Thermoreversible-mucoadhesive Gel for Nasal Delivery of Sumatriptan

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Rita J. Majithiya,[1] Pradip K. Ghosh,[1] Manish L. Umrethia,[1] and Rayasa S. R. Murthy[1]

[1]Drug Delivery Research Laboratory, Center of Relevance and Excellence in New Drug Delivery Systems (NDDS), Pharmacy Department, G. H. Patel Building, Donor's Plaza, Fatehgunj, M. S. University of Baroda, Vadodara-390002, Gujarat, India

ABSTRACT

The purpose of the present study was to develop intranasal delivery systems of sumatriptan using thermoreversible polymer Pluronic F127 (PF127) and mucoadhesive polymer Carbopol 934P (C934P). Formulations were modulated so as to have gelation temperature below 34°C to ensure gelation at physiological temperature after intranasal administration. Gelation temperature was determined by physical appearance as well as by rheological measurement. The gelation temperatures of the formulations decreased by addition of increasing concentrations of Carbopol (ie, from 29°C for 18% PF127 to 23.9°C for 18% PF127, 0.5% Carbopol). The mucoadhesive force in terms of detachment stress, determined using sheep nasal mucosal membrane, increased with increasing concentration of Carbopol. The results of in vitro drug permeation studies across sheep nasal mucosa indicate that effective permeation coefficient could be significantly increased by using in situ gelling formulation with Carbopol concentration 0.3% or greater. Finally, histopathological examination did not detect any damage during in vitro permeation studies. In conclusion, the PF127 gel formulation of sumatriptan with in situ gelling and mucoadhesive properties with increased permeation rate is promising for prolonging nasal residence time and thereby nasal absorption.

KEYWORDS: Carbopol, migraine, mucoadhesive, nasal, Pluronic F127.

INTRODUCTION

Sumatriptan is a 5-HT1D(5-hydroxy tryptamine 1D)-receptor agonist, used in the treatment of migraine and cluster headache. Sumatriptan is generally given by oral or parental routes. However, a substantial proportion of patients suffer severe nausea or vomiting during their migraine attack, which may make oral treatment unsatisfactory. Moreover, sumatriptan has previously been shown to have a low oral bioavailability in human volunteers (15%).[1] Subcutaneous administration is an alternative; however, dislike of injections or inability to self-administer by this route makes subcutaneous treatment unacceptable to some individuals. The intranasal route may be a viable alternative for self-administration, whereby these limitations could be overcome. However, the problem associated with nasal delivery of sumatriptan solution is lower retention time of solution in nasal cavity (15 minutes) resulting in lower bioavailability as well as lower transfer of sumatriptan directly to the brain through the olfactory pathway. After 15 minutes, sumatriptan solution is swallowed and it enters the gastrointestinal tract (GIT), where remaining dose is absorbed. Although sumatriptan nasal spray provides faster onset of effect than the tablet, it produces a similar headache response at 2 hours.[2] Recently, an intranasal-microemulsion drug delivery system to accomplish rapid delivery of sumatriptan to the brain in acute attacks of migraine has been reported.[3] Hence, a formulation that would increase residence time in the nasal cavity and at the same time increase absorption of the drug would be highly beneficial in all respects.

The use of bioadhesive polymers can lengthen the residence time and enhance bioavailability of drugs delivered to the nasal cavity.[4,5] Our research group has previously shown the use of chitosan-based mucoadhesive formulation to enhance the retention time and bioavailability of antibiotic to stomach.[6] Polymer gels and mucoadhesive polymers have been studied for the mucosal delivery of various compounds ranging from small molecule to macromolecular drugs. The nasal bioadhesive gels might be used to provide an enhanced bioavailability compared with oral delivery.[7] A very good example for such a system is EnerB (Nature's Bounty Inc, Bohemia, NY), a vitamin B_{12} supplement available in gel form. Application of in-situ gelling solutions of tri-block copolymers of poly(ethylene oxide) and poly(propylene oxide) (Pluronic) exhibiting thermoreversible properties have been proposed to lower the viscosity of the nasal formulations below the body temperature.[5] By modulating the gelation temperature of different PF127 solutions, liquid bases for nasal use can be formulated that form a gel in the nasal cavity at body temperature with suitable gel strength resulting in enhancement of the residence time in the nasal cavity.[4] Gelation of concentrated solutions of Pluronics is a well-documented phenomenon related to the appearance

Corresponding Author: Rayasa S. R. Murthy, Drug Delivery Research Laboratory, Center of Relevance and Excellence in NDDS, Pharmacy Department, G. H. Patel Building, Donor's Plaza, Fatehgunj, M. S. University of Baroda, Vadodara-390002, Gujarat, India. Tel: 91-265-2794051; Fax: 91-265-2423898; E-mail: m_rsr@rediffmail.com

of tightly packed cubic liquid crystalline micellar phases.[8] The high solubilizing capacity and nontoxic properties of PF127 make it suitable for nasal drug delivery. The use of thermoreversible PF127 gels of vitamin B_{12} for nasal delivery has been reported by Pisal et al.[9]

The objective of this study was to develop an intranasal delivery system of sumatriptan succinate using thermoreversible polymer PF127 and mucoadhesive polymer C934P, which would enhance nasal residence time and absorption of drug across nasal-mucosal membrane.

Materials and Methods

Materials

PF127 was procured from Sigma (St Louis, MO). C934P was supplied from Hi-Media Lab Pvt Ltd (Bombay, India). Sumatriptan as succinate salt was received as gift sample from Natco Fine Pharmacis Pvt Ltd (Hyderabad, India). All other chemicals and reagents used in the study were of analytical grade.

Methods

Preparation of Mucoadhesive Polymer-based Thermoreversible Nasal Formulations

PF127 and sumatriptan were solubilized in distilled water containing 1% propylene glycol. The PF127 vehicles used throughout this study were composed of 18% wt/vol of PF127. The concentration of PF127 was selected so as to obtain thermoreversible gel at minimum possible concentration. PF127 vehicles with concentration varying from 16% wt/vol to 20% wt/vol were screened preliminarily to decide lowest possible concentration. PF127, 18% wt/vol, was found to be lowest concentration (when formulated in addition of sumatriptan succinate) that exhibited thermoreversible property below 34°C (temperature of the nasal cavity). Hence, 18% wt/vol of PF127 was selected for further studies. The liquid was left at 4°C until a clear solution was obtained. Thermoreversible gels were prepared using cold method.[10] Bioadhesive anionic polymer C934P was slowly added to the solution with continuous agitation. C934P was added in concentration range of 0.1% wt/vol to 1% wt/vol to PF127 solution.

Measurement of Gelation Temperature by Visual Inspection

Gelation temperature, defined as the temperature at which the liquid phase makes a transition to gel, was determined as described previously.[11] In brief, a 10-mL transparent vial containing a magnetic bar and each formulation were placed in a water bath. The vial was heated at a constant rate while stirring. The gelation temperature was measured when the magnetic bar stopped moving due to gelation. Each preparation was tested thrice to control the repeatability of the measurement.

Gelling Temperature Determination by Rheological Method

Rheological studies were performed with a thermostatically controlled Brookfield Programmable Rheometer (Brookfield LVDV III, Brookfield Engineering Laboratories, Middleboro, MA) fitted with CP-52 spindle. The cone/plate geometry was used. The cone had a 1.2-cm radius and an angle of 3°. The shear stress was controlled to maintain a shear rate of 10 seconds^{-1} shear rate. This value was chosen to allow precise determination of the gelling temperature. The temperature was increased in steps of 1°C/minute, from 20°C to 40°C to locate the solution/gel transition point. The gelling temperature was determined graphically as the inflection point on the curve of the apparent viscosity (mPa s) as a function of the temperature (°C). Each preparation was tested thrice to control the repeatability of the measurement.

Evaluation of the Mucoadhesive Strength

The mucoadhesive potential of each formulation was determined by measuring the force required to detach the formulation from nasal mucosal tissue using a modified method described by Jones et al.[12] In brief, nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissues were immediately used after separation. At the time of testing, a section of nasal tissue was secured (keeping the mucosal side out) to the upper probe using a cyanoacrylate adhesive. The upper probe was attached to precalibrated force displacement transducer SS12LA, (BIOPAC Systems Inc, Santa Barbara, CA) connected to the Biopac MP-30 data acquisition system (BIOPAC Systems). The surface area of each exposed mucosal membrane was 0.785cm^2. At room temperature, fixed amount of samples of each formulation were placed on the lower probe. The probes were equilibrated and maintained at 34°C. Probe with nasal tissue was lowered until the tissue contacted the surface of the sample. Immediately, a force of 0.1 N was applied for 2 minutes to ensure intimate contact between the tissues and the samples. The probe was then moved upwards at a constant speed of 0.15 mm/s. The bioadhesive force, expressed as the detachment stress in dyne/cm^2, was determined from the minimal weights that detached the tissues from the surface of each formulation using the following equation.[13]

$$\text{Detachment Stress (dyne/cm}^2) = \frac{mg}{A}, \qquad (1)$$

where m is the weight added to the balance in grams; g is the acceleration due to gravity taken as 980 cm/s^2; and A is

the area of tissue exposed. Measurements were repeated thrice for each of the gel preparations, but before each measurement a fresh smooth gel surface was created.

Effect of Initial Contact Time on Mucoadhesive Strength

Effect of varying contact time (1, 2, 3, 5, and 10 minutes) was investigated for some of the gel preparations to optimize initial contact time. In brief, formulations were allowed to be in contact with mucosa for carrying contact times (1, 2, 3, 5, and 10 minutes), and the bioadhesive force was determined as discussed above. Contact time that resulted in maximum bioadhesive strength was selected as optimum contact time required for adequate adhesion.

In Vitro Permeation Studies

Fresh nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissue samples were inserted in Franz diffusion cells displaying a permeation area of 0.785 cm^2. Twenty milliliters of phosphate buffer saline (PBS) pH 6.4 at 34°C was added to the acceptor chamber. To ensure oxygenation and agitation, a mixture of 95% O_2 and 5% CO_2 was bubbled through the system. The temperature within the chambers was maintained at 34°C. After a pre-incubation time of 20 minutes, pure drug solution and formulation equivalent to 2.5 mg of sumatriptan was placed in the donor chamber. At predetermined time points, 1-mL samples were withdrawn from the acceptor compartment, replacing the sampled volume with PBS pH 6.4 after each sampling, for a period of 4 hours. The samples withdrawn were filtered and used for analysis. Blank samples (without sumatriptan) were run simultaneously throughout the experiment to check for any interference. The amount of permeated drug was determined using a UV-visible spectrophotometry at 283 nm (Linearity range = 1.25 μg/mL to 100 μg/mL, $R^2 = 0.9998$).

Data Analyses of Permeation Studies

The effective permeability coefficients (Peff) (cm s^{-1}) under steady-state conditions across excised mucosa can be calculated according to Equation 2.

$$Peff = \left(\frac{dC}{dt}\right)_{ss} \frac{V}{AC_D} \qquad (2)$$

where $(dC/dt)_{ss}$ (μg mL^{-1} s^{-1}) is the time-dependent change of concentration in the steady-state; A (cm^2) is the permeation area; V (mL) the volume of the receiver compartment; and C_D(ug mL^{-1}) is the initial donor concentration.[14]

Histopathological Evaluation of Mucosa

Histopathological evaluation of tissue incubated in PBS (pH 6.4) after collection was compared with tissue incubated in the diffusion chamber with gel formulation (PLC-3). Tissue was fixed in 10% buffered formalin (pH 7.2), routinely processed and embedded in paraffin. Paraffin sections (7 μm) were cut on glass slides and stained with hematoxylin and eosin (HE). Sections were examined under a light microscope, to detect any damage to the tissue during in vitro permeation by a pathologist blinded to the study.

Statistics

Data were expressed as mean ± SD. Statistical analysis of data was performed using analysis of variance (ANOVA) followed by the Dunnett multiple comparison test. A P value <.001 was considered significant.

Results and Discussion

Gelation Temperature

Thermoreversible polymer-based liquid formulations that provide in situ gelling property in nasal cavity were designed to delay clearance of the formulations from the nasal cavity.

In the preliminary studies, the minimum concentration of PF127 that formed gel below 34°C was found to be 18% wt/vol. In general, the gelation temperatures have been considered to be suitable if they are in the range of 25°C to 37°C. If the gelation temperature of a thermoreversible formulation is lower than 25°C, a gel might be formed at room temperature leading to difficulty in manufacturing, handling, and administering. If the gelation temperature is higher than 37°C, a liquid dosage form still exists at the body temperature, resulting in the nasal clearance of the administered drugs at an early stage. As the temperature of the nasal cavity is 34°C,[15] this study aimed at preparing the liquid formulations of PF127 that may gel below 34°C. It is evident from the data shown in Table 1 that the gelation temperature obtained using 2 different methods (visual inspection and rheological method) did not vary more than ±1.5°C. Figure 1 shows the viscosity versus temperature curves of all the formulations. The gelation temperatures of PF127 vehicles as determined by rheological method were lowered from 29°C to 28.5°C by the presence of 0.1% mucoadhesive polymer C934P (Table 1). It is to be noted that the addition of increasing concentrations of C934P from 0.1% to 0.5% further lowered the gelation temperature from 28.5°C to 23.9°C. The gelation of PF127 vehicles is known to result from the change in micellar number with temperature. With increasing temperature, the number of micelles formed increases as a consequence of the negative coefficient of solubility of block copolymer

Table 1. Gelling Temperature Determined Rheologically and by Visual Inspection and Effective Permeability Coefficient Determined for Various Formulations Across Sheep Nasal Mucosal Membrane*

Sample	Composition	Peff ($\times 10^{-5}$) (cm s^{-1})	Solution-Gel Transition Temperature (°C)	
			Rheology	Visual Inspection, Mean ± SD (n = 3)
Pure Drug	Pure Drug Solution	5.58 ± 0.365	—	—
PLB	18% PF127	4.46 ± 0.275†	29	28.2 ± 1.04
PLC-1	18% PF127, 0.1% C934P	4.51 ± 0.174†	28.5	27.3 ± 0.58
PLC-2	18% PF127, 0.2% C934P	5.81 ± 0.276	26.7	25.8 ± 0.29
PLC-3	18% PF127, 0.3% C934P	6.61 ± 0.170†	25.9	24.8 ± 0.76
PLC-4	18% PF127, 0.5% C934P	7.00 ± 0.364†	23.9	23.0 ± 1.00

*Peff indicates effective permeability coefficients; PF127, Pluronic F127; C934P, Carbapol 934P; —, not applicable.
†$P < .001$ versus pure drug.

micelles. Eventually the micelles become so tightly packed that the solution becomes immobile and gel is formed.[16] Recently, Cabana et al[17] suggested a mechanism of gelation based on micelles packing and entanglements. Also, conformational changes in the orientation of the methyl groups in the side chains of poly(oxypropylene) polymer chains, constituting the core of the micelle, with expulsion of the hydrating water from the micelles will contribute to the gelation phenomenon.[18] The gelation temperature-lowering effect of mucoadhesive polymers might be caused in part by the increased viscosity after dissolution of mucoadhesive polymers. The formulations containing concentration of C934P higher than 0.5% were found to have very high viscosity, and so were difficult to administer into the nostril. Regardless of the concentration of mucoadhesive polymers, all the formulations gelled at the temperature ranging from 23.9°C to 29°C. These temperatures seem to be proper for in situ gelling of the various vehicles at the nasal cavity, minimizing the loss of administered drug caused by clearance from the site of application.

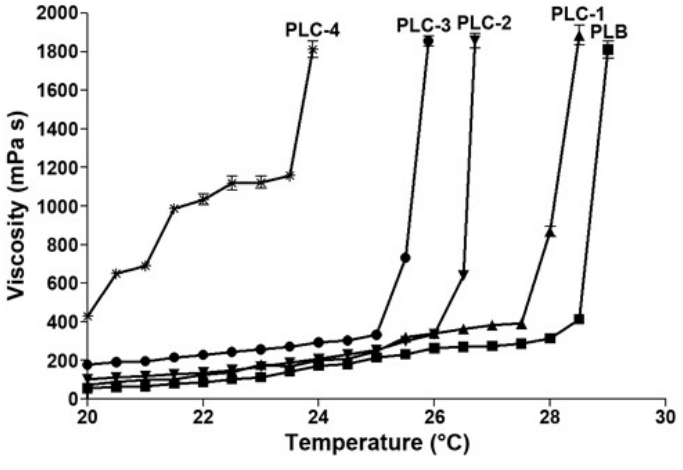

Figure 1. Effect of temperature on the viscosity of various Pluronic gels with varying concentration of C934P (0.1% to 0.5%) measured at 10 $seconds^{-1}$ shear rate. Values are expressed as mean ± SD (n = 3).

Mucoadhesive Strength

Two minutes of contact time was found to give optimum mucoadhesive strength. Further increase in contact time did not affect the mucoadhesive strength, whereas decreased contact time resulted in less mucoadhesive strength resulting from insufficient time for entanglement of polymer chains with mucin. Assessment of the mucoadhesive strength in terms of detachment stress showed that the PF127 preparations possessed adhesive properties that increased with the addition of C934P concentration (Figure 2). Mucoadhesive strength for formulations PLC-2, PLC-3, and PLC-4 with 0.2%, 0.3%, and 0.5% C934P concentration, respectively, increased significantly ($P < .001$) with respect to PLB, whereas increase in mucoadhesive strength of PLC-1 (0.1% C934P) was not significant. Earlier work with Carbopol polymers has clearly indicated that it is the availability of carboxyl groups that determines bioadhesion,[19] Carbopol has a very high percentage (58%-68%) of carboxylic groups that gradually undergo hydrogen bonding with sugar residues in oligosaccharide chains in the mucus membrane, resulting in formation of a strengthened network between polymer and mucus

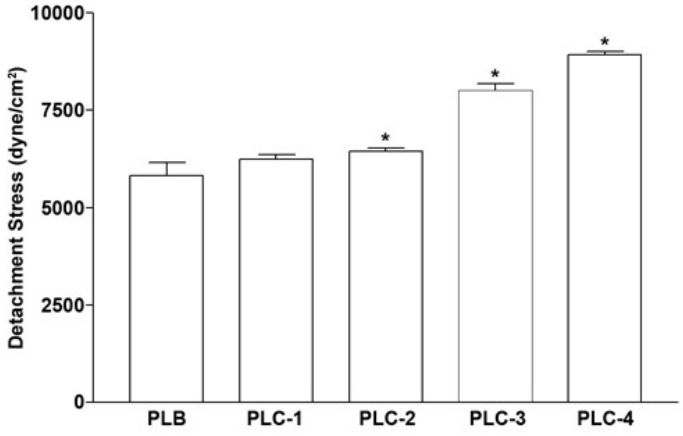

Figure 2. Influence of C934P concentration on the detachment stress measured in vitro. Values are expressed as mean ± SD (n = 3);*$P < .001$ versus PLB (18% PF127 concentration).

membrane. Thus, Carbopol having high density of available hydrogen bonding groups would be able to interact more strongly with mucin glycoproteins. In addition, Carbopol may also adopt more favorable macromolecular conformation with increased accessibility of its functional groups for hydrogen bonding. It is speculated that the higher mucoadhesive strength of the delivery system may lead to the prolonged retention and increased absorption across mucosal tissues.[20]

In Vitro Permeation Study

Our previous experiments studying the effect of calcium chelator ethylene-glycol-bis-[β-aminoethylether]-N,N,N', N'-tetraacetic acid (EGTA) on permeability of sumatriptan across sheep nasal mucosal membrane exhibited increased permeability of sumatriptan in pure drug solution on exposure of membrane to EGTA, indicated by the upward shift of time-permeation profile, which was significant over the whole time period of 240 minutes (unpublished data, R.J. Majithiya and R.S.R. Murthy, 2005). This study confirms the importance of chelating calcium for enhancing permeability of sumatriptan. Anionic polymers such as polycarbophil or Carbopol are reported to demonstrate permeation enhancing properties. These polymers were shown to express a high Ca^{2+} binding ability. Therefore, it was important to address the question as to what extent an increase in in-vitro permeation across the nasal membrane could be attained by thermoreversible gels of PF127 and Carbopol. Peff determined for sumatriptan in each of the gel formulations is given in Table 1. Effective permeability of sumatriptan reported in literature was found to be 1.4×10^{-5} (cm s^{-1})[21]; in this study effective permeability of 5.58×10^{-5} (cm s^{-1}) was obtained. This difference in the reported and obtained value may be owing to different mucosal membranes used (reported method used bovine nasal mucosa); also the diffusion cell as well as diffusion medium were different in the 2 methods. Moreover, the reported method determined effective permeability using sumatriptan base, whereas in this study succinate salt of sumatriptan was used. The formulation was to be selected based on the maximum effective permeability obtained. It is evident from the results that the effective permeability coefficient for sumatriptan was significantly lower for formulation PLB and PLC-1 ($P > .001$) as compared with that of pure drug solution. Since PF127 gels are viscous, isotropic liquid crystals consisting of micelles, it was hypothesized that the drug is released by diffusion through the extramicellar water channels of the gel matrix. Peff was not significantly different from the pure drug solution in case of PLC-2 ($P > .001$), while Peff was significantly higher for formulation PLC-3 and PLC-4. Addition of anionic polymer (above 0.3%) to the PF127 has dramatically increased permeation coefficient. Presence of Carbopol results in very rapid dissolution and release of highly soluble drug due to rapid swelling and dissolution of Carbopol at pH 6.4. However, the presence of PF127 in the gel retards the drug release rate slightly owing to reduction in dimension of water channels resulting for enhanced micellar structure. As seen from the results (Figure 3), addition of 0.1% Carbopol to PF127 had no permeation enhancing effect owing to insufficient concentration of Carbopol (0.1%) to enhance drug dissolution from the gel by circumventing PF127 gel network. Although addition of 0.2% Carbopol had permeation enhancing effect as compared with PLB, it was not significantly greater than that obtained with pure drug solution. The addition of 0.3% and 0.5% Carbopol enhanced the permeation of drug from gel significantly. This result could be attributed to increase in concentration of ionized carboxyl group to a level required to cause conformational changes in the polymer chain. Electrostatic repulsion of the ionized carboxyl group results in decoiling of the polymer chain, resulting in the relaxation of the polymer network.[22] At this stage, drug is rapidly dissolved and released from the gels as a result of very high swelling (or fast dissolution) of the ionized Carbopol.[22] Increase in permeation of the drug from the formulation can be further explained on the basis that increase in Carbopol concentration will not only result in an increase in Ca^{2+} binding sites but also in an increase in interaccessibility of Ca^{2+} binding sites owing to relaxation of the polymer network. Similar results have also been obtained in previous studies, where the depletion of Ca^{2+} ions from the extracellular cell medium has been shown to increase the permeation of sodium fluorescein, bacitracin, a vasopressin analog, and insulin.[23,24] Indications also exist that Pluronic poly(acrylic acid) (PAA) can also bind Ca^{2+} in biological milieu.[25] Enhancement of the absorption of drugs loaded into Pluronic-PAA microgels through the epithelial cell monolayer of the upper small intestine has been reported by one of the research groups.[26]

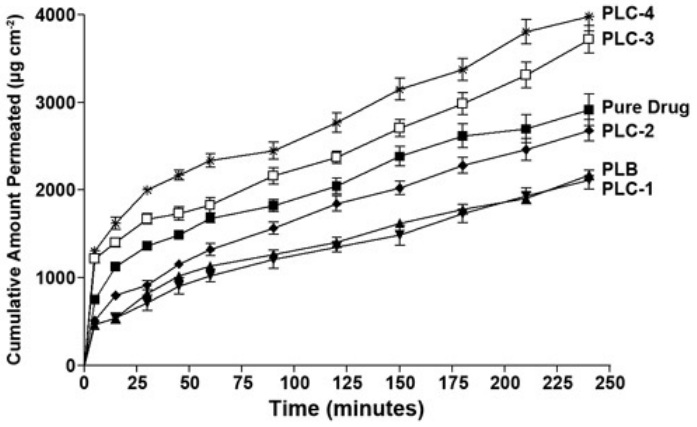

Figure 3. Cumulative amount of sumatriptan permeated across sheep nasal mucosal membrane from pure drug solution and from various PF127 gel formulations at 34°C in Franz diffusion. Data are expressed as mean ± SD (n = 3).

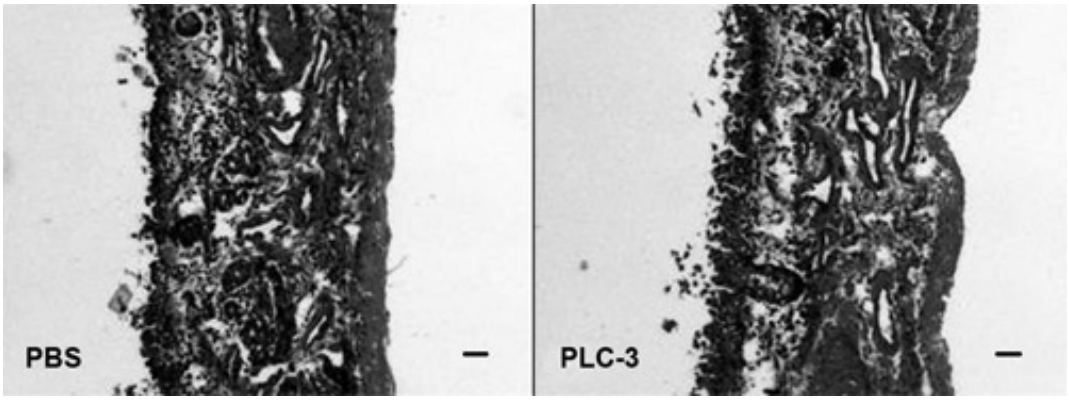

Figure 4. Histopathological evaluations of sections of sheep nasal mucosal membrane. (PBS) Mucosal layer after incubation with PBS (pH 6.4) in diffusion chamber; (PLC-3) Mucosal layer after incubation in diffusion chamber with gel formulation (PLC-3). HE stain; line = 20 μm.

Histological Evaluation of Mucosa After In Vitro Permeation Study

The microscopic observations indicate that the optimized formulation has no significant effect on the microscopic structure of mucosa. As shown in Figure 4, neither cell necrosis nor removal of the epithelium from the nasal mucosa was observed after permeation of PLC-3. The epithelium layer was intact and there were no alterations in basal membrane and superficial part of submucosa as compared with PBS-treated mucosa. Thus, gel formulations seem to be safe with respect to nasal administration.

In brief, PF127 gel (PLB) had favorable rheological and mucoadhesive properties to allow the formulation to gel and adhere to the nasal mucosa after intranasal instillation, thereby decreasing clearance of the formulation at the site of instillation and enhancing drug absorption. Addition of anionic mucoadhesive polymer C934P resulted in reduction of gelling temperature along with increased mucoadhesive potential. The effective permeation coefficient was found to be significantly increased by incorporating 0.3% and 0.5% Carbopol. Taking into consideration all of the aforementioned results, PLC-3 and PLC-4 were found to be suitable in situ gelling formulations with good mucoadhesive potential as well as permeation enhancing effect. However, for formulation PLC-4, gelling temperature was below 25°C, which would make handling of the formulations difficult at room temperature. Thus, PLC-3 with 0.3% Carbopol and 18% PF127 was found to be the best formulation for intranasal delivery of sumatriptan succinate. Histopathological study of the nasal mucosa after permeation study suggested that the formulation was safe for nasal administration.

CONCLUSION

Pluronic F-127 gel formulation with 0.3% Carbopol is a promising nasal drug delivery system for the antimigraine drug sumatriptan succinate, which would enhance nasal residence time owing to increased viscosity and mucoadhesive characteristics; furthermore, it also exhibited a permeation enhancing effect. In conclusion, this study demonstrated that the use of in situ gelling vehicles of PF127 incorporating mucoadhesive polymer Carbopol could effectively and safely improve the nasal residence time and absorption of sumatriptan succinate.

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