CHAPTER-I

1. Introduction

Cancer is one of the fatal diseases and leading cause of death in the world (Ferlay et al., 2013). Accounting to statistical analysis of American Cancer Society at National Cancer Institute of the USA, 580350 worldwide deaths in 2013 was due to cancer (Atlanta, 2013). 14.1 million new cases of cancer per year in the world was estimated by GLOBOCAN 2012 which expected to be increased by 19.3 million new cases of cancer per year by 2025 (Ferlay et al., 2013). There are mainly four methods to treat cancer including Surgery, chemotherapy radiation, and immunotherapy (Masood, 2016). However, poor prognosis, poor bioavailability, lack of selectivity, inconsistency in the systemic circulation, non-specific biodistribution and severe side effects are major limitations of conventional cancer therapy (Cho et al., 2008; Soni and Yadav, 2015). Chemotherapy has a number of advantages, however it is associated with a variety of challenges due to lack of selectivity and associated high toxicity. The main reasons for the failure of chemotherapy includes poor solubility of drug, narrow therapeutic index and efflux of drug from cells by several efflux transporters leading to hampered pharmacokinetic profile (Bansal et al., 2009; Parhi et al., 2012; Saneja et al., 2014). Therefore, a harmless, efficient and novel treatment strategy, especially for chemotherapeutic agents are immediately needed to fight against cancer.

Among the chemotherapeutic agents for cancer, nucleoside analogs constitute one of the most important classes of drugs (Jordheim et al., 2013). Gemcitabine (2’,2’-difluoro-2’-deoxycytidine) (dFdC) is a prototype drug of this class which is active against a wide range of solid tumors including pancreatic, non-small cell lung, breast, bladder, ovarian, thyroid and multiple myelomas (Abdayem et al., 2014; Carballido and Rosenberg, 2014; Chanpanitkitchot et al., 2014; Khare et al., 2014a; Oettle, 2014; Wu et al., 2014). Gemcitabine hydrochloride is marketed clinically, in more than 70 countries, as a parenteral formulation (Gemzar®), administered via intravenous (i.v.) infusion at a dose of 1000-1250 mg/m² over 30 min, on a weekly basis, for 3-4 weeks (Bender et al., 2009). Its clinical benefit, however, is limited by its short plasma half-life (about 15 min) (Heinemann et al., 1992); it undergoes rapid deactivation by the
action of deoxycytidine deaminase, present in the blood and liver (Reid et al., 2004b), followed by renal excretion. Such pharmacokinetic (PK) and pharmacodynamic (PD) profiles suggest that more frequent dosing is required. In some cases, daily administrations or prolonged infusions are required so that a therapeutic effect of the drug can be obtained (Boven et al., 1993b; Moog et al., 2002). Higher doses of gemcitabine are associated with serious side effects such as myelosuppression, thrombocytopenia, mild and transient neutropenia and anemia (Dasanu, 2008a). In addition to the PK/PD complexity, nucleoside transporters are needed for intracellular internalization of gemcitabine and so drug resistance develops over time, diminishing its therapeutic efficacy (Hui and Reitz, 1997; Ueno et al., 2007).

The nanotechnology based drug delivery in cancer chemotherapy made many successful sign of progress in last decades. The field of nanotechnology specially targeted delivery system showed potential to enhance the therapeutic outcome of gemcitabine by selectively killing of cancerous cells while preventing the normal cells. A number of attempts have been made in recent years for the targeted delivery of gemcitabine including nanoparticles (Arias et al., 2011; Ji et al., 2012; Lee et al., 2013), liposomes (Dalla Pozza et al., 2013; Paolino et al., 2010; Papa et al., 2013), polymersomes (Nahire et al., 2014), supramolecular vesicular aggregates (Paolino et al., 2012), nanovesicles (Jia et al., 2010), polymeric micelles (Chitkara et al., 2013; Wang et al., 2014) and polymer-drug conjugates (Bender et al., 2009; Tsume et al., 2014). The advantages of targeted delivery of gemcitabine include protection of the drug from enzymatic degradation, prolonged drug exposure times and selective drug accumulation in tumor tissue, which may result in better therapeutic effect and mitigation in toxicity and drug resistance.

In order to overcome the limitations associated with gemcitabine we have investigated two different novel drug delivery based approaches. In our first approach, gemcitabine loaded folate conjugated bovine serum albumin nanoparticles (Fa-Gem-BSANPs) were developed by chemical conjugation of folate to the surface of the Gem-BSANPs. The Fa-Gem-BSANPs were characterized for various parameters including size, polydispersity, zeta potential, morphology, encapsulation efficiency and drug release profile. The cytotoxicity of Fa-Gem-BSANPs and Gem-BSANPs was
determined in the folate receptor over-expressing cell lines (MCF-7 and Ovcar-5 cells) and folate receptor-deficient cell line (MIAPaCa-2). In addition, cellular uptake and apoptosis activity of the developed formulations were investigated in order to correlate the cytotoxicity of the nanoparticles with their folate receptor targeting potential. Further, in vivo anticancer activity Fa-Gem-BSANPs were determined in a solid tumor model in mice.

In our second approach, polymer-Gem conjugates have been investigated, as one of the solutions, to improve gemcitabine plasma stability and therapeutic index. In this approach, hyaluronic acid (HA) was used as a macromolecule for conjugation of gemcitabine, via an amide linkage as a more stable analogue in vivo. Conjugation of HA to gemcitabine using the amide linkage has not been reported before. In general amide linkages are more stable than ester linkages so it is hypothesized that this strategy is likely to improve the plasma stability of the drug; prolong its biological activity, and if successfully delivered to solid tumors, could delay tumor growth of CD44-expressing solid tumor models, following i.v. administration. The HA was individually conjugated, via amide coupling, to gemcitabine (HA-Gem), 4’-(aminomethyl)fluorescein hydrochloride (HA-4’-AMF) or tris(hydroxypyridinone) amine (HA-THP) for cancer therapy, in vitro tracking of HA pathway and in vivo biodistribution coupled with single photon emission computed tomography/computed tomography (SPECT/CT) imaging, respectively. Chemical characterization, in vitro cell tracking and cytotoxicity studies, were performed in a range of cancer cells. In vivo tumor biodistribution studies were carried out in two CD44-expressing tumor-bearing mice; the highly vascularized murine colon cancer (CT26) and the highly resistant human pancreatic cancer (PANC-1) mice models. Tumor accumulation was quantified by gamma scintigraphy. In addition, an in vivo therapeutic efficacy study was performed in the CT26 tumor model. A series of in vitro and in vivo studies were performed to assess the potential of this conjugate for improvement in gemcitabine delivery. This is the first report of conjugation of a THP chelator to HA for CD44 mediated SPECT imaging. This work is likely to open new avenues for the application of HA-based macromolecules in the field of image-guided delivery in oncology.
In the third approach, we developed a nano-sized formulation of pentacyclic triterpenediol (TPD), an institutional anticancer lead from the plant *Boswellia serrata*. The TPD was encapsulated in biocompatible and biodegradable PLGA polymer (TPD NPs) to further improve its anticancer potential by selective accumulation at desired site through EPR effect. The prepared NPs were characterized for different parameters including particle size, zeta potential, morphology, physical state, hemolytic activity and in vitro release profile. The *in vitro* cytotoxicity of the NPs was performed on two different cancer cell lines (MCF-7 and OVCAR-5). To confirm the mechanism of cells cytotoxicity of the formulation we have performed different assay including apoptosis, mitochondrial membrane potential (MMP) loss and reactive oxygen species (ROS) generation. The *in vivo* anticancer potential of the developed TPD NPs was performed in solid tumor model and finally, hematology, biochemistry and histology of the treated animal were investigated.

The objectives of the undertaken works are as following:


2. Synthesis and evaluation of hyaluronic acid conjugated gemcitabine for cancer chemotherapy.

3. Preparation and evaluation of pentacyclic triterpenediol (TPD)-loaded polymeric nanoparticles for improved anticancer potential.