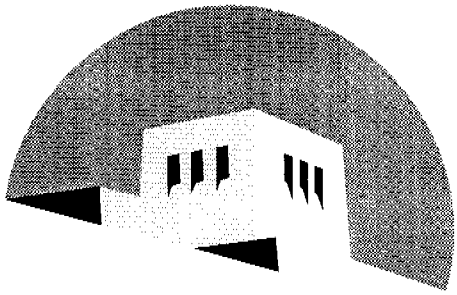

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Correspondence Continuing Education Courses for Nuclear Pharmacists and Nuclear Medicine Professionals

VOLUME III, NUMBER 6

Pharmacologic Enhancement of Radioimmunotherapy

By:

Kenneth T. Cheng, Ph.D., B.C.N.P.

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PHARMACOLOGIC ENHANCEMENT OF RADIOIMMUNOTHERAPY

STATEMENT OF OBJECTIVES

The goal of this correspondence continuing education lesson is to increase the reader's knowledge in the use of pharmacologic agents to enhance the efficacy of RADIOIMMUNOTHERAPY (RIT). This lesson is intended for nuclear pharmacists and nuclear medicine professionals who have an interest in the use of pharmacologic agents to increase the therapeutic efficacy of radiolabeled antibodies in treating cancer patients.

Upon successful completion of this course, the reader should be able to:

1. List the four major causes of physiologic difference between normal tissue perfusion and tumor perfusion.
2. Explain the difficulty involved with the process of radiolabeled antibody crossing the interstitial fluid space.
3. List three pharmacologic agents which can increase tumor blood flow and explain their mechanisms of action.
4. List two pharmacologic agents which can modulate microregional heterogeneity in tumor blood flow and explain their mechanisms of action.
5. Name the pharmacologic agent which can enhance tumor antigen expression.
6. Explain the rationale of using pharmacologic agents to sensitize tumor to radiolabeled antibodies.
7. List the five classes of agents which can sensitize tumor cells to radiation.
8. Explain the importance of oxygen in radiation therapy.
9. Describe the mechanism of action of hypoxic cell sensitizer.
10. Explain the mechanism of radiation sensitization of thiol-depleting agents.
11. Explain the mechanism of radiation sensitization of potentially lethal damage (PLD) repair inhibitors.
12. Explain the mechanism of radiation sensitization of halogenated pyrimidines.
13. Describe the mechanism of action of Taxol and how it sensitizes tumor cells.
14. Describe the mechanism of radiation sensitization of 5-fluorouracil (5-FU).
15. Describe the mechanism of radiation sensitization of hydroxyurea.
16. Describe the mechanism of radiation sensitization of cisplatin.

COURSE OUTLINE

I. INTRODUCTION

II. TUMOR PATHOPHYSIOLOGY

- A. Tumor Vasculature
- B. Tumor Perfusion
- C. The Interstitial Fluid Space Barrier
- D. Tumor Antigen Expression

III. PHARMACOLOGIC ENHANCEMENT OF RADIOLABELED ANTIBODY LOCALIZATION IN THE TUMOR

- A. Pharmacologic Agents Which Elicit Indirect Effects on Tumor Blood Flow
  1. Angiotensin II
  2. Beta-adrenergic Blocking Agents
  3. Anesthetics
- B. Pharmacologic Agents Which Modulate Microregional Heterogeneity in Tumor Blood Flow
  1. Nicotinamide
  2. Calcium Channel Blockers
- C. Agents Which Enhance Tumor Antigen Expression

IV. PHARMACOLOGIC ENHANCEMENT OF TUMOR SENSITIVITY TO THE RADIATION DOSE DELIVERED BY THREE RADIOLABELED ANTIBODIES

- A. Hypoxic Cell Sensitizers
- B. Thiol-Depleting Agents
- C. PLD Repair Inhibitor
- D. Halogenated Pyrimidines
- E. Chemotherapeutic Agents
  1. Paclitaxel (Taxol)
  2. 5-Fluorouracil (5-FU)
  3. Hydroxyurea
  4. Cisplatin

V. SUMMARY

PHARMACOLOGIC ENHANCEMENT OF RADIOIMMUNOTHERAPY

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INTRODUCTION

Targeted cancer therapy using radiolabeled antibodies has been of great interest in nuclear medicine for many years, and the production of monoclonal antibodies (MoAbs) using hybridoma technology has led to a great surge of research in this field (1). The basic theory behind radioimmunotherapy (RIT) is that radiolabeled antibodies can selectively seek out antigen-positive cancer cells *in vivo* and deliver a therapeutic dose of radiation to the whole tumor. As more experience has been gained from both basic and clinical research studies, it has become clear that the *in vivo* behavior of radiolabeled MoAbs is very complex (2). The fact is that the development of radiolabeled MoAbs for tumor imaging and therapy has been hindered by complex problems associated with tumor pathophysiology and related radiolabeled antibody biodistribution (3).

The successful treatment of tumor with radiolabeled antibody depends largely on the achievement of a high target (tumor) to nontarget (normal tissue) ratio. In order to be effective, radiolabeled antibodies have to destroy tumor cells without harming the surrounding normal tissues. Thus, the target-to-nontarget ratio must be particularly high when dealing with normal tissue (non-target tissue) that include radiosensitive organs such as bone marrow. It is only by achieving a very high ratio that the lethal consequences of radiation can be avoided. Unfortunately, such high target-to-nontarget ratios have been difficult to achieve with radiolabeled antibodies because of various complex and unfavorable *in vivo* conditions related to tumor pathophysiology.

The biodistribution of radiolabeled MoAbs may differ from patient to patient depending on many factors (4). For example, one of these factors is the tumor mass. If the tumor mass is large and the anti-

tumor MoAb has some affinity for normal tissues, then the amount of radiolabeled antibody localized in normal tissues will vary inversely with the tumor mass. Other obvious factors are the tumor vascularity and vascular permeability. Uptake of radiolabeled antibody by tumor is not only dependent on vascular supply but also on vascular permeability. Thus, very small tumors may have relatively poor uptake of radiolabeled antibodies because of their poor vascular supply while larger tumors may show significantly higher uptake of radiolabeled antibodies due to their increased vascular permeability.

All of these complex tumor pathophysiologic factors have contributed significantly to the inconsistent results of current clinical RIT studies. Since tumor pathophysiology is not controllable in patients, even a perfect radiolabeled antibody is therefore not likely to produce consistent therapeutic efficacy in all patients.

Among different methods that have been studied to increase the therapeutic efficacy of radiolabeled antibodies, one potential area that deserves our attention is the utilization of pharmacologic agents to enhance RIT efficacy. Presently, there are two major areas that are under active investigation. One area is the utilization of pharmacologic agents to enhance localization of radiolabeled antibodies in tumors (5). For example, radiolabeled antibody delivery to tumor cells may be improved by exploiting the differences between tumor blood vessels and normal blood vessels. The expression of cellular antigen may also be improved by pharmacologic means to enhance the tumor binding of radiolabeled antibody.

Another area is the use of pharmacologic agents to enhance radiosensitivity of tumor cells to the radiation dose delivered by RIT (6). Pharmacologic agents can render tumor cells more sensitive to radiation than normal cells based on the growth and cell cycle activity differences between tumor cells and normal cells.

TUMOR PATHOPHYSIOLOGY

Tumor Vasculature

Tumor physiology is very different from that of normal tissue. Normal tissues are governed by natural physiologic laws and orders, but tumor is a place of chaos. As tumor cells multiply to form a solid mass they become increasingly dependent on the development of a vascular system (3). The proximity of cells to the functional vasculature is very important because it will determine their supply of oxygen and nutrients. During the progressive growth of a tumor, the host vessels are replaced by new nutrient vessels under stimulation by angiogenetic factors. These neoplastic vessels may develop anatomically, but they do not retain normal physiological function.

Tumor Perfusion

In general, normal tissues have a good blood flow in relation to their needs (3). On the other hand, blood flow to tumors is poor. Studies in animal tumors and human malignancies have shown the presence of low O_2 levels, acidic pH, and overt necrosis (7). It is evident that the vascular system does not adequately supply all the cells within a tumor. This vascular insufficiency is also more pronounced in larger tumor masses for which response to RIT is often poor. A deficiency in functional vasculature (and thus blood supply for antibody delivery) plays an important role in RIT treatment failures.

Despite the growth of new neoplastic vessels, there can be a rapid and drastic reduction in the blood flow as the tumor mass increases. One reason for the reduction of flow is that the number of vessels in the tumor decreases proportionately as the tumor enlarges. This is caused by the fact that tumor cells divide several times faster than endothelial cells. This decrease in the number of tumor capillaries may vary from one tumor type to another and even within the same tumor type.

In addition, as tumors enlarge, the actual diameter of the tumor capillaries increases dramatically. As a result, there is a decrease in the total vascular cross-sectional area of the tumor and an increase in the efferent flow resistance. Much of this blood is static in tumor vessels, and does not exchange well with blood from the systemic vascular compartment. Under these circumstances, some areas of the tumor will turn out to be fairly well perfused and others virtually nonperfused. This poor profusion is especially prominent in the central portion of the tumor.

Arteriovenous shunts also occur in tumors (3). The entering blood is shunted back into the systemic circulation before it has a chance to go through a capillary bed. In this situation, the radiolabeled antibodies in the shunted blood will not exchange with the tumor cells during the pass.

Blood flow is also affected by pH. The pH in a tumor is relatively low (acidic), causing the capillaries to lose their flexibility. There is also high pressure within tumors due to the lack of lymphatics. The thin walls of the tumor capillaries often collapse under this high pressure which further decreases perfusion (3).

Poorly perfused tumor regions are much more resistant to RIT than well-perfused regions. It is well known that hypoxic cells in poorly perfused areas are three times more resistant to radiation treatment than well oxygenated ones (8). Such regions are also poorly accessible by radiolabeled antibodies.

The Interstitial Fluid Space Barrier

Once the radiolabeled antibody leaves the capillary,

it is met by the interstitial fluid surrounding the cells (3,4). At a few microns distance, two major forces move the antibody molecule to the cells. The first is diffusion, and the second is convection. Convection refers to movement of the antibodies along with movement of interstitial fluid. The effect of these two forces on the molecule will depend on molecular size. Small molecules are highly affected by diffusion and penetrate the interstitium of tissues primarily by that mechanism. As the molecules get larger, diffusion becomes less important, and convection becomes the dominant moving force. Antibodies have a large molecular size and thus depend more on convection than diffusion. For the vast majority of the radiolabeled antibodies, penetration will be poor. Since tumors have an interstitial fluid space two to four times greater than normal tissues, it is also a bigger obstacle to move the radiolabeled antibody from a capillary to a tumor cell.

In animal tumor models, the majority of capillaries in the tumor are found at the surface in the area of the highly viable tissue. As expected, studies in human tumor models have shown that there is a great decrease in radioactivity in the interior of the tumor due to the poor penetration power of the radiolabeled antibody.

Tumor Antigen Expression

A tumor antigen is an antigen expressed as a consequence of a malignant transformation event (9). Tumor-specific antigens are antigens that are uniquely expressed by tumors and not by normal tissues. Cell surface antigens are ideal targets for systemically circulating radiolabeled antibodies. Some antigens are modulated and released from the cell surface as a consequence of antibody binding. Many other cell surface antigens are not affected. There is some evidence that antigen concentration may vary with different phases of the cell cycle. However, antigen production remains basically a mystery. In cell culture, the production of antigen can be very fast. Regardless of how it is produced, the expression of antigen on tumor cells is critically important to the binding of antibody.

PHARMACOLOGIC ENHANCEMENT OF RADIOLABELED ANTIBODY LOCALIZATION IN THE TUMOR

Awareness of the unfavorable consequences of various aspects of tumor pathophysiology has led to investigations to study methods of improving *in vivo* local antibody concentration in tumors (10). In one approach to facilitate the localization of radiolabeled antibodies in tumors, a number of pharmacologic agents have been identified which produce selective modulation of tumor blood flow. Studies have suggested that neoplastic vessels lack the ability to react to vasoactive agents. However, vasoactive agents can be manipulated to exert an indirect influence to increase the tumor blood flow.

In another approach, pharmacologic agents are used to modulate the tumor surface antigens for radiolabeled antibody binding. Heterogeneity in the expression of tumor-associated antigens, as defined by the binding of MoAbs, is a characteristic common to most human carcinoma cell populations. Making a human tumor cell population more homogeneous for the expression of an antigen could enhance antibody binding and local tumor concentration. Human natural and recombinant interferons can change the surface antigenic phenotype and increase the amount of tumor antigen expressed (11). Thus, concomitant administration of interferon and radiolabeled antibody may effectively enhance the *in vivo* tumor binding of radiolabeled antibody. This will lead to increased antibody localization in the tumor due to enhanced tumor antigen expression.

Pharmacologic Agents which Elicit Indirect Effects On Tumor Blood Flow

The effects of vasoactive drugs on tumor blood flow have been investigated extensively over the past 40 years. Many agents have been identified which produce transient (minutes to hours) changes in tumor blood flow. These vasoactive drugs increase the ratio of tumor blood flow to surrounding normal tissue blood flow as a result of differences in vascular structure and function between normal and tumor tissue (12).

Neoplastic vessels do not retain normal physiological function. Regulation of blood flow velocity, direction, pressure, and capacity is lost (10). In addition, tumor tissue lacks vascular smooth muscle and possesses many areas in which interstitial fluid pressure is elevated. Tumor vessels are therefore not very responsive to the pharmacologic effects of vasoactive drugs directly. Instead, the influence of vasoactive drugs on tumor circulation is most likely to be mediated indirectly via effects on flow in surrounding normal tissue, or on systemic blood pressure. Hence, vasodilators (such as papaverine) have not been found to be effective in increasing tumor blood flow. In fact, some vasodilators (such as hydralazine) have been found to decrease tumor blood flow by selectively dilating surrounding normal tissue vessels and shunting blood away from the tumor (13). Conversely, vasoconstrictive agents hold much promise as pharmacologic agents of choice in increasing tumor blood perfusion.

Angiotensin II. Vasoconstrictive agents have been tested, with varying success, to determine their

capacity to increase blood flow to tumors. Adrenaline, noradrenaline, oxytocin and vasopressin have produced conflicting results in different animal models. This is probably a result of the presence of different receptor populations in different organ systems. Among vasoconstrictors, angiotensin II (angiotensin amide, Hypertensin™), a vasoconstrictor peptide, has been shown to be most promising and appears to consistently increase blood flow in experimental tumors (14-15). It increases the peripheral resistance, mainly in cutaneous, splanchnic, and renal blood vessels, and acts both directly and via the sympathetic nervous system. The increased blood pressure is accompanied by a reflex reduction in heart rate, and cardiac output may also be reduced.

Suzuki, et al. (16) described an approximately six-fold selective increase in blood flow to subcutaneous tumors without increasing blood flow to normal tissue. Angiotensin II has been investigated as an adjunctive agent in renal pharmacoangiography to reduce blood flow in normal tissue, while allowing pooling of contrast medium in tumor vessels (17). Specifically, the effect of angiotensin II and tolazoline (a vasodilator) was compared in 18 patients with bone and soft-tissue tumors. Angiotensin was found to be the drug of choice in increasing diagnostic information in angiographic procedures. Ten to 15 ug of angiotensin II appeared to be a convenient dose in the axillary, iliac, and femoral arteries. In the same study, tolazoline was found to be totally ineffective.

Mechanistically, angiotensin II causes vasoconstriction by binding to specific saturable receptors present in vascular smooth muscle. These receptors are primarily distributed in the precapillary sphincter arterioles, and their quantity is tissue-dependent. There are fewer arterioles incorporated into the tumor mass than are present in the surrounding normal tissue. Angiotensin II acts more peripherally in the vascular bed than other vasoconstrictors and, therefore, should have less effect on the arteries feeding the tumor while still providing a net decrease in flow to the smaller vessels of the normal tissue. The increase in tumor blood flow following angiotensin II is most likely to result from the elevation in systemic blood pressure which it induces in the host animal. The absence of vascular smooth muscle and the occurrence of high interstitial pressure in tumors results in increased perfusion pressure. Consequently, the infusion of angiotensin II will cause significant reduction in normal tissue blood flow, whereas tumor blood flow will either be decreased to a significantly lesser degree or actually increased if the arterial pressure is sufficiently elevated. In all cases, the ratio of blood flow in the tumor versus that in the normal tissue will be significantly increased.

Although no study has been performed with radiolabeled antibody, Burton, et al. (15) have shown that intravenous (IV) infusion of angiotensin II increased the number of radioactive microspheres gaining arterial access to the central portions of experimental hepatic tumors in rats and rabbits. In terms of radiolabeled antibody therapy, the results would indicate a substantially enhanced dose reaching tumor tissue after angiotensin II infusion, while relatively sparing the surrounding normal tissues.

Beta-adrenergic blocking agents. Bomber and colleagues (18) have shown that the injection of propranolol HCl (Inderal™) increased the uptake of Ga-67 in tumor relative to normal tissues. In this study, the effect of propranolol hydrochloride on the blood perfusion of a mouse sarcoma and other tissues was studied. The maximum increase in relative tumor perfusion (2X control) occurred 15 minutes after IV administration of 10 mg/kg propranolol hydrochloride. The propranolol hydrochloride was also given 10 mg/kg 10 minutes before administering Ga-67 citrate. Four hours after Ga-67 administration, the tumor-to-blood ratio increased from 1.16 in controls to 3.41 in test animals. Propranolol hydrochloride blocks the beta adrenoreceptors to produce a decrease in heart rate and blood pressure. The increased tumor perfusion is thought to be due to the change in the relative perfusion in muscle and of other tissues as a result of the decreased cardiac output with compensatory sympathomimetic vasoconstriction to maintain blood pressure. Tumor blood vessels lack smooth muscle and do not respond to the peripheral vasoconstriction feedback.

Other nonselective and cardioselective beta-adrenergic blocking agents at therapeutic doses have also been shown to increase twofold to fourfold tumor-to-blood and tumor-to-liver ratios of I-125 labeled anti-Ly-2.1 antibody uptake in mouse E3 thymoma (19). The increased ratio was caused by a decreased accumulation of I-125 labeled antibody in normal organs (resulting from a reduced cardiac output) and a relative increased in tumor uptake. Using the same tumor animal model, propranolol, pindolol, and oxprenolol were also found to increase the antitumor efficacy of Ida-anti-Ly-2.1 conjugate. By contrast, prazosin HCl (alpha-adrenergic blocking agent) and cyclandelate (Cyclospasmol™, peripheral vasodilator) did not enhance the tumor perfusion and antibody target-to-nontarget ratio.

Unlike angiotensin II, not all studies with beta blockers have been positive. A study by Pimm (20) using nude mice with human tumor xenografts indicated that both propranolol and pindolol might not give highly effective or consistent increases in tumor blood flow, and therefore might not be very effective

in enhancing tumor localization of MoAbs for tumor therapy.

Anesthetics. Zanelli and colleagues (21) have shown that the anesthetic agents pentobarbitone sodium and urethane increased the relative tumor perfusion in experimental tumor in mice through a decrease in blood flow to muscle. These agents appeared to cause a decrease in cardiac output, with compensatory sympathomimetic vasoconstriction taking place in an effort to maintain the blood pressure. Pentobarbitone was found to increase the relative blood perfusion by a factor of 1.3 to 2.0 in tumors but muscle perfusion fell to 0.3 to 0.5 that of controls. The effects of urethane were found to be smaller and dose dependent. Despite this positive report, no other studies have repeated or confirmed the results of this study.

Pharmacologic Agents which Modulate Microregional Heterogeneity in Tumor Blood Flow

In tumors, hypoxia can result from transient alterations in blood flow (10). The mechanism for such blood flow changes has not yet been clearly elucidated. Temporary plugging of blood vessels by circulating cancer cells or white blood cells may play a role. In addition, if the interstitial tumor pressure exceeded the intravascular pressure in some tumor microregions, blood flow stasis would result. These cessations in microregional flow have been shown to involve up to 10% of tumor vessels at any one time, and to last for at least several minutes. Moreover, this can affect several vessels in the same microregion, resulting in relatively large patches of tissue hypoxia. Thus it has become evident that agents which could prevent the transient microregional alterations in tumor blood flow, could provide another class of agents with a defined use in RIT.

Two classes of pharmacologic agents have been found to be active in reducing such subtle blood flow changes (5): the calcium channel blockers and nicotinamide. These agents have been shown to improve the response of tumors to radiation if administered prior to therapy and to provide small improvements in tumor blood flow. Studies have also shown that enhanced radiation response can be achieved by combining nicotinamide with therapies such as Fluosol DA and carbogen which improve the oxygen-carrying capacity of blood. Such an approach improves the oxygenation status of hypoxic cells resulting from either blood flow fluctuations (i.e., perfusion limitations) or location relative to vasculature (diffusion limitations).

Nicotinamide. Chaplin and Trotter (22) have demonstrated that prior treatment with nicotinamide prevents the opening and closing of blood vessels known to occur in the SCCVII tumor implanted in C_3H/He mice. Nicotinamide was found to be more effective in reducing tumor acute hypoxia than flunarazine (a calcium channel blocker) and Fluosol-DA (20%). It has little or no significant radiosensitizing effect on hypoxic cells *in vitro* but does modify tumor blood flow. The results are consistent with other studies that nicotinamide reduces the dynamic changes in microregional tumor perfusion and, as a consequence, decreases the amount of acute hypoxia in the tumor.

Nicotinamide alone is not known to have any vasoactive properties, although nicotinic acid, for which nicotinamide is a precursor, does have some weak vasodilator effects. Also of interest is the fact that nicotinamide, at a dose of 500 mg/kg, has been reported to reduce mortality in endotoxin-induced shock in rats. Lethality in endotoxin-induced shock is due, at least in part, to ischemia in normal tissues. This phenomenon suggests that nicotinamide may have the ability to reduce transient ischemia in tumor tissue.

Calcium Channel Blockers. Calcium channel blockers are a diverse group of compounds with the general property of uncoupling calcium-mediated cellular processes by blocking the uptake of this ion through the calcium channels of the plasma membrane (23). They are particularly effective on vascular smooth muscle, and for this reason have found widespread use in the treatment of cardiovascular disease. The calcium antagonists may be divided into a number of subgroups, according to their chemical structure and preferential site of activity. They may act upon the tumor vasculature in a manner independent of the systemic circulation.

Four different calcium channel antagonists were studied by Wood and colleagues (24) in SCCVII/St tumor implanted in C_3H/Km mice. Verapamil, diltiazem, nifedipine, and flunarizine, representing four different main subgroups of calcium blockers, were studied. Verapamil is a phenylalkylamine and primarily targets the cardiac conduction mechanism and coronary blood vessels. It has activity at the large systemic blood vessels at high doses. Diltiazem is a benzothiapine and has activity primarily on coronary vessels. Nifedipine (1,4-dihydropyridine) targets the large systemic blood vessels. Flunarizine, a diphenylpiperazine, is active on peripheral vessels and on blood cellular components at concentrations which have little cardiac effect. All of these agents have different effects on tumor perfusion over a large dose range.

Among these four agents, flunarizine was found to be most effective in increasing tumor blood flow. Flunarizine sensitized tumors to external irradiation over the dose range 0.005-500 mg/kg I.P. Increases (20% to 30%) in tumor perfusion were seen at doses

of 0.05-5 mg/kg. Verapamil increased tumor radioresistance at doses of 20 mg/kg and above. It actually reduced tumor perfusion at 50 mg/kg. Below 10 mg/kg, verapamil sensitized tumors to external irradiation, with little or no increase in tumor perfusion. Nifedipine at 10 mg/kg and above produced very radioresistant tumors, with correspondingly large reductions in tumor perfusion. At doses below 0.5 mg/kg sensitization was seen, but no increased tumor perfusion. Diltiazem at 50 mg/kg also increased tumor radioresistance, with a reduction in tumor perfusion. At lower doses, it sensitized tumors to irradiation, with small increases in tumor perfusion. Between 0.05 to 5 mg/kg, flunarizine increased tumor perfusion by 30%. All of these agents have activity over a large dose range. The similarity between the tumor radiation responses among these four calcium blockers suggests that they may be acting upon the tumor vasculature in a manner independent of the systemic circulation. They may prevent the rigidification of red blood cells and permit them to pass through more tortuous blood vessels within the tumor. This phenomenon may explain why verapamil and diltiazem did not increase tumor blood flow yet sensitized tumors to external irradiation.

Flunarizine shows the best potential to be used clinically since it gives the flattest dose-response curve within the clinical range. Flunarizine is active on peripheral vessels and on blood cellular components at concentrations which have little cardiac effect. The increase in tumor perfusion is probably mediated through prevention of the naturally-occurring constriction of small vessels in or around the tumor, and subsequently reduces the acutely hypoxic cell population.

Agents Which Enhance Tumor Antigen Expression

The problems of insufficient local concentration of radiolabeled MoAbs in tumor may be partially compensated by enhancing the expression of tumor-associated antigens. Heterogeneity of antigen expression is characteristic of human carcinoma cell populations. This phenomenon has important implications for the application of radiolabeled antibodies for therapy. Rendering a human tumor cell population more homogeneous for the expression of an antigen could result in an augmentation of radiolabeled antibody binding.

Human natural and recombinant interferons can alter the surface antigenic phenotype of various human target cells *in vitro*. They can modulate the level of class I and class II histocompability antigens, and certain tumor-associated antigens (24-25). Both the type I and type II interferons (IFNs) can induce or amplify expression of the major histocompability complex (MHC) antigens. Human immune (gamma) IFN has been shown to be a potent inducer of de novo expression of the class II human leukocyte antigens (HLA) and can also ampiify expression of the class I HLA surface glycoproteins. The type I leukocyte (alpha) and fibroblast (beta) human IFNs (Hu-IFNs) play a more restrictive regulatory role in the modulation of these antigens.

Hu-IFNs are potent regulators of antigenic expression on the established human carcinoma cell lines. Studies have shown that Hu-IFN treatment could also augment tumor antigen content, amplifying the signal emitted by radiolabeled MoAbs localized to the tumor site. Among all Hu-IFNs tested, Hu-IFN-gamma appeared to be most potent *in vitro*. An important difference in the abilities of Hu-IFNs to augment tumor antigen and normal HLA expression seems to be the requirement for constitutive gene expression for an Hu-IFN to amplify the surface antigen. Hu-IFN-gamma is an inducer of gene expression for the class II MHC antigens. In addition, Hu-IFN-gamma seems to amplify the expression of actively transcribed genes, resulting in the augmentation of the gene product, the tumor antigen. Studies have shown that the Hu-IFN treatment of a variety of human cancer cells resulted in an increase in antigen density per cell as well as increase in the proportion of the cell population that was antigen positive.

Greiner and colleagues (26) have shown that administration of IFN-gamma effectively increased the amount of antigen expressed in patients with either gastrointestinal or ovarian carcinoma. This enhanced antigen expression has demonstrated a subsequently increased tumor localization of I-131 B72.3 MoAb.

PHARMACOLOGIC ENHANCEMENT OF TUMOR SENSITIVITY TO THE RADIATION DOSE DELIVERED BY THE RADIOLABELED ANTIBODY

Another way to increase therapeutic efficacy of RIT would be making the tumor more sensitive to the radiation dose delivered by the antibody. There are currently five classes of pharmacologic agents that can enhance tumor sensitivity to radiation (6).

Hypoxic Cell Sensitizers

The first class of agents consists of compounds which sensitize hypoxic cancer cells to radiation. The application of these agents was started approximately 30 years ago with the discovery of hypoxic cells within tumors (27). It is now recognized that 1%-30% cells in rapidly growing tumors could become deprived of oxygen through the abnormality of the tumor blood

supply and the rapidity of tumor cell growth versus capillary proliferation. Hypoxic cells are very resistant to radiation therapy.

A therapeutic dose of ionizing radiation is of sufficient energy to eject electrons from target tissues (28). After exposure to radiation, these free electrons interact with various intracellular molecules to form many very short-lived free radicals. These unstable and highly reactive radicals are formed in water, DNA, and other cellular molecules. When present, oxygen can interact with the DNA radical and form a more permanent DNA-peroxy radical (Figure 1). These DNA-peroxy radicals can decay and produce DNA lesions by fragmenting DNA. The mechanism responsible for cell death is often the presence of critical lesions of double-strand breaks in DNA induced by either radiation directly or DNA-radicals indirectly. A therapeutic dose of ionizing radiation can produce a sufficient number of these lesions in DNA leading to cell death.

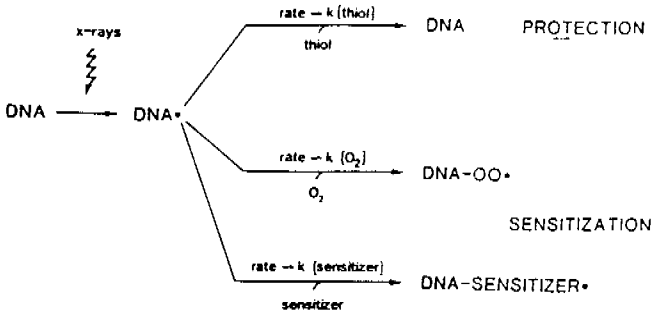

Figure 1. The interaction between oxygen and DNA radical (DNA·) produced by radiation. In the presence of thiols, the DNA radical can be chemically restored. In the absence of oxygen, a hypoxic cell sensitizer can bind to the DNA radical to produce DNA lesions.

The presence of oxygen to produce peroxy-DNA radicals is extremely important for cell killing. In the absence of oxygen (0.01%), it requires between two and three times the radiation dose to produce the same fraction of cell killing as obtained in ambient air. In theory, an oxygen-mimetic hypoxic-cell sensitizer can take the place of oxygen, leading to a stable DNA lesion. Since oxygen is much more reactive than the sensitizer, the sensitizer will not increase the damage formed in cells in the presence of oxygen. Instead, it will sensitize cells that are lacking oxygen. The process in which DNA radicals are stabilized by either oxygen or an oxygen-mimetic sensitizer (hypoxic cell sensitizer) is referred to as sensitization. The ratio of radiation dose required to produce a certain fraction of cell killing under hypoxia compared with dose required to kill the same fraction in air is the oxygen enhancement ratio (OER). This ratio is known as the sensitizer enhancement ratio (SER) in the presence of hypoxic cell sensitizers.

Many compounds are able to enhance the radiation response of hypoxic cells *in vitro*. Most of these belong to the electron-affinic class (29) because there is a direct relationship between radiation sensitizing ability and the electron affinities of the compound. However, only a few of these agents are active *in vivo* due to either toxicity limitations or to poor tumor penetration. The hypoxic sensitizers that have received the most attention are the 2-nitroimidazole compounds. As with oxygen, the sensitizer must be present at the time of irradiation to achieve its oxygen-mimetic sensitization. The efficacy of a 2-nitroimidazole sensitizer increases with increasing drug dose.

One compound that has been introduced for clinical testing is misonidazole. The dose-limiting toxicity of this drug is peripheral sensory neuropathy. Half of the patients in one clinical trial had this toxicity at a cumulative dose of 10 to 12 g/m^2. Misonidazole produces an SER of 1.5 at the dose of 2 g/m^2. At this dose level, only five to six doses of misonidazole could be administered. Given the toxicity limitations, most of the clinical trials did not show an advantage using misonidazole. The most encouraging results have been reported from a head and neck cancer trial that demonstrated a statistically significant increase in survival for men with pharyngeal squamous cell cancer.

A series of less lipid-soluble misonidazole analogs and nonnitrosensitizers have been produced in the past 10-15 years. Some of their structures are presented in Figure 2. They are as potent as misonidazole but less neurotoxic. There are presently at least 20 different groups worldwide attempting to develop hypoxic cell radiosensitizers that are potent enough at clinically acceptable doses.

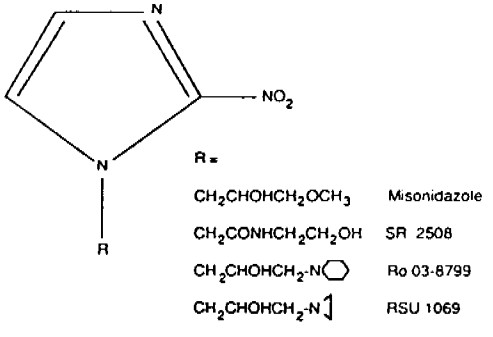

Figure 2. Structures of misonidazole and its analogs.

Thiol-Depleting Agents

The second class of agents is composed of the thiol depleting agents. All cells contain varying degrees of non-protein reducing species such as sulfhydryls (thiol, -SH) which act to protect the DNA. When DNA radicals are produced by radiation, DNA can be protected and chemically restored by naturally existing thiols inside the cell (Figure 1). These thiols act as free radical scavengers and serve to protect cells against radiation damages by competing with oxygen for DNA radicals. Thus, as the level of these reducing species is reduced to low concentrations either through chemical suppressors such as N-ethylmaleimide or through synthesis blockers such as L-buthione-sulfoximine (L-BSO), sensitization of the cell occurs (6).

The most promising agent in this class has been L-BSO. L-BSO acts to deplete cellular glutathione (GSH) which has been shown to play a crucial protective role against cellular injury produced by ionizing radiation (30).

PLD Repair Inhibitor

The PLD (potentially lethal damage) repair inhibitors (6) comprise the third class of agents. It has been shown in several *in vitro* study systems that increased survival will occur under certain conditions after radiation exposure and before plating or other assay of cell survival. These studies provide evidence of the process of PLD repair. It is not known to what extent PLD occurs in normal tissues and whether or not compounds inhibiting it in tumors would also inhibit it to the same degree in normal tissues.

There are a number of mechanisms of action of PLD repair inhibition, and a number of drugs shown to be active either in cells in culture or *in vivo* in tumor systems. The best agents appear to be purine analogs such as 3-deoxyadenosine and 3-deoxyguanosine, which have been shown active both *in vivo* and *in vitro*. These agents alter the intracellular nucleotide pool in cancer cells, block DNA synthesis and thus inhibit repair of radiation-induced lesions.

Halogenated Pyrimidines

The fourth class is the brominated and iodinated pyrimidines. These halogenated pyrimidines were designed as thymidine analogues that would incorporate into the DNA of cycling cells (31). The ease of substitution of halogenated pyrimidines is due to the stereochemical similarity in atomic radius between the methyl group of thymidine (2.0 A) and the bromide (1.95 A) and iodine (2.15 A) atoms of the halogenated pyrimidines.

The mechanism of sensitization is attributed to enhanced susceptibility of substituted DNA to the induction of the radiation lesion and effects on repair. The degree of radiosensitization actually increases as the percentage of thymidine replacement increases. The thymidine substitution weakens the DNA chain. As incorporation into DNA is important for sensitization, the cells must be exposed to halogenated pyrimidines for a sufficient period.

Studies have recently been published using 5-iododeoxyuridine (IUdR) and bromodeoxyuridine (BUdR) (Figure 3) to enhance the efficacy of radioimmunotherapy (32-34). IUdR and BUdR are halogenated pyrimidine analogues. These compounds can be incorporated into DNA in place of thymidine. They are taken up preferentially by the more rapidly dividing tumor cells. Tumor Cells are sensitized to a degree dependent on the amount of analogue incorporated. However, conflicting results have been reported from Santos, et al. (32) and Pedley, et al. (33). Pedley and colleagues reported that IUdR at a very low dose of 200 mg/kg (total) resulted in radioresistance of tumors treated with I-131 anti-CEA antibodies. However, Santos et al. showed that IUdR at 1,200 mg/kg effectively increased therapeutic effectiveness of RIT.

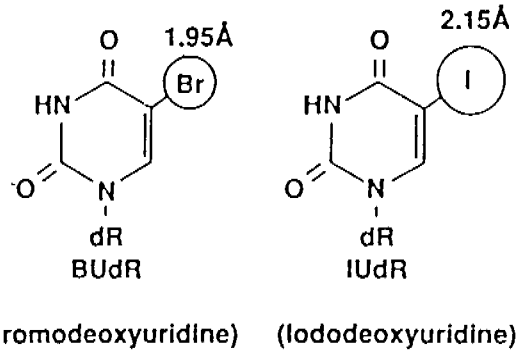

Figure 3. Structures of IUdr and BUdr.

Chemotherapeutic Agents

The fifth class of agents consists of currently approved chemotherapeutic agents that are not only cytotoxic to cancer cells, but also can sensitize cancer cells to radiation. All of these agents have unique mechanisms of killing cancer cells and sensitizing cells to radiation (35).

Paclitaxel (Taxol). Taxol, a novel antineoplastic agent, has recently been approved for use in ovarian and breast cancers (36). Taxol is a natural product derived from the needles and bark of the western yew (Figure 4), Taxus Brevifolia (37). It has a unique mechanism of action. Taxol acts as a mitotic inhibitor by inducing formation of microtubles and then prevents microtubular depolymerization (38). Research studies performed in our own research laboratories have

shown that paclitaxel could be a very effective radiosensitizer. (unpublished data)

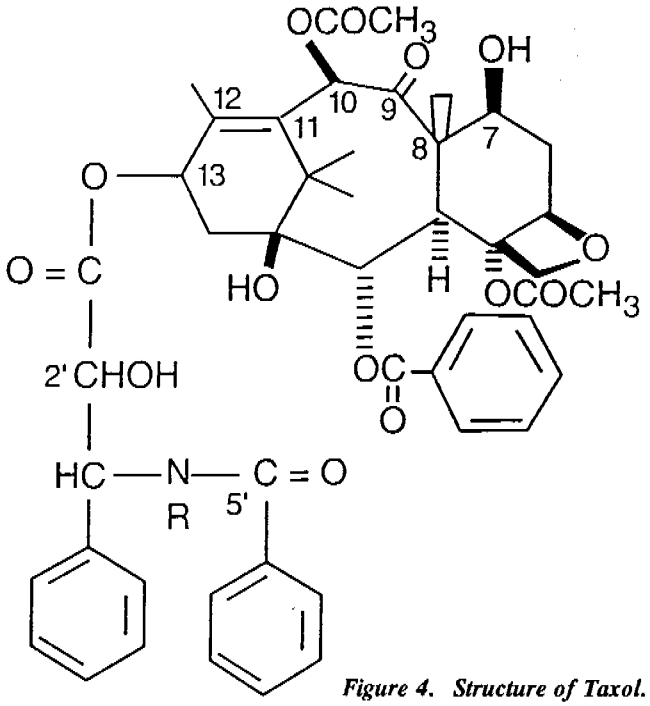

Figure 4. Structure of Taxol.

Taxol's mechanism of action essentially blocks cells in the G_2/M phase of the cell cycle (Figure 5); therefore, cells cannot form a competent mitotic spindle or dissociate a spindle.

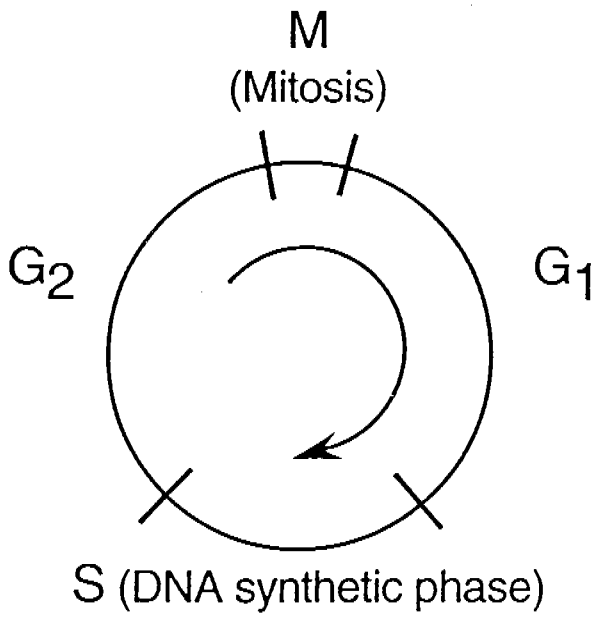

Figure 5. The cell cycle.

Studies of radiosensitivity in mammalian cells have determined that the G_2/M phase of mitosis in the cell cycle is most sensitive to radiation (39-40). The synchronization or blocking of cells in the G_2/M phase, induced by Taxol, can provide an effective means to increase the efficacy of radiation treatment of cancer cells (41-45).

In our first *in vitro* study using Taxol, we incubated CEA secreting TS-174 human carcinoma cells with I-131 labeled anti-CEA monoclonal antibody (I-131 anti-CEA MoAb actively binds to TS-174) without or with different concentrations (1, 2, 5, 7, 10 nM) of Taxol. The fraction of cells surviving after the two different treatments are plotted against Taxol concentrations (Figure 6). Taxol appeared to effectively enhance the cell killing effect of I-131 anti-CEA MoAb at concentrations as low as 5 nM. We also repeated the study using different radioactivities (25, 50, 100, 200, and 300 uCi I-131 anti-CEA MoAb). In this study (Figure 7), Taxol effectively enhanced the cell killing effect of I-131 anti-CEA MoAb at radioactivities as low as 50 uCi.

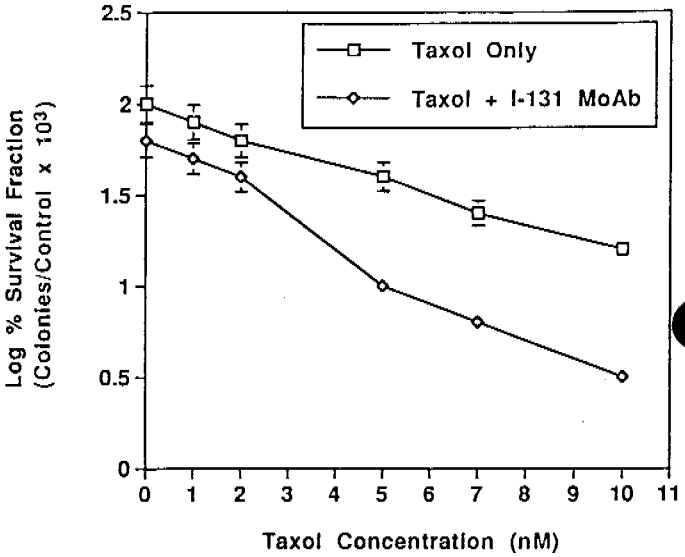

Figure 6. Survival curve of TS-174 human colon carcinoma cells at various concentrations of Taxol with and without I-131 anti-CEA MoAb.

In our *in vivo* study, we treated human colonic carcinoma implanted in nude mice (N=6 for each treatment group) with (a) I-131 MoAb (500 uCi) alone, (b) Taxol alone, (c) Taxol + I-131 MoAb, and (d) saline (control). The results are expressed as tumor growth ratios (tumor size increase/original tumor size at the beginning of treatment) versus time in days (Figure 8). The study shows Taxol increased the effectiveness of 500 uCi of I-131 anti-CEA MoAb in suppressing human colonic carcinoma growth in nude mice when compared to the results of animals treated with I-131 anti-CEA MoAb alone.

The results of our study have supported the hypothesis of a synergistic effect between Taxol and radiolabeled antibody in colon cancer cells *in vitro* and *in vivo*. Taxol has shown evidence of antitumor

activity in a variety of solid tumors, such as ovarian and breast carcinoma and malignant melanoma. Clinical investigations are evaluating Taxol's role in

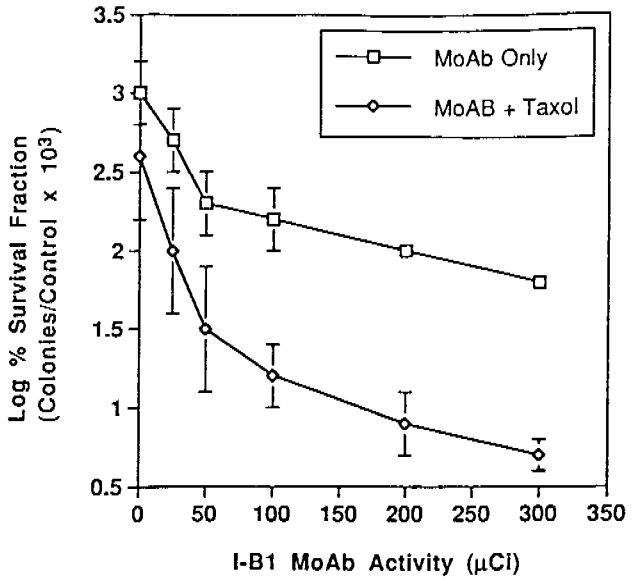

Figure 7. Survival curve of TS-174 human colon carcinoma cells at various radioactivities of I-131 anti-CEA MoAb with and without Taxol.

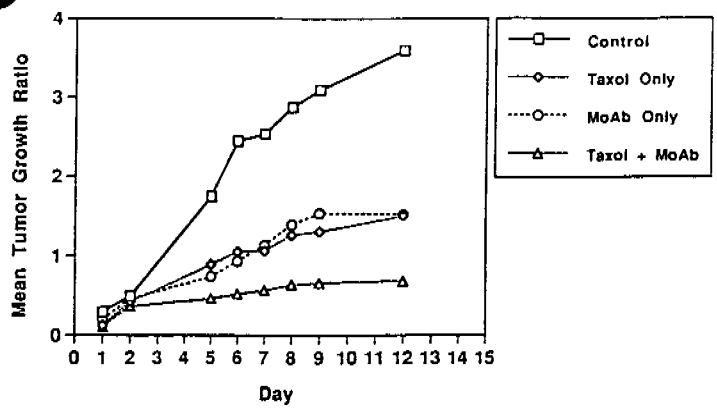

Figure 8. Suppression of tumor growth in nude mice receiving different treatments (N=6).

refractory acute leukemias, non-small cell lung carcinoma, as well as gastric, colon, and cervical carcinomas (37). Taxol's limitations in cancer therapy are its dose-related toxicities (myelosuppression, bradycardia, peripheral neuropathy, myalgia, arthralgia, nausea/vomiting, diarrhea, and alopecia) (38). The *in vivo* Taxol concentrations resulting in these toxicities are much higher than those in our studies. For example, a phase I study of Taxol reported peak plasma concentrations ranging from 2-10 uM with doses ranging from 175-275 mg/m^2 (41). Toxicities reported most commonly from this regimen included bone marrow suppression and alopecia, with both toxicities now commonly seen in clinical practice. The same study also reported a large volume of distribution (60 L/m^2) and accumulation of Taxol in the ascitic fluid of one patient, with maximum ascitic concentrations of 0.25 uM (250 nM), then stabilizing at a level of 40% above plasma concentrations for approximately 12 hours. The use of low dose Taxol in combination with radiation or RIT (radiolabeled antibody) may therefore enhance tumor cell killing effects without inducing the dose-related toxicities seen clinically.

5-Fluorouracil (5-FU). 5-FU has also emerged as one of the most promising clinical radiosensitizers now available (46). The chemical structure of 5-FU is shown in Figure 9. 5-FU is an analogue of the naturally occurring pyrimidine uracil with a fluorine atom substituted at the 5 carbon position of the pyrimidine ring in place of hydrogen. 5-FU is indicated for the palliative treatment of carcinoma of colon, rectum, breast, stomach, and pancreas that is not amenable to surgery or irradiation. It is also used as an adjunct to surgery for the treatment of various solid tumors (e.g., adenocarcinoma of the colon, rectal carcinoma, breast cancer).

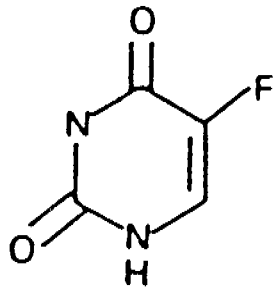

Figure 9. Structure of 5-FU.

The anti-cancer mechanism of action of 5-FU is related to its antimetastatic activity. 5-FU is thought to function as an antimetabolite in at least three different ways (47). It inhibits thymidylate synthetase, incorporates onto RNA, and blocks uracil phosphatase. Its radiation sensitization effect appears to come mainly from the inhibition of thymidylate synthetase by 5-fluoro-2'deoxyuridylate, a metabolite of 5-FU. This inhibition results in a loss of the de novo source of thymidine necessary for DNA synthesis. This causes imbalances in triphosphate pools and subsequent altered DNA damage repair (48). Clinical trials with 5-FU and radiotherapy are reporting impressive results with a variety of tumors.

Hydroxyurea. Hydroxyurea is the first clinically available derivative of urea to show antineoplastic activity (Figure 10). The drug is structurally similar to urea and acetohydroxamic acid, and is also a urease inhibitor (49). Hydroxyurea is indicated in the treatment of melanoma, resistant chronic myelocytic (granulocytic) leukemia, and recurrent, metastatic, or inoperable ovarian carcinoma. It is also used in combination with radiation therapy for local control of primary squamous cell (epidermoid) carcinoma of the head and neck.

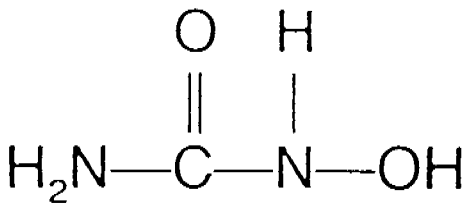

Figure 10. Structure of hydroxyurea.

The primary site of cytotoxic action for hydroxyurea is inhibition of the ribonucleotide reductase system. This highly regulated enzyme system is responsible for the conversion of ribonucleotide diphosphates to the deoxyribonucleotide form, which can subsequently be utilized in either de novo DNA synthesis or DNA repair.

In 1965, hydroxyurea was shown to selectively kill cells that were synthesizing DNA and to block cells at the G_1-S border (50). This capacity to sensitize cells occurred when hydroxyurea was continually present or was added after irradiation. It has been shown that the time course of repair of radiation-induced, single strand DNA breaks is partially inhibited by exposure to hydroxyurea before and after irradiation (51). Hydroxyurea and radiotherapy have been used in patients with advanced stages of cervical cancer. A survival advantage was noted in a small, prospective, randomized trial comparing radiotherapy/hydroxyurea with radiotherapy alone.

Cisplatin. Cisplatin is a platinum-containing antineoplastic agent (52). The drug is an inorganic complex that contains a platinum atom surrounded in a plane by 2 chloride atoms and 2 ammonia molecules in cis position. It is used for the treatment of metastatic testicular tumors, metastatic ovarian tumors, advanced bladder carcinoma, and a wide variety of other neoplasms. Cisplatin is often used as a component of combination chemotherapeutic regimens.

The exact mechanism of cytotoxic action of cisplatin has not been conclusively determined. Cisplatin binds to DNA and inhibits DNA synthesis. It also inhibits protein and RNA synthesis. The drug also produces intrastrand and interstrand crosslinks in DNA. DNA with these crosslinks are more sensitive to radiation damage (53). This is thought to be the mechanism responsible for radiation sensitization. Unfortunately, the effect of radiation sensitization of cisplatin is still inconclusive. Cisplatin has been shown to potentiate radiation damage in some, but not all, *in vitro* culture cell lines and in some, but not all, *in vivo* experimental tumor models.

SUMMARY

Clinical applications of cancer therapy with radiolabeled monoclonal antibody are hindered considerably by the complex *in vivo* behavior of antibody and by the pathophysiology of tumor. Pharmacologic enhancement using vasoactive drugs, calcium channel blockers, nicotinamide and interferons have offered some potential solutions to the problems by enhancing the local concentration of radiolabeled antibodies. Alternatively, pharmacologic agents can be used to sensitize tumor cells to radiation. The most promising group of drugs in this approach is the currently-approved chemotherapeutic agents including paclitaxel, 5-FU, hydroxyurea and cisplatin. In the future, it would seem logical to integrate these two different approaches in a single treatment plan so that the efficacy of RIT could be effectively improved.

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QUESTIONS

1. What is the major requirement for a successful application of radiolabeled antibody therapy in cancer?

   a. low target (tumor) to non-target (normal tissue) ratio
   b. low chemical toxicity
   c. The capability of the radiolabeled antibody to destroy tumor cells without harming normal tissue.
   d. The presence of hypoxic cells in the tumor.

2. What is causing the inconsistent results from current clinical studies with radiolabeled antibodies for cancer therapy?

   a. complex tumor pathophysiologic factors
   b. The gamma isotopes are not ideal.
   c. complicated dosing schemes
   d. Radiolabeled antibodies are totally ineffective.

3. What is the major hindrance of developing radiolabeled antibody for cancer therapy?

   a. There is no acceptable therapeutic radioisotope.
   b. The radiolabed antibody preparation is too expensive.
   c. The tumor pathophysiology and *in vivo* radiolabeled antibody biodistribution are very complex.
   d. The radiolabeling techniques are too difficult.

4. How is the blood perfusion in tumor different from blood perfusion in normal tissue?

   a. It is better and more rapid.
   b. It is poorer and less uniform.
   c. It is especially good in the central portion of the tumor.
   d. There is not that much difference.

5. What is causing the rapid and drastic reduction in tumor blood flow as the tumor mass increases?

   a. There is not enough systemic pressure.
   b. All tumor vessels are constricted by the stimulation of excessive sympathomimetic stimulation.
   c. The number of vessels in the tumor decreases proportionately as the tumor enlarges.
   d. Tumor cells grow too slowly.

6. How does the pH in a tumor affect the tumor capillaries?

   a. The pH is acidic and causes the capillaries to lose their flexibility.
   b. The pH is alkaline and causes the capillaries to expand.
   c. The pH is neutral and causes the capillaries to grow faster than the surrounding tissue.
   d. The pH does not affect the capillaries in the tumor.

7. What is the major method of transporting radiolabeled antibody molecules across the interstitial fluid barrier to reach the cancer cells?

   a. convection
   b. active transport
   c. diffusion
   d. molecular linking

8. What is the basis for using vasoactive drugs to increase tumor blood flow?

   a. Tumor tissue lacks vascular smooth muscle.
   b. Normal tissue does not have receptors to these drugs.
   c. Tumor blood vessels respond to vasoconstriction only.
   d. Tumor tissue has low interstitial pressure.

9. What is the beta-adrenergic blocking agent that has been shown to be useful to enhance the tumor perfusion?

a. nembutal
b. hydralazine
c. isoproterenol
d. propranolol

10. How does pentobarbitone sodium increase tumor perfusion?

a. It causes vasolidation of tumor vessels.
b. It stimulates the production of catecholamines.
c. It causes compensatory sympathomimetic vasoconstriction.
d. It modulates microregional perfusion.

11. Which of the following pharmacologic agents may prevent the transient mircroregional alterations in tumor blood flow?

a. urethane
b. angiotensin II
c. calcium channel blockers
d. epinephrine

12. How does nicotinamide improve tumor perfusion?

a. It decreases the amount of acute hypoxia in the tumor.
b. It increases mortality in endotoxin-induced shock in rats.
c. It can cause vasoconstriction.
d. It can induce transient ischemia in tumor tissue.

13. What is the pharmacologic effect of flunarizine:

a. It can enhance tumor antigen expression.
b. It is a cytokine.
c. It is a calcium channel blocker.
d. It increases the acutely hypoxic cell population.

14. Which of the following statements is true about the expression of tumor antigen?

a. It can be modulated by the recombinant interferon.
b. It is not important for antibody binding.
c. It is sensitive to verapamil.
d. It is proportional to tumor blood flow.

15. Which of the following agents has been shown to be effective in increasing blood flow in experimental tumors?

a. vasopressin
b. epinephrine
c. angiotensin II
d. nifedipine

16. Which of the following agents is a hypoxic cell sensitizer?

a. benzamide
b. bromodeoxyuridine
c. misonidazole
d. taxol

17. What is the mechanim of radiation sensitization of L-buthione-sulfoximine (L-BSO)?

a. It minimics oxygen.
b. It depletes cellular glutathione.
c. It promotes the synthesis of thiols.
d. It acts as a free radical.

18. How does oxygen sensitize cells to radiation?

a. It increases cellular tension.
b. It interacts with the DNA radical to form DNA-peroxyl radical.
c. It produces interstrand and intrastrand crosslinks in DNA.
d. It impairs the PLD repair mechanism.

19. What kind of pharmacologic agent is Taxol?

a. a novel antineoplastic agent
b. monoclonal antibody against CEA
c. purine analog
d. murine protein

20. The is the major mechanism of action of Taxol?

a. a pyrimidine analog to replace DNA to cause cell toxicity
b. a potentially lethal damage repair inhibitor
c. a nuclear ADP-ribosyl transferase inhibitor
d. a mitotic inhibitor by inducing formation of microtubules

21. How does Taxol sensitize cancer cells:

a. It binds to hypoxic cells.
b. It blocks cancer cells in the G_2/M phase of the cell cycle.
c. It causes synchronization of cells in the G_1/S phase of the cell cycle.
d. It causes depolymerization of the cell membrane.

22. What is the mechanism of radiation sensitization of halogenated pyrimidines?

a. They can inhibit RNA synthesis.
b. They can be incorporated into DNA in place of thymidine to weaken the DNA.
c. They block cells in the G_1 phase of the cell cycle.
d. They are preferentially taken up by the cancer cell nuclei.

23. Which of the following chemotherapeutic agents can sensitize tumor cells to radiation?

a methotrexate
b. BCNU
c. 5-FU
d. Cytoxan

24. What is the mechanism of radiation sensitization of hydroxyurea?

a. It inhibits repair of single strand DNA breaks.
b. It promotes membrane synthesis.
c. It replaces thymidine incorporation into DNA.
d. It sensitizes hypoxic cells to radiation.

25. What is the probable radiation sensitization mechanism of cisplatin?

a. It inhibits the thiols production.
b. It produces intrastrand and interstrand crosslinks in DNA.
c. It protects the calcium channels.
d. It binds up all RNA.