International Journal of Drug Delivery 5 (2013) 245-256

http://www.arjournals.org/index.php/ijdd/index

Review Article

ISSN: 0975-0215

Deep Percutaneous Penetration into Muscles and Joints: Update

Vladyslava Doktor[1,2], Christine M. Lee[2], Howard I.Maibach[2]

***Corresponding author:**

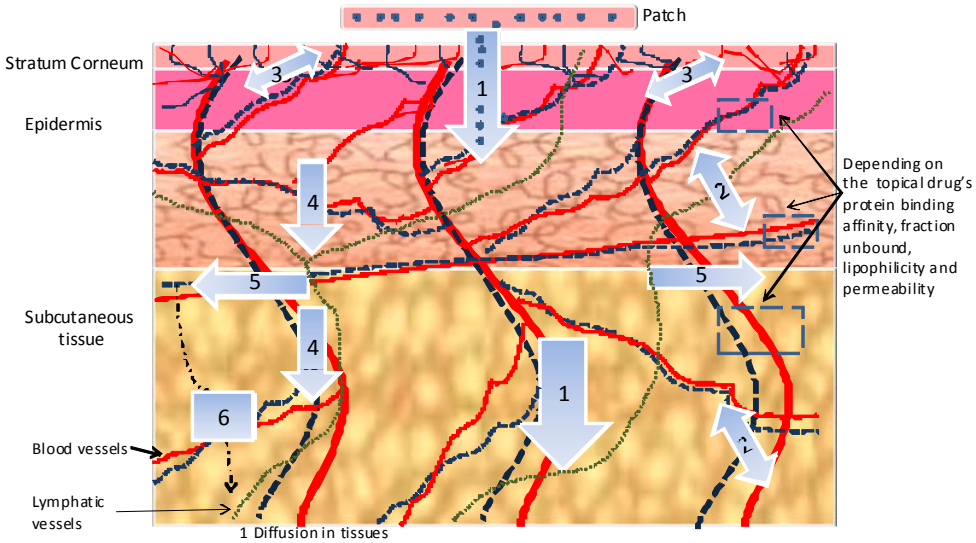
Vladyslava Doktor

[1]Touro College of Osteopathic Medicine, New York
[2]Department of Dermatology, University of California San Francisco, 90 Medical Center Way, Box 0989, San Francisco, CA 94143-0989

Abstract

Transdermal drug delivery is a multifactorial process with variable penetration mechanisms. Adverse effects associated with nonsteroidal anti-inflammatory drugs' (NSAIDs) use in treatment for joint and muscle diseases are sufficiently severe to consider topical application. A drug's lipophilicity, fraction unbound and permeability found in the viable skin are some of the physiochemical factors influencing the delivery mechanism of transdermal absorption. These and other variables play a role in determining if the drug reaches the deep tissues via direct penetration from dermal or systemic blood redistribution. Pharmacokinetic models have been developed to help elucidate penetration routes and efficacy for various drugs. Improvements in modes of transdermal delivery through active research projects including relevant animal models and human translational research may introduce advances in clinical development of treatments.

Keywords: topical administration; non-steroidal anti-inflammatory agents; skin absorption; percutaneous drug delivery



Introduction

Percutaneous delivery of drugs into deeper tissues for the treatment of inflammatory muscle, joint and tendon diseases is a much discussed, but not fully educated topic. With many now living past the 70s; the age when the risk levels for inflammatory diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA) increase, the need for an efficient and transdermaly delivered medication is substantial.[1] Since oral NSAIDs pose a risk of adverse effects such as renal, hepatic, cardiac, and

gastrointestinal toxicity, topical use of NSAIDs is necessary. The benefit of a transdermal therapeutic system (TTS) results in a bypass of the gastrointestinal tract and associated side effects. Steps the topical compounds take to reach the joints and underlying muscular tissue are partition and diffusion, primarily through the outermost skin layer, stratum corneum (SC), epidermis, dermis and subcutaneous fat. The challenge remains to overcome the resistance of SC and of course, to reach the deeper tissue without losing much of the compound's concentration to cutaneous microcirculation. However, earlier work showed that local subcutaneous drug delivery is viable and can be effective.[2] Novel approaches to enhance the penetration of the SC and transdermal drug delivery are now available.[3] Penetration mechanisms and efficacy of absorption of topically applied drugs have reached new heights. Pharmokinetic properties of topical NSAIDS have been studied in length as they are the main focus for the local treatment of several inflammatory diseases of joints and muscles.[2] Some common NSAIDS are salicylic acid (SA), diclofenac, ketoprofen and naproxen. Diclofenac is the only drug in the group of NSAIDS approved for use in the United States. Available formulations are diclofenac epolamine topical patch 1.3%, diclofenac sodium topical gel 1%, and diclofenac sodium topical solution 1.5% w/w in 45.5 % dimethyl sulfoxide (DMSO). The latter two are specifically approved by the United States Food and Drug Administration for osteoarthritis treatment.[4]

Following from a previously published review of percutaneous penetration new advances in skin barrier function, physicochemical, pharmokinetic and physiological factors that ground the transdermal administration have been made and are discussed in detail here.[5] The groundwork for determining bioequivalence via kinetics has not been laid.

Drug Properties Affecting Distribution

As a drug transports through the first barrier, SC, passive diffusion serves as a main mode. Whether it is via intercellular or transcellular pathway, factors mostly involved are lipophilicity and permeability.[6] Other important factors affecting absorption include drug molecular size and water solubility, vehicle, skin integrity, which mainly is affected by the disease, body site and age.

Lipophilicity

Lipophilicity is defined by log P, the logarithmic octanol-water coefficient.[7] High lipophilicty is needed to overcome SC barrier and to penetrate to deeper tissues. The higher the lipophilicty the deeper the solute will be transported. It is also directly proportional to increase in log P.[8] For example, the lipophilic salt of SA, triethanolamine salicylate (TEA), penetrated more efficiently into muscle compared to ionized SA.[9] Another study performed *in vivo* on male rats demonstrated almost two- fold greater penetration of a lipophilic derivative of SA (10% TEA) compared to the less lipophilic derivative than TEA, 10% methyl salicytate, with the topical application of the two compounds on the abdomen of male rats.

If the solution is not sufficiently lipophilic to penetrate the SC enhancers such as propylene glycol, alcohol, dimethyl must be utilized. Diclofenac sodium 1% gel uses isopropyl alcohol, propylene glycol and water to accelerate drug penetration of the skin. The alcohols typically promote drug solubility and also aid in permeation of the SC. However, higher concentration of alcohol may cause SC dehydration, which will in return impact drug permeability.[10]

Permeability

Another drug property contributing to the penetration ability to underlying muscles and joints is permeability,[11] which can be measured by the permeability coefficient, k_p. Permeability coefficient increases with an increase in the fraction of unionized drug,[12] and has a parabolic relationship with log p, the lipophilicity of a compound[2] for several NSAIDs, as reported in an *in vitro* study of human cadaveric skin from the mid abdomen following delivery of the drug via a donor compartment. An optimal log p value will yield a peak permeability coefficient. This observation suggests that a hydrophilic-lipophilic balance is required for deep penetration of drugs.[2] By taking the product of k_p and the solubility of the drug in a given vehicle, the maximum flux, J_{max}, through the skin can be calculated. However, a study showed that molecular weight (MW) is a more significant determinant of maximal flux than k_p.[13]

Molecular Weight

Previously, MW was not believed to be a significant determinant of transdermal drug delivery. Even though earlier studies showed that with increasing MW, clearance of the drug from viable skin into the muscle decreases, results were not statistically significant.[14] A review of J_{max} versus several parameters of the drug including MW, solubility in octanol, octanol/water partition coefficient, ultimately showed that MW was the dominant determinant of J_{max}, with the following regression relationship: log J_{max} = -3.90 to 0.0190 MW(r^2=0.847, p<0.001).[13] Data was gathered from several experiments performed on human skin in vitro. The results changed previous views on which factors affect the maximal flux and transdermal drug distribution.

Fraction Unbound Drug in Viable Skin

Interestingly, unbound fraction of drug in viable skin (fu_{vs}) had statistically significant positive correlation (p<0.005, R^2 = -0.63) with the drug clearance from viable skin to the muscle (Table 1).[14] As fu_{vs} increased, an increased in clearance from the viable skin into the muscular layer was observed. The study was performed on stripped-skin rats in vivo after application of drug to the rat's abdomen via a donor cell for 0.5,1, 2, 3, 4, and 7 h. In viable skin, drugs may bind to the cytosolic components, influencing direct penetration.[15] When a drug is not bound to

proteins or lipids, the molecule diffuses more easily into deeper tissues including the muscle. However, it is not known why the increase in fraction of unbound drug in viable skin is not an important factor for systemic absorption. Perhaps the equilibrium between the unbound fraction among the viable skin, muscular layer, and plasma may play a role.[14]

Relative Importance of the Physicochemical Factors

Among the factors presented above, it seems that the fraction unbound in viable skin is important for the direct penetration of a compound. Penetration rate was characterized by a kinetic parameter, k_{direct}, which was found to correlate with the clearance from the viable skin to muscle.[14] Multiple linear regression analysis was then used to determine the relative contribution of MW, log p, and fu_{vs} on the penetration rate. The results were 0.1588 for MW, 0.2686 for log p, and 0.5726 for fu_{vs}.[14] This analysis showed that fu_{vs} had the greatest contribution to the penetration rate, as measured by k_{direct}.

Pharmacokinetic Models

One of the first pharmacokinetic models was developed in 1982, which was a four compartment model with the epidermis, dermis, capillaries, and urine using first order kinetics. Two fixed rate constants were used to characterize absorption and elimination, while two variable rate constants helped define the penetration through the viable skin and the competition for the drug between the viable skin and the stratum corneum. Guy et al. found that topical delivery produces high local subcutaneous levels of drug despite reduced blood concentrations.[16] Direct deep penetration is thus the mechanism implicated. The blood flow in skin, particularly in dermis, is a crucial factor in deciding the direct penetration of drugs into muscle. On the one hand, blood vessels in the dermis absorb and dilute most compounds passing the epidermis, keeping a "sink" condition and promoting percutaneous absorption.[17] On the other hand, blood flow prevents drugs from directly penetrating into deeper tissues by removing them to the systemic circulation. Absorption can also be modified by vasoconstrictors. A recent vivo study on rats by Higaki et al. showed that topical application of a vasoconstrictor, phenylephrine, enhanced the direct penetration of the drugs into viable skin and muscle.[18] Phenylephrine was co administered with several drugs via a donor cell, and the drugs' concentrations measured after 2 h of application time on the rat's abdomen. Distribution of antipyrine ($p<0.001$), SA ($p<0.001$), and diclofenac ($p<0.01$) into viable skin yielded statistically significant increase in penetration when phenylephrine was co administered versus control. Similar results were seen for distribution into the muscle layer: antipyrine ($p<0.001$), SA ($p<0.01$), and diclofenac ($p<0.05$).[18]

Multiple groups have analyzed drug distribution kinetics following percutaneous delivery. Singh et al. developed a pharmacokinetic model using multiple differential equations to estimate the drug concentrations found below the epidermis after topical application of a drug.[19] Importance of the pharmacokinetic model is that it can predict the amount of drug that eventually will reach the deep tissue compartments, providing useful information for the development of controlled dosing methods.

Distribution to underlying tissues was modeled as multiple compartments in series, with the dermis and deep muscle in contact with the systemic circulation. Separate differential equations were developed to characterize drug concentration in four or more compartments: the application cell, the dermis, the underlying tissue, and the systemic circulation.[19]

Experimental Methods

Drug solutions were applied to rats and subsequently the underlying tissue concentrations and clearance values were taken. Since rats and humans have somewhat similar permeability characteristics, animal models were used to examine the pharmacokinetic properties of the drug after percutaneous delivery.[20] Solutions were applied to exposed rat epidermis in absence of SC. A glass cell containing the drug solution was attached to the exposed dermis, and was removed at predetermined times to measure solute concentration. Blood samples were taken from the tail vein. Afterwards, the animals were sacrificed and tissues below the treated site were dissected to analyze drug concentrations.[7]

Table 1. Effect of Physiochemical Prameters on $CL_{vs\ m}$ and Peak Muscle Concentration*

Drug	MW (g/mol)	fu_{vs}	$CL_{vs\ m}$ (mL/h)	Peak Muscle Concentration (nmol/g)[a]
Diclofenac	295.1	0.592	0.008±0.000	6.0±0.5
Salicylic acid	138.1	0.870	0.085±0.004	40±5
Ketoprofen	254.3	0.701	0.133±0.069	15±2
Felbinac	212.2	0.667	0.043±0.000	19±2
Flurbiprofen	244.3	0.401	0.032±0.000	33±5

The clearance of a drug from the viable skin into the muscle, $CL_{vs\ m}$, and peak muscle concentration are examined in relation to the molecular weight, MW, and the fraction of unbound drug found in viable skin, fu_{vs}. The study was performed on stripped-skin rats *in vivo* after application of the drug to the rat's abdomen via a donor cell. The rats were sacrificed at 0.5, 1, 2, 3, 4, and 7h.

*Data from [14]; [a]Peak muscle concentration estimated from Higaki et al., Figure 3.[14]

Model Predictions

The model predicted tissue concentrations of different NSAIDs, in some cases within 90% of actual value. With indomethacin, however, there was a higher observed value than predicted, suggesting that indomethacin has poor diffusiblity and accumulates in the dermis. [7] Inaccuracies in the predictions may be due to inherent differences in tissue affinities for different solutes, nonlinearities in tissue binding (although trace concentrations of all solutes were used to avoid the nonlinearities in plasma and tissue binding), variations in plasma protein binding, dermis water partitioning, tissue-plasma partitioning, and possible drug effects on membrane or blood flow. The apparent tissue–tissue clearances may also vary for different compound[7].

Table 2. Relative Contributions of Different Penetration Mechanisms for NSAIDs*

Drug	Direct Penetration (%)	Distribution from Systemic Circulation (%)
Diclofenac	79.0	21.0
Salicylic acid	72.0	28.0
Ketoprofen	69.4	30.6
Felbinac	43.3	56.7
Flurbiprofen	62.5	37.5

The relative contribution of two delivery routes was calculated based on curves derived from Higaki's six-compartment pharmacokinetic model. The study was performed on stripped-skin rats *in vivo* after application of the drug to the rat's abdomen via a donor cell.
*Taken from Higaki et al.[14]

Table 3. Comparison of Two Different Pharmacokinetic Models

	Singh and Roberts [7]	Higaki et al. [14]
No. of compartments	9	6
Model arrangement	Donor cell and deep tissue compartments in series arranged in parallel to plasma	Donor cell, viable skin, and muscle in series arranged in parallel to both plasma nad contralateral skin, muscle
Experimental studies	*In vitro* human study on epidermal penetration	*In vitro* human study on epidermal penetration
	In vivo rat study on transdermal absorption	*In vivo* rat study on transdermal absorption
Parameters from in vitro studies	k_p (epidermal permeability coefficient) Log p (lipophilicity)	k_{direct} (penetration rate constant from viable skin to muscular layer) fu_{vs}
Application	To predict deep tissue concentrations and compare with observed values	To formulate standard curves to approximate the decreasing concentrations in deep tissues
Statistical significance	N/A	CV ranges from 0.1% to 13%, R^2 greater than 0.994 for standard curves

Two of the pharmacokinetic models used to approximate deep tissue drug contcentrations with differential equations and nonlinear regression analysis. The experimental methods, applications, and statistical sifnificance of the results are examined.

Higaki et al. also developed a pharmacokinetic model, this time with six compartments including
contralateral skin and contralateral muscle, which were not included in the Singh et al. model.[14] Inclusion of the contralateral side allows the contribution of the absorption from the systemic circulation to the muscular disposition to be evaluated more precisely. In vivo rat transdermal studies were performed by comparing model predictions to experimental results.

Unlike Singh et al., linear differential equations were used to plot standard curves (drug concentration over time) rather than estimate deep tissue concentrations. For each standard curve, coefficient of variations ranged from 0.1% to 13.0%, and the squared correlation coefficient was over 0.994 for all the model drugs used.[14] The standard curves fit the lines well, and

correlate significantly with the observed values for all six drugs. Having the standard curves enabled the calculation of the relative contribution of direct penetration of the drug based on the area under the curve values (Table 2). Thus, the two models appear useful for analyzing and describing the transdermal disposition of drugs after topical application, though there may be slight variations in the way the differential equations are utilized (Table 3).

New Physiological Models

Earlier pharmokinetic models created by Singh and Roberts, and Higaki et al. assumed that for solute is transported to deeper layers by molecular diffusion only. Kretsos et al. reanalyzed the data and confirmed the consistency with their own similar distributed diffusion-clearance model.[21] Following the previous research, Kretsos and Kasting created a new model to explain the dermal capillary clearance process based on assumed periodic microscopic distribution of dermal capillaries in three-dimensional space. The downfall of this model is that it only applies to the steady-state scenario and explains localized concentration in dermis.[22] Bound by the inability to recreate *in-vivo* conditions using *in-vitro* experiments and invasiveness associated with the collection of such data via biopsy the contributory research is difficult to carry out. However, a breakthrough came when Anissimov and Roberts determined that the deep percutaneous transport of drug cannot be attributed to diffusion alone and offered a new two-compartment model that considers blood and/or lymphatics, in addition to molecular diffusion to be involved in the transport to deeper tissues. More specifically, they focused on the effect of blood flow, blood protein binding and dermal binding exert on the rate and depth of percutaneous penetration of topical drugs. Unlike Singh and Roberts, and Higaki et al., in applying their model they used the combination of human biopsy data collected by Schaefer and colleagues[23-27] and their own human in-vitro dermis penetration experiments to obtain dermal diffusion/dispersion coefficient, and dermal blood clearance rate of 6 solutes. Schaefer and colleagues collected human tissue concentration-depth profile of drugs *in-vivo* after topical application.

The term dispersion implies transport of solute by both blood and diffusion in the dermis. They recognized that in order for convective blood flow transport to significantly impact the transport to deeper tissues, there must be sufficient binding to plasma proteins and blood flow, as the surface area of blood vessels is much less than that of the dermal matrix through which diffusion transport will occur. Therefore, the contribution of dermal blood flow transport is likely to be noticeably decreased when there is vasoconstriction.[28] They analyzed human dermal distribution data from previous micro dialysis experiments and recorded similar findings. One limitation of their analysis was the assumption of no contribution of topically absorbed drug into the systemic circulation contributing to the underlying tissue concentration on recirculation. It is considered to occur at long times and most considerable contribution to tissue concentrations deep below the treated site.[28]

Dancik et al. further expanded on the findings by Anissimov and Roberts' physiological pharmokinetic model in that it recognized the interstitial convection associated with capillary flow and draining of the interstitial space. Their new comprehensive model described drug diffusion in the extravascular tissue space tissue and vascular binding, axial (into the tissue) vascular, lymphatic and interstitial convection transport and constant radial (clearance) vascular transport, with the assumption of high capillary permeability. This was done by comparing the in-vitro penetration lag times of diclofenac and nicotine gathered from their in-vitro experiments to those reported in-vivo penetration lag times in the dermis and deeper tissues.[29]

They concluded that transport of highly plasma protein bound drugs into deeper tissues increases by several orders of magnitude faster than predicted by passive dermal diffusion. Although considerable concentration-depth gradient is evident for poorly protein bound drugs, it is nonexistent for highly bound drugs in the papillary dermis and small in the reticular dermis.[29] Highly protein bound drugs bind to collagen and albumin in the dermis. The convective transport of albumin into lymphatic vessels that run deeper in subcutaneous tissue encourage deeper transport (Fig. 1 abstract).

Distribution in Deeper Tissues

Direct Versus Indirect Penetration

NSAIDs directly penetrate to a depth of 3–4 mm, with the systemic blood supply accounting for penetration into the deeper, underlying tissues.[19] Drug levels peaked between 2 h and 4 h due to direct NSAID penetration. At around 10 h, drug levels peaked again, this time due to the systemic blood supply redistributing the drug.[19]

Higaki et al. also examined drug penetration distribution after topical application.[14] Similar to Singh and Roberts' findings, also performed in vivo on rats,[19] they determined that direct penetration was the predominant mechanism during the early period after starting the absorption study. There is variability between the drugs in regards to concentration of the direct penetration into muscle after topical application. For instance, the muscular disposition of diclofenac was almost all attributed to the direct penetration (90.8%), but felbinac was distributed to the muscle via the systemic circulation (>50%) (Table 2).[14]

Contrary to Singh and Roberts' conclusions, Higaki et al. found that SA in the muscle layer was mainly from direct penetration (72.0%) and less from systemic distribution (28%).[14] This is almost the reverse of Singh and Roberts' observations in1993 (80% due to systemic blood supply, 20% due to direct penetration).[19] Subsequent research confirmed that most of the

drug in muscle is due to direct migration and not from the systemic circulation following topical application.[30]

Penetration Efficacy

SA yielded the highest local tissue levels, followed by piroxicam, naproxen, indomethacin, and diclofenac (Table 4). Note that aqueous solutions were used for estimating tissue concentrations, but in clinical practice, NSAIDs may be administered as partially nonaqueous creams, ointments, or gels. The observed differences may also reflect the differences in formulation, patches, application method (solution, ointment, or cream with or without rubbing), duration, application site, or species studied.[7]

Table 4. Estimated Concentrations of Various NSAIDs Found in Muscle at Maximal Flux

Drug	Fraction of Initial Concentration[a]
Salicylic acid	10^{-6}
Piroxicam	10^{-7}
Naproxen	$10^{-7.8}$
Indometacin	$10^{-9.5}$
Diclofenac	$10^{-9.7}$

Drug concentration found in muscle at maximal flux after topical, aqueous drug delivery.
Singh and Roberts' estimated concentrations were obtained using pharmokinetic equations and experimental k_p values.
[a]Taken from Singh and Roberts, Figure 6.[7]

Table 5 examines the relative drug concentrations below the site of application and in the plasma of different studies. In general, the results agreed with one another, though the relative concentration observed may be different. This may be attributed to variations in topical application methods and the tissue location where the sample was taken. Diclofenac, SA, ketoprofen, all have a greater concentration found in plasma than in muscle, where as naproxen has a higher drug amount in the muscle than in plasma.

Table 5. Comparison of NSAID Concentrations 2 h Following Topical Application *

	Reference	Donor Cell	Viable Skin	Plasma	Muscle
Diclofenac	13	1	0.50±0.02	(1.70±0.09) x 10^{-3}	(1.00±0.05) x 10^{-3}
	2	1	0.10±0.01	(4.5±0.2) x 10^{-3}	(1.00±0.05) x 10^{-3}
Salicylic Acid	13	1	0.22±0.01	(2.3±0.1) x 10^{-2}	(1.00±0.08) x 10^{-2}
	2	1	0.080±0.004	(1.00±.05) x 10^{-2}	(2.50±0.12) x 10^{-3}
Ketoprofen	13	1	0.20±0.01	(5.7±0.3) x 10^{-3}	(1.00±0.15) x 10^{-3}
Naproxen	2	1	0.08±0.004	(2.5±0.1) x 10^{-2}	(4.00±0.15) x 10^{-3}
	26[a]	1(Epidermis)	0.22±0.01(Dermis)	(1.3±0.1) x 10^{-3}	(4.60±0.23) x 10^{-3}

The drug concentration profiles from the donor cell to the deep tissues from several experimental findings are compared.
*Results are expressed as a fraction of initial donor cell or epidermis concentration.
[a]Concentration profile taken 3 h postapplication.

Distribution into Joints

Diclofenac Controversy

In a human study of percutaneous penetration into the joints, diclofenac gel was applied to one knee and a placebo gel to the other knee of patients with bilateral knee joint effusions.[31] Drug distribution through synovial fluid was mainly through the systemic blood supply. Direct penetration, if at all, was minimal.[31] These results differ from other studies involving diclofenac, including the aforementioned study by Higaki et al., which found that 90.8% of diclofenac's distribution to muscle was due to direct penetration.[14]

However, Higaki et al. used a six-compartment model based on steady-steady kinetics in collecting their data. Other literature supports direct penetration as the main delivery mechanism for diclofenac, including one which observed 219.68 mg/mL of diclofenac in skeletal muscle allowing topical administration, whereas only18.75 mg/mL of the drug was found in plasma, where the study was also done in vivo patients, but diclofenac was applied to the anterior thigh.[32] Recent study of diclofenac concentration in soft tissues after oral versus topical diclofenac administration in 14 patients prior to knee orthoplasty reported

that the diclofenac concentration was significantly lower in synovial membrane and synovial fluid in topically applied diclofenac than in oral administration (P=0.0181for topical diclofenac group and P=0.004 for oral diclofenac group). The authors could not identify the difference as the concentration-time curves of plasma and synovial tissues or the peak values after administration were not determined.[33] On the contrary, using equivalent study methods and materials on 16 human subjects prior to ACL reconstruction surgery with the same study participation exclusion criteria, Kai et al. have found no significant difference in flurbiprofen concentration in synovial tissue after oral and topical administration of NSAID. They postulated that the role of dermal vessels in the delivery of NSAID to the bone is reduced since the bone tissue is surrounded by a dense calcified matrix.[33-34]

The minimal concentration of NSAID formulation that has anti-inflammatory effects in the synovial tissue remains unknown. Additionally, the question of whether injection site and/or injection needle serve as contaminants in the studies ought to be given special attention. Precision of measurement of diclofenac concentration inside the synovial tissue and plasma is imperative in drawing the appropriate conclusion of studies.

Further investigations into this apparent contradiction of results are required, including whether the topical application site would make a difference as this has not been assessed properly to date to the best of our knowledge. In addition, note that higher relative drug concentrations in the synovial fluid were measured in the smaller joints, such as finger and wrist joints, after topical delivery, which probably reflects the shorter diffusion distance that is needed to reach the deeper tissues.

Difference in drug prescription, formulation and measurement play a role in the outcomes of all studies. It is vital to collect more human data and develop more standardized animal/human models that will take into account not just one dimensional kinetics but the blood flow and lymphatic distribution of the microcirculation in the dermal layers of skin.

Salicylic Acid

SA penetrates into the synovial fluid. Rabinowitz et al. found high levels of salicylate in local tissues after transdermal application of its triethanolamine salt to knees of dogs. After a 60 min administration period, 1.18±0.84 mmol/g was found in the synovial fluid, significantly more than the 0.0094 mmol/g observed in serum.[35] The greater tissue penetration of salicylate (used as a triethanolamine salt) is probably the result of the more lipophilic nature of TEA compared with SA. Similarly, Mills et al. also demonstrated higher concentrations of SA and methyulsalicylate (MeSA), its commercial ester, in the synovial fluid after topical drug application to affected joints in greyhound dogs. They used combined experimental procedure of micro dialysis and direct tissue concentrations to measure penetration of a commercial SA ester. This technique allowed plasma drug concentration measurement from both systemic circulation and regional vascular drainage. Mills et al. credited direct diffusion and local blood redistribution to be responsible for the results.[36]

Modes of Delivery

In recent years, the delivery of SA following intracutaneous (i.c.), subcutaneous (s.c.), intramuscular injections (i.m.), and also after topical application were examined in rats.[37] A pharmacokinetic model was employed to calculate the rate constants between the skin, muscle, central, and peripheral compartments of the systemic circulation. For calculations of intramuscular drug delivery, a two muscle compartment model was used, with the skin compartment eliminated. The first order differential equations were also fitted to the concentration data of SA using the nonlinear least-square method. However, for the i.c. and s.c. injection data, the fitted lines were a little bit higher than observed for early period measurements in the skin.[37] From this observation, it appears that the first-order kinetics model for the drug migration may be too simple to express the complex migration process of SA. However, the model should be sufficient to illustrate the general differences in skin dispositions of SA following i.c., s.c., and i.m., injections, as well as after topical application. The group concluded that i.c. injection was the best for localizing the drug to the muscle while maintaining an effective drug concentration (Table 6).[37] Their results corresponded with previous studies where most of the SA in the muscle following cutaneous injections was due to direct delivery from the injection site, and not from systemic redistribution.

Table 6. Salicylic Acid Clearance into Muscle for Various Delivery Routes*

Delivery Rate	% Injected Dose	$K_{s\,m}$
i.c. injection	10± 1	8.24×10^{-3}
s.c. injection	9± 1	7.58×10^{-3}
i.m. injection	2.0 ± 0.1	2.94×10^{-3}
Topical application	1.0± 0.1	4.95×10^{-3}

The percent of the injected drug concentration found in the muscle 2h following delivery for different delivery routes are compared. $K_{s\,m}$ is the first-order rate constant from skin to muscle, obtained through curve-fitting using values obtained from several experiments.[37]

Transdermal Drug Delivery Enhancements

Benefits of transdermal delivery of drugs include the following: Pre-systemic metabolism is eliminated, which allows for reduction in the daily dosage levels. Blood or plasma levels of the drug can be retained within the therapeutic window for prolonged periods of time. Patient's compliance is improved and the drug administration can be aborted by removal of the patch. On the other hand the limitation is that the transdermal delivery only works for potent drugs with daily dose of the order of 10 mg or less. They must be "small" lipophilic molecules with molecular weight no greater than 500Da. The drug must also be free of local irritation.
Therefore, it comes as to no surprise that non-invasive approaches to enhance and control the drug transport in transdermal manner has been a hot topic for some time now.

Those drug molecules that exceed the size of 500Da need enhancements to aid in their passing through the main security gate, SC barrier. Most current tactics encompass: chemical penetration enhancers, iontophoresis, transporter carriers, ultrasound/microneedles/thermal poration.

Transporter Proteins

Transporter mechanisms have been implicated in affecting the drug absorption, disposition and elimination. One such is a P-glycoprotein (P-gp), a member of the ABC transporter family, was recently found expressed in human skin in addition to already known expression in other human tissues (liver, blood-brain-barrier). By comparing mRNA, protein expression and localization of P-gp in several skin tissues to human liver tissues, Skazik et al. determined strong P-gp protein expression within sweat ducts, vessels, nerve sheaths and most importantly muscles of human skin.[38] P-gp appears capable of transporting itraconazole within dermal tissue. This new knowledge can possibly play a role in drug development and increasing the efficiency of delivery to specific sites in deep layers of skin such as muscles and joints. Data on this mechanism and deep penetration is in its early stage.

Ultrasound guided percutaneous drug delivery

High-frequency sonophoresis, HFS, (0.7 MHz) has been used for five decades to aid percutaneous delivery of corticosteroids. With the discovery of the cavitation effect within the skin that can impact skin permeability, thermal and convective effects can also play a role in increasing solute partitioning into the SC. Most compounds delivered by HFS are small molecules, with only a handful of drugs having molecular weights greater than 1000 Da tested. Because HFS is safe and FDA approved, many studies that include treatment protocols have been done testing the use of various drugs including NSAIDs and the efficacy of transdermal delivery.[39]

Combination of iontophoresis, terpene and hypothermia

Kigasawa et al. showed that using new iontophoresis device with terpene, like geraniol, in rats the percutaneous penetration of diclofenac was amplified. Plasma concentration of diclofenac was increased 20-fold based on time-dependent delivery. This new device uses an ion-exchange membrane that when combined with geraniol improves the penetration of diclofenac into the stratum corneum. Overall there was no reported skin irritation.[40] Another study demonstrated the synergistic effect of iontophoresis and regional cutaneous hypothermia on trandermal delivery of diclofenac and prednisone to synovial fluid in rats. The study showed 3- fold increase in biovailability of both drugs by decreasing the dermal clearance of the drug via vasoconstrictive effects of hypothermic reaction.[41] The results of both studies are promising and warrant further investigation to determine the mechanism and efficacy in human subjects.

Efficacy and Safety of Percutaneous Drug Delivery in Humans

While research in humans regarding percutaneous drug delivery to muscle, tendon and joint is limited, more than a dozen studies assess the efficacy and safety of topical NSAIDs in several pain conditions. Two randomized double-blinded controlled studies by Tugwell et al. and Simon et al. assessed efficacy and safety of a topical diclofenac solutions compared to an oral diclofenac solution, as well as the ability to alleviate the primary osteoarthritis symptoms in the knee in a 12 week period. The first study by Tugwell et al. was performed on 622 female and male patients presenting with radiographic evidence of knee OA. Three efficacy measures were pain, physical function and patient global assessment, which were measured on a nominal index scale. Results demonstrated no clinically significant difference between the two treatment arms in treating pain associated with osteoarthritis ($p=0.10$), asserting that a topical diclofenac solution is as effective as an oral diclofenac treatment.[42] In the second study Simon et al. assessed pain scale, physical function as well as patient overall health assessment on ordinal scale. The patient population consisted of 775 females and males also presenting with radiographic evidence of knee OA. However, in addition to topical and oral diclofenac solutions the subjects were blindly given either placebo solution or a diclofenac solution in DMSO vehicle or a mixture of topical and oral diclofenac solutions. The results were similar to the first study in that the efficacy of topical diclofenac was comparable to that of oral diclofenac treatment ($p=.429$).[43] Overall both studies recommended that topical NSAIDs, specifically daclofenac, are indeed able to provide the therapeutic relief of osteoarthritis pain. Moreover, when comparing the safety of diclofenac in both studies, it is clear that topical diclofenac treatment demonstrated a lower incidence of GI side effects such as dyspepsia, diarrhea, abdominal distention, abdominal pain and nausea. Treatment with oral diclofenac showed an association with significantly greater increases in liver enzymes and creatinine, and greater decreases in creatinine clearance and hemoglobin ($p<0.001$ for all). The most common

adverse effect associated with topical diclofenac solution was dry skin. In fact the dryness and irritation of the skin was similar in patients receiving the vehicle alone and topical solution with DMSO vehicle. This finding might be possibly explained by the fact that vehicle dissolved lipids on the skin surface.[44] Typically, in clinical setting the use of emollients parallel to the main treatment of topical NSAID is encouraged but was not allowed in the trials. Also, it was reported previously that DMSO can cause halitosis and body order in some patients as a result of its metabolite dimethyl sulfide producing a garlic-like order.[45]

Elderly patients exhibit predisposition for topical NSAIDs based on the perception that a lower dose of the medication would result in less toxicity and would provide quick effect without affecting the rest of the body based on localized application.[46] In order to elicit the best advice more longitudinal studies (>12 weeks) are needed with participants that have non-limited co-morbid conditions and concomitant medications.[47]

New and Future Research Direction

Photoacoustic Spectroscopy

One limitation in human studies on topical penetration of anti-inflammatory drugs is the inability to quantitatively and non-invasively measure the formulation penetration rate through the tissue where the topical compound is applied. Photo acoustic Spectroscopy (PAS) is one technique used in dermatological research to analyze the depth of penetration of a compound. It has been shown to measure accurately penetration and distribution of various compounds through skin *in vitro*, *ex vivo* and *in vivo*.[48] PAS measures the effect of absorbed light through the skin layers with and without the applied compounds. Oliveira et al. demonstrated with PAS that deep percutaneous infiltration of Helicteresgardneriana (EEHg) crude extract reduced significantly the croton oil-induced auricle inflammation in mice. Doses of 5.0mg and 7.5 mg of the EEHg created 61% and 75% decrease in edema of the auricle ($P<0.001$). High resolution and the low cost and the effectiveness of PAS might be the future of instrumentalization of human dermatological research studies.[49]

Compound Transdermal Patch

Xi et al. explored the potential of compound transdermal patch containing teriflunomide (TEF) and lornoxicam (LOX), both recommended for treatment of rheumatoid arthritis (RA). TEF is an active metabolite of leflunomide, a disease-modifying anti-rheumatic drug (DMARD) and lornoxicam is a NSAID. This is the first of its kind study examining compound topical agent for RA as it has not been investigated before in either animals or humans. The challenge is the delivery of drugs into the synovial tissues. Xi et al. first used in vitro permeation animal experiments to optimize the formulation of the compound patch and then delivered it to inflammation induced two knee joints of rabbits. The goal was to observe whether the drug released into the articular cavity via direct diffusion on the application or via systemic circulation. After applying the transdermal compound patch, authors noted the isochronous rates of penetration for TEF and LOX. Moreover, direct diffusion of transdermal application of the patch with the medicine was more successful for the superficial joint tissues than for the deeper tissue synovial fluid as correlated to the drug concentration measured in the extracellular synovial fluid at 2h and 6h. Additionally, drug concentrations were detected in the contralateral skin that did not receive the direct application of the transdermal patch. This signified that the systemic blood supply played part in it. As with any topical application of patches there is a risk for the local area irritation. This experiment also showed that applying the compound patch at non-inflammatory ski of the rats' knee and abdominal skin was sufficient to have drug's concentration detected in the inflamed bilateral hind paws of each rat.[50] These findings are important for future research as they continue to contribute to the next generation of researchers who like the idea of combining treatment methods to increase the efficacy for given disease. The query of topical site application remains to be explored further with the next way of research studies.

Improvements To Future Research

Unequivocally all the studies presented above continue propelling forward the research on percutaneous drug penetration into deeper underlying tissues. Recent findings of involvement of dermal blood flow, lymphatic flow and convective transport in the transdermal delivery mechanism of NSAIDs added another dimension to consider for researchers. It is possible that the newly formulated physiological pharmokinetic model represents more sound explanation for variations of transdermal transport of NSAIDs to muscle, tendon and joint in handful human studies that have been completed thus far. Perhaps the questions of direct versus indirect drug penetration should be attributed to percutaneous drug penetration into muscles, tendon and periosteal tissues rather than all the way to bone tissue. A new classification including the contribution of local blood flow system might be useful in applying to mechanism of delivery of NSAIDs into bone tissues.

Questions raised by the studies primarily focus on the need of standardization of study procedures and increase the number of human studies to reciprocate the findings from *in vivo* and *in vitro* animal studies. t cannot be emphasized properly the imperative nature of development not only for longitudinal studies in studying the safety and efficacy of topical NSAIDs, but also appropriate study protocols that will focus on standardization of anatomical sites and techniques used to collect the data. For instance, clinical techniques routinely used to clean or prepare skin can significantly affect the rate and extent of penetration of a topically applied drug. This may sway the results and affect our precise understanding of the percutaneous drug delivery into deeper tissues. Further investigations into the NSAIDs, diclofenac specifically, deep penetration needed in order to resolve the conflicting results concerning the drug distribution mechanisms.

Conclusion

The current focus in research of transdermal drug transport remains on finding ways to effectively control drug dosing, as well as targeting and retention into the site of interest. Taken together, topical therapy for deep tissues has demonstrated efficacy and safety advantage. Yet much remains to be done to clarify the mechanisms so as to permit further clinical development of treatments of various chronic local conditions in muscles and joints. Confirming the clinical relevance of animals will be a major step forward.

Author's contribution

VD- drafted the manuscript (acquired, analyzed the data, designed the layout)
CL- assisted in drafting the manuscript (acquired and analyzed the data)
HM- revised the draft versions of manuscript with contributions of ideas and concept evolution
All three authors have made significant contributions to the final version of this manuscript, which was also equally approved and read by them.

Declaration of interest:

No conflict of Interest

References

[1]. Prieto-Alhambra D, Judge A, Javaid MK, Cooper C, Diez-Perez A, Arden NK. Incidence and risk factors for clinically diagnosed knee, hip and hand osteoarthritis: influences of age, gender and osteoarthritis affecting other joints. Ann Rheum Dis 2013. doi:10.1136/annrheumdis-2013-203355

[2]. Singh P. Percutaneous penetration as a method of delivery to skin and underlying tissues. In: Bronaugh RL, Maibach HI, editors. Percutaneous Absorption: Drugs-Cosmetics-MEchanisms-Methodology. 7th ed. New York: Marcel Dekker Inc.; 1999. p. 597-613.

[3]. Guy RH. Transdermal drug delivery. In: Schafer-Korting M, editor. Drug Delivery. 197th ed. Vol. 197. Springer; 2010. p. 399–410.

[4]. Stanos S P. Osteoarthritis guidelines: a progressive role for topical nonsteroidal anti-inflammatory drugs. Journal of multidisciplinary healthcare. 2013; 6:133–7.

[5]. Lee CM, Maibach HI. Deep percutaneous penetration into muscles and joints. Journal of pharmaceutical sciences, 2006; 95(7): 1405–13.

[6]. Jepps OG, Dancik Y, Anissimov YG, Roberts MS. Modeling the human skin barrier--towards a better understanding of dermal absorption. Advanced drug delivery reviews. 2013;65(2): 152–68.

[7]. Singh P, Roberts MS. Skin permeability and local tissue concentrations of nonsteroidal anti-inflammatory drugs after topical application. The Journal of pharmacology and experimental therapeutics. 1994a; 268(1): 144–51.

[8]. Singh P, Roberts MS. Deep tissue penetration of bases and steroids after dermal application in rat. The Journal of pharmacy and pharmacology.1994b; 46(12): 956–64.

[9]. Baldwin JR, Carrano R A, Imondi AR. Penetration of trolamine salicylate into the skeletal muscle of the pig. Journal of pharmaceutical sciences. 1984; 73(7): 1002–4.

[10]. Herndon C M. Topical delivery of nonsteroidal anti-inflammatory drugs for osteoarthritis. Journal of pain & palliative care pharmacotherapy. 2012; 26(1): 18–23.

[11]. Roberts MS, Anderson RA, Swarbrick J. Permeability of human epidermis to phenolic compounds. The Journal of pharmacy and pharmacology.1977; 29(11): 677–83.

[12]. Swarbrick J, Lee G, Brom J, Gensmantel N P. Drug permeation through human skin II: Permeability of ionizable compounds. Journal of pharmaceutical sciences.1984; 73(10): 1352–5.

[13]. Magnusson BM, Anissimov Y G, Cross SE, Roberts MS. Molecular size as the main determinant of solute maximum flux across the skin. The Journal of investigative dermatology. 2004; 122(4): 993–9.

[14]. Higaki K, Asai M, Suyama T, Nakayama K, Ogawara K, Kimura T. Estimation of intradermal disposition kinetics of drugs: II. Factors determining penetration of drugs from viable skin to muscular layer. International journal of pharmaceutics. 2002;239(1-2): 129–41

[15]. Yagi S, Nakayama K, Kurosaki Y, Higaki K, Kimura T. Factors determining drug residence in skin during transdermal absorption: studies on beta-blocking agents. Biological & pharmaceutical bulletin.1998; 21(11): 1195–201.

[16]. Guy RH, Hadgraft J, Maibach HI. A pharmacokinetic model for percutaneous absorption. International Journal of Pharmaceutics.1982; 11(2): 119–129.

[17]. Barry B. Transdermal drug delivery. In: Aulton M, editor. Pharmaceutics: the science of dosage form design. London: Harcourt Publishers Ltd.; 1988. p. 499–533.

[18]. Higaki K, Nakayama K, Suyama T, Amnuaikit C, Ogawara K, Kimura, T. Enhancement of topical delivery of drugs via direct penetration by reducing blood flow rate in skin. International journal of pharmaceutics.2005; 288(2): 227–33.

[19]. Singh P, Roberts MS. Dermal and underlying tissue pharmacokinetics of salicylic acid after topical application. Journal of pharmacokinetics and biopharmaceutics.1993a; 21(4): 337–73.

[20]. Singh P, Roberts MS. Iontophoretic transdermal delivery of salicylic acid and lidocaine to local subcutaneous structures. Journal of pharmaceutical sciences. 1993b;82(2): 127–31.

[21]. Kretsos K, Kasting GB, Nitsche JM. Distributed diffusion-clearance model for transient drug distribution within the skin. Journal of pharmaceutical sciences. 2004;93(11): 2820–35.

[22]. Kretsos K, Kasting GB. A geometrical model of dermal capillary clearance. Mathematical biosciences.2007; 208(2): 430–53.

[23]. Schaefer H, Stüttgen G. Absolute concentrations of an antimycotic agent, econazole, in the human skin after local application. Arzneimittel-Forschung. 1976;26(3): 432–5.

[24]. Schaefer H, Zesch A. Penetration of vitamin A acid into human skin. Acta dermato-venereologica. Supplementum. 1975; 74: 50–5.

[25]. Zesch A, Schaefer H. [Penetration of radioactive hydrocortisone in human skin from various ointment bases. II. In vivo-experiments (author's transl)]. Archiv für dermatologische Forschung; 1975;252(4): 245–56.

[26]. Schaefer H, Zesch A, Stüttgen G. Penetration, permeation, and absorption of triamcinolone acetonide in normal and psoriatic skin. Archives for dermatological research. Archiv für dermatologische Forschung.1977; 258(3): 241–9.

[27]. Schaefer H, Stüttgen G, Zesch A, Schalla W, Gazith J. Quantitative determination of percutaneous absorption of radiolabeled drugs in vitro and in vivo by human skin. Current problems in dermatology.1978; 7:80–94.

[28]. Anissimov YG, Roberts MS. Modelling dermal drug distribution after topical application in human. Pharmaceutical research.2011; 28(9):2119–29.

[29]. Dancik Y, Anissimov YG, Jepps O G, Roberts MS. Convective transport of highly plasma protein bound drugs facilitates direct penetration into deep tissues after topical application. British journal of clinical pharmacology.2012; 73(4): 564–78.

[30]. Yanagimoto G, Hayashi T, Hasegawa T, Seki T, Juni K, Sugibayashi K, Morimoto Y. Skin disposition of drugs after topical application in hairless rats. Chemical & pharmaceutical bulletin.1999; 47(6): 749–54.

[31]. Radermacher J, Jentsch D, Scholl M A, Lustinetz T, Frölich J C. Diclofenac concentrations in synovial fluid and plasma after cutaneous application in inflammatory and degenerative joint disease. British journal of clinical pharmacology.1991; 31(5): 537–41.

[32]. Müller M, Rastelli C, Ferri P, Jansen B, Breiteneder H, Eichler HG. Transdermal penetration of diclofenac after multiple epicutaneous administration. The Journal of rheumatology.1998;25(9): 1833–6.

[33]. Miyatake S, Ichiyama H, Kondo E, Yasuda K. Randomized clinical comparisons of diclofenac concentration in the soft tissues and blood plasma between topical and oral applications. British journal of clinical pharmacology.2009; 67(1): 125–9.

[34]. Kai S, Kondo E, Kawaguchi Y, Kitamura N, Yasuda K. Flurbiprofen concentration in soft tissues is higher after topical application than after oral administration. British journal of clinical pharmacology.2013; 75(3): 799–804.

[35]. Rabinowitz JL, Baker D. Absorption of labeled triethanolamine salicylate in human and canine knee joints. II. Journal of clinical pharmacology.1984;24(11-12): 532–9.

[36]. Mills PC, Magnusson BM, Cross SE. Penetration of a topically applied nonsteroidal anti-inflammatory drug into local tissues and synovial fluid of dogs. American journal of veterinary research. 2005;66(7):1128–32.

[37]. Yoshida D, Hasegawa T, Sugibayashi K.Targeting of salicylate to skin and muscle following topical injections in rats. International journal of pharmaceutics. 2002; 231(2): 177–84.

[38]. Skazik C, Wenzel J, Marquardt Y, Kim A, Merk HF, Bickers DR, Baron JM(2011). P-glycoprotein (ABCB1) expression in human skin is mainly restricted to dermal components. Experimental dermatology. 2011; 20(5): 450–2.

[39]. Polat BE, Hart D, Langer R, Blankschtein D. Ultrasound-mediated transdermal drug delivery: mechanisms, scope, and emerging trends. Journal of controlled release : official journal of the Controlled Release Society.2011; 152(3): 330–48.

[40]. Kigasawa K, Kajimoto K, Watanabe M, Kanamura K, Saito A, Kogure K. In vivo transdermal delivery of diclofenac by ion-exchange iontophoresis with geraniol. Biological & pharmaceutical bulletin.2009;32(4): 684–7.

[41]. Sammeta SM, Murthy SN. "ChilDrive": a technique of combining regional cutaneous hypothermia with iontophoresis for the delivery of drugs to synovial fluid. Pharmaceutical research.2009; 26(11): 2535–40.

[42]. Tugwell PS, Wells GA, Shainhouse JZ. Equivalence study of a topical diclofenac solution (pennsaid) compared with oral diclofenac in symptomatic treatment of osteoarthritis of the knee: a randomized controlled trial. The Journal of rheumatology.2004; 31(10):2002–12.

[43]. Simon LS, Grierson LM, Naseer Z, Bookman AAM, Zev Shainhouse J. Efficacy and safety of topical diclofenac containing dimethyl sulfoxide (DMSO) compared with those of topical placebo, DMSO vehicle and oral diclofenac for knee osteoarthritis. Pain. 2009;143(3):238–45.

[44]. Roth SH, Fuller P. Diclofenac topical solution compared with oral diclofenac: a pooled safety analysis. Journal of pain research.2011; 4: 159–67.

[45]. Bookman AAM, Williams KSA, Shainhouse JZ. Effect of a topical diclofenac solution for relieving symptoms of primary osteoarthritis of the knee: a randomized controlled trial. Canadian Medical Association journal.2004; 171(4): 333–8.

[46]. Carnes D, Anwer Y, Underwood M, Harding G, Parsons S. Influences on older people's decision making regarding choice of topical or oral NSAIDs for knee pain: qualitative study. BMJ.2008;336(7636): 142–5.

[47]. Makris UE, Kohler MJ, Fraenkel L. Adverse effects of topical nonsteroidal antiinflammatory drugs in older adults with osteoarthritis: a systematic literature review. The Journal of rheumatology.2010; 37(6): 1236–43.

[48]. Haisch C. Photoacoustic spectroscopy for analytical measurements. Measurement Science and Technology. 2012; 23.

[49]. De Melo JO, Pedrochi F, Baesso ML, Hernandes L, Truiti MCT, Barons S, Bersani-Amado CA. Evidence of deep percutaneous penetration associated with anti-inflammatory activity of topically applied Helicteres gardneriana extract: a photoacoustic spectroscopy study. Pharmaceutical research. 2011; 28(2): 331–6.

[50]. Xi H, Cun D, Xiang R, Guan Y, Zhang Y, Li Y, Fang L.Intra-articular drug delivery from an optimized topical patch containing teriflunomide and lornoxicam for rheumatoid arthritis treatment: Does the topical patch really enhance a local treatment? Journal of controlled release : official journal of the Controlled Release Society.2013; 169(1-2):73–81.