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THE ROLE OF MITOMYCIN ANTIBIOTICS IN THE CHEMOTHERAPY OF SOLID TUMORS

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Solid tumors have inadequate vascular networks; thus, significantly less blood flows through these tumor vascular beds than through those of the tissue of origin, and as neoplastic masses enlarge, the blood supply to these tumors further decreases [1–3]. This phenomenon is believed to be due in part to a slower growth rate for endothelial cells of blood vessels than for malignant cells; such a differential results in an inability to maintain an adequate vascular supply [4]. In addition, as tumor masses enlarge, changes in interstitial pressure are possible which can cause compression of tumor capillary beds and regional changes in tumor blood flow. The end result of these oxygen deficit producing effects is the formation of chronic and acute hypoxia in the neoplastic cell population (Fig. 1), which may have significant impact on the curability of malignant lesions.

There is evidence that hypoxic cell populations exist in both animal [5] and human (see ref. 6 for appropriate references) tumors. Characterization of hypoxic cell populations in transplanted animal tumors has shown that most tumors contain between 5 and 30% of the malignant cell population in an oxygen-deficient state [5]. Although tumor enlargement increases the quantity of hypoxic cells, it is clear that micrometastases also contain oxygen-deficient cell populations.

Oxygen-deficient malignant cells are significantly more resistant to ionizing radiation than their aerobic counterparts (see for example refs 7 and 8) and, in addition, hypoxia may limit the efficacy of chemotherapy. This latter phenomenon is due to the fact that hypoxic cell populations may be composed of cells which are either blocked or are slowly progressing through the cell cycle [9–11]. Thus, cell cycle active agents may have a relatively low efficacy against hypoxic tumor cell populations. Chemotherapeutic agents may also have difficulty in reaching hypoxic regions, since they must diffuse into the mass to reach oxygen-deficient cells. In this regard, drugs cannot be equated with the diffusion properties of oxygen, which has a limited capacity to penetrate the tumor mass due to rapid utilization by cells of the malignant population; thus, certain agents are clearly capable of reaching hypoxic cell regions (see for example refs 12–15). For these reasons, the hypoxic cell component of solid tumors must be considered in the design of curative therapeutic regimens.

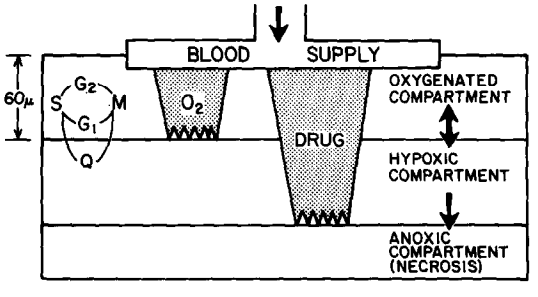

Fig. 1. Diagramatic representation of the oxygen containing compartments of a solid tumor. Reproduced from ref. 42.

The oxygen deficit of solid tumors may be considered a site of vulnerability that is amenable to selective therapeutic attack; to exploit this property, we formulated the concept of bioreductive activation [16–18]. This approach envisions a greater capacity for reductive activation by hypoxic tumor cells than by normal oxygenated cells in a manner analogous to that observed for anaerobic microbial cultures, which have lower half-wave potentials (greater capacities for reductive reactions) than those of aerobic cultures [19, 20]. The concept of bioreductive activation to generate reactive electrophiles has been expounded in elegant reviews by Moore [21, 22].

Mitomycin C, an antitumor antibiotic isolated from *Streptomyces caespitosus*, may be considered to be the prototype bioreductive alkylating agent available for clinical use [23]. To evaluate the potential of this antibiotic to eradicate preferentially hypoxic tumor cells, studies were conducted to ascertain the effects of oxygen deficiency on both its cytotoxic activity and its activation to reactive metabolites in EMT6 mammary carcinoma cells [24, 25]. The sensitivity of hypoxic EMT6 cells was evaluated in cultured cells continuously gassed with a humidified mixture of 95% N_2–5% CO_2. These cells, as well as comparable cells gassed with 95% air–5% CO_2, were exposed to various concentrations of mitomycin C for various periods of time [14, 26, 27]. The antibiotic was preferentially cytotoxic to hypoxic cells under all conditions of concentration and time, causing about a one log differential kill of the oxygen deficient cells compared to their normally aerated counterparts. That this is a general phenomenon was demonstrated in studies with a variety of cultured neoplastic cell lines which have shown that this agent is more cytotoxic to hypoxic cells than to aerobic cells [25–29].

Reductive activation of the mitomycin antibiotics has been shown to produce reactive intermediates with the capacity to produce interstrand cross-links between complementary strands of DNA; these cross-links are considered to be the lesions responsible for the cytotoxicity produced by these agents (see for example refs 30–32). The differential toxicity

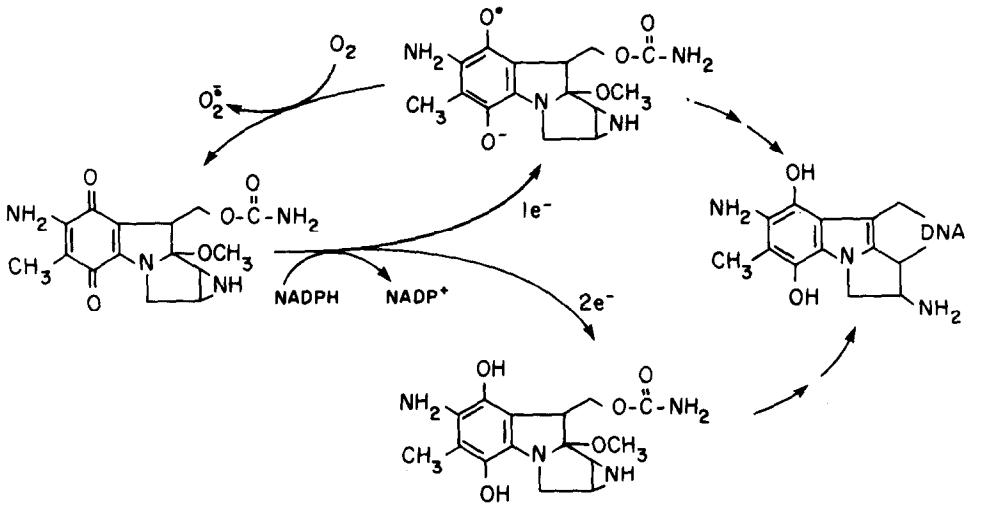

Fig. 2. Potential pathways for the bioactivation of the mitomycins. Reproduced from ref. 42.

of mitomycin C to hypoxic and oxygenated cells appears to be most reasonably explained by the formation of a fully reduced bifunctional alkylating agent which cross-links DNA following either a 1 or 2 electron reduction as shown in Fig. 2. In the presence of oxygen, the semiquinone radical formed after a one electron reduction can transfer its electron to oxygen to generate superoxide anion and regenerate the oxidized quinone. The detection of superoxide and hydroxyl radicals following the reduction of mitomycin C in the presence of oxygen provides evidence for the existence of such a reaction mechanism [33]. Under these conditions, the generation of oxygen-containing radicals partially serves as a protective device to minimize exposure of cells to the more toxic bifunctional alkylating form of the antibiotic. The oxygen-containing radicals thereby act as the forms that generate aerobic cell toxicity.

A variety of studies have shown that NADPH-cytochrome *c* reductase and xanthine oxidase are capable of activating mitomycin C [25, 34–36]. Studies with liver homogenates have shown that NADPH is required for the anaerobic production of reactive products from mitomycin C, and that enzymes with similar properties are present in the microsomal and nuclear subcellular fractions that catalyze the activation of this class of antibiotics [37–41]. Work conducted in our laboratory and by others has shown that NADPH-cytochrome *c* reductase alone is sufficient for the reductive activation of mitomycin C both in the presence and in the absence of oxygen [25, 34, 35]. Addition of cytochrome P-450 to an NADPH-cytochrome *c* reductase containing reaction mixture increases the anaerobic metabolism of mitomycin C twofold [25]. Cytochrome P-450 does not participate directly in the reduction, however, but acts as a modulator of NADPH-cytochrome *c* reductase activity.

To gain information on the enzyme systems that activate mitomycin C in neoplastic cells, we have measured the degree of activation of this antibiotic by sonicates of Sarcoma 180 and the EMT6 mammary carcinoma cells; both Sarcoma 180 and EMT6 cells consumed mitomycin C under anaerobic conditions in the presence of NADPH to generate reactive products that could be measured by conjugation with the nucleophile 4-(*p*-nitrobenzyl)-pyridine [25]. Furthermore, this process correlated with the toxicity of this agent to hypoxic cells. Studies with inhibitors of NADPH-cytochrome *c* reductase have suggested that an additional enzyme(s) is involved in the reductive activation of mitomycin C [25]. To gain information on possible candidates, we measured the activity of several reductase systems in EMT6 cells. EMT6 tumor cells contained, in addition to NADPH-cytochrome *c* reductase, significant amounts of NADPH-cytochrome *c* reductase, NADPH-cytochrome b_5 reductase, cytochrome b_5, and DT-diaphorase activity; however, no xanthine oxidase activity and cytochrome P-450 were detected. To evaluate the role of DT-diaphorase in the reductive activation of mitomycin C by EMT6 cell sonicates, the effects of dicumarol on the activity of DT-diaphorase and on the generation of reactive products from mitomycin C were measured [14]. Dicumarol completely blocked the DT-diaphorase activity of EMT6 sonicates but did not decrease the formation of reactive species from mitomycin C; instead, an increase in the generation of reactive products was produced. Although the mechanism by which dicumarol increases the generation of alkylating species from mitomycin C under anaerobic conditions is unknown, it seemed reasonable to attempt to exploit these findings. To ascertain whether the increased generation of reactive metabolites from mitomycin C was capable of increasing the cytotoxicity of the antibiotic under hypoxic conditions, the effects of this combination on the viability of EMT6 cells was measured *in vitro* under hypoxic and aerobic conditions. Dicumarol increased significantly the cytotoxicity of mitomycin C to these malignant cells under conditions of oxygen deficiency and decreased slightly the toxicity of the antibiotic to aerobic cells. Furthermore, treatment of mice bearing established EMT6 intradermal solid tumors with both dicumarol and mitomycin C produced a

decrease in the survival of hypoxic cells over that produced by the antibiotic alone [14]. This enhancement of the action of mitomycin C occurred with no major increase in the leukopenia produced by the combination over that caused by the antibiotic alone. Thus, a major increase in the efficacy of mitomycin C was attained by the inclusion of the anticoagulant dicumarol in the therapeutic regimen.

In the search for agents that are more preferentially toxic to hypoxic cells than mitomycin C, we have found that the related agent porfiromycin was similar to mitomycin C in its cytotoxicity to hypoxic EMT6 cells *in vitro* but was considerably less toxic than mitomycin C to aerobic cells [15]. This finding corresponded to the rate at which EMT6 cell sonicates reduced mitomycin C and porfiromycin to reactive products, for similar quantities of electrophiles were formed from molar equivalent quantities of these two antibiotics under anaerobic conditions. Extension of these findings to mice bearing solid implants of EMT6 tumors corroborated the differential effects observed *in vitro*, in that porfiromycin was much less efficacious than mitomycin C in killing neoplastic cells in the absence of X-irradiation, a measure of aerobic cell kill, while both drugs were equally efficacious against hypoxic tumor cells, as measured by the addition of sufficient total body X-irradiation to eliminate the majority of the aerobic tumor cell population [15]. Since the LD_{50} for mitomycin C in mice is one-fifth of the LD_{50} for porfiromycin [15], it is clear from the similar kill of hypoxic cells produced by equivalent concentrations of these agents, that the therapeutic index for porfiromycin against hypoxic cells is significantly greater than that of mitomycin C. These collective findings suggest that the use of porfiromycin to eradicate hypoxic cell populations and X-irradiation to preferentially kill aerobic tumor cells would be a particularly attractive combination to employ against localized cancer; furthermore, the capacity of added dicumarol to this regimen to increase the potency of porfiromycin against the hypoxic tumor cell population would be expected to yield further gain.

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