4 Combinations of Topoisomerase Inhibitors and Ionizing Radiation

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4.1 Introduction

Radiation has been used in the treatment of cancer since its discovery and has recently undergone significant innovations through increased technical refinements. With increased computing power, three-dimensional radiation therapy, intensity-modulated radiation therapy, tomotherapy, and stereotactic radiation have emerged as tools to increase the therapeutic ratio. While safe implementation of such programs has resulted in improvements in local control through pure dose escalation and/or alterations in fraction sizes, limitations still exist secondary to adjacent normal tissue toxicity. Furthermore, radiation has never been more than a local therapy, unable to affect disease distant to the treatment field. Chemotherapy agents, in contrast, act systemically, although they are unlikely to control completely gross solid tumors. Combining highly effective local therapy and systemic therapy might enhance the overall chance of cure, especially in disease entities known to harbor microscopic metastatic deposits frequently. Additional benefit in terms of local tumor control might be derived from an additive effect of chemotherapy; thus, cytotoxic agents might provide several advantages over radiation alone for improved local, regional, and systemic disease control.

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4.2 Topoisomerases

DNA topoisomerases (Topo) function to regulate the topology of DNA to ensure correct DNA metabolism. Currently five human topoisomerases for DNA have been identified, Topo1, Topo2α, Topo2β, Topo3α, and Topo3β (Wang 2002). They serve in a vital capacity for successful DNA synthesis (Wang 1985; Wang 1991). As such, DNA topoisomerases I and II (Topo I and II) are important targets for cancer chemotherapeutic agents. These nuclear enzymes are essential for DNA replication, RNA transcription, chromosomal condensation, and mitotic chromatid separation (Wang and Sinha 1996). The level of Topo I is independent of cell cycle phases, although cytotoxicity is manifested only in proliferating cells (Choy and Macrae 2001). Topo II, in contrast, is cell-cycle dependent. It increases at the onset of G2 and S phases and disappears in G0/G1 phase (Heck and Earnshaw 1986). Topoisomerases also have activity in G1 cells or cells held in plateau phase (Ng et al. 1994). The difference between Topo I and Topo II is number of DNA strands involved. The human DNA topoisomerase I is a monomeric 100 kDa protein that is able to relax supercoiled DNA. This is achieved through the introduction of a single stranded break in DNA followed by the passing of the intact strand through the break prior to religation. This activity is key in many aspects of DNA

metabolism including transcription, replication and the regulation of DNA supercoiling, which is important in maintaining genomic stability. It is believed that the camptothecins function by stabilizing a topoisomerase I-DNA intermediate called the cleavable complex such that the 5'phosphoryl end of the DNA single-stranded break is bound covalently to a topoisomerase-I tyrosine residue (Chen et al. 1999). It is believed that collision of this drug-trapped complex with the DNA replication machinery will lead to G2 phase cell-cycle arrest and cell death (Chen and Liu 1994; Cheng et al. 1994). Topo II acts similarly, but on two strands of DNA (Wang 1985). Topo II binds covalently to double-stranded DNA, cleaves both strands, and reseals the cleaved complex. Collisions of Topo II–etoposide cleavable complexes with DNA tracking enzymes, such as polymerases or helicases, generate DNA DSBs. The resulting DNA DSBs may lead to cell death by apoptosis. Through analysis of their dysfunction in otherwise normal cells, other duties involving DNA repair have been implicated. When not performing properly, mutation (Bae et al. 1988), sister chromatid exchange (Pommier et al. 1984, 1998; Downes et al. 1991) illegitimate recombination (Bae et al. 1988), DNA fragmentation (Kaneko and Horikoshi 1987), and tumor promotion (Downes et al. 1994; Andoh et al. 1987) may occur.

4.3 Topoisomerase-I Inhibitors

Camptothecin, the parent compound (see Fig, 4.1), was initially isolated from the tree, *Camptotheca acuminata*, and was found to have a broad spectrum of activity in a variety of solid tumors through inhibition of Topo I (Chen and Liu 1994); however, early clinical trials with the ring-open form of the drug showed excessive toxicity and the trials were terminated (Muggia et al. 1972). More recently, interest has been rekindled in these drugs with the advent of derivatives that have significant antitumor activity and much less toxicity. Irinotecan, one of these derivatives, is actually a prodrug which is metabolized intracellularily into SN-38 (Takimoto et al. 1998). SN-38 is approximately a 1000 times more potent inhibitor of Topo I than irinotecan (Kawato et al. 1991). All of the camptothecins have a terminal lactone ring with can be hydrolyzed to a less active carboxylate species; however, under acidic conditions, like those expected in a tumor's microenvironment, the active lactone species is favored (Takimoto et al. 1998). After an intravenous infusion, SN-38 can have a plasma half-life of 5.9–13.8 h and this certainly can have implications in terms of both direct cytoxicity and radiosensitization abilities. The major method of SN-38 elimination is through hepatic glucuronidation, and it is felt that a decreased ability to glucuronidate the drug correlates with increased gastrointestinal side effects (Takimoto et al. 1998). One of the major side effects of irinotecan is late onset diarrhea. This is felt to be related to the high S-phase fraction of the intestinal mucosa as well as action of intestinal flora glucoronidase in cleaving the camptothecin-glucuronidase conjugate leading to the drug's release into the intestinal lumen (Araki et al. 1993). Other common toxicities include neutropenia, nausea, vomiting, anorexia, fatigue, asthenia, and elevation of hepatic transaminases.

Currently available camptothecin drugs include irinotecan (CPT-11), topotecan (9-aminocamptothecin; Lamond et al. 1996), 7-ethyl-10-hydroxycamptothecin (Kohara et al. 2002), and 9-nitro-20(S)-camptothecin (RFS-2000; Amorino et al. 2000; see Table 4.1).

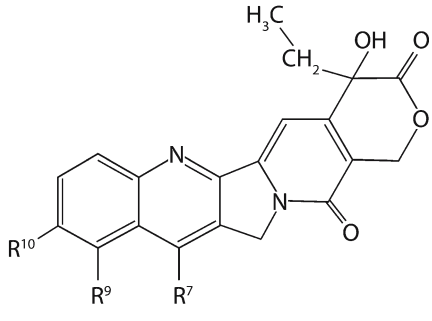

Fig. 4.1. Chemical structure of camptothecin

4.4 Topoisomerase-II Inhibitors

Etoposide (VP-16) is one of the most frequently used Topo-II inhibitors with specific action in late S or early G_2 phase of the cell cycle. Etoposide forms a ternary complex with Topo II and DNA (Sakamoto et al. 2001). An early change in etoposide treated cells is an interruption in the transition from S phase prior to G_2 arrest. Coinciding with this S-phase delay is a selective inhibition of thymidine incorporation into DNA and a severing of DNA strands. Very low doses of etoposide can initiate DNA strand inter-