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Short Communication

Antibacterial activity study of a mucoadhesive suspension containing ciprofloxacin

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Considering various effects of mucoadhesive suspension containing Ciprofloxacin, it was found that newly designed mucoadhesive formulation was better than its conventional immediate release preparation. Due to that their *in vitro* antibacterial activity was also studied and compared. It was found that the newly designed mucoadhesive formulation showed much larger zones of inhibition against all the strains used in the study than the conventional immediate release preparation. In addition, this novel formulation and even the standard discs of Ciprofloxacin produced more or less similar zones of inhibition. However, the conventional immediate release preparation was much inferior to the standard discs as far as the zones of inhibition were concerned.

Key words: Ciprofloxacin, mucoadhesive formulation, conventional immediate release preparation, antibacterial activity.

INTRODUCTION

Extended-release (mucoadhesive suspension) formulation of Ciprofloxacin provides more systemic drug exposure comparable with that achieved with twice-daily administration of conventional, immediate-release Ciprofloxacin. Moreover, the mucoadhesive suspension helps in attaining higher maximum plasma concentrations with less inter-patient variability and by achieving constant blood level, drug benefit is maximized while its potential toxicity is minimized (Dong et al., 2006). Considering those factors, a mucoadhesive suspension of Ciprofloxacin was prepared by using Carbopol 940 (C 940). C 940 consists of chains of polyacrylic acid cross linked with allyl ethers of Pentaerythritol. Carbopol polymers are pH sensitive, environmentally responsive polymer along with other additives (Bettini et al., 1995; Galaev et al., 1999; Jeong et al., 2001; Qiu et al., 2001). After preparation of this novel formulation, pharmaceutical optimization was carried out. Chemical compatibility, crystallinity and particle size were studied using FTIR, XRD and SEM analyses, respectively. Franz diffusion cell was used with the excised goat stomach mucosal layer for the study of permeability of above selected formulation along with the marketed ñCIPLOXôô immediate release suspension. From the permeability study it has been found that this mucoadhesive formulation shows higher cumulative percentage drug release as compared to the marketed formulation. By radio labeling of Ciprofloxacin with Tc^{99} preparing active formulation and administering to rats, gamma scintigraphic study was performed. From that investigation it was clear that the prepared formulation showed better biodistribution and GI retention property over the conventional dosage form of Ciprofloxacin. Considering several beneficial effects of the present formulation over conventional immediate release form of the drug, it was necessary to compare their antibacterial activity as well. Since the main purpose of using any ciprofloxacin containing preparation is to get the benefit of its antibacterial actions, the antibacterial activity study

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of a new formulation should be compared with the conventional immediate release form of the drug. Accordingly, *in vitro* antibacterial activity of the mucoadhesive suspension containing ciprofloxacin was evaluated. From that study report we would be able to communicate whether this formulation is actually a better option to eradicate several bacterial infections or not.

MATERIALS AND METHODS

Materials

Ciprofloxacin hydrochloride was obtained from Dr. Reddyôs Lab, Hyderabad, India, as a gift sample. Carbopol 940, Pluronic F68 and Soya lecithin were purchased from HiMedia Laboratories Pvt. Ltd., India. Citric acid, Sodium citrate, Glycerol, Methyl praraben, Propyl paraben, Sorbitol solution I.P., Sucrose were kindly supplied by Cosmo Chem. Laboratory, India. Ultra pure water was obtained from a Millipore Milli-Q UV water filtration system.

For antibacterial activity study neutrient agar, Mueller-Hinton broth and Mueller-Hinton agar were obtained from HiMedia, Mumbai.

Methods

Preparation of formulation

Praparation of bulk A: In a beaker, 6 ml. of water was taken and heated up to 80°C. Sucrose (10 g) was added under continuous stirring. The temperature was monitored in such a way so that it should not fall bellow 70°C till the sucrose was completely dissolved. The prepared syrup was cooled properly to room temperature and kept overnight. Syrup was filtered using 120 mesh nylon cloth.

Preparation of bulk B: 5 ml of ultra pure water was taken in a beaker to which 1.8 ml of sorbitol solution and 0.2 ml glycerin were added. The mixture was stirred properly. To this solution pluronic-F-68 (3% w/w-0.038 g), soya lecithin (2% w/w-0.025 g) and carbopol 940 (5% w/w-0.063 g) in w/w of drug were added with continuous stirring.

Preparation of mucoadhesive suspension and ultrasonication: 5 ml of water was taken in another beaker to which 1.25 g of ciprofloxacin HCl was added with continuous stirring. To the drug suspension the bulk B and bulk A were added with continuous stirring. Methyl paraben sodium (0.015% w/v) and Propyl paraben sodium (0.08% w/v) were added as preservative. The volume was adjusted up to 25 ml by ultra pure water. The pH was adjusted by adding citrate buffer (0.75 M) to pH 5.0.

Homogenization was carried out for at least 15 min by ULTRASONIC HOMOZENIZER LABSONIC^R M (SARTORIUS) having operating frequency 30 KHZ and line voltage 230 V/50HZ, using the probe made up of Titanium having diameter 7 mm and length 80 mm. The setting knob ñcycleò was adjusted to 0.8 indicating sound was emitted for 0.8 s and paused for 0.2 s. In this manner, we could expose our sample with 100% amplitude, while reducing the heating effect to 80%. This LABSONIC^{R}M generates longitudinal mechanical vibrations with a frequency of 30,000 oscillations/sec (30KHZ). The probes bolted to the sound transducer are made of high-strength Titanium alloys, built as a ½ oscillator. It amplifies the vertical oscillation, and transfers the ultrasonic energy through its front surface with extremely high power density into the sample that is to be subjected to ultrasonic waves. Here, stress applied was sound wave and mild rise in temperature of the sample occurred during ultrasonication.

Antibacterial activity study

Stock cultures of *Escherichia coli (*ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) were maintained at 4°C on slants of nutrient agar. Active cultures for the study were prepared by transferring a loopful of cells from the stock culture to test tubes of Mueller-Hinton broth (MHB) and those tubes were incubated without agitation for 24 h at 37°C. After getting growth, the cultures were diluted with fresh MHB to an inoculum size of 10^6 colony forming units/ml (Collins et al., 1995). The disc diffusion method was used to know the antibacterial activity of the drug (Bauer et al., 1966). *In vitro* antibacterial activity was determined by using Mueller-Hinton Agar (MHA) medium. The MHA plates were prepared by pouring 15ml of sterile molten medium into sterile Petri dishes. The plates were allowed to solidify and a lawn of bacterial culture was made on MHA plates (Bauer et al., 1966). Identical concentration of Ciprofloxacin [5 mcg/disc- standard supplied disc; 5 mcg/disc-Ciprofloxacin containing conventional immediate release form (suspension) of the drug; 5 mcg/disc- Ciprofloxacin containing new formulation under investigation] was loaded on 6 mm sterile filter paper (Whatman no. 1) discs. In addition, some sterile filter paper discs were soaked in sterile distilled water. Those discs were placed on the surface of the dried inoculated MHA plates and the drug was allowed to diffuse for 2 h at room temperature (Bhaskar et al., 2007) during which time diffusion of the antibiotic into the medium occurred. Then the plates were kept for incubation at 37°C for 24 h. At the end of incubation period, inhibition zones formed around the discs were measured with a ruler in mm. For each strain, three MHA plates were used in a similar fashion. The results were represented as average zone of inhibition of all the strains of bacteria used in this study. The antibacterial activity was evaluated by measuring inhibition zones of bacterial growth. In the study, discs soaked in distilled water served as negative control and Ciprofloxacin disc was used as positive control.

RESULTS AND DISCUSSION

From the antimicrobial efficacy study (Table 1), it was found that the final formulation (mucoadhesive suspension) showed much larger zones of inhibition against all the strains (*E. coli, P. aeruginosa* and *S. aureus*) used in the study than the conventional immediate release preparation. In addition, the present formulation and even the standard discs produced more or less similar zones of inhibition. However, the conventional immediate release preparation was much inferior to the standard discs as far as the zones of inhibition were concerned. From the above-mentioned result, it is clear that the present formulation is a better option to control several bacterial infections, in spite of using polymer to get sustained release effects. In addition, considering other beneficial effects, it may be mentioned that the present formulation is probably a superior product than the conventional immediate release preparation. So, at present, developing several such sustained release formulations, detailed investigations are needed to overcome the limitations of the conventional immediate release preparations.

Table 1. Comparative antibacterial study of different forms of Ciprofloxacin and distilled water.

Name of micro- organism	Zone of inhibition (mm)			
	S.D[a]	C.R.I[b]	F[c]	D.W[d]
E. coli (ATCC 25922)	34	25	31.5	0
P. aeruginosa (ATCC 27853)	33	22	29	0
S. aureus (ATCC 25923)	30	20	27.5	0

a - Standard Ciprofloxacin disc, b - Conventional immediate release suspension, c - Newly designed formulation, d - distilled water.

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