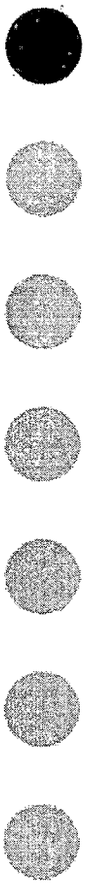
Introduction



1. INTRODUCTION

Cancer is one of the most dreadful diseases to the mankind. It is a generic term for large group of diseases' that can affect any part of the body. Other terms used are malignant tumors and neoplasms. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. This process is referred to as metastasis. Metastases are the major cause of death from cancer.

Global burden of cancer

More than 11 million people are diagnosed with cancer every year. WHO estimates that by 2020 there will be about 16 million new cancer patients each year? Cancer is a leading cause of death worldwide. The disease accounted for 7.4 million deaths (or around 13% of all deaths worldwide) in 2004. Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030. The main types of cancer leading to overall cancer mortality each year are:

- Lung (1.3 million deaths/year)
- Stomach (803 000 deaths)
- Colorectal (639 000 deaths)
- Liver (610 000 deaths)
- Breast (519 000 deaths)

Treatment may involve surgery, radiation therapy, chemotherapy, hormonal therapy, or a combination of these. Excision of the tumor and radiation therapy is useful where cancer cells have not metastasized and single or combination of chemotherapy is used when cancer cells have metastasized. The most advanced forms of treatment may produce a 5-year survival rate of 75% or more for certain types of cancer, e.g. cancer of the uterine corpus, breast, testis, and melanoma. Survival rates in cancer of the pancreas, liver, stomach, and lung are generally less than 15% (www.who.org).

Cancer chemotherapy and multidrug resistance

In a past few decades the development and use of anticancer agents has become one of the most important ways of controlling the malignant diseases. They end up with the tumor cell death by necrosis (premature or unnatural death of cell) or apoptosis (programmed cell death). Multidrug resistance (MDR) is a major obstacle hindering the success of the cancer chemotherapy. It is defined as the resistance of the tumor cell to a broad spectrum of structurally and mechanistically diverse antitumor agents (Vyas and Khar, 2002). Tumor cells carrying MDR phenotype are often associated with over expression of some of the drug efflux pumps, known as P-glycoprotein (Pgp) pumps and multidrug resistance associated protein (MRP) pumps (Kartener et al., 1985). It prevents intracellular accumulation of many anticancer agents (that are its substrates) and hence causes a reduction in their cytotoxic activity mainly by increasing cellular efflux of positively charged amphipathic drugs in an ATP-dependent manner. The prime requirement for effluxing the drug out of the cell is ATP hydrolysis which provides the energy (Schinkel, 1999). In the structure of Pgp the transmembrane domains act as drug translocating sites whereas the ATP-binding sites that have the ATPase activity will provide the metabolic energy by ATP hydrolysis with a net outcome of drug efflux (Azzaria et al., 1989; Ambudkar et al., 1992). This kind of phenomenon is more pronounced with drugs which appear to enter the cell by passive diffusion through the lipid bilayer for example doxorubicin, paclitaxel etc. Pgp plays a crucial role in the pharmacokinetics of many clinically important therapeutic substrates (Endicott and Ling, 1989; Pastan and Gottesman, 1991; Gottesman and Pastan, 1993).

Overcoming MDR of cancer cells

In order to overcome MDR in cancer cells various strategies can be used that will inhibit or bypass (circumvent) Pgp so that the antitumor agent does not get effluxed out of the cell for an effective treatment. The different strategies that can be used to inhibit or circumvent the Pgp pump are as described below:

- Formulation of anticancer agent into Novel Drug Delivery Systems like nanoparticles, liposomes, micelles, solid lipid nanoparticles etc.
- Non substrate strategy (Borowski et al., 2005)
- By interfering with ATP hydrolysis (Shapiro and Ling, 1997)

- By altering integrity of cell membrane lipids (Drori et al., 1995)
- Use of modulators or inhibitors (Soma et al., 2003).
- Controlling the expression of MDR proteins.

Novel Drug Delivery Systems in circumventing Pgp mediated MDR

Instead of direct inhibition of Pgp, another way of bypassing resistance is to protect the drug against the pumping action of Pgp by means of chemical modifications or by association with colloidal carriers (nanoparticles or liposomes). The rationale behind association of drugs with colloidal carriers for reversal of MDR is the fact that Pgp probably recognizes the drug to be effluxed out of the tumoral cell only when it is present in the plasma membrane and not in case when it is located in the cytoplasm or lysosomes after its endocytosis. The other advantage of using these colloidal carriers are firstly the toxic side effects of the anticancer agents will be reduced due to specific and direct targeting of cancer cells. Pgp interacts directly with non-polar substrates within the membrane environment moving drugs from the inner to the outer leaflet of the lipid bilayer (Sharom, 1997). It can be assumed that the Pgp substrates that are not able to access the transporter protein (Pgp) from within the plasma membrane are not recognized by Pgp efficiently. Hence the use of a drug delivery system to transport a Pgp substrate across the plasma membrane would allow to by-pass Pgp and result in intracellular drug accumulation.

Nanoparticles

Nanoparticles are colloidal systems that range in size typically from 10 to 1000 nm in diameter, and are formulated from a biodegradable polymer in which the therapeutic agent is entrapped in, adsorbed or chemically coupled onto the polymer matrix (Labhasetwar, 1997). The most promising application of nanoparticles is their possible use as carriers for antitumor agents (Couvreur et al., 1990; Kreuter, 1991). Cancer-selective delivery systems are highly desired for chemotherapeutic agents for their ability to efficiently deliver the drug load to the tumor site. By confining the cytotoxic activity of the drug to within the malignant tissues, such delivery systems are envisaged to minimize indiscriminate drug distribution and lead to a focused destruction of the cancerous tissues. Drugs loaded in the nanoparticles are effective in a number of

multidrug resistant tumors. The polyalkycyanoacrylate (PACA) nanoparticles have been used successfully on a wide range of resistant tumor cell lines. These nanoparticles get located in the lysosomes which protects the loaded drug from the direct action of the Pgp as they do not come in immediate contact with the Pgp that is located in the plasma membrane. Nanoparticulates cause cell sensitization. Also it was assumed that the nanoparticles protect the drug from the action of Pgp as they enter the cell by endocytosis. (Cuvier et al., 1992; Nemati et al., 1994; Benis et al., 1994).

Paclitaxel (PTX) is a widely used antitumor agent that is effective in a broad spectrum of cancers. The limiting factor for using PTX in treatment of tumors is the multidrug resistance effect associated with it. It is effluxed out of the tumor cells by the Pgp present on the cell membranes resulting in a decreased efficacy of the drug. The present work is designed to evaluate the possibility of drug loaded nanoparticles to overcome multidrug resistance of tumor cells.

Hypothesis

It can be hypothesized that the nanoparticulate delivery system would overcome MDR to PACLITAXEL by bypassing Pgp present in the plasma membrane. Nanoparticles protect the drug from the action of Pgp as they enter the cell by endocytosis. By use of appropriate polymers it will be possible to have controlled drug delivery to the cells depending on the rate of degradation of the polymer. By conjugating the surface with transferrin, active targeting to the tumor cells can be achieved resulting in enhanced antiproliferative action due to receptor mediated endocytosis. Also it can help reduce the overall toxic effects of the anticancer agents due to specific and direct targeting of cancer cells and secondly cellular uptake of the drug delivery system will be promoted once the system has reached the target.

Aims and objectives

The purpose of this study is formulation of an anticancer agent (Paclitaxel) into novel drug delivery systems like nanoparticles and solid lipid nanoparticles in order to overcome Pgp mediated multidrug resistance associated with it.

- Formulation of polymeric biodegradable poly(lactide-co-glycolide), poly(n-butyl cyanoacrylate) nanoparticles and solid lipid nanoparticles loaded with PTX.
- Optimization of the various formulation and process parameters.
- Modification of the surface of PLGA NPs by
    - ✓ Coating the surface by Pluronic®P85 (Pgp inhibitor)-Passive targeting
    - ✓ Conjugation of transferrin to poly(lactide-co-glycolide) NPs loaded with PTX – Active targeting.
- Study the DSC, XRD, FTIR and NMR patterns of excipients and nanoparticles.
- To characterize the prepared formulations for entrapment efficiency (%), particle size, zeta potential and its morphological properties by transmission electron microscopy.
- To carry out *In-vitro* drug release from the formulation in pH7.4 phosphate buffer containing 20% ethanol.
- To carryout stability studies at various environmental conditions.
- To carry out In-vitro cell cytotoxicity and cell uptake studies in Pgp expressing cell line namely C6 rat glioma cell line.
- To perform the in-vivo pharmacokinetics and biodistribution studies in subcutaneous C6 glioma induced in rats and to study the tumor distribution of the NPs in comparison to free drug solution.

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