



DEVELOPMENT & EVALUATION OF CLOZAPINE LOADED MUCOADHESIVE MICROSPHERES FOR BRAIN TARGETING

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ABSTRACT:

In the present work, mucoadhesive nasal microspheres containing clozapine were formulated and evaluated for increasing its bioavailability at cerebrospinal fluid. Clozapine is antipsychotic agent which is of BCS class II, hence need to improve its bioavailability at central nervous system (CNS). To make the drug delivery system safer, natural ingredients were used. Various natural polymers and cross-linkers were screened. Starch is a natural polymer crosslinked with citric acid as a natural crosslinker. Clozapine loaded cross-linked starch microspheres (CSMs) were successfully developed for intranasal delivery for CNS targeting using single emulsion cross-linking method. Ex-vivo mucoadhesion study and In-vivo brain targeted study has been carried out using prepared CSM's. More than 90% mucoadhesive strength was achieved using starch as a natural polymer. Noncompartmental analysis was carried out to calculate the pharmacokinetic parameters. Clozapine concentration was analysed in both plasma and CSF using HPLC analysis. There was 1.5-fold increase in the bioavailability of clozapine when administered intranasally. Drug targeting efficiency (DTE %) and Drug targeting potential (DTP %) was calculated. There was a 2.4-fold increase in % DTE and 2.04-fold increase in % DTP for CSM's as compared to clozapine. In-vivo studies showed increased relative bioavailability with nasal route as compared to the oral route. Intranasal route has helped to attain significant therapeutic levels of the antipsychotic drug in the Cerebrospinal fluid by surpassing the blood brain barrier and hepatic first pass effect.

KEYWORDS: Clozapine, Mucoadhesion, Intranasal, Bioavailability.

I. INTRODUCTION:

Schizophrenia is a major psychotic disorder that frequently has devastating effects on various aspects of the patient's life carries a high risk of suicide and other life-threatening behaviours. In schizophrenia patients experiencing an acute exacerbation of psychotic symptoms, the primary goal of disease management is to achieve optimal control of symptoms¹.

Clozapine (CLZ) is a prototype atypical antipsychotic drug used to treat patients with schizophrenia. These patients are generally unresponsive or intolerant to typical antipsychotics. Clozapine is found to effective in resistant schizophrenia. It also helps to reduce the suicidal behaviour of patients. Clozapine acts through a combination of antagonistic effects at D2 receptors in the mesolimbic pathway and 5-HT_{2A} receptors in the frontal cortex. D2 antagonism relieves positive symptoms while 5-HT_{2A} antagonism alleviates negative symptoms². Oral tablets of clozapine are available but it shows very poor bioavailability (<27%) at brain due presence of blood brain barrier and extensive first pass metabolism.

The major challenge in CNS drug delivery is the blood-brain barrier, which limits the access of drugs to the brain. The diffusion of drugs from the blood into the brain depends mainly upon the ability of the biologically active molecule to traverse lipid membranes. Blood cerebrospinal fluid (BCB) barrier is the second barrier which restricts the entry systemically administered drug molecule in the CNS. The BCB is located in the epithelium of the choroid's plexus. It is arranged in order to limits the passage of molecules and cells into the CSF¹¹. Macromolecular drugs like peptides and proteins are not only too large but also too hydrophilic to penetrate the BBB from the systemic circulation.

As per BCS classification system clozapine is Class II drug having low solubility and high permeability. Now a days, many formulations development was done to enhance the solubility of clozapine and reduce the side effects associated with clozapine. In order to improve the patient compliance research has been done to prepare formulations such as nanoparticles for injectable drug delivery⁴, Proniosome for transdermal drug delivery⁵ etc., but it shows the drawbacks including particle aggregation, limited drug loading and leakage on storage⁶. These adverse events beside the need of prompt therapeutic action make clozapine suitable candidate for development of intranasal formulation. Intranasal formulations also overcome the blood brain barrier, avoids GI first pass metabolism and hance improves the drug bioavailability at brain.

Intranasal drug delivery is now recognized to be a useful and reliable alternative to oral and parenteral routes. The nasal mucosa has been considered as a potential administration route to achieve faster and higher level of drug absorption²⁶. This is due to large surface area of nose and the thin, porous, highly vascularized nasal epithelium which ensures high absorption and rapid transport of the absorbed substances directly into the systemic blood circulation avoiding drug metabolism in the liver. Structurally, the nose is divided into two nasal cavities via a midline septum. The nasal cavity is about 12 cm long; the volume of each nasal cavity is 13 ml and has a surface area of around 150 cm². Each cavity consists of three different regions, namely the vestibule, the olfactory region and the respiratory region. The olfactory region is located in the roof of the nasal cavity and extends a short way down the septum and lateral wall. Its neuroepithelium is the only part of the CNS that is directly exposed to the external environment. Similarly, to the respiratory epithelium, the olfactory one is also pseudo stratified but contains specialized olfactory receptor cells important for smell perception^{9,10}. If drugs are

absorbed in the olfactory region, they will be absorbed directly into the central nervous system bypassing the tight blood brain barrier⁵.

The use of pharmaceutical micro/nanocarriers (micro/nanoparticles, micro/nano emulsion, micelles and liposomes) to enhance the in vivo efficiency of many drugs well established itself over the past decade both in pharmaceutical research and clinical setting. These systems can include- Microspheres, Nanoparticles, Liposomes, Nasal inserts, Nasal Drops, Nasal Sprays, Nasal Gels, Nasal Powders. Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 μm to 1000 μm). Mucoadhesive microspheres enhance the intimate contact with the mucus layer. Tailored mucoadhesive microspheres offers the possibilities of localized as well as controlled release of drugs by adherence to any mucosal tissue present in eye, nasal cavity, urinary, and GI tract. Mucoadhesive microspheres are prepared by using mucoadhesive polymers. Mucoadhesive polymers can be of either natural or synthetic in origin. The microspheres of natural polymers are prepared by single emulsion technique. The natural polymers are dissolved/ dispersed in aqueous medium followed by dispersion in the non-aqueous medium (example- oil). In the second step, cross linking of the dispersed globule is carried out either by means of heat or by using chemical cross linkers. Among various polymeric microspheres, starch microspheres have been widely investigated as haemostatic, embolic, agents and drug carriers, because of their total biodegradability, biocompatibility, non-toxicity, as well as cost-effectiveness. The cross-linking has been a predominant approach of solidification¹³. Due to above mentioned benefits of 'starch' in the present study green technology-based synthesis of microspheres was carried out using natural polymer and cross-linker¹⁴.

Nasal mucoadhesive microspheres of clozapine has not been investigated till now. Hence for brain targeting via olfactory nerve of nasal cavity this drug delivery system prepared and evaluated for its bioavailability study by comparing it with intravenous route. The present study carried out to determine ex-vivo mucoadhesive strength at nasal mucosa and in vivo pharmacokinetic parameters related to both plasma and CSF.

II. MATERIALS & METHODS:

Clozapine was obtained as a gift sample from Om Sai Pharmaceuticals Limited, India. Starch, Citric acid and soluble starch were procured from Loba Chemicals, India. All other excipients and solvents used in the experiment were of analytical grade.

A) Animals:

In vivo experiments were performed in male Wistar rats procured from the DY Patil medical college, Pune (one week prior to experiments). During this one-week period animals were allowed to acclimatize to the experimental conditions of temperature and humidity. Animals were housed together in plastic cages under standard conditions of temperature (24 ± 1 °C), relative humidity ($55 \pm 10\%$) and 12hr light/dark cycles throughout the experiment. Rats were fed with standard pelletized diet (Pranav Agro Industries, Sangli, Maharashtra, India) and filtered water but abstained overnight prior to experimentation. During this acclimatization period, the health status of the animals was monitored daily.

B) Preparation of Clozapine Loaded Crosslinked Starch Microspheres (CSMS):

Clozapine loaded CSMS were prepared by single emulsion crosslinking method. Briefly, aqueous phase prepared by dissolving starch in water (15% W/V) and heated to 60°C to form a gel and then it is allowed to cool at room temperature. Drug was added into the gel matrix. Drug loaded gel matrix was added drop wise using syringe into the external organic phase chloroform containing span 80 (0.2% V/V) as a surfactant under constant mechanical stirring and temperature of the system was maintained about 40°C to form w/o emulsion. After complete homogenization of w/o emulsion, 1ml of citric acid solution (6% W/V) as a cross-linker was added drop wise. Organic phase was allowed to evaporate completely and obtained microspheres were dried by lyophilisation technique and further characterized.

C) *Ex-Vivo* Mucoadhesion Study:

Percent mucoadhesion was calculated by using falling film technique²⁴. 100mg of microspheres were placed over the piece of nasal mucosal surface mounted on a tilted slide at an angle of 45°. The effluent was run over the mucosal surface. The effluent was collected on a whatmann filter paper and weight of detached particles was determined. Percentage of mucoadhesion (% M) was determined using following formula:

$$\% \text{ Mucoadhesion} = \frac{\text{Wt. of CSMS taken} - \text{Wt. of detached CSMS}}{\text{Wt. of CSMS taken}} * 100$$

D) *In-Vivo* Pharmacokinetic Study:

a) Administration of pure drug and drug loaded CSM's & collection of biological fluids:

Male Wistar rats weighing 200-250g were used in the study. Animals were divided into two groups each for intranasal (IN) and intravenous administration (IV) as follows:

Group 1: Standard drug (n=8)

Group 2: Formulation (n=8).

The animals are administered with a single dose of 0.9 mg/kg of CLZ and CLZ loaded cross-linked starch microspheres by intranasal route and intravenous route. The blood samples were collected from retro orbital plexus of rat at 0.5, 1, 2, 3, 6, 9, 12, 24 hours post administration and plasma separated for analysis. Brain samples (CSF) were isolated from the animals at different set time points by decapitation method.

b) Determination of Clozapine Conc. In Plasma and CSF:

Clozapine concentration in both plasma and CSF is determined by HPLC method. Chromatography was performed using Jasco PU 1580 series chromatographic system. The chromatographic system operation and recording of data were performed using Borwin software. Chromatographic separations were achieved on Thermo C-18 column. The mobile phase composition included phosphate buffer (pH 6.5): acetonitrile (50:50) (pH was adjusted with triethylamine)²³. Before delivering the mobile phase into the system, it was filtered through 0.45µm filter using vacuum and degassed for 15min by bath sonication. Clonazepam was used as an internal standard (IS). Compounds were monitored by UV detection at 259 nm with a flow rate of 1 ml/min.

and volume of injected sample was 20 μ l. Standard solution of CLZ was prepared by dissolving 5mg in 5ml methanol and standard solution of clonazepam was prepared by dissolving 5mg in 5ml chloroform, separately (1000ppm).

For calibration curve estimation in both plasma and CSF, serial dilutions of CLZ were made from the standard solution using mobile phase to obtain concentrations ranging from 1 μ g/ml - 5 μ g/ml, 100 μ l of plasma or CSF and 10 μ l (1000 μ g/ml) of IS was added to all the dilutions. The samples were injected into HPLC system and peaks were recorded. To 100 μ l of standard pure plasma and CSF, 10 μ l of IS (clonazepam of concentration 1000 μ g/ml) was added. The samples were vortex for 15 min., filtered through 0.22 μ m syringe filters and then subjected to HPLC analysis.

c) Data Analysis:

Noncompartmental analysis was carried out to calculate the pharmacokinetic parameters (C_{max} , T_{max} , AUC, K_e , MRT, $T_{1/2}$, etc.) for each individual set of data using the pharmacokinetic software, WinNonlin version 4.0 (Pharsight, Mountain View, CA, USA).

The absolute CNS bioavailability 'F' was determined using $AUC_{0-\infty}$ values obtained from CNS concentration-time curves following intravenous and intranasal administration of pure CLZ and CSMs²⁵.

$$F_{abs.} = AUC_{0-\infty} (IN) / AUC_{0-\infty} (IV)$$

To evaluate brain targeting after nasal dosing, two indexes were calculated²⁶: Drug targeting efficiency (DTE %) represents time average partitioning ratio and Drug targeting potential (DTP %) represents direct nose to brain transport and can be calculated by using following formulas:

$$DTE \% = \left[\frac{\left[\frac{AUC_{brain}}{AUC_{blood}} \right]_{IN}}{\left[\frac{AUC_{brain}}{AUC_{blood}} \right]_{IV}} \right] \times 100$$

$$B_x = (B_{i.v} / P_{i.v}) \times P_{i.n}$$

$$DTP \% = \frac{(B_{i.n} - B_x) \times 100\%}{B_{i.n}}$$

Where,

B_x is the brain AUC fraction contributed by systemic circulation through the blood brain barrier (BBB) following IN administration;

B_{IV} is the AUC_{0-360} (brain) following IV administration of pure drug solution or CSMs;

B_{IN} is the AUC_{0-360} (brain) IN administration of pure drug solution or CSMs;

P_{IN} is the AUC_{0-360} (blood) following IN administration of pure drug solution or CSMs.

P_{IV} is the AUC_{0-360} (blood) following IV administration of pure drug solution or CSMs.

III. RESULTS & DISCUSSION:

A) *Ex-Vivo* mucoadhesion study:

Mucoadhesive strength can be defined as the potency of adhesive bonds between two materials. The % mucoadhesive strength of CLZ loaded CSMs were found to be 93.46% which is more than 90 %.

Higher mucoadhesion offers long duration of residence time of drug delivery system at nasal mucosa and leads to increase the efficacy of drug.

B) *In-Vivo* Pharmacokinetic study:

The clozapine calibration curve in plasma ($Y=1.520X + 0.003$) was found to be linear over the concentration range 0 – 6 $\mu\text{g/ml}$ with R^2 0.993. Brain tissue calibration curve of clozapine ($Y = 0.009X - 0.010$) also found to be linear within the concentration range of 0 – 6 $\mu\text{g/ml}$ with R^2 0.997.

The retention times for CLZ and clonazepam were found to be 8.1 and 4.6 minutes respectively.

Following IN administration, at each time point studied concentration of CLZ was higher in CNS from CSMs than pure CLZ. Also, significant increase in C_{max} was observed for CSMs (35.34 $\mu\text{g/ml}$) as compared to pure CLZ (15.73 $\mu\text{g/ml}$) in CNS.

Table 1: Pharmacokinetic parameters after single dose IN administration of CLZ and CSMs.

Parameters	Plasma		CNS	
	Pure CLZ	CSMs	Pure CLZ	CSMs
C_{max} ($\mu\text{g/ml}$)	12.30	27.33	15.73	35.34
T_{max} (hour)	8	6	2	2
$AUC_{0-\infty}$ ($\text{hr}\cdot\mu\text{g/ml}$)	110.7	177.64	131.2	192.5
K_e (hr^{-1})	0.18	0.02	0.09	0.07
MRT (hour)	1.35	3.55	1.7	4.17
$T_{1/2}$ (hour)	3.7	15.3	7.7	9.9

A 2.2-fold increase in the C_{max} value and approximately 1.4-fold increase in the $AUC_{0-\infty}$ values for CLZ following IN administration of CSMs demonstrated improvement in CNS bioavailability which can be attributed to smaller particle size and type of mucoadhesive system. The $AUC_{0-\infty}$ values suggest that CLZ surpassed the BBB more effectively as compared to pure CLZ. However, there was no difference in the T_{max} of pure CLZ and CSMs (2 h). A significant decrease in the rate of elimination (K_e) resulted in significant increase in $t_{1/2}$ (0.07) with 2.45-fold increase in mean residence time of the drug from CSMs than pure CLZ. This increase in MRT can also be correlated to increased magnitude of $AUC_{0-\infty}$. As shown in Table 1 similar 2.2-fold increase in C_{max} was observed for CSMs as compared to pure CLZ in plasma by IN route with a significant lag time of 6h and 8h respectively. Less amount of drug in plasma as compared to CNS indicates more amount of drug distributed in brain tissue when administered as CSMs by intranasal route.

Following IV administration, 1.4-fold increases in the $AUC_{0-\infty}$ was observed for CSMs with C_{max} of 27.94 $\mu\text{g/ml}$ as compared to C_{max} of 19.8 $\mu\text{g/ml}$ for pure CLZ in the CNS. However, time (T_{max}) to achieve this C_{max} was same for both pure CLZ and CSMs. Furthermore, higher plasma (C_{max}) was observed for CSMs (83.46 $\mu\text{g/ml}$) than pure CLZ (47.30 $\mu\text{g/ml}$) indicates about 1.7-fold increase in C_{max} which is due to increase in solubility of CLZ in hydrophilic polymeric network. Less amount of drug in CNS as compared to plasma was detected due to presence of BBB following IV administration.

Table 2: Pharmacokinetic parameters after single dose IV administration of CLZ and CSMs.

Parameters	Plasma		CNS	
	Pure CLZ	CSMs	Pure CLZ	CSMs
C_{max} ($\mu\text{g/ml}$)	47.30	83.46	19.83	27.94
T_{max} (hour)	0.5	1	1	1
$AUC_{0-\infty}$ ($\text{hr} \cdot \mu\text{g/ml}$)	30.74	103.623	144.5	169.37
K_e (hr^{-1})	0.19	0.13	0.11	0.09
MRT (hour)	1.10	2.43	1.05	2.69
$T_{1/2}$ (hour)	3.54	5.18	5.92	7.53

It was also observed that IN administration of CSMs increased the bioavailability in the CNS compared to IV administration (Table 1 & 2).

CNS and plasma concentration-time curves of pure CLZ and CSMs after single dose IV and IN administration (0.9 mg/kg) are depicted in Figure 1.

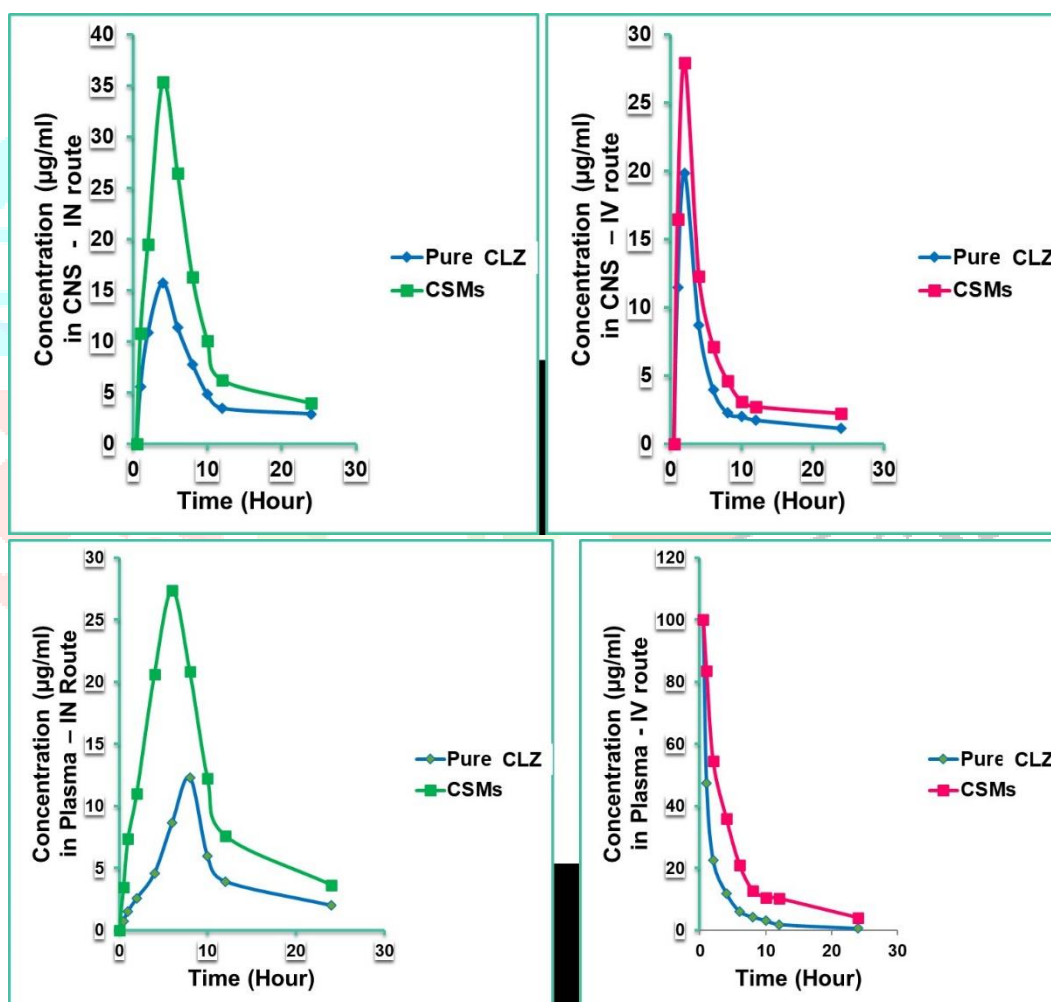


Figure 1: Plasma and CNS concentrations of CLZ after the administration of pure CLZ and CSMs by intranasal and intravenous routes (n=8).

CNS bioavailability was found to be increased from 0.9 to 1.45 for pure CLZ and CSMs respectively, reveals that 1.5-fold increase in bioavailability achieved by administering the formulation by intranasal route.

% DTE and % DTP were calculated for pure CLZ and CSMs using brain and blood concentration of the drug after single dose IV and IN administration as summarized in Table 3.

Table 3: % DTE and % DTP following intranasal administration of CLZ and CSMs.

Batch	DTE (%)	DTP (%)
Pure Drug	34.82	45.45
CSMs	83.55	92.43

There was a 2.4-fold increase in % DTE and 2.04-fold increase in % DTP for CSM's as compared to pure CLZ. Conversion of pure drug into CSMs which consisted of a hydrophilic strong polymeric matrix with increased encapsulation efficiency could have resulted in higher % DTE and % DTP. Animal studies thus demonstrated the potential of mucoadhesive drug delivery systems in nose to brain delivery of drugs when compared to equivalent drug solution formulation.

IV. CONCLUSION:

In the present study the potential of CSMs in targeted brain delivery of CLZ to schizophrenia and other psychotic disorders too was investigated for the first time. The CSMs were successfully prepared by single emulsion cross-linking technique using natural polymer and crosslinker.

Administering the formulation through IN route has helped to attain significant therapeutic levels of the drug in the CSF by surpassing the BBB. CSMs offered a potential approach for enhancing the bioavailability of antipsychotic agents which are of BCS class II category. Further investigations in humans need to be carried out to establish the clinical potential of the nasal mucoadhesive systems in brain targeting.

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