Therapeutic Attack of Hypoxic Cells of Solid Tumors: Presidential Address

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Abstract

Hypoxic cells of solid tumors are relatively resistant to therapeutic assault. Studies have demonstrated that oxygen-deficient tumor cells exist in an environment conducive to reductive reactions making hypoxic cells particularly sensitive to bioreductive alkylating agents. Mitomycin C, the prototype bioreductive alkylating agent available for clinical use, is capable of preferentially killing oxygen-deficient cells both in vitro and in vivo. This phenomenon is at least in part the result of differences in the uptake and metabolism of mitomycin C by hypoxic and oxygenated tumor cells, with the ultimate critical lesion being the cross-linking of DNA by the mitomycin antibiotic. The combination of mitomycin C with X-irradiation, to attack hypoxic and oxygenated tumor cell populations, respectively, has led to enhanced antitumor effects in mice bearing solid tumor implants and in patients with cancer of the head and neck. More efficacious kill of hypoxic tumor cells may be possible by the use of dicoumarol in combination with mitomycin or by the use of the related antibiotic porfiromycin. The findings support the use of an agent with specificity for hypoxic tumor cells in potentially curative regimens for solid tumors.

Introduction

Solid tumors with a low growth fraction are relatively resistant to therapeutic assault. A variety of factors are involved in the lack of responsiveness of these neoplasms, including cellular heterogeneity due to genetic differences between cells and physiological factors created by inadequate vascular networks. Thus, properties such as the nutritional status of cells, the pH of the environment, and the degree of oxygenation, factors which often go hand in hand, markedly influence therapeutic response. In addition, the penetration of tumor masses by adequate concentrations of drugs constitutes a significant factor. These considerations make it unlikely that a simple in vitro screen will select for agents with specificity for the major nonresponsive human cancers, and they imply that a strategy must be devised to cope with the diversity of cell types in solid tumors and the mechanical problems created by the presence of a tissue mass. The strategic approach must consider the physiological environment of the tumor cells within the mass and if possible exploit the environment in designing therapy.

Our laboratory has been concerned with one aspect of such a strategy to approach the treatment of solid tumors and that is to develop a selective attack of the hypoxic cell component of these neoplasms. There is evidence that hypoxic cells exist in both animal (1) and human (2, 3) tumors and that oxygen-deficient malignant cells are significantly more resistant to ionizing radiation than their aerobic counterparts (4-7). More efficacious cell kill of hypoxic tumor cells may be possible by the use of dicoumarol in combination with mitomycin or by the use of the related antibiotic porfiromycin. The findings support the use of an agent with specificity for hypoxic tumor cells in potentially curative regimens for solid tumors.

Metabolic Activation of Mitomycin C

The antibiotic mitomycin C can be considered to be the prototype bioreductive alkylating agent. This antibiotic causes a preferential kill of hypoxic EMT6 mammary tumor cells in vitro compared to their normally aerated counterparts under all conditions of concentration and time (24-29). This result is not unique to EMT6 cells and occurs with other cultured cell lines (27, 30).

Early work demonstrated that the reductive activation of the mitomycin antibiotics resulted in the formation of reactive intermediates that produced interstrand cross-links between complementary strands of DNA, an action considered to be at least partially responsible for the cytotoxicity of this antibiotic (31-34). The DNA cross-linked adduct of mitomycin C has been isolated and characterized by Tomaz et al. (35) as an interaction between the 2-amino group of two deoxyguanosine residues and activated mitomycin C bound through the C-1 and C-10 positions of the antibiotic. This bioadduct was demonstrated to be capable of snugly fitting into the minor groove of the DNA double helix with minimal distortion of the nucleic acid. Schwartz (36) provided the first demonstration of the metabolism of the mitomycin antibiotics in mammalian systems. Using rat liver microsomes, mitomycin C was shown to be metabolized in a reaction requiring anaerobiosis and an NADPH-generating system. We and others have confirmed and extended these findings using mouse liver, demonstrating that both microsomes and nuclei were capable of converting the antibiotic to a reactive metabolite(s) (28, 37, 38). The enzymes able to convert the mitomycin antibiotics to reactive electrophiles have been identified as xanthine oxidase and NADPH-cytochrome c reductase (37, 39, 40). The anaerobic metabolism of mitomycin C is increased 2-fold by the addition of cytochrome P-450 to the NADPH-cytochrome c reductase reaction mixture; however, this action is not due to the direct participation of cytochrome P-450 in the transfer of electrons but is the result of the modulation of the activity of NADPH-cytochrome c reductase by the cytochrome P-450 (27).
have demonstrated that, following the reduction of mitomycin antibiotics to the corresponding hydroquinone, it was possible through oxidation of this intermediate to recover the mitomycin form. When the mitomycin quinone and hydroquinone forms were present simultaneously, however, a semiquinone radical was generated which subsequently rearranged to the form that generated the alkylating species. To explain the greater toxicity of the antibiotic to hypoxic cells relative to their oxygenated counterparts, it is envisioned that oxygen decreases the formation of the reduced intermediate that is responsible for the production of what is considered to be the most cytotoxic lesion, the cross-linking of the DNA double helix. This is envisioned to occur through the scavenging of the semiquinone radical form of the mitomycin antibiotic by oxygen to form the superoxide radical anion and regenerate the mitomycin molecule. Evidence to support this redox cycling includes the facts that superoxide and hydroxyl radicals are present in oxygenated reaction mixtures containing mitomycin C (48–55), the semiquinone radical can be detected by electron paramagnetic resonance spectrometry (53), and unaltered mitomycin C can be recovered quantitatively from reduction mixtures when oxygen is present (50, 56, 57). Thus, although oxygen radicals generated from the mitomycin antibiotics can be cytotoxic, they are apparently much less so than the cross-links in DNA produced by these agents, contributing to the differential kill of oxygenated and hypoxic cells produced by these agents. Also of major significance in the oxygen-dependent sensitivity of neoplastic cells to the mitomycin antibiotics is the rate of uptake of these agents. Using the methylated analogue of mitomycin C, porfiromycin, we have demonstrated that the uptake of this material into hypoxic EMT6 tumor cells is more rapid than into their oxygenated counterparts, and that the antibiotic is preferentially retained in oxygen-deficient cells (58).

Mitomycin antibiotics are available that have different degrees of inhibitory activity to oxygenated and hypoxic tumor cells (55, 59); the structures of some of these agents are shown in Fig. 3. Porfiromycin is equivalent to mitomycin C in its toxicity to hypoxic EMT6 cells but is much less active against oxygenated cells. In contrast, the semisynthetic mitomycin antibiotic BMY-25282 is considerably more toxic to aerobic cells than hypoxic cells, and the toxicity of BL-6783, the porfiromycin-like analogue of BMY-25282, is equivalent to oxygenated and hypoxic cells. BMY-25282 and BL-6783 generated more hydroxyl and superoxide radicals than either porfiromycin or mitomycin C, an effect that may contribute to the

![Fig. 1. Schematic representation of the compartments of a solid tumor. Reprinted with permission from Keyes et al. (59).](image-url1)

![Fig. 2. Schematic representation of possible pathways for the bioactivation of the mitomycin antibiotics. Reprinted with permission from Keyes et al. (59).](image-url2)

![Fig. 3. Structural formulae of mitomycin antibiotics. Reprinted with permission from Pritsos and Sartorelli (55).](image-url3)
greater toxicity of these semisynthetic analogues to aerobic cells than that of mitomycin C and porfiromycin (55).

In Vitro Activity of Mitomycin Antibiotics

The capacity of the mitomycin antibiotics to eradicate hypoxic cells in solid tumors has been assessed in well-established implants of the EMT6 mammary carcinoma in BALB/c mice. In these experiments, X-irradiation was used to selectively destroy the aerobic tumor cell population. Colonies formed from single cell suspensions made from these neoplasms primarily represent hypoxic cells that survived the ionizing irradiation, and treatment of these tumor-bearing animals with a mitomycin antibiotic prior to the X-rays provides an indication of the effect of the agent under evaluation on the hypoxic fraction of the tumor. It is critical for the interpretation of experiments of this kind to evaluate the capacity of the agent under study to sensitize cells to the action of the ionizing irradiation. Importantly, in vitro studies have demonstrated that neither mitomycin C nor porfiromycin sensitizes EMT6 cells to X-irradiation (60, 61). Studies with solid tumor implants of the EMT6 carcinoma have demonstrated that both mitomycin C (62–64) and porfiromycin (65) are capable of killing hypoxic tumor cells spared by radiotherapy. Analogous results in other experimental tumor systems have demonstrated the efficacy of combinations of mitomycin C with X-irradiation (30, 66–68).

These findings have led to a clinical trial at Yale University of mitomycin C in combination with X-irradiation in newly diagnosed untreated patients with head and neck cancer (69). Patients were stratified into groups consisting of planned preoperative and prophylactic postoperative therapy, postoperative therapy for known residual disease after surgery or those receiving primary radiotherapy for known residual disease or those receiving radiation without surgery greater than 60 Gy of total X-irradiation. Mitomycin C was given i.v. at a dose of 15 mg/m² on Day 5 of the radiotherapy and patients with known residual disease after surgery or those receiving primary radiotherapy received a second dose of the antibiotic 6 wk later. Disease-free survival at 60 mo was significantly better in patients receiving mitomycin and X-rays (75%) than in those individuals receiving X-irradiation alone (55%), and therapy was well tolerated. The results are consistent with the concept that the antibiotic functioned to attack the irradiation-resistant hypoxic tumor fraction.

Current research is aimed at (a) seeking approaches that increase the degree of differential toxicity of mitomycin C to aerobic and hypoxic cells, since the greater the specificity for hypoxia the larger the dose of bioreductive alkylating agent that can be administered without compromising normal tissues, a factor of considerable importance, since the chemotherapeutic agent must diffuse from vascular areas to hypoxic regions of the tumor; and (b) using the hypoxic fraction as a metabolic factory to activate prodrugs to exceedingly toxic but nonreactive materials that have the capacity to diffuse from the hypoxic cells into other regions of the tumor mass. We have called antimetabolites of this kind bioreductive antimetabolites. An approach we have employed to increase the differential toxicity of the mitomycins to oxygenated and hypoxic tumor cells is to use these bioreductive alkylating agents in combination with another agent which preferentially increases the toxicity of the mitomycin antibiotic to oxygen-deficient cells. Dicoumarol is capable of accomplishing this, increasing significantly the toxicity of mitomycin C to hypoxic EMT6 cells, while decreasing the toxicity of this antibiotic for aerobic cells both in vitro and in vivo (64). The dicoumarol acts at least in part to increase the enzymatic activation of mitomycin C to reactive metabolites in hypoxic cells (59). Clinical trials have been initiated at this institution to evaluate the effectiveness of dicoumarol added to the combination of mitomycin C and X-irradiation.

A second approach seeks new agents that produce a differential kill that is greater than that caused by mitomycin C. As indicated previously, the related antibiotic, porfiromycin, accomplishes this, being similar to mitomycin C in its toxicity to cultured hypoxic cells but markedly less toxic than mitomycin C to aerobic cells (29, 59). Extension of in vitro findings to mice bearing solid EMT6 tumors have demonstrated that porfiromycin plus radiation produces superadditive cytotoxicity (65). In addition, since the 50% lethal dose for mitomycin C in mice is one-fifth of the 50% lethal dose for porfiromycin, the similar kill of hypoxic cells produced by molar equivalent concentrations of these agents indicates that the therapeutic index for porfiromycin when used against hypoxic cells is significantly greater than that of mitomycin C.

The findings encourage the use of a bioreductive alkylating agent with specificity for hypoxic cells in potentially curative regimens for solid tumors.

References


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