UGC MAJOR RESEARCH PROJECT

(2013 – 2017)

SUMMARY

FORMULATION AND EVALUATION OF SELECTED DRUGS THROUGH NASAL DRUG DELIVERY SYSTEM

Principal Investigator

Dr. V.P.Pandey
Professor,
Department of Pharmacy,
Faculty of Engineering & Technology
Annamalai University, Annamalai Nagar,
Tamilnadu – 608 002.

Co-investigator

Dr. M.Menaka
Assistant Professor,
Department of Pharmacy,
Annamalai University, Annamalai Nagar,
Tamilnadu – 608 002.
INDIA

ANNAMALAI UNIVERSITY

DEPARTMENT OF PHARMACY

FACULTY OF ENGINEERING AND TECHNOLOGY
ANNAMALAI NAGAR - 608 002, TAMIL NADU, INDIA

SUMMARY

Formulation and evaluation of ondansetron loaded chitosan nanoparticles

The ondansetron loaded nanoparticles was prepared by ionic gelation method. The average particle size, polydispersibility index, zeta potential, entrapment efficiency and loading capacity of ondansetron loaded chitosan nanoparticles were performed and the results were summarized.

The IR spectral analysis of ONDNP shows a sharp peak at 3405.16cm^{-1} representing O-H stretch and H-bonded aromatic ring, which indicates the formation of hydrogen bond between chitosan (CHI) and OND. The peak at 1623.63 cm^{-1} showed the N-H bend 1577.91 cm^{-1}, 1481.68 cm^{-1}, 1420.69 cm^{-1} show the C-C stretch in rings referring aromaticity. C-N stretch, =C-H bend also observed through peaks at 1196.20 cm^{-1},1090.74 cm^{-1} and 960.96 cm^{-1} respectively. The DSC analysis was recorded between 80°C to 280°C approximately. No peaks of OND and CHI was visible in the OND loaded NPs. This finding suggests that OND is molecularly dispersed within the CHI NPs showing the amorphous nature that further authenticates the entrapment of OND. The XRD analysis of ONDNP was performed. The XRD patterns of OND-CHI NP, showed peaks at 26.96°, 35.63°, 45.70°, 53.85°, and 68.59° corresponding to the crystal lattice constant 3.30, 2.52, 1.98, 1.70 and 1.36Å, whereas the characteristic peaks of CHI at 2θ at 10.279°,19.767°, and 21.534° attributing to the crystalline structure is missing in OND encapsulated NP. The particle size and shape of the formulated ONDNP determined by transmission electron microscopy (TEM) analysis. The TEM image of the prepared ondansetron loaded chitosan nanoparticles indicates that the nanoparticles were roughly spherical in shape with size of 100 nm.

The *in vitro* drug release study of ondansetron loaded chitosan nanoparticles showed an initial release of 22.8% over the period of 30min, followed by slow release upto 24h. The *in vitro* drug release of ondansetron from ONDNP showed an increased release in aqueous media from 22.8 to 71.2% was due to the swelling property of the polymer. There was no further significant release were observed. Among various release kinetics models, the coefficient of correlation (R2) for the Higuchi model was near to unity (i.e, 986), therefore the best fit model for release from the NP was found to be Higuchi model.

The *in vitro* antiproliferative effect of prepared ondansetron hydrochloride nanoparticles on RPMI 2650 human nasal epithelial cell line was determined by MTT assay and results were depicted. The study indicated that OND encapsulated chitosan nanoparticles exhibit lower cytotoxicity with high CTC_{50} value compared to free OND.

The ciliotoxicity studies of prepared ONDNP were performed by using goat nasal mucosa. The study revealed that ONDNP is capable of providing direct nose-to-brain delivery, by without producing toxic effect to the nasal mucosa.

The *Ex vivo* nasal permeation study was carried out in isolated porcine nasal mucosa as it's histological and biochemical aspects are closely resembles to human. The ondansetron nanosuspension exhibits maximum permeation in 240 min found to be 71.72% whereas ONDS was only 35.36%. The increase in permeation of ONDNP could be attributed to an interaction of a positively charged amino group CHI with negatively charged sites on the mucosal membranes.

The *in vivo* toxicity study of OND nanoformulation showed no mortality, haematological changes, body weight variations and histopathological changes in animals, when formulation was administered in different doses as compared to OND solution.

The pharmacokinetic kinetic study of the formulated ONDNP was studied in Wistar rats. The AUCs of ondansetron nanosuspension and ondansetron suspension were compared and it depicted that the OND nanosuspension group had higher AUC as 562.34±14.81 ng h/mL in plasma and 324.36±15.28 ng h/mL in brain when compared to OND suspension which was 286.72±13.26 ng h/mL in plasma and 92.67±7.85 ng h/mL in brain when administered by intranasal route. When the C_{max} and T_{max} were compared, nasally administered free drug reached its peak within 5 minutes (C_{max} = 94.38±7.12 ng h/mL) and the OND nanosuspension reached its peak within 5h (C_{max}=210.62±11.28 ng h/mL) in plasma and the concentration found in brain when OND drug suspension administered intranasally and reached its peak within 8h (C_{max} =12.84±1.37 ng h/mL) and for OND nanosuspension the drug reached its peak within 2h (178.56±18.42 ng h/mL).

Intranasal OND nanosuspension group was compared with the intranasal OND suspension group and it was observed that OND nanosuspension formulation had higher C_{max}. This phenomenon could be explained by the fact that the OND suspension reaches the systemic circulation rapidly via nasal route whereas OND nanosuspension the presence of mucoadhesive biodegradable polymer leads to accumulation into the nasal mucosa and released slowly into the

circulation delaying the T_{max} compared to OND suspension. The results of the study indicated that OND loaded nanosuspension is capable of providing nose to brain delivery, thereby enhancing drug concentration in the brain.

Formulation and evaluation of olanzapine loaded chitosan nanoparticles

The olanzapine loaded chitosan nanoparticle was prepared by ionic gelation method. The results of Particle size, polydispersibility index and zeta potential of olanzapine loaded chitosan nanoparticles were summarized. The entrapment efficiency and loading capacity of formulated OLA nanoparticles increased from 33.42 % to 69.82% and 23.31% to 31.72%, depending upon the ratio of drug: polymer.

The IR spectrum of OLANP shows peaks at 3440.86 cm^{-1}, 2924.25 cm^{-1}, 1623.64 cm^{-1}, 1556.78 cm^{-1}, 1416.99 cm^{-1}, 1090.83 cm^{-1} due to N-H, C-H, N-H bend, C-C, C- N stretches revealing the amine, alkane and aromatic nature respectively which indicates the formation of hydrogen bond between CHI and OLA.

The DSC of OLANP showed its curve between 8-10 min. of its operation referring temperature 80°C to 300°C approximately. This curve is because of release of water molecule and reaching of melting point of OLANP thereafter its decomposition. Analysis of the results of thermal analysis reveals the fact that there were no peaks of OLA and CHI was visible in the OLA loaded NP. This finding suggests that OLA is molecularly dispersed within the CHI NP showing the amorphous nature that further authenticates the entrapment of OLA.

The XRD pattern of OLANP showed the characteristic peaks at 2θ =6.464°, 26.954°, 31.37°, 35.55°, 37.75°, and 53.76° corresponding to the crystal lattice constant of 13.66, 3.31, 2.85, 2.53, 2.38, and 1.70Å respectively. Chitosan has a strong reflection at 19.77°, and relative weak reflections at 10.28° and 21.53° as which is missing in OLA encapsulated NP, OLA probably formed a molecular dispersion or an amorphous nanodispersion within the CHI matrix of the NP. The particle size of formulated OLANP was determined by transmission electron microscopy (TEM) analysis. The TEM photograph of olanzapine loaded chitosan nanoparticles indicates that the nanoparticles were roughly spherical in shape with size of 100 nm.

The *in vitro* release profile of olanzapine showed a cumulative percentage release of 14.32% to 74.38% for 24h for olanzapine nanoparticles suspension. Olanzapine loaded chitosan

nanoparticles showed a biphasic release pattern. Initial burst release effect occurred within 30min and the remaining amount of drug was released in a sustained manner for a period of 24h. The percentage of drug release for different batches of olanzapine loaded chitosan nanoparticles was given.

The antiproliferative activity of olanzapine drug suspension and olanzapine loaded chitosan nanoparticles against RPMI 2650 cells (CTC_{50}>1000 µg/mL) was studied and the results indicates that nanoformulation has more antiproliferative effect in less concentration compared with plain olanzapine drug suspension. The cytotoxic effect of the olanzapine and olanzapine nanoparticles on RPMI Cell line was depicted. The histopathological studies revealed that the condition of goat nasal mucosa after treatment with PBS pH 6.4 a negative control, isopropyl alcohol (IPA) a positive control, OLA solution and OLANP dispersion. The results showed that there is no cell necrosis or cilia detachment from the nasal mucosa was observed after treating with OLANP dispersion. It indicates that the microscopic structure of nasal mucosa shows no significant harmful effects**.**

The diffusion of drug across the nasal mucosa was fast from nanosuspension compared with OLA drug suspension. Almost 67.92 ± 0.753% drug diffused within 240 min from nanosuspension, while in the case of OLA drug suspension, slower diffusion was clearly observed with a diffusion of 15.84 ±1.59% in 240 min.

The *in vivo* toxicity results indicated that OLA nanoformulation was able to show no mortality, haematological changes, body weight variations and histopathological changes in animals, when formulation was administered in different doses as compared to OLA solution.

The pharmacokinetic kinetic study of the formulated OLANP was studied in Wistar rats. The AUCs of OLA nanosuspension and OLA suspension were compared and it was depicted that the OLA nanosuspension group had higher AUC in plasma and in brain when compared to OLA suspension when administered by intranasal route. Intranasal OLA nanosuspension group was compared with the intranasal OLA suspension group and it was observed that OLA nanosuspension formulation had higher C_{max}. This phenomenon could be explained by the fact that the OLA suspension reaches the systemic circulation rapidly via nasal route whereas OLA nanosuspension the presence of mucoadhesive biodegradable polymer leads to accumulation into the nasal mucosa and released slowly into the circulation delaying the T_{max} compared to OLA suspension.

Formulation and evaluation of Memantine Hydrochloride (MEM) loaded chitosan nanoparticles

Memantine loaded chitosan nanoparticles were prepared and optimized with various concentrations of the drug and polymer ratio by ionic gelation method. The average particle size of memantine loaded chitosan nanoparticles range from 149.7±5.46nm to 324.30±11.24 nm. Polydispersibility index is an essential tool to investigate the characteristics of nanoparticles in dispersion. Formulation MEMNP4 has 0.523±0.06 (poly dispersibility index <1) it has narrow dispersion. The zeta potential of memantine nanoparticles ranging from 23.8±0.4 to 54.0±0.5mV. Nevertheless, memantine was entrapped in the matrix of nanoparticles to an appreciable extent (56.00±2.74% to 80.72±1.84%). The loading capacity of MEMNP was found to be 34.86±4.16%.

The IR spectral interpretation shown that the spectra obtained from the memantine nanoformulation matches with original spectra of memantine. Similarly characteristic peaks, for the polymers chitosan were also notices in the formulation spectrum. DSC curve of MEMNP exhibited a large endothermic peak between 50°C and 145°C due to evaporation of water molecules. A small exothermic peak was also found for MEMNP between 250°C and 270°C approximately. X-Ray powder diffraction (XRD) patterns of memantine nanoparticles were recorded. The main peaks of the XRD patterns can be indexed in the original structure, although sometimes small transient peaks could be observed. These results were in good support indicating that the MEM encapsulated in core of NP and conversion of drug from crystalline to amorphous state.

The particle size of the formulated MEMNP was determined by (TEM) analysis. The TEM images of the prepared memantine loaded chitosan nanoparticles indicate that nanoparticles were roughly spherical in shape with size of 100 nm.

The *in vitro* drug release of memantine loaded chitosan nanoparticles showed initial release 21.44% which may be accounted for the memantine adsorbed to the surface. The i*n vitro* drug release of MEMNP. After 24h, 86.51% of memantine was released, followed by slow release up to 24h.

The cytotoxic effect of the memantine and memantine loaded chitosan nanoparticles on RPMI cell line showed, the cells treated with pure MEM exhibited cytotoxicity to some extent

with CTC_{50} value of 86.67±2.9 µg/mL & growth inhibitory effect was four times lesser than MEMNP indicating that the MEMNP shows lesser toxic effects when compared to pure MEM.

Nasal ciliotoxicity study and nasal permeation study was carried out in goat nasal mucosa and the results showed that there was no toxicity of nasal mucosa with high permeation of memantine nanosuspension when compared with memantine drug suspension. The *in vitro* toxicity study results supported the fact that MEMNP did not have any toxic effect on animals when nasally administered. The pharmacokinetic kinetic study of the formulated MEMNP was studied in Wistar rats and the results showed that MEM nanosuspension had higher AUC and C_{max}. as in brain when compared to MEM suspension. This phenomenon could be explained by the fact that the MEM suspension reaches the systemic circulation rapidly via nasal route whereas MEM nanosuspension the presence of mucoadhesive biodegradable polymer leads to accumulation into the nasal mucosa and released slowly into the circulation delaying the T_{max} compared to MEM suspension.

Abbreviations used for Ondansetron, Olanzapine and Memantine and its nanoparticles in this Study

1. OND- ondansetron, ONDNP- ondansetron nanoparticles
2. OLA- olanzapine, OLANP- olanzapine nanoparticles
3. MEM- memantine, MEMNP- memantine nanoparticles

<u>Summary of Comparative Study of Ondansetron, Olanzapine and Memantine for Nose to Brain Targeting</u>

Nose to brain delivery of drugs has attracted as a potential route of drug delivery to the brain as it bypasses first pass metabolism, prevents enzymatic and chemical degradation of drugs and provides fast onset of action due to high permeable and vascularized site. Moreover being non invasive in nature, nasal route provides an alternative to injectable formulations and enhances patient compliance because drugs can bypass the BBB during this transport and enter the CNS (**Djupesland *et a,l* 2014)**, (**Talegonkar and Mishra 2004**). Polymeric biodegradable nanoparticles have been extensively reported for encapsulation of drugs and nose-to-brain drug delivery. The present investigation was hypothesized that intranasal chitosan nanoparticles could

serve as a noninvasive drug delivery carrier for ondansetron, olanzapine and memantine hydrochloride.

Description

OND - white to off-white powder, OLA - yellow crystalline solid, MEM- white amorphous powder

Odor

OND, OLA and MEM - odorless

Solubility

OND - sparingly soluble in water and in alcohol, OLA - Practically insoluble in water, MEM - The solubility of memantine hydrochloride in water at room temperature is about 3.5%.

Molecular Mass

OND - 293.63g/mole, OLA - 312.432 g/mole, MEM- 179.3 g/mole.

pKa

OND - 7.34, OLA - 7.24, MEM- 10.5

Half Life

OND - 5-7 hours, OLA - 21 to 54 hours, MEM - 60-100 hours

Indications

OND - For treatment of chemotherapy induced nausea and vomiting. Antiemetic. OLA - Olanzapine is used for the acute and maintenance treatment of schizophrenia and related psychotic disorders. MEM - For the treatment of moderate to severe dementia of the Alzheimer's type.

Side Effects

OND - Confusion, dizziness, fast heartbeat, fever, headache, shortness of breath, weakness. OLA - Include somnolence, dizziness, weight gain, constipation, akathisia (restlessness), and increased ALT. MEM - Side effects include pain, abnormal crying, leg pain, fever, increased apetite. Adverse drug reactions include: dizziness, confusion, headache, hallucinations, tiredness. Less common side effects include: vomiting, anxiety, hypertonia, cystitis, and increased libido.

Dosage Forms

OND - Tablets, oral Solution and orally disintegrating tablets. OLA - Tablets: 2.5, 5, 7.5, and 10 mg, orally disintegrating tablets: 5 and 10 mg. MEM - Solution 10 mg/5 mL, tablets, film coated 5 mg, 10 mg.

Particle Size

ONDNP - 141.4±8.62. OLANP - 183.1±4.99. MEMNP - 149.7±5.46

Polydispersibility Index

ONDNP - 0.270±0.04. OLANP - 0.122±0.12, MEMNP- 0.523±0.06

Zeta Potential

ONDNP - 54.10±0.8, OLANP -52.1±0.8 MEMNP - 54.0±0.5

Entrapment Efficiency

ONDNP-74.49±2.62, OLANP- 69.82±4.12, MEMNP - 80.72±1.84

Loading Capacity

ONDNP -22.78±1.78, OLANP-26.64±3.45, MEMNP - 34.86±4.16

Surface Morphology by Scanning Electron Microscopy

ONDNP, OLANP, MEMNP- shows distinct, spherical shaped particles with the average diameter around 100nm.

Particle Size by Transmission Electron Microscopy

ONDNP, OLANP, MEMNP - roughly smooth and spherical around 100nm

Drug Release

OND-71.2%, OLA-74.38% for 24 h, MEM - 86.51% for 24 h

Mechanism of Drug Release

ONDNP, OLANP, MEMNP-Higuchi followed by korsmeyer's Peppas.

Cell line Toxicity MTT Assay

OND- CTC_{50}=550.00±10.00µg/mL, ONDNP- CTC_{50} >1000µg/mL, OLA- CTC_{50} >1000µg/mL, OLANP- CTC_{50} >1000µg/mL, MEM - CTC_{50}= 86.67±2.9 µg/ml, MEMNP- CTC_{50}= 340±10.00 µg/mL.

Nasal Ciliotoxicity study (Goat nasal mucosa)

ONDNP, OLANP, MEMNP – No toxic effects on nasal mucosa.

***Ex vivo* Permeation Study (Franz diffusion cell)**

OND- 35.36±5.63, ONDNP- 71.72±5.78 (after 240 min), OLA- 15.84±1.94, OLANP- 67.92±1.72 (after 240 min), MEM-14.18±1.37, MEMNP- 52.53±1.35 (after 240 min)

Stability Study

ONDNP, OLANP, MEMNP-possess good stability.

Subacute Toxicity Study

ONDNP, OLANP, MEMNP- There were no treatment related mortalities, clinical signs of toxicity throughout the course of the study. No gross pathological changes, body weight changes, hematological changes, biochemical and histopathological changes in the treated groups implies no toxic effects.

Drug loaded chitosan nanoparticles were successfully developed for ondansetron, olanzapine, and memantine. *In vitro* and *ex vivo* drug release studies supported the controlled drug release from nanoparticles. Toxicity study showed a very good and acceptable toxicity profile and proven to be safety when administered via nasal route. Cytotoxic concentration of developed nanoparticles and plain drug solution were comparable on nasal epithelial cell lines RPMI 2650. The pharmacokinetic study of the formulated nanoparticles showed that the drug administered via nasal route delivers rapidly and more effectively in brain.