Chapter 1

Introduction

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1.1 INTRODUCTION

Cancer represents a complex and multifaceted group of diseases characterized by the uncontrolled proliferation of abnormal cells, coupled with their propensity to invade adjacent tissues and metastasize to distant anatomical sites. This pathological process presents formidable challenges to global health systems, underscoring the urgent need for effective intervention strategies [1, 2]. The global burden of cancer as of 2020 is particularly alarming, with the World Health Organization reporting approximately 1.93 Crore new cancer cases and 1 Crore cancer-related deaths worldwide [1-3]. These statistics highlight the critical public health implications of cancer and the necessity for ongoing research and development in oncological therapies. Current therapeutic modalities for cancer treatment are diverse and include surgical resection, chemotherapy, radiation therapy, hormonal therapies, and innovative targeted therapies [1-4]. Advancements in research continue to unveil new therapeutic strategies and enhance existing treatments, aiming to improve patient outcomes and reduce the burden of this disease on healthcare systems globally [1-4].

Breast cancer represents a prevalent and concerning malignancy that impacts a substantial number of individuals, particularly women. It is recognized as the most commonly diagnosed cancer among females worldwide and is the leading cause of cancer-related mortality in this population [5, 6]. In 2020, there were approximately 685,000 reported fatalities attributable to breast cancer, alongside the identification of 2,300,000 new cases among women globally [5-7]. Data from the World Health Organization (WHO) indicates that breast cancer has ascended to the status of the most prevalent cancer on a global scale, affecting an estimated 7,800,000 women who received a diagnosis within the preceding five years and were alive as of December 2020 [5-8]. Projections for the year 2040 suggest an alarming increase, with expectations of over 3,000,000 new diagnoses and around 1,000,000 deaths due to breast cancer, thereby underscoring the significant challenges anticipated in the future [5-8]. Furthermore, the disparities in survival rates across different regions of the world highlight the grim reality of breast cancer. High-income countries report an estimated five-year survival rate of approximately 80 %, whereas low-income nations experience a considerably lower survival rate of around 40 %. This stark divergence emphasizes the critical issues of healthcare access and resource availability that persist on a global scale [5-8].

The trajectory of breast cancer, which has been a subject of significant concern for healthcare professionals and policymakers alike, reveals a troubling global trend characterized by an alarming escalation in the incidence of this devastating disease, as evidenced by a staggering increase of more than 20 % worldwide since the year 2008, highlighting the urgent need for action [3, 4]. Equally troubling is the equally significant 14 % surge in mortality rates associated with breast cancer that has occurred over the same time frame, further emphasizing the gravity of the situation and the necessity for immediate intervention [3-5]. These concerning figures serve to underscore the critical and pressing need for the implementation of comprehensive strategies aimed at addressing various elements such as prevention, early detection, and effective treatment on a global scale, as it is clear that the time for action is now [3, 4]. These compelling statistics not only highlight the alarming reality we face but also underline the essential requirement for concerted global efforts in areas such as research initiatives, public awareness campaigns, and strategic resource allocation, all of which are crucial to effectively confront and combat the multifaceted challenges that cancer presents in its numerous and varied forms [6-9].

Effective drug delivery has played an undeniably significant role in substantially enhancing and advancing the overall effectiveness of cancer treatment methodologies, contributing to better patient outcomes and improved survival rates [10, 11]. On the contrary, ineffective drug delivery mechanisms have led to a variety of detrimental consequences, such as insufficient tumour response, the occurrence of severe and debilitating adverse effects, and the unfortunate emergence of well-known and notorious cancer drug resistance, which complicates treatment strategies [10, 11]. Given that anticancer drugs are generally known to be toxic not only to cancerous cells but also to healthy proliferating cells that are essential for normal body functions, it is crucial that the dosage of these medications be meticulously restricted in order to prevent the onset of potentially life-threatening adverse effects that could compromise patient safety [11, 12]. Furthermore, several factors, including but not limited to limited systemic circulation lifetime, undesirable biodistribution patterns, non-specific cellular uptake mechanisms, and inadequate tumour vascularisation, can significantly further diminish the therapeutic efficacy of such a low administered drug dose, rendering treatment less effective [10-15]. As a direct consequence of these challenges, each course of chemotherapy has typically resulted in only partial treatment outcomes, which creates a selective pressure that inadvertently favours the development of mutations and drug

resistance among the surviving cancer cells, ultimately complicating future treatment options [12-16]. Even drugs that initially demonstrate a favourable response in terms of effectiveness are frequently rendered ineffective after multiple administrations, thus making the ongoing treatment of recurrent tumours considerably more challenging and complex for healthcare professionals [10-14].

The alarming increase in resistance to chemotherapeutic drugs has been intricately connected to the gradual emergence of various mutations, which can significantly impair the effectiveness of cancer treatments. Among the diverse types of mutations that have been identified, we can find compromised apoptotic signalling pathways, which hinder the natural process of programmed cell death, alongside enhanced mechanisms for repairing cellular damage, which effectively allow cancer cells to recover from the detrimental effects of treatment; additionally, there are increased rates of drug metabolism that facilitate the breakdown of therapeutic agents, altered drug targets that prevent the drugs from binding effectively, and an up-regulation of drug efflux pumps that expel drugs from the cancer cells, all contributing to this complex resistance phenomenon [12-16]. Consequently, to develop an effective strategy aimed at improving the treatment outcomes for cancer cells, it would be essential to activate multiple signalling pathways simultaneously in order to thwart tumor cells from acquiring the necessary mutations that lead to drug resistance [12-16]. Furthermore, it is noteworthy that the majority of malignancies have been associated with a multitude of genetic alterations or abnormalities that collectively contribute to the significant phenomenon of tumor heterogeneity, presenting an additional challenge in the effective treatment of cancer patients [12-16].

Utilizing a singular chemotherapeutic agent to treat various forms of cancer has unfortunately led to the pervasive development of drug resistance, which has become a significant and formidable barrier to achieving the desired success of cancer therapy, as documented in various studies [12-16]. In order to effectively decrease the emergence of drug-resistance phenotypes that complicate treatment outcomes, an efficient and innovative method for enhancing the overall therapeutic efficacy would be to implement treatment strategies that incorporate agents capable of acting through multiple, diverse mechanisms of action [12-16]. The delivery of multiple therapeutic agents through the use of highly efficient nanocarrier platforms is emerging as a particularly promising and advantageous strategy for successfully

overcoming all of the aforementioned challenges and obstacles currently faced in cancer treatment [12-17].

Paclitaxel, which is more commonly referred to by its abbreviated form PAC, represents a particularly potent and effective chemotherapeutic agent that plays a crucial role in the treatment and inhibition of a diverse range of cancer types, among which are notably included ovarian cancer, breast cancer, and lung cancer [18, 19]. However, despite its remarkable efficacy in combating these serious diseases, the limited solubility of PAC in aqueous environments poses significant challenges that can hinder the optimal delivery of this important medication to the target sites within the body [19-21]. Given that Paclitaxel exhibits reduced solubility in water, it is standard practice for this drug to be prepared in a specific formulation that consists of an equal mixture of dehydrated ethanol and Cremophor EL, with a volume ratio of 50:50, which is commonly recognized by the brand name TAXOL [22]. Nevertheless, it is imperative to note that the utilization of both ethanol and Cremophor EL in this formulation has been associated with a range of adverse effects that can negatively impact patient health and treatment outcomes [20-22]. Specifically, Cremophor EL, which functions as a non-ionic surfactant and serves the purpose of acting as a carrier for various medications that are poorly soluble, has been correlated with the occurrence of severe hypersensitivity reactions of the anaphylactoid type, as well as conditions such as hyperlipidaemia, unusual lipoprotein patterns, aggregation of erythrocytes, and peripheral neuropathy, as documented in several studies [19-25].

Cyclophosphamide (CYC), an alkylating agent, is an effective cancer treatment option because it weakens and destroys malignant cells by interfering with their genetic material. Cyclophosphamide is still one of the most successful and commonly used antineoplastic medicines. However, it has a number of adverse effects and interactions with other drugs [25-27]. Cyclophosphamide is available in dry powder form for intravenous injection. That requires a total reconstitution time of 5 to 15 minutes. Moreover, Cyclophosphamide is difficult to store as a liquid due to deterioration in solution form. Therefore, no "ready-to-use" liquid formulations are available in the market [25-27].

The chemical stabilities associated with solutions of Cyclophosphamide have been thoroughly documented and extensively reported in the scientific literature, demonstrating a

comprehensive understanding of their behavior under various conditions [28-30]. In order to evaluate the properties of Cyclophosphamide solutions, a series of examinations and tests were conducted, focusing on those solutions that were prepared through the methods of reconstitution and/or dilution of both available formulations, with careful observations made at three distinct temperature settings: 4°C, 22°C, and 37°C, allowing for an in-depth analysis of their stability profiles [28-31]. It was determined that Cyclophosphamide solutions exhibited their peak stability when stored at 4°C in a dark environment, maintaining this optimal condition for a maximum duration of seven days, thereby highlighting the importance of temperature control in preserving the integrity of the compound [31-33]. Conversely, at elevated temperatures, degradation became evident throughout the testing period, specifically demonstrating a 10% reduction in the concentration of the medication after a span of seven days at an ambient temperature of 37°C, and a more pronounced 50% reduction after the same duration of storage at this higher temperature, indicating a significant loss of potency. Comparable findings were also observed with admixtures that contained 5% dextrose and 0.9% sodium chloride, which were prepared with an initial concentration of the medication set at 1mg/mL, reinforcing the notion that these combinations did not alter the stability outcomes significantly [28-31]. Moreover, it is abundantly clear that the chemical stability characteristics do not differ markedly between the solutions that are produced through the processes of reconstitution and dilution of both dry-filled and lyophilized formulations, suggesting a consistent behavior across these preparation methods [24-31]. Ultimately, it becomes strikingly apparent that Cyclophosphamide exhibits a precarious nature when dissolved in aqueous solutions, rendering it particularly susceptible to hydrolysis, as evidenced by multiple studies and reports in the existing literature [24-31].

Referring specifically to the Product Information Leaflet of the Reference Standard known as CYTOXAN, which is the common name for Cyclophosphamide for Injection USP, it has been observed that lyophilized vials containing this particular medication are susceptible to melting under various conditions [28-31]. The vials in question may hold either a clear or a yellowish viscous liquid, which is typically observed as a connected phase or as droplets that can be found within the affected vials, indicating that the contents may not be in their optimal state [31-33]. In order to ensure proper reconstitution, lyophilized Cyclophosphamide may necessitate vigorous shaking in order to achieve a complete dissolution, and it is important to note that this process may not happen instantaneously or entirely at once, thereby requiring a

certain period of time to settle for complete dissolution to occur [28-33]. Furthermore, the lyophilized formulation of Cyclophosphamide, often abbreviated as CYC, necessitates additional dilution with one of several specific solutions, which may include Dextrose Injection, USP (5% dextrose), Dextrose and Sodium Chloride Injection, USP (which combines 5% dextrose and 0.9% sterile sodium chloride), 0.9% Sodium Chloride Injection USP, or Sodium Chloride Injection, USP (which contains 0.45% sterile sodium chloride). Once diluted, these solutions exhibit stability that can last for as long as 24 hours when stored at room temperature, and they can remain stable for up to 36 hours when placed in refrigeration at temperatures ranging from 2 to 8 degrees Celsius [28-33].

Nanostructured Lipid Carriers (NLCs), which represent the innovative second generation of lipid-based nanocarriers, are intricately constructed from a carefully orchestrated mixture of both solid and liquid lipids, resulting in an unstructured matrix that arises from the diverse chemical moieties of the various constituents that make up these NLCs [34]. These NLCs are skilfully comprised of biocompatible solid lipid matrices combined with liquid lipid components, exhibiting a particle diameter that remarkably ranges from as small as 10 nanometers to as large as 1000 nanometers [35-38]. The incorporation of liquid lipids featuring different fatty acid carbon chain lengths leads to the formation of NLCs that possess a less organized crystalline structure, which, in turn, enhances their capacity for accommodating a greater volume of drugs [35-38]. These specialized carriers are formulated using physiological and biodegradable lipids that are characterized by their low systemic toxicity as well as their minimal cytotoxicity, making them an appealing option in the realm of drug delivery systems [33-38].

NLCs have been meticulously chosen as the preferred vehicles for the parenteral administration of chemotherapeutic agents primarily due to the fact that they represent a highly sophisticated and remarkably efficient carrier system that comes with a multitude of advantages, including but not limited to, enhanced physical stability, the convenience of straightforward preparation and scalability, improved dispersability within an aqueous environment, exceptional capacity for the entrapment of lipophilic drugs, meticulous control over particle size, prolonged release profiles for the drug, a significantly improved benefit-to-risk ratio, minimal side effects, extended circulation time of the drug within the body, reduced cytotoxic effects, superior bioavailability of the drug, and greatly enhanced drug

permeability and retention specifically within tumor tissues [38]. Furthermore, NLCs have risen to prominence as a highly promising carrier system for the effective delivery of pharmaceutical compounds, utilizing various routes of administration such as oral, parenteral, ocular, pulmonary, topical, and transdermal pathways, as supported by numerous studies and references [38-42].

Microemulsions (MEs) can be described as isotropic systems that possess thermodynamic stability, comprising a carefully balanced mixture of oil, water, and surfactant, and are often found to be combined with a cosurfactant; the droplet size within these systems typically falls within the range of 20 to 200 nanometers, which is supported by various studies and documented references [43-45]. These homogeneous systems, which can be synthesized and prepared across a broad spectrum of surfactant concentrations and varying oil-to-water ratios, consistently exhibit characteristics of low viscosity, making them fluid in nature [44-46]. When considering the application of microemulsions as a potential drug delivery mechanism, they demonstrate a range of advantageous properties, including notable thermodynamic stability, which contributes to an extended shelf-life, ease of formation characterized by the presence of zero interfacial tension leading to almost spontaneous formation, optical isotropy that enhances their usability, the capability to undergo sterilization via filtration methods, a high surface area that correlates with an impressive solubilization capacity, and exceptionally small droplet sizes that facilitate their effectiveness in various applications [43-47].

1.2 AIMS & OBJECTIVES

The present research focused on the development of nanostructured lipid carriers and microemulsion for the simultaneous delivery of Paclitaxel (PAC) and Cyclophosphamide (CYC) for the treatment of Breast Cancer. Thus, development of Paclitaxel-Cyclophosphamide dual drug NLCs and microemulsion may provide a new attractive treatment option.

The objectives of present work were:

(i) To design and develop NLCs and microemulsions for the effective co-delivery of PA) and Cyclophosphamide (CYC) in breast cancer treatment.

(ii) To improve the solubility, and stability of PAC and CYC using advanced nanocarrier systems.

(iii) To enhance targeted delivery PAC and CYC.

(iv) To overcome drug resistance mechanisms in breast cancer treatment through co-delivery of PAC and CYC with nanocarriers, aiming to improve therapeutic efficacy and patient outcomes.

(v) To develop scalable carriers for the treatment of breast cancer ensuring effectiveness and safety.

(vi) To optimize composition and process parameters of the developed formulations and to characterize their physico-chemical properties.

(vii) To carry out in-vitro cell line studies and in vivo studies of drug loaded nanocarriers.

(viii) To assess the stability of drug loaded nanocarriers.

1.3 PLAN OF WORK

a) Literature review
b) Procurement of excipients and therapeutic moieties
c) Preformulation studies
d) Formulation, optimization and characterization of drug-loaded carriers
e) In-vitro cell line and In-vivo evaluation of nano-carriers
f) Stability studies of formulated nano-carriers

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