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REVIEW

Skin penetration and tissue permeation after topical administration of diclofenac

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ABSTRACT

Objective: Topical delivery of drugs is an alternative to oral administration, often with similar efficacy but potentially a more favorable tolerability profile. However, topical formulations need to be able to penetrate the skin and permeate to the target areas in quantities sufficient to exert a therapeutic effect. Many factors can affect this process, including the physicochemical properties of the drug, the formulation used, and the site and mode of application. It is believed that measurement of drug concentrations at the sites of action may be an indicator of their likely efficacy. This review addresses these issues, with reference to topically administered diclofenac in osteoarthritis.

Methods: Articles relevant to this review were identified after a systematic search of Medline and Embase, using the key words "diclofenac", "topical administration" and "osteoarthritis" in the search strategy.

Results: The sparse data available indicate that topical diclofenac can penetrate and permeate to deeper tissues, with a lower plasma to tissue ratio than oral diclofenac. The tissue diclofenac levels after topical delivery are sustained over time (at least several hours). However, there is not enough data to establish how diclofenac levels in the joint compare with IC_{50} levels (50% of the maximum inhibition of prostaglandin synthesis) established following oral administration.

Conclusions: After topical application, diclofenac can penetrate the skin and permeate to deeper tissues, where it reaches a concentration that appears to be sufficient to exert a therapeutic effect. More robust methods are required for in vivo characterization to better estimate the clinical efficacy of topically applied drugs.

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Introduction

Topical administration of drugs can be a practical alternative to oral delivery, not least because they avoid first-pass metabolism, are associated with a lower rate of systemic adverse events, and allow direct application over the target areas[1]. Topical formulations should be easy and acceptable to use, but importantly need to be able to penetrate the skin and permeate to the target areas in quantities sufficient to exert a therapeutic effect. Topical analgesics are often used in acute and chronic painful conditions, delivering non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen, diclofenac, and acetylsalicylic acid directly to the site of injury to relieve pain. They can be particularly useful in the management of osteoarthritis (OA), a chronic condition where a regular intake of oral NSAIDs to control painful flares may be associated with systemic adverse events, especially in the older population that typically suffers from OA[2] and may be more prone to adverse events[3–5]. Topical NSAIDs have been proven to be as effective as and better tolerated than oral NSAIDs in the treatment of OA[4,6–8], and are recommended in certain international guidelines before the use of oral NSAIDs in OA of the knees or hands[9–11]. In a preference study, almost three times as many OA patients chose to use a topical rather than oral NSAID, particularly those who were more concerned about toxicity such as the elderly[12,13]. It seems that topical NSAIDs are currently underutilized[14], and their efficacy in pain relief remains debatable[15]. Clinicians still seem to be unsure of the value of topical NSAIDs[16], with many regarding them as little more than placebo[15]. Indeed, a large placebo effect of around 50% (after 12 weeks) has been observed in studies of topical NSAIDs, twice as high as that in studies with oral placebo (25% in a pooled analysis)[15]. Despite this, real-life studies in OA indicate that topical NSAIDs are as effective as oral NSAIDs over 1 year of treatment[4].

To relieve pain effectively, topical NSAIDs need to work at the appropriate site of action. However, in OA there is still uncertainty regarding the target tissues and how OA-associated pain is generated. There is often a disparity between the degree of pain perception or functional impairment and the extent of damage in the OA joint, and the pain mechanisms are likely to be complex[17]. Pain perception appears to be influenced by peripheral factors (e.g. damaged structures

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Supplemental data for this article can be accessed here.

impinging on other local structures), as well as activation of central pain-processing pathways[17]. And there is evidence that there is an inflammatory component to OA, including the activation and release of local proinflammatory mediators such as cytokines or prostaglandins[17–19]. Topical NSAIDs appear to work on peripheral pain receptors in OA, with relatively few central effects[20]. They relieve peripheral pain by inhibiting the cyclooxygenase (COX)-2 enzyme, thereby reducing the production of prostaglandins that would otherwise increase sensitivity to pain by sensitizing peripheral nociceptors to painful stimuli[21]. This is the established mechanism of action, but there are putative mechanisms that include inhibition of leukotriene synthesis, inhibition of phospholipase A2, modulation of arachidonic acid levels, inhibition of the N-methyl-D-aspartate (NMDA) pathway and increase in plasma β-endorphin levels[22,23]. Emerging mechanisms include inhibition of peroxisome proliferator-activated receptor gamma, reduction in plasma and synovial substance P and interleukin-6 levels, inhibition of the thromboxane-prostanoid receptor, and inhibition of acid-sensing ion channels. There are no nociceptors in articular cartilage, the main structure affected morphologically in OA. Thus, the pain that occurs when the cartilage wears away must instead originate from other structures within the joint, such as the synovial membrane or tissue, bone, or periarticular muscles and ligaments, for example[17,24–27]. Nociceptors are abundant in many articular tissues[21] that are in contact with the intra-articular environment[28]. A clearer understanding of the drug concentrations that can be achieved in synovial tissue and fluid following application of a topical NSAID, particularly in relation to plasma levels, may provide a useful insight into the potential therapeutic effect in OA and the potential liability for systemic adverse events.

The current review considers the general characteristics that influence the effectiveness of topical products, with specific illustration using diclofenac in the treatment of OA-related pain. Factors addressed include issues regarding skin penetration and tissue permeation, and the concentrations that have been reached in and around the articular joint and in plasma in published studies. We chose diclofenac because analysis suggests that it is the most potent COX-2 inhibitor compared with other commonly used NSAIDs[22,29–31]. Topical diclofenac has been widely available since its first approval in 1985, and is one of the most extensively investigated NSAIDs, often used as a benchmark in clinical studies in OA[32]. Topical diclofenac is proven to be effective in relieving the pain of OA[4,15,33], and is the only NSAID approved for topical treatment of OA pain in the United States[33]. Specific aims of the review are to determine: (1) whether topical NSAIDs, specifically diclofenac, can penetrate through the skin and permeate to deep sites of action; (2) whether the amount of diclofenac at the site of action is known, and how the formulation can be optimized and measured; (3) whether topically administered diclofenac is pharmacologically effective. To identify relevant articles, a systematic search of Medline and Embase was performed (to October 2016), using the key words "diclofenac", "topical administration" and "osteoarthritis" in the search strategy. Further details of the search strategy used can be found in the Supplementary Appendix.

Table 1. Advantages of using topical NSAIDs in preference to oral NSAIDs in osteoarthritis.

- Administration directly at the site of pain
- Avoidance of first-pass metabolism
- Reduced systemic exposure, with a resultant lower risk of systemic adverse events (e.g. gastrointestinal, cardiovascular or renal complications)
- Ability to use in patients unable to tolerate oral NSAIDs
- Avoidance of drug–drug interactions
- Potential dose-sparing effect when used with oral NSAIDs
- Patient preference, with the potential for increased compliance

Abbreviations. NSAIDs; nonsteroidal anti-inflammatory drugs.

Rationale for using topical vs. oral NSAIDs

Topical products were developed to provide well tolerated, effective targeted therapies, based on the drug's pharmacokinetics and penetration to the site of action. There are various reasons to use a topical NSAID in preference to an oral NSAID (Table 1).

Topical therapies are delivered to the site of action, avoiding the first-pass metabolism of oral drugs[34]. Most importantly, topical NSAIDs were developed to reduce the risk of gastrointestinal (GI), cardiovascular (CV), and renal adverse events associated with oral NSAIDs[33,35]. Although some of the drug does enter the systemic circulation from the dermal microcirculation, systemic exposure to NSAIDs is reduced. For example, plasma levels after topical administration have been reported to fall within a range of 0.2% to 8% of those achieved after oral administration[33,36,37]. Thus, complications such as GI bleeding and gastric ulcerations associated with oral administration of NSAIDs[38,39], as well as CV and renal toxicity[40], are less common following use of topical NSAIDs[41–43]. This is important in older patients who form the OA population, who often have co-morbid conditions or an increased risk for GI, CV or renal complications[3–5]. A meta-analysis of the safety data for topical diclofenac found that although the overall risk of adverse events was similar between topical diclofenac and oral comparators, the risk of systemic effects was significantly lower with topical administration[44]. The risk of local effects was significantly higher with topical delivery, however[44], and future development should aim to minimize local effects with better topical formulations. Use of a topical NSAID may also have a treatment-sparing effect, such that it may be possible to substantially reduce the overall dose of concomitant oral NSAIDs required to manage OA by 40%, and thereby reduce the risk of systemic adverse events[4]. In older OA patients, who are likely to have high concomitant medication use, the low systemic exposure associated with topical NSAIDs will reduce the potential for clinically relevant drug–-drug interactions (e.g. with warfarin, antihypertensives or low-dose aspirin used for cardioprotection) associated with oral NSAIDs[3,45].

Although there is a sound rationale for using a topical NSAID, questions remain as to how the drug reaches the target tissues. In OA, it is important that a topically applied drug reaches the target tissues in sufficient amounts for there to be a pharmacodynamically active concentration

present, that it is unequivocally effective in the disease indicated, and that it has no local toxic or allergic effects or undesirable, dose-related systemic effects[46].

Pathways used by topical drugs to reach target tissues

The efficacy of a topical drug at relieving pain and inflammation is dependent on its ability to penetrate skin and permeate to the target tissues. It has been suggested that topical NSAIDs exert their action locally at structures that surround superficial joints such as the knee or hand and within the joint itself[15,47], and must reach a concentration in those areas that is sufficient to inhibit the COX enzymes[46].

Oral drugs are dependent on absorption into the circulation and subsequent distribution to the peripheral tissues. The pharmacological action of topical drugs instead relies on penetration through the stratum corneum and permeation into the lower layers of the skin, illustrated in Figure 1.

The stratum corneum is the outermost, horny layer of skin that limits penetration of substances to protect the more delicate structures underneath. Therefore, it can be very difficult to penetrate passively, and is the rate-limiting step for epidermal drug transport[48]. Topically applied drugs may have a depot effect, such that they accumulate for a prolonged time in the stratum corneum, epidermis, dermis and subcutaneous fatty tissue to form a reservoir, from which there is a sustained release of drug into the surrounding tissues[49–54]. The efficiency of the reservoir is dependent on the active pharmaceutical ingredients (APIs), including lipid/water solubility, protein-binding capacity, percutaneous absorption, compound concentration, clearance, application time, and application mode[51]. The highly bound nature of diclofenac may contribute to the formation of a reservoir, with retention occurring when diclofenac is highly bound in tissues underlying the topical absorption site[53]. Movement of the drug through the skin is a passive diffusional process, relying on diffusion down concentration gradients (following Fick's Law[55]) and partitioning into tissues and solutes[1,56,57]. Active transport processes can occur, but the mechanisms have not been identified[1,58]. Some of the drug may travel from the surface of the skin via hair follicles or sweat ducts to reach the lower layers[59,60].

At the dermal level (Figure 1), the drug may enter the local blood vessels for distribution to deeper tissues[60]. Uptake of the drug from the dermal microcirculation into the systemic circulation may also occur (for example, diclofenac has been found in treated and untreated tissues after topical application[56,61,62]) – although total systemic exposure is low[33]. Alternatively, the drug may diffuse deeper into inflamed tissues[56,63,64] and/or be absorbed via lymphatic drainage[58]. It should be noted that the exact mechanisms involved remain unclear[65].

Factors affecting penetration and permeation

Several factors can affect penetration of the drug through the stratum corneum and permeation to the underlying tissues (outlined in Table 2), which must be considered when choosing a topical NSAID.

Permeation through the layers of skin, and the factors that influence this process, are commonly assessed using the Franz cell[78–80]. This technique is an efficient in vitro method of evaluating drug movement through excised human or animal skin or synthetic skin; it can be used to identify the changing concentrations of APIs in different physiological layers of the skin, and can be combined with a variety of imaging techniques to visualize the movement of drug through the skin[79]. Physiologically, the Franz cell assesses the penetration of APIs through the skin, the first step in the passage of the APIs to the site of action. Yet permeation through the tissue layers to the deeper sites of action still needs to be evaluated.

Properties influencing penetration through the skin

Innate drug properties

Topical drugs must be small enough to pass through the skin (molecular weight <500 g/mol)[1], and molecular size is probably the main determinant of flux across the skin

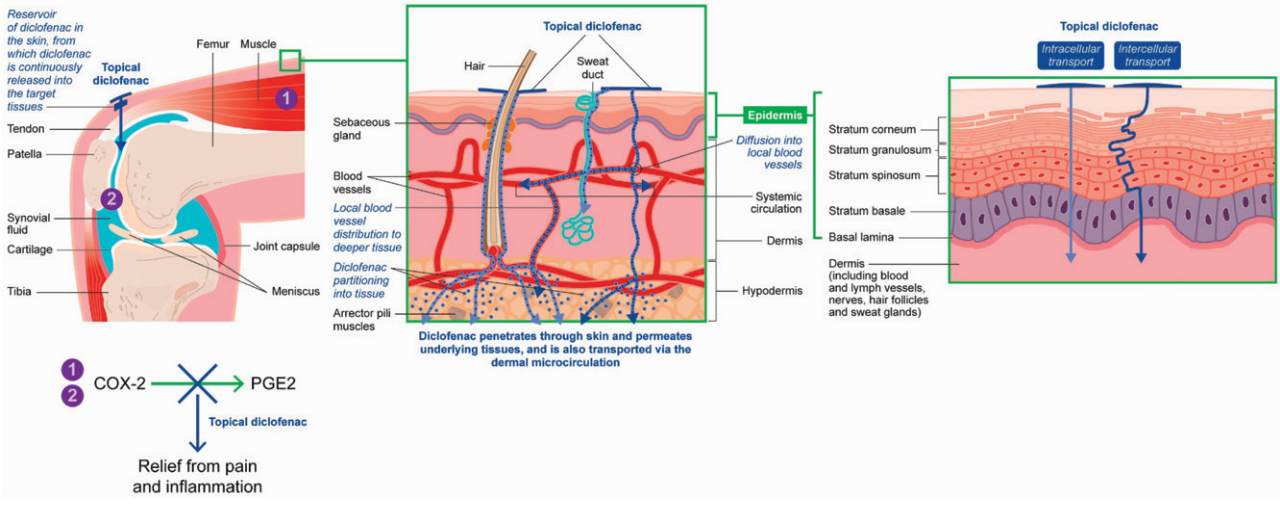

Figure 1. Penetration of topical diclofenac through the skin and permeation through the deeper layers to the inflamed joint.

Table 2. Overview of the factors affecting the ability of a topical drug to optimally penetrate skin and permeate through the underlying tissues.

Factor	Considerations	Topical diclofenac
Molecular size	• Small molecules (<500 g/mol[1]) pass through stratum corneum more easily • Probably the main determinant of flux across skin[66]	• Small molecular weight (296 g/mol)[67]
Water solubility	• Must be water soluble[68], but also lipophilic enough to penetrate lipid matrix of stratum corneum[1,56]	• Has both lipophilic and hydrophilic properties; can access all tissues[33,50,63]
Acidity	• Inflamed tissues have acidic microenvironment • More acidic NSAIDs (lower pKa values) will be un-ionized and able to cross membrane barriers • Acidic NSAIDs will reach higher concentrations at cell membranes and in neutral intracellular spaces containing COX-2 enzymes than in the relatively acidic extracellular space of inflamed tissue[56,63]	• Weak organic acid (pKa 3.9[69])
Vehicle used	• Solubility, molecular mass, depth of penetration, pharmacology and toxicology of its components need to be considered[70,71]	• Diclofenac gel has higher flux than diclofenac solution or patch[72]
Penetration enhancer	• Can greatly increase penetration through stratum corneum[42,47,73] • Should ideally be inactive with no adverse effects on skin	• Dimethylsulfoxide (DMSO) often used with diclofenac – but can cause skin irritation[73]
Site and method of application	• Topical NSAIDs more likely to reach superficial joints (e.g. finger, knee) than deeper structures (e.g. hip joint)[15] • Prolonged rubbing increases flux through skin[74] • Occlusion hydrates the stratum corneum, often facilitates penetration through the skin and into the underlying tissues[75,76] • Repetitive administration can greatly increase bioavailability of drug[77]	• Topical diclofenac indicated for finger or knee osteoarthritis • Recommended to use gentle massage upon application of diclofenac gel
Protein binding	• Concentration will be higher where albumin concentrations are higher • In an inflamed joint, the concentrations of albumin increase in synovial tissue and fluid	• Highly bound to plasma albumin (99.4%[69]) • Diclofenac concentration five times higher in synovial fluid than plasma after one day, seven times higher at steady state after eight days[50]

Abbreviations. COX, cyclooxygenase; NSAID, non-steroidal anti-inflammatory drug.

(i.e. the amount of drug that can penetrate the skin per unit of time)[66]. Diclofenac is a small molecule (296 g/mol[67]) (Supplementary Table 1), allowing it to pass more easily through the stratum corneum. The drug must be water soluble[68], yet also have adequate lipophilicity to penetrate through the lipid matrix of the stratum corneum[1,56]. Diclofenac is a weak organic acid (pKa 3.9[69]) and thus amphiphilic, with both lipophilic and hydrophilic properties that allow it to access all tissues including the stratum corneum and other skin tissues, but also cell membranes such as in the synovial lining of joints[33,50,63]. Diclofenac has a low molecular weight, high lipophilicity, and has been shown to have the highest in vitro permeation rate constant compared to other NSAIDs that were studied[81]. However, it also has a modest flux (evaluated in the Franz cell) and a relatively low clearance from skin into muscle[64,81]. The physicochemical characteristics of diclofenac allow diffusion of the drug through the skin although with the need for permeation enhancers.

Topical formulation properties

The topical formulation is also important, and affects the ability of a drug to penetrate the skin. Few drugs readily penetrate skin when used alone and so topical delivery can be locally enhanced by altering a drug's formulation, which can have a substantial impact on the rate of skin absorption and on the subsequent penetration depth[42,82]. Characteristics of the vehicle used to carry the drug need to be considered, such as the solubility, molecular mass, depth of penetration, pharmacology and toxicology of its components[70,71]. Gels, sprays and microemulsions may be absorbed through the skin more effectively than creams[41], and an in vitro study has suggested that diclofenac gel has faster flux than a diclofenac solution or patch[72]. An aqueous solution of diclofenac has been shown to penetrate to a depth of around 3–4 mm into the underlying dermis and subcutaneous tissue[56]. It should be noted that different formulations of topical diclofenac may penetrate more deeply and at different velocities, reflecting the varying effects of excipients[56,65,83].

Formulations providing faster skin penetration would seem desirable, seemingly promising faster absorption to sites of action. Penetration-enhancing factors can affect the formation of a skin reservoir, resulting in faster formation and emptying of the reservoir[51]. However, faster penetration (assessed in vitro) can also have the unintended consequence of increasing plasma exposure (and thus the risk of systemic adverse events), while leaving tissue levels largely unchanged (cf. Brunner *et al.*[83], Nivsarkar *et al.*[84]). For example, the pharmacokinetic parameters of diclofenac in skeletal muscle were variable and indistinguishable following topical administration of diclofenac diethylamine gel compared to that of a novel diclofenac formulation (whose skin transport of diclofenac was reported to be ten-fold faster than the gel)[83]. Furthermore, the bioequivalence 90% confidence intervals of the novel formulation versus the gel included 100% in all instances, indicating that there were no significant differences in exposure. Although skin penetration

speed had no effect on tissue permeation, it did lead to higher levels of diclofenac in the plasma: diclofenac exposure following topical administration of diclofenac diethylamine gel is around 6% of that observed compared to three times daily oral administration of diclofenac 50 mg; in contrast, the plasma exposure following administration of the novel formulation was 2.8–4.8 times higher[83]. Similar outcomes were seen with a comparison of four topical ibuprofen products[65,85]. In all of these studies with diclofenac and with ibuprofen, the speed of penetration through the skin – as measured in the Franz cell – did not translate to proportional changes in tissue permeation.

Use of penetration enhancers

A penetration enhancer may be used to encourage local absorption through the skin into the underlying tissues and could increase the depth of local direct penetration[42,47,73]. It should be noted that the vehicle and enhancers themselves can have a clinical effect, for example topically administered dimethylsulfoxide (DMSO) used to treat veterinary systemic inflammation[15,73,86]. The vehicles/enhancers should ideally be inactive with no adverse effects on the skin – the high concentrations of DMSO used for enhancing penetration of diclofenac can cause erythema and wheals of the stratum corneum and may denature some proteins[73].

Site and mode of application

The site of application affects how readily the drug can reach the target tissues, and a topical NSAID is more likely to reach superficial joints such as the finger or knee, rather than deeper structures such as the hip joint[15]. In addition, the mode of application of the drug influences penetration. For example, rubbing or local heat can increase local blood flow and facilitates uptake into the blood, maintaining the concentration gradient that drives the passive diffusion[87]. Prolonged rubbing has been shown to greatly increase the flux of diclofenac gel through the skin[74].

Once in the blood circulation, the drug can be redistributed into local tissues or into other body compartments. Skin surface occlusion, which hydrates the stratum corneum, often facilitates penetration through the skin and into the underlying tissues[75,76]. Repetitive administration can greatly increase the bioavailability of the drug[77]. Single-dose topical administration of diclofenac resulted in drug levels in the plasma and tissue indicative of direct penetration (pathways in Figure 1), while multiple-dose administration led to redistribution from the systemic circulation to the tissue[88]. Furthermore, repeated daily applications of NSAIDs provide potentially effective concentrations of the drug in skeletal muscle[89]. Interindividual variability is often reported in in vivo studies of topical drugs[90], because of differences in skin permeability due to age, disease or damage, hydration of the epidermis, local blood flow, for example, or the presence of metabolic skin enzymes that may break down the drug and reduce its potency[91].

The penetration of topical products through the skin is dependent on the active drug substance and the formulation, yet optimal skin penetration may not consistently result in the optimal downstream delivery to tissue. While skin penetration assays are well understood, broadly used and robust, our evaluation of the available evidence – and that of other authors[80,92,93] – indicates that their results may not be suitable for anticipating tissue penetration. This is in contrast to establishing that in vitro methods can reflect systemic bioavailability[94], as this is not consistently reflective of target tissue permeation. Several issues regarding the active drug need to be determined, including how fast it can penetrate to the site of action, how deep it can directly penetrate, and how its delivery to the site of action can be optimized.

Distribution to the target tissues

It is important to note, as demonstrated above, that the rate of absorption is not the only factor that is important in the efficacy of a topical NSAID. Physiology and the API characteristics already mentioned also play an important role[45,56,64]. However, these factors do not always lead to understood outcomes; data on topical ibuprofen, for example, shows that even if topical NSAIDs with the same APIs have the same rate of skin penetration, they do not necessarily reach the target tissues in the same concentrations[65,85,95]. Ultimately, it is the concentration of the drug at the joint which is of paramount importance.

Preferential distribution of diclofenac to the sites of inflammation, the "effect" compartments, is influenced by several factors. For example, like all NSAIDs diclofenac is bound to plasma proteins, primarily albumin, and concentrations of the drug should be higher and persist where there is a larger concentration of albumin. Furthermore, the partition coefficient (K_s) of a drug indicates the ratio of the mean concentration in synovial fluid and plasma over long-term administration[96]. Thus, drugs that have a K_s value greater than one, such as diclofenac after multiple administration (K_s 1.1[96]), should be present in greater amounts in synovial fluid than in plasma.

The pH of topical drugs may also be a factor that could promote uptake and retention of the drug in the acidic microenvironment of inflamed tissues. In an acidic environment, protein binding is decreased and the more acidic NSAIDs (those with low pKa values, such as 3.9 for diclofenac[69]) will be un-ionized and able to cross membrane barriers; such NSAIDs will thus reach a higher concentration at cell membranes and in neutral intracellular spaces containing COX-2 enzymes than in the relatively acidic extracellular space of inflamed tissue[56,63,97].

Preferential distribution of diclofenac to areas of inflammation rather than plasma is facilitated by its lipophilicity and higher distribution coefficient, and thus a greater likelihood of penetrating lipophilic membranes such as the synovium[63]. Despite the short half-life and relatively fast elimination of diclofenac from plasma, there are persisting therapeutic concentrations of the drug in areas of inflammation[34,63]. The synovial mean transit time has been estimated as 2–2.5 h[98] and its effect can be observed in the time course

Table 3. Ratios of average plasma to tissue concentrations for topical versus oral diclofenac after administration over 3 days (data adapted from Brunner *et al.*[83,90]).

	Skeletal muscle	Subcutaneous fat
Oral diclofenac tablets (150 mg/day)[90]	0.006	0.006
Topical diclofenac 4% spray gel (144 mg/day)[90]	0.51	0.74
Topical diclofenac diethylamine 1.0% gel (40 mg/day)[83]	0.27	0.40
Topical diclofenac 1% ± menthol and eucalyptus oil (5 mg/day)[83]	0.11	0.12

reported by Liauw and colleagues[99], where the plasma to synovial fluid ratio of diclofenac levels increases for approximately 8 h (signaling a loss of drug from plasma with a slower loss from synovial fluid). A similar effect is seen for soft tissue, where muscle levels are sustained longer than those in plasma[90]. The duration of effect in tissue may be sustained by the reservoir of diclofenac that accumulates in the skin after repeated application[100], from which diclofenac is continuously released into the target tissues and the relatively slower clearance from tissues[90].

As already indicated, the penetration of diclofenac into tissue depends on the formulation and route of administration – but the speed of skin penetration (as measured by the Franz cell) does not consistently translate into tissue concentrations[83]. This implies that other formulation characteristics must also be influential. Similar data collected on topical diclofenac compared with oral diclofenac tablets provides additional complexity[83,90]. Comparison of these topical products shows that they have different ratios of tissue exposure relative to plasma exposure (Table 3).

A higher ratio, which derives from higher diclofenac levels in the tissue relative to plasma, is indicative of better penetration. The ratios following topical application contrast greatly with tissue exposure following oral administration; plasma levels are significantly higher while tissue levels are similar, thus yielding a significantly lower partition into tissue (i.e. a much smaller ratio). This may reflect direct penetration of topical products from the site of administration and minimal systemic redistribution. Optimizing this direct penetration requires more than fast in vitro skin penetration – data with the same APIs suggests a role for excipients in influencing tissue penetration alongside skin permeation, requiring further understanding and evaluation techniques in addition to in vitro data.

Suitability of diclofenac for topical administration

The described characteristics of diclofenac are thought to be optimal for penetration and uptake into local sites (Table 2)[50], although methods are needed to fully evaluate its potential. Topical diclofenac has been determined to exhibit acceptable efficiency for external use, based on the ratio between skin penetration to concentration in the target tissue, called the index of topical anti-inflammatory activity (ITAA)[101]. The ITAA takes into account both biopharmaceutic (i.e. the facility to reach the target in the skin) and pharmacodynamic aspects (i.e. demonstration of a local therapeutic effect) of a drug and may give an indication of the anti-inflammatory efficacy of a topical NSAID[101]. Diclofenac has been shown to have a high transdermal penetration[81], and was the most potent inhibitor of COX-2 activity[101], and therefore had the highest ITAA at 50%, 75% and 90% of COX-2 inhibition. However, the dermis was the target tissue[101], and it is less clear whether the concentrations of topical diclofenac are sufficient in peri- and intra-articular tissues.

Forecasting whether a formulation and its APIs led to those APIs achieving active concentrations in target tissue may not be consistently accurate, as demonstrated in our previous examples and in the seeming dislocation of rate of skin penetration and tissue penetration/API pharmacokinetics. There are limitations with in vitro data[80,92], and in silico based physiologically based pharmacokinetic (PBPK) modeling is relatively recent and focuses on predicting rates of penetration and plasma exposure rather than target tissue exposure[58,102,103]. Alternatives include more labor intensive in vivo approaches that can measure at the site of action, including microdialysis and (specific to knee OA) joint sampling techniques like synovial biopsies and arthroplasties[80,104,105]. Animal models can also be used to anticipate human results[106]. However appropriate these methods are, they all require data to establish the bioactivity of forecast drug levels in tissue.

Concentration of topical diclofenac in target tissues compared with plasma

Data obtained after oral application

The extent to which an NSAID reaches inflamed tissues gives an indication of the likely efficacy of the drug[107,108]. The data obtained following oral administration of diclofenac provides some characterization of the drug levels required in the knee joint. This oral data provides a context for understanding diclofenac levels achieved in the knee. They also provide context for levels that need to be achieved when developing new products. Most of the recent data is collected in studies where knee bioactivity and diclofenac levels in the knee and plasma are measured exclusively, forgoing the chance to identify the relationships between systemic drug levels, site of action drug levels, and the bioactivity they provide. Prostaglandin production (specifically PGE_2) is a surrogate measure of COX-2 activity[109]. Therefore, inhibition of PGE_2 may be regarded as an indicator of COX-2 inhibition. Few studies have measured the therapeutically active concentrations of diclofenac in vivo or ex vivo. Chlud and Wagener determined that 100–500 ng/ml of diclofenac reduced PGE_2 in the OA synovium and was "therapeutically effective"[110]. Liauw et al. investigated the concurrent diclofenac levels in plasma and synovial fluid and PGE_2 levels in synovial fluid after oral treatment with 75 mg diclofenac tablets[99]. The diclofenac IC_{50} (i.e. the concentration that produces 50% of the maximum inhibition of prostaglandin synthesis[31]) for PGE_2 in synovial fluid was calculated to be 45 ng/ml[99]. Using this IC_{50}, the therapeutically active concentrations of 100–500 ng/ml predict PGE_2 reductions of 85–100%, consistent with the effects noted by Chlud and Wagener[110].

Table 4. Minimal effective therapeutic concentrations of diclofenac in target tissues, as reported in the literature.

Reference	Method used	Minimal therapeutic concentration for 50% PGE_2 inhibition (IC_{50}), ng/ml (mean ± SE)
Cordero *et al.*, 2001[101]	*In vitro*, human fibroblasts culture	8.9 ± 3.0
Kato *et al.*, 2001[112]	Bioassay, human peripheral monocytes (healthy volunteers)	7.7 ± 3.0
Riendeau *et al.*, 2001[113]	*In vitro*, human whole blood assays (healthy volunteers)	14.8 ± 3.0
Giuliano and Warner, 1999[29]	*In vitro*, washed human platelets (healthy volunteers)	0.47
Warner *et al.*, 1999[114]	Human whole blood assay	11.2
	Human modified whole blood assay	5.9
Cryer and Feldman, 1998[115]	Human whole blood assay (healthy volunteers)	2.96
Pairet *et al.*, 1998[116]	Human whole blood assay (healthy volunteers)	21.0

Abbreviations. COX, cyclooxygenase; IC_{50}, minimal concentration of an active substance that brings about a 50% reduction in the prostaglandin synthesis; PGE_2, prostaglandin E_2; SE, standard error of the mean.

Martel-Pelletier and colleagues confirmed the effects of active diclofenac levels by spiking synovial tissue samples (ex vivo, stimulated with lipopolysaccharide stimulation [LPS]) with 125 and 250 ng/ml diclofenac, leading to >90% inhibition of PGE_2 synthesis[111]. Therefore, based on our own calculations (cf. Liauw *et al.*[99], Chlud and Wagener[110], Martel-Pelletier *et al.*[111]), a diclofenac concentration in synovial tissues of 45 ng/ml is associated with a 50% reduction (IC_{50}) in PGE_2 (acting as a surrogate for inhibition of COX-2), while >100 ng/ml is associated with >80% reduction in PGE_2. This data and the corresponding IC_{50} values for the synovial tissue differ significantly from those estimated in whole blood (which can be found in Table 4); the in vivo IC_{50} levels are much higher than these in vitro levels, and higher diclofenac concentration levels would be therefore required to reach in vivo levels.

The inconsistency between synovium and whole blood results may be due to differences in experimental process and/or differences in the biological environment. The in vitro whole blood evaluation of PGE_2 following LPS stimulation follows a set protocol[109], whereas the clinical data followed an alternative methodology as the patient data from Liauw *et al.* did not require LPS stimulation[117]. The data from Martel-Pelletier *et al.* (in synovial tissue with LPS stimulation, similar to whole blood assay) was consistent with the patient data[111] and not the whole blood IC_{50} results. Another possible reason for the difference is the biological environment. The whole blood assay is performed using 10 μg/mL LPS in heparinized whole blood and so contains albumin, lymphocytes, etc. associated with diclofenac disposition and the immune response. Conversely, synovial fluid does not contain the same amount of lymphocytes and has a small and significant difference in plasma protein binding[118]. Whether due to assay or environment, the differences indicate that the in vitro estimates of COX-2 inhibition through inhibition of PGE_2 production greatly overestimate the inhibition caused by diclofenac in the synovial environment. The PGE_2 whole blood assay is a convenient in vitro method for assessing COX-2 potency in blood and it matches the ex vivo inhibition seen in human subject blood following administration of NSAIDs. However, the observed human in vivo/ex vivo potency is shown to be different, so evaluation of COX-2 inhibition should be done with human samples in vivo or by replicating the assay in synovial explanted tissue[104,111,117].

This potency data for diclofenac in OA knee tissue following oral administration provides context to the diclofenac levels achieved following topical administration. This would allow an initial evaluation, based on COX-2 and PGE_2 pharmacology, of whether the levels achieved are active or not. Further context to expected bioactivity/efficacy would also be provided by considering the downstream therapeutic and biochemical consequences of the PGE_2 synthesis inhibition, with effects on therapeutic endpoints such as the Western Ontario and McMaster Universities Arthritis (WOMAC) index and/or inflammatory cytokines in the PGE_2 signal transduction pathway[104].

Inflammatory cytokines are downstream of PGE_2 within the signal transduction pathway[119]. The PGE_2 levels can correlate with inflammatory cytokines, including interleukin (IL)-6 and tumor-necrosis factor (TNF)α – reduction of PGE_2 synthesis coincides with reduction in these cytokines[120,121]. Gallelli and colleagues demonstrated that IL-6 and TNFα reduced in a dose-dependent manner in OA patient synovial tissue when patients were treated orally with either 75 mg or 150 mg diclofenac per day[104]. This reduction in cytokines matched the reduction in PGE_2 observed at the same dose, and also matched an improvement in the composite OA score, the WOMAC index. As might be expected, there was a smaller improvement in WOMAC score with oral diclofenac 75 mg/day compared with 150 mg/day, but the improvement was nonetheless clinically meaningful. A dose of 75 mg/day diclofenac represents the over-the-counter (OTC) limit in many countries. It is associated with an approximate average synovium diclofenac concentration of 50–175 ng/ml (which we estimated from Elmquist *et al.*[98], Liauw *et al.*[99] and Fowler *et al.*[122] [Supplementary Tables 2–4], assuming linear pharmacokinetics) following single and steady state dosing respectively associated with estimated peak PGE_2 inhibition of >50%.

Topical versus oral application

In the studies that directly compared topical and oral administration, maximum plasma concentrations of diclofenac after topical application were generally lower than after oral administration, falling within a range of 0.4–2.4% of those achieved after oral administration (Supplementary Table 3). This observation is in agreement with the 0.2–8% reported in other reviews[33,36,37]. The mean plasma concentrations and plasma AUC values were also lower after topical versus oral administration (between 6–71% and 0.6–21% lower, respectively; Supplementary Tables 2 and 4), indicating that plasma

diclofenac concentrations were lower over time with topical administration than with oral administration. Furthermore, maximum plasma diclofenac concentrations were achieved more slowly after topical administration (1.25–30 hours) compared with oral administration (20 minutes to 6.5 hours). The results from Brunner *et al.*[90] indicate a steep tissue-to-plasma gradient; the relative bioavailability of diclofenac in the target tissue (subcutaneous adipose and skeletal muscle) was substantially higher after topical dosing (324%) than oral dosing (29%), whereas relative plasma bioavailability was 50-fold lower.

Topical application

Topical NSAIDs are considered effective in treating joint pain[15,43,45]. This efficacy was established in clinical trials using endpoints such as the WOMAC score, for which the oral data suggests a link with joint drug levels and their effects on PGE_2. Evaluating the joint concentrations of diclofenac, in the context of the oral data, may shed insight into how these products have an effect and what might be target levels of drug.

In OA, topical NSAIDs seem to work by permeating through the skin to reduce inflammation in periarticular structures, and travelling via the local blood supply to reduce inflammation within the joint itself[15]. Therefore, it is important to have a clear understanding of the concentrations of topical diclofenac that can be reached in synovial fluid and synovial tissue. Accordingly, we searched the literature to identify studies that measured the concentration of diclofenac in various compartments after topical administration (Supplementary Tables 2–4). It is important to note that there were many inconsistencies in methodological approaches between these topical diclofenac studies, which makes it difficult to draw firm conclusions on concentrations in the target tissues. The available data is variable due to differences in study designs (e.g. dose size, regimen, etc.), but also between similar trials and between subjects within the trials. It was not possible to differentiate the data, thus the concentration data discussed below does not reflect the dose or formulation used and only general trends can be observed. Future studies should adopt a standardized approach using consistent criteria to enable a more robust comparison.

Despite the above-mentioned shortcomings, it has been demonstrated that topically administered diclofenac penetrates through the skin and permeates to the target tissues in appreciable amounts, with different concentrations within the knee (Supplementary Tables 2–4). The mean diclofenac concentrations varied across tissues, from 90.6 ng/ml in subcutaneous tissue to 9.3–63.3 ng/g in muscle, 4.99–20.4 ng/g in synovial tissue, 1.02–25.5 ng/ml in synovial fluid, and 1.42–40.6 ng/ml in plasma (Supplementary Table 2), with a similar pattern in the maximum concentrations obtained (Supplementary Table 3). Overall, the mean concentrations of diclofenac after topical administration appear to be similar in synovial fluid and plasma. Diclofenac concentrations were generally higher in synovial tissue than in synovial fluid or plasma after topical administration. Although there is little data, it appears that after topical administration the concentration of diclofenac declined from subcutaneous tissue > muscle > synovial tissue > synovial fluid ≈ plasma. As might be expected, repeat dosing led to higher levels than following single dose administration.

A typical topical OTC dose is 160 mg of diclofenac per day. There is sparse joint concentration data, with most obtained from single time points and few at relevant doses. The scarcity of data means that it is not possible to draw any conclusions about what levels of diclofenac are reached for comparison with oral data or evaluation of prostaglandin inhibition – further studies are required.

Duration of exposure in target tissue

The tissue concentrations of diclofenac achieved after oral administration demonstrate a durable and sustained exposure compared to plasma levels (Supplementary Table 4). A similar trend in joints is expected following topical administration, where soft tissue exposure is similarly durable and consistent and independent of the dose route[96]. This was observed in an in vivo comparison of oral vs. topical diclofenac[90]. Diclofenac concentrations were sustained over 48 h in subcutaneous tissue and muscle after both topical and oral administration, although higher levels were observed after topical delivery; plasma levels were significantly lower after topical administration[90]. Supplementary Table 4 indicates that the mean diclofenac concentration AUC values over the 12 hour period after topical application ranged 1.41–8867 ng·h/ml in the subcutaneous tissue, 1.09–18.2 ng·h/ml in muscle, and 7.30–1224.19 ng·h/ml in plasma, with a median value between 93 and 142 in synovial fluid (Supplementary Table 4). In general, the AUC declined from plasma > synovial fluid > subcutaneous tissue ≈ muscle. This order was also observed after oral administration, but the AUC was much higher in plasma while the AUC was much lower in subcutaneous tissue and muscle compared with topical application.

Effective concentrations in target tissues after topical application

To determine whether the concentrations of diclofenac reported throughout the skin and joint after topical application are sufficient to exert a therapeutic effect, they may be compared against the minimal concentrations of diclofenac (IC_{50}) that have been reported to have a therapeutic effect. The human data, which provides a more cautious IC_{50} compared to in vitro data, suggests a higher IC_{50} of approximately 45 ng/ml. It should be noted that the mechanisms of COX inhibition are variable and complex and there should be a certain degree of caution when interpreting the IC_{50} values[123]. The contributing data for diclofenac levels in joints following topical administration are sparse and insufficient to estimate the levels of PGE_2 inhibition. We know the relationship between synovium drug levels and PGE_2 from oral studies, but there is poor data on either endpoint for topical NSAIDs. Furthermore, we are unable to determine the degree of effectiveness of the diclofenac concentrations observed in

Supplementary Tables 2–4. We know that topical diclofenac is effective, but we can't associate this effectiveness with a joint diclofenac concentration or reduction in PGE_2 levels. There is a high placebo effect, but nevertheless topical NSAIDs are as effective as oral diclofenac in OA[4]. There is a link to clinical outcomes such as the WOMAC scale, but it needs to be finalized. More data is required to define a minimum effective drug concentration in the synovium and see how current or future topical diclofenac concentrations compare.

Even with low systemic availability, topical diclofenac can be effective in OA, supporting the notion that plasma concentrations are not necessarily an indication of efficacy[124]. For example, in one study plasma levels of diclofenac were undetectable after topical administration for up to 4 hours; however, the antihyperalgesic effect 1 hour after dosing was 2.2-fold greater with topical diclofenac than oral diclofenac, corresponding to a subcutaneous tissue AUC value that was 2.6-fold higher[20]. Topical diclofenac has been used effectively for many years in the management of OA[4,15,33], with a lower rate of systemic adverse events than oral diclofenac[44].

We noted that in some studies after topical administration, diclofenac levels were higher in plasma than in synovial fluid or tissue levels. This may reflect the fact that faster penetration can increase plasma exposure, which is an undesirable situation for a topical drug as there is a potentially greater risk of systemic adverse events. Patient variability is also an important factor that influences how quickly effective concentrations are reached, with intra- and interindividual skin properties influencing percutaneous absorption[42,90]. Interindividual variability has been shown to result in different concentrations of diclofenac in subcutaneous tissues, with a subsequent antihyperalgesic effect that was highest in patients with the highest tissue AUC values[20]. For this reason, it is difficult to accurately compare different formulations of a topical NSAID in clinical trials.

A preferred trial approach would include a design where the plasma levels of drug are collected in parallel with concentrations at the sites of action and measures of bioactivity, i.e. PGE2, inflammatory cytokines, etc., in an approach similar to that used by Liauw and colleagues[99]. This could be performed using a combination of synovial biopsy and arthroplasty. The patients would already be scheduled for arthroplasty, making it unnecessary to schedule a significant intervention that is not beneficial to the patient. Use of synovial biopsy prior to surgery can strengthen the study design by allowing a repeated measures approach, where the biopsy provides the baseline followed by arthroplasty to evaluate the drug effect. Additionally, the arthroplasty could provide tissue suitable for advanced imaging techniques such as MALDI mass spectrometry. This approach would provide a holistic characterization of drug exposure and effect, increase the value of data provided, and provide important context between target drug levels, systemic exposure and drug effect.

Summary

Topical products were developed to reduce the potential for systemic effects that have been reported with orally administered drugs, and to deliver the active drug locally to the site of injury to relieve pain. They can be an effective alternative to orally administered drugs, and topical NSAIDs are recommended before the use of oral NSAIDs in the treatment of knee OA. The pharmacological action of topical drugs relies on penetration and permeation through the skin into the lower layers. Many factors can affect this process and need to be considered in the topical administration of NSAIDs, including the innate properties of the drug, the formulation used, the methods of application, and patient inter- and intraindividuality.

Taking these factors into consideration, there is a sound rationale to use topical diclofenac to relieve pain and inflammation in OA. The available evidence suggests that after topical application, the drug can penetrate the skin and permeate to deeper tissues, with generally higher levels in muscle than in plasma compared with oral administration. The concentrations achieved in the target tissues appear to be sufficient to exert a therapeutic effect, although these may be minimally effective levels. Repeat dosing is beneficial. Nevertheless, there is room for improvement with future formulations.

More data is required to evaluate the penetration and permeation after topical delivery. The available data for the concentration of diclofenac within various tissues after topical administration is old, sparse and inconsistent. Use of the Franz cell alone, although useful, only evaluates penetration and possibly systemic exposure, and does not provide an insight regarding the likely site of action levels and resulting efficacy of the drug. A better screening cascade incorporating Franz cell and other assays is needed. The absence of additional in vitro or in silico methods means downstream tissue permeation requires in vivo characterization of tissue concentrations and bioactivity. The synovium IC_{50} (approximately 45 ng/mL) is higher than that determined using the whole blood PGE_2 assay. To estimate the clinical efficacy, the synovial approach is better than the whole blood approach as the latter will overestimate the efficacy. Thus, COX-2 inhibition should be done with human samples in vivo or replicating the assay in synovial explanted tissue. The use of IC_{50} needs to be clarified, particularly regarding the differences between in vitro and in vivo values.

Despite uncertainty regarding the concentration of diclofenac required to inhibit COX-2, it is clear that topically administered diclofenac is pharmacologically effective, and patients report significant pain relief in mild to moderate OA that extends beyond the placebo effect and is comparable to oral diclofenac. Thus, combined with its more favorable safety profile, there is a sound basis to use diclofenac administered topically rather than orally.

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