Chapter – 4

COURSE OF WORK DONE
4.1 PREFORMULATION

4.1.1 Selection of the Drug (API)

Paclitaxel is the selected drug as discussed before in chapter 2. In the further chapters we will discuss its formulation alone as well as in combination with a polyphenolic compound from a herbal component. The studies are discussed using Ellagic acid as a model drug and the study is extendable to all polyphenolics with similar solubility. The reason for making such a combination attributes to the fact that many plant-derived polyphenols have been keenly studied for their possible chemopreventive properties and are found to be pharmacologically safe. These compounds include genistein, curcumin, resveratrol, silymarin, caffeic acid phenethyl ester, flavopiridol, emodin, green tea polyphenols, piperine, oleandrin, ursolic acid, Punicalagin and with other ellagitannins, ellagic acid and betulinic acid. Recent research has suggested that these plant polyphenols might be used to sensitize tumor cells to chemotherapeutic agents and radiation therapy by inhibiting pathways that lead to treatment resistance. For instance, the ubiquitously expressed transcription factor NF-kB is involved in a wide spectrum of cellular responses, including cell cycle control, apoptosis, and stress adaptation. Punicalgins, ellagitannins inhibited NF-kB and cell viability of prostate cancer cell lines in a dose-dependent manner in vitro. In the LAPC4 xenograft model, pomegranate extracts rich in ellagitannins delayed the emergence of LAPC4 androgen independent xenografts in castrated mice through an inhibition of proliferation and induction of apoptosis. They have also been studied in colon cancer where they caused inhibition Wnt signaling pathways. Still another mechanism by which these ellagitannins can sensitize the chemotherapy is by COX-2 inhibition. COX-2, an
enzyme expressed mainly in response to inflammatory disorders and cancer, mediate prostaglandin production and is currently under clinical investigation for anticancer therapy. Its presence has been linked with more aggressive tumor phenotypes and inferior outcome for patients with breast cancer, colon cancer, head and neck cancers, lung cancer, and pancreatic cancer. Selective inhibitors of COX-2, such as SC-236 and celecoxib, have been used in vitro and in vivo to test if its inhibition may sensitize tumor cells to chemotherapy and radiotherapy and it was found that SC-236 increased tumor radiosensitivity in murine tumor models and in a human glioma xenograft in nude mice, and celecoxib enhanced the response of A431 human tumor xenografts in nude mice to radiation and docetaxel chemotherapy, radiotherapy, or both.

The current study involves making a formulation for the co-administration of paclitaxel and a polyphenolic compound (Ellagic acid being taken as model drug) which can act through any of the above suggested pathways and help in sensitizing the cell towards paclitaxel.

a. Role of NFκB in Onogenesis

NF-κB activation has been associated with multiple aspects of oncogenesis, including the directing of apoptosis, the cell cycle, differentiation, and cell migration. Additionally, activation of NF-κB in cancer cells by chemotherapy or by radiation can decrease the ability of the cancer therapy to induce cell death.

Numerous studies have showed that NF-κB activation can suppress cell death pathways and that NF-κB activation is required to protect cells from the apoptotic cascade induced by TNF and other stimuli. NF-κB suppression of apoptosis is a transcriptional event since it activates expression of TRAF1 and 2 and c-IAP1 and 2 to block caspase-8.
activation. Other antiapoptotic genes that are activated by NF-κB includes Bcl-2 homologues A1/Bfl-1 and Bcl-xL, IEX-1, and XIAP. Of latent interest concerning inhibition of apoptosis is the scrutiny that NF-κB can antagonize p53 function, possibly through the cross-competition for transcriptional coactivators. On the other hand, p53 may in some cases act through NF-κB to induce apoptosis. It has long been conjectured that oncogenesis is associated with antiapoptotic mechanisms. It was found that inhibition of NF-κB, via expression of the repressor form of IκBα, led to the induction of apoptosis when an oncogenic allele of H-Ras (RasV12) was expressed. Other evidence said that NF-κB played a survival role in oncogenesis since inhibition of NF-κB in transformed cells induced apoptosis. Activation of NF-κB by growth factors also suppressed the apoptotic response induced by c-myc expression, although it is unclear which of the antiapoptotic mechanisms that are regulated by NF-κB perform this function. Moreover, members of the IAP family of antiapoptotic proteins were upregulated in a variety of cancers, possibly by the NF-κB pathway. Moreover, to being required to suppress transformation-induced apoptosis NF-κB promoted cell growth by upregulating transcription of cyclin D1, an event that was associated with Rb hyperphosphorylation, progression into the S phase of the cell cycle, and inhibition of apoptosis. Importantly, cyclin D1 was upregulated in a number of human tumors. In light of its potent effects on cell survival and growth, NF-κB was found to control other aspects of cell cycle progression as well.

b. Chemotherapy And NFκB

Fact that inhibition of NF-κB in cells induced to express an oncogenic form of Ras or in some cancer cell lines led to an apoptotic response increased the likelihood that NF-κB
inhibitors may function as individual cancer therapy or as cancer-preventive compounds. Concerning the first hypothesis, it was assumed that inhibition of NF-κB alone will not dramatically affect most solid tumors, since these cells expressed other antiapoptotic factors. Nevertheless, NF-κB inhibition proved to be therapeutic in certain leukemias or lymphomas (such as Hodgkin’s lymphoma), where NF-κB played a unique survival role. Unfailing with the hypothesis that NF-κB may be an important target for chemopreventive compounds, NF-κB was inhibited by aspirin or other nonsteroidal anti-inflammatory drug (NSAID) treatments, which blocked the initiation and/or progression of certain cancers, particularly colorectal cancer. Amusingly, aspirin and sulindac both were reported to inhibit activation of the IκB kinase complex. Several dietary chemopreventive compounds, including flavonoids, curcumin, and resveratrol, are known to block NF-κB activation. These studies strongly proped up the hypothesis that NF-κB is a functionally relevant target of chemopreventive drugs and dietary compounds, possibly indicating that the role of NF-κB is primarily in the earliest stages of oncogenesis.

Working with HT1080 fibrosarcoma cells exposed to ionizing radiation or to the chemotherapeutic agent daunorubicin, it was found that these treatments induced strong nuclear accumulation of the NF-κB p50-p65 heterodimer. Inhibition of NF-κB by expression of the super-repressor form of IκBα dramatically improved the apoptotic response to ionizing radiation or daunorubicin. HT1080 fibrosarcoma-derived tumors grown in nude mice were resistant to the drug CPT-11 but were induced to undergo apoptosis following treatment if they were infected with an adenovirus expressing the modified form of IκBα. Other tumors (for example, those derived from the colorectal
tumor cell line LoVo) showed nearly identical responses to the combined treatment. In fact, LoVo tumors could be eliminated with CPT-11 systemic treatment and with adenoviral delivery of IκBα either every 5 or 10 days.\textsuperscript{16,17} Importantly, the systemic use of inhibitors of NF-κB in conjunction with CPT-11 treatment also led to significantly improved chemotherapeutic responses through enhanced apoptosis.\textsuperscript{12} The form of chemotherapy used may determine whether NF-κB is pro- or antiapoptotic. For example, the majority of chemotherapies studied induce some form of DNA damage; inhibition of NF-κB activation, conversely, promotes cell death. However, it was reported recently that NF-κB may be required for cell death induced by the microtubule-binding agent Taxol\textsuperscript{18} which does not affect DNA stability, but engages the mitotic spindle. In light of data given from another group showing that NF-κB inhibition can enhance Taxol-induced cell death.\textsuperscript{19}

4.2 CHARACTERIZATION OF DRUG

- Organoleptic properties
- Solubility
- Log p/Pk\textsubscript{a}
- Melting point
- FTIR spectra analysis
- UV spectral analysis
- DSC analysis
- Stability studies
- Drug-excipient compatibility study
4.3 ANALYTICAL METHODOLOGY FOR ROUTINE ANALYSIS

Discussed in Further Chapters; chapter 5 and 6

4.4 DESIGN, DEVELOPMENT AND OPTIMIZATION OF THE PARTICULATE NOVEL DRUG DELIVERY SYSTEMS

Two systems were developed:

➢ Thermosensitive Nanomicellar system
➢ Solid lipid nanoparticles

4.5 CHARACTERIZATION OF THE OPTIMIZED FORMULATIONS USING DIFFERENT TECHNIQUES.

a) Surface morphology

b) Particle size and size distribution

➢ Dynamic light scattering

c) Surface charge and Zeta potential

➢ Laser Doppler Anemometry or Velocimetry

d) Crystallinity

e) Drug Content and encapsulation efficiency

f) In-vitro dissolution studies of the optimized formulations and its Characterization

g) Stability studies of the optimized formulation.

h) Ex-vivo studies
References:


