

Radiation Sensitizers: A Selective Review of Molecules Targeting DNA and non-DNA Targets

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The ideal radiation sensitizer would reach the tumor in adequate concentrations and act selectively in the tumor compared with normal tissue. It would have predictable pharmacokinetics for timing with radiation treatment and could be administered with every radiation treatment. The ideal radiation sensitizer would have minimal toxicity itself and minimal or manageable enhancement of radiation toxicity. The ideal radiation sensitizer does not exist today. This review outlines the concept of combining 2 modalities of cancer treatment, radiation and drug therapy, to provide enhanced tumor cell kill in the treatment of human malignancies and discusses molecules that target DNA and non-DNA targets. Combining drugs that have unique mechanisms of action and absence of overlapping toxicities with systemically administered radiotherapy should be exploited in future clinical trials. This is an exciting time in clinical oncology research, because we have a plethora of new molecules to evaluate.

Key Words: radiation sensitizers; 5-fluorouracil; platinum; gemcitabine; topoisomerase; epidermal growth factor inhibitors; vascular endothelial growth factor inhibitors

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Surgery, radiation, and chemotherapy have been the mainstays of treatment for human malignancies for more than 40 y. The use of a combination of radiation and chemotherapy is often called chemoradiation in the medical literature. For most of the last 4 decades, this has involved the use of cytotoxic agents with external beam radiation. Recently, however, with newer molecules that target very specific pathophysiology or molecular pathways and the use of radiation delivered systemically by antibodies or hormones labeled with radionuclides, the concept of radiation sensitizers has been expanded.

Heidlberger's preclinical studies in 1958 were the first to establish the concept of giving drugs concomitantly with radiation to enhance the effect of radiation (1). In the 1960s, Moertel et al. from the Mayo Clinic reported improved survival of patients with stomach and pancreatic cancer

when the 2 modalities were combined (2). Initial reports showed only modest improvement; however, with a disease that had such a dismal prognosis, any improvement was welcome. In 1974, Nigro's trial of 5-fluorouracil (FU) in combination with mitomycin C as concurrent therapy with radiation for cancer of the anal canal demanded the attention of both the radiation and medical oncology communities (3). Combined modality therapy is now well established in the following cancers: head and neck, esophagus, lung, stomach, pancreas, anal canal, and cervix (4–14).

External beam radiation therapy and the combination of drugs and systemically administered radiation show interesting pharmacokinetic differences. Unlike drugs and systemically administered radiation, external beam radiation will penetrate tissue and cellular boundaries without any of the usual pharmacokinetic barriers. The dose delivered can be preplanned with external beam radiation and brachytherapy. Chemotherapy and radionuclides attached to carrier molecules, such as monoclonal antibodies or peptides, require distribution from the site of administration to the blood, tissue, interstitial space, cell, and subcellular target.

An ideal radiation sensitizer would reach the tumor in adequate concentrations and act selectively in the tumor compared with normal tissue. It would have predictable pharmacokinetics for timing with radiation treatment and could be administered with every radiation treatment. The ideal radiation sensitizer would have minimal toxicity itself and minimal or manageable enhancement of radiation toxicity. The ideal radiation sensitizer does not exist today.

This review will discuss the concept of combining 2 modalities of cancer treatment, radiation and drug therapy, to provide enhanced tumor cell kill in the treatment of human malignancies. These drugs may be traditional chemotherapeutic agents or some of the newer molecular targeting agents. Much of the published clinical research has reported on the traditional cytotoxic agents, nucleoside analogs and platinum compounds. Substantially more information is currently available from basic and clinical research with these agents in combination with standard external beam radiation therapy than with systemically administered therapy, such as radiolabeled peptides or radiolabeled monoclonal antibodies. The concepts, however, should be applicable in both arenas.

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Some of the newer agents, such as growth factor inhibitors, cyclooxygenase enzyme 2 (COX-2) inhibitors, farnesyltransferase inhibitors, and inhibitors of new vessel formation, will also be reviewed in this paper.

HOW DO CONVENTIONAL CHEMOTHERAPY DRUGS BRING ABOUT RADIOSENSITIZATION?

5-Fluorouracil

One of the first agents to be exploited as a radiation sensitizer was 5FU, and the basis for its action is currently thought to be primarily from thymidilate synthase inhibition (15). Interestingly, noncytotoxic concentrations of 5FU can also increase radiation sensitivity *in vitro*, but only when cells are incubated with drug before radiation. Because of the short half-life of 5FU in plasma, these laboratory studies have suggested that the drug should be given by continuous intravenous infusion (CIVI) during a course of fractionated radiation if radiosensitization of most fractions is to be achieved. In fact, the use of CIVI of 5FU with radiation has become the preferred therapy for both pancreatic and rectal cancer (16,17).

Of course, this approach requires long-term venous access and specialized pumps over 5–6 wk, which can predispose the patient to thrombosis or infection. An oral form of 5FU, the prodrug capecitabine, may prove to make the protracted combined modality therapy easier and safer in the clinic, but additional studies are necessary.

Analogues of Platinum

The platinum analogues include cisplatin, carboplatin, and oxaliplatin. These are used clinically in combination with radiation in a variety of solid tumors. When given before or after radiation, these analogues are believed to enhance cell killing by one of several mechanisms. These mechanisms include enhanced formation of toxic platinum intermediates in the presence of radiation-induced free radicals, inhibition of DNA repair, radiation-induced increase in cellular platinum uptake, and cell cycle arrest (18–22).

The concomitant use of cisplatin or carboplatin has been shown to improve clinical outcome for non-small lung cancer, cervical cancer, and cancers of the head and neck (23–25).

Oxaliplatin is a third-generation cisplatin analogue that has recently been approved for use in colorectal cancer. Freyer et al. (26) have reported using oxaliplatin along with 5FU and folinic acid and concomitant radiation for rectal cancer. The Eastern Cooperative Oncology Group and Cancer and Leukemia Group B also have studies underway looking these same combinations in rectal cancer.

Gemcitabine

Gemcitabine is an analogue of cytarabine (cytosine arabinoside) with a broad spectrum of clinical activity against human cancers, particularly pancreatic and non-small cell lung cancer (27–30). Gemcitabine is a potent radiosensitizer in both laboratory studies and clinical trials. In the labora-

tory, there was no evidence of radiosensitization when cells were radiated before gemcitabine exposure, and the greatest enhancement ratio was seen when cells were incubated for 24 h before irradiation (31). Maximum sensitization appears to require simultaneous redistribution into S phase along with deoxyadenosine triphosphate (dATP) pool depletion (32). The dATP pool depletion is a result of ribonucleotide reductase inhibition.

Minimally cytotoxic concentrations of gemcitabine can radiosensitize, and unlike 5FU, do not have to be given continuously. Clinical trials evaluating once- or twice-weekly gemcitabine along with radiation in head and neck cancer and pancreatic cancer are in process (33).

DNA Topoisomerase I-Targeting Drugs

The camptothecin derivatives, topotecan and irinotecan, target the topoisomerase enzyme. The activities of topoisomerase I are important for many aspects of DNA metabolism, including initiation and elongation of RNA transcription, DNA replication, and the regulation of DNA supercoiling, which is essential for maintaining the stability of the genome (34).

Drug interference with topoisomerase I-mediated cleavage/rejoining of DNA strands is thought to be the common mechanism of action of these drugs. The presence of up-regulated levels of topoisomerase in tumor cells compared with normal cells suggests a therapeutic advantage of topoisomerase I-targeting drugs selective against slow-growing as well as rapidly proliferating tumors.

Chen et al. (35) conducted clonogenic survival assays using cultured mammalian cells. They found that drug incubation with camptothecin derivatives radiosensitized log-phased human MCF-7 breast cancer cells in a schedule-dependent manner. The radiation sensitization effect was observed when the cells were exposed to drug treatment before or concurrent with radiation treatment but not after radiation treatment. The implication of these observations is that camptothecin derivatives should be administered before or concurrently with radiation to optimize the radiosensitizing effect during chemoradiation trials.

A wide range of clinical antitumor activity, including activity against colorectal cancer, ovarian cancer, both small cell and non-small cell lung cancer, and malignant lymphomas, has been seen with the camptothecin derivatives. Based on clinical success as systemic therapy, chemoradiation trials are ongoing in a variety of solid tumors.

MOLECULES THAT ARE RADIOSENSITIZING BUT DO NOT TARGET DNA

Most of our chemotherapeutic agents and radiation therapy have focused on DNA as the target. Non-DNA targets may be effective in killing the cell or modifying the cell in such a way that it is more susceptible to cell killing after radiation-induced damage.

Epidermal Growth Factor Receptor Blockade

The ErbB family is a group of 4 structurally similar growth factor receptors with tyrosine-kinase activity (epidermal growth factor receptor [EGFR], HER2/neu, ErbB-3, ErbB-4), which dimerize on binding with several ligands, including EGF and transforming growth factor (TGF), allowing downstream transduction of mitogenic signals. New agents developed to inhibit EGFR function include monoclonal antibodies and small-molecule receptor tyrosine-kinase inhibitors. In this review, the emphasis will be on results of *in vivo* and *in vitro* studies with the monoclonal antibody, C225 (cetuximab), and the tyrosine-kinase inhibitor CI-1033 (gefitinib, Iressa; AstraZeneca plc) as radiation sensitizers.

Squamous cell carcinomas (SCC) arising in the head and neck have high expression of EGFR. Overexpression of this receptor often accompanies growth and development of these malignant tumors. The anti-EGFR monoclonal antibody, C225, is a potent antiproliferative agent in these tumors. It is capable of inhibiting tumor cell growth kinetics. In addition, preclinical studies have demonstrated the capacity of C225 to enhance *in vitro* radiosensitivity and to promote radiation-induced apoptosis (36). In studies using a xenograft model system, human head and neck cancer cells are particularly sensitive to radiation damage when the EGFR signaling pathway in these cells is blocked by C225. Most impressively, the *in vivo* tumor response after combined administration of C225 and radiation was dramatic and long lasting. Such profound antitumor activity *in vivo* appeared to derive not only from proliferative growth inhibition (with associated cell cycle redistribution) but also from inhibition of postradiation damage repair and inhibition of tumor angiogenesis (37). Because locoregional disease recurrence remains the dominant form of treatment failure for these patients, the results of phase 3 clinical trials evaluating this approach are eagerly awaited.

Although C225 is a reversible inhibitor that exhibits receptor selectively, CI-1033 appears to bind to all tyrosine-kinase receptors irreversibly and thus may have a larger spectrum of activity in the clinic.

Farnesyltransferase Inhibitors

Activation of Ras by mutation, overexpression, or signaling through tyrosine-kinase receptors is associated with radioresistance. It follows that therapies which inhibit Ras function could be effective means to radiosensitize certain solid tumors. Brunner et al. (38) used clonogenic assays with human and rodent tumor cell lines and transfected cell lines in the testing of radiosensitivity. Xenograft tumors were treated with farnesyltransferase inhibitors and radiation and assayed for *ex vivo* plating efficiency and regrowth of tumors. Blocking the prenylation of Ras proteins in cell lines with Ras activated by mutations or receptor signaling resulted in radiation sensitization *in vitro* and *in vivo*. The PI3 kinase downstream pathway was identified as a contributor to Ras-mediated radiation resistance. In a phase 1 trial

of the farnesyltransferase inhibitor, L-778-123, in advanced head and neck cancer and non-small cell lung cancer, the same investigators demonstrated a high response rate coupled with mild toxicity (39).

COX-2 Inhibitors

Prostaglandins have been known to impact the radiosensitivity of cells and tissues, and many studies have centered on exploiting nonspecific prostaglandin inhibitors such as nonsteroidal antiinflammatory drugs for therapeutic gain. These studies have ultimately been unsuccessful because of a lack of targeted specificity against the tumor. The discovery of the inducible COX-2 and development of some highly selective inhibitors (which spare the constitutive COX-1 activity) have renewed excitement for modulating tumor prostaglandins as a method of specific radiosensitization of tumors, while at the same time sparing normal tissues (36). Celecoxib is the selective COX-2 inhibitor that has been studied in non-small cell lung cancer and in upper gastrointestinal tract cancers.

Targeting Tumor Vasculature

The progressive enlargement of a tumor mass requires the formation of new blood vessels to facilitate delivery of nutrients and oxygen. This process is called angiogenesis, and all types of solid tumor cells promote new blood vessel formation by releasing endothelial cell growth factors. Two critically important growth factors are basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). These support endothelial cell proliferation and migration of blood vessels.

Several approaches targeting bFGF and VEGF have been developed and include the use of antibodies such as bevacizumab and thalidomide. Bevacizumab has recently been approved for use in colorectal cancer, and thalidomide has been shown to have activity in Kaposi's sarcoma, multiple myeloma, prostate cancer, and islet cell carcinomas.

Some investigators have expressed concern that inhibition of tumor angiogenesis could increase the fraction of hypoxic tumor cells and, as a result, induce radiation resistance. Accordingly, future clinical trials with this class of agents must keep this in mind and be closely monitored.

CONCLUSION

The use of traditional cytotoxic chemotherapy drugs to augment the effectiveness of external beam radiation therapy in the treatment of solid tumors is established and well documented in the medical literature. The remarkable success of radiolabeled antibodies and radiolabeled peptides as systemic therapy for hematologic malignancies and neuroendocrine malignancies begs for even further improvement by evaluating the approaches reviewed in this article. Virtually no malignancies have been cured using single modalities of treatment. Therefore, studies are needed to explore combinations of systemically administered radiotherapy with one or a combination of the molecular targeted

therapies. Combining drugs that have unique mechanisms of action and absence of overlapping toxicities with systemically administered radiotherapy should be exploited in future clinical trials. For example, once individual drug toxicities in combination with radiation have been established in humans, it would be interesting to explore various combinations such as cisplatin, EGF inhibitors, and antiangiogenic agents. This is an exciting time in clinical oncology research, because we have a plethora of new molecules to evaluate.

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