SYNOPSIS

1. TITLE

DEVELOPMENT OF SITE SPECIFIC DRUG DELIVERY SYSTEM OF QUERCETIN USING BIO-LIGANDS FOR TREATING ULTRAVIOLET RADIATION INDUCED SKIN CARCINOMA

INTRODUCTION

Oxidative stress (OS) results from excessive exposure of the skin to environmental insults including sun and air pollution. Under these conditions, excessive reactive oxygen species (ROS) can form. The presence of large amounts of ROS leads to oxidative stress that can damage cell membranes, lead to DNA breakage and inactivation of free radical scavenger enzymes, eventually resulting in skin damage. Recent studies have shown that natural flavonoids possess the ability to mitigate OS-induced skin damage. Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone), which is a natural flavonoid with a variety of biological activities and pharmacological actions, has been shown to have the highest anti-radical activity compared to other flavonoids. Quercetin has also been reported to act as a scavenger of free radicals and inhibit lipid peroxidation. The ability of quercetin to mitigate ROS-mediated skin damage has led to considerable interest in the field of topical delivery of quercetin. However, quercetin has a low solubility in physiologically relevant media, which contributes to low absorption in vivo. As a result, clinical application of quercetin is greatly restricted and the development of a targeted dermal formulation that can deliver quercetin into the skin would be beneficial.

In recent years, drug targeting to specific organs and tissues has become one of the critical endeavors of the new century. The search for new drug delivery approaches and new modes of action represent one of the frontier areas which involves a multidisciplinary scientific approach to provide major advances in improving therapeutic index and bioavailability at site specific-delivery. These new systems can hinder solubility problems, protect the drug from the external environment such as photodegradation and pH changes, while reducing dose dumping by controlling the release profile. Moreover, controlled targeting at the site of action and reduced

time of exposure at non-targeting tissues increases the efficacy of treatments and reduce toxicity and side effects thus improving patient compliance and convenience.

Folate and the folate receptor

The folate receptor, a glycosylphosphatidylinositol an-chored cell surface receptor, is overexpressed on the vast majority of cancer tissues, while its expression is limited in healthy tissues and organs (9). Folate recep-tors are highly expressed in epithelial, ovarian, cervical, breast, lung, kidney, colorectal, and brain tumors. Sarcomas, lymphomas, and cancers of the pancreas, testicles, bladder, prostate, and liver often do not show elevated levels of folate receptors. When expressed in normal tissue, folate receptors are restricted to the lungs, kidneys, placenta, and choroid plexus. In these tissues, the receptors are limited to the apical surface of polarized epithelia. Folate, also known as pteroylglutamate, is a non-immunogenic water-soluble B vitamin that is critical to DNA synth-esis, methylation, and repair (folate is used to synthesize thymine). Folic acid is small (441 Da), stable over a broad range of temperatures and pH values, inexpensive, and non-immunogenic, and it retains its ability to bind to the folate receptor after conjugation with drugs or diagnostic markers. After folate attaches to the receptors located within caveolae, it is internalized through the endocytotic pathway. As the pH of the endosome approaches five, the folate dissociates from the receptor and the drug is released.

These include solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and nanoemulsions (NE). SLNs are produced by replacing the liquid lipid (oil) of an o/w emulsion by a solid lipid or a blend of solid lipids where the lipid particle matrix is a solid at both room and body temperature. NLCs are mixtures of solid and liquid lipids. Some of the advantages of SLN and NLC include the use of physiological lipids in the composition, the avoidance of organic solvents, the possibility to produce concentrated lipid suspensions and the availability of established scale-up processes. Lipid based nanoparticles can increase the apparent solubility of incorporated drugs, leading to the formation of a high concentration gradient on the skin and facilitating drug permeation into the skin layers. The small particles in the nanometer size range can tightly adhere to the surface of the skin and transport drugs in a more controlled manner.

Lipid based nanosystems have also been shown to exert an occlusive effect on the skin, leading to improved skin hydration and facilitating drug permeation. Additionally, the lipids and

surfactants that form an integral part of these systems can act as permeation enhancers to some extent to loosen or fluidize the lipid bilayers of the stratum corneum.

In this proposal, lipid based nanosystems will be evaluated for the targeted delivery of quercetin. SLN and NLC systems will be developed using commonly used lipids and surfactants considered acceptable in dermal formulations. Systematic screening of the formulation and process parameters will be carried out for the development of a physically stable nanosystem. Characterization of the systems including particle size, zeta potential, morphology, crystallinity, in vitro release rates of quercetin and physical stability will be performed.

2. OBJECTIVE OF RESEARCH

The objective of the proposed research work is to evaluate the feasibility of development of lipid based nanosystems of quercetin with folic acid and to assess the potential of these systems to deliver quercetin dermally. Towards this goal, the research is divided into four specific aims:

1. To conceptualized and design a targeted drug delivery system for dermal delivery of photoprotective agent and to overcome the drawbacks faced in topical delivery of quercetin.
2. To design, prepare and characterize quercetin-folic acid conjugated solid nanoparticles.
3. To evaluate the feasibility of the manufacturing of nanosystem containing formulations of quercetin-folic acid complex for targeted drug delivery.
4. To evaluate the ability of the developed nanosystem containing formulations of quercetin for dermal delivery against photocarcinoma.

3. BRIEF REVIEW OF THE WORK ALREADY DONE IN THE FIELD

The exhaustive literature survey furnished the information about complexation of natural bioactives with folic acid with different polymers and their derivatives for enhancement of solubility, stability, bioavailability, dissolution etc. Till date, very little work, related to targeted drug delivery of natural bioactive agent as photoprotectives have been done, which can give a fruitful contribution in this area, is available. Some of the important work that has been carried out are summarized below:

1. **H. Misak et.al, 2013**, has carried out a study in which oil-in-oil emulsion/ solvent evaporation method was used to prepare Albumin or drug loaded magnetic nanocomposite spheres and tested on a mouse model to determine the efficacy of the drug delivery system (DDS) on skin cancer.
2. **Manuela Curcio et.al in 2012** reported in there work, molecularly imprinted nanospheres for controlled/sustained release of quercetin were synthesized employing methacrylic acid and ethylene glycoldymethacrylate as functional monomer and crosslinking agent, respectively.And the applicability of the obtained materials as drug delivery devices was evaluated by performing in vitro release studies in plasma simulating fluids and cytotoxicity testson HeLa cells.
3. **Khoee S *et al.* in 2012**, in their study a novel nanocarrier was synthesized based on methacrylated poly(lactic-co-glycolic acid) (mPLGA) as a lipophilic domain, acrylated methoxy poly(ethylene glycol) (aMPEG) as hydrophilic part and N-2-[(tert-butoxycarbonyl)amino] ethyl methacrylamide (Boc-AEMA) as pH-responsive segment. Radical polymerization of the above-mentioned three modified monomers produces amphiphilic brush-like copolymer. Nanoprecipitation method was used to prepare quercetin-loaded nanoparticles. Dynamic light scattering (DLS) analysis showed that the produced nanoparticles had nanometric size (<100nm) and low polydispersity in size at different pHs. Higuchi and Korsmeyer-Peppas models were applied to evaluate release mechanisms and kinetics. Based on in-vitro degradation study, we found that the brush-like copolymer underwent a rapid weight loss in acidic pH.

4. In an another study by **Shutava *et al***. in 2012 reported that Nanoparticle-encapsulated EGCG protects its biological activity and blocked hepatocyte growth factor (HGF)-induced intracellular signaling in the breast cancer model.
5. **Aniket S. Wadajkar et.al, 2012** , proposed through their work the development of New magnetic-based core–shell particles (MBCSPs) to target skin cancer cells while delivering chemotherapeutic drugs in a controlled fashion. In his study a thermo-responsive shell of poly(N-isopropylacrylamide–acrylamide–allylamine) and a core of poly(lactic-co-glycolic acid) (PLGA) embedded with magnetite nanoparticles were fabricated for desired result against skin cancer cells.

6. Similarly, **Dhruba J. Bharali *et.al*, 2011** demonstrated in his study of exploiting nanotechnology to increase the systemic delivery and bioavailability of any natural product. In their study they employed nanoparticle-mediated delivery for sustained release of epigallocatechin-3-gallate (EGCG), a polyphenol from green tea and reported remarkable results.
7. **Narayanan S et.al, 2010,**in their study reported Poly (lactide-co-glycolide) (PLGA) nanoparticles encapsulating a well known nutraceutical namely, grape seed extract (GSE)-'NanoGSE'-was prepared by a nanoprecipitation technique. The drug-loaded nanoparticles of size approximately 100 nm exhibited high colloidal stability at physiological pH.
8. Similarly, in a study carried out by **R.K Das, et.al**, 2010 showed that curcumin when nanoformulated with three different biocompatible polymers namely alginate, chitosan, and pluronic enhanced the solubility of curcumin and facilitated cellular internalization of curcumin-loaded composite nanoparticles.

9. In continuation with the same, **A. Kumari et.al, in 2010** emphasized over the utilization of biodegradable nanoparticles for various alinimaents. In an another study by the same researcher, they demonstrated the poly-D,L-lactide (PLA) nanoparticles formation for better therapeutic activity and improved aquaeous solubility of Quercetin, a natural antioxidant.

10. **Shao et al. in 2009**, through their study suggest that resveratrol-loaded nanoparticles has the potential to can become a better alternative in treating various types of pathological manifestation associated with tumour.

11. **Sahu *et al*.in 2008** synthesized a novel curcumin conjugated polymeric amphiphile with methoxy poly(ethylene glycol) (mPEG) and palmitic acid (PA).

12. **Farnaz Esmaeili et.al, in 2008,** reported For folate-receptor-targeted anticancer therapy, docetaxel (DTX) nanoparticles (NPs) were produced employing polylactide-co-glycolide–polyethylene glycol-folate (PLGA–PEG–FOL) conjugate. The FOL-conjugated di-block

copolymer was synthesized by coupling the PLGA–PEG–NH2di-block copolymer with an activated folic acid. It was expected that FOL moieties were exposed on the micellar surface**.** The conjugates assisted in the formation of DTX NPs with an average size of 200 nm in diameter through an emulsification/solvent diffusion method. The FOL-targeted NPs showed a greater extent of intracellular uptake in FOL-receptor-positive cancer cells (SKOV3) in comparison with the non-targeted NPs, indicating that the FOL-receptor-mediated endocytosis mechanism could have a role in the cellular uptake of NPs. These results suggested that FOL-targeted DTX NPs could be a potentially useful delivery system for FOL-receptor-positive cancer cells.

13. In another study, by **Italia *et al*.** 2008 suggested the potential of biodegradable nanoparticles to improve the therapeutic efficacy of EGCG .
14. In a pioneering study by **Bisht *et al*.**,2007 curcumin was effectively incorporated with cross-linked and random copolymers of N-isopropylacrylamide (NIPAAM), with N-vinyl-2-pyrrolidone (VP) and poly(ethylene glycol) monoacrylate (PEG-A) nanoparticles and showed excellent results against pancreatic cancer.

15. **Stella B, et.al, 2000**, in their study developed a new concept for the design of poly(ethylene glycol) (PEG)-coated biodegradable folic acid coupled nanoparticles for targeting the folate-binding protein. For this purpose a novel copolymer, namely, the poly[aminopoly(ethylene glycol)cyanoacrylate-co-hexadecyl cyanoacrylate] [poly(H(2)NPEGCA-co-HDCA)] was synthesized and characterized. (R. Duncan,et.al,2006)

4. NOTEWORTHY CONTRIBUTION IN THE FIELD OF PROPOSED WORK

From the exhaustive literature review, it was observed that the complexation of quercetin with folic acid coupled with an appropriate polymer, for targeted drug delivery can be prove beneficial in the field of photoprotection. However, very few literatures are available and reported on the development of novel delivery system of these folic acid conjugated natural bioactives. Despite the great potential of natural photoprotectants, no drug formulation based on

this technology is yet available in Indian market for ultraviolet radiation cancer treatment. Therefore, keeping in mind this concept, the proposed research work will contribute in the development of quercetin-folic acid based 'green' chemotherapy can be fruitful by employing targeted nanoparticle mediated delivery to enhance the efficacy of phytomedicines in the field of photocarcinoma.

5. PROPOSED METHODOLOGY DURING THE TENURE OF THE RESEARCH WORK

The proposed research work will be performed as per the following:

1. Review of literature
2. Selection and procurement of drug and polymers.
3. Preformulation Study
4. Compatibility studies for drug, ligand and polymers.
5. Characterization of the prepared drug-ligand conjugate.
6. In vitro drug release test
7. In vitro characterization of nanosystem.
8. Optimization of various parameters.
9. Preparation and characterization of dosage form/delivery system
10. Primary Biochemical study
11. Histopathological Study
12. Compilation and presentation of data
13. Stability Studies
14. Compilation of data and results.

6. EXPECTED OUTCOME OF THE PROPOSED WORK

The aim of this proposed research work lies in the development of a tissue selective and specific drug delivery system to provide therapeutic concentrations of natural anticancer agent at the site of action of dermal and epidermal junction in skin cancer therapies. The folate receptor appears to be a promising target for delivery of small molecules and have tremendous targeting potentials

in cancer imaging and treatment. Folate receptors are highly overexpressed on the surface of many tumor types. This expression can be exploited to target therapeutic compounds directly to cancerous tissues using many avenues. Meanwhile, the drug quercetin exhibits antioxidant, chemoprotective, antiproliferative, photoprotective activities etc. The conjugation of folic acid with quercetin will play a dual role in combaiting the deleterious effects of ultraviolet radiations and in mininmizing the progression of photocarcinoma.

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