Literature Review

Cytotoxic agents and radiation therapy: mechanisms of action and clinical applications

Amanda Marrone[1], William T. Tran[2]

[1]*Department of Radiation Therapy, Royal Victoria Hospital, Barrie, ON, Canada,* [2]*Department of Radiation Oncology, Sunnybrook Health Sciences Centre, Toronto, ON, Canada*

Abstract

Background: The combination of radiation therapy and chemotherapy is rooted in its ability to help achieve locoregional and systemic control, therefore increasing the overall disease-free survival of patients. Understanding the mechanistic actions of cytotoxic agents and their targets on the cell cycle, as well as the governing pharmacokinetic principles can improve treatment delivery. The adjuvant treatment setting can overcome barriers such as hypoxia and genetically driven treatment resistance.

Purpose: The purpose of this review is to present theoretical frameworks behind the chemoradiation paradigm and to describe current chemoradiation practices in radiation oncology.

Methodology: A review was conducted using the US National Library of Medicine, National Institutes of Health database (PubMed) using the following search keywords: chemoradiation, spatial cooperation, chemotherapeutic agents, pharmacokinetics, anti-vascular agents, tumour vasculature and tumour hypoxia.

Results and conclusions: Current research has reported several rationales for the beneficial combination of radiation and chemotherapy to eradicate oncological diseases. Mechanisms of action and biological approaches are showing that concurrent treatments, as well as novel agents such as anti-vascular and anti-angiogenic agents may benefit improved treatment outcomes by reducing micro hypoxic environments in tumours. In addition, chemotherapy administered in tandem with radiation enhances cell-killing effects by targeting the cell cycle.

Keywords: chemoradiation; chemotherapeutic agents; spatial cooperation; tumour hypoxia; tumour vasculature

INTRODUCTION

The use of primary, neoadjuvant, adjuvant and concurrent chemoradiotherapy has been proven to be efficacious in treating cancer.[1] Several mechanisms of action are responsible for the advantageous combination of chemotherapy and radiation therapy. These mechanisms are rooted in disrupting the cell cycle, and both chemotherapy and radiotherapy act on similar cytotoxic response pathways. In 1979, a theoretical framework was

Correspondence to: Amanda Marrone, Department of Radiation Therapy, 201 Georgian Drive, The Royal Victoria Hospital, Barrie, L4M 6M2 ON, Canada. Tel: (705) 728 9802, Ext. 43425. E-mail: marronea@rvh.on.ca

introduced by Steel and Peckham that described the chemoradiation paradigm.[1] The term 'spatial cooperation' was used to describe systemic and local disease control when chemotherapy and radiation are used, respectively.[2] Combined therapies could potentially improve overall disease-free survival in patients who have both locoregional and micrometastatic cancers.[2] In addition, local control can be reached if both modalities can target the tumour through its respective mechanisms of action while minimally impacting normal tissue.[3] The enhancement effect where one agent increases the effects of the other agent is referred to as *additive* or *supradditive*.[2,3] An increasing number of treatments in radiation oncology are applying these frameworks to achieve better outcomes. The treatment of brain, rectum, cervical, breast, and head and neck cancer are examples of disease sites where radiotherapy is delivered in a timely sequence with chemotherapy. Understanding the underlying mechanisms and rationale for chemoradiotherapy is essential in optimising treatment efficacy.

Treatment efficacy is impacted by several biological and treatment factors. Biological factors include inefficient tumour vasculature and hypoxia that ensues within the tumour microenvironment. Oxygen deficiency increases radiation resistance and inefficient tumour vessels limit the delivery of chemotherapeutic agents into tumours.[4] Treatment factors involve patient compliance to treatment and the timely administration of chemoradiation regimens. The purpose of this review is to examine the theoretical frameworks behind the chemoradiation paradigm and to describe current chemoradiation practices in radiation oncology. A review of the mechanisms of these cytotoxic agents is presented with respect to hypoxic microenvironments, pharmacokinetic frameworks and the potential for causing serious normal tissue side effects when escalating dose.

The chemoradiation paradigm mechanisms of chemoradiation action

Several rationales exist for combining chemotherapy and radiation therapy, with the first being the preservation of organ function and improved cosmesis when compared with surgery. Additional benefits of combinatory treatments
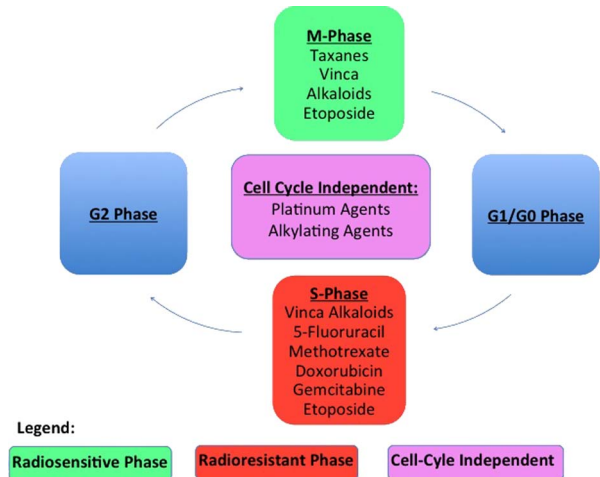

Figure 1. Combination treatments and the cell cycle. Combinatory treatments capitalise on tumour cells in different phases of the cell cycle. Cells in the G2/M phase are more radiosensitive than cells in the radioresistant S phase. Therefore, treatment strategies have used chemotherapy to interrupt mitotic programming by arresting cells in the more radiosensitive G2/M phases.

include chemotherapy-driven cell cycle disruption and radiosensitization by reducing the hypoxic microenvironments within the tumour, and the capability of targeting both local and systemic disease when administered concurrently.[1] These rationales stem from the conceptual framework outlined by Steel and Peckham,[3] which analysed chemotherapy and radiation interactions. Within this context, a major benefit to combinatory treatments is the advantageous targeting of tumour cells in different phases of the cell cycle.[5] Cells in the G2/M phase are more radiosensitive than cells in the radioresistant S phase.[5] Therefore, treatment strategies have used chemotherapy to interrupt mitotic programming by arresting cells in the more radiosensitive G2/M phases[5] (Figure 1).

The main clinical objective for combining chemotherapy and radiation therapy is to increase overall survival and quality of life in patients while minimising or reducing potentially life-threatening side effects.[3] In order to achieve this, combined treatment regimens need to be tailored according to the therapeutic ratio. The therapeutic ratio is commonly described as two sigmoid-shaped dose curves that define both the lethal dose and therapeutic dose. It can be described as the dose ratio that results in tumour

64

and normal tissue damage.[5] The amount of tumour control that can be expected from radiation administration depends on the dose tolerance of normal tissues.[3] The therapeutic ratio is an important consideration when choosing the appropriate anti-cancer treatments, where the intention to increase tumour control is influenced by the potential side effects caused by normal tissue damage. These side effects can be dose limiting, especially when critical anatomical structures are involved.

Additive effects from vascular normalisation

Tumour vasculature plays an important role in radiation sensitivity, drug delivery and tumour survival.[4] Tumour blood vessels are immature, and structurally disorganised throughout the tumour.[4] Tumour vasculature has weak endothelial cell connections, abnormal basement membranes and exhibits large variances in diameter and length.[4,6,7] The abnormal scaffolding of vessels creates hypoxic regions from insufficient blood flow into the tumour stroma.[8] Hypoxia, as a result of inefficient tumour vasculature, plays a principal role in the biological effectiveness of radiation therapy. During radiation exposure to the cell, fast charged particles are produced.[9] These particles are responsible for the production of ion pairs, which then lead to the production of reactive oxygen species, otherwise known as free radicals.[9] Free radicals are known for their lethality to cellular DNA owing to the production of organic peroxide from molecular oxygen, which has been known to cause irreparable DNA damage.[9] Molecular oxygen is a powerful radiosensitiser.[10] The method of radiosensitization is the result of oxygen's high electron affinity and its involvement in cascading reactions that lead to DNA damage.[10]

Similar to radiotherapy, chemotherapeutic treatment success is partly dependent on perfusion and oxygen saturation. This is commonly a net result of vascular disorganisation within tumours.[11] The unstable vascular network ultimately results in poor perfusion and thus, delivery of chemotherapy.[11] Harrison and Blackwell[11] noted that hypoxic conditions cause cells to cycle slower than normal and get 'stuck' in the radio-resistant S phase of the cell cycle. This hinders the efficacy of certain chemotherapeutic agents, such as alkylating agents and antimetabolites, whose main targets are cells in S phase.[11] In recent years, newly discovered classes of drugs known as vascular targeting agents have gained notoriety in their ability to normalise the erratic vascular architecture within tumours. Anti-angiogenic agents are responsible for targeting new tumour vessel growth, a process termed angiogenesis, while anti-vascular agents eradicate pre-existing tumour vessels.[6,8,12] Inefficient vessels in the network are eliminated, therefore improving blood flow, drug delivery and oxygen levels.[8,12] The increase in oxygen availability within the tumour microenvironment has been described to enhance sensitization to both radiation[8] and chemotherapy.[7]

Additive effects from cell cycle disruption

Chemotherapeutic agents act specifically at different points in the cell cycle. Each agent has a unique mechanism of action that is targeted to various cellular components, ultimately resulting in cell death. The exact mechanism of action of the alkylating agents, antimetabolites and taxanes are described in the following section (Table 1).

Alkylating agents modulate DNA, by way of cross-linking and DNA strand breaks. These effects lead to inhibition of cell division, abnormal base pairing and ultimately cell death.[13] These agents typically affect cells in all phases of the cell cycle, and are beneficial in the treatment of slow growing cancers such as leukaemia.[13] There are three distinct mechanisms associated with DNA damage: (1) formation of cross-bridges, which prevents DNA strands from being separated for synthesis or transcription,[13] (2) mismatch of nucleotides, thus leading to mutations,[13] and (3) attachment of alkyl groups to DNA bases. This activates DNA repair enzymes, which attempt to replace the alkylated bases, therefore causing the DNA to become fragmented.[13]

Antimetabolites typically affect the S phase of the cell cycle by inhibiting the assembly of nucleic acids.[14] Antimetabolites can be classified as: (1) antifolates, (2) purine analogues, (3) pyrimidine analogues and (4) nucleoside (sugar-modified) analogues.[14] Antifolates such as Methotrexate

Table 1. *Summary of cytotoxic agents and their mechanisms of action*

Class of chemotherapy	Drug example	Mechanism of action	Reference
Alkylating agents (cell cycle independent—affect all phases of the cell cycle)	Cyclophosphamide Temozolomide Chloroambucil	Three distinct mechanisms associated with DNA damage: Formation of cross-bridges Mismatch of nucleotides Attachment of alkyl groups to DNA bases	13
Antimetabolites (affect the S phase of the cell cycle)	Fluorouracil Capecitabine Gemcitabine Pemetrexed Methotrexate	Antimetabolites affect the S phase of the cell cycle by inhibiting the assembly of nucleic acids Classified as: Antifolates Purine analogues Pyrimidine analogues Nucleoside (sugar-modified) analogues	1 14 16 17
Taxanes (affects G2/M phase of the cell cycle)	Paclitaxel and Doxetaxel	Bind to the β subunits of tubulin Results in: An increase of tubulin polymer mass Formation of microtubule bundles Inhibit microtubule depolymerisation	1 18

interfere with cell activity by targeting folate-dependent enzymes.[14] Pyrimidine analogues, such as Gemcitabine, are known to prevent DNA synthesis and repair by exhausting deoxynucleoside triphosphates. These are essential for maintaining DNA polymerase and ribonucleotide reductase.[1] Gemcitabine has been shown to induce radiosensitization in cells when administered 24 hours before radiation therapy, with lasting effects for up to 48 hours.[1] Nucleoside analogues such as 5-fluorouracil (5-FU) prevent nucleic acid production.[14] This mechanism of action relies on cleavage into the DNA and RNA, resulting in disrupted DNA synthesis and transcription, thus affecting protein synthesis.[14] 5-FU is used widely with radiation in the treatment of rectal and stomach cancer, and mainly affects cells in the radioresistant S phase of the cell cycle.[15] Typically, 5-FU is administered continuously owing to its short half-life in plasma, thus not necessitating radiotherapy treatment time frames for optimal results.[1,16,17] The suggested administration method for 5-FU is through intravenous administration.[16,17] However, issues with infection and long-term venous access leading to thrombosis can complicate the course of treatment for patients, as well as the need for specialised medical equipment (i.e., pumps), which can be costly.[17] To counteract these issues, 5-FU is also available in pill form and is known as Capecitabine.[17] Hydroxyurea, another example of an antimetabolite, is a known radiosensitiser and is commonly used in the treatment of head and neck cancer.[1] It has been shown to also affect cells at the G1/S checkpoint in the cell cycle.[1]

Taxanes are microtubule-stabilising agents that are widely used for the treatment of metastatic breast and head and neck cancers.[18] Paclitaxel and Doxetaxel are common taxanes that are similar in function where both agents bind to the β subunits of tubulin, resulting in an increase of tubulin polymer mass, formation of microtubule bundles and inhibit microtubule depolymerisation.[1,18] As a consequence of this interaction, the cell cycle comes to a halt at the G2/M phase that leads to cell death and enhances radiation lethality.[1,18]

Clinical applications of chemoradiation

Chemoradiation has been used in various tumour sites as standard therapy. These tumour sites include: brain, rectum, cervix, breast, and head and neck.

For brain lesions, such as glioblastoma, Temozolomide (TMZ) is typically administered daily at 75 mg/m^2 of body surface area, 7 days a week for the entire course of radiation therapy.[19] Following this, six additional cycles are given at 150–200 mg/m^2 for 5 days, every 28 days.[19] It has been noted that the optimal concentration of TMZ in a patients' plasma is approximately
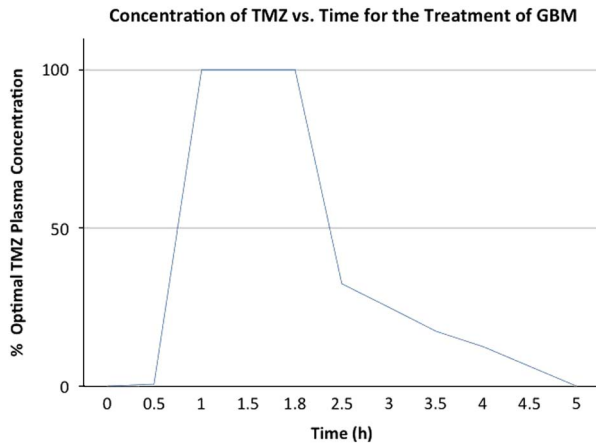
66

Figure 2. Concentration of Temozolomide (TMZ) versus time for the treatment of glioblastoma multiforme (GBM). TMZ is used in the adjuvant treatment of GMB. The per cent optimal concentration of TMZ in a patients' plasma is approximately within 1 hour of consumption, and is eliminated within ~1·8 hours.[20]

within 1 hour of consumption, and is eliminated within ~1·8 hours[20] (Figure 2). Pharmacokinetic drug effects are most optimal when patients take TMZ continuously and ~1 hour before radiation treatments.[19–21] The continuous dose administration of TMZ enables an increase in the dose intensity by almost two-fold, without increasing toxicity to the patient, and enables the reduction of the amount of a particular enzyme that is responsible for the repair of DNA damage caused by alkylating agents such as TMZ.[19]

It has been shown that Capcetabine in combination with radiation therapy is effective in the treatment of advanced rectal cancer.[22] It is typically absorbed by the body in the gastrointestinal tract and converted to 5-FU by a particular enzyme (thymidine or uridine phosphorylase).[22] Typically, the time frame for administration of Capcetabine is orally 1 hour before radiation therapy treatments in order to maximise the radiosensitization of cells, therefore increasing the effectiveness of radiation therapy treatments.[22]

Other disease sites do not necessitate timing regimens in order to achieve optimal radiotherapy results, as drug concentrations are steadily maintained by continuous infusion. Cervical cancer has been shown to benefit from chemoradiation, and progression free survival is increased when concomitant treatments are delivered.[23] A

study by Rose et al.[23] showed that concomitant radiation and cisplatin, fluorouracil, and hydroxyurea were beneficial in the treatment of locally advanced cervical cancer.

For head and neck cancers, Calais et al.[24] showed that overall and disease-free survival increased for patients who received chemotherapy and radiation therapy as opposed to the cohort of patients who received radiation therapy alone for squamous cell carcinoma of the oropharynx. In a similar study conducted by Brizel et al.,[25] it was also shown that the combination of chemotherapy and hyperfractionated radiation therapy was proven to be more beneficial versus the administration of radiation alone. The common aetiology of cervical and head and neck cancers involves the expression of human papilloma virus (HPV), suggesting that HPV-driven cancers may have a potential biological susceptibility to chemoradiation. However, there is limited data describing the biological interactions.

Emerging data is demonstrating novel treatment paradigms for disease sites that have been traditionally treated by mono-modalities. Lee et al.[26] demonstrated local disease control for triple-negative breast cancer patients undergoing salvage treatment for resistant disease. Cisplatin was delivered weekly, with a median dose of $30\,mg/m^2$ concurrent with external beam radiotherapy (total dose 65 Gy).[26] A large percentage of patients in this observational study demonstrated complete clinical response.[26]

Although the timing of chemotherapy and radiotherapy is not necessitated, or explicitly stated/determined in the cases of cervical, breast, and head and neck cancer, it is clear that the administration of both treatment modalities offers improved survival and disease-free progression because of the enhancement of normal tissue effects that can be observed with simultaneous drug and radiation therapy administration.[27]

CONCLUSION

Understanding the mechanistic activity of chemotherapy and radiation therapy, and the effects these treatments have on the cell cycle is crucial to finding a balance between increasing dose to

patients while minding normal tissue side effects. The administration of chemotherapy in conjunction with radiation therapy is dependent on treatment timing and type of agents. There is great opportunity to explore the clinical application of combining radiation and chemotherapy, as novel therapies are emerging within the treatment landscape.

Acknowledgements

Financial Support

Conflicts of Interest

None.

References

1. Seiwert T Y, Salama J K, Vokes E E. The concurrent chemoradiation paradigm—general principles. Nat Clin Pract Oncol 2007; 4 (2): 86–100.

2. Bentzen S M, Harari P M, Bernier J. Exploitable mechanisms for combining drugs with radiation: concepts, achievements and future directions. Nat Clin Pract Oncol 2007; 4 (3): 172–180.

3. Steel G G, Peckham M J. Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. Int J Radiat Oncol Biol Phys 1979; 5 (1): 85–91.

4. Tran W T, El Kaffas A, Al-Mahrouki A, Gillies C, Czarnota G J. A review of vascular disrupting agents as a concomitant anti-tumour modality with radiation. J Radiother Pract 2013; 12 (3): 255–262.

5. Nishimura Y. Rationale for chemoradiotherapy. Int J Clin Oncol 2004; 9 (6): 414–420.

6. Tozer G M, Kanthou C, Baguley B C. Disrupting tumour blood vessels. Nat Rev Cancer 2005; 5 (6): 423–435.

7. Shannon A M, Bouchier-Hayes D J, Condron C M, Toomey D. Tumour hypoxia, chemotherapeutic resistance and hypoxia-related therapies. Cancer Treat Rev 2003; 29 (4): 297–307.

8. Wachsberger P, Burd R, Dicker A P. Tumor response to ionizing radiation combined with antiangiogenesis or vascular targeting agents: exploring mechanisms of interaction. Clin Cancer Res 2003; 9 (6): 1957–1971.

9. Hall J, Giaccia A. Radiobiology for the Radiologist. Philadelphia, PA: Lippincott Williams & Wilkins, 2006.

10. Rockwell S, Dobrucki I T, Kim E Y, Marrison S T, Vu V T. Hypoxia and radiation therapy: past history, ongoing research, and future promise. Curr Mol Med 2009; 9 (4): 442–458.

11. Harrison L, Blackwell K. Hypoxia and anemia: factors in decreased sensitivity to radiation therapy and chemotherapy? Oncologist 2004; 9 (suppl 5): 31–40.

12. Tozer G M, Bicknell R. Therapeutic targeting of the tumor vasculature. Semin Radiat Oncol 2004; 14 (3): 222–232.

13. Ralhan R, Kaur J. Alkylating agents and cancer therapy. Expert Opin Ther Patents 2007; 17 (9): 1061–1075.

14. Scagliotti G V, Selvaggi G. Antimetabolites and cancer: emerging data with a focus on antifolates. Expert Opin Ther Patents 2006; 16 (2): 189–200.

15. Gez E, Sulkes A, Yablonsky-Peretz T. Combined 5-fluorouracil (5-FU) and radiation therapy following resection of locally advanced gastric carcinoma. J Surg Oncol 1986; 31: 139–142.

16. Kvols L K. Radiation sensitizers: a selective review of molecules targeting DNA and non-DNA targets. J Nucl Med 2005; 46 (1): 187S–190S.

17. Lawerence T S, Blackstock A W, McGinn C. The mechanism of action of radiosensitization of conventional chemotherapeutic agents. Semin Radiat Oncol 2003; 13 (1): 13–21.

18. Frame D. Introduction to taxane pharmacokinetics and pharmacodynamics. J Oncol Pharm Pract 2000; 6 (3): S22–S27.

19. Stupp R, Mason W P, van den Bent M J et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005; 352: 987–996.

20. Brada M, Judson I, Beale P et al. Phase I dose-escalation and pharmacokinetic study of temozolomide (SCH 52365) for refractory or relapsing malignancies. Br J Cancer 1999; 81 (6): 1022–1030.

21. Portnow J, Badie B, Chen M, Liu A, Blanchard S, Synold T W. The neuropharmacokinetics of temozolomide in patients with resectable brain tumors: potential implications for the current approach to chemoradiation. Clin Cancer Res 2009; 15 (22): 7092–7098.

22. Yu C K, Kim T W, Kim J H et al. Optimal time interval between capecitabine intake and radiotherapy in preoperative chemoradiation for locally advanced rectal cancer. Int J Radiat Oncol Biol Phys 2007; 67 (4): 1020–1026.

23. Rose P G, Bundy B N, Watkins E B et al. Concurrent cisplatin-based radiotherapy and chemotherapy for locally advanced cervical cancer. N Engl J Med 1999; 340: 1144–1153.

24. Calais G, Alfonsi M, Bardet E et al. Randomized trial of radiation therapy versus concomitant chemotherapy and

radiation therapy for advanced-stage oropharynx carcinoma. J Natl Cancer Inst 1999; 91: 2018–2086.

25. Brizel D M, Albers M E, Fisher S R et al. Hyperfractionated irradiation with or without concurrent chemotherapy for locally advanced head and neck cancer. N Engl J Med 1998; 338: 1798–1804.

26. Lee W, Brackstone M, Gandhi S, Arce S C, Dinniwell R. Salvage radiotherapy and cisplatin for triple negative breast cancer: a multi-centre study. Cancer Res 2012; 72 (24 suppl): Abstract nr P4-16-14.

27. Fu K K. Biological basis for the interaction of chemotherapeutic agents and radiation therapy. Cancer 55: 2121–2130.