NANOPARTICLE ENCAPSULATED BIOACTIVE FORMULATIONS FOR DRUG DELIVERY

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by

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Submitted
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Dedicated

to

My Family

CERTIFICATE

This is to certify that the thesis entitled "**Nanoparticle Encapsulated Bioactive Formulations For Drug Delivery**", being submitted by **Ms. Swati Jaiswal** to the Indian Institute of Technology, Delhi, for the award of degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by her, which has been prepared under our supervision and guidance in conformity with the rules and regulations of "Indian Institute of Technology, Delhi". The research reports and the results presented in this thesis have not been submitted in part or full to any other University or Institute for the award of any degree or diploma.

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ABSTRACT

Nanoparticle based formulations of drug delivery systems have shown their potential in improving the efficacy of existing drugs. Delivery of therapeutic agents to cells can be highly inefficient because of problems like rapid degradation, need for repeated injections, low bioavailability and poor transport of drugs through biological barrier. In order to achieve efficient drug delivery, delivery of therapeutic molecules via various nanocarrier systems such as biodegradable polymeric nanoparticles, liposomes, nanocapsules, nanospheres have been extensively investigated. Nanoparticles have also been used in combating antibiotic resistance which has necessitated search for new antibacterials. Silver nanoparticles, a known antimicrobial agent are toxic to human cells at higher concentrations. Since combination of antibiotic and silver nanoparticles have shown encouraging results, we have used bioactive compound curcumin to prepare silver nanoparticles.Curcumin has been reported to exhibit anti-proliferative, antiangiogenic, anti-inflammatory, and anti-oxidative properties through down-regulating transcription factors, production of ROS, damage to DNA. Nonetheless, curcumin remains insoluble in aqueous solution and has issues related to its stability over a period of time. Silver nanoparticles (Cur-Ag nanoensembles) of size 25-35 nm, produced by green synthesis using curcumin, were effective against both Gram positive and Gram negative bacteria and less toxic to human keratinocytes. These nanoensembles had very low total silver content and high stability. The antibacterial activity of Cur-Ag nanoensembles, as studied by minimum inhibitory concentration, time kill kinetics and post agent effect, was better than silver nanoparticles (AgNPs, size ≈35 nm). The inhibitory effect of Cur-Ag nanoensemble on biofilm formation was also better than AgNPs as supported by live dead imaging and scanning electron microscopy. Cur-Ag nanoensembles gave bacterial inhibition at very low silver concentration (0.01 mg of silver/g of nanoparticle weight). These nanoensembles were cytotoxic to skin keratinocytes (HaCaT) at a concentration much higher than the bacterial MIC.

In addition to selective toxicity of these Cur-Ag ensembles to bacterial cells over mammalian cells, subdued inflammatory response to mammalian cell line was observed suggesting their potential application as wound dressing agent. Curcumin was also employed to prepare anti-cancer nanoparticles using anti-inflammatory enzyme Serratiopeptidase by desolvation method. Here serratiopeptidase acted as a carrier as well as bioactive molecule in the nanoformulations. In this work, for the first time anti-inflammatory molecule, Curcumin has been combined with another anti-inflammatory molecule, Serratiopeptidase in nanoparticulate form. Serratiopeptidase not only provided stability to curcumin but also increase its effectiveness against cancer cells. These nanoparticles had anti-cancer activity in MCF and HeLa cell lines as shown by cytotoxicity assay, DAPI nuclear staining, ROS production and DNA damage. The immunomodulatory tests showed that Cur-SPD nanoparticles reduce level of IL6 but increase TNFα level in THP1 cell lines. This is due to structural similarity of serratiopeptidase to MMPs, matrix metalloproteases, which have been found to induce TNFα production and play tumour suppressive role in certain cancers. Cur-SPD nanoparticles may have potential application as anti-cancer agent. Thus results in present investigation provide an insight towards properties and drug delivery potential of nanoparticles consisting of novel combination of bioactive molecules.

सार

इस उपन्यास में दवाइयों के लिए डिलीवरी सिस्टम के नैनोपार्टिकल आधारित योगों से मौजूदा औषधियों की प्रभावकारिता में सुधार की अपनी क्षमता को दिखाया है । कोशिकाओं के लिए चिकित्सीय एजेंटों की डिलीवरी समस्याओं की वजह हैं- तेज़ी से गिरावट, दोहराए इंजेक्शन की आवश्यकता, कम जैव-उपलब्धता और जैविक बाधा के माध्यम से दवाओं के खराब परिवहन । दवाओं के प्रभावशाली वितरण विभिन्न नैनोकायरियर सिस्टम जैसे बायोडिग्रेडेबल पॉलिमेरिक नैनोपार्टिकल, लाइपोसोम, नैनोकैप्सूल, नैनो गेंद का उपयोग बड़े पैमाने पर एंटीबायोटिक प्रतिरोध से निपटने में किया गया है ।चांदी नैनोकणों (AgNPs), एक ज्ञात रोगाणुरोधी एजेंट, मानव कोशिकाओं के लिए विषाक्त हैं। एंटीबायोटिक और रजत नैनोकणों के संयोजन ने उत्साहजनक परिणाम दिखाए हैं, इसलिये हमने बायोएक्टिव चांदी नैनोकणों को तैयार करने के लिए Curcumin का इस्तेमाल किया है । Curcumin को DNA को नुकसान, ROS का उत्पादन, ट्रांसक्रिप्शन कारकों को कम करने, antiproliferative, antiangiogenic, anti-inflammatory, और anti-oxidative गुण प्रदर्शित करने के लिए सूचित किया गया है। बहरहाल, Curcumin जलीय समाधान में अघुलनशील रहता है और इसकी अवधि के दौरान इसकी स्थिरता से संबंधित समस्याएं हैं। 25-35 nm आकार के सिल्वर नैनोपार्टिकल (Cur-Ag nanoensembles), , ग्राम पॉजिटिव और ग्राम नेगेटिव जीवाणु दोनों के खिलाफ प्रभावी थे । यह बैक्टीरिया के खिलाफ अधिक और मानव केरेटिनोसाइट्स से के खिलाफ कम विषैले थे । इन नैनोसेन्स्ब्लल्स में बहुत कम कुल चांदी और उच्च स्थिरता थी । Cur-Ag nanoensembles की जीवाणुरोधी गतिविधि, न्यूनतम निरोधात्मक संकेंद्रण (MIC), time kill kinetics और पोस्ट एजेंट प्रभाव, के माध्यम से अध्ययन किया गया है और पाया गया की Cur-Ag nanoensembles, चांदी नैनोकणों (AgNPs, आकार ≈35 nm) से बेहतर थे । Cur-Ag nanoensembles का निरोधात्मक प्रभाव बायोफिल्म के निर्माण पर AgNPs से भी बेहतर था, यह live-dead इमेजिंग और स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोपी द्वारा समर्थित है। Cur-Ag nanoensembles बैक्टीरियल MIC की तुलना में बहुत अधिक संकेंद्रण पर त्वचा केराटिनोसाइट्स (HaCat) के लिए कोशिकाविषी है। इसके अलावा anti-inflammatory एंजाइम Serratiopeptidase और Curcumin को desolvation द्वारा एंटीकैंसर नैनोकणों को तैयार करने के लिए नियोजित किया गया । यहां serratiopeptidase ने एक वाहक और bioactive अणु के रूप में काम किया है। इस काम में, पहली बार anti-

inflammatory अणु, Curcumin को एक अन्य anti-inflammatory अणु के साथ जोड़ा गया है। Serratiopeptidase ने Curcumin को स्थिरता ही प्रदान नहीं की बल्कि इसकी वृद्धि भी की है। कैंसर कोशिकाओं HeLa और MCF-7 के खिलाफ प्रभावशीलता इन नैनोकणों में cytotoxicity assay, DAPI nuclear staining, ROS production और DNA damage परीक्षणों से पता चला है ।इम्युनोमोडायलेटरी परीक्षणों से पता चला है कि Cur-SPD NPs, THP1 सेल लाइनों में TNFα स्तर की वृद्धि और IL-6 स्तर को कम करते हैं जिसका कारण serratiopeptidase की Matrix metalloprotease से संरचनात्मक समानता है। वर्तमान जांच नैनोकणों के गुणों, बायोएक्टिव अणुओं का संयोजन, दवाओं की वितरण क्षमता और कैंसर में ट्यूमर दबाने वाली भूमिका हेतु प्रस्तुत किया है।

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List of Abbreviations

NLT	N lobe tether
WHO	World Health Organization
ROS	Reactive oxygen species
MMP	Matrix metalloprotease
NP	Nanoparticle
NPs	Nanoparticles
FDA	Food and Drug Administration
BSA	Bovine serum albumin
MW	Molecular weight
DMEM	Dulbecco's Modified Eagle's Medium
EDTA	Ethylene diamine tetra acetic acid
FBS	Fetal bovine serum
HPLC	High performance liquid chromatography
Da	Dalton
kDa	kilo Dalton
SDS-PAGE	Sodium dodecyl sulfate poly acrylamide gel electrophoresis
Vol	Volume
rpm	Revolutions per minute
CO_2	Carbon dioxide
v/v	Volume per unit volume
w/v	Weight per unit volume
psi	pounds per square inch
LPS	Lipopolysaccharide
PMA	Phorbol-12-Myristate-13-Acetate
ELISA	Enzyme linked immunosorbent assay

List of Symbols

%	percent
°C	degree Celsius
µ	micro
µL	microliter
cm	centimeter
nm	nanometer
G	gram
M	Molar
m	milli
mg	milligram
ml	milliliter
mM	millimolar
µm	micrometer
λ	wavelength
mm	millimeter
µg	Microgram
s	second
pg	picogram