1.1. Uterine Disorders

Poor hormonal control over uterus and/or malfunctioning of endometrial linings of the uterus may result in abnormal uterine conditions - termed as uterine disorders. Abnormal vaginal bleeding, dysmenorrhea and menorrhagia are the key signs and symptoms of the uterine disorders.

The most common types of uterine disorders are:

Non-cancerous uterine conditions

- Uterine fibroids
- Endometriosis
- Adenomyosis
- Uterine polyps
- Endometrial hyperplasia

Uterine cancers

- Endometrial cancer
- Cervical cancer
- Uterine sarcomas

1.1. Endometrial cancer (EC)

1.1.1. Statistics

Endometrial cancer is the sixth most common type of cancer of the female reproductive organs worldwide, with 320,000 new cases diagnosed in 2012. About 53 percent of EC cases occurred in more developed countries. The peak prevalence of endometrial cancer was in Northern America and Europe; and the lesser incidence in Africa and Asia. The American Cancer Society has estimated 63,230 new cases and 11,350 deaths in the United States in the year 2018 [1, 2].

1.1.2. Risk factors

Prolonged unopposed oestrogen exposure is associated with most endometrial cancers. Oestrogen replacement therapy prescribed to control menopausal symptoms increases the risk of developing endometrial cancer by 2 to 20 fold, with an increasing risk correlating with the duration of use [3]. Long-term exposure to the endogenous

oestrogen, as happens in oestrogen-producing tumours, and with enhanced peripheral conversion of androgens to oestrone in adipose tissue, is also linked with an increased risk for developing endometrial hyperplasia and cancer. Apart from these factors, high blood pressure and diabetes mellitus may increase the risk of endometrial cancer [4].

Tamoxifen (a selective oestrogen receptor modulator) is extensively used for the treatment of breast cancer. It works as an oestrogen antagonist in breast tissues and as an agonist in bone and endometrial tissues [5]. The use of tamoxifen is associated with a 6 to 8 fold surge in the occurrence of endometrial cancer [6]. Age also represents a significant risk factor for developing endometrial cancer. Most women are diagnosed after menopause, with only 15% diagnosed before the age of 50 years and only 5% before 40 years of age [7]. Nulliparous and obese younger women are more likely to develop EC and have well differentiated endometrioid histology than older women [8, 9].

1.1.3. Treatment Modalities

Current therapies available for EC management are medical, surgical, radiological, and genetic modalities. The surgical procedures include hysterectomy (removal of the uterus), fallopian tubes, and ovaries. Pelvic lymph nodes may also be removed and/or sampled to be examined for the spread of the cancer. Depending on the spread of the cancer, other treatments, such as radiation and/or chemotherapy are recommended. The invasive surgical procedure has its own complications. Hysterectomy is not an appropriate option for women who wants to have pregnancy or wants to avoid surgery. If the cancer is not benign, then surgery may not be helpful, and so chemotherapy or other treatments may be used [10].

1.1.4. Chemotherapy

The International Federation of Gynaecology and Obstetrics (FIGO) staging systems, classified endometrial cancer in four classes. Chemotherapy is used as an effective treatment option at all the stages of EC, either as an adjuvant or main therapy [11-14]. Different treatment regimens containing various combinations of chemotherapeutic agents to treat EC are in practice. The combination therapy of Paclitaxel and

Carboplatin is most widely used treatment considering its advantages over other combination chemotherapies. The results of clinical studies suggest that Paclitaxel and Carboplatin have significant effect in advanced endometrial cancer with minimal side effects. 3 year progression free survival and overall survival rates were 50% and 75%, respectively, in Paclitaxel and Carboplatin receiving group and 37.5% and 50%, respectively, in the cisplatin, doxorubicin, and cyclophosphamide receiving group [15-18].

1.2. Mechanism of Action

1.2.1. Paclitaxel

PTX binds specifically to β-tubulin present in the microtubules and stabilizes it. Paclitaxel causes mitotic arrest since, the microtubules appear in the M phase of the cell cycle. Thus, chromosomes are not able to attain a metaphase spindle configuration. This blocks advancement of mitosis, and prolonged triggering of the mitotic checkpoint activates apoptosis or return to the G-phase of the cell cycle without cell division [19].

1.2.2. Carboplatin

Inside the cell, carboplatin undergoes hydrolysis of 1,1-cyclobutane dicarboxylate ring, becoming positively charged. This allows carboplatin to interact with nucleophilic molecules within the cell, including DNA, RNA and protein, which results into the formation of platinum adducts. This process occurs through covalent binding of carboplatin to the N7 site of purine bases, forming DNA-protein or DNA-DNA complexes. These complexes prevent DNA replication and transcription, results in breaks and miscoding, and if identified by p53 and other checkpoint proteins, induces apoptosis [20].

1.3. Presently available forms of Paclitaxel and carboplatin and limitations thereof

Paclitaxel- It is available in the market in the form of Paclitaxel Injections. As Paclitaxel is highly hydrophobic molecule, lipid-based solvents are used as a vehicle. Solubility of paclitaxel is enhanced using a mixture of 50:50 Cremophor EL® (CrEL, a non-ionic

surfactant polyoxyethylated castor oil; BASF, Florham Park, NJ, USA) and ethanol. Before administration via intravenous infusion, it must be further diluted 5 to 20 fold with normal saline or 5% dextrose solutions.

Cremophor EL is biologically and pharmacologically active and leaches plasticizers from standard intravenous tubing, releasing di-(2-ethylhexyl) phthalate (DEHP). Its infusion produces histamine release with consequent well-described hypersensitivity reactions, including anaphylaxis. Moreover it has been also associated with hyperlipidaemias, abnormal lipoprotein patterns, aggregation of erythrocytes, and prolonged, sometimes irreversible sensory neuropathy which may be associated with demyelination and axonal degeneration. CrEL can also cause neutropenia [21].

A different type of marketed formulation is Albumin bound paclitaxel (Abraxane®; Abraxis Bio Science and AstraZeneca). It works on the principle of EPR assisted uptake of nanovectors. Various nanotechnologies are also studied *viz* liposomes [22], dendrimers [23], super paramagnetic nanoparticulates [24], gold nanoshells [25], silicon- and silica-based nanoparticles [26] and nanocrystals [27]. All these preparations are IV administered and thus associated with diverse chemotherapeutic side effects such as Low blood counts, Alopecia, Peripheral neuropathy, Arthralgias and myalgias, Abnormal ECG, Weakness and fatigue etc.

Carboplatin-

Carboplatin possess good solubility in water (14mg/ml). On account of its water solubility, various IV injectable formulations available in the market. Various nano-carrier based systems i.e. Nanoparticles [28], Liposomes [29], Nanocapsules [30] etc, are reported in literatures in order to achieve tumour targeting and to reduce systemic side effects. However, due to IV administration of these formulations, non-specific distribution of the drug occurs, which ultimately leads to severe systemic side effects.

Paclitaxel and carboplatin intravenous injections are administered as combination chemotherapy. Carboplatin as a single drug administered as 400 mg/m^2 IV and in combination with paclitaxel, it is administered to achieve AUC of 5-6 IV over 1 h. Paclitaxel is administered via IV injection of dose 175 mg/m^2 over 3 hours. Cycle is

repeated every 3 weeks for 6 to 9 cycles [31]. A weekly low-dose PTX (80 mg/m^2) and CBP (AUC 2) regimen is also well tolerated and has activity in patients with advanced or recurrent endometrial cancer [32]. Intravenous chemotherapy causes phlebitis at the site of injection that leads to poor patient compliance and more typical chemotherapeutic systemic side effects related with the drug and/or excipients.

1.4. Importance of Tumour Targeting

The long-standing problem of chemotherapy is the lack of tumour-specific treatments. Conventional chemotherapy depend on the premise that quickly proliferating cancer cells are more likely to be killed by a cytotoxic agent. In reality, however, cytotoxic agents have very less or no specificity, which leads to systemic toxicity, causing severe side effects. Therefore, various "molecularly targeted cancer therapies" have been developed for use in specific cancers.

1.4.1. Approaches for targeting

Passive targeting

Administered nanocarriers accumulate in tumours mostly because of the enhanced permeability and retention effect (EPR effect), which is due to angiogenic processes that produce highly permeable blood vessels in tumours and their characteristic abnormal lymphatic drainage that leads to the accumulation of the nano-scale particles in them, allowing the release of the cytotoxic drugs close to the tumour cells. Nanocarriers of size range 100-200 nm with prolonged blood circulation time, leads to effective targeting via EPR effect [33, 34].

Active targeting

Active targeting is achieved by attaching specific ligands to the nanocarrier structures, allowing a selective recognition of different receptors or antigens overexpressed in the tumour cell surfaces, increasing the cytotoxicity of the anticancer agents in tumours and avoiding most of their side effects, since the exposure of healthy cells to the drug is minimized [35]. Various methods are used to target cancer cells e.g. Folate conjugated systems [36], Albumin bound systems [37], Hyaluronic acid based targeting

systems [38], Transferrin based targeting systems [39], mAbs and Peptide targeting systems [40, 41] etc.

Uterine targeting

Endometrial cancer is associated with a 50% relapse rate and had a 5-year survival of 18–27%. Moreover, recent studies shows that, the survival of women with stage I–II EC is 35–50% and for stage III–IV EC is 0–15% [42]. These figures illustrate the dire need for a deeper understanding of the methods for uterine targeting for the treatment of EC, as well as the necessity to develop novel and more effective therapeutic modalities against recurrent disease. Uterine targeting may represent a reasonable and innovative approach for the treatment of Endometrial Cancer refractory to standard treatment modalities [43].

1.5. Vaginal Route for drug administration

The vaginal route for drug administration has been well established through its long and well-studied history of safety and efficacy. Drugs are easily and rapidly absorbed through the vaginal epithelium, and there are no adipose tissue or other cell layers with metabolic enzymes to traverse as with the transdermal or oral routes. Vaginal administration allows low, continuous dosing, which results in stable drug levels and may, in turn, achieve a lower incidence of side effects and improve patient compliance. Various vaginal formulations such as pessaries, vaginal films, foams and gels have been used to administer drugs using this route. However, anatomical structure of vaginal canal does not allow these formulations to stay longer in its place. The most common problem is leakiness of the vaginal route. However, vaginal ring technology makes drug administration easy and discreet for patients, giving them complete control over the treatment method and its reversibility [44].

1.6. First Uterine Pass Effect

In recent years, the existence of direct transport mechanisms between the vagina and uterus has been demonstrated, resulting in preferential uterine delivery of hormones that are administered vaginally [45]. By analogy to hepatic effects seen after oral administration, this phenomenon is termed as the first uterine pass effect [46].

This locally functional "portal" system occurs through four proposed mechanisms by which drug can pass from vagina to uterus [47].

- Diffusion through tissues
- Passage through the cervical lumen from vagina to the uterus
- Transport via venous or lymphatic circulatory system
- Countercurrent vascular exchange with diffusion between utero-vaginal veins and/or lymph vessels and arteries.

The passive targeting of the drug could also be assisted by the EPR effect. The advantages of First Uterine Pass Effect [46] are summarized below –

- Targeting the drugs directly to the site of action and hence achieving maximum therapeutic effect by lowest possible dose
- Reduction in side effects of drugs by avoiding the systemic absorption
- Increasing the patient compliance by avoiding painful injectable route and also self-medication being made possible by vaginal administration
- Passive targeting to uterus can be possible

Intra-vaginal drug delivery leads to drug targeting to the uterus by the means of functional mechanism called First Uterine Pass Effect. The drug can be targeted to uterus when administered intra vaginally.

1.7. Formulation Development

1.7.1. Ultra deformable vesicles

Ultradeformable vesicles comprising phospholipids and an edge activator (EA) is a novel formulation in vesicular drug delivery. Ultradeformable vesicles by the virtue of their enhanced elasticity compared to the conventional liposomes, are more amenable to the transport of therapeutic agents across the biological membranes [48]. Elasticity in these vesicles is attributed to the presence of an EA, which is generally a single chain surfactant with a high radius of curvature, capable of weakening the lipid bilayers of the vesicles and increasing their deformability. During destabilization and owing to their affinity for curved configurations, surfactants attempt to gather at the location of enhanced pressure and consequently lead to a decrease in the energy needed for the

change of shape. The maximum deformability is obtained when the vesicular membrane attempts to optimize its local composition in response to an external, spatially anisotropic stress [49]. The properties and applications of ultradeformable vesicles are summarized below [50]

- They are able to transport therapeutic agents through very narrow pathways between most cells in the biological membranes (e.g. Stratum Corneum).
- Unlike Nanoparticles, Liposomes and other nano-carrier systems, UDVs are able to penetrate vaginal mucus through low viscosity channels.
- UDVs are biocompatible, biodegradable and are capable of protecting the encapsulated drug from metabolic degradation.
- UDVs have also been used as transporters for various macromolecules, including peptides, proteins (insulin, albumin), DNA, antigens, corticosteroids etc, and have been proven to significantly augment amount of drug permeated.
- With their great penetration abilities, UDVs offer a non-invasive means of drug delivery.

Intra Vaginal Rod Inserts

The vaginal route has been extensively studied as a site for drug administration. As a consequence, a novel controlled release system known as intra vaginal rings recently been studied by researchers. Vaginal rings are torus shaped devices formed by a polymeric material (e.g. poly-dimethylsiloxane/silicone, ethylene vinyl acetate copolymer (EVA) etc.) which holds the active ingredient [51]. The ring is simply inserted into the vagina and it releases the drug in a controlled manner. The rate of release is controlled by several factors like the relationship between the characteristic dimensions, the initial load of active ingredient, the presence of excipients, the design of the IVRs etc [52]. The use of vaginal rings presents numerous advantages [51] like,

- It permits the controlled release of drug
- Avoids daily administration
- Allows the use of low drug dose and the simultaneous administration of several drugs by the same device

- It is user controlled

A novel vaginal ring device, the 'insert vaginal ring' (InVR), comprising a ring body into which various drug- loaded inserts can be placed has been recently developed. The ring device comprises one or more drug-loaded rods, fabricated from either modified silicone elastomer, a compressed solid tablet, or a lyophilized gel, that are inserted into cavities contained within a non- medicated silicone elastomer ring carrier [53].

1.8. Aim and Objectives

The aim of the study is to target the drugs available for Endometrial Cancer by vaginal route to achieve the following:

- Targeting the drugs directly to the site of action and hence achieving maximum therapeutic effect by lowest possible dose.
- Reduction in side effects of drugs by targeting it directly to the site of action.
- Increasing the patient compliance by avoiding painful injectable route and self-medication being made possible by intra vaginal rings.

1.9. Hypothesis

The ultradeformable vesicles loaded with Paclitaxel and Carboplatin are expected to target in uterus to act upon endometrial cancer via intravaginal route of administration. The ultradeformable vesicles, by virtue of its flexibility will localize drugs in the uterus to achieve augmented tissue concentrations thereby overcoming the limitations of existing formulations.

1.10. Plan of work

- Review of Literatures
- Procurement of Drugs and Excipients
- Preformulation studies to confirm the identification, purity of drugs and to ensure drug-excipient compatibility
- Development of analytical methods and validation thereof
- Formulation Development using statistical optimization of PTX loaded UDNVs
- Formulation Development using statistical optimization of CBP loaded UDNVs

- Characterization of the developed formulations of PTX and CBP
- Biocompatibility and safety assessment of the developed formulations
- Short term stability studies as per ICH guidelines
- Cytotoxicity studies
- In vivo biodistribution and efficacy studies

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