



## EVALUATION OF BRAIN-TARGETING FOR THE NASAL DELIVERY OF MIDAZOLAM

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### ABSTRACT

Gelatin-chitosan mucoadhesive microspheres of midazolam were prepared using the emulsion cross linking method. In vivo CNS drug distribution studies were carried out in albino rats by administering the midazolam microspheres intranasally and midazolam solution intravenously. From the drug levels in plasma and CSF, drug targeting index and drug targeting percentage were calculated. Results obtained indicated that intranasally administered midazolam microspheres resulted in higher brain levels with a drug targeting index of 2.12 & drug targeting percentage of 62.52%. Gelatin-chitosan cross linked mucoadhesive microspheres have the potential to be developed as a brain-targeted drug delivery system for midazolam.

**Keywords:** Gelatin- chitosan cross linked microspheres, Mucoadhesive microspheres, Midazolam, Brain targeted drug delivery.

### INTRODUCTION

Midazolam is chemically 8-chloro- 6-(2-fluorophenyl)- 1-methyl- 4H-imidazo[1,5-a] [1,4]benzodiazepine . It is used to produce sleepiness or drowsiness and to relieve anxiety before surgery. Midazolam is also given to produce amnesia so that the patient will not remember any discomfort or undesirable effects that may occur after a surgery or procedure. Midazolam undergoes first pass metabolism and it is generally given by oral or parenteral routes. An alternative route of drug delivery is needed since oral and intravenous routes for delivering drugs are sometimes impractical and/or inconvenient. Direct transport of drugs to the brain circumventing the brain-barriers following intranasal administration provides a unique feature and better option to target drugs to brain. The plasma half life of midazolam is 4 hrs and that is the reason it was used for the nose to brain drug delivery and the use of bioadhesive microspheres gives more residence time to facilitate absorption. Chitosan-gelatin was used to prepare the midazolam microspheres for the nose to brain drug delivery so as to increase the residence time and by pass the first pass metabolism by liver. Intranasal administration has drawn considerable interest in the last decade since it provides a non-invasive method for bypassing hepatic first-pass effect and possibly the blood brain barrier (BBB). Therefore, it might be a feasible way of both enhancing midazolam bioavailability and achieving its brain-targeting. Drugs administered to the nasal cavity can travel along the olfactory and trigeminal nerves to reach many regions within CNS. Many substances, including tracer materials, heavy metals, low molecular weight drugs and peptides have been shown to reach the CSF, the olfactory bulb (OB) and in some case other parts of the brain, after nasal administration. Drugs have been shown to reach the CNS from the nasal cavity by a direct transport across the olfactory region situated at the loft

of the nasal cavity. It is the only site in the human body where the nervous system is in direct contact with the surrounding environment. The nasal route could be important for drugs that are used in crisis treatments, such as for pain, and for centrally acting drugs where the putative pathway from nose to brain might provide a faster and more specific therapeutic effect. The nasal route, therefore, offers a potential for drugs targeting the brain providing more opportunities for midazolam to enter the CNS and then act on CNS disorders.<sup>1-10</sup> However, whether this kind of direct delivery route exists or not for midazolam has not yet been investigated. In order to clarify this issue, midazolam formulations for nasal administration were prepared and the prepared microspheres were evaluated with respect to the particle size, encapsulation efficiency, shape and surface properties, mucoadhesive property and in vitro drug release. The aim of present study was to carry out the *in vivo* studies and the midazolam concentration in plasma and CSF samples was determined by reversed phase-high pressure liquid chromatography (RP-HPLC) with fluorescence detection.<sup>11-21</sup>

### MATERIALS AND METHODS

#### Materials

Midazolam was obtained as a gift sample from Sun Pharmaceutical Industries Ltd. Vadodara, Gujarat. Chitosan was obtained as a gift sample from Central Institute of Fisheries Technology, Cochin, India. Glutaraldehyde, heavy paraffin, light Paraffin, Tween 80 was procured from Loba Chem. Mumbai. Gelatin was obtained from S.D Fine.

Male Wister rats weighing 250–300 g were provided by the animal house of Pioneer Pharmacy Degree College, Vadodara, Gujarat, India (CPCSEA Registration number-1247/ac/09/CPCSEA dated 21/5/2009 and the approved



protocol number is: OGECT/PPDC/IAEC/2011/6/2). These animals were allowed to acclimatize in environmentally controlled area ( $24 \pm 1^\circ\text{C}$  and 12:12 h light–dark cycle) for at least 5 days before being used for experiments.

### Preparation of Microspheres

Chitosan-gelatin based microspheres were prepared using emulsion cross linking method using different ratios of Gelatin/Chitosan (6:0, 8:0, 10:0, 6:4, 8:2). Chitosan solution was prepared in 1% w/v glacial acetic acid was first mixed with the gelatin solution. The drug was dissolved into it, and then the solution was extruded through a glass jacketed syringe in 50 mL of liquid paraffin (heavy and light 1:1 mixture) containing 0.1% surfactant, with continuous stirring on Remi stirrer at 2000 rpm. After 3 hrs, 1 mL of glutaraldehyde (25% solution, as crosslinking agent) was added and stirring was continued for 2 hrs. Microspheres obtained were filtered and washed several times with petroleum ether to remove oil, and finally washed with water to remove excess of glutaraldehyde. Different amount of drug and polymers were used to obtain the microspheres of optimum properties and characteristics.<sup>22-31</sup>

### Drug Administration and Collection of Biological Samples

For nose to brain administration, microsphere dispersion was instilled into one of the nares towards the roof of the nasal cavity at a dose of 0.5 mg/ kg and for intravenous (i.v) administration midazolam solution at a dose of 0.5 mg/ kg was injected through tail vein. Blood samples were collected and plasma was separated. Plasma and CSF samples were withdrawn at set time points. Drug content was analyzed in both the fluids.<sup>32-40</sup>

### Assay of Midazolam in Plasma and CSF Samples

Midazolam was assayed using an HPLC method. An approximately 0.5 mL sample of plasma and CSF as mixed with 50 $\mu\text{L}$  of the internal standard solution, Montelukast ( $10\mu\text{g mL}^{-1}$ ), and the mixture was extracted with 10mL ethyl acetate. After 5 min centrifugation (3500 rev min), the samples were frozen at  $-28^\circ\text{C}$ , and the organic layer was collected and evaporated under vacuum at  $45^\circ\text{C}$ . The drug residue was reconstituted in 100mL mobile phase and injected into the HPLC system for analysis. Concentration of Midazolam in the samples was determined using an HPLC apparatus (Shimadzu SCL-10A VP, Japan) equipped with a 15 cm X 4mm i.d. VP-ODS C18 column (Shimadzu, 5 $\mu\text{m}$ , Japan), C18 precolumn and Shimadzu SPD- 10A VP-UV detector. Calibration curves of Midazolam were prepared with plasma and CSF mixed with known amounts of the drug, utilizing its HPLC peak area ratios to the internal standard. The mean best fit linear regression equation was used to estimate the concentrations of Midazolam at different time intervals. The conditions of the HPLC method were as follows: The mobile phase was prepared with 0.065 M ammonium acetate buffer, adjusted to pH  $5.5 \pm 0.02$  with orthophosphoric acid: methanol: acetonitrile (30:20:50, v/v/v), flow rate was set at  $1\text{ mL min}^{-1}$ ; volume of the

injected sample was 10 $\mu\text{L}$ ; detector wavelength set at 220 nm. Under these conditions the retention times were 3.2 min for midazolam and 5.6 min for Montelukast.<sup>16</sup>

### Assay Validation

The mean calibration curve for midazolam ( $Y = 28932 X + 1198.1$ ) ( $n > 6$ ) was linear within the concentration range between 2- 30  $\mu\text{g/ml}$  with  $r^2$  values of 0.9990. The mean extraction recovery of midazolam from plasma or CSF was more than 101.45% and 99.15%. The intra-assay coefficients of variation (CV) for the standard of the calibration curve ( $n > 4$ ) ranged from 0.36 to 7.1%. The values for the inter-assay CV for the same concentrations ( $n > 6$ ) were between 1.9 and 4.3 %. The inter and intraday accuracy (expressed as the percentage error of the determined concentration compared with the theoretical concentration) was lower than 1.6 and 8.3 %, respectively. The percentage plasma or CSF recovery of midazolam and internal standard as well as the inter and intra-assay CV were confirmed to the requirements for validation.<sup>41</sup>

### Data Analysis

The area under the plasma concentration–time curve  $AUC_{\text{plasma}}$  value and CSF concentration–time curve  $AUC_{\text{CSF}}$  values were calculated using the trapezoidal rule. The degree of microsphere dispersion targeting to CSF after intranasal administration can be evaluated by the drug targeting index (DTI) which can be described as the ratio of the value of  $AUC_{\text{CSF}} / AUC_{\text{plasma}}$  following intranasal (i.n) administration to that following intravenous injection.

The higher the DTI is, the further degree of microsphere dispersion targeting to CSF can be expected after intranasal administration.

$$DTI = \frac{(AUC_{\text{CSF}}/AUC_{\text{plasma}}) \text{ i.n}}{(AUC_{\text{CSF}}/AUC_{\text{plasma}}) \text{ i.v}}$$

In order to more clearly understand the direct transfer of microsphere dispersion between nose and brain after i.n. administration, drug targeting percentage (DTP) was introduced, the equations are as follows:

$$Bx = (Bi.v / Pi.v) \times Pi.n$$

$$DTP\% = \frac{(Bi.n - Bx) \times 100\%}{Bi.n}$$

Where, Bx =  $AUC_{\text{CSF}}$  fraction contributed by systemic circulation through the blood–brain barrier (BBB) following intranasal delivery, Bi.v =  $AUC_{\text{CSF}}$  following intravenous administration, Pi.v =  $AUC_{\text{plasma}}$  after intravenous administration, Bi.n =  $AUC_{\text{CSF}}$  flowing intranasal delivery, Pi.n =  $AUC_{\text{plasma}}$  by intranasal administration. Statistical differences between i.n. and i.v. administration were concluded.<sup>42</sup>

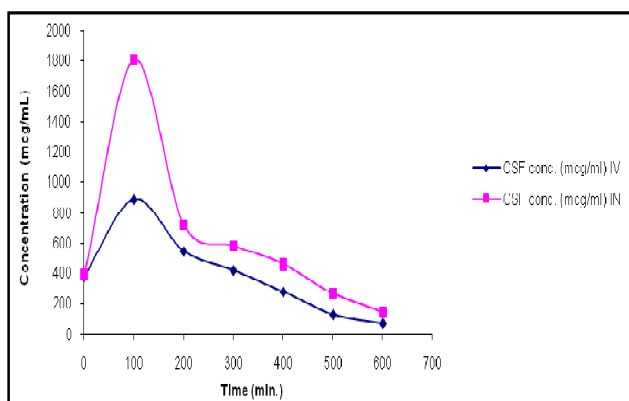


**RESULTS**

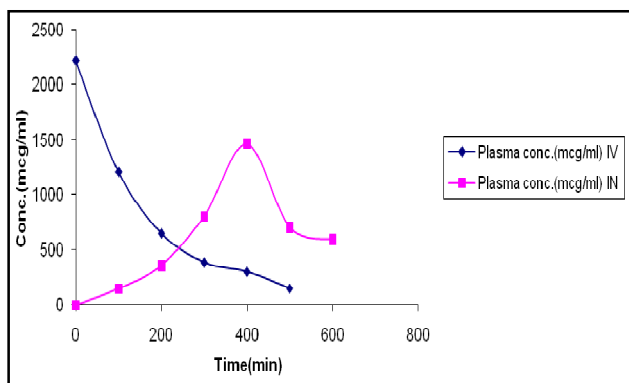
**Brain-Targeting Study**

To determine whether or not midazolam loaded microspheres is transported from the nasal cavity into the CSF via the olfactory neurons, it was administered intranasally and drug solution was administered intravenously in the same set of rats. The calculated concentrations of midazolam in CSF after intranasal and intravenous administration are shown in Figure 1 & 2. It was found that the midazolam microsphere levels in CSF of nasal route were significantly higher than those obtained after i.v. injection of midazolam solution.

As seen in Table 1, DTI was 2.50, greater than 1, and DTP was 62.52%, we can observe a measurable degree of drug loaded microsphere targeting to CSF following intranasal administration.



**Figure 1:** CSF concentration time profile of Midazolam after intranasal and intravenous administrations at 0.2-0.5 mg/kg dose in rats



**Figure 2:** Plasma concentration time profile of Midazolam after intranasal and intravenous administrations at 0.2-0.5 mg/kg dose in rats.

**Table 1:** DTI and DTP of Midazolam loaded microspheres by intranasal administration (mean ± SD, n = 5)

| Administration | AUC <sub>CSF</sub> (mcg/ml) | AUC <sub>plasma</sub> (mcg/ml) | AUC <sub>CSF</sub> /AUC <sub>plasma</sub> | DTI  | DTP (%) |
|----------------|-----------------------------|--------------------------------|---|------|---------|
| i.n.           | 5.423                       | 2.654                          | 2.04                                      | 2.50 | 62.52   |
| i.v.           | 4.845                       | 6.325                          | 0.817                                     |      |         |

**DISCUSSION**

The BBB presents a challenge for the treatment of several CNS disorders, since many drugs are not able to effectively transverse the vascular endothelium to reach their target in the parenchyma. It is therefore thought that treatment of these diseases could be improved if drug delivery across the BBB could be increased. Additionally, there is marked variability in patient response to drugs that have been in use for epilepsy for several years. It is well established that a formidable membranous barrier severely hampers the access of many drugs into the brain and central nervous system. Three elements underlie the BBB function: a physical barrier comprised of tight junctions, who form a tight seal to intercellular diffusion; the cells themselves, which exhibit a low rate of endocytosis; and a metabolic barrier, consisting of specific membrane transports expressed by endothelial cells. The olfactory route of drug administration provides a route of entry to the brain that circumvents the BBB, and this neuronal connection constitutes a direct pathway to the brain. Rats have been used widely for nasal drug delivery studies. Rats are classified as macrosomatic i.e. the olfactory epithelium occupies a large area (50% for the rat) of the total nasal epithelium. However, man is classified as microsomatic with a total olfactory area of approximately 3%. Further, in man, the olfactory region is located in the roof of the cavity, while in rats the olfactory area is spread throughout the whole cavity, as it is in many other common animal models. It is important to take these anatomical differences between species under consideration when results are interpreted and compared. High brain midazolam concentration was attained following intranasal administration. This was followed by a gradual decline in brain drug concentration. *In vivo* studies indicated higher concentration of Midazolam microsphere dispersion in CSF when administered intranasally and this contributed towards a good release. A drug targeting index value more than one, indicates direct transport to brain. Hence, a formulation was developed for better drug distribution into the brain, increasing the therapeutic index, reducing side effects with decreased dose for Midazolam.<sup>43-46</sup>

**CONCLUSION**

The drug loaded microspheres gained higher concentration in CSF at each sampling time following intranasal delivery, resulting in higher AUC<sub>CSF</sub> value, which proved that microspheres are a more suitable formulation for midazolam to be transported into CNS. It can be explained as follows: chitosan-gelatin microspheres can bind strongly to negatively charged materials such as cell surfaces and mucus. Mucus contains mucins that have different chemical constitutions, but some contain significant proportions of sialic acid. At physiological pH, sialic acid carries a net negative charge and, as a consequence, mucin and chitosan can demonstrate strong electrostatic interaction

in solution. It is evident that chitosan-gelatin behaves as a mucoadhesive material increasing significantly the half-time of clearance.

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