DIFFUSION OF DICLOFENAC AND PIROXICAM FROM COMMERCIALLY AVAILABLE GELS THROUGH HUMAN SKIN

VAN DER BIJL P, BChD, PhD, DSc[1]
VAN EYK AD, PhD[2]
SEIFART HI, Dr rer nat[2]
DE JAGER R, Physiotherapy student[3]
NEL CMM, Physiotherapy student[3]

[1] Professor and Head of the Department of Pharmacology, Faculty of Health Sciences, University of Stellenbosch.
[2] Senior Researchers, Department of Pharmacology, Faculty of Health Sciences, University of Stellenbosch.
[3] Physiotherapy Students, Department of Physiotherapy, Faculty of Health Sciences, University of Stellenbosch.

ABSTRACT: *Continued interest in the use of topical non-steroidal anti-inflammatory drugs (NSAIDs) by physiotherapists prompted us to investigate the in vitro transdermal diffusion of diclofenac from Voltaren Emulgel® and Zeroflam Gel® and piroxicam from Rheugesic Gel®. Human skin (snap-frozen in liquid nitrogen, stored at -85°C) was used for permeability experiments. The permeation of diclofenac and piroxicam from the gels through thawed/frozen skin was determined using a flow-through diffusion apparatus (37°C, 24h). Diclofenac and piroxicam concentrations were measured using high-pressure liquid chromatography (HPLC). An unpaired t-test with Welch's correction was used to test for steady state and for differences between the mean flux values at each 2-h time point (significance level of 5%). No statistically significant differences were found between the flux rates across skin for diclofenac from the two formulations and it was therefore suggested that the cheaper of the two formulations be used in physiotherapy practice. Although the flux rates of piroxicam were significantly lower than those of diclofenac across human skin, it does not necessarily follow that the former drug is clinically less efficaceous. The results from this study have demonstrated the usefulness of a flow-through diffusion system for enabling physiotherapists to study the trancutaneous diffusion kinetics of NSAIDs in vitro. Further studies with other topical NSAIDs are therefore warranted.*

KEY WORDS: NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS), DICLOFENAC, PIROXICAM, HUMAN SKIN, PHYSIOTHERAPY PRACTICE.

INTRODUCTION

The topical non-steroidal anti-inflammatory drugs (NSAIDs) form part of the armamentarium available to the physiotherapist for treating patients with certain musculoskeletal and arthritic conditions. These drugs, which are well recognized for their analgesic properties as well as for reducing swelling and inflammation, increase mobility of muscles or joints thereby facilitating the efficacy of and compliance with physiotherapy treatment (Whelan and Walker 1996). Using this combined-modality drug/physiotherapy treatment approach, pain and inflammation can be reduced and patients maintained at their optimal functional level (Moncur and Williams 1995).

Topical NSAIDs, although expensive, have better safety profiles than their systemically administered counterparts due to their lower plasma concentrations, an observation in accord with their dose-dependent toxicity. However, side-effects due to these topically administered drugs do occur and may include the full spectrum of adverse reactions including gastrointestinal, renal, cardiac, cutaneous and hypersensitivity reactions. Although NSAIDs penetrate skin slowly, they accumulate in the dermis and achieve levels in the muscle tissue below the site of application which are at least equivalent to those obtained with oral administration (Heyneman et al 2000).

Although a wide variety of NSAIDs, including indomethacin, ketoprofen, diclofenac, piroxicam, tenoxicam, ketorolac and aceclofenac have been studied with regard to their transdermal diffusion characteristics, their passage across skin was not always found to be optimal (Singh and Roberts 1994; Cordero et al 1997; Wenkers and Lippold 2000). In this respect, product formulation may have a dramatic impact, not only on transcutaneous absorption rates, but also on depth of penetration into the underlying tissues (Heyneman et al 2000).

Previous *in vitro* permeability studies have enabled us to thoroughly evaluate a continuous flow-through perfusion system to determine the diffusion characteristics of a wide variety of therapeutic agents and other chemical compounds through fresh and frozen human vaginal, buccal and intestinal mucosa, skin and cornea (Van der Bijl et al 1997; 1998a,b,c; 2000; 2001; Van der Bijl and Van Eyk 2002). The main advantages of this system include the use of small tissue samples and the maintenance of a continuous high gradient of permeant across the biological barrier to be studied. Furthermore, *in vitro* systems of this nature are being considered for the

CORRESPONDENCE TO:
Professor P van der Bijl
Department of Pharmacology,
Faculty of Health Sciences,
University of Stellenbosch
Private Bag X1, Tygerberg 7505
South Africa
Tel: +27-21-938 9331
Fax: +27-21-932 6958
E-mail: pvdb@sun.ac.za

purpose of bioavailability/bioequivalence studies in the regulatory process for therapeutic agents (Van der Bijl and Van Eyk 2002).

In view of the above considerations, the potential clinical implications for physiotherapy practice and the costs involved for patients, the *in vitro* diffusion system described was used to study the diffusion characteristics of two different commercially available topical formulations of diclofenac and one of piroxicam, across frozen/thawed human skin.

METHODS AND MATERIALS

3.1 Skin

Skin specimens were obtained from excess tissue removed from 12 females, mean age 40 ± 17 SD (range: 17 - 75) years, during breast reduction procedures at the Louis Leipoldt Hospital, Bellville, South Africa. No specimens were obtained where there was clinical evidence of any disease that might have influenced the permeability characteristics of the skin.

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

3.2 Opical NSAID preparations used

Voltaren Emulgel® was obtained from Novartis SA (Pty) Ltd, Rivonia and Zeroflam Gel®, as well as Rheugesic Gel®, from Cipla Life Sciences (Pty) Ltd, Bellville. Both Voltaren Emulgel® and Zeroflam Gel® contained 1.16g diclofenac diethylammonium equivalent to 1g diclofenac sodium/ 100g (ie 10mg/g). Rheugesic Gel® contained 5 mg/g of piroxicam.

3.3 Permeability Experiments

All skin specimens were immediately placed in a transport fluid after removal and transferred to our laboratory within two hours. The transport fluid consisted of a stock solution of Eagle's Minimum Essential Medium (MEM) without L-glutamine and sodium bicarbonate (Gibco, Paisley, Scotland), to which the latter as well as an antibiotic (penicillin/streptomycin, 100 IU/ml) and an antimycotic (amphotericin-B, 2.5 μg/ml) were added prior to using it for the transport of tissue specimens. In the laboratory, excess connective tissue was trimmed away, and specimens from each patient cut into 10 mm diameter disks and snap-frozen in liquid nitrogen and stored at -85°C. Prior to use the frozen samples were thawed and hydrated in MEM for at least 24 hours at 4°C. Thawed tissue disks were mounted in flow-through diffusion cells (exposed areas 0.196 ± 0.002 SD cm^2) as previously described (van der Bijl et al 2000), and permeation studies performed on 7 tissue replicates for each patient. Tissue disks were equilibrated for 10 minutes with phosphate-buffered-saline (PBS, pH 7.4) at 37°C in both the donor and receiver compartments of the diffusion cells. Following equilibration, the phosphate-buffered-saline (PBS) was removed from the donor compartment and replaced with either 0.5ml of Voltaren Emulgel®, Zeroflam Gel® or Rheugesic Gel®. The gels were covered with a teflon disk and 0.5ml of PBS. PBS at 37°C was pumped through the acceptor chambers at a rate of 1.5ml/h and collected, by means of a fraction collector, at 2-h intervals for 24 hours. The permeability study was performed under sink conditions, i.e. at the com pletion of each run the concentration of NSAID in the acceptor chamber never reached 10% of that in the donor compartment. The diclofenac and piroxicam were quantitated by means of high-pressure liquid chromatography (HPLC) analysis.

3.4 HPLC detection of diclofenac and piroxicam

Permeant-containing effluent samples, collected from the acceptor compartments of the perfusion apparatus over the two hour sampling intervals, were analysed using an Hewlett Packard 1100 series high-performance binary liquid chromatograph (Agilent Technologies, Waldbronn, Germany) with an Agilent Eclipse (XDB-C18) Zorbax analytical column (5μm particle size), 150 mm x 4.6 mm (ID). The latter column was preceded by a 30 x 2.1 mm (ID) C18 guard column (40μm particle size). 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 two solvents, A (50mM 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. All reagents used for the mobile phase were HPLC grade (Burdick & Jackson, Honeywell International Inc, Muskegon, MI, USA) and were filtered through a 0.45μl filter. Deionized water was used for preparing all aqueous standard and buffer solutions. Aliquots (50μl) from each sample were injected directly into the column. Diclofenac and piroxicam were detected at 273 nm (retention time 2.3 minutes) and 354 nm (retention time 2.2 minutes), respectively. Total run time was 3.5 minutes. Recording and integration of peaks was performed by means of an Agilent Chem Station. Spiked standards over the expected concentration range (0.5-20μg/ml) were randomly included in each batch.

3.5 Calculation of Flux values

Flux (J) values across membranes were calculated by means of the relationship: $J = Q / A \times t$ (μg x cm^{-2} x min-1), where Q = quantity of substance crossing membrane (μg), A = membrane area exposed (cm^2) and t = time of exposure (min).

3.6 Steady State Kinetics

It was assumed that when no statistically significant differences at the 5% level (t-test with Welch's correction) between flux values were obtained over at least two consecutive time intervals, a steady state (equilibrium kinetics) had been reached for a particular tissue specimen and permeant.

3.7 Statistical Analysis

An unpaired t-test with Welch's correction was used to investigate possible differences between flux means of diclofenac and piroxicam from the gels, across skin at 2-h intervals. A significance level of 5% was used for all tests and comparisons.

3.8 Cost

Ten retail pharmacies in the Cape Peninsula were randomly selected from the 2002-2003 Telkom Yellow Pages. These pharmacies were telephonically contacted and the retail prices of Voltaren Emulgel® (50g), Zeroflam Gel® (40g) and Rheugesic Gel® (40g)

Figure 1: Mean flux values for diclofenac ex Voltaren Emulgel ® and Zeroflam Gel ® and piroxicam ex Rheugesic Gel ® across the human skin.

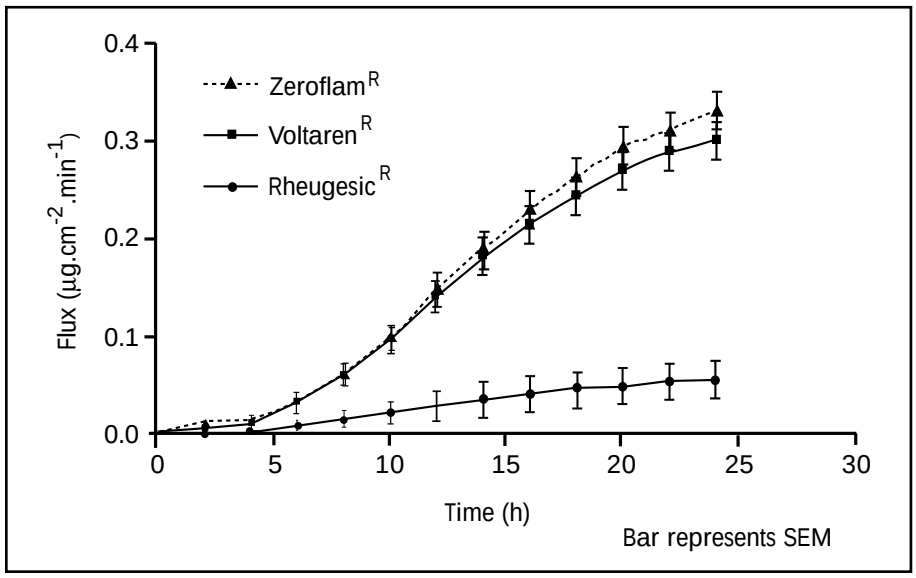
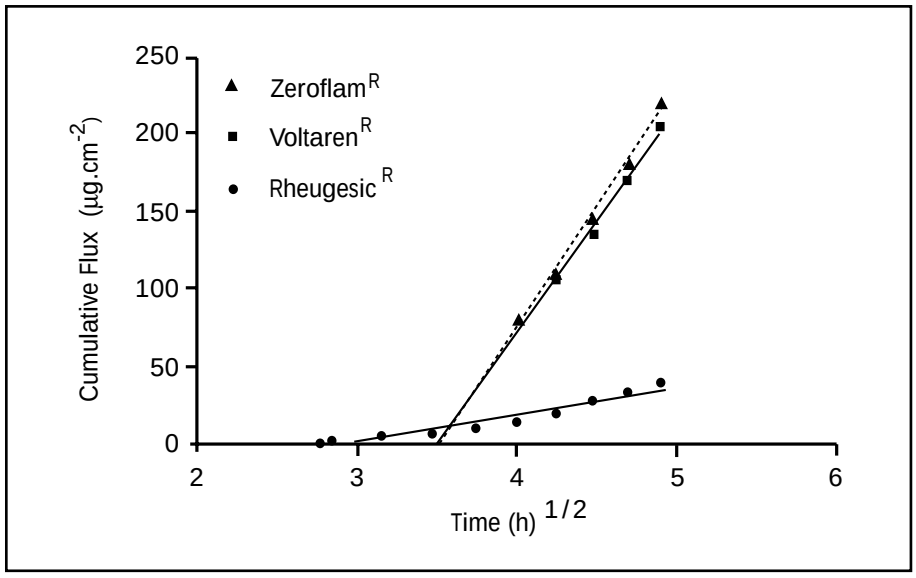


Figure 2: Cumulative flux values for diclofenac ex Voltaren Emulgel ® and Zeroflam Gel ® and piroxicam ex Rheugesic Gel ® across the human skin.



obtained. Hereafter, the average price per gram for each of these gels was calculated.

RESULTS

Mean flux values for diclofenac from Voltaren Emulgel® and Zeroflam Gel®, as well as piroxicam from Rheugesic Gel®, across human skin are shown in Figure 1. Steady-state flux conditions were reached between approximately 12 to 14 hours for diclofenac from Voltaren Emulgel® and Zeroflam Gel® and after 6-8h for piroxicam from Rheugesic Gel®, and were estimated to be approximately to be 0.251 ± 0.019 (SEM), 0.271 ± 0.022 (SEM) and 0.032 ± 0.005 (SEM) $\mu g.cm^{-2}.min^{-1}$, respectively. Although there was a tendency for the flux rates of diclofenac from Zeroflam Gel® to be 5 to 10% higher than that from Voltaren Emulgel® between 12 and 24h , these differences were not statistically significant ($p>0.05$). However, the steady state flux values between the diclofenac released from Voltaren Emulgel® and Zeroflam Gel® were were approximately 8 times higher than those of piroxicam from the Rheugesic Gel® and statistically significantly different. 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 ($h^{1/2}$) (Higuchi 1962, Guy and Hadgraft 1990, Rahman et al 1990). For diclofenac from Voltaren Emulgel® and Zeroflam Gel® and piroxicam from the Rheugesic Gel®, apparent release constants were 143.2 ± 3.5, 153.8 ± 3.9 and 17.42 ± 1.33 $\mu g.cm^{-2}.h^{-1/2}$, lag times were 3.49, 3.50 and 2.90 $h^{1/2}$ while r^2-values (goodness of fit of data points to the linear regression line) were 0.9976, 0.9974 and 0.9557, respectively (Figure 2). The non-linearly related portions of the data curves, ie <4 $h^{1/2}$, for Voltaren Emulgel® and Zeroflam Gel® and <3 $h^{1/2}$ for Rheugesic Gel®, were excluded from the linear regression plots and hence are not shown in the figures.

Mean retail prices for Voltaren Emulgel®, Zeroflam Gel® and Rheugesic Gel® per gram were R1.10 (range: R1.00 - R1.30), R0.88 (range: R0.57 - R1.03) and R0.90 (range: R0.72 - R1.23), respectively.

DISCUSSION

In addition to the wide range of activities in which physiotherapists engage to restore, maintain and improve the health and physical function of their patients, they can contribute further to the well-being of their patients by the judicious use of topical NSAIDs in acute musculoskeletal and, possibly, rheumatic conditions. These drugs can reduce pain, stiffness, movement limitations or swelling associated with either soft tissue injuries or rheumatic dysfunction. The topically applied NSAIDs may be of particular benefit in older patients because they are more prone to developing toxic reactions due to the systemic forms of these drugs. Moreover, the elderly are also particularly subject to toxic reactions as well as to drug interactions due to the multiple therapeutic agents which they often receive.

Apart from the efficacy of the topically applied NSAIDs, it is incumbent on the physiotherapist to select those agents which are most cost-effective. Several grams of the topical agent are usually applied over a relatively large skin area, making the use of this form of administration expensive. This may impose certain limitations on the use of these agents, particularly in the under-

privileged elderly, who often fall in the category of patients for which these drugs are indicated. Furthermore, the topical NSAIDs may be excluded from certain medical aid benefits, making the access to these drugs difficult for those patients who need them most. The cost savings incurred by using cheaper, equivalent agents are therefore significant.

It is clear from the present study that although the flux rates at steady state (>12-14h) of diclofenac from Zeroflam Gel® were 5 to 10% higher than those from Voltaren Emulgel®, no statistically significant differences between these two formulations could be demonstrated. Furthermore, the lag times (corresponding to time taken for drugs to be released from the gels and to diffuse across the membrane, as well as representing steady state when squared) and mean apparent release constants (release rates of diclofenac from the gels), were also comparable. However, when comparing the retail cost of each preparation per gram, the Zeroflam Gel® was at least 20% cheaper than the Voltaren Emulgel®.

The steady state flux rates, lag times and mean apparent release constants of piroxicam were statistically significantly lower than those of diclofenac across human skin, steady state flux rates being reached after approximately 6-8h. However, diclofenac is usually systemically administered in doses ranging from 75 to 100mg, a therapeutic dose range which is approximately 4 times higher on a w/w basis than the usual systemic doses of piroxicam (20mg) (O'Hanlon et al 1996). When compared to diclofenac, piroxicam has a much longer plasma half-life (50h vs 1-2h) and can also inhibit activation of neutrophils, independent of its ability to inhibit the cyclooxygenase enzyme (Roberts and Morrow 2001). Hence, additional modes of anti-inflammatory action on this drug have been proposed including inhibition of proteoglycanase and collagenase in cartilage (Roberts and Morrow 2001). Furthermore, the concentration of piroxicam in Rheugesic Gel® is only 5mg/ml as opposed to the 10mg/ml of diclofenac in Voltaren Emulgel® and Zeroflam Gel®. The lower, concentration-driven flux rates of piroxicam compared to diclofenac, may be offset by the additional modes of anti-inflammatory action of the former drug, its longer plasma half-life as well as its more rapid achievement of steady state, and consequently these differing flux rates may be clinically unimportant.

In conclusion, diffusion rates through human skin of diclofenac from two different formulations commercially available on the South African market, were indistinguishable. Price would therefore be the major determinant in choosing between these two formulations. Although piroxicam was found to have flux rates across human skin which were significantly lower than those of diclofenac, this does not necessarily imply that the former drug is clinically less efficacious. Furthermore, we also demonstrated the usefulness of the *in vitro* flow-through diffusion system to study the diffusion characteristics of NSAIDs through human skin. Bioavailability/bioequivalence studies on drugs using a system of this nature are much cheaper to perform than clinical studies and enable physiotherapists to compare different commercially available topical formulations of NSAIDs in order to make rational choices between these products. Further studies using a variety of other topically available forms of this class of drugs are therefore warranted.

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