Transmucosal Permeation of Topically Applied Diclofenac and Piroxicam

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ABSTRACT

Nonsteroidal antiinflammatory drugs (NSAIDs) are frequently used for the treatment of acute myalgias, orthopedic injuries, postoperative pain, chronic rheumatoid arthritis, and osteoarthritis. This study involves the permeation of 2 NSAIDs, diclofenac and piroxicam, from topically applied solutions and gels through human vaginal mucosa as a model of buccal mucosa. Permeation of diclofenac and piroxicam from the solutions and gels through human vaginal mucosa was determined using a flow-through diffusion apparatus. Vaginal specimens were obtained from 8 postmenopausal patients aged 57 ± 16 years (mean ± standard deviation [SD]; range, 32–76 y) after vaginal hysterectomies. Experiments were conducted at 20°C and over a time period of 24 hours. High-pressure liguid chromatography (HPLC) analysis was used as a detection method. Statistical tests used included analysis of variance (ANOVA) and Duncan's multiple range test for determination of steady state and an unpaired *t* test with Welch's correction for comparing differences between the mean flux values at each time point. Flux rates of both diclofenac and piroxicam from aqueous solutions were significantly higher than those from the gels. Steady-state flux rates of the 5 mg/mL diclofenac solution were approximately half that of the 10 mg/mL solution, whereas maximal partitioning to the mucosa from the vehicle and/or saturable diffusion kinetics was achieved at concentrations of 5 mg/mL or less for the piroxicam solutions under the experimental conditions used. The diffusion of diclofenac and piroxicam across mucosal surfaces appears to be more efficient when aqueous solutions of these compounds are used as opposed to gel formulations. This must be considered when vehicles for both these NSAIDs are chosen. There also appeared to be a limit to the release of piroxicam from the vehicle and/or its flux rate across mucosal tissues.

INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAIDs) belong to the most frequently used drugs worldwide, with as many as 8% of the global adult population taking prescribed forms of these agents at any given time.[1] However, it is estimated that they are also responsible for approximately 25% of all adverse drug reaction reports.[2] In this respect, NSAID-related gastrointestinal toxicity is the most frequently observed adverse event, and it is a significant cause of morbidity and mortality.[3] The ingestion of NSAIDs increases the relative risk of upper

gastrointestinal tract bleeding 5-fold,[4] is elevated in the elderly,[5] and might be even higher for certain NSAIDs.[6,7] The NSAIDs are often the first choice of treatment for patients with acute myalgias, orthopedic injuries, postoperative pain, chronic rheumatoid arthritis, and osteoarthritis.[8]

In an attempt to reduce the relatively high incidence of serious adverse effects associated with the systemic use of NSAIDs, a growing number of topical formulations of these drugs have become commercially available. These topical formulations, either on their own or as adjuncts to reduced dosages of systemic agents, have proven to be useful in the management of a variety of musculoskeletal and rheumatic diseases.[2,9] Although the topical NSAIDs have mainly been studied regarding their transdermal diffusion kinetics, these agents might also have useful applications when topically applied to mucous membranes. In this respect, piroxicam, benzydamine, ketoprofen, flunoxaprofen, and diclofenac have all been topically applied to mucosal membranes for a variety of conditions ranging from pain to inflammation.[10–15] However, the majority of the topically available NSAIDs on the market have been formulated for cutaneous application and hence contain components and enhancers suitable for improving skin, but not necessarily mucosal, absorption.

We have previously shown that human vaginal mucosa can be used as a model for the buccal mucosa for in vitro permeability studies of a wide variety of chemical compounds.[16–21] Furthermore, we have demonstrated that both these tissues can be snap-frozen in liquid nitrogen and stored at –85˚C for many months and thereafter used for permeability experiments without significant changes in permeability characteristics.[22]

In view of the fact that the therapeutic efficacy of NSAIDs depends on their penetration into the mucosal and underlying tissues, it was the objective of the present study to investigate the permeability of human vaginal mucosa, as a model of buccal mucosa, to aqueous and commercially available gel forms of diclofenac and piroxicam.

MATERIALS AND METHODS

Vaginal Mucosa

Specimens were obtained from excess tissue removed from 8 postmenopausal patients aged 57 ± 16 years (mean ± standard deviation [SD]; range, 32–76 y) after vaginal hysterectomies at the Louis Leipoldt and Panorama Mediclinic Hospitals, Bellville, South Africa. Surgical specimens were immediately placed in a transport fluid, prepared as previously described,[16–21] and transferred to our laboratory within 1 hour. Excess connective tissue was trimmed away and all specimens were snap-frozen in liquid nitrogen and stored at -85˚C for periods up to 6 months.[22] No specimens were obtained in which there was clinical evidence of any disease that might have influenced the permeability characteristics of the vaginal mucosa.

The study was approved by the Ethics Committee of the University of Stellenbosch and the Tygerberg Hospital.

Permeability Experiments

Before each permeability experiment tissue specimens were thawed at room temperature in phosphate-buffered saline (PBS, pH 7.4). Thereafter the specimens were cut into 5-mm^2 sections and mounted in flow-through diffusion cells (exposed areas 0.039 cm^2) as previously described,[16–21] and permeation studies performed on 7 tissue replicates for each patient. Before beginning each permeability experiment, tissue disks were equilibrated for 10 minutes with PBS (pH 7.4) at 20^{0}C in both the donor and receiver compartments of the diffusion cells. After equilibration, the PBS was removed from the donor compartment and replaced with either 1 mL of 10 mg/mL or 5 mg/mL diclofenac solution in PBS containing 10% ethanol or 0.5 mL of 10 mg/g diclofenac gel (Voltaren Emulgel®, Novartis SA [Pty] Ltd, Rivonia, South Africa). Alternatively, 1 mL of 5 mg/mL, 10 mg/mL of piroxicam solution, or 0.5 mL of the 5 mg/g piroxicam gel

(Rheugesic gel®, Cipla-Medro [Pty] Ltd, Bellville, South Africa) was used. The gels were covered with a Teflon disk and 1 mL of PBS. PBS at 37°C was pumped through the receiving chambers at a rate of 1.5 mL/h and collected, by means of a fraction collector, at 2-hour intervals for 24 hours. The permeability study was performed under sink conditions, ie, at the completion of each run the concentration of diclofenac or piroxicam in the acceptor chamber never reached 10% of that in the donor compartment. The permeant was detected by means of HPLC analysis. Crystalline diclofenac was obtained from Sigma Chemical Company (St. Louis, MO). For the piroxicam solution, a readily available IM solution (Feldene® IM, Pfizer Laboratories [Pty] Ltd, Sandton, South Africa) was used. Each mL of Feldene® IM solution contains 20 mg of piroxicam, 2% m/v benzyl alcohol, and 10% m/v ethanol.

HPLC Detection of Permeants

Permeant–containing effluent samples, collected from the acceptor compartments of the perfusion apparatus over the 2-hour sampling intervals, were analyzed using an Agilent 1100 series high-performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) with a Zorbax XDB C_{18}, 150 mm × 4.6 mm (ID), 5-µm column. The temperature was maintained at 40°C and flow rates of 1.0 mL/min and 1.2 mL/min were used for diclofenac and piroxicam, respectively. The mobile phase consisted of a mixture of 2 solvents, A (50 mM KH_2PO_4, pH 5.42) and B (acetonitrile–isopropanol; 4:1 v/v). Isocratic mixtures of A:B were 65:35 and 58:42 for diclofenac and piroxicam, respectively. Solvents were "pro analisi" (E Merck, Darmstadt, Germany) and were filtered through a 0.45 µm filter. Aliquots of 50 µL from each sample were injected directly into the column. Diclofenac and piroxicam were detected at 273 nm (retention time 2.3 min) and 354 nm (retention time 2.2 min), respectively. Total run time was 3.5 minutes. Recording and integration of peaks was performed by means of an Agilent Chem Station (Agilent Technologies, Waldbronn, Germany). Spiked standards over the expected concentration range (0.5–20 µg/mL) were randomly included in each batch.

Calculation of Flux Values

Flux (J) values across vaginal tissue were calculated by means of the relationship

$$J = Q / A \times t \ (\mu g \times cm^{-2} \times min^{-1})$$

where Q = quantity of substance crossing vaginal tissue (in µg), A = tissue area exposed (in cm^2) and t = time of exposure (in min).

Steady-State Kinetics

When no statistically significant differences ($P < 0.05$) (ANOVA and Duncan's multiple range test) between flux values were obtained over at least 2 consecutive time intervals, a steady-state (equilibrium kinetics) was assumed to have been reached for a particular specimen and diclofenac or piroxicam.

Statistical Analysis

An unpaired *t* test with Welch's correction was used to investigate possible differences between flux means of vaginal tissues at 2-hour intervals. A significance level of $P < 0.05$ was used for all tests and comparisons.

RESULTS

Mean flux values for solubilized diclofenac (5 mg/mL and 10 mg/mL) and gel diclofenac (Voltaren Emulgel®, 10 mg/g) through frozen/thawed vaginal mucosa versus time are shown in Figure 1. Steady-state flux conditions were reached after approximately 9 hours across vaginal mucosa for both the diclofenac solutions and gel. Mean steady-state flux values of 1.682 ± 0.164 (standard error of mean [SEM]) µg/cm^2/min and 3.969 ± 0.292 (SEM) µg/cm^2/min were found for the 5 mg/mL and 10 mg/mL solutions, respectively. The diclofenac gel (10 mg/g) yielded a mean steady-state flux value of 0.626 ± 0.058 (SEM) µg/cm^2/min, which is found to be on average 6.3 times lower than the 10 mg/mL diclofenac solu-

tion used in the permeability experiments. Statistically significant differences ($P < 0.05$) between flux rates of both diclofenac concentrations (5 mg/mL and 10 mg/mL) and diclofenac gel (10 mg/g) across vaginal mucosa were found after approximately 6 hours. The diclofenac solution had higher flux rates than the gel form, whereas the flux rates of the 5 mg/mL diclofenac solution were found to be approximately half that of the 10 mg/mL diclofenac solution (Fig. 1). Mean apparent release constants (slopes) and lag times (x-axis intercepts) were obtained by linear regression analysis of plots of cumulative amount of released drug ($\mu g/cm^2$) versus square root of time ($h^{1/2}$).[23–25] For diclofenac 5% and 10% solutions and gel, apparent release constants were 751.9 ± 34.8, 1826 ± 74.9, and 288.6 ± 9.7 $\mu g/cm^2/h^{1/2}$, lag times were 2.91, 2.87, and 2.66 $h^{1/2}$, and r^2-values were 0.9873, 0.9900, and 0.9931, respectively (Fig. 2). The non-linearly related portions of the data curves, ie, <3 $h^{1/2}$, were excluded from the linear regression plots for both diclofenac and piroxicam and hence are not shown in the figures (Figs. 2 and 4).

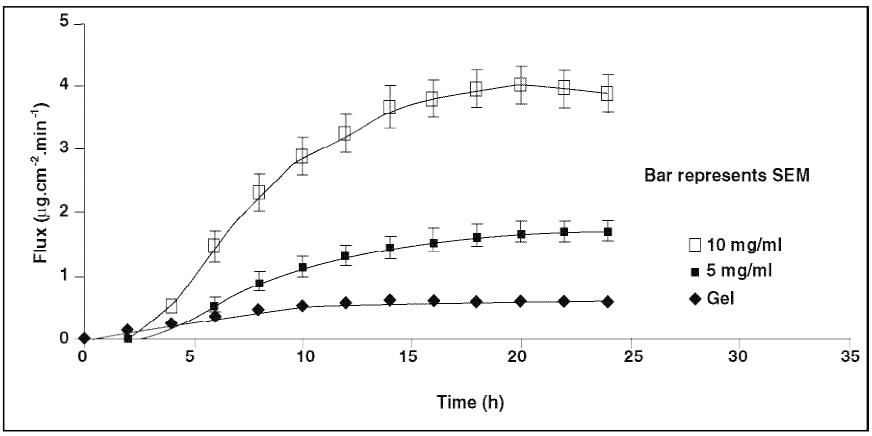

Figure 1. Mean flux values for diclofenac solutions (5 mg/mL and 10 mg/mL) and gel (10 mg/g) across human vaginal mucosa.

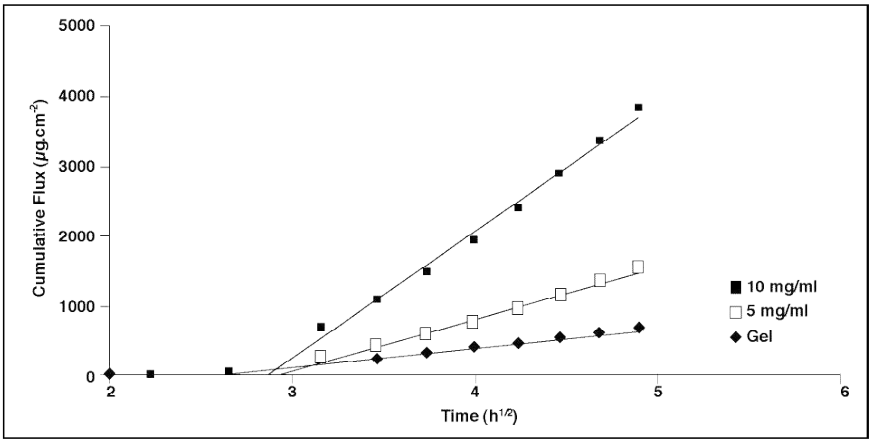

Figure 2. Cumulative flux values for diclofenac solutions (5 mg/mL and 10 mg/mL) and gel (10 mg/g) across human vaginal mucosa.

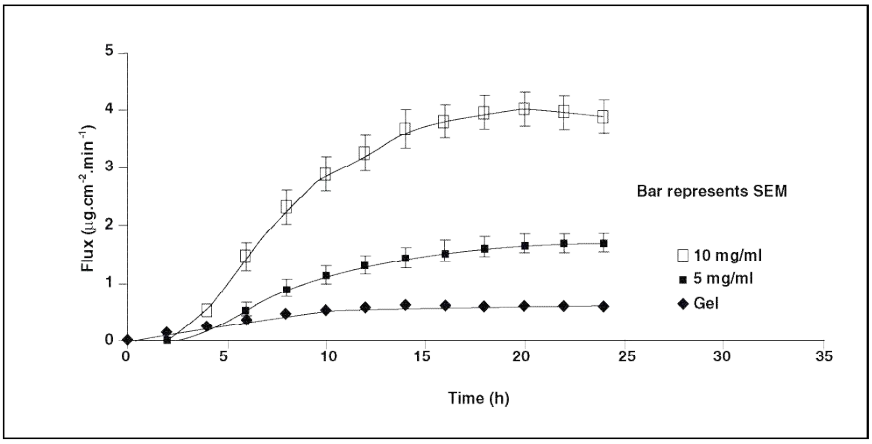

Figure 3. Mean flux values for piroxicam solutions (5 mg/mL and 10 mg/mL) and gel (5 mg/g) across human vaginal mucosa.

Overall mean flux values for piroxicam intramuscular injectable solution (Feldene®, 5 mg/mL and 10 mg/mL) and piroxicam gel (Rheugesic gel®, 5 mg/g) across vaginal mucosa over time are shown in Figure 3. Steady-state flux values were achieved after approximately 6 hours across the vaginal mucosa for both the piroxicam solutions and

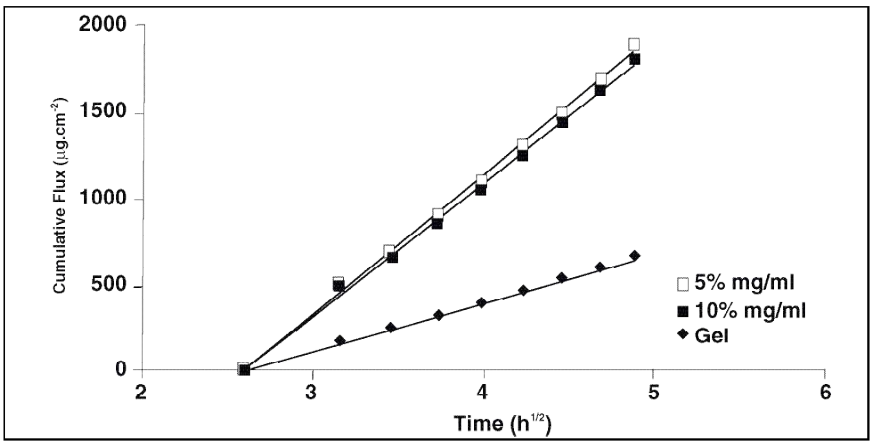

Figure 4. Cumulative flux values for piroxicam solutions (5 mg/mL and 10 mg/mL) and gel (5 mg/g) across human vaginal mucosa.

the gel. Mean steady-state flux rates of 1.665 ± 0.160 (SEM) μg/cm²/min and 1.578 ± 0.128 (SEM) μg/cm²/min were obtained for the 5 mg/mL and 10 mg/mL piroxicam solutions, respectively. The piroxicam gel (5 mg/g) yielded a mean steady-state flux rate of 0.589 ± 0.038 (SEM) μg/cm²/min, which was approximately 2.8 times lower than the flux rate obtained for the 5 mg/mL piroxicam solution. Significant differences (P <0.05) between flux rates across vaginal mucosa of the gel and either of the 2 solutions were found from approximately 4 hours onward. However, no statistically significant differences in flux values were obtained between the 2 piroxicam solutions (5 mg/mL and 10 mg/mL) used. For piroxicam 5% and 10% solutions and gel, apparent release constants were 808.1 ± 21.6, 778.3 ± 18.0, and 286.2 ± 6.1 μg/cm²/h$^{1/2}$, lag times were 2.59, 2.59, and 2.61 h$^{1/2}$, and r^2-values were 0.9957, 0.9968, and 0.9973, respectively (Fig. 4).

DISCUSSION

Mucosal delivery of therapeutic agents involves the penetration of a drug into mucosal surfaces either for the purpose of treating diseases or alleviating symptoms in deeper lying tissues, or to treat systemic disease by achieving systemically active levels of the agent. To achieve the required degree of penetration, not only the properties of the membranes involved, but also the chemical nature, size and conformation, lipid/water partition coefficient, and degree of ionization of the permeant molecules are important.[26] The transmembrane diffusion process is passive in nature and depends on a concentration differential as the driving force, each molecule requiring kinetic energy to effect a net movement down this gradient. Permeant molecules must therefore diffuse through the vehicle in which they are contained to the mucosal interface and have to partition from the formulation into the upper layers of the tissue. From here molecules must diffuse within the mucosa, equilibrating laterally, and must emerge, eventually under steady-state conditions, from the distal surface of the tissue. Adsorptive interaction might be extensive in this layer, forming a reservoir of the permeant molecules. Further partitioning into neighboring tissue strata or into the receptor fluid then takes place under the influence of the concentration gradient, and adsorption might occur once again. Diffusion through any one of the layers or any of the partitioning events might form the rate-limiting step controlling the overall rate of permeation. Initially the concentration gradient across the mucosa will not be linear as the permeant equilibrates within the tissue. However, after sufficient time has elapsed, steady state will be achieved and the effective permeant concentration at all points in the tissue will remain constant.

Penetration of drugs into tissues is dependent on the influence of the vehicle on the thermodynamic activity of the active ingredient. Diclofenac and piroxicam are sparingly soluble in water, hence their release is favored from aqueous solutions and hydrophilic bases such as gels, which are poor solvents for these drugs. As a result, there are less drug–vehicle interac-

tions in these solvent systems and this leads to improved partitioning into the mucosa. The pKa-value of diclofenac is 4, whereas that of piroxicam is 6.3 (dioxane:water 2:1).[27] At the pH values of the PBS buffer system used in this study, ie, 7.4, approximately 99.9% of the diclofenac and approximately 92.6% of the piroxicam are present in their dissociated forms. Although this improves diffusion through the hydrophilic outer layers of the epithelium, it does not facilitate penetration of the lipoidal layer, which is thought to constitute the major permeability barrier of vaginal and buccal mucosa and is located in the outer third of the epithelium.[26,28]

It is clear that the flux rates across vaginal mucosa of the aqueous solutions of diclofenac are statistically significantly higher than those of the gel (Fig. 1). This is also reflected in the relative apparent release constants, showing that the release of diclofenac from the gel formulation is well described by the "Higuchi" model in which the rate-controlling step is the process of diffusion through the gel matrix (Fig. 2).[23–25] This relationship exists for formulations in which the drug is fully dissolved or is in suspension. Hence, the mucosa has no significant effects on drug release, the latter being controlled mainly by the properties of the formulation. This might be attributed to the higher viscosity of the gel matrix compared with the aqueous solutions as well as the presence of a fatty emulsion base containing isopropanol and propylene glycol, all of which retard partitioning of the diclofenac to the mucosal surface. The effect of these cosolvents on the penetrability of diclofenac has been well documented.[29] Similar observations for flux rates and apparent release constants were made for the injectable piroxicam solutions and the gel (Figs. 3 and 4). Although the piroxicam gel contained 0.01% m/m benzyl alcohol as a preservative, the injectable solutions had a considerably higher total alcohol content (benzyl alcohol, 2% m/v and ethyl alcohol, 10% m/v). Both of these compounds can be classified as chemical penetration enhancers, ie, they might improve drug diffusion by modifying the thermodynamic properties of the drug or by violating the mucosal barrier making it more permeable. However, the point at which substances become enhancers has not been well defined. Both of these alcohols are also cosolvents and aid solubilization of the almost water-insoluble piroxicam into the gel vehicle, thereby negatively affecting its partitioning into the mucosa. The net effect of these complex interactions between the constituents of the mucosa, piroxicam, and the components of the donor vehicle that might enhance drug permeability or retain it in the formulation are difficult to predict. It is also interesting to note that notwithstanding the fact that the concentration of piroxicam in the 10 mg/mL solution was double that of the other solution, no statistically significant differences between the steady-state flux rates for these 2 solutions were found. This appears to indicate that maximal partitioning to the mucosa from the vehicle and/or saturable diffusion kinetics for piroxicam across the tissue is achieved at concentrations of 5 mg/mL or less under the experimental conditions used.

CONCLUSION

We have demonstrated that diffusion of diclofenac and piroxicam into mucosa appears to be more efficient when aqueous solutions of these compounds are used instead of gels formulated for transcutaneous use. This aspect should be kept in mind when vehicles for both these NSAIDs intended for intraoral, or even intravaginal, transmucosal use are chosen. Furthermore, there appears to be a limit to the release of piroxicam from the vehicle and/or its flux rate across mucosal tissues. These aspects will, however, require further investigation.

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