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Tamoxifen and Sulphoraphane for the breast cancer management: A synergistic nanomedicine approach

Bharti Mangla[a], Yub Raj Neupane[b], Archu Singh[a], Kanchan Kohli[a],*

[a] Department of Pharmaceutics, School of Pharmaceutical Education & Research, Jamia Hamdard, New Delhi 110062 India
[b] Department of Pharmacy, National University of Singapore, Singapore 117559, Singapore

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ABSTRACT

Breast cancer is second most leading cause of death in all over the world and not only limited to the females. Tamoxifen has been considered as the gold line therapy for estrogen receptor positive breast cancer. However, this chemopreventive approach has been focused at individuals in high risk group and limits its clinical applications to moderate and/or lower risk groups. Moreover, Tamoxifen treatment is associated with a dose related hepatotoxicity and nephrotoxicity and eventually results in poor quality of life of patients. Sulphoraphane, a naturally occurring isothiocyanate derivative has been investigated for its numerous potential biological activities including anticancer effects. The present hypothesis aims to put forward in which Tamoxifen is combined with a natural bioactive Sulphoraphane, both incorporated into a novel lipid based nanocarrier at a reduced dose, which would eventually shuttle the cargo to the target site. At the breast cancer, Sulphoraphane sensitizes the estrogen receptors and ameliorates the binding affinity of Tamoxifen to these receptors, thereby potentiating the anticancer efficacy and reducing the offsite toxicity of Tamoxifen. This dual loaded zero-dimension lipid carrier would be a value addition to the current treatment regimen for breast cancer management.

Introduction:

Since 1500 BC, Egyptians reported the first case of breast cancer remains as the most leading cause of death worldwide after lung cancer [1,2]. According to the cancer statistics, about 5 lakh deaths occur due to cancer in 2018 in which 2 lakh deaths were due to breast cancer and most of them were due to metastasis of breast cancer to vital organs like bones, lung, liver and brain [3]. Breast cancer is not only limited to females but also accounts 0.8–1% cases in males [4]. Among various types of breast cancer, Estrogen receptor positive breast cancer is the most common invasive cancer in women and its formation is encouraged by Estrogen Receptors as it regulates the cyclin D1, Myc, Bcl-2 and VEGF levels which are important for cell cycle, cell survival and stimulation of angiogenesis [5].

Targeted therapy, chemotherapy, endocrine therapy, surgery and radiation therapy are most commonly used for the treatment of breast cancer in modern medicine. Among them, most common treatment for estrogen positive breast cancer includes chemotherapy with Tamoxifen a selective estrogen receptor modifier, aromatase inhibitor like letrozole and selective estrogen receptor down regulator, such as fulvestrant [6].

Tamoxifen has been used as a chemotherapy for breast cancer since its approval by U.S. FDA in 1988 [7] and reported to reduce the annual mortality rate reduced by 51%, being considered as gold line drug for the treatment for estrogen receptor positive breast cancer in post and pre-menopausal women [8]. Tamoxifen acts by binding to the estrogen receptors and induces the conformational change in the receptors and subsequently change in the expression of estrogen dependent genes [9]. Tamoxifen stops the cancerous cells in the G_0 and G_1 phase of the cell cycle and avert their multiplication. Moreover, Tamoxifen can reduce the circulating levels of insulin-like factor IGF-I [10] which is responsible for controlling the growth of breast tumour which may act by paracrine, autocrine and endocrine routes to stimulate their growth. It increases sex hormone binding globulin [11] level in postmenopausal cancer patient which in turn lowers the amount of free estradiol in the serum [12].

Despite being a promising molecule, Tamoxifen possess several biopharmaceutical and toxicological issues [13,14]. Nevertheless, it shows poor oral bioavailability nearly 30% with large inter-individual variation [15], due to precipitation as free base in acidic environment of the stomach [13], extensive first pass metabolism in the liver [16] and effluxes out through P-glycoprotein pump present in the intestine [17]. Furthermore, extensive first pass metabolism of Tamoxifen leads to formation of toxic metabolites and free radicals [18,19], leading
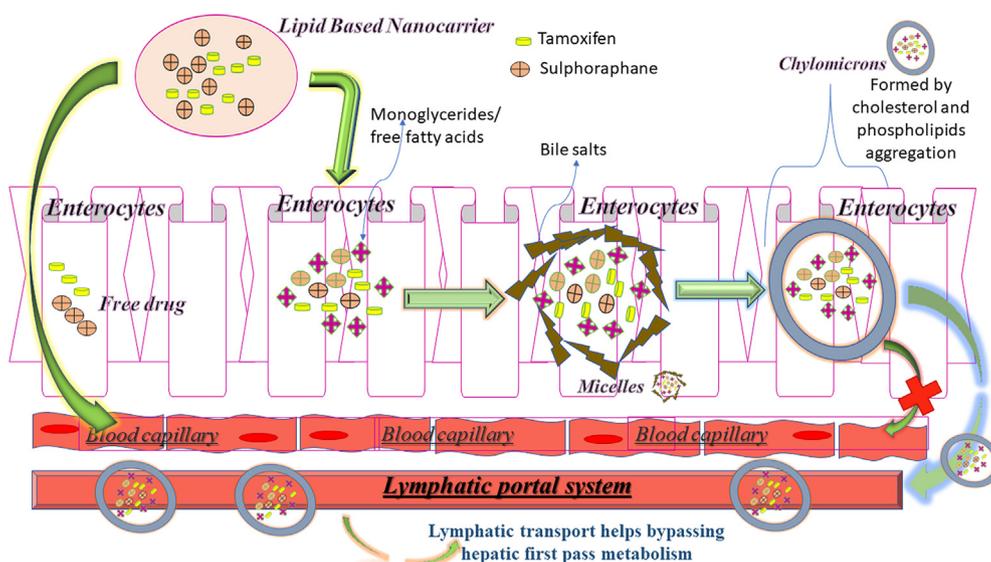
Fig. 1. Absorption mechanism of lipid based nanocarrier: Drug loaded lipid based nanocarrier undergoes lipid digestion and converted into micelles, mixed micelles and chylomicrons in the presence of lipid and bile salts in the intestine. Then micelles converted into chylomicrons with aggregation of cholesterol and phospholipids. Finally, chylomicrons are targeted to lymphatic system and bypass first pass metabolism.

patients at risk of endometrial cancer and oxidative stress mediated hepatotoxicity due to dose accumulation [20,21]. It is also reported that, high dose of tamoxifen induce liver diseases like steatosis and cholestatic syndrome [22,23]. Moreover, Saleh et al. demonstrated that nephrotoxicity may develop in association with dose of Tamoxifen therapy [24] resulting to decreases the hexose monophosphate shunt and thereby increasing the incidence of oxidative stress in the cells. Similar study reported that breast cancer patient who have received oral tamoxifen with the dose of 40 mg causes fatty liver which was confirmed by abdominal computed-tomography (CT) examinations [25].

Sulphoraphane, is a natural bioactive compound present in many cruciferous vegetables like broccoli, choy, cauliflower, cabbage and kale which is the safe and most abundant dietary phytochemical [26]. It has been reported to demonstrate a variety of biological effects in humans like antioxidant, antimicrobial, anti-inflammatory, anti-aging, neuroprotective and antidiabetic, and also found to be very promising chemopreventive agent against variety of cancers such as breast, prostate, colon, skin, lung, stomach, and bladder [27]. Sulphoraphane works primarily through HDAC (Histone DeACetylase) inhibition by increasing Nrf2 activity. Histones (proteins) make DNA more compact, however Suphoraphane increases the activity of an entire genetic signalling pathway whose main function in the body is to protect against cancer formation, toxins, and excessive oxidation [28]. Moreover, a study performed in estrogen receptor breast cancer cell lines have been shown the anticancer activity of via adjusting cytochrome P450 involved in oestrogen metabolism [29], Nrf2 activator [30], (mTORC1)–p70 ribosomal protein kinase 1 (S6K1) pathway [31], ERK1/2-IKKα (extracellular signal-regulated kinase-1/2) and NAK-IKKβ (nuclear factor-kappaB -activating kinase) [32], ErbB2/ER-PI3K/Akt-mTOR-S6K1 signalling (phosphoinositide 3 kinase) [33] and CSCs pathway [34].

Oral conventional chemotherapy is considered as the ideal way for treatment of cancer. It is due to the fact that oral chemotherapy is non-invasive, easy of drug administration and with better patients compliance [35]. Some of the oral chemotherapeutics available in market are 6-mercaptopurine, methotrexate, busulphan and cyclophosphamide have been given orally for many years for different types cancer [36]. However, poor oral bioavailability of most anticancer drugs limit the approach to oral chemotherapy in clinical settings [37] due to poor aqueous solubility and low permeability of the drugs.

Lipid based nanocarrier hold promising applications in the nascent field of oral drug delivery including both hydrophilic and lipophilic drugs and are considered as an alternative delivery system to the traditional colloidal drug delivery systems [38,39]. Sandimmune™ and Neoral™ are reported as the successful examples of lipid based nanocarrier for the oral delivery of Cyclosporine A, which are available in the current clinical settings [18].

Nanocarrier based approach has been considered as stupendous advantages in oral delivery of various combination therapeutic regimens [40,41]. The potential drug-drug interactions between various combinatorial therapeutic regimen of anticancer drugs would fruitfully avoided by utilization of lipid nanocarrier-based approach where drugs are encapsulated within carrier matrices and also prevent from degradation in the GIT, enhanced absorption and enhanced oral bioavailability with reduced toxicity and offsite target [42,43].

Hypotheses

In this study, we hypothesize to design and fabricate lipid based nanocarrier scaffold for the co delivery of Tamoxifen (a synthetic chemotherapeutic agent) and Sulphoraphane (a natural chemosensitizing agent) for their enhanced and efficient delivery to the breast cancer. This study hypothesizes 1) enhanced oral delivery of Tamoxifen through intestine in the presence of Sulphoraphane in lipid based nanocarrier thereby preventing the first pass metabolism and increasing the lymphatic uptake of Tamoxifen 2) increased sensitivity of the estrogen receptors in the breast cancer cells by Sulphoraphane and subsequently promote the binding of Tamoxifen to the estrogen receptors 3) reduced dose of Tamoxifen through synergistic effect when co delivered with Sulphoraphane and subsequently reduce the offsite toxicity of Tamoxifen 4) inhibition of p-glycoprotein efflux pump and multi drug resistance by Sulphoraphane and ameliorate the cellular uptake of Tamoxifen by estrogen receptor positive breast cancer cells 5) surfactants (Tween 80, poloxamer 188) used in the lipid based nanocarrier as a stabilizer enhanced the permeation Tamoxifen by inhibiting p-glycoprotein efflux pump present in the intestinal milieu (Fig. 1).

Evaluation of hypothesis

An entire filed of cancer research dedicated towards the combination of herbal bioactive with synthetic chemotherapeutics has spawned in recent years to circumvent the offsite toxicity of conventional chemotherapy. Number of natural bioactive which have been investigated for their chemosensitization effect along with their synergistic effect to the synthetic chemotherapeutics includes curcumin, resveratrol, genistein, CAPE, emodin, flavopiridol, silymarin, Suphoraphane and reported that these natural herbal molecules have multiple foci of action

to the tumour cells [44–46]. Sulphoraphane, a naturally occurring isothiocyanate derived herbal bioactive molecule which has been extensively investigated for chemosensitization and synergistic effect when combined with a synthetic chemotherapeutic agent proved to be effective in cancer treatments with ameliorated therapeutic effect and reduced dose related toxicity.

It has been reported that doxorubicin-mediated cardiotoxicity which is considered as the serious side effect of doxorubicin was significantly reduced when Sulphoraphane combined with doxorubicin [35]. In another study, it is also reported that Sulphoraphane potentiates the anticancer effects of doxorubicin and reduces the risk of cardiotoxicity by reducing nuclear Nrf2 binders [36] and restoring cardiac expression of Nrf2-regulated genes at Protein and RNA levels [35,37]. Lee et al. observed that significant cytotoxicity towards malignant mesothelioma cells using a combination of cisplatin with Sulphoraphane and observed that the effect could be due to pro-oxidant activity of Sulphoraphane [38]. Similar study conducted by Danafar and co-workers revealed the synergistic and antiproliferative action of curcumin upon combining with Sulphoraphane against breast cancer cells using PEGylated Iron Oxide-Gold Core Shell Nanoparticles [39]. Another study performed by Srivastava et al. unveiled that Sulphoraphane in combination with Quercetin had increased effect on pancreatic cancer stem cell increasing its self-renewal capacity [40].

However, it has been also reported that 70–75% of the Sulphoraphane was found in urine as N-acetylcysteine–SFN conjugate after 24 h of a single dose administration of Sulphoraphane in rats [41], indicating that there is a need of maintaining therapeutic levels of Sulphoraphane for a prolonged period to provide an improved anticancer efficacy.

A hypothesis by Verma et al. proposed that a synthetic chemotherapeutic agent when combined with natural chemosensitizer and loaded them into nanoparticulate system deliver the drug cargo at the target site with certitude and subsequently the loaded natural bioactive would then favourably act on the tumour milieu through multiple portals and would chemosensibilize the cells towards cytotoxic action of the synthetic drug moiety. Also, they hypothesized that the combined approach along with the application of nanometric particles would yield a multipronged tool to target the multifactorial disease and play a pivotal role in better cancer management [47]. Another hypothesis proposed by Siddiqui et al. the combinatorial approach between Irinotecan and Quercetin to circumvent drug resistance and target to CD44 receptor of colon cancer cells through surface modification of nanoparticles with a ligand hyaluronic acid [48].

Metastasis is one of the major reasons for mortality during Breast Cancer. Regional lymph nodes are prime sites for metastasis. Migration of tumour cells to lymph nodes increases in rate due to formation of lymph vessels leading to spread of tumorous cells systemically. Hence, a strategy effective in targeting lymphatic tissues would be needed to combat the toxicological and biopharmaceutical issues of Tamoxifen and Sulphoraphane.

Lipid based nanocarrier has been extensively investigated in the nascent filed of drug delivery to deliver the drugs to target site through oral route [49]. As intestinal lymphatic system targeting helps to reduce the hepatotoxicity, improved bioavailability and systemic toxicity profile and delivered significant drug amount through lymphatic system which prevent metastasis [13]. The poorly aqueous soluble drugs exhibit dissolution limited absorption which consequently results poor absorption from gastrointestinal tract and subsequently results in poor oral bioavailability. To circumvent these biopharmaceutical challenges of Tamoxifen and Sulphoraphane and to deliver them simultaneously to the target site we proposed these zero-dimension lipid based nanocarrier would be beneficial.

These lipid nanocarrier has successfully encapsulated poor aqueous soluble drug and enhanced their oral bioavailability along with improved pharmacokinetic profile of the drugs [50–53]. Their ability to enhance the oral bioavailability of the poor aqueous soluble drugs could be enhanced solubilization of the drugs in the intestinal milieu through the formation of micelles, mixed micelles and chylomicrons in the presence of bile salts and efficient uptake of these micelles and chylomicrons through enterocytes to the lymphatic circulation [50,54].

Taking together the aforementioned combination of Tamoxifen and Sulphoraphane and leverage the lipid based nanocarrier, we hypothesize to codelivery of Tamoxifen and Sulphoraphane to circumvent the systemic toxicity profile of Tamoxifen. Such a combined approach would better target to the breast cancer and would be beneficial than the conventional combined chemotherapy.

Experimental proof of the hypothesis

With main aim to circumvent the hepatotoxicity and nephrotoxicity and offsite target associated with Tamoxifen, Tamoxifen would be co delivered with Sulforaphane in lipid based nanocarrier in reduced dose. Combined reduced dose would be determined by utilizing combination index developed by the Chou-Talalay [55] through MTT assay.

According to Chou-Talalay method, the effect is considered additive when combination ratio is 1. The effect is considered negative (sub additive) when the ratio is far more than 1 whereas supra-additive if the ratio is far less than 1.

Combination index: $CI = (D)_1/(D50)_1 + (D)_2/(D50)_2$

whereas, $(D)_1$ and $(D)_2$ represent the concentration of Tamoxifen and Sulphoraphane in the combination system at the IC50 value, respectively. $(D50)_1$ and $(D50)_2$ represent the IC50 value of Tamoxifen and Sulphoraphane, respectively [56].

Tamoxifen and Sulforaphane at reduced does obtained from the combination index would be incorporated into lipid based nanocarrier stabilized with various surfactants. Lipid based nanocarrier would be produced through melt emulsification method where melted lipids along with drugs are emulsified in hot aqueous surfactant phase and size reduced with ultrasonication.

Prepared lipid based nanocarrier would be characterized in terms of hydrodynamic diameter, zeta potential, drug loading capacity, *in vitro* drug release performance and stability of these nanocarrier.

For proof of concept of our hypotheses, these lipids based nanocarrier would be evaluated for *in vitro* anticancer potential in human breast cancer cell lines which are estrogen receptor positive like MCF-7, T47D and ZR-75-1 cell line. In addition, cellular uptake and its mechanism would be assayed by confocal laser scanning microscopy and flow cytometry [57,58]. Moreover, the antitumor efficacies of these lipid nanocarrier would be determined by inducing the tumour either in the BALB/c or nude mice or in the wistar rats using Ehrlich ascites tumour (EAT) cells or chemicals and further measurement of tumour volume reduction [59] and eventually tissue biodistribution study. Toxicity study would also be conducted to evaluated the reduction of toxicity of Tamoxifen in combined with Sulphoraphane in lipid nanocarrier by liver and kidney function test as well biochemical assays.

Conclusion

Cancer biology being heterogeneous in nature, conventional chemotherapy is far from successful in the management of this flagitious disease and is being impossible to forecast which patient will respond to which treatment regime. Synthetic chemotherapeutics alone in conventional treatment regime has progressed to have severe life-threatening significances. Thus, from this proposed hypothesis we expect that combined nanomedicine approach would target this heterogenous disease and play a vital role in the cancer management.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

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