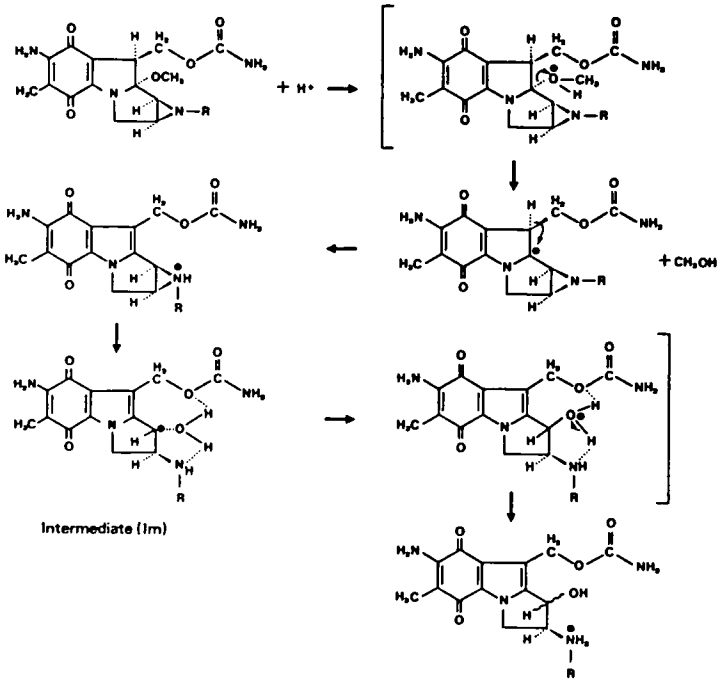

Figure 2. Mechanism of acid-catalyzed hydrolysis of mitomycin C (R = H).

alkylation.[8] Bifunctional alkylation is thought to lead to crosslinking of strands of double helical DNA.[9] A second DNA alkylation promoting mechanism, which appears to be less important, is acidic activation.[10,11]

The reduction of MMC which is necessary for alkylating reactions has led to the introduction of the term 'bioreductive alkylation' to describe the mechanism of action.[12] MMC is considered to be the prototype of the bioreductive alkylating agents.

Under anaerobic conditions a one- or two-electron reduction with subsequent spontaneous loss of methanol leads to the formation of an unstable reactive intermediate. The formation of a quinonemethide[13] then results from rearrangement of the hydroquinone followed by a nucleophilic addition of DNA leading to a mono-alkylated product.[14] Intramolecular displacement of the carbamate group would then result in the cross-linked adduct (Figure 3). Gradual addition of a reducing agent[15] or excess addition[16] results in a higher binding frequency. These conditions are favorable for the maintenance of the semiquinone radical, the intermediate which is formed by the first electron uptake of MMC. The semiquinone is therefore believed to initially bind to DNA. The existence of this radical during the process of MMC reduction has been proven and evidence is increasing that one-electron reduction is sufficient to activate both the C_1 and C_{10} electrophilic centers.

The fate of MMC after reductive metabolization under aerobic conditions is different. Either the

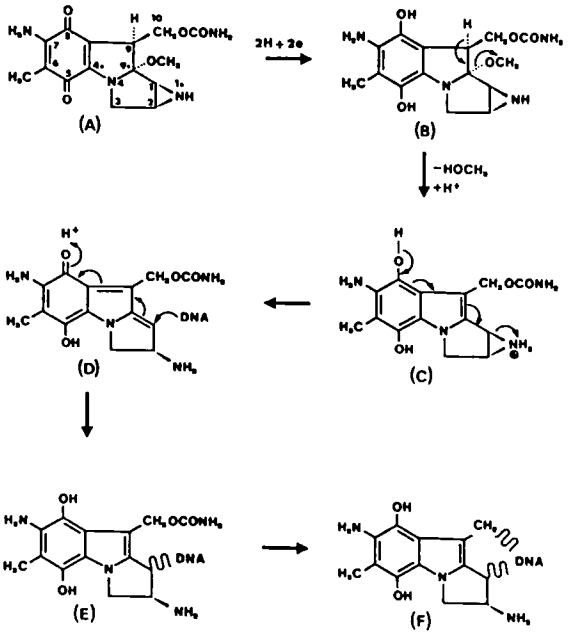

Figure 3. Reductive activation and DNA alkylation of mitomycin C.

semiquinone radical[17–21] or the hydroquinone reacts with molecular oxygen to generate superoxide radical anions,[17–21] hydroxyl radicals[22,23] or hydrogen peroxide.[24] Cytotoxicity of these highly reactive forms may be exerted through lipid peroxidation or nucleic acid damage, which can in turn be prevented by free radical scavengers such as mannitol as well as by protective enzymes such as superoxide dismutase or catalase. The type of reactive MMC intermediate is a result of the half-life of the semiquinone radical: in an aprotic environment this is the semiquinone itself due to a long half-life; in protic media the semiquinone exists only a few milliseconds before rapid uptake of a second electron results in the formation of the hydroquinone. Besides, O_2 will play a role because it inactivates the semiquinone and this inhibits a further reduction.

The reductive activation of MMC can be initiated or modulated by enzyme systems such as DT-diaphorase,[25,26] NADPH-cytochrome P-450 reductase,[27] NADPH-cytochrome C reductase,[25] xanthine-oxidase[28] and some flavoprotein transhydrogenases,[29] and also by chemical reducing agents such as sodium thiosulfate[15,30] and sodium borohydride.[30]

Our knowledge on the process of alkylation of DNA nucleotides by MMC has been extended by mimicking the *in vivo* process by *in vitro* work with model nucleophiles in aqueous solution under reducing condition.[31,32] Potassium ethyl xanthate[30] and potassium ethyl monothiocarbonate[32] appeared to be ideal nucleophiles. Both mono- and disubstituted mitosenes with nucleophiles at C_1 and/or C_{10} of MMC were identified.

By decreasing the temperature during the alkylation reaction it is possible to discriminate between these two reaction sites.[32] Such mild conditions lead to preferential substitution at C_1, while under more reductive conditions displacement of the carbamate group at C_{10} in the monofunctionally alkylated molecule is readily observed,[32] which implies that after monofunctional alkylation of MMC at C_1, secondary activation of monofunctionally bound MMC at C_{10} is probably the main route for the bifunctional binding of MMC to nucleophiles, and for cross-linking of DNA.[20,26]

Studies on covalent interactions between MMC and DNA or DNA fragments characterized adducts as mono- or bifunctional mitosene derivatives, similar to the observations with model nucleophiles. The binding sites of MMC in DNA are the N_6 position of adenine residues or either the N_2 or N_7 position of guanine residues.[9,11,33,34] The formation of O^6-guanine-MMC adducts may also be important.[34,35] Acid-activated MMC was found to alkylate preferentially the guanine N_7 position, in contrast to reductively activated MMC, which preferentially alkylates the guanine N_2 position,[11] possibly by the different electronic structures of acid- and reduction-activated MMC. Evaluation of the activation mechanism of MMC can presumably now be deduced from analysis of the DNA adducts formed *in vivo*.

Activated MMC is most effective in the late G_1 and S phases of the cell cycle.[36] MMC does not reduce the size of nucleotide pools[37] and has no effect on DNA polymerase from HeLa cells.[36] DNase activity has not been observed.[38] Consequently, besides the specific inhibition of DNA synthesis secondary to alkylation, inhibition of both RNA synthesis inhibition and protein synthesis inhibition seems to be non-specific manifestations of cell toxicity.[39,40] There are several indications that DNA repair is not inhibited by MMC.[41–43] MMC has been shown to produce chromosomal abnormalities, such as chromatid exchanges in leukocytes, but whether these are related to mitotic inhibition, or inhibition of DNA synthesis, is unclear.[36] MMC is teratogenic[36] as it increases the incidence of tail and paw abnormalities, as well as the incidence of spontaneous abortions, in rats and mice. MMC is also carcinogenic.[44,45]

Although a definitive proof is still lacking, there is strong evidence that MMC is preferentially activated to a cytotoxic intermediate in hypoxic environments.[46–50] *In vitro* a preferential activation of MMC to cytotoxic metabolites in hypoxic tumor cells has been demonstrated in the EMT-6 and S-180 murine cell lines.[23] On the other hand, such selective toxicity could not be observed *in vivo*.[46,47] However, recently it was shown that dicoumarol, an inhibitor of DT-diaphorase, increases the cytotoxicity of MMC to hypoxic EMT-6 cells *in vitro* and *in vivo*,[51] possibly by inhibition of enzymes involved in the activation and inactivation of MMC. The increase in cytotoxicity to hypoxic tumor cells did not coincide with an increase of toxicity to well-oxygenated tissues such as the bone marrow.[51] In view of this preferential toxicity for hypoxic cells, a combined regimen of MMC and radiation is considered interesting for the treatment of solid tumors, because radiation will be more effective to aerobic tumor cells.

The mechanism(s) of resistance to MMC is not completely understood but probably involves changes in drug accumulation, bioactivation of the alkylating species, and DNA excision repair.[52] In Chinese hamster ovary cell mutants increasing

drug-resistance was related to a progressive loss of MMC activation capacity and increasing capacity for excision repair of DNA. The specific bioactivation enzyme system deficient in these resistant cells has not yet been identified. Also MMC appears to share in the multidrug resistance phenotype that encompasses doxorubicin, vincristine and other natural products, and appears to be mediated by amplification of the P-170 transmembrane drug efflux glycoprotein.

Clinical pharmacology

Extensive pharmacokinetic data on MMC have become available since the introduction of different modifications of a high performance liquid chromatography (HPLC) assay.[53–58] All these assays have a detection limit of approximately 1 ng/ml sample. There is a biexponential decline of the plasma concentration time curves, indicating a two-compartment model with linear pharmacokinetics up to doses as high as 60 mg/m^2. After a rapid half-life of distribution (2–10 min), the elimination half-life is 25–90 min (mean 54 min). No correlations have been found between pharmacokinetic data of MMC and a wide variety of clinical parameters.[53–55] Most importantly, impaired liver and renal function do not appear to change the pharmacokinetic behavior of MMC and therefore do not require dose reductions. Two studies reported an unexplained increased total body clearance and decreased area under the plasma concentration time curve of MMC following combination chemotherapy also including 5-fluorouracil and doxorubicin.[54,59]

Because urinary recovery after i.v. administration ranged from 1–20%, which cannot explain the rapid plasma clearance, it has been suggested that MMC is rapidly cleared from plasma by biodegradation. The liver is thought to be the major organ of biotransformation but the spleen, kidney, brain and heart may also be involved in the process.[60,61] The presence of oxygen markedly reduced the rate of metabolism of MMC in liver homogenates, as compared to the metabolism in a similar anaerobic system.[60] As biotransformation is required for activity, this supports the theory of a more pronounced activity under anaerobic conditions. After intra-arterial hepatic infusions, only a three-fold greater regional exposure was found,[56] which in view of the hepatic extraction of MMC, which is only about 20%, suggests a very limited benefit of this technique with respect to reduced systemic toxicity. A variety of microspheres have been used in an attempt to increase local exposure to MMC and although they resulted in a reduced systemic exposure with a decrease in systemic peak levels and AUC[62–65] the increase in tumor exposure was insufficient to enhance tumor regression.[62]

Both the intraperitoneal[66–68] and intravesical[69] route of administration result in a significant local exposure advantage and very low plasma levels. While intravesical administration has been successful in treating bladder cancer, the precise role of intraperitoneal administration has yet to be delineated.

Although MMC is absorbed after oral administration, absorption has been shown to be rather erratic by this route.[70–72] MMC is usually administered as a 10–15 mg/m^2 bolus i.v. injection once every 6 weeks, because more frequent administration will result in severe bone marrow toxicity.

Toxicity

The most frequent side effect of MMC is a delayed myelosuppression, which appears to be directly related to schedule and total dose.[36] Thrombocytopenia is more frequent than leucocytopenia and anemia. Other toxicities include usually mild and infrequent anorexia, nausea, vomiting and diarrhea. Alopecia, stomatitis and rashes also occur infrequently. Extravasation results in tissue necrosis with very disabling ulcers that may require plastic surgery.[73] Extremely high doses of MMC (60 mg per dose) may result in lethal veno-occlusive liver disease.[74] Other infrequent, but potentially lethal, side effects include hemolytic uremic syndrome, interstitial pneumonitis and cardiac failure.

The pathogenesis of the MMC-induced hemolytic uremic syndrome[75] still remains unclear, although prostacycline deficiency may play a role.[76] The incidence appears to be less than 10%[75,77] and was suggested to be dose-dependent.[77] There is no consistently effective treatment for this syndrome.

Pulmonary toxicity of MMC consists of an interstitial pneumonitis.[75] Discontinuation of MMC administration may occasionally lead to recovery from this side effect, but usually there will be a progressive respiratory failure. Corticosteroid treatment may be helpful in preventing progression of pulmonary dysfunction. The incidence of pulmonary toxicity is approximately 7% of the treated population; cardiac failure secondary to MMC occurs in a similar percentage of treated patients, and rises with cumulative doses above 30 mg/m^2.[78,79]

Clinical antitumor activity

MMC has been studied extensively in advanced neoplasms in humans. The initial studies reported on the use of daily low dose schedules, which resulted in unacceptably severe and cumulative myelosuppression. For this reason, an intermittent dosing schedule was introduced, using bolus injections every 4–8 weeks, resulting in manageable hematologic toxicity. The following review will only refer to studies using the intermittent schedule (Table 1).

Single agent activity in gastric cancer was reported to be 29%.[80–82] Combination chemotherapy incorporating MMC achieves slightly higher response rates and also improves survival in the responding patients.[83–85]

Single agent treatment with MMC in pancreatic cancer achieves a response rate of 27%.[86] This indicates that MMC is one of the most active drugs against this neoplasm presently available. Unfortunately, combination chemotherapy does not appear to improve these results.[84,86–92]

Single agent activity in 293 breast cancer patients treated with MMC was 20%,[93–96] with a wide variation in response rates in different studies (0–31%), as a result of the fact that MMC was used either as first-, second- or third-line treatment. Recent studies indicate that second- and third-line treatment with MMC are far from rewarding.[96] In this tumor type, combination chemotherapy including MMC was found to be superior to single agent treatment with MMC, but response rates in larger series remain lower compared to more active combinations such as cyclophosphamide/methotrexate/fluorouracil (CMF) or fluorouracil/doxorubicin/cyclophosphamide (FAC).[97–102]

Treatment with MMC as a single agent in 148 patients has resulted in an overall response rate of 28% in non-small-cell lung cancer (NSCLC).[103–106] Data on survival are frequently not indicated. Data on combination chemotherapy with MMC included are quite inconsistent, with response rates varying from 20-59%.[107–112] However, as for other drug combinations, responses are usually short and survival benefit is not obtained.

In squamous cell cancer of the uterine cervix, single agent MMC treatment achieved an overall response rate of 36% in 173 patients.[113,114] Combination chemotherapy including MMC yields even higher responses. However the most important drug in this disease appears to be cisplatin and the decision as to whether to add other drugs such as MMC to cisplatin is presently under investigation in an ongoing European Organization for Research and Treatment of Cancer (EORTC) study. Survival for complete responders to combination chemotherapy is 12–30 months and for partial responders 6–15 months, both compared to a median survival of 3–6 months for progressive disease, which at least suggests a benefit.

In superficial bladder cancer intravesical instillations of 30–40 mg MMC in 20–40 ml sterile water and retained in the bladder over 2–3 hours have achieved an overall response of 67% in 276 patients[115–118], approximately two-thirds of them achieving a complete remission of long duration up to 25 months or more. Epodyl, thiotepa, and doxorubicin appear to be equally active in destroying superficial bladder tumors, but MMC probably is the least toxic. Data on i.v. treatment in more advanced disease are anecdotal.

MMC is one of the few agents with very modest activity against colorectal cancer. In a compilation of 272 patients, 44 responders were found (16%) with single agent treatment.[119] Inconsistent data on combination chemotherapy with MMC show only minor improvement of results compared to single agent treatment. All treatments including MMC do not create any survival benefit for responding patients. For this reason, it is not recommended to apply MMC in colorectal cancer.

In prostatic cancer, two studies applying MMC/DX/5-FU have achieved response rates of 44% and 60% in a relatively small number of patients, but those studies did not apply measurable disease as eligibility criterion, and used hardly evaluable criteria such as serum acid phosphatase levels, and sclerotic healing of lytic bone lesions, for follow-up.[120,121] The EORTC has studied MMC single agent treatment in 30 patients with *measurable* disease, achieving a 36% response rate.[122].

In view of this wide spectrum of antitumor activity, a widespread use may be expected. However, MMC is not a curative drug and its earlier

Table 1. Single agent activity of mitomycin C

Tumor type	No. of evaluable patients	Response rate (%)
Gastric cancer	343	29
Pancreatic cancer	44	27
Breast cancer	293	20
Non-small-cell lung cancer	148	28
Cervical cancer	173	36
Colorectal cancer	272	16
Prostatic cancer	30	36

mentioned rare but severe side effects prevent more frequent use. For similar reasons, use in adjuvant chemotherapy regimens cannot be advocated. Obviously there is a need for analog development, aimed at retaining the antitumor effect and diminishing the side effects. In view of its unique activity, MMC-analog development warrants thorough investigation.

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