Chapter 1
Introduction

1.0 Introduction

Leukaemia, the cancer of the blood is characterized by the widespread uncontrolled proliferation of large number of abnormal blood cells, usually of the white cell lineages, which take over the bone marrow and often spill out into the blood stream. In leukaemia, non- functioning cells accumulate in the marrow and blood. Etoposide and Cytarabine are two of the most commonly used drugs used in chemotherapy of leukaemia.

Etoposide is a semisynthetic podophyllotoxin derived from the root of Podophyllum peltatum. It is known to cause single-strand breaks in DNA. Etoposide also causes DNA damage through inhibition of topoisomerase II and activation of oxidation-reduction reactions to produce derivatives that bind directly to DNA. Etoposide is cell cycle phase specific with predominant activity occurring in late S phase and G2. It is primarily used for acute lymphocytic leukemia and acute myelogenous leukaemia (www.bccancer.bc.ca). Etoposide has variable oral bioavailability ranging from 24-74% and has terminal half life (a) of 1.5 hours by intravenous route and 0.44 hours by oral route (Henwood, 1990).

Cytarabine is an antimetabolite used primarily for acute myelogenous leukaemia and meningeal leukemia. It is metabolized intracellularly into its active triphosphate form (cytosine arabinoside triphosphate). This metabolite then damages DNA by multiple mechanisms, including the inhibition of alpha-DNA polymerase, inhibition of DNA repair through an effect on beta-DNA polymerase, and incorporation into DNA. The latter mechanism is probably the most important. Cytotoxicity is highly specific for the S phase of the cell cycle (Chabner, 1989). Cytarabine is poorly absorbed from gastrointestinal tract with less than 20% bioavailability and has a short half life of 2-4 hours (Ho et al, 1971; Knoester et al., 1993; Balis et al., 1989).

Both these drugs are usually required to be administered intravenously and are available as multidose vials. The conventional parenteral therapy with these drugs is painful to the patients even with as little as effective concentration and cause severe side effects. These side effects are mainly caused by the lack of specificity of these anticancer drugs, that is, the anticancer drugs not only kill cancer cells but also inhibit normal cell growth and eventually lead to necrosis of normal cells. Moreover, the conventional drugs kill the

leukaemia cells in the blood but are not effective in penetrating into the spinal chord or brain. Leukaemia cells flourish into these central nervous systems hideouts, eventually causing fatal complications. Other crucial problem in conventional drug delivery systems is that drugs cannot be released in a sustained manner. Also, the amount of released drug decreases with time, which plays a detrimental role in maintaining a constant level of drug in blood.

Thus improvement of treatment modalities for leukaemia requires a drug delivery system which is long circulating in blood and can provide sustained release of the drug. The polymeric materials used for the carrier have to be biodegradable and biocompatible.

Colloidal drug carriers such as polymeric nanoparticles have recently gained attention for targeting and sustaining the release of the drug. Nanoparticles are solid or semisolid colloidal particles ranging in size from 10 to 1000 nm (Couvreur et al., 1995). Nanoparticles (NP) have the ability to deliver a wide range of drugs to varying areas of the body for sustained periods of time (Prabha et al., 1994). Polymeric particles can be administered by intravenous and oral routes to increase the bioavailability and reduce the associated adverse effects. The potential applications of nanoparticles by the intravenous route can be summarized in terms of the concentration of drugs in accessible sites; the rerouting of drugs away from sites of toxicity; and increasing the circulation time of labile or rapidly eliminated drugs. Nanoparticle preparations, mainly used for intravenous injection, are employed for the sustained release of drugs, and for the passive targeting of anticancer drugs. Moreover, it has been shown that the irritation at the injection site can be minimized by using smaller particles. Narrowly distributed stable polymeric nanoparticles with an average diameter less than 200 nm are ideal for intravenous injection because they can easily pass through the blood capillary (Davis, 1997). Polymeric nanoparticles have shown to be good carriers for cellular drug delivery because they improve drug stability and availability at the intracellular site of action (Chavanpatil et al., 2006). Nanoparticles of anticancer drugs penetrate into tumour tissues across the wall of blood vessel which have loosened cell contact, but would not penetrate into normal tissues across the wall of blood vessel which have tight cell contact, therefore, the lack of specificity of anticancer drugs can be successfully overcome due to the structural difference.

The most widely used polymers for biodegradable nanoparticles have been poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and their co polymers, poly(lactide-coglycolide) (PLGA) (Gliding et al., 1979). These polymers are known for their biocompatibility and resorbability through natural pathways. Additionally, the degradation rate and accordingly the drug release rate can be manipulated by varying the ratio of PLA to PGA (Wise et al., 1979). Since the biodegradable polymers are degraded in a certain period of time, they are not harmful to the human body. PLGA is degraded into non-toxic lactic acid and glycolic acid in the body. Therefore, drug delivery systems based on these polymers can be applied in the sustained release of drugs (Anderson et al., 1997). Conventional polymeric nanoparticles are rapidly removed from the blood stream after I.V. administration by the macrophages of the mononuclear phagocyte system (MPS), which prevents their application in sustained delivery. The presence of hydrophilic coating on nanoparticles sterically stabilizes them, avoids MPS uptake and hence prolongs circulation in body (Illum, 1984). Pluronic is a triblock copolymer composed of polyethyleneoxide and propyleneoxide (PEO-PPO-PEO). Some authors have shown that Pluronics makes the PLGA particles hydrophilic and avoid rapid recognition by MPS and makes them long circulating in the blood (Illum et al., 1984). Pluronic block copolymers have good biocompatibility and can be used for sustaining the release of the drug (Xiong et al., 2006).

Polyethyleneglycol (PEG) modified biodegradable polymer is one of the most popular materials to prepare the stealth nanoparticles which could avoid, or at least reduce the uptake by phagocytes and prolong the time of drug in effective concentration in blood circulation. (Gref et al., 1994). Nanoparticles prepared from polyethyleneglycol-modified poly (D, L-lactide-co-glycolide) (PEG-PLGA) have been extensively investigated as drug carriers due to their controlled release, biodegradability and biocompatibility (Avgoustakis et al., 2003). After intravenous administration, the PLGA–PEG nanoparticles remain in the systemic circulation for hours, whereas the PLGA nanoparticles are removed from blood within few minutes. The PEG layer provides a steric barrier to the particle and its opsonization is reduced. As a result, these particle have been shown to remain in circulation for an extended period of time. Long-circulating nanoparticles made of methoxypoly(ethylene glycol)- poly(lactide-co-

glycolide) (mPEG-PLGA) also have a good safety profiles and provide drug-sustained release.

Against this background, the present investigation was aimed at developing etoposide and cytarabine loaded PLGA based biodegradable nanoparticles which would have sustained release, have steric barrier for longer blood circulation time, provide distribution of the drug to brain and bones and increase the half life of the drugs. The prolonged drug release with the PLGA, PLGA-MPEG and PLGA-PLURONIC nanoparticles would reduce the side effects associated with the conventional leukaemia therapy by reducing dosing frequency and reducing pain at the site of injection. The drug delivery would be given as a single shot injection by IV route that would release the drug for a number of days, hence would be beneficial in better control of leukaemia therapy.

1.1 Aims and Objectives

The present study was undertaken with the following objectives-

1. To develop sustained release biodegradable nanoparticles of two antileukaemic drugs- Etoposide and Cytarabine.
2. To develop PLGA based nanoparticles that could effectively prolong the circulation of drug in the blood, provide sustained release.
3. To develop a formulation that would help in improving the therapy of leukaemia and also reduce its side effects and dosing frequency.

1.2 Plan of Work

The plan of work includes the following-

- To prepare etoposide loaded PLGA nanoparticles and optimize them on the basis of their mean particle size and entrapment efficiency.
- To prepare etoposide loaded PLGA-Pluronic nanoparticles and optimize them on the basis of mean particle size and entrapment efficiency.
- To prepare etoposide loaded PLGA-mPEG nanoparticles and optimize them on the basis of mean particle size and entrapment efficiency.
- To prepare cytarabine loaded PLGA nanoparticles and optimize them on the basis of mean particle size and entrapment efficiency.
- To prepare cytarabine loaded PLGA-mPEG nanoparticles and optimize them on the basis of mean particle size and entrapment efficiency.
- To evaluate the prepared nanoparticles for surface charge (Zeta potential), DSC, XRD and surface morphology (SEM).
- To carry out in-vitro drug release studies of the NP.
- To study the stability of the NP at 2-8, 25 and 40°C for 1, 2 and 3 months.
- To study in vitro cytotoxicity of the prepared nanoparticles and determine their IC_{50} values using L1210 and DU145 cell lines.
- To visualize the intracellular uptake of the drug loaded nanoparticles by confocal microscopy and flow cytometry using L1210 and DU145 cell lines.
- To radiolabel the free drug and drug loaded nanoparticles using ^{99m}Tc and study their biodistribution in mice.
- To study blood clearance of radiolabeled free drugs (etoposide and cytarabine) and radiolabeled drug loaded nanoparticles in rats.

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