Available online on 25.04.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Pharmacokinetics and Pharmacodynamic evaluation of Nizatidine microspheres on experimental animals

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ABSTRACT

Mucoadhesive microspheres overcome the physiological adversities like short gastric residence time promoting the drug bioavailability. In the present investigation Nizatidine loaded Eudragit RS 100 microspheres were prepared by solvent evaporation technique using disparate drug polymer ratios with Eudragit RS 100 polymer and Nizatidine loaded chitosan microspheres were prepared by coacervation phase separation method. The prime objective was to enhance the absorption and bioavailability by prolonging the gastric residence time. Totally six formulations RES1- RES6 Eudragit RS 100 microspheres and six formulations RCP1 – RCP6 Eudragit RS100 microspheres were prepared and evaluated. RES3 and RCP3 showed nearly 90% drug release after 24 h. Hence the Nizatidine loaded Eudragit RS100 microspheres and Chitosan microspheres were further studied for the in vivo study for the suitability of the formulation. The bioavailability of nizatidine after oral ingestion is about 50% and is absorbed via the small intestine and it may be attributed to permeability of the intestine is relatively slow in nature. Hence the current research was to widen the therapeutic efficacy of Nizatidine by formulating microspheres enhance the intestinal permeability as well as bioavailability by performing the anti-ulcer activity.

Keywords: Microspheres, Eudragit RS 100, Chitosan, Bioavailability.

Article Info: Received 17 Feb 2019; Review Completed 20 March 2019; Accepted 16 April 2019; Available online 25 April 2019

Cite this article as:

Mishra SK, Rajput S, Jain AK, Pharmacokinetics and Pharmacodynamic evaluation of Nizatidine microspheres on experimental animals, Journal of Drug Delivery and Therapeutics. 2019; 9(2-A):39-43

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INTRODUCTION

Peptic ulcer is a very common disease escalating throughout the world. A peptic ulcer is an erosion or sore in the wall of the gastrointestinal tract. The mucous membrane being delicate in nature which lines the digestive tract erodes and leads to a slow breakdown of tissue. This breakdown yields a burning pain in the upper middle part of the belly (abdomen). Peptic ulcer disease (PUD) occurs to the following: H pylori infection Drugs Lifestyle factors Severe physiologic stress Hypersecretory states (uncommon) Genetic factors Nizatidine restrain histamine stimulation of the H2 receptor in gastric parietal cells, which consecutively reduces gastric acid secretion, gastric volume, and hydrogen ion concentrations.[1,2] Nizatidine hydrochloride by inhibiting basal gastric acid secretion cause depletion of volume of acid and pepsin secretion. Nizatidine is administered as often in tablet, capsule, syrup and injection but the bioavailability is less. Hence to elevate the peptic ulcer pain the present work comprises of formulating Nizatidine as mucoadhesive microspheres since mucoadhesive microspheres form an integral part of novel drug delivery systems. Mucoadhesive microspheres provides numerous advantages such as effective drug absorption and intensified bioavailability of drugs due to a high surface-tovolume ratio and enormous intimate contact with the mucus layer, and specific targeting of drugs to the absorption site. Mucoadhesive microspheres being adhered to the stomach wall and thereby remain in the gastrointestinal tract for a longer time period. In recent years, mucoadhesive microspheres attention has been greatly focused since drug releases to the need of the body throughout the period of treatment and it provides the active entity solely to the site of action.[3, 5]

MATERIALS AND METHODS

Chemicals

Nizatidine was obtained from SMS Pharmaceuticals Pvt. Ltd. (Hyderabad, India) as free gift sample. PEG-400 was purchased from BD Pharmaceuticals Ltd. (Kolkata, India), and Tween80 was purchased from Merck Specialties Pvt. Ltd. (Mumbai, India). All other chemicals used in this study were obtained commercially and were of analytical (AR) grade.

Preparation of microspheres by solvent evaporation technique

Nizatidine loaded Eudragit Rs 100 microspheres were prepared by solvent evaporation. The Nizatidine microspheres were prepared with various ratios of drug and Eudragit RS100 polymer as shown in Table 1 using solvent evaporation technique. The method is a modification of emulsion solvent evaporation technique and involves preparation of o/w emulsion between organic phase consisting of Nizatidine and Eudragit RS 100 in dichloromethane (DCM) and aqueous phase, 1% w/v aqueous solution of polyvinyl alcohol (PVA).The dichloromethane solution of Nizatidine and Eudragit RS100 was emulsified by using probe homogenizer (Virtis Cyclone IQ, USA).The dichloromethane was completely evaporated by stirring overnight(12 to 16 hrs) at room temperature (250C±20C). The prepared microspheres were recovered by centrifugation for 20 minutes at 15,000rpm [Sorvall Ultracentrifuge, USA]. The precipitate was washed repeatedly with ice cold water to remove the traces of polyvinyl alcohol. Finally, the product was dispersed in cold water and recovered by lyophilisation (Labconco Lyophilisor, USA). Six batches of Eudragit microspheres were prepared keeping organic phase to aqueous phase at 1:5, and varying drug: polymer ratios. The biopharmaceutical parameters of dosage forms can be achieved directly by measuring the drug blood levels as a function of time or indirectly by measuring pharmacodynamic response as a function of time.[6]

Preparation of Nizatidine microspheres by coacervation- phase separation method

The Nizatidine loaded chitosan microspheres were prepared by coacervationphase separation technique using various ratios of drug and polymer. The polymeric solution was prepared by dissolving the chitosan in DCM. Then Nizatidine was dissolved in the polymeric solution and sonicated for 5 min. The organic non-solvent liquid paraffin was added to the polymeric solution at the rate of 1ml/min under continuous stirring using mechanical stirrer with 600 rpm. The slow addition of liquid paraffin coacervates the polymer in the mixture. The coacervate phase is then added to the hexane (non-solvent) under gentle stirring to harden the coating layer. The formed microspheres were then centrifuged for 10 min at 10,000 rpm. The precipitate was then collected and washed at least three times with distilled water. The prepared microspheres were lyophilized and stored in container for further studies. Six batches of microspheres were prepared using various ratios of drug: polymer Selected formulations of Nizatidine microparticles prepared by Eudragit RS100 and Chitosan were subjected to in vivo studies in animal model. The in vivo anti ulcer activity and bioavailability study were carried out in rat model. Rats are widely used for in vivo bioavailability study as well as anti ulcer activity study, as its physiological structure is similar to human model. Moreover rats are easy to handle and are low in cost.[7]

Animals

Albino rat of either sex weighing 400 – 450 gm were chosen for this experiment. Institutional animal ethics committee approved the experimental protocol; animal were maintained under standard condition in an animal house approved by committee for the purpose of control and supervision on experiment on animal. All animal experiments were approved by Institutional animal ethics committee (IAEC) of Registration No. 1546/PO/E/S/11/CPCSEA.

Animals were kept in standard cages for constant room temperature at 25 ± 1 ^{0}C. Rats were kept in Fasted condition for 18 hour where no food but water was provided ad-libitum and had free access to water.

In vivo anti-ulcer activity

The anti-ulcer activity of the formulation was carried out in Albino rat. The oral dose of 20 mg/kg was chosen for this purpose. The healthy rats were divided into four groups with five animal each. The animals in the test groups were administered 1ml / 100 gm of rat with necrotizing agent (80% ethanol) orally which is known to produce gastric lesions. The dosage schedule for the study is as follows

Group 1: Animals were given the normal saline with dose of 10 ml / Kg and served as negative control. **Group 2**: Animals were administered with Ethanol (80%) orally and served as positive control. **Group 3**: Animals were administered ethanol 1 ml /100gm and treated with pure Nizatidine 20mg/kg. **Group 4:** Animals were administered with ethanol and treated with formulation (equivalent 20 mg Nizatidine).**Group 5:** the animals administered with ethanol and treated with RCP3 (equivalent of 20 mg Nizatidine)

According to the gastric emptying in fasted rats, formulations was given 30 minute before the ethanol administration. Animals were sacrificed under anesthesia 1 hr after treatment with formulation. The stomach was excised and opened along the greater curvature and after washing with normal saline, the gastric lesions were quantified using a magnifying glass. and ulcer index (UI) was estimated.

$$UI = \frac{\text{Ulcerated area (mm}^2\text{)}}{\text{Total stomach area (mm}^2\text{)}}$$

In vivo Bioavailability study

The bioavailability study was carried out in albino rats of either sex weighing 200 – 250 gm. The animal were divided into three groups of five animals each and were fasted overnight before starting the experiment with free access to water. The pure Nizatidine and prepared microspheres formulation was administered orally with dose 20mg/kg body weight with the help of cannula after anaesthetizing for a very short period of time with diethyl ether , after administration 0.3 ml blood samples were collected from retro- orbital plexus into the heparinized tubes at pre set period of 0.5, 1,2,4,8,12,24h. The blood samples were centrifused at 4000rpm for 10 minutes and the separated samples were stored at - 20 ^{0}C until analysis had completed.

Estimation of Nizatidine in plasma sample by RP-HPLC analysis

The amount of Nizatidine in blood samples was measured by RP-HPLC method (Haque et al., 2011.The method was validated prior estimation. The measurement was carried out at 280 nm .The mobile phase used consist of mixture of 0.1(M) orthophosphoric acid (P^H 3.0) and methanol in the ratio of 30:70 and the pump flow rate was 1ml/min and C18 (250mm × 4.6 mm) column was used..The mobile phase was filtered with nylon membrane filter and degassed before use.

To 0.1 ml plasma 50 µL of standard nizatidine (50 ng/ml) was added in a micro centrifuge tube and volume was made up to 2 ml with acetonitrile to precipitate the protein. Then tha sample was centrifuged at 4000 rpm for 25 min. The supernatant was collected and transferred into an eppendorf tube and was dried. The residue was dissolved in 200 µL of mobile phase and 10 µL was injected to the HPLC system. The analysis was carried out by

RP-HPLC method using flow rate 1.0 ml/min and measurement was made at 280 nm. The amount of the nizatidine in the sample was determined from the peak area ratio correlated with standard curve prepared under the same identical condition.

Pharmacokinetic Analysis

Determination of C_{max} and T_{max}

The peak plasma concentration (C_{max}) and the time of peak plasma concentration (T_{max}) were determined from the plasma drug concentration vs time plot for the pure drug and prepared microspheres.

Determination of area under curve (AUC):

The area under the time versus plasma concentration curve (AUC) was measured by applying trapezoidal rule . $(AUC)_{0-\alpha}$ was calculated as given below

$$(AUC)_{0-t} = \int_0^t C(t)\,dt$$

$$(AUC)_{0-\alpha} = (AUC)_{0-t} + C_t / K_{el}$$

Determination of relative Bioavailability

The relative bioavailability (F_r) of Nizatidine was calculated using the following equation:

$$F_r\,(\%) = \frac{\text{AUC (Nizatidine microspheres)}}{\text{Pure nizatidine suspension}}$$

RESULTS AND DISCUSSION

Optimized formulation In the present study mucoadhesive microspheres using chitosan and Eudragit RS 100 of Nizatidine hydrochloride were successfully prepared by solvent evaporation technique. The formulations RES3 and RCP3were selected as an ideal formulation based in vitro drug release tests. Formulation and evaluation of Nizatidine loaded chitosan and Eudragit RS 100 microspheres for controlled release was found to be potential and effective in terms yield, encapsulation efficiency, particle size distribution. RES3 formulation drug release was slow and extended beyond 12 hr up to 24 hr which could be the constant release of drug which has been loaded near the surface of microspheres. Kinetic results reveals microspheres obeyed Higuchi and Peppas model.RCP3formulations showed slow drug release which extended beyond 12 hr up to 24 hr. The formulation showed rapid drug release after 4 hr and this could be result of polymer erosion in the surface of microspheres and obeyed Higuchi and as well as Peppas model indicating the diffusion drug release mechanism. Hence the optimized RES3 and RCP3 formulations of Nizatidine mucoadhesive microspheres were selected for invivo anti ulcer activity study and invivo bioavailability study.

***In-vivo* anti-ulcer activity study**

Ethanol is considered a major risk factor for the gastric ulcer. The ethanol penetrates the gastric mucosa in the stomach due to its ability to solubilize the mucous on the membrane. Once the mucous get solubilized the membrane becomes exposed to the proteolytic enzyme pepsin and hydrochloric acid in the stomach causing the damage to the membrane. The rat model is widely used for antiulcer activity as its physiological structure is resembled to the human. In the present study the anti-ulcer activity of the prepared microspheres formulations (RES3 and RCP3) was evaluated in the ethanol induced ulcerative rats by measuring the ulcer index. The ulcer index is given in table 1. The ulcer index value in the ethanol treated group was 23.92 ± 0.58, whereas, the normal saline treated group showed no ulcer owing to the lower value. The ulcer index of the formulations RES3 and RCP3 was notably reduced as compared to ethanol treated group, but this value is remarkably reduced for the pure drug treated group as compared to the positive control group. The percentage of ulcer protection of the pure Nizatidine and the formulations RES3 and RCP3 was given in figure 1.

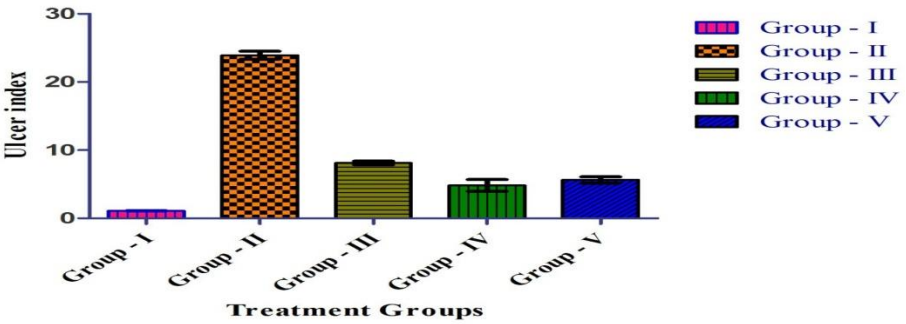

Figure - 1: Anti-ulcer activity of the Nizatidine formulations RES3 and RCP3

The ulcer protection of the microspheres formulations RES3 and RCP3 were 79.84% and 76.49% respectively as compared to the Nizatidine pure drug (66.05%) in ulcer induced rats. The pure Nizatidine showed poor activity against mucosal damage as compared to the microspheres. The improve activity of the microspheres was because of sustained release property as well as mucoadhesive property of the RES3 and RCP3, which suggests that Nizatidine microspheres strengthens and protects the gastric mucosal barrier 3.3. In vivo bioavailability study The availability of the drug to the biological system and its therapeutic effectiveness is the goal of any dosage form design. The bioavailability study of the two microsphere formulations RES3 and RCP3 were carried out in the rat model. The rat model is widely used for bioavailability study for many drugs. The plasma drug concentration-time profile of Nizatidine was constructed following the oral administration of pure Nizatidine and Nizatidine loaded microspheres at a dose of 20 mg/kg to rats (table 2). The plasma concentration-time profile is illustrated in figure 2 and the pharmacokinetic parameters are listed in table 3. The plasma concentration time profile of Nizatidine pure drug showed the higher peak than that of formulation RES3 and RCP3. The C max value of Nizatidine as obtained from the graph was 575.14 ± 55.43 ng/ml with T max value 2 h and for formulations RES3 and RCP3 C max were 206.58 ± 7.71 and 221.52 ± 9.42 ng/ml respectively.

Table 1: Anti-ulcer activity of the Nizatidine formulations RES3 and RCP3 in ethanol induced ulcer in rat

Groups	Induction	Dose	Ulcer Index
Group – I	Normal Saline	10ml/kg	1.090 ±0.04
Group –II	Ethanol	1ml/gm	23.92 ± 0.58
Group –III	Nizatidine	20 mg/kg	8.12 ± 0.28**
Group – IV	RES3	20 mg/kg	4.83± 0.86*
Group – V	RCP3	20 mg/kg	5.63 ±0 .46*

Note : Values express mean ± SEM ; n=4; *p<.0.05 bversus control, **p<0.01versus control

Table 2: Drug Plasma concentration vs. Time data following oral administration of pure Nizatidine (Standard) to rat.

	Plasma Concentration in ng / ml vs. Time		
Time (Hrs.)	Pure Nizatidine	RES3	RCP3
0	0	0	0
1.5	269.65± 42.31	45.250±8.42	40.250 ± 5.560
1	391.72±56.25	69.250± 9.81	79.760 ± 10.03
2	575.14±55.43	138.26±13.94	169.50 ± 13.26
4	283.5±33.960	189.250 ±7.03	197.75 ± 14.23
8	61.790±7.720	206.58± 7.71*	221.52 ± 9.42*
12	16.430±4.570	97.750±10.38	112.75 ± 12.37
24	_	22.500± 9.600	29.500 ± 7.920

Note : Values express mean ± SEM ; n=4; *p<.0.05 versus control,

Table 3: Phamacokinetic Profile of pure Nizatidine and Nizatidine loaded Microspheres after oral administration in rats.

Pharmacokinetics Parameters	Units	Nizatidine Standard	RES3	RCP3
C_{max}	ng / ml	575.14±55.43	206.58±7.71	221.52 ± 9.42
T_{max}	H	2 ± 0	8 ± 0	8 ± 0
AUC $_{(0-24)}$	h x (ng / ml)	2064.07	2272.22	2408.46
Fr	(%)	_	110.07	116.68

The drug release from the ranitidine microspheres extend up to 24h as the formulations showed sustained release properties as evidenced from the in vitro drug release study. The AUC (0-24) of the formulations were higher than the AUC (0-24) of the pure ranitidine, which proves the better bioavailability of the microspheres and better therapeutic effect for the management of gastric and peptic ulcer.

CONCLUSIONS

In the present investigation, the utility of microsphere as carrier for oral delivery of Nizatidine was studied. The Nizatidine loaded microspheres were prepared by emulsion solvent evaporation method and coacervation phase separation method. The pharmacodynamic study (ethanol-induced ulcer model) revealed that Nizatidine microspheres showed lower incidence of mucosal damage when compared with standard drug, both administered orally, indicating the superiority of oral Nizatidine microspheres over standard ranitidine. The pharmacokinetic studies reveal that the oral administration of Nizatidine microspheres sustained the release of drugs over 24 hrs. The drug release from the microspheres showed controlled drug release mechanism. Enhanced antiulcer efficacy was obtained with reduction in gastric acid secretion. As a consequence of this, decrease in the dose and frequency of administration for drugs is possible to achieve the desired therapeutic activity.

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