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RESEARCH PAPER

Comparison of the Transdermal Absorption of Nimesulide from Three Commercially Available Gel Formulations

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ABSTRACT

*Nimesulide is a non-steroidal anti-inflammatory drug (NSAID) applied topically for a variety of conditions characterized by pain and inflammation. One of the aims of this study was to compare the permeation profile of nimesulide from the commercially available transdermal gel formulations across dermatomed porcine and human skin. The in vitro transdermal absorption of nimesulide formulations across porcine skin and human skin was studied for 24 hr using a continuous flow-through diffusion cell. The three commercial gels used in this study were Nimulid®, Nise*Gel®, and Orthobid®. All gels contained 1% (w/w) nimesulide. An infinite dose of nimesulide gel (about 300 mg) was applied on the skin over 0.636 cm^2 surface area. The rank order for the drug permeation from these formulations using porcine skin was: Nimulid > Orthobid > Nise*Gel. The rank order of the permeation across human skin was: Nimulid > Nise*Gel > Orthobid. The permeation profiles followed zero-order kinetics without any significant lag time. The steady-state flux of nimesulide from Nimulid was significantly higher than that of Nise*Gel and Orthobid in both porcine and human skin ($p < .05$). However, there were no significant differences in the delivery of nimesulide (24 hr) from Nise*Gel and Orthobid across both human and porcine skins. The results suggest that the Nimulid gel may have a greater bioavailability of nimesulide compared to the other gels. In addition, permeation profiles of the various gels across porcine skin did show a positive profile behavior to human skin.*

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However, the in vitro drug release of nimesulide gels across a synthetic membrane did not correlate with skin permeation profiles.
Key Words: *Drug release; Human skin; Nimesulide gels; Porcine skin; Transdermal absorption*

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used medications in the world. They act by inhibiting the enzyme cyclooxygenase (COX), which is responsible for the formation of prostaglandins, and are important mediators of inflammation. Major limiting side effects of chronic NSAID use include gastrointestinal symptoms (10–20% chronic duodenal ulcers) and renal dysfunction.

There are two COX isoforms, COX-1 and COX-2. They differ mainly in their pattern of expression. COX-1 is constitutively expressed in most tissues and initiates the production of prostaglandins that regulate gastrointestinal function. It also initiates thromboxane A_2 that stimulates platelet aggregation and helps maintain normal homeostasis. COX-2 is induced during inflammatory states and leads to the production of prostaglandins that mediate inflammation, pain, and fever. The recent identification of COX-1 and COX-2 isoforms, together with the demonstration that COX-2 predominates in inflammatory conditions, has led to the discovery and development of COX-2-selective NSAIDs with less deleterious effects (1–5).

Nimesulide (4-nitro-2-phenoxymethanesulfonanilide) is one of the newer NSAIDs that acts through selective inhibition of COX-2. It is used in the treatment of various inflammatory diseases and is chemically unrelated to the other acidic NSAIDs such as acetylsalicylic acid and indomethacin. Nimesulide is weakly acidic (pK_a=6.5) and contains a sulfonanilide moiety as the acidic group (1,2). Its potent analgesic activity and lack of topical irritation has led to the development of several topical formulations in Europe and Asia. The potential advantage associated with topical drug delivery includes avoidance of first-pass gut and hepatic metabolism, and adverse side effects (including gastrointestinal reactions and idiosyncratic drug reactions). Specific advantages of topical applications of NSAIDs include dose-dependent reduction of localized pain and edema, and systemic benefits in the management of inflammatory conditions such as osteoarthritis, rheumatic arthritis, and fever.

In transdermal therapy, the formulation composition may have a significant effect on the drug delivery and bioavailability. In the present study, we investigated the permeation profiles of nimesulide from three popular commercially available transdermal gels in the Indian market across dermatomed porcine and human skins. Furthermore, we studied whether the various formulations exhibited a similar skin permeation behavior with porcine and human skin, and the suitability of using porcine skin as a substitute for human skin in skin permeation studies. Many researchers have suggested that porcine skin is a good animal model for human skin, citing that porcine and human skin have similar surface lipids, barrier thickness, and morphological aspects (6–8). Access, cost, and availability of human skin have been a barrier for many researchers.

Another objective was to characterize the release profile of nimesulide from the commercial gels. Unlike permeation studies, which represent an overall physicochemical diffusion of a drug, in vitro drug release reflects the ease with which the drug leaves its vehicle. An optimized formulation delivers a thermodynamically stable release of the drug, while optimizing its absorption through the skin. Permeation and drug release studies are important in the evaluation of bioavailability and batch-to-batch quality control, respectively.

MATERIALS AND METHODS

Materials

A synthetic hydrophilic membrane (GH Polypro filter, Waters Corporation, Philadelphia, PA) with a pore size of 0.45 μm was used for in vitro drug release studies. The commercial nimesulide gels used in this study were Nimulid® (Panacea Biotec., New Delhi, India), Orthobid® (Nicholas, Deonar, India), and Nise*Gel® (Dr. Reddy's Laboratories, Bollaram, India). All gels contained 1% w/w nimesulide. All other chemicals were of reagent grade and

used as received from the suppliers. Human skin of uniform thickness (500 μm) was obtained commercially from NDRI (National Disease Research Interchange, Philadelphia, PA). Porcine skin was obtained from a local slaughterhouse (Bradley's Country Store, Tallahassee, FL) and dermatomed (Padgett dermatone, Kansas City, MO) to 500 μm.

In Vitro Drug Release Study

The synthetic membrane was mounted onto a Franz diffusion cell (PermeGear, Riegelsville, PA). The receiver compartment contained 6.5 mL of phosphate buffer (pH 7.4). A dose (~300 mg) was applied to the synthetic membrane over a 1.131 cm^2 area in the donor compartment. The donor cell was exposed to ambient temperature and covered with parafilm in order to prevent evaporation. The temperature of the receiver compartment was maintained at 32°C, and the buffer solution was stirred continuously with a Teflon-coated magnetic bar. Samples were collected at 0.5, 1, 2, 4, 6, and 12 hr. At each sample interval, 6.5 mL of the buffer solution was removed and placed with fresh solution to maintain sink conditions. The samples were analyzed by high-performance liquid chromatography (HPLC).

Skin Permeation Studies

All epidermal preparations were mounted onto an automated, temperature-controlled, continuous flow-through diffusion cell system maintained at 32°C (PermeGear, Riegelsville, PA). An infinite dose of nimesulide gel (~300 mg) was applied to the skin over a 0.636 cm^2 area. A phosphate buffer solution (pH 7.4) was passed through the receptor chamber at a controlled rate using an Ismatec IP multi-channel peristaltic pump. Samples were collected at 1, 2, 4, 6, 8, 12, 18, and 24 hr using a retriever IV fraction collector (Gilson, Inc., Middleton, WI) operated by an index controller (PermeGear, Riegelsville, PA). The samples were analyzed by HPLC.

HPLC Analysis

A Waters Corporation HPLC with a 717 autosampler operated by Millennium 32 software was used for analyzing the samples. The mobile phase consisted of a mixture of 60% water and 40% acetonitrile containing ammonium phosphate dibasic salt. Prior to use, the mobile phase was filtered through a 0.45 μm filter and degassed in an ultrasonic bath for 30 min. The ultraviolet (UV) detector was set at a wavelength of 230 nm, and a flow rate of 1 mL/min was equilibrated using a C18 column.

Data Analysis

Concentrations of nimesulide samples were analyzed, and the cumulative amount permeated was plotted against time. The steady-state fluxes (μg/cm^2/hr) were determined by taking the slope of the linear portion of the plot according to Fick's equation (9). Analysis of variance (ANOVA) was performed for multiple comparisons followed by Tukey's test. The data was considered to be significant at $P < .05$. The drug release rate constant was determined by taking the slope of the plot of cumulative amount released vs. square root of time.

RESULTS AND DISCUSSION

In Vitro Drug Release of Nimesulide Through Synthetic Membrane

The in vitro drug release reflects the thermodynamic driving force for the release of nimesulide from its gel vehicle and its diffusion across a synthetic membrane. The in vitro release profiles of nimesulide from the various gels using Franz diffusion cells are shown in Fig. 1 and the drug release rate constants are given in Table 1. The cumulative amount of nimesulide released through the synthetic membrane vs. square root of time showed a linear relationship, indicating matrix diffusion-controlled release kinetics. There was a significant correlation with in vitro drug release as a function of square root of time for all three formulations of nimesulide, and their correlation coefficients are as follows: Nimulid (r^2=0.9915, $P < .0001$); Orthobid (r^2=0.9991, $P < .0001$); Nise*Gel (r^2=0.9864, $P < .0001$). The in vitro drug release of nimesulide was highest from Orthobid followed by Nise*Gel and Nimulid. The data obtained show that Orthobid released three to four times more nimesulide from its vehicle compared to Nise*Gel and Nimulid. The differences in drug release rates may be attributed to the different composition of their vehicles. The excipients in the three formulations are proprietary and are not listed in the label. Nimesulide is relatively lipophilic with low aqueous

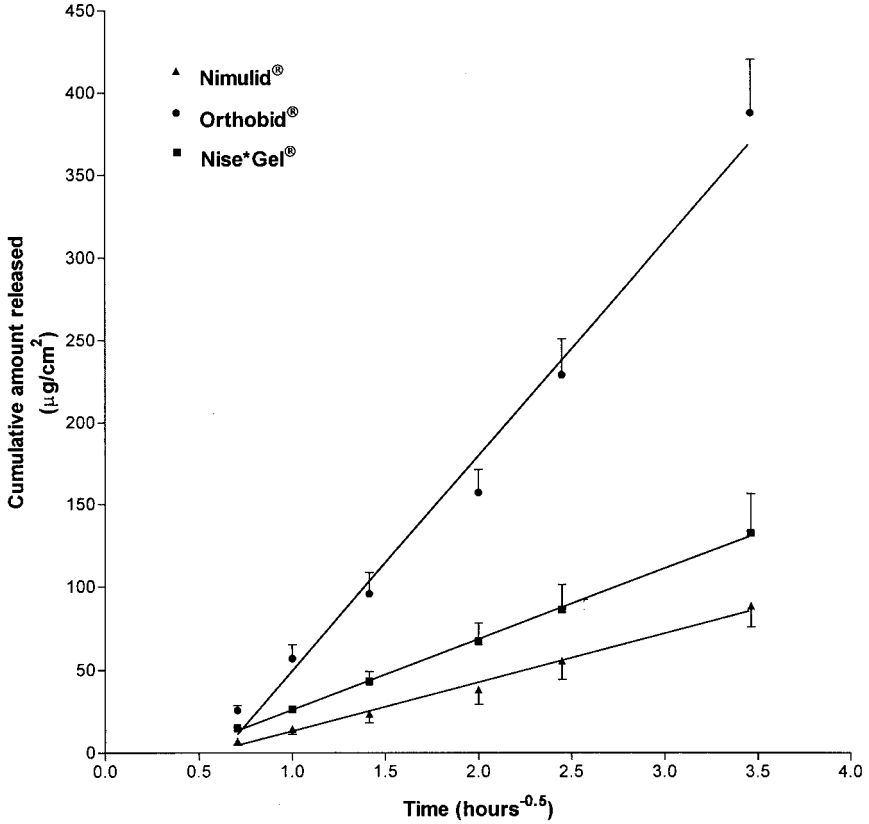

Figure 1. In vitro release profiles of nimesulide from commercial gels across synthetic membrane (GH Polypro filter, Waters Corporation, Philadelphia, PA). Each data point represents the mean and SD (n=6).

Table 1

Steady-State Flux Values of Nimesulide Across Porcine and Human Skin and Release Rate Constants of Nimesulide from Three Commercial Gels. Each Data Entry Represents the Mean and SD. The Value in Parentheses Represents the Number of Replicates

Nimesulide Gels	Steady-State Flux Values (μg/cm^2/hr)		Release Rate Constant (μg/cm^2/hr$^{-0.5}$)
	Porcine Skin	Human Skin	
Nimulid	1.626 ± 0.06 (13)	1.586 ± 0.11 (14)	29.40 ± 3.43 (6)
Orthobid	1.372 ± 0.059 (11)	1.1251 ± 0.02 (7)	130.00 ± 7.93 (6)
Nise*Gel	1.418 ± 0.062 (12)	1.188 ± 0.041 (14)	42.47 ± 4.81 (6)

solubility (0.01 mg/mL). The label contents are listed as 1% w/w nimesulide in a soluble gel base or polymeric aqueous base. In addition, Nimulid lists the contents of ethanol (66% v/v) in an aqueous polymeric base. We suspect that the other preparations contain significant amounts of ethanol to increase its solubility in the aqueous gel. The solubility of nimesulide in these vehicles might be different, resulting in different thermodynamic activities of nimesulide. The thermodynamic activity of the drug in the formulation can be manipulated by using a binary mixture in which one component is a good solvent and the other is a non-solvent. Slow addition of the non-solvent to the solvent creates a

saturated formulation that can have a significant driving force for the release and penetration of the drug across synthetic membranes, as well as into skin. Increasing the thermodynamic release of a drug from its vehicle might improve percutaneous absorption, however, in vitro drug release cannot be used to predict permeation across the skin. This is due to the barrier properties of the stratum corneum, which can be altered by the composition of the vehicle. This superficial layer is composed of distinct protein and lipid domains. The major protein component of the stratum corneum is composed of intracellular keratin. Both keratin and the viable epidermis are hydrophilic in nature and can be rate-limiting to the absorption of lipophilic drugs. The lipid domain comprises a highly structured distribution of intercellular lamellae (10), and constitutes a highly effective lipophilic barrier to polar chemical penetrants and permeability. In order to enhance the drug percutaneous absorption, the vehicle should reasonably increase the solubility of the drug in both the hydrophilic and hydrophobic media of the skin. Our data shows that the in vitro drug release profiles did not correlate with any skin permeation profiles. Nimulid's in vitro drug release profile revealed that it released the least amount of nimesulide from its vehicle compared to the other gels, despite having the highest permeation rates in both human and porcine skin. This confirms the complicated diffusional barrier the skin possesses to the inward movement of xenobiotics.

Permeation of Nimesulide Formulations Across Porcine Skin

The permeation profiles of nimesulide from the gels across porcine skin followed near zero-order kinetics without any significant lag time (Fig. 2). There was a significant correlation between the amount permeated with time for all three formulations across porcine skin, and their correlation coefficients are as follows: Nimulid (r^2=0.9962, $P < .0001$); Orthobid (r^2=0.9991, $P < .0001$); Nise*Gel (r^2=0.9990, $P < .0001$). The steady-state flux values of nimesulide across porcine skin are shown in Table 1. From the porcine studies, statistical comparison using one-way ANOVA showed that there was a significant difference between the amounts of drug permeated across porcine skin from the three formulations over a 24-hr period ($F_{(2,32)}$=131.6, $P < .001$). Permeation of nimesulide across porcine skin was significantly higher from Nimulid compared to Orthobid and Nise*Gel ($P < .001$). Nimulid exhibited about 20% greater percutaneous (24-hr) delivery of nimesulide than the other two gels. The permeation rates of nimesulide across porcine skin from both Nise*Gel and Orthobid were similar, and indicated no significant difference ($P > .05$).

Permeation of Nimesulide Formulations Across Human Skin

The permeability of isolated human skin has been well characterized and the in vitro procedures used in this work have been shown to provide reliable prediction of in vivo performance (11–14). Although such data cannot be extrapolated to a clinical situation, the differences in nimesulide delivery from these formulations across the skins may markedly affect its therapeutic efficacy. As observed with porcine skin, the permeation profiles of nimesulide from the gels followed near zero-order kinetics without any significant lag time (Fig. 3). Also, there was a significant correlation between the amount permeated with time for all three formulations across human skin, and their correlation coefficients are as follows: Nimulid (r^2=0.9974, $P < .0001$); Orthobid (r^2=0.9980, $P < .0001$); Nise*Gel (r^2=0.9984, $P < .0001$). The permeation of nimesulide across human skin was significantly different among the three formulations ($F_{(2,33)}$=61.93, $P < .001$). Nimulid exhibited 30% greater percutaneous delivery of nimesulide over a 24-hr period compared to the other two gels. The permeation rates of nimesulide across human skin were similar from Nise*Gel and Orthobid ($P > .05$). Ethanol has several functions in topical formulations, including solubility, preservative, and skin penetration-enhancing effects. Broad studies have shown the effect of ethanol in skin permeation is dependent on its concentration in topical vehicles, as well as the lipophilicity of the drug used. Several studies have tried to elucidate the enhancing mechanisms of ethanol. They include: delipidization and pore formation of the stratum corneum at high concentrations, lipid fluidization and solvent drag at low concentrations (15–17). This may have contributed to the greater flux for Nimulid gel. This in vitro study proves that vehicle composition possesses a

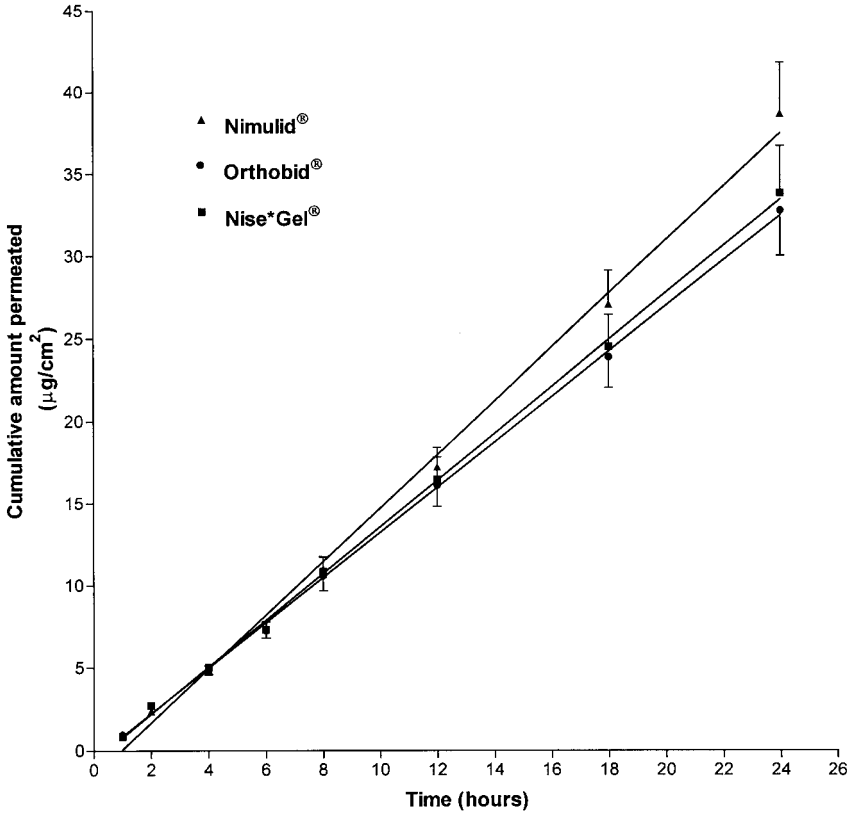

Figure 2. Permeation profiles of nimesulide from commercial gels across porcine skin. Each data point represents the mean and SD (n=11–13).

strong activity in the transdermal absorption of this drug. The rat carrageen-induced pleurisy and carrageen in-paw edema is a well-tried model of acute inflammation in which a variety of NSAIDs have been shown to reduce a variety of inflammatory parameters (18–20). The topical application of many NSAIDs, including nimesulide gels, has shown similar log-linear dose–response curves in the management of inflammation and moderate pain (21,22). Thus, the present study suggests that Nimulid may deliver more nimesulide in vivo and may provide better therapeutic levels of the drug.

Human vs. Porcine Skins: In Vitro Profiles

Porcine skin has been reported to have the characteristics closest to those of human skin (23). Although the anatomical site of application can influence skin absorption rates (24), porcine ear skin has been reported to be a good anatomical region, and its permeation characteristics are close to those of human skin (25). In our laboratory, we have studied the permeability characteristics of several chemicals across human and porcine skins. Our results show that the flux across porcine skin is similar to that of human skin for melatonin and tridecane (26,27). One of our objectives was to check whether the flux of nimesulide from formulations across porcine skin is similar to that across human skin. In this study, we observed that the flux values of nimesulide from Nimulid across both human and porcine skins were similar, and indicated no significant difference ($P > .05$). However, Orthobid and Nise*Gel flux values of nimesulide were slightly higher than those of human skins. Compared with human data, the flux values of nimesulide through porcine skin were within a 20% variation. In transdermal delivery via passive diffusion, there are usually large variations in absorption rates among

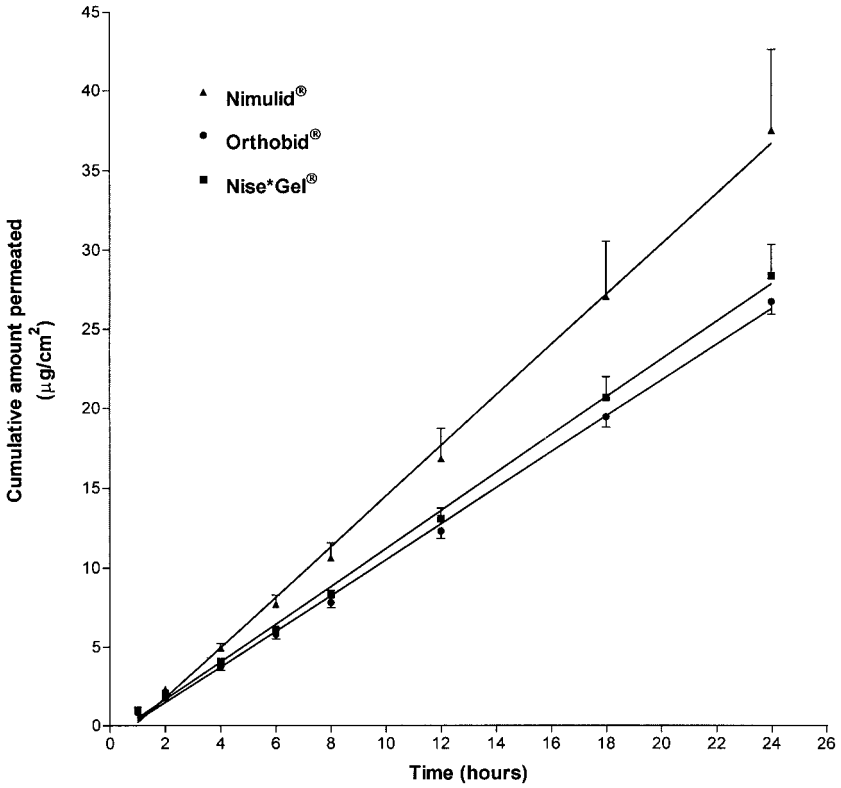

Figure 3. Permeation profiles of nimesulide from commercial gels across human skin. Each data point represents the mean and SD (n=7–14).

individuals, even with comparative sites of application. Our results using porcine skin were close to those with human skin, and are in agreement with other researchers (8,25). Porcine skin reproducibility and its similarity to human skin provide further evidence that porcine skin can be considered as an animal skin model when human skin is unavailable.

Skin appendages are an easily identified break in the continuity of the stratum corneum that can act as a low resistance conduit for drugs to reach the vasculature of the skin (28). This could have contributed to the greater permeability rates in porcine skin. Although human skins are relevant for comparative diffusional studies, porcine skin appears useful for estimation of in vitro human skin permeation behavior. In addition, porcine skin can be useful in preformulation work, to help establish if adequate quantities of a drug can feasibly be administered by a transdermal route.

In conclusion, we have demonstrated that the permeation of nimesulide is formulation-dependent. The percutaneous absorption of nimesulide gels across porcine skin shows a positive correlation with human skin. Our results provide further evidence that porcine skin can be used as an in vitro model to replace human skin for diffusional studies. Nimulid had the most significant permeation rates of nimesulide across both human and porcine skins. However, the in vitro drug release of nimesulide gels across a synthetic membrane did not correlate with skin permeation profiles.

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