J. Pharm. Pharmacol. 1998, 50: 153–158
Received July 30, 1997
Accepted October 28, 1997

Transdermal Delivery and Accumulation of Indomethacin in Subcutaneous Tissues in Rats

STEPHEN A. MIKULAK*, C. THOMAS VANGSNESS* AND MARCEL E. NIMNI

*Department of Biochemistry & Molecular Biology and *Department of Surgery and Orthopaedics, School of Medicine, University of Southern California, Los Angeles, CA 90033, USA*

Abstract

Oral non-steroidal anti-inflammatory drugs (NSAIDs) are effective pharmacotherapy for a wide variety of painful, inflammatory disorders. Development of an efficient means of topical administration of NSAIDs could increase local soft-tissue and joint concentrations while reducing systemic distribution of the drug, thereby reducing side-effects. With this in mind we studied the effects of a novel topical penetration enhancer for lipophilic compounds, a trans-phase delivery system (TPDS), a solution of benzyl alcohol, isopropanol and acetone, on the distribution of indomethacin in various tissues locally and remote from the site of application. We compared the TPDS with a 50 : 50 (v/v) mixture of propylene glycol and ethanol, a commonly used penetration enhancer, and with oral administration.

We found that the TPDS was significantly superior to the other approaches at achieving high local-tissue concentrations in the vicinity of the site of application. In addition, comparison of these two carrier systems seems to clarify the different aqueous and hydrophobic pathways of drug penetration which emerge from various experimental findings and theoretical considerations.

Our results suggest that this non-aqueous solvent system, and benzyl alcohol in particular, because of its unique physicochemical and solvating characteristics, might be able to deliver therapeutic levels of indomethacin to tissues close to the site of application in a safer and more effective manner than presently accepted forms of delivery.

For decades, soft-tissue problems associated with trauma, osteoarthritis and rheumatoid arthritis have been treated successfully with non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin (Van Marion 1973). Unfortunately, the occurrence of many side-effects (Borda & Koff 1992) has lessened the appeal of NSAIDs. Gastrointestinal bleeding and exacerbation of renal insufficiency occur secondarily to the high plasma concentrations necessary to achieve therapeutic concentrations at the site of action. One proposed method for reducing plasma levels while achieving therapeutic tissue levels is administration of NSAIDs percutaneously rather than orally (Guy & Maibach 1983). Many investigators have employed compounds that interact with the stratum corneum barrier of the skin, enhancing the penetration of co-administered compounds (Hori et al 1989; Idson 1985). Penetration enhancers such as piperidone and pyrrolidone derivatives (Quan et al 1990; Sasaki et al 1991), oleic acid (Francoeur et al 1990; Takahashi et al 1991), 1-dodecylazacycloheptan-2-one (azone; Stoughton 1982), cetyl lactate (Kaiho et al 1989), propylene glycol (Nomura et al 1990), and transparent oil-water gels (DeVos et al 1991) have been proven to aid the passage of NSAIDs across the stratum corneum. Although literature comparing the efficacy of topical NSAIDs with oral preparations is sparse (Kroll et al 1989; Russell 1991), studies have demonstrated equal efficacy for topical and oral administration of indomethacin in rat models (Fregnan et al 1974; Heilman 1975). Because the true efficacy of percutaneously delivered NSAIDs remains to be proven in a properly designed clinical comparison with commonly used oral NSAIDs, over-the-counter topical salicylates in a triethanolamine vehicle (Golden 1978; Rabinowitz et al 1982) presently remain one of the few alternatives to oral NSAID therapy in the US.

Correspondence: M. E. Nimni, USC/Children's Hospital Los Angeles, Division of Surgical Research, 4650 Sunset Blvd., mailstop #35, Los Angeles, CA 90027, USA.

The data, or lack thereof, suggest the need for a more effective penetration enhancer. Benzyl alcohol preparations have shown promise as such (Jimbo et al 1983; Hiramatsu et al 1990). Physicochemical studies have demonstrated that benzyl alcohol can solvate compounds, forming a micelle, while retaining contact with an aqueous solution (Kaneshina et al 1984), a property conducive to penetration of the stratum corneum. The current study compares the levels of [2-^{14}C]indomethacin in blood, urine, faeces and several tissues after delivery of one oral and two topical preparations to rats. Our results show that, compared with a standard 50 : 50 (v/v) propylene glycol–ethanol vehicle and an oral corn-oil solution, a trans-phase delivery system (TPDS; Nimni et al 1990) comprising a mixture of benzyl alcohol, isopropanol and acetone results in high levels of indomethacin in tissue in the vicinity of the site of application while delaying systemic absorption.

Materials and Methods

Composition and preparation of indomethacin solutions

[2-^{14}C]Indomethacin (100 μCi; specific activity 53 mCi mmol^{-1} = 147 μCi mg^{-1}, radiochemical purity 84%) was purchased from Amersham International, UK. Ethanol (150 μL) was added to dissolve the radioactive indomethacin. Unlabelled indomethacin, propylene glycol, ethanol, acetone, isopropyl alcohol and benzyl alcohol were purchased from Sigma (St Louis, MO). A solution (1%) of indomethacin was prepared in the TPDS (10% benzyl alcohol, 40% acetone and 50% isopropyl alcohol) or in a 50 : 50 (v/v) mixture of propylene glycol and ethanol for topical application. Radioactive indomethacin solution (50 μL, i.e. 33 μCi) was added to 1·65 mL of the 1% solutions of indomethacin in the TPDS and propylene glycol–ethanol, resulting in a solution that contained 2 μCi in each 0·1-mL dose. A 0·25% solution of indomethacin for oral ingestion was prepared by mixing with corn oil over mild heat. For this purpose radioactive indomethacin solution (50 μL) was added to 6·6 mL of 0·25% indomethacin in corn oil, resulting in a solution that contained 2 μCi in each 0·4-mL dose.

Animal studies

These were conducted on 3-month-old male Long-Evans rats, 300–350 g, from Simonsen Industries. The rats were housed in metabolic cages enabling separate collection of faeces and urine. Each value in this study represents triplicate assays from three different rats (nine rats per time-interval). Rats were tranquillized by inhalation anaesthesia with halothane USP. Their right shoulders were shaved so that an approximately 1 cm^2 square could be drawn on the intact skin over the right glenohumeral joint. The first rat received 0·1 mL radioactive 1% indomethacin in the TPDS solution (approximately 2 μCi) in three separate applications of 33 μL by means of a micropipette. The solution was left to dry and penetrate the skin for 30 min, after which time the skin was thrice blotted dry with paper towel. The rat was placed in a metabolic cage and left to recover from the anaesthesia. At various times (0·5, 2, 4, 8, 24 and 48 h) after the initial application rats were re-anaesthetized with halothane and a sample of blood (1 mL) obtained by cardiac puncture. Rats were killed with a 1-mL intraperitoneal injection of Eutha-6 and the skin wiped with ethanol to remove any residual surface radioactivity. The rat was dissected and several tissues were removed for assay of radioactivity: skin below the application area, deltoid (full-thickness sections 3 mm above and 3 mm below the glenohumeral joint), rotator cuff (subscapularis, supraspinatus, infraspinatus and teres minor, sectioned at the humeral insertions and 3 mm proximal), joint capsule (devoid of muscle fibres), liver, kidney, stool from the ascending colon, urine from the bladder and from the collection beaker at the base of the metabolic cage. Samples were diced with a scalpel into the smallest possible fragments. Instruments and dissecting area were cleaned with ethanol before harvesting and dicing to prevent transfer of radioactivity between tissues. Samples, including blood and urine, were weighed and transferred to centrifuge tubes for addition of ethanol (2–4 mL). Tissue samples were homogenized in a Polytron homogenizer to enable ethanol extraction of indomethacin from tissues. The resulting emulsion was centrifuged at 3500 rev min^{-1} for 5 min and a sample of the indomethacin-containing supernatant was counted for radioactivity in a Beckman liquid-scintillation counter.

During the same experiment, another group of rats underwent the previously described procedure after receiving topical application of 0·1 mL radioactive 1% indomethacin (approximately 2 μCi) in propylene glycol–ethanol solution. A third and final group was intubated with an oral-gastric feeding tube for gavage with 0·4 mL radioactive 0·25% indomethacin solution in corn oil.

Measurements and analysis

As a means of standardizing and comparing results, counts min^{-1} (mg tissue)$^{-1}$ were calculated taking into account the volume of ethanol added to the tissue, blood or urine, and the weight or volume of the material, and a sample of extract or solution was counted. This calculation was performed after

triplicate analysis of all the tissues from three rats and data were analysed by one-way analysis of variance to establish statistical significance. Tissue levels of indomethacin are expressed as counts min^{-1} (mg tissue)$^{-1}$.

Results and Discussion

The presence of benzyl alcohol in formulations used for transdermal delivery of indomethacin significantly increased the retention of this drug in subcutaneous tissues beneath the site of application (Figure 1, A–C). The concentration achieved peaked at 2 h for the deltoid muscle and at 4 h for the rotator cuff structures and the joint capsule, reflecting the pathway followed during penetration. Similar patterns were observed for indomethacin dissolved in the propylene glycol solvent mixture, but levels achieved were always significantly lower. Both forms of transdermal delivery resulted in much higher tissue levels than those achieved when a similar dose was administered orally. At the peak periods differences are statistically significant ($P < 0.05$).

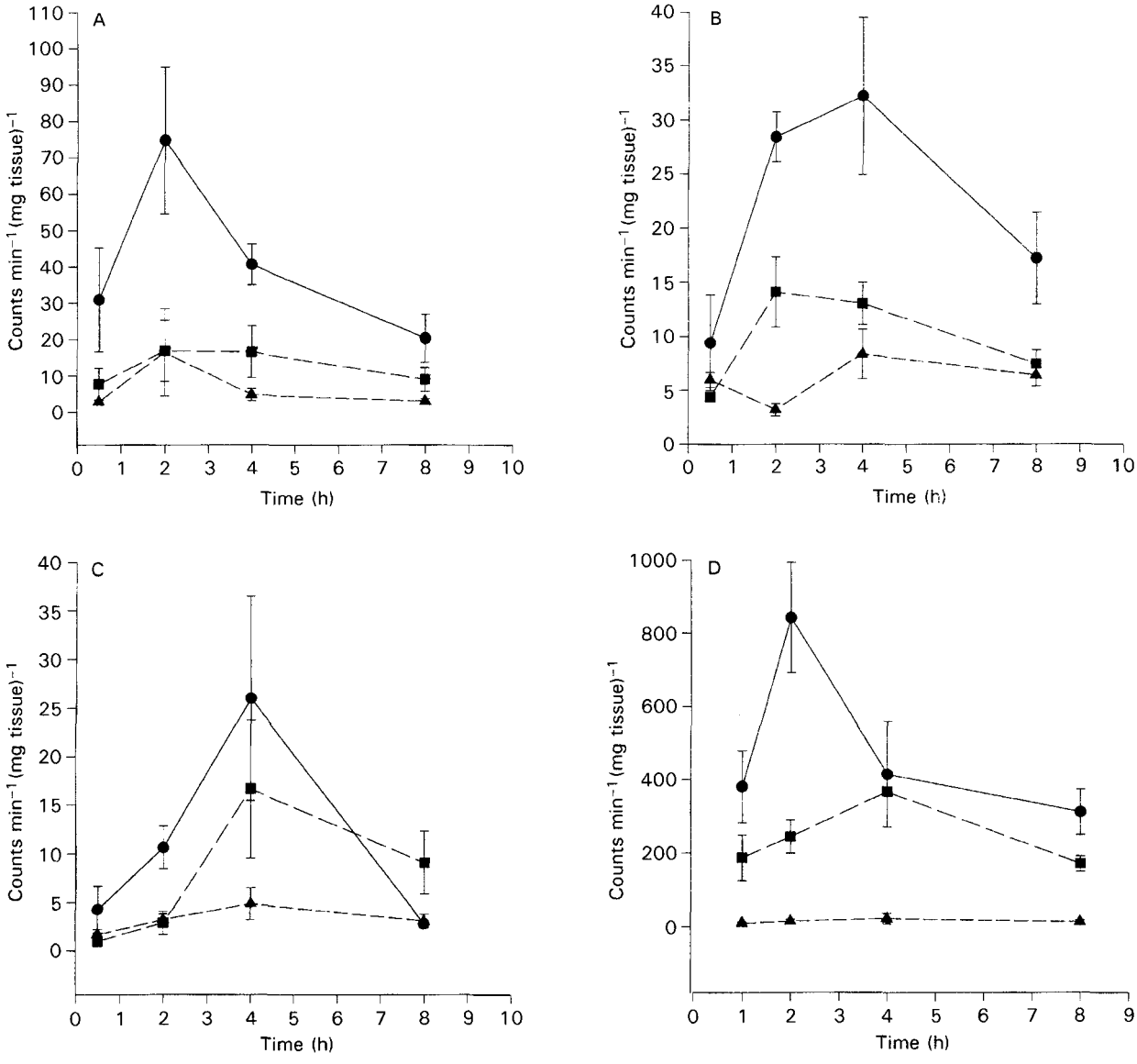

Figure 1. Radioactivity of [2-^{14}C]indomethacin in subcutaneous tissues after topical application of a solution of indomethacin (1%; 0·1 mL) containing 2 μCi radiolabelled compound dissolved either in the TPDS (●) or in propylene glycol–ethanol (■) or after oral administration of equivalent amounts of radioactive material and carrier dissolved in corn oil (▲). The values are expressed as counts min^{-1} (mg original tissue)$^{-1}$. The perpendicular lines represent the standard error of values determined in triplicate from three different animals for each time point (0·5, 2, 4 and 8 h) after either topical or oral administration. A, deltoid muscle; B, joint capsule; C, rotator cuff; D, skin at the site of application or, for oral administration, from the dorsum of the animal above the deltoid muscle.

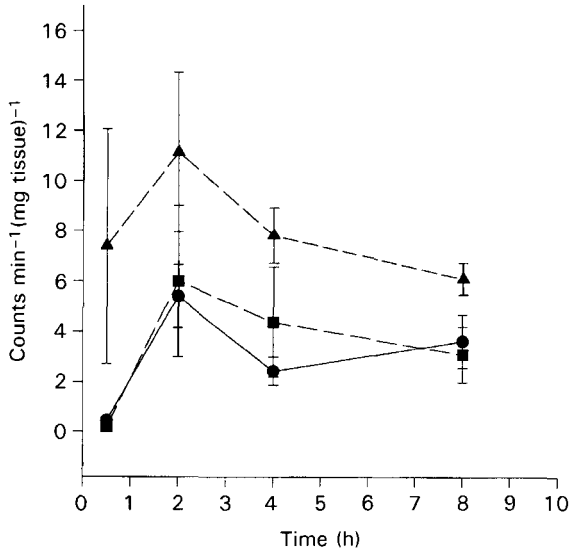

Figure 2. Radioactivity present in the liver after topical or oral administration of radioactive indomethacin: ●, TPDS; ■, propylene glycol–ethanol; ▲, oral.

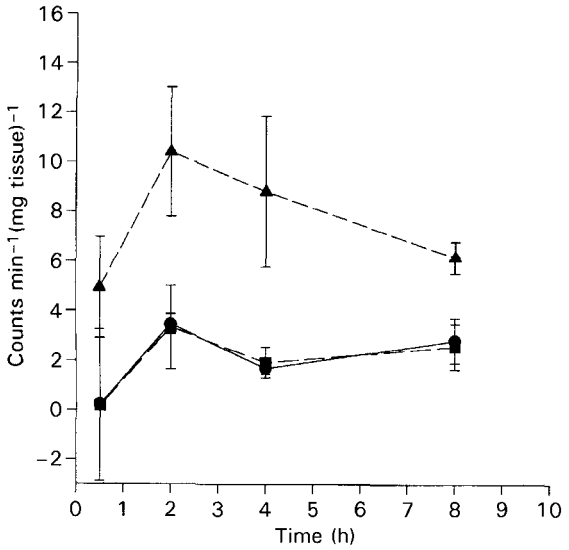

Figure 3. Radioactivity present in the kidney after topical or oral administration of radioactive indomethacin: ●, TPDS; ■, propylene glycol–ethanol; ▲, oral.

The skin underlying the site of application, which included both dermis and epidermis, seemed to retain more indomethacin when the vehicle contained benzyl alcohol (Figure 1D). Essentially no significant amount of drug reached the skin when the drug was fed orally. On the other hand, higher levels were, as expected, measured in the other organs (liver, kidney and blood) in the animals that received the drug orally; these levels peaked approximately 2 h after administration (Figures 2–4).

The amount excreted (in urine and faeces combined) was also much greater when the drug was

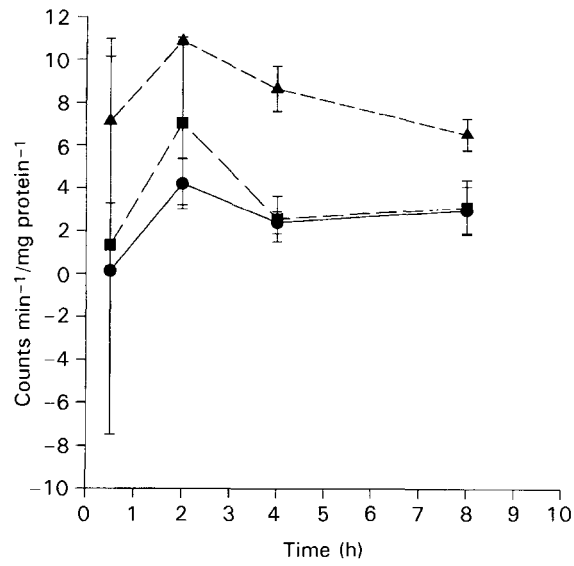

Figure 4. Radioactivity present in the blood after topical or oral administration of radioactive indomethacin. Radioactivity is expressed as counts min^{-1} (mg serum protein)$^{-1}$: ●, TPDS; ■, propylene glycol–ethanol; ▲, oral.

administered orally. The values for combined excretion in Figure 5 (which was extended to the full 48 h) clearly show that by 8 h most of the absorbed drug has been eliminated from the body. The results in Figure 5 represent cumulative values for combined excretion during the 48-h period of investigation.

The amphopathic properties of benzyl alcohol, i.e. its strong solvation of hydrophobic compounds and its moderate hydrophilicity, are probably

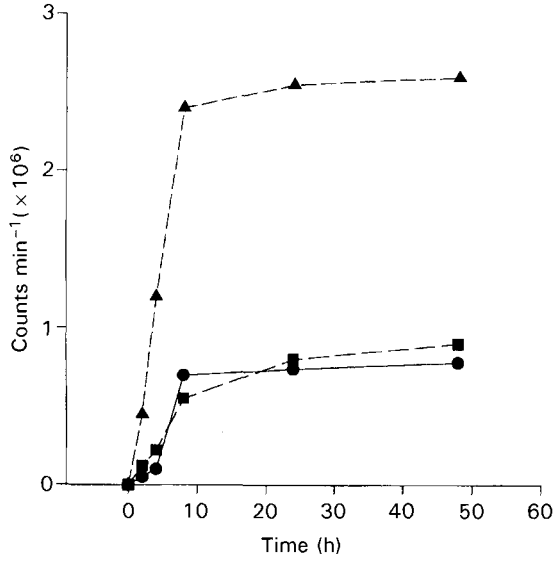

Figure 5. Combined values of urinary and faecal excretion of radioactivity after topical or oral administration of radioactive indomethacin. The values are cumulative and represent total radioactivity excreted without correction for quenching: ●, TPDS; ■, propylene glycol–ethanol; ▲, oral.

responsible for generating the pattern of tissue distribution of indomethacin observed in this study. Most compounds, particularly those with marked aqueous solubility, penetrate the epidermis with difficulty because water is readily excluded from the body by a barrier effect.

Various penetration enhancers, including benzyl alcohol, penetrate the skin, disrupting the skin structure or fluidizing its lipids; this results in increased rates of absorption (Knutson et al 1985; Woodford & Barry 1986; Smith & Maibach 1995). On the other hand, vehicles such as polyethylene glycol seem to slow the rate of absorption by a mechanism which is not known (Katz & Dalvi 1983).

The data from Figures 1-4 illustrate a trend showing that the topical TPDS was superior to topical propylene glycol–ethanol and oral delivery systems at achieving higher local tissue indomethacin concentrations, while maintaining lower concentrations in remote tissues, excrement and the circulation. Keeping liver-indomethacin levels to a minimum is the key of reducing enterohepatic recirculation, which can be extensive (Duggan & Kwan 1979; Schneider et al 1990) and which contributes to the dose-dependent gastrointestinal toxicity of the drug. In contrast, in man drug diffusion through the skin, from which hair follicles are relatively absent, seems be approximately 2–4 times lower in 24 h (Rougier et al 1987; Illel et al 1991). Our data indicate that the TPDS enabled deep penetration of indomethacin with delayed systemic absorption; this accords with the finding that a topical benzyl alcohol–indomethacin gel inhibits vascular permeability to systemically administered dye by 25% in rats (Hiramatsu et al 1990). The same study also shows this formulation to be efficacious in animal inflammatory models.

The unique physicochemical properties of benzyl alcohol make it compatible with both aqueous and lipophilic environments. This biphasic behaviour might explain the penetration of the TPDS through the stratum corneum, conceivably by disorganizing its multilaminate hydrophilic-lipophilic layer (Dohi et al 1990; Boden et al 1991). Even though our experiments showed deep tissue concentrations to be comparable when the two different topical formulations were studied, accumulation in the target tissues (i.e. joint capsule and proximal musculature) was significantly higher in TPDS-treated animals.

The method proposed for transdermal drug delivery employing benzyl alcohol and fugitive solvents such as acetone and isopropyl alcohol is based on a simple principle which we have termed the trans-phase system. The compounds in question, indomethacin or other NSAIDs, are most suitable for delivery by this method when they are significantly more soluble in organic solvents such as benzyl alcohol than in aqueous solvents. Acetone and isopropyl alcohol are used as co-solvents and because of their greater volatility rapidly evaporate from the skin. As this happens the drug is transferred to the less volatile phase, benzyl alcohol, which has very rapid permeation characteristics (Nimni et al 1990). As part of a study of the rate of penetration of 18 different fragrance materials used in cosmetics benzyl alcohol was found to penetrate man's epidermis most rapidly (Jimbo 1983). When the drug has been carried across the epidermal barrier, the dermis with its meshwork of collagen, elastin and proteoglycans, a high water content and a sparcity of cells offers little resistance to flow (Nimni 1997).

It is clear from these studies that benzyl alcohol is an excellent penetration enhancer. Because of its penetrant and solvent characteristics it prevents the deposition of solutes on the skin surface, as would happen if acetone or isopropyl alcohol alone were used as carriers.

In summary, the TPDS was significantly superior to oral indomethacin and topical propylene glycol–EtOH–indomethacin at achieving high local-tissue concentrations relative to levels in plasma and remote tissues. Indomethacin in the TPDS is readily absorbed and results in plasma indomethacin levels comparable with those which result from a variety of other enhancers (Ginsberg & Famaey 1991). Its property of selective concentration of the drug in the target tissues seems to be associated with intrinsic characteristics of the benzyl alcohol molecule. Despite indications of skin toxicity associated with transdermal delivery reported by investigators who in some instances exposed skin for up to 24 h to solvents by means of retention cups (Lashmar et al 1989; Doucet et al 1990), we found no qualitative evidence of erythaema, oedema, or skin anomalies secondary to topical delivery of indomethacin.

References

Boden, N., Jones, S. A., Sixl, F. (1991) On the use of deuterium nuclear magnetic resonance as a probe of chain packing in lipid bilayers. Biochemistry 30: 2146–2155

Borda, I. T., Koff, R. S. (1992) NSAIDs, A Profile of Adverse Effects, Hanley & Belfus, Pennsylvania

DeVos, F., DeMuynck, C., Geerts, M., Remon, J. P. (1991) Percutaneous absorption of indomethacin from transparent oil/water gels in rabbits. J. Pharm. Pharmacol. 43: 237–241

Dohi, M., Kaiho, F., Suzuki, A., Sekiguchi, N., Nakajima, N., Nomura, H., Kato, Y. (1990) Enhancing effects of myristyl lactate on percutaneous absorption of indomethacin. Chem. Pharm. Bull. 38: 2877–2879

Doucet, O., Hagar, H., Marty, J. P. (1990) Enhancement of indomethacin percutaneous absorption effect of 2-*n*-nonyl-1,3-dioxolane. Travaux Originaux Research Papers

Duggan, D. E., Kwan, K. C. (1979) Enterohepatic recirculation of drugs as a determinant of therapeutic ratio. Drug Metab. Rev. 9: 21–41

Francoeur, M. L., Golden, G. M., Potts, R. O. (1990) Oleic acid: its effects on stratum corneum in relation to transdermal drug delivery. Pharm. Res. 7: 621–627

Fregnan, G. B., Torsello, A. L., Brunet, S. (1974) Local anti-inflammatory properties of etoclofene. A new fenamate derivative. Pharmacology 11: 213–219

Ginsberg, F., Famaey, J. P. (1991) Double-blind randomized crossover study of the efficacy and tolerability of a topical indomethacin spray versus placebo in the treatment of tendinitis. J. Int. Med. Res. 19: 131–136

Golden, E. L. (1978) A double-blind comparison of orally ingested aspirin and a topically applied salicylate cream in the relief of rheumatic pain. Curr. Ther. Res. 24: 524–529

Guy, R. H., Maibach, H. L. (1983) Drug delivery to local subcutaneous structures following topical administration. J. Pharm. Sci. 72: 1375–1380

Heilman, R. D. (1975) Effect of topical administration of antiinflammatory drugs to rats with adjuvant arthritis. Arthritis Rheum. 18: 519–523

Hiramatsu, Y., Akita, S., Salamin, P. A., Maier, R. (1990) Assessment of topical non-steroidal anti-inflammatory drugs in animal models. Arzneim. Forsch. 40: 1117–1124

Hori, M., Satoh, S., Maibach, H. I. (1989) Classification of percutaneous enhancers: a conceptual diagram. In: Bronaugh, R. L., Maibach, H. I. (eds) Percutaneous Absorption. 2nd Edn, Marcel Dekker, New York, pp 40–55

Idson, B. (1985) Percutaneous absorption enhancers. Drug Cosmet. Ind. 137: 30–45

Illel, B., Schaefer, H., Wepierre, J., Doucet, I. (1991) Follicles play an important role in percutaneous absorption. J. Pharm. Sci. 80: 424–427

Jimbo, Y. (1983) Penetration of fragrance compounds through human epidermis. J. Dermatol. 10: 229–239

Jimbo, Y., Ishihara, M., Osamura, H., Takano, M., Ohara, M. (1983) Influence of vehicles on penetration through human epidermis of benzyl alcohol, isoeugenol and methylisoeugenol. J. Dermatol. 10: 241–250

Kaiho, F., Koike, R., Nomura, H., Hara, H., Maruoka, K., Dohi, M., Kato, Y. (1989) Enhancing effect of cetyl lactate on the percutaneous absorption of indomethacin in rats. Clin. Pharm. Bull. 37: 1114–1116

Kaneshina, S., Kamaya, H., Issaku, U. (1984) Benzyl alcohol penetration into micelles, dielectric constant of the binding site, partition coefficient and high-pressure squeeze-out. Biochim. Biophys. Acta 777: 75–83

Katz, J. L., Dalvi, U. G. (1983) Evaluation of solvent-skin interaction in percutaneous absorption. J. Soc. Cosmet. Chem. 34: 327–338

Knutson, K., Potts, R. O., Guzek, D. B., Golden, G. M., McKie, J. E., Lambert, W. J., Higuchi, W. I. (1985) Macro- and molecular physical-chemical considerations in understanding drug transport in the stratum corneum. J. Contr. Rel. 2: 67–80

Kroll, M. P., Wiseman, R. L., Guttadauria, M. (1989) A clinical evaluation of piroxicam gel: an open comparative trial with diclofenac gel in the treatment of acute musculoskeletal disorders. Clin. Ther. 11: 382–391

Lashmar, U. T., Hadgraft, J., Thomas, N. (1989) Topical application of penetration enhancers to the skin of nude mice: a histopathological study. J. Pharm. Pharmacol. 41: 118–122

Nimni, M. E. (1997) Collagen, structure and function. In: Dulbecco, R. (ed.) Encyclopedia of Human Biology. Vol. 2, Academic Press, New York, in press

Nimni, M. E., Ertl, D., Oakes, R. A. (1990) Distribution of griseofulvin in the rat: comparison of the oral and topical route of administration. J. Pharm. Pharmacol. 42: 729–731

Nomura, H., Kaiho, F., Sugimoto, Y., Miyashita, Y., Dohi, M., Kato, Y. (1990) Percutaneous absorption of indomethacin from mixtures of fatty alcohol and propylene glycol (FAPG bases) through rat skin: effects of oleic acid added to FAPG base. Chem. Pharm. Bull. 38: 1421–1424

Quan, D., Higuchi, R. I., Takayama, K., Higashiyama, K., Nagai, T. (1990) Enhancing effect of piperidone derivatives on the percutaneous absorption of indomethacin. Drug Des. Del. 6: 61–71

Rabinowitz, J. L., Feldman, E. S., Weinberger, A., Schumacher, H. R. (1982) Comparative tissue absorption of oral ^{14}C-aspirin and topical triethanolamine ^{14}C-salicylate in human and canine knee joints. J. Clin. Pharmacol. 22: 42–48

Rougier, A., Lotte, C., Maibach, H. I. (1987) The hairless rat: a relevant animal model to predict in vivo percutaneous absorption in humans? J. Invest. Dermatol. 88: 577–581

Russell, A. L. (1991) Piroxicam 0·5% topical gel compared to placebo in the treatment of acute soft tissue injuries: a double-blind study comparing efficacy and safety. Clin. Invest. Med. 14: 35–43

Sasaki, H., Kojima, M., Mori, Y., Nakamura, J., Shibasaki, J. (1991) Enhancing effect of pyrrolidone derivatives on transdermal penetration of 5-fluorouracil, triamcinolone acetonide, indomethacin, and flurbiprofen. J. Pharm. Sci. 80: 533–538

Schneider, H. T., Nuernberg, B., Dietzel, K., Brune, K. (1990) Biliary elimination of non-steroidal anti-inflammatory drugs in patients. Br. J. Clin. Pharmacol. 29: 127–131

Smith, E. W., Maibach, H. I. (1995) Percutaneous Penetration Enhancers, CRC Press, Boca Raton, Florida

Stoughton, R. B. (1982) Enhanced percutaneous penetration with 1-dodecylazacycloheptan-2-one. Arch. Dermatol. 118: 474–477

Takahashi, K., Tamagawa, S., Katagi, T., Yoshitomi, H., Kamada, A., Rhtting, J. H., Nishihata, T., Mizuno, M. (1991) In vitro percutaneous transport of sodium diclofenac and diclofenac from oleaginous vehicle. Chem. Pharm. Bull. 39: 509–511

Van Marion, W. F. (1973) Indomethacin in the treatment of soft tissue lesions: a double-blind study against placebo. J. Int. Med. Res. 1: 151–158

Woodford, R., Barry, B. W. (1986) Penetration enhancers and the percutaneous absorption of drugs: an update. J. Toxicol. Cutan. Ocul. Toxicol. 5: 167–178