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Metronidazole has also been proposed as a radiation sensitizer for hypoxic cells

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Abstract
A radio sensitizer is a drug that makes tumor cells more sensitive to radiation therapy. One of the major limitations of radiotherapy is that the cells of solid tumors become deficient in oxygen. Solid tumors can outgrow their blood supply, causing a low-oxygen state known as hypoxia. Oxygen is a potent radiosensitizer, increasing the effectiveness of a given dose of radiation by forming DNA-damaging free radicals. Tumor cells in a hypoxic environment may be as much as 2 to 3 times more resistant to radiation damage than those in a normal oxygen environment. Much research has been devoted to overcoming this problem including the use of high pressure oxygen tanks, blood substitutes that carry increased oxygen, hypoxic cell radiosensitizers such as misonidazole and metronidazole, and hypoxic cytotoxins, such as tirapazamine. A newer approach involves the use of an oxygen diffusion-enhancing compound to re-oxygenate hypoxic tumor tissue. Extensive studies show that Metronidazole increased by more than two times the radiosensitivity of cells from the central zones of the tumor and did not influence the radiation response of cells from the peripheral zones. Metronidazole was shown to inhibit the repair of potentially lethal radiation damages.

Keywords: - Radiation mitigators, radioprotectors, radiosensitizers, radiotherapy.

Introduction

Radiotherapy is regarded as one of the most important therapeutic modality for the treatment of malignant lesions. This field is undergoing rapid advancements in the recent times. With the use of radiosensitizers and radioprotective agents, the course of radiotherapy has improved the sensitization of tumor cells and protection of normal cells, respectively. The aim of this paper was to critically review and analyze the available compounds used as radiosensitizers, radioprotectors, and radiation mitigators. For reviewing, the author used the electronic search for the keywords 'Radiosensitizers', 'Radioprotectors', 'Radiation mitigators' on PubMed for inclusion of previously published articles and further search of reference papers on individual radiosensitizing and radioprotecting agents was done. Radiosensitizers are agents that sensitize the tumor cells to radiation. These compounds apparently promote fixation of the free radicals produced by radiation damage at the molecular level. The mechanism of action is similar to the oxygen effect, in which biochemical reactions in the damaged molecules prevent repair of the cellular radiation damage. Free radicals such as OH^+ are captured by the electron affinity of the radiosensitizers, rendering the molecules incapable of repair. Radioprotectors are compounds that are designed to reduce the damage in normal tissues caused by radiation. These compounds are often antioxidants and must be present before or at the time of radiation for effectiveness. Other agents, termed mitigators, may be used to minimize toxicity even after radiation has been delivered. This article tries to discuss the various aspects of radiosensitizers, radioprotectors, and radiation mitigators including the newer agents.

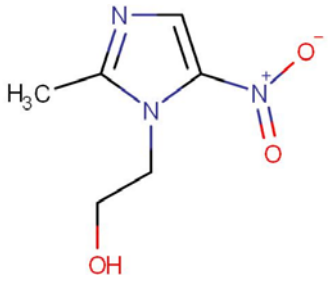

Fig: Metronidazole Molecule

Tumor hypoxia is the situation where tumor cells have been deprived of oxygen. As a tumor grows, it rapidly outgrows its blood supply, leaving portions of the tumor with regions where the oxygen concentration is significantly lower than in healthy tissues. Hypoxic micro-environements in solid tumors are in result of available oxygen being consumed within 70 to 150 µm of tumour vasculature by rapidly proliferating tumor cells thus limiting the amount of oxygen available to diffuse further into the tumor tissue. In order to support continuous growth and proliferation in challenging hypoxic environments, cancer cells are found to alter their metabolism. Furthermore, hypoxia is known to change cell behavior and is associated with extracellular matrix remodeling and increased migratory and metastatic behavior. Cells undergo a variety of biological responses when placed in hypoxic conditions, including activation of signalling pathways that regulate proliferation, angiogenesis and death. Cancer cells have adapted these pathways, allowing tumours to survive and even grow under hypoxic conditions, and tumour hypoxia is associated with poor prognosis and resistance to radiation therapy. Many

elements of the hypoxia-response pathway are therefore good candidates for therapeutic targeting. Hypoxia is a common characteristic of locally advanced solid tumors that has been associated with diminished therapeutic response and, more recently, with malignant progression, that is, an increasing probability of recurrence, locoregional spread, and distant metastasis. Emerging evidence indicates that the effect of hypoxia on malignant progression is mediated by a series of hypoxia-induced proteomic and genomic changes activating angiogenesis, anaerobic metabolism, and other processes that enable tumor cells to survive or escape their oxygen deficient environment. The transcription factor hypoxia-inducible factor 1 (HIF-1) is a major regulator of tumor cell adaptation to hypoxic stress. Tumor cells with proteomic and genomic changes favoring survival under hypoxic conditions will proliferate, thereby further aggravating the hypoxia. The selection and expansion of new (and more aggressive) clones, which eventually become the dominant tumor cell type, lead to the establishment of a vicious circle of hypoxia and malignant progression.

Hypoxia-Induced Mechanisms for Cell Survival, Invasion, and Metastasis

Hypoxia (oxygen tension [pO_2] <7 mmHg) can induce changes in the proteome of tumor cells that lead to impaired growth or to cell death, including cell-cycle arrest, differentiation, apoptosis, and necrosis [8-12]. Alternatively, however, hypoxia can induce proteomic changes that allow the tumor cells to successfully adapt to or overcome their O_2- and nutrient-deprived state and to survive in or escape from their hostile environment. This is accomplished through hypoxia-stimulated angiogenesis, glycolysis, inhibition of apoptosis, and upregulation of growth factors (e.g., platelet-derived growth factor-B [PDGF-B], transforming growth factor beta [TGF-β], insulin-like growth factor-2 [IGF-2], epidermal growth factor [EGF]) and other proteins involved in tumor invasiveness (e.g., urokinase-type plasminogen activator). Systemic responses leading to an elevation in the hemoglobin level, and thus improvement in the O_2 transport capacity of the blood, can support the local mechanisms mentioned within tumors (e.g., through activation of the genes for erythropoietin, transferrin, and transferrin receptors) [6, 13]. Additionally, hypoxia may induce downregulation of adhesion molecules, thereby facilitating tumor cell detachment [14, 15]. Many of these hypoxia-inducible genes are controlled by hypoxia-inducible factor 1 (HIF-1).

Role of Metronidazole as Novel Radiation Sensitizers Targeting Tissue Hypoxia

Classic experiments performed in the early part of this century first established that the absence of oxygen diminished the lethal effects of radiation therapy. In general, under anaerobic conditions, the radiation dose must be increased by a factor of 2.5 to 3 to achieve the same degree of cytotoxicity that occurs under oxygenated conditions. The radiosensitivity of cells increases as the partial pressure of oxygen increases from 0 to 20 to 40 mmHg. Cells at oxygen tensions of 20 to 40 mmHg demonstrate radio sensitivities that are nearly equivalent to those of cells exposed to 100% oxygen. Therefore, increasing oxygen pressure beyond this minimal level is not necessarily beneficial [1]. - Hypoxic conditions are present in tumors and, based on experimental studies, hypoxia appears to be a major cause of treatment failure with radiation therapy and chemotherapy. In animal models, 10% to 20% of tumor cells are generally found to be hypoxic [2, 3]. Direct oxygen measurements in human tumors have confirmed tumor hypoxia in glioblastoma multiforme and in carcinomas of the breast, uterine cervix, and head and neck [2, 3]. Potential mechanisms of chronic or transient hypoxia include obstruction of blood flow, inadequate or defective (malignant) angiogenesis, and failure of cellular growth control, allowing the cell population to outstrip the capacity of the capillary blood supply. In general, tumor cells are oxygenated up to a distance of about 150 mm from capillaries; beyond this distance, tumor cells become oxygen-depleted and either die or survive in a hypoxic state [4-8]. Since hypoxic cells are substantially more resistant to radiation than are oxygenated cells, even a small hypoxic fraction in a tumor will dominate the overall response to radiation by increasing the probability that some viable tumor cells will survive the treatment. Conversely, few hypoxic cells exist in normal tissues. Therefore, therapies that increase the delivery of oxygen to hypoxic cells are not expected to increase the toxicity of radiation to normal tissues. Several clinical studies have demonstrated that tumors with low median partial pressures of oxygen have a higher in-field failure rate after radiation therapy. For example, compared with well-oxygenated tumors of similar size and stage, tumors of the uterine cervix have been found to have a higher rate of recurrence if the median partial pressure of oxygen in tissue is < 10 mmHg [9]. A similar phenomenon has been noted in patients with head and neck cancer [10].

Radiation sensitizers mimic the effects of oxygen to increase radiation damage. The most common class of radiation sensitizer that has been evaluated in clinical studies is the nitroimidazoles (eg, misonidazole). However, their major limitation is neurotoxicity, which has prevented the delivery of effective doses with conventional daily fractionated radiation. One randomized trial suggested improved survival when the radiosensitizer nimorazole was used to treat head and neck cancer [22], but thus far, radiation sensitizers have not led to consistent improvements in the therapeutic index compared with optimal fractionation schedules of radiation used alone [23].

Genomic Instability

The tumor microenvironment is considered hostile, being characterized by areas of chronic or transient hypoxia, low pH, nutrient deprivation, and energy depletion. In a classic study, *Reynolds* and colleagues examined the consequences of tumor growth under these conditions, using a tumorigenic cell line carrying a recoverable, chromosomally based lambda phage shuttle vector designed to identify mutations without the need for a genetic selection of mutant cells [42]. The cells were grown concurrently either in culture or as tumors in nude mice. The frequency of mutations in the cells within the murine tumors was found to be five times that of the comparator cultured cells (9.3×10^{-5} versus 1.8×10^{-5}, respectively; $p<0.0001$). Moreover, the mutation patterns of the two cell groups differed, with the tumor-grown cells displaying significantly more deletions and transversions than those grown in culture. Particularly noteworthy is the finding that exposure of cultured cells to hypoxic conditions produced an elevated mutation frequency and a mutation pattern similar to those observed in the tumor-grown cells. These findings suggest that the type of genetic

instability found in malignant tumors may in part be the consequence of specific mutagenic properties of the hypoxic microenvironment [43].

Point mutations may develop in tumor cells exposed to hypoxia and reoxygenation through several mechanisms, including insufficient DNA repair, errors in DNA replication, or both [42, 46]. Metabolic damage to DNA bases may also play a role in point mutations, since a hypoxia-reoxygenation sequence may cause oxidative damage. Such damage has the potential to lead to various pyrimidine- and purine-derived lesions in DNA. The most abundant of these is the generation of 8-hydroxyguanine, which has been shown to mispair with adenine and lead to G: C to T: A transversions [47, 48].

Several studies have demonstrated that hypoxia followed by reoxygenation can lead to gene amplification [49], which, together with chromosomal rearrangements, can be caused by DNA strand breaks or decreased repair of DNA strand breaks [45]. The strand breaks may occur as a result of increased expression of endogenous endonuclease [50]. Hypoxia-induced point mutations, chromosomal rearrangements, and gene amplification may, in turn, promote development of metastatic disease by several mechanisms, including inactivation of metastasis suppressor genes or increased expression of oncogenes involved in the metastatic process, for example, genes encoding for angiogenesis and growth factors.

The overall effect of hypoxia-induced mutation and gene amplification is an increase in the number of gene variants. It has also been suggested that hypoxia exerts a strong selection pressure on malignant cells [5, 6, 51, 52]. Thus, any malignant cells with proteomic or genomic adaptive changes favoring survival under hypoxic conditions (e.g., decreased capacity for cell-cycle arrest, differentiation, or apoptosis, or increased angiogenic potential) will have selection advantages over nonadapted cells. The progeny of the adapted cells will increase at a greater rate than those of the nonadapted cells and eventually will become the dominant cell subpopulation within the tumor. Moreover, these cells are likely to have more favorable traits related to invasion, metastasis capability, and aggressiveness, providing the basis for the clinical findings of increased locoregional spread, distant tumor metastasis, and treatment resistance in advanced disease. Additionally, hypoxia-mediated clonal selection of tumor cells with genomic changes leading to apoptotic insensitivity, and possibly increased angiogenic potential, further aggravates tumor hypoxia and establishes a vicious circle of hypoxia and malignant progression that is considered a pivotal biological mechanism of advanced (and often incurable) disease [52].

Reoxygenation and Malignant Progression

Results of several preclinical studies have provided evidence that hypoxia, with or without reoxygenation, may result in malignant progression and poor prognosis. In the *Reynolds et al*. study discussed above, the frequency and pattern of mutations in hypoxically cultured cells were similar to those observed in the tumor-grown cells [42]. The mutation frequency of the cultured cells continued to rise with repeated exposure to hypoxia followed by reoxygenation, suggesting impairment of cellular repair capabilities. It has been suggested that repeated hypoxia-reoxygenation cycles may function as a mutagenic force by increasing the levels of superoxides and other O_2 radicals [53]. Cycles may also lead to chromosomal rearrangements and gene amplification [43]. As stated in a lecture given by *P.W. Vaupel, M.D.* (1994), at the Ernst Schering Research Foundation in Berlin, it is well recognized in the clinical setting that patients receiving blood transfusions experience intermittent hypoxia and reoxygenation [54, 55]. Reoxygenation-related increases in free radical formation can, in turn, activate stress response genes, such as heat shock protein 70 (which is an effective inhibitor of apoptosis), or stress-response transcription factors, such as NF-κB (which regulates numerous genes including VEGF), potentially leading to malignant progression.

Summary and Conclusions

Because of its demonstrated impact on malignant progression and therapeutic response, leading to a poor long-term disease outcome, tumor hypoxia is a growing concern in the oncology setting. Results of preclinical and clinical investigations during the past decade have established that tumor hypoxia may promote malignant progression by several mechanisms, including an increased expression of transcription factors and gene products involved in tumor propagation and induction of genomic instability (e.g., point mutations, deletions, and gene amplification). In those investigations, the transcriptional factor HIF-1 has emerged as a major regulator of adaptive processes (including angiogenesis) that can support tumor cell survival, proliferation, invasion, and metastatic spread. Also, it has been shown that hypoxia can enhance malignant progression and increase aggressiveness through clonal selection. Therefore, in developing treatment strategies for cancer patients, it is reasonable to consider approaches aimed at ameliorating tumor hypoxia in an effort to maximize the effects of cancer therapy.

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