Design and Evaluation of Certain Oral Mucoadhesive Nano-drug Delivery Systems

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A Thesis Presented by

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Abstract

Oral cancer is a global health problem with increased incidence and high rate of recurrence and metastasis. There are different protocols for treatment of cancers of the oral cavity including surgery, radiotherapy, and, the most frequently applied approach, chemotherapy. However, chemotherapy results in severe adverse effects such as nausea, vomiting, constipation, diarrhea, fatigue, hair loss, and mucositis. Cyclooxegenase-2 (COX-2) enzyme is over expressed in oral cancer cells and affects a number of carcinogenesis processes. The inhibition of COX-2 enzyme could be an intriguing remedial target. Celecoxib (CXB), a selective COX-2 inhibitor, is considered an effective chemopreventive therapeutic target for oral carcinoma. Unfortunately, its clinical application is vulnerable due to its poor aqueous solubility and hence, low absorption and bioavailability. In addition, the systemic side effects of CXB would further limit its potential. For these reasons, encapsulating CXB in nano-drug delivery systems could offer a promising strategy to circumvent these drawbacks. Additionally, loading the prepared nanocarrier on a mucoadhesive polymer would further enhance the localized delivery of CXB by increasing its intimate contact with the buccal mucosa. Hence, this would increase the drug tissue accumulation and avoid the systemic side effects associated with high dosing.

The aim of the present work is to formulate and evaluate oral mucoadhesive nano-drug delivery systems of CXB for localized treatment of oral carcinoma with minimized drug dosing and reduced associated systemic adverse effects.

The work in this thesis was divided into three chapters:

Chapter One: Development and *In-vitro* Evaluation of Mucoadhesive Celecoxib-loaded Cubosomal Sponges as Novel Nanocarriers for Mucosal Drug Delivery

Different CXB-loaded cubosomes (3, 6, and 12 mg CXB) were prepared through fragmentation method using glyceryl monooleate (GMO) and water. Poloxamer-407 was added at two different ratios with respect to GMO (5:1, 2.5:1) in order to investigate its effect on stability of the dispersions. The developed cubosomal dispersions were evaluated for their particle size, polydispersity index, zeta potential, drug content, and *in-vitro* drug release. FT-IR spectra were tested to study if there were any molecular interactions between CXB and GMO. TEM micrographs were also obtained for structural examination.

Selected cubosomal dispersions containing 6, and 12 mg CXB and prepared at GMO:P-407 (2.5:1), were loaded on HPMC polymer to prepare mucoadhesive cubosomal sponges with the addition of mannitol and glycerol as a cryoprotectant and plasticizer, respectively. Additionally, sponges loaded with CXB powder were investigated for comparison. They were evaluated for their physical appearance, porosity, hydration capacity, and surface pH. Solid state characterizations were performed through XRD and FT-IR studies. SEM micrographs were obtained for topographic examination.

Results of this chapter showed that:

- All cubosomes demonstrated nano-sized particles in the range of 130.00 – 191.40 nm, with negative surface charge. The lower ratio of GMO:P-407 revealed significant lower values ($P \leq 0.05$) of particle size as compared to those resulted by the higher ratio.
- FT-IR spectra showed that there were no molecular interaction between CXB and GMO, or P-407.
- The TEM micrographs revealed the cubic structure of the developed cubosomal dispersions with no aggregates.

- *In-vitro* release studies of the cubosomes resulted in significant slower release rate ($P \leq 0.05$)of CXB as compared to the drug solution and higher release rate as compared to the drug suspension.
- The prepared cubosomal-loaded sponges were cotton-like, with uniform weight and thickness.
- Incorporation of both mannitol and glycerol resulted in more flexible and non brittle surface.
- FT-IR spectra of the physical mixture and cubosomal-loaded sponge indicated no molecular interaction between any of the sponge ingredients.
- Surface pH values of all developed sponges were in the range of salivary pH.
- Hydration capacity of CXB-loaded sponges were higher than those of CXB cubosomal sponges.
- SEM micrographs revealed more porous structure of sponges loaded with CXB as compared to those loaded with the cubosomal dispersions.
- *In-vitro* release studies of CXB-loaded cubosomal sponges showed slower and sustained release rate pattern with respect to CXB-loaded cubosomes.
- Additionally, release rate profile of CXB from sponges loaded with CXB cubosomes was higher than that from sponges loaded with CXB powder. Thus, this indicates the efficacy of cubosomal dispersions for encapsulating and solubilizing CXB.

In conclusion, CXB was successfully loaded in low doses in cubosomes as nano-drug delivery carriers that resulted in sustained drug release. Moreover, mucoadhesive CXB-loaded cubosomal sponges were developed for further investigation in experimental *in-vivo* studies.

Chapter Two: Potential Application of Celecoxib-loaded Cubosomal Sponges for management of Oral Carcinoma: *Ex-vivo* and *In-vivo* studies

Selected CXB-loaded cubosomal sponges from the previous chapter, corresponding to 6, 12 mg CXB, were evaluated through *ex-vivo* residence, and *ex-vivo* permeation and deposition studies. CXB-loaded cubosomes and CXB-loaded HPMC gels, at the corresponding CXB concentrations, were also examined.

In-vivo studies were carried out on female albino rats. The tested animals were divided into 5 groups; the first group received no treatment, the second group received low dose (6 mg) CXB-loaded HPMC gel, the third group received high dose (12 mg) CXB-loaded HPMC gel, the fourth group received low dose (6 mg) CXB-loaded cubosomal sponges, and the fifth group received high dose (12 mg) CXB-loaded cubosomal sponges. Induction of the tumor was performed by local application of 0.5% w/v 4-NQO in the oral cavity. Serum samples were assessed for the tumor markers; CEA, SCCAg, IAP, and VEGF and tissue markers were evaluated for the presence of COX-2, and caspase-3. In addition, excised tumors were examined through histopathology by H&E staining and Annearoth's grading system was used for staging histopathologhy of the developed tumors. Furthermore, immunohistochemical staining of the proliferative marker; Ki-67, in tumor tissue was assessed.

Results of this chapter showed that:

- *Ex-vivo* mucoadhesion residence time of the sponges loaded with cubosomal dispersions was significantly higher ($P \leq 0.001$) than that resulted by either CXB-loaded cubosomes or CXB-loaded HPMC gels.
- The amount of CXB deposited on the buccal mucosa from cubosomal-loaded sponges was significantly higher ($P \leq 0.001$) than that deposited from CBX-loaded cubosomes and CXB-loaded HPMC gels.

- The combined assay of the serum tumor markers CEA, SCCAg, and IAP showed a significant reduction ($P \leq 0.001$) in the mean value of each tumor marker following treatment with SP27, SP28 (6, 12 mg CXB, respectively) compared to the corresponding CXB-loaded HPMC gels at the same doses of CXB.
- Determination of VEGF in serum samples showed that rats treated with CXB-loaded HPMC gel (6 mg CXB) revealed the significantly highest values ($P \leq 0.001$) with respect to other treated groups. Meanwhile, SP28, equivalent to 12 mg CXB, demonstrated the least value of VEGF.
- Assessment of tumor tissue markers COX-2 and caspase-3 showed the statistically significant difference ($P \leq 0.001$) between treated groups and untreated rats.
- Histologic staining and grading of tumors' tongues and palates was reduced from the fourth grade in the untreated rats to the third, second, and first grades in CXB-loaded HPMC gels (6 or 12 mg CXB), SP27 (6 mg CXB), and SP28 (12 mg CXB), respectively.
- Immunohistochemical staining of Ki-67 as a proliferative marker revealed that CXB-loaded cubosomal sponges (SP27, SP28) significantly reduced ($P \leq 0.001$) Ki-67 expression, through decreased stain positivity, compared to CXB-loaded HPMC gels.

In conclusion, encapsulating CXB into mucoadhesive cubosomal sponges as novel nano-drug carriers resulted in enhanced CXB permeation to tumor tissues, and achieved a promising local treatment of the developed oral tumors in small doses of CXB.

Chapter Three: Design of Novel Celecoxib-loaded Fucoidan Nanoparticles: *In-vitro* Evaluation of Anticancer Efficacy, and Study of Pharmacological and Chemical Interactions

Blank fucoidan (FCD) nanoparticles were developed through electrostatic interactions between the positively charged chitosan (CS) and negatively charged fucoidan (FCD). Five different formulations were prepared at different CS:FCD weight ratios, and evaluated for their particle size, PDI, and zeta potential. The optimum weight ratio was then selected for preparation of CXB-loaded FCD NPs. The developed NPs were evaluated for PS, PDI, ZP, entrapment efficiency, and *in-vitro* release of CXB. FT-IR spectra were tested to investigate if there was any interaction between CXB and FCD and/or CS. TEM micrographs were obtained for structure elucidations. *In-vitro* anticancer studies of CXB-loaded FCD NPs were carried out on SCC-4 cell lines in order to investigate their anticancer activity against oral carcinogenesis through; MTT cell viability test, apoptosis detection assay, detection of cell proliferation assay, and cell cycle arrest pattern. CXB solution, FCD solution, and plain FCD nanoparticles were also tested for comparison.

Results of this chapter showed that:

- Weight ratio of CS:FCD (1:5) demonstrated optimum values of both particle size and PDI, and hence, it was selected for loading CXB in the developed NPs.
- CXB-loaded FCD NPs revealed 76.87 ± 0.81% entrapment efficiency of CXB.
- The obtained TEM micrographs indicated the spherical shape of the developed NPs with an average diameter of 218 ± 4.72 nm.
- FT-IR spectra indicated the absence of any chemical molecular interaction in the prepared CXB-loaded FCD NPs.
- The *in-vitro* release rate data showed a sustained release rate pattern of CXB from the developed NPs with no burst release, as compared to the drug solution. Additionally, CXB release from the developed NPs was faster than that from the drug suspension.
- The cytotoxic activity of CXB-loaded FCD NPs was more potent (with lower doses) than that resulted by single application of both CXB and FCD solutions.

- The combination index (CI) analysis of the prepared NPs enhanced the cellular inhibition, as compared to CXB and FCD solutions, and indicated the synergistic effect obtained by combining CXB along with FCD.
- The dose reduction index (DRI) analysis of CXB-loaded FCD NPs demonstrated a desirable reduction of CXB and FCD doses by 1.625 and 2.98 folds, respectively.
- Flow cytometer assay for detection of apoptosis showed that the prepared NPs resulted in significant increase ($P \leq 0.001$) in both early and late apoptotic stages in SCC-4 cells as compared to the control untreated cells. Moreover, CXB-loaded FCD NPs at the corresponding concentrations of CXB and FCD showed more apoptosis than that attained by both CXB and FCD solutions.
- Detection of cell proliferation by Ki-67 staining assay showed the significant inhibitory effect of CXB-loaded FCD NPs on proliferation of SCC-4 cells with respect to that of the control untreated cells. Meanwhile, the antiproliferative effect of SCC-4 cells obtained by either CXB or FCD solutions wasn't significantly different than the control untreated cells.

In conclusion, CXB was successfully encapsulated in FCD NPs as a novel delivery approach to reduce CXB dose and achieve synergistic effect with FCD. The developed nanoparticles of the combined CXB and FCD showed synergistic effect on the viability of SCC-4 cells. In addition, they exhibited potent *in-vitro* anti cancer effects through induction of apoptosis, inhibition of cellular proliferation, and cell cycle arrest.