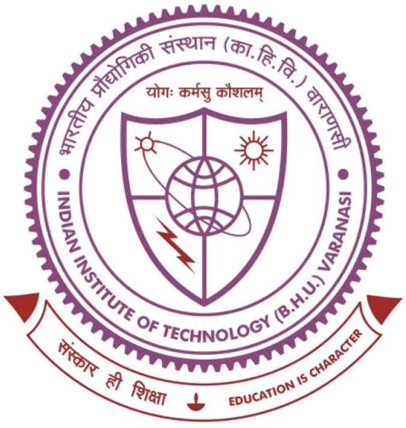
Development and Evaluation of Lapatinib-Loaded Nanocolloidal Micellar Drug Delivery System for Efficient Treatment of Breast Cancer



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By

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6 Summary and Conclusions

6.1 Summary

Globally, breast cancer (BC) is the most common type of cancer among women, constituting 6.53 % of 9.6 million cancer deaths in 2018. Among the types of BC, 30% of instances were metastatic HER2-positive BC. Generally, its treatment includes anti-HER2 monoclonal antibodies viz. Herceptin and tyrosine kinase inhibitors (TKIs), e.g., dasatinib, lapatinib (LP), that were developed as targeted therapies for HER2-positive BC. In 2007, USFDA approved LP in combination with capecitabine for the management of HER2-positive BC; however, less drug loading, lower oral bioavailability, its poor aqueous solubility, higher plasma protein binding (about 99%) requires higher doses, thus limits its clinical use. Therefore, the potential drug lacks patient compliance due to associated side-effects and demands a higher cost for treatment. To date, Tykerb®, oral tablets (GlaxoSmithKline) is the only commercially available dosage form. Thus, we aim to develop a novel nanocarrier system of LP to address the above issues.

Taking into considerations the advantages of nanocolloidal micelles as drug delivery systems for their ability to provide sustained release, to improve stability in aqueous dispersion and their potential of passive targeting the tumors, we had proposed to encapsulate Lapatinib in the form of nanocolloidal micelles and deliver the drug by an alternate route, i.e. intravenous route. Micelles with following compositions were fabricated by thin-film hydration technique: (1) Lapatinib-loaded polymeric micelles using Soluplus as an polymer; (2) Lapatinib loaded binary micelles using Soluplus and Solutol HS 15 as a combination of polymers. The fabricated micelles were extensively evaluated for different *in-vitro* as well as *in-vivo* characterizations.

Micelles were optimized and fabricated using Quality by Design approach.The prepared micelles had smooth and spherical surface. The results of DLS and HR-SEM revealed their spherical shape and size less than 100 nm with uniform size. The size indicated their higher potential of targeting the tumors passively. The solid-state characterizations of prepared micelles using FTIR, XRPD and EDX pointed towards the encapsulation of drugs inside the micelles with traces on their surface without any physical and chemical interactions.

High entrapment efficiency confirmed their greater drug-carrying capacity. The higher but sustained release at pH 5.0 than pH 7.4 indicated their preferential drug release at the pH condition resembling acidic tumor environment. The stability studies of LPPMs showed that the stability of its dispersion was compromised due to aggregation at room temperature and further lyophilized LPPMs enhanced the stability due to conversion into solid form. Further, the binary micelles using the combination of two polymers were prepared to address the stability issue of dispersion at room temperature and the results evidenced their higher stability at room temperature in dispersion form.

Further, the hemolysis less than 1 % and the absence of platelet aggregation confirmed their safety to patients after IV administration of prepared micelles. The enhancement of mean residence time and more sustained blood concentration in rats post-intravenous administration as compared to marketed tablets demonstrated the higher potential of drug delivery and suggested the possibility of lowering the effective dose of a drug. The observation was then supported by a marked increase in inhibition rate of tumor proliferation in *in-vivo* studies in xenograft mice model bearing cancer tumors. The prepared micelles induced very milder liver toxicity as compared to pure drug. The results of different *in-vitro* and *in-vivo* characterizations are shown in Table 1.

Table 6.1: Findings of various *in-vitro* and *in-vivo* characterizations

Parameters	**Lapatinib-loaded polymeric micelle (LP-PMs)**	**Lapatinib-loaded binary micelle (LP-BMs)***
Morphology	Spherical shape	Spherical shape
Surface Texture	Smooth surface	Smooth surface
Particle size	92.9±4.07 nm	93.53 ± 3.56 nm
Polydispersity index	0.093±0.083	0.22 ± 0.02
Particle size by HR-SEM	81.85±12.44 nm	67.14 ± 10.19 nm
Zeta potential	5.06 Mv	-12.30 mV
FT-IR & XRPD	No physico-chemical interaction was reported between drug and polymer(s) except hydrogen bonding between polymer and drug	
Entrapment efficiency	87.23% ± 4.85%	89.45%±1.98%
***In-vitro* release profile in 48 h**	Sustained release of drug pH 7.4 = 36% pH 5.0 = 60%	Sustained release of drug pH 7.4 = 35% pH 5.0 = 57%
Stability (Lyophillized form)	30±2°C/65±5% RH = 11 months 5±3°C = 15 months	30±2°C/65±5% RH = 15 months 5±3°C = 19 months

***In-vitro* cytotoxicty by MTT assay**	1.60 µg/mL	1.22 µg/mL
***In-vitro* cytotoxicty by microscopic observations**	SKBr3 cells showed indications of apoptosis like change in morphology, more granular cytoplasm, cell fragments, etc.	
Hemocompatibility	Yes	Yes
Platelet Aggregation	No	No
Pharmacokinetic studies	Extended the mean residence time as compared to oral marketed tablets with enhanced AUC at 10 times less dose than oral tablet.	
***In-vivo* anti-cancer study**	Nearly 45 % reduction in the growth rate of tumor at 10 times less dose than oral tablet	Nearly 72 % reduction in the growth rate of tumor at 10 times less dose than oral tablet
Hepatotoxicity	Very low	Very low

*All data presented as Mean ± SD, n=3.

6.2 Conclusions

The current research focused on the development of nanocarriers for the encapsulation of poorly water-soluble anti-cancer drug, Lapatinib. Considering the advantages of micelles, we proposed to fabricate a nanocolloidal micelles loaded with lapatinib by thin film hydration method. Two different types of micelles (polymeric micelles and binary micelles) were fabricated one by one to circumvent the shortcomings of the previous one. The polymeric micelles were aggregated at room temperature within a short period. Therefore, binary micelles with a combination of two polymers were prepared to enhance the stability in the second part of research work. On the basis of research findings of the various *in-vitro* and *in-vivo* evaluations, a higher efficacy of prepared micelles for breast cancer treatment was evidenced. The hemocompatibility studies also demonstrated their safety for intravenous administration. Although both the prepared formulations showed comparable efficacies, binary micelles induced more tumor inhibition and had longer stability than polymeric micelles, even at room temperature. Conclusively, this study endorses the use of Lapatinib-loaded nancolloidal micelles as a better alternative for efficient breast cancer treatment by intravenous route in contrast to the oral tablets.

Future perspective

Although current research supports the application of LP-BMs for treatment of breast cancer. However, it further requires efficacy determination by well-controlled clinical study, dose titration studies for human and the suitability of the method of preparation for technology transfer at pilot scale and manufacturing scale at industry. After obtaining appropriate results, the LP-BMs can be endorsed for human application and commercial manufacturing.