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FORMULATION AND EVALUATION OF PIROXICAM LOADED SOLID LIPID NANOPARTICLES FOR TOPICAL DELIVERY

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ABSTRACT

During the recent years, there has been growing attention to the development of topical delivery systems to facilitate drug permeation through the skin. The drugs commonly used are those with debatable oral administration. Piroxicam is a valuable anti-inflammatory, antipyretic and analgesic drug, however, its long-term oral administration is limited due to the various gastrointestinal side effects. The main purpose of this study was to prepare and assess a topical formulation of piroxicam, based on solid lipid nanoparticles (SLNs), to improve its percutaneous permeation rate. Topical Nanolipidic gel of piroxicam was formulated and its pharmaceutical characteristics were evaluated. Piroxicam loaded SLNs were formulated by solvent emulsification/evaporation method. Particle size assessment, entrapment efficiency assessment, *in vitro* release study and skin permeation of the piroxicam were carried out to characterize the SLNs. These SLNs were then formulated in gel as a topical delivery system to assess percutaneous permeation of piroxicam. The SLNs were prepared in different size ranges from 100 to 300 nm and drug release behavior from two different nano-sized SLN suspensions was evaluated. Piroxicam nanolipidic gel exhibited increased skin permeation of the drug over commercial piroxicam gel formulation and also mean particle size of formulated SLNs had a significant effect on permeation rates.

KEYWORDS: Topical Nanolipidic gel, SLNs, piroxicam gel, particle size, gastrointestinal, anti-inflammatory.

INTRODUCTION

Piroxicam is a highly effective non-steroidal anti-inflammatory, anti-rheumatoid arthritis and

analgesic agent. Due to its significant side effects on the gastrointestinal system and some undesirable physicochemical properties such as its poor solubility, it has limited use in therapeutic regimens. A promising approach to overcome piroxicam's limitations is to develop a topical delivery system using an appropriate carrier.

Solid lipid nanoparticles (SLNs) are one of the submicron colloidal carrier systems, with many reported advantages for dermal drug delivery, both for hydrophilic and lipophilic drugs. The advantages attributed to SLNs as a delivery system include the feasibility of controlled and modified drug release system, improvement on drug stability, high drug loading, permeation enhancement and increase in skin hydration. The small drug loaded particles enhance a close contact with the stratum corneum and increase skin penetration which in turn contribute to the increased targeted effect and decreased systemic effect.

SLNs general structure is composed of a solid lipid core e.g. triglyceride, stearic acid, waxes and emulsifiers. Several methods were considered in the preparation of SLNs, with the most relevant of which being solvent emulsification/evaporation method described by Sjostrom and associates. Accordingly, the lipophilic material is dissolved in water-miscible organic solvent (such as acetone). Then, the lipid containing phase is emulsified in an aqueous phase and through the evaporation of solvent, the nanoparticle dispersion is formed by solidification of the lipid in the aqueous medium after evaporation of the organic solvent and diminishing the temperature of the medium to room temperature. In the present study, nanolipidic gel is a term assigned to a system consisting of the SLNs incorporated into a gel base. This system is considered for the delivery of piroxicam through the percutaneous route of administration, given its acceptable biosafety, good stability and low cost of SLNs in comparison with the liposomal dosage form.

MATERIALS AND METHODS

Piroxicam was procured as a gift sample from AlkemLaboratories, Sikkim, India. Phospholipon 80 was obtained as gift sample from Lipoid, Ludwigshafen, Germany. Stearic acid and Plu- ronic F68 were bought from Himedia Chemicals, Mum-bai, India. All reagents and chemicals used in this study were of analytical grade and used as received withoutadditional purification.

In vitro Drug Release Study

In vitro release study of piroxicam SLNs was studied using the Franz diffusion apparatus

method. Typically, 1mL of the centrifuged SLN sediment (As mentioned in the SLN preparation method) was placed on the dialy- sis membrane having a molecular weight cutoff: 10,000-12,000 (HiMedia, Mumbai, India). The membrane was adjusted between the donor and receptor sectionsof the diffusion apparatus which was then filled with 200 mL phosphate buffer, pH 5.8, which served as therelease medium. The release medium was stirred at 175 rpm in a magnetic stirrer (Remi, Mumbai, India) and the temperature was kept at 37 ± 0.5ºC during the experi-mental procedure. At specific time intervals, 1 mL ofaliquot was withdrawn and reinstated with equal volume of fresh buffer to preserve the sink condition. Aliquots were diluted suitably with acetonitrile and the quantity of piroxicam contained in the samples was evaluated using a UV-Visible spectrophotometer (UV-1700, Shi- madzu, Tokyo, Japan) at? max of 230 nm. The releaseof individual SLN formulations was done in triplicate.

Drug Release Kinetics

The *in vitro* drug release pattern from piroxicam SLNs was predicted by applying the drug release data to dif- ferent release kinetic models like: zero-order, first-order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell. For each release kinetics model, the values of R^2 (Correla-tion coefficient) and n (Release exponent of Korsmeyer- Peppas kinetics), K (Rate constant) and SSR (Sum of squared residual) were determined for each formulation and all the models and the possible methods of drug release were identified.

Optimization of Suitable SLN Formulation

Suitable formulation from amongst the prepared piroxi- cam SLN formulations was selected, based on their optimum particle size and zeta potential, high drug loading, entrapment efficiency and better *in vitro* drug release properties. The optimized formulation was fur-ther scrutinized and used for additional studies.

Incorporation of Piroxicam-loaded SLN into GelSystem

Gel containing appropriate formulation of piroxicam SLN and pure piroxicam were made by using 0.5% w/v of carbopol 934P as gel forming polymer. Firstly, gelwas prepared by diffusing carbopol 934P in distilled water containing glycerol (10%) and kept for saturation for 3 h. Lyophilized piroxicam-loaded SLN was admixedwith aqueous carbopl 934P dispersion under controlled stirring using a magnetic stirrer (Remi, Mumbai, India) to obtain uniform and smooth dispersion containinga final concentration of 20% w/w piroxicam-loaded SLN. The pH of the piroxicam-loaded SLN enriched gel was adjusted to 6.0 using sufficient quantity

of tri- ethanolamine. Piroxicam conventional gel was preparedin similar process and was used as reference during effi-cacy evaluation.

Permeability parameter calculation Steady-state flux

Flux is defined as the rate of diffusion or transport of a sub- stance across a permeable membrane. After drug permeation has reached steady state, the steady- state flux was calculated using the following equation.

Steady state flux (Jss) $\lceil dM/S.Dt$ (1)

Where dM is the amount of drug that permeates through a unit cross section area, S, per unit time, t.

The slope of the steady-state portion of the permeation curve created by plotting the cumulative amount of drug permeated in micrograms versus time in hours is the flux.[23]

Permeability Coefficient

The permeability coefficient through the membrane (Kp) was determined according to the following equation.

Permeability coefficient (Kp) $\lceil (Jss.H)/C_0$ (2)

Where H is the thickness of membrane and., C_0 is the initial drug concentration.

Enhancement ratio

This factor was calculated to find the relative enhancement in the flux of formulations in respect to the reference enhance- ment ratio. The enhancement ratio was estimated according to the following equation.

Enhancement ratio (Er) $\lceil Jss$ formulation/Jss reference (3)

Anti-inflammatory activity

The anti-inflammatory activity of the tested preparations and the commercially available preparation were measured and compared. Male Sprague-Dawley rats weighing 180–200 g were used in this experiment. Measurements of the in vivo anti- inflammatory and analgesic activities of the formulae conformed with the guidelines and practices of the Animal Ethics Committee of Universiti Sains Malaysia, and had its approval. The anti-inflammatory action was evaluated using the carrageenan-induced hind paw edema method with a slight modification.[24] Rats were randomly selected and divided into four groups of six animals each. These groups were divided, according to the formulae administered, into control

(vehicle base), F2, F3, and reference gel groups. The animals were housed in polypropylene cages at 25 1 C and 60 5% rela- tive humidity, with free access to food and water.

One day prior to application of the trial formulation, the hair on the dorsal surface of the rat was shaved. F2, F3, refer- ence gel, and control formulae were applied on the shaved dorsal surface by gentle rubbing for 15 seconds. After five hours, 0.1 mL of 1% w/v suspension of carrageenan in normal saline was injected into the subplantar region of the right hind paw of all control and treated rats. Edema volume, in terms of thickness, was measured in all four groups at hours 2, 4, and 6 after carrageenan injection using a micrometer (Ozaki Ltd, Tokyo, Japan).[25] The induced thickness was measured by placing the foot of the rat between the anvil and spindle of the micrometer.

Mathematically, the degree of swelling can be expressed as.

% change in hind paw thickness $(C_t - C_0)/C_0 \times 100$ (4)

Where C_t is hind paw thickness at hours 2, 4, and 6 after injec- tion of carrageenan, and C_0 is the initial hind paw thickness before injection of carrageenan.

Analgesic Activity

This study was conducted similarly to the anti-inflammatory study, except that the pain threshold response of the rat right hind paw was measured instead of edematous thickness. The pain threshold was measured prior to and at hours 2 and 4 intervals after injection of carrageenan using a portable pain threshold device (YMF-P1) equipped with a data acquisition system (developed by the Department of Pharmacology, School of Pharmaceutical Sciences, Uni- versiti Sains Malaysia, Penang, Malaysia). The pressure (g) applied to the edematous hind paw caused the rat to withdraw its hind paw. The vocalization or struggle of the rat was recorded as the pain threshold, as described by Yam et al.[26] Change in pain threshold (g) was calculated as the difference in pain threshold before and after injection of carrageenan.

RESULTS AND DISCUSSION

Droplet size measurements were found to be less than 140 nm for the F1, F2, and F3 formulae as determined by photon correlation microscopy. Droplet size was also measured for formula F3 by TEM.[17] Formula F3 was found to be stable for a three-month period at 40 C, 25 C, and 5 C (data not shown).[18]

Formulation F1 was excluded from this study because it showed drug precipitation within seven days of preparation.[18] Only the F2 and F3 formulae were assessed for their in vitro activity. The polydispersity index of for- mulations F2 and F3 were 0.037 0.006 and 0.052 0.009, respectively.

Drug transfer across cellulose acetate membrane

A drug must be released from its vehicle prior to penetration and partition into the skin. For certain formulations, drug release from the topical preparation is the rate-limiting step for drug absorption. Therefore, to ascertain that drug release from the vehicle was not the rate-limiting step for absorption, diffusion studies through an artificial synthetic membrane using the Franz diffusion cell has been proposed by earlier researchers. The membrane used must be inert and porous so as to allow drug passage in accordance with molecular weight. When drug molecules have a molecular weight as small as the pores of the synthetic membrane, they are able to pass through it.

Drug transfer rates through the cellulose membranes of both nanocream formulae were compared with the transfer rate of the commercially available 0.5% piroxicam gel. Figure 1 shows a considerably higher and faster drug transfer rate across the membrane for nanocream F3 than for nano- cream F2. It can be observed that drug transfer through the membrane is affected by the pH of the external phase of the nanocream.

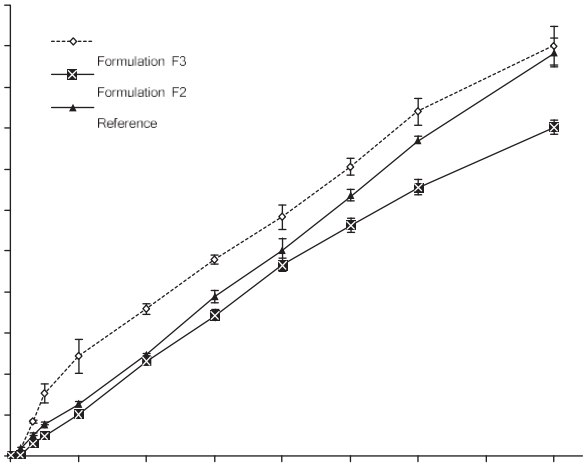

Figure 1: Comparative mean in vitro cellulose acetate membrane transport profiles of piroxicam from formulations F2, F3 and reference gel.

to the difference in the solubility of piroxicam at different pH conditions. Piroxicam with its weak acidic properties is more soluble in pH 7.4 buffer than in pH 6 buffer. Due to its high

solubility at pH 7.4, partitioning of piroxicam from the oil phase to pH 7.4 buffer would be higher, hence leading to a higher drug transfer rate.

Diffusion of the drug incorporated into an oil-in-water cream or nanocream system is affected by partitioning of the drug between the internal oil phase and the external water phase. This means that the drug must diffuse from the internal phase to the external phase where the drug molecules are free to be released. Hence, by increasing drug solubility in the external phase, ie, enhancement of drug partitioning from the internal oil phase to the external aqueous phase, it is possible to observe the driving force for enhanced drug release.[29]

Because the solubility of piroxicam in the mixed surfac- tants system of Tween 80 and Span 20 is higher compared with that in the POEs and buffers pH 6 and 7.4, the piroxicam molecules would then be mainly integrated at the oil water interface.[30] Therefore, it is expected that drug diffusion to the external phase would be faster. The solubility of piroxicam in pH 7.4 buffer is about 10 times higher than that in pH 6 buffer.[17] Presumably, partitioning of piroxicam from the oil to the pH 7.4 buffer would also be about 10 times higher than that in pH 6 buffer. This higher solubility and partitioning would be the driving force for the drug to be transferred through the membrane at a faster rate. Figure 1 shows that 100% of the drug was transferred from formulation F3 within eight hours, while only 80% of the drug was transferred from formulation F2 over the same time period.

Transfer of the drug through the membrane from formula F3 was faster, even during the initial hours, than that from.

CONCLUSION

Formula F3 had a high drug transfer rate through a cellulose acetate membrane compared with F2 and the reference gel during the initial hours of permeation. Both F3 and reference gel showed 100% drug release after eight hours of permeation. However, formulation F2 and F3 exhibited a higher skin per- meation flux at steady state compared with the reference gel. This may be attributable to the reduced droplet size, along with the synergistic effect of the nanocream components which may enhance drug permeability. Formula F3 also dem- onstrated a higher flux compared with F2. This is perhaps due to a difference in partitioning of the drug from the internal oil phase to the external buffer phase of the emulsion system. The higher pH value of the external phase of formula F3 relative to that of F2 leads to higher availability of piroxicam in the external phase, probably leading to a higher release of the

drug from formula F3. Furthermore, the study discovered that the prepared nanocream formulation of F3 and F2 had higher analgesic and anti-inflammatory activity compared with the currently marketed gel. The enhanced reaction may be due to the smaller droplet size along with increased permeability.

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