Gellan Gum Based Microparticles of Metoclopromide Hydrochloride for Intranasal Delivery: Development and Evaluation

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The purpose of this study was to develop nasal microparticles of metoclopromide employing gellan gum as a polymer by spray drying method. This method of microencapsulation is particularly less dependent on the solubility characteristics of the drug and polymer and is simple, reproducible, and easy to scale up. The microparticles were evaluated for characteristics like particle size, incorporation efficiency, swelling ability, zeta potential, mucoadhesion, thermal analysis, X-ray diffraction (XRD) study and *in vitro* drug release. The microparticles so prepared had irregular shape and smooth but distorted surface morphology. They were negatively charged. The particle size ranged from 9.38 to 10.67 μm. Differential scanning calorimetry (DSC) studies revealed that metoclopromide was molecularly dispersed inside the microparticles. The swelling was increased with increase in amount of polymer. The release of drug from microparticles was moderately sustained without lag time and attributed to formation of hydrogel; ionically cross linked hydrogel was hypothesized. The formulation was found to be non toxic to nasal tissue. These *in vitro* preliminary results show that spray dried microparticles based on gellan gum could be suitable nasal delivery system for the administration of metoclopromide.

Key words gellan gum; microparticle; spray drying; metoclopromide hydrochloride

Microparticles, in general are investigated for targeted and controlled release drug delivery. A polymeric device allows for slow, controlled and predictable drug release over a period of time and hence reduces the overall amount of drug needed.[1] In nasal drug delivery, coupling of bioadhesive properties to microparticles is of great importance because of additional advantages like intimate contact with the mucus layer, reduction in frequency of drug administration due to the reduction in mucocilliary clearance of drug delivery system adhering to nasal mucosa. The aim of this work was to develop bioadhesive microparticles for nasal delivery of metoclopromide, a potent antiemetic effective in the treatment of nausea and vomiting associated with cancer therapy, pregnancy, migraine *etc.* Gellan gum is an anionic heteropolysaccharide produced by aerobic fermentation of the bacterium sphingomonas elodea (formerly known as pseudomonas elodea).[2] The chemical structure made up of repeating units of a tetrasaccharide is composed of β-D-glucose, β-D-glucuronic acid and α-L-rhamnose residues in the molar ratio of 2 : 1 : 1. Because of its ability to form strong clear gels at physiological ion concentration, it can provide a longer contact time for drug transport across the nasal membrane, before the formulation is cleared by the mucocilliary clearance mechanism single. These features along with biodegradability, biocompatibility and absence of toxicity of the polymer, attracted widespread interest in gellan gum as drug carrier. Microparticles were produced by spray drying. The drug encapsulation efficiency, size and morphology, zeta potential, swelling ability and bioadhesive properties were studied.

Experimental

Materials Gellan Gum (Deacetylated): Burzin and Leons, CPKelco division of the Monsanto Company (U.S.A.), metoclopromide hydrochloride (Ipca Laboratory, India). All other chemicals were of analytical grade and used without any purification.

Preparation of Microparticles Metoclopromide hydrochloride loaded microparticles were prepared using gellan gum in different drug to polymer ratios. Their compositions were reported in Table 1. Gellan gum was added in double distilled water and dissolved by heating to 100 °C with moderate stirring. Metoclopromide hydrochloride was added to above polymer solution after cooling to about 40 °C. Microparticles were obtained by spraying the feed with spray drier (LU222, Labultima, India) using a standard 0.7 mm nozzle. The solution was fed to nozzle with a peristaltic pump, atomized by the force of compressed air and blown together with heated air to the drying chamber where the solvent in the droplets was evaporated. The dried microparticles were harvested from the collector. The process conditions of the spray drying were inlet temperature 110—120 °C; outlet temperature 80—90 °C; feed rate of 4—5 ml/min; spray pressure 2 kg/cm^2. The total volume of solution used for preparation of each batch was 200 ml.

Characterization of Microparticles. Scanning Electron Microscopy The morphology of microparticles was examined by scanning election microscopy. A small amount of powder was spread on an aluminum stub, which was placed after gold sputtering in SEM chamber (JSM 6390®, U.S.A.). Photographs were taken at an acceleration voltages of 20 kV electron beam.

Particle Size Analysis A microscopic image analysis technique for determination of particle size was applied. The morphology and particle sizes were determined in a Motic DMW2-223 digital microscope (Motic Instruments Inc., Canada) equipped with a 1/3″CCD camera imaging accessory and computer controlled image analysis software (Motic images 2000, 1.3 version). The microparticles were dispersed on a microscope slide. A microscopical field was scanned by video camera. The images of the scanned field are analyzed by the software. In all measurements at least 100 particles were examined.

Percentage Production Yield and Incorporation Efficiency The percentage of production yield was calculated as the weight of final product obtained after drying, with respect to the total amount of dry starting materials. Metoclopromide hydrochloride in microparticles of each formulation was extracted in phosphate buffer (pH 6.6). The concentration of metoclopromide hydrochloride was determined using a UV-spectrophotometer, at a

Table 1. Formula for Different Batches of Metoclopromide Hydrochloride Loaded Gellan Gum (GG) Microparticles

Formulation code	Metoclopromide hydrochloride : GG	Metoclopromide hydrochloride (% w/w)	GG (% w/w)
GGM 1	1 : 1	50	50
GGM 2	1 : 2	33.3	66.7
GGM 3	1 : 3	25	75
GGM 4	1 : 4	20	80
GGM 5	1 : 5	16.6	83.4

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wavelength 307 nm (UV-spectrophotometer-1700, Shimadzu, Kyoto, Japan). The percent incorporation efficiency was calculated from actual drug content in weighed quantity (10 mg) of powder of microparticles (Ma) and theoretical amount of drug in microparticles calculated from the quantity added in the fabrication process (Mt) as the following equation.[3)]

$$\% \text{ incorporation efficiency} = \frac{\text{Ma}}{\text{Mt}} \times 100 \quad (1)$$

Zeta Potential Study The microparticles were dispersed in distilled water. Then this dispersion was filled in zeta cell and placed in the Zeta Sizer (Nano ZS, Malvern Instruments, U.K.).

***In Vitro* Swelling Studies** The ability of the microparticles to swell in phosphate buffer pH 6.6 was determined by swelling them to their equilibrium. Accurately weighed amount of microparticles (10 mg) were placed on Millipore filter NY 11, 0.22 μm placed on a Franz diffusion cell (16 ml capacity) filled with phosphate buffer pH 6.6 and kept for 3.5 min.[4)]

The following formula was used for calculation of degree of swelling:

$$\alpha = (\text{Ws} - \text{Wo})/\text{Ws} \quad (2)$$

where α=degree of swelling, Wo=initial weight of microparticles, Ws=weight of microparticles after swelling.

***In Vitro* Mucoadhesion Studies** A freshly cut 2 cm^2 piece of sheep nasal mucosa was obtained and cleaned by washing with isotonic saline solution. One hundred milligrams of microparticles were placed on mucosal surface was fixed over polyethylene support. The microparticles were brought in contact with simulated nasal electrolytes (SNES: aqueous solution containing 8.77 mg/ml NaCl, 2.98 mg/ml KCl and 0.59 mg/ml $CaCl_2$ per liter). The nasal mucosa was washed with phosphate buffer (pH 6.6) at the rate of 5 ml/min using a peristaltic pump. Sixty minutes after application of microparticles, the concentration of drug in collected perfusate was determined spectrophotometrically. The amount of microparticles corresponding to the drug amount in perfusate was calculated. The amount of adhered microparticles was estimated from the difference between the applied microparticles amount and the amount of flowed microparticles. The percent mucoadhesion was determined using following equation.[5)]

$$\% \text{ mucoadhesion} = \frac{\text{amount of drug in washout liquid}}{\text{actual amount of drug in applied microparticles}} \times 100 \quad (3)$$

Thermal Analysis Differential scanning calorimetry (DSC) was performed on metoclopromide hydrochloride (A), blank microparticles (B), and metoclopromide hydrochloride loaded microparticles (C). DSC measurements were done on a Mettler Toledo DSC 822c. The thermograms were obtained at a scanning rate of 10 °C/min over a temperature range of 40 to 3000 °C under an inert atmosphere flushed with nitrogen at a rate of 20 ml/min.

X-Ray Diffraction (XRD) Studies The crystallinity of metoclopromide and metoclopromide loaded microparticles was evaluated by XRD pattern recorded for metoclopromide, bland microparticles and metoclopromide loaded microparticles using an X-ray diffractometer (Brucker Axs, 08 Advance) scanning was done upto 2θ of 70°.

***In Vitro* Drug Release Study** *In vitro* drug release test of the microparticles was performed using Franz diffusion cell with dialysis membrane (cut off mol. weight, 12000). The membrane was equilibrated carefully with phosphate buffer (pH 6.6) before dispersing the sample equivalent to 15 mg of drug onto the donor compartment. The donor compartment contained 3 ml of SNES and receiver compartment was filled with phosphate buffer solution pH 6.6 that was within the pH range in nasal cavity and maintained at 37±0.5 °C.[6)] Samples were periodically withdrawn from the receptor compartment, replaced with the same amount of fresh buffer solution, and assayed by a spectrophotometer at 307 nm.

Histopathological Evaluation of Mucosa The histopathological evaluation of tissue incubated in phosphate buffer (pH 6.8) for 6 h after collection was compared with tissue incubated in diffusion chamber with formulation. Tissue was fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Sections were cut on glass slides and stained with hematoxylin and eosin. Sections were examined under a light microscope to detect and damage to the tissue.[7)]

Results and Discussion

Five formulations of metoclopromide loaded microparticles were prepared by spray drying method. Spray drying is a good technique for the preparation of microparticles. It is one step process, easy and rapid, as it combines drying of the feed and embedding of the drug into a one step operation. All gellan gum microparticles were spherical with smooth surfaces (Fig. 1). These microparticles had no hole or rupture on the surface, such morphology would result in slow clearance and good deposition pattern in nasal cavity.[8)] Particle size of microparticles is one of the most important characteristics as a nasal drug delivery system. The mean particle size of microparticles ranged from 9.38 to 10.67 μm suitable for nasal administration (Table 2). It has been suggested that 4 μm is sufficient particle size for intranasally administered drug.[9)] Particles smaller than 1 μm pass the nasal cavities with the inspired air, whereas particles larger than 10 μm deposits at the anterior parts of the nose and thus avoids ciliated absorption areas.[10)] The yield of production was relatively low. Similar yields were already reported for this method.[11)] The loss of material during spray drying process is mostly due to powder adhering to the cyclone walls.[12)] In this work low values of yield could also be attributed to the small

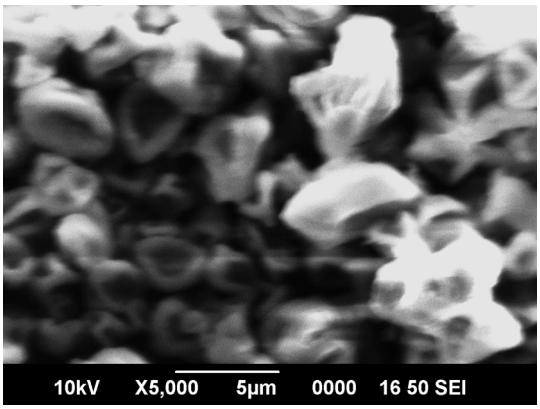


Fig. 1. Scanning Electron Micrograph of Metoclopromide Hydrochloride Loaded Microparticles

Table 2. Characteristics of Prepared Metoclopromide Hydrochloride Loaded Microparticles

Formulation code	Production yield (%)	Incorporation efficiency (%±S.D.)[a)]	Mean particle size (μm±S.D.)[a)]	*In vitro* mucoadhesion (%±S.D.)[a)]	Degree of swelling (α)
GGM 1	22.95	86.93±1.35	10.1±1.39	85.47±0.30	0.81
GGM 2	26.72	89.98±2.57	9.61±1.21	86.25±0.36	0.89
GGM 3	25.50	94.03±2.05	9.73±1.28	87.39±0.16	0.98
GGM 4	28.36	91.66±1.39	9.38±1.15	87.70±1.20	1.08
GGM 5	31.40	94.28±1.68	10.67±1.52	88.66±1.36	1.17

a) n=3.

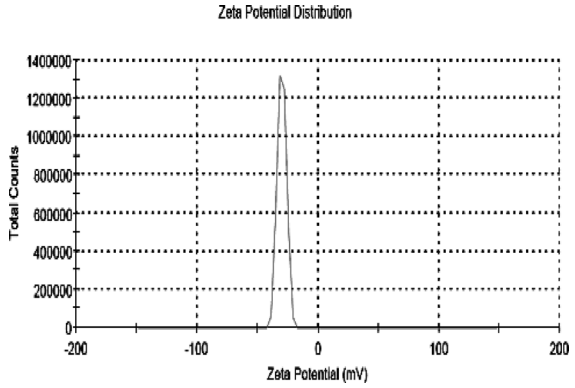


Fig. 2. Zeta Potential Distribution Curve of Metoclopromide Hydrochloride Loaded Gellan Gum Microparticles (GGM-3)

amount of materials processed in each batch as well as to the loss of the smallest and highest particles through the exhaust of the spray dryer apparatus as it is not equipped with a trap to recover the lighter and smaller particles. The determination of drug content shows good uniformity. In addition, they were close to their percentages of theoretical content which were 7.5 to 2.5% for drug to polymer ratio 1 : 1 to 1 : 5. All microparticles had good incorporation efficiency between 86% and 97% (Table 2). These results indicate very good reproducibility of the spray drying method. All the microparticles prepared were negatively charged; indicating the presence of gellan gum at the surface of all microparticles formed (Fig. 2). Studies have shown that polymers with charge density can serve as good mucoadhesive agents. It has also been reported that poly anion polymers are more effective bioadhesive than poly cations or non ionic polymers.[13,14] *In vitro* swelling properties of the spray dried microparticles were expressed as degree of swelling estimated by use of Eq. 3. The maximum swelling (degree of swelling) was observed with microparticles containing highest concentration of gellan gum (Table 2). The swelling study was an important attribute of studying clearance of drug from nasal cavity. It was suggested that administration of microparticles lowers clearance of the microparticles systems which may be probably due to the fact that the microparticles undergo a process of taking up water and swelling, which results in polymer/mucus mixture leading to reduced mucocilliary clearance.[10,15] Mucoadhesion studies were carried out to ensure the adhesion of the formulation to the mucosa for a prolonged period of time at the site of absorption. Percent mucoadhesion was increased with increase in polymer concentration (Table 2). In mucoadhesion process, both weak and strong interactions (*i.e.* van der Waals interaction, hydrogen bonding and ionic bonding) can develop between certain types of functional groups on the polymer (*e.g.* hydroxyl or carboxyl groups) and glycoprotein network of the mucus layer or the glycoprotein chains attached to the epithelial cells for example in the nose. In order to develop strong adhesive bonds, the establishment of strong intimate molecular contact between the polymer and glycoprotein chain is essential.[16] Thus an important requirement for polymer is their ability to swell by absorbing water (here from mucus layer in the nasal cavity) thereby forming a gel like layer in which the interpenetration of polymers and glycoprotein chains can take place and the binding can form rapidly. Gellan gum form gels in the presence of mono and divalent cations, here with physiological cations from nasal electrolytes have a key role in mucoadhesion strength.[17] All the prepared microparticles showed high degree of mucoadhesion. The swelling index is shown in terms of fluid intake capacity depends upon polymer content. The rapid fluid uptake from mucus layer enabling the polymer chain to penetrate mucin network and establish adhesive bond has a key role in mucoadhesion. Linear relationship has observed between polymer concentration, swelling index and mucoadhesion. Gellan gum microparticles after uptake of fluid transform into gel matrix and as swelling behaviour plays an important role in the *in situ* gel formation on nasal mucosa hence retard the release rate. DSC thermograms of (A) pure metoclopromide hydrochloride, (B) blank microparticles and (C) metoclopromide hydrochloride loaded microparticles (GGM 3) are presented in Fig. 3. The blank microparticles has shown an exothermic peak at 249 °C, indicating the decomposition of the polymer without melting. For pure metoclopromide, an endothermic peak was observed at 182 °C due to melting of the drug, but this has not appeared in the metoclopromide-loaded microparticles, indicating an amorphous dispersion of the drug into the polymer matrix.[18] The X-ray diffraction spectra recorded for pure (A) metoclopromide hydrochloride, (B) gellan gum blank microparticles, (C) drug loaded microparticles are presented in Fig. 4. These studies are useful to investigate the crystallinity of drug in the polymeric microparticles. Metoclopromide hydrochloride has shown characteristic intense peaks between 2θ of 10 and 60 but in case of blank microparticles and drug loaded microparticles no intense peaks were observed between 2θ of 10 and 60, indicating amorphous nature of drug after entrapment into the gellan gum microparticles by spray drying. Figure 5 shows the drug release profiles from various formulations of microparticles. Microparticles prepared with gellan gum moderately sustained the drug release to 5 h without any lag time. The rate and extent of metoclopromide release from microparticles significantly decreased with an in-

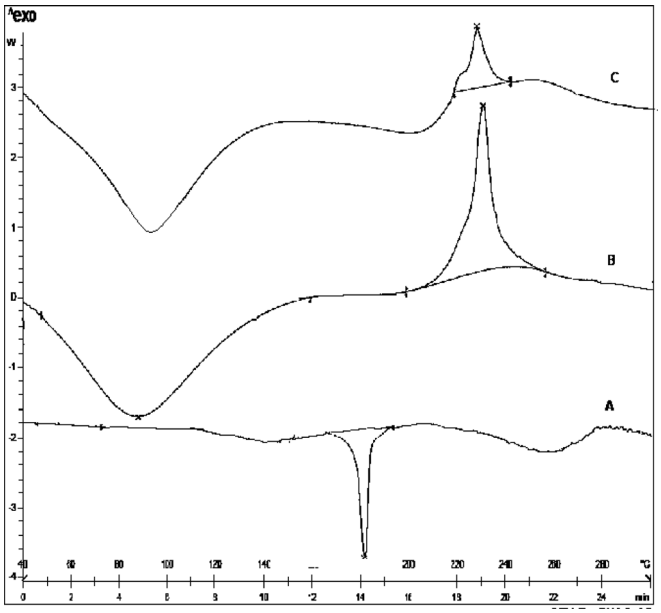


Fig. 3. DSC Spectra of Metoclopromide Hydrochloride (A), Gellan Gum Blank Microparticles (B) and Metoclopromide Hydrochloride Loaded Microparticles (C)

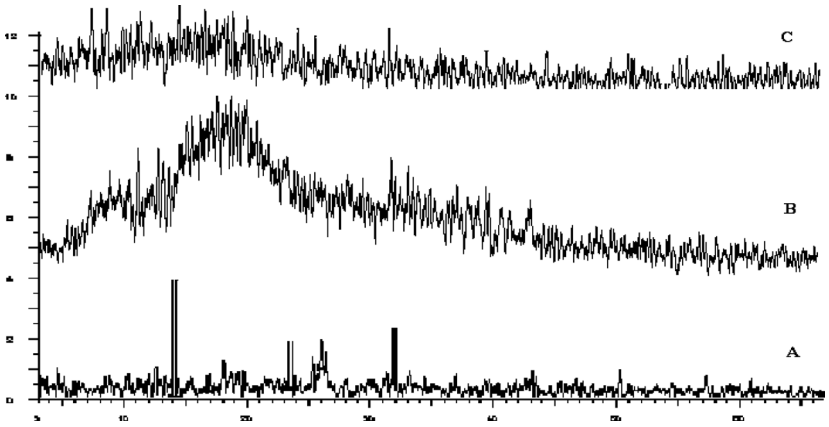


Fig. 4. X-Ray Diffractogarm of Metoclopromide Hydrochloride (A), Gellan Gum Blank Microparticles (B) and Metoclopromide Hydrochloride Loaded Microparticles (C)

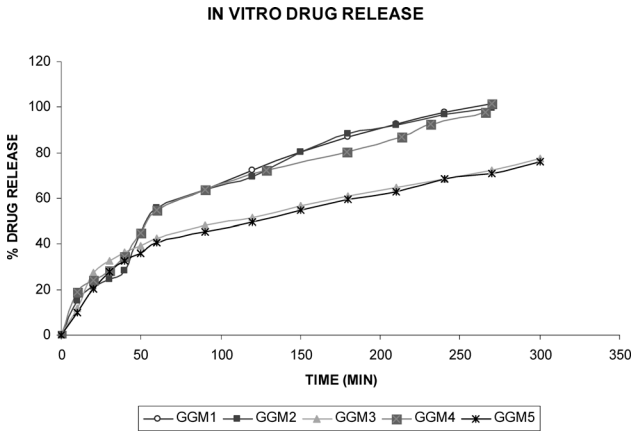
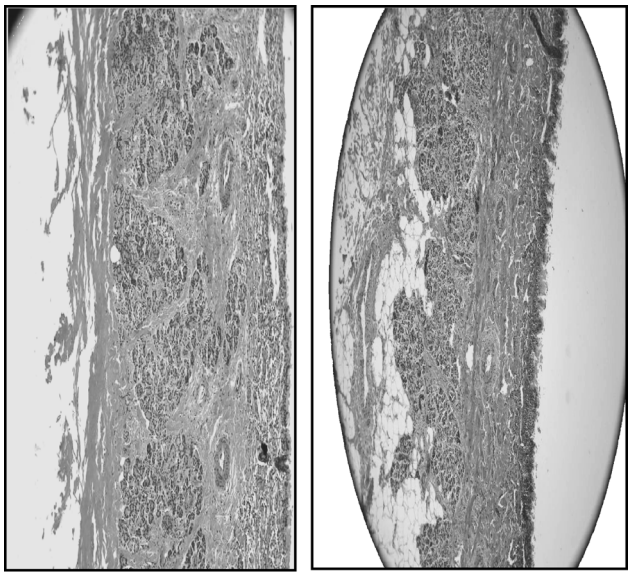


Fig. 5. *In Vitro* Drug Release of Metoclopromide Hydrochloride from Microparticles



(a) (b)

Fig. 6. Light Photomicrograph of the Nasal Mucosa Normal Mucosa (a) and Gellan Gum Microparticles Treated Mucosa (b)

crease in gellan concentration. All the formulations showed dissimilar gelation characteristic depending upon polymer concentration. Formulation GGM1, GGM2 showed weakest gelation while GGM3, GGM4 moderately stiff gelation and GGM5 showed very stiff gelation. The jumping release of GGM1, GGM2 were rapid due to incomplete (weak) gel formation as compared to GGM3 and GGM5 having stiff gelation property. Despite of moderate stiff gel formation GGM4 showing rapid release, the reason behind was unclear. The drug release from the microparticles was at slower rate due to ionic gelation (cross linking of gellan with cations in SNES). On the basis of results of characterization of microparticles and *in vitro* drug release study GGM3 was selected as optimum formulation. To investigate the drug release mechanism, the release data of GGM3 were analyzed using models representing zero order, first-order and Higuchi's square root of time. The examination of coefficient of determination vales indicated that the drug release from the microparticles followed the diffusion control mechanism since the R^2 values for Higuchi's square root of time (0.9876) was always higher as compared to zero-order (0.8556) and first order (0.9741). A more stringent test was used to distinguish between the mechanisms of drug release; the data were fitted to the Peppas exponential model.[19] $Mt/M_\infty = Kt^n$, where Mt/M_∞ is the fraction of drug released after time t, k is the kinetic constant and n is the release exponent which characterizes the drug transport mechanism. The n value was 0.4897 indicating that GGM3 formulation followed the anomalous (non-Fickian) transport mechanism of drug release. The light micrograph was taken of nasal mucosa following incubation with microparticles formulations for more than 6 h. Examination of tissue showed ciliated respiratory epithelium and normal goblet cell appearance. None of the severe signs such as appearance of epithelial necrosis, sloughing of epithelial cells was detected (Fig. 6). In conclusion, the results of our present study clearly indicated promising potentials of gellan gum microspheres for delivering drug intranasally and could be viewed as attention with to conventional dosage form, could be prepared by a conventional spray drying method. After getting contact with the nasal mucosa, microspheres formulations are believed to form viscous gel by withdrawing water from the nasal mucosa and interaction with cations present in nasal secretions. The resultant gel formation decreases the ciliary clearance rate and as a consequence the

residence time of the formulation at the nasal mucosa is prolonged. The mucoadhesive properties of microspheres were attributed to spontaneous gel formation on nasal mucosa. Gellan gum is a biocompatible polymer, it does not cause any deleterious effect or toxic response in the nasal mucosal cavity even if used for prolonged periods was evaluated by histopathological studies. However extensive pharmacokinetics and pharmocodynamic studies are required to establish a correlation, if any, before establishing nasal delivery as an alternative.

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