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Research Article

COLLOIDAL DISPERSIONS AS A POTENTIAL NASAL DRUG DELIVERY SYSTEM FOR ONDANSETRON HYDROCHLORIDE – *IN VITRO* AND *IN VIVO* PROPERTIES

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ABSTRACT

The influence of the particle size of colloidal dispersion on nasal absorption and the localization of Ondansetron hydrochloride in the dispersion have been investigated in rats. Using scanning electron microscopy, it was found that the freeze-drying process, which was used to load Ondansetron hydrochloride into the colloidal dispersions, had a significant impact on the integrity and surface properties of the nasal epithelium in rats. The optimized formulation was analyzed for the sterility by (total bacterial count) and stability study, which proved that the formulation does not contain bacteria and fungi, found to be stable up to 3 years. Pharmacokinetics study was carried out in Wistar rats for C_{max}, t_{max} and AUC, which was found to be 2 h, 0.5 h and 0-2 h respectively. It has been concluded that the tendency of the particle size influence the kinetics of the Ondansetron effect curve.

Keywords: Nasal spray; Colloidal dispersion; Ondansetron; kinetic study, stability study.

INTRODUCTION

Systemic delivery of drugs via the nasal route as an alternative, has many advantages, it is usually limited by the specific nasal morphological and physiological characteristics [1]. One of the most important disadvantages is a nasal mucociliary clearance that limits the time allowed for drug absorption to occur. Thus, the mucous adhesive colloidal dispersion has been developed in order to decrease the effect of mucociliary clearance [2]. Colloidal dispersions also exert a direct effect on the mucosa resulting in the opening of tight junctions between the epithelial cells. Colloidal dispersions are an alternative process that is cheaper and faster than all other techniques to prepare nasal particles from mucoadhesive polymers.

β-cyclodextrin derivatives are biodegradable, biocompatible, bioadhesives properties of colloidal dispersion due to its hydrophilic. It is a polymer of choice, because it enhances the nasal absorption of low molecular weight molecules. Ondansetron is a serotonin (5-hydroxytryptimine) subtype 3 (5-HT) receptor antagonists used in the management of nausea and vomiting [3]. Oral administration of Ondansetron hydrochloride is well with bioavailability of 60% with half life of 3.1h [4]. The aim of this work is to evaluate the optimized formulation [5] which was developed with trails of Ondansetron hydrochloride for nasal absorption and the localization of the drug in the dispersions has been investigated in rats [6].

MATERIALS AND METHODS

Ondansetron hydrochloride USP was obtained as a gift sample from Secure healthcare Chennai, Pvt. Ltd. β-cyclodextrin and hydroxyl propyl β-cyclodextrin were purchased from Signet chemicals, Mumbai. All animal experiments adhered to the principles for biomedical research involving animals developed by council for international organizations of medical sciences. Wistar rats weighing 180-200g were received from the central animal house (CAH), Annamalai University after getting approval from the institutional animal ethical committee (IAEC no. 160/1999/CPCSEA/829). All surgical procedure was performed under anesthesia with ether and an intraperitoneal injection of 0.2ml/kg Xylocaine.

Characterization of colloidal dispersions

Morphological studies of colloidal dispersions

The structural feature of forming a colloidal dispersion after lyophilization was observed by SEM6700F scanning electron microscopy (SEM, JEOC, Japan). The sample was fixed on a metal stub with conductive carbon type and was coated with gold under vacuum by an ion sputter (JFC-1600, JEOL, Japan).

Particle size

It is important that the size of the colloidal dispersion for nasal delivery should above 10µ, if the particle size is less than 10µ, this size would able to be carried by the air stream down into the trachea bronchial region [7]. Larger particles will remain deposited in the anterior insulated portion of the nose and thus a size range of 40-60µ was chosen for colloidal dispersion, which is observed by the particle size analyzer (Malvaniser Particle size analyzer, UK). The particle size was expressed as mean volume diameter.

Total microbial count

It was carried out by using the filter paper disc diffusion method [7], employing 24h culture of the *Salmonella Sp (ATCC* 35664*),* Escherichia *coli* (*ATCC* 35218), *Staphylococcus Sp* (*ATCC* 43957) *and Pseudomonas Sp* (*ATCC* 15915). The test organisms were seeded in sterile nutrient agar mediums by uniformly mixing one ml of inoculum with 20 ml sterile melted nutrient agar cooled to 48-50ºC in a sterile petridish. The medium was allowed to solidify. The optimized formulation with a concentration of 5%v/v was prepared in a sterile dimethyl formamide (DMF) as solvent. The optimized formulation and Ciprofloxacin (100µg/ml) standard drug as well as blank was impregnated in Whatman filter paper disc and placed on solidified medium in the petridish and the petridish was left undisturbed for two hours at room temperature. The petridish was then incubated at 37ºC for 24h and zone of inhibition was measured.

Sterile yeast nitrogen base (Himedia) with 2% agar was inoculated with *Aspergillus niger(ATCC* 1015*), Microsperum gypserm* (*ATCC* 24102), *Candida albicans* (*ATCC* 10231) by rotating swab (soaked in standard inoculum suspension) over the surface of the media. The solvent DMF was used as a control. The optimized formulation and Flucanazole (100mµg/ml) standard drug as well as blank was impregnated in Whatman filter paper disc and placed on solidified medium in the petridish and the petridish was left undisturbed for two hours at room temperature. The petridish was then incubated at 37ºC for 24h and zone of inhibition was measured. All the operations were carried out in a laminar air flow chamber. The results were recorded as colony forming units per gram of material.

Pharmacokinetic studies

Rats were divided into three groups of six animals each for nasal, intravenous and oral. Each group comprised of six samples for each time point. Blood samples were withdrawn from the orbital plexus of rat and collected in an EDTA coated vacuette tube and centrifuged at 3000 RPM for about 10 minutes to separate the serum for analysis. The brain was removed immediately and weighed. One gram of the brain sample was stored in 10% formalin solution at -70^{0}C until analyzed.

For intravenous administration of Ondansetron hydrochloride 8 mg was dissolved in 9 ml of normal saline, filtered through 0.2µ membrane filter, and injected through the jugular vein which was cannulated using polyethylene tube (0.76 mm i d × 1.22m od). Prior to oral administration, the animals were fasted overnight and kept under fasted conditions until 4h, after oral administration of a single dose of 4mg/ml Ondansetron hydrochloride. Blood samples were collected at 10, 15, 20, 25, 30, 45, 60 minutes and used for further studies.

The nasal absorption study of Ondansetron hydrochloride was conducted using an *in vivo* experimental technique [9]. A single dose of the constant volume of 2ml/kg was administered into one nostril using a micro syringe. Blood samples were collected at predetermined time intervals 10, 15, 20, 25, 30, 45, 60 minutes and used for further studies.

Pharmacokinetic parameters

The first order terminal elimination rate constant (ke) was estimated by linear regression from the points describing the elimination phase on a log linear plot. Half life ($t_{1/2}$) was derived from the rate constant ($t_{1/2}$= in (2)/ke). The maximum observed plasma concentration (C_{max}) and time taken to achieve this concentration (T_{max}) were obtained directly from curves. The AUC (0-2h) was calculated using the trapezoidal formula.

The pharmacokinetic variables from three dosage forms were compared with a one way ANOVA, which followed by a posterior testing with an unpaired t-test using the Bonferroni correction. A 'P' value of less than 0.05 was considered significant.

Estimation of Ondansetron hydrochloride

Plasma sample 5 ml was mixed with 100 µl of working IS [internal standard] solution and alkalized with 20 µl of 2M sodium hydroxide solution. The mixer was added to 50 ml of acetonitrile, vortexed for 10min, and centrifuged for 15 min. 5ml of organic phase was back extracted into 100 µl of 0.05% phosphoric acid by vortex mixing for 3 min and then 20 µl of the aqueous phase was injected directly into the above HPLC system.

Evaluation of nasal tissue morphology and mucociliary clearance

Several absorption enhancers were investigated in animal tissue for histological effects. PEG, PVP, MCC, β-cyclodextrin, hydroxyl propyl β-cyclodextrin has been investigated in animal tissue for the histological effects using 5-10min contact time with the nasal epithelium. The toxicity of nasal absorption enhancer has been estimated in recent years by measuring their effect on the mucociliary transport rate nasal morphology and ciliary beat frequency [10]. The potential toxicity of some absorption enhancer has been tested with the wistar rat model [11]. Measuring the effect of absorption enhancer by mucociliary transport rate, before and after application of the formulation.

Stability studies

Stability studies were conducted for the optimized formulation, which was subjected to the following long term stability studies at 30^{0}C±2^{0}C/65%RH±5%RH and accelerated stability studies at 40^{0}C±2^{0}C/75%RH±5%RH and analyzed for drug content, pH viscosity, clarity, crystallization, weight loss, (formulation stored under upward , inverted, horizontal, position leakage, water loss) and particle size. From the results obtained from accelerated stability studies the shelf life of the product was determined. The optimized formulation was placed at different temperature conditions as per ICH guidelines. Samples were withdrawn at regular intervals and the percentage of drug content was determined. Shelf life was estimated with upper and lower acceptance criteria based on assay at 40^{0}C±2^{0}C/75%RH±5%RH. Drug content was estimated by HPLC method, (Shimadzu, Japan) was estimated at regular intervals of time. The samples were checked for clarity for crystal growth, pH changes using a digital pH meter and viscosity by Viscometer (DV model).

RESULTS AND DISCUSSION

Morphological studies of colloidal dispersions

Examination of the structural feature of forming a colloidal dispersion after lyophilization was observed by scanning electron microscope. It reveals that the changes in the structure of the colloidal dispersion, which was analyzed before and after the stability studies. The structure of the colloidal dispersions was smooth surface and no obvious pores were observed on the surface which could be shown in Figure 1. The smooth surface was altered and a significant number of large holes could be seen on the surface spherical form dramatically changed after stability studies shown in Figure 2.

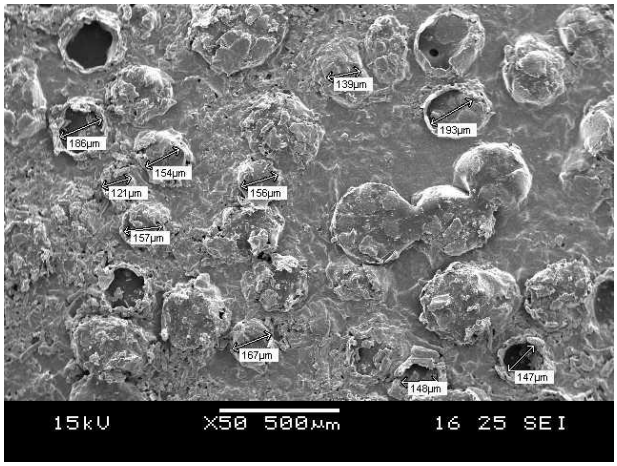

Figure 1: SEM photomicrograph of colloidal dispersion prior to stability study

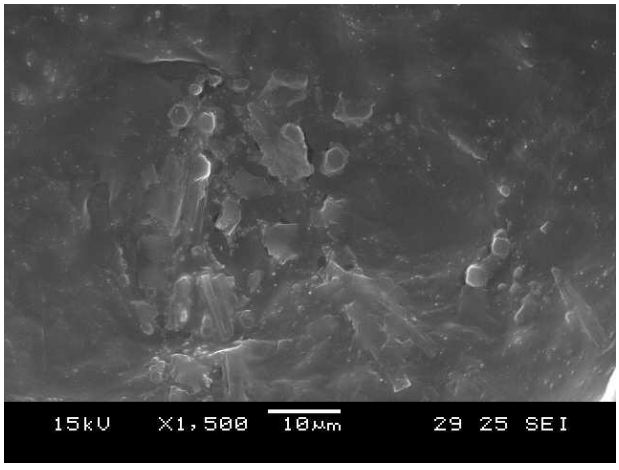

Figure 2: SEM photomicrograph of colloidal dispersion after the stability study

Particle size

Examination of the colloidal particles in particle size analyzer and it showed an increase in particle size after freeze drying, whereas larger size was not influenced (Table 1 and 2). The two different qualities of the colloidal dispersions, before and after the stability study revealed the significant difference between size and structure. Particle size and morphology are also important to minimize the feel

of grittiness and possibly irritating to nasal cavity. Too fine particles, below five microns may be inhaled into lungs and deposited in the nostrils so that to be avoided for nasal products.

Total microbial count

The results were recorded as colony forming units per gram of material. It was observed that, both the bacteria and fungi colony forming unit was found to be less than 5cfu/ml. Hence it is inferred that the formulation does not contain any microorganism. Benzalkonium chloride 0.02%, sodium chloride 0.09% plays vital roles in as antimicrobial agent.

***In vivo* pharmacokinetics results**

Based on the *in vitro* results from stability of Ondansetron hydrochloride nasal delivery systems, aqueous solution containing PEG, PVP, β-cyclodextrin, hydroxyl propyl β-cyclodextrin, was employed as biocompatible polymers for the *in vivo* pharmacokinetic study of Ondansetron hydrochloride nasal delivery system.

As shown in Table 1 Ondansetron hydrochloride was rapidly absorbed through nasal route. The mean C_{max} of Ondansetron by nasal route was found to be 2 times higher than that by oral route, which was statistically significant (P<0.5). The AUC (0-2h) after nasal administration was significantly higher than that after oral administration (P 0.01) and comparable to intravenous administration. The time to reach (C_{max}) after nasal administration was found faster than that by oral administration, even though, the difference was significant.

Table1: Pharmacokinetic parameters of Ondansetron hydrochloride after intravenous, intranasal and oral administration in rats.

Parameter	Nasal	Oral	I.V
N	5	5	5
C_{max}(ng/ml)	49.2±20.5	28.6±10.1	--
T_{max}	8.5±1.92	10.6±1.82	--
AUC_{0-2h}	53.9±8.2	22.5±10.6	49.04±7.8

Data were expressed as the mean ±SD. Statistically significant difference formed administration.(p*<0.05,**p<0.01)

Effects on nasal tissue morphology and mucociliary clearance

Immediately after the application of the formulation, no effect was observed on the mucociliary transport route. All these enhancers were reported more or less severe epithelium disruption. The effect of several enhancers was studied on the surface morphology of rat nasal mucosa. The optical microscope is used to characterize the gross structural and cellular changes. After the exposure of 5-10 min to the enhancer, micrographs of the nasal tissue were evaluated mainly for surface integrity, ciliary morphology, mucus, extracellular debris.

For each absorption enhancers investigated, the duration of exposure did not result in large differences in score numbers [score 0: No erythema, score 1: Very slight erythema, score 2: Well defined erythema, score 3: Moderate to severe erythema and score 4: Severe erythema (beet redness)].

The observation was performed at 30 min, 1 h, 3 h, 12 h & 24 h and the erythema was determined. From the data mucosa irritation index was calculated. As evident of Table 4, the order of increasing morphological damage caused by enhancer was PEG (2%) <<PVP (2%) << MCC (2%) << β-cyclodextrin (4%) << hydroxyl propyl β-cyclodextrin (5%), which was discussed in Table 2. PEG showed the lowest scoring, almost similar to the control phosphate buffer, but exposure to PVP, MCC, β-cyclodextrin and hydroxyl propyl β-cyclodextrin resulted in severe erosion of the nasal mucosa within 5-10 min (mean scores 4.6 to 5) indicating total loss of cells and ciliary identity as well as prevalence of extracellular debris. Even though β-cyclodextrin moderate irrational, it is temporally for 5-10 min and this polymer maintain pH, viscosity, stability of the formulation.

Table 2: *In vivo* effects of absorption enhancer on surface morphology of rat nasal mucosa

Absorption enhancer	Exposure time (min)	Mean scores of mucous irritation index (cat-I-III)
Phosphate buffer	10-May	1.3-1.6
PEG(polyethylene glycol)	10-May	1.5-1.8
PVP(polyvinyl pyrolidine)	10-May	1.9-2.8
MCC(micro crystalline cellulose)	10-May	2.7-3.3
β-cyclodextrin	10-May	3.0-3.5
Hydroxyl propyl β-cyclodextrin	10-May	4.6-5.0

Mean score of categories I (mucosal surface integrity), II (ciliary morphology) and III (mucus /extra cellular debris); optical microscopy of rat nasal mucosa were scored from 0 (No erythema), 1 (Very slight erythema), 2 (Well defined erythema), 3 (Moderate to severe erythema), 4 (Severe erythema (beet redness).

Stability studies

From the study it was revealed that the drug content was found to be 95.9% and 100.26% receptively, the pH of the formulation was found to be 5.4, the viscosity of the formulation was found to be 4.4cps, there was no crystal growth and weight loss (formulation stored under upward, inverted, horizontal position no leakage, water loss), which indicates that the formulation is stable in long and short term stability studies, since all the results are within the limit. The marginal particle size increases in average, possibly due to clumping of colloidal dispersions. This is probably due to stress, increasing temperature which precipitates the formulation shown in Figure 2.

Colloidal dispersion was placed at different temperature conditions and the samples were withdrawn at regular interval of time and the percentage of drug remaining was determined. The shelf life was estimated with upper and lower acceptance criteria based on assay at 40ºC±2ºC & 75%RH±5%RH. From the data it was found to be that the developed optimized formulation was stable up to 3.2 years. The optimized formulation was found to be more stable, when it is subjected to the short term and long term stability study.

Table 3: Long term stability study for optimized formulation

Months	Drug content%	Clarity	Viscosity	pH	Average size (µm)
0	102.9±1.1	-	4.42	5.61	8.9
3	100.4±0.5	-	4.43	5.55	9.9
6	99.5±2.6	-	4.40	5.44	10.0
9	99.2±0.6	-	4.43	5.25	10.2
12	99.1±1.1	-	4.3	5.21	10.1
	100.26	-	4.4	5.4	9.82

n=3; - no turbid forms.

Table 4: Accelerated stability study for optimized formulation

Months	Drug content%	clarity	Viscosity	pH	Average size (µm)
0	102.7±0.1	-	4.42	5.61	8.9
2	99.73±1.2	-	4.43	5.55	10.6
4	94.42±1.9	-	4.40	5.44	11.3
6	87.01±4.0	-	4.33	5.25	10.6
	95.9		4.4	5.4	10.35

n=3; - no turbid forms.

CONCLUSION

In conclusion as a novel nasal drug delivery system the colloidal dispersion should meet several requirements, especially low cyto and systemic toxicity. PEG, PVP, β-cyclodextrin, hydroxyl propyl β-cyclodextrin, used in this formulation is non toxic and biocompatible polymers. β-cyclodextrin even at high concentration not relevant, because the residence time in the nose is very limited. Optimized

formulation of Ondansetron hydrochloride nasal delivery system was sterile, posses better pharmacokinetics activity, stable for 3 years. It is feasible for nasal administration at low concentration of the drug and rapidly its antiemetic effect.

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