CHAPTER I

INTRODUCTION

The skin is particularly effective as a selective barrier to the penetration of a diverse range of substances. The epidermis is the major element in this control as illustrated by the evidence that most small, water-soluble nonelectrolytes can diffuse into the capillary system a thousand times more rapidly when the epidermis is absent, damaged or diseased, than when it is present and intact. Permeability of the skin can be predicted and controlled by relating the physiological and physicochemical attributes of the skin to the properties of the penetrant in a vehicle. Scientists are now gathering data to relate the intrinsic properties of the skin barrier to the molecular requirements for modifying the interactions with topical vehicles. The ultimate aim in dermatological biopharmaceutics is to design active drug molecules with selective permeability to be incorporated into vehicles which enable the medicament to arrive at the active site in the biophase at a controlled rate and there, to maintain a sufficient concentration for the required time (Barry, 1983).

When the molecule moves onto the intact skin, either from the external environment or from a vehicle, it first makes contact with the sebum. The diffusant then has three potential routes of entry to the subepidermal tissue-through the hair follicles with their associated sebaceous glands; via the sweat ducts; or across the continuous stratum corneum

between these appendages. The actual pathway for penetration via the pilosebaceous apparatus could be through the hair fiber itself, through the outer root sheath of the hair into the viable cells of the follicle, or through the air-filled canal and into the sebaceous gland. The route for the sweat duct may be through either the lumen or the walls to below the epidermis and through the thin ring of keratinized cells. Dense capillary networks closely envelop the bases of both the sweat ducts and the hair follicles. Most molecules reaching these highly permeable vascular regions would immediately sweep into the systemic circulation.

The stratum corneum is a multicellular membrane. Electron microscopic evidence has implied that the intercellular regions are filled with a lipid-rich amorphous material. In the dry membrane the intercellular volume may reach 5% of the total volume. In the hydrated tissue, the intracellular keratin primarily absorbs the water so that the ratio of intercellular to intracellular volume may drop to 1%. Thus, two possible pathways for diffusion exist, i.e. between or across the cells. Although the intercellular volume is small, it is still able to provide a significant route in theory, provided that the diffusion coefficient for this pathway is large enough. Many topical drugs may find that the stratum corneum provides too great an impediment to their diffusion and that the only significant route by which they may reach the dermis is via the appendages. Very large molecules should penetrate the stratum corneum with difficulty, although the critical size which restricts diffusion have not been established. It appears that electrolytes and polar molecules with three or more polar groups,

particularly NH_2 and OH, require the shunt route to penetrate to any clinically significant extent (Barry, 1983).

There are several methods for assessing the efficacy of anti-inflammatory drugs in man. They generally consist of treating the skin by either physical or chemical means to produce an inflammatory reaction on which the efficacy of the preparation is tested. This is evaluated by allocating a score to the clinical reaction observed, or by measurement of other parameters of inflammation, e.g. redness or blood flow. The inflammatory reaction can be induced by various means, including the application of croton oil, kerosene, or by irradiation with ultraviolet A, B or C. To date, the only non-invasive technique available is the measurement of the degree of blanching produced by the steroids or NSAIDs. The other techniques cause durable, sometimes painful lesion, and the inflammatory mechanism is poorly defined.

Since there is an increased interest in drug administration via the skin, the extent and the rate of percutaneous absorption have therefore become important factors in determining the substance efficacy and pharmacology. The most desirable approach to studying percutaneous absorption is to perform human *in vivo* experiments. Some studies use the indirect radiochemical method or the skin punch biopsy method, which are invasive and have severe technical limitations. Chambin-Remoussenard et al (1993) evaluated the nonradiolabeled caffeine percutaneous absorption from two vehicles by two non-invasive *in vivo* techniques. One method was a

surface recovery technique, in which accurate quantities of caffeine in emulsion and acetone are applied on several skin areas. Then with a recovery solvent, the unabsorbed product was totally removed at different times. HPLC was used to determine the unabsorbed caffeine on the skin surface. The difference between the applied and the recovered amounts corresponded to the absorbed amount. The second technique was a stripping method. Furthermore, the treated and surrounding areas were stripped to check whether there was lateral spread of caffeine within the stratum corneum.

Currently, there are many topical preparations commercially available in the market with numerous indications for use. Evaluation of their *in vivo* performance or topical bioavailbility is a formidable task due to the variety of products as well as difficulties in finding the appropriate evaluation systems for each drug. One of the most interesting groups of drugs available for topical application is the NSAIDs. Its main therapeutic indications are for the treatment of surface and deep skin inflammation as well as the inflammation of joints and muscles.

Piroxicam is one of a series of N-heterocyclic carboxamides of 1,2 benzothiazine 1,1-dioxide which have recently been named by the Committee on United states Approved Names (USAN), as oxicams. It is a nonsteroidal anti-inflammatory agent with potent analgesic and antipyretic activities. In nonspecific animal models piroxicam has been shown to possess anti-inflammatory activity as evidenced by inhibition of carrageenan-induced paw

edema, adjuvant-induced arthritis and granuloma tissue formation in rats, urate crystal-induced synovitis in dogs, ultraviolet-induced erythema in guinea pigs, and leukocyte migration in inflammatory exudates (Brogden et al, 1981).

Oral piroxicam suppresses both primary and secondary lesion of adjuvant-induced arthritis at dosages of 0.3 to 3.3 mg/kg (5 to 20% inhibition of swelling). Topical piroxicam has activity comparable with that of equal oral doses when applied the day before or 15 days after adjuvant challenge. In carrageenan paw edema test, rectally administered piroxicam suspension was equipotent with the orally administered drug (Schiantarelli et al, 1981).

Piroxicam is well absorbed following the oral administration. However, its use has been associated with a number of gastro-intestinal disorders including bleeding and ulceration. To overcome these side effects, Babar et al (1990) undertook a study to develop dosage from using various polymeric gels and ointment bases. Gels and ointment bases containing 1% piroxicam were studied for the *in vitro* release of drug. The general rank order for the *in vitro* drug release from all the bases evaluated was: gel base > hydrophilic base > emulsion base. Among the formulations evaluated, the gel base containing DMSO gave the best *in vitro* drug release both through the cellulose membrane and the hairless mouse skin.

Topical delivery of nonsteroidal anti-inflammatory drugs (NSAIDs) directly to the site of localized distress pain is a desirable feature because

local pain can be alleviated without adverse systemic side effects. However, there has been considerable debate as to whether the topical effect is due to direct penetration or is secondary to systemic absorption and recirculation (Monteiro-Riviere et al, 1993). The studies in male rats strongly implied that topical administration of piroxicam resulted in a high concentration of the drug in the underlying musculature. Thus, cutaneous vasculature apparently does not function as an infinite sink that removes all topically applied drugs to the systemic circulation (Riviere and Williams, 1992). Other investigations have alluded to this mechanism of local topical drug delivery. It has been demonstrated that local modulation of the cutaneous vasculature by co-iontophoresis of vasoactive compounds could affect drug distribution to underlying tissues. The local vasculature underlying the topical application site creates a convective force carrying the topical by applied drug down into the underlying tissues without being absorbed into the systemic circulation (Monteiro-Riviere et al, 1993).

Monteiro-Riviere et al (1993) studied using *in vivo* pig skin treated with topical ^{3}H-piroxicam gel to assess the role of systemic absorption on its delivery to deep tissues. Further, the role of the structure of the cutaneous vasculature (e.g., direct cutaneous of musculocutaneous) was studied. Finally piroxicam delivery was measured using *in vitro* diffusion cells with pig skin obtained from the same sites to determine the inherent permeability independent of vascular anatomy. These studies showed that penetration of the radiolabel occurred in subcutaneous and muscle tissues only under the dose sites and as a prerequisite for local delivery. In contrast, *in vitro* flux

was identical in skin harvested from the two vascular sites, suggesting that the vasculature plays a pivotal role in deep tissue penetration of piroxicam. In conclusion, local delivery of topical drugs occurred independent of systemic absorption and the nature of cutaneous vasculature at different sites must be taken into consideration for optimal delivery.

The parallel trial was conducted to compare the efficacy of piroxicam 0.5% gel and diclofenac 1.16% topical gel preparations in the treatment of 173 patient with well-defined, acute sprains and tendinitis of ankle, shoulder, or elbow (Kroll et al, 1989). Piroxicam gel was applied to the injured area four times daily. There was no difference in the response to treatment or in the global impressions of efficacy between piroxicam and diclofenac. Toleration was regarded as good or excellent by 98% of patients receiving piroxicam and 94% of patients receiving diclofenac. The results of this study show that piroxicam 0.5% gel and diclofenac 1.16% gel are equally effective and well tolerated in the treatment of selected acute sprains and tendinitis.

The pharmacokinetic parameters of a piroxicam 0.5% topical gel were determined in 20 healthy volunteers. Following application of multiple doses (20 mg piroxicam daily) over 14 days, blood samples were drawn from each patient beginning just before application of the first dose, with the final sample taken 14 days after application of the last dose. Plasma concentrations of piroxicam were determined by HPLC using UV detection at 340 nm. There was considerable inter-individual variation in piroxicam

half-lives with a mean of 79 hr. Mean piroxicam plasma concentrations at steady-state were between 300 and 400 ng/ml, which is about 5% of those observed after equivalent doses of oral or intramuscular piroxicam. No adverse experiences were reported during the study period. Thus, the results of this study confirm the minimal systemic absorption of piroxicam during multiple-dose application of the 0.5% topical gel and the excellent tolerability of this mode of piroxicam therapy.

One site of action for NSAIDs is the synovium. However, synovial tissue sampling to determine drug concentrations at the affected site is impractical and therefore synovial fluid is often sampled to examine the penetration of the drug into the joint (Elmquist, 1994). Yet, withdrawal of synovial fluid is still very painful and cannot be commonly practiced. It is therefore employed only in clinical situation under close supervision of a physician.

A new non-invasive technique enabling the efficacy of nonsteroidal anti-inflammatory drugs (NSAID) to be quantified in terms of their effect on a cutaneous challenge is by the use of a nicotinic ester. It is well known that the application to the skin of methyl nicotinate solution rapidly produces a visible vasodilatation, the duration of which varies from subject to subject and depends on the concentration used. The interest of this method is that the extent of the inflammatory reaction can be quantified in terms of cutaneous blood flow by means of a Doppler laser (Poelman, 1989).

The *in vivo* investigation in animals or humans is preferable to the *in vitro* methods, with the most reliable method for the *in vivo* bioavailability assessment generally being the measurement of drug concentration in plasma. However, the very low amount of the drug detected in the systemic circulation may not always represent the amount actually present at the site of action for topically applied drugs, let alone the technical difficulties associated with the plasma drug analysis (Radermacher et al, 1991). The use of radiolabeled compound may increase the detection sensitivity but may not be ethical when applied to humans. More importantly, the target organ of these topical NSAIDs products are the muscles and joint tissues that mostly lie just underneath the skin. Therefore, measurement of the drug level in the skin appears to be more relevant and may better represent the *in vivo* percutaneous absorption (Zesch, 1982). The amount of drug in the skin, especially in the stratum corneum, should reflect the amount of drug at the site of action that are relatively close to the skin surface. Analysis of drug in the skin at various times may give the rough indicator of the rate of percutaneous absorption.

Other *in vivo* models for evaluating topical NSAIDs commonly involves measurements of pharmacological outcomes in animals. The animal models are mostly used for the evaluation of drug action since they often are the necessary tools for mechanistic studies leading to knowledge of the various events occurring during skin inflammation. The increasing need for the proof of ethical use of animals has already resulted in the establishment of a variety of regulations for the control and type of animals models used in

pharmacology. Nevertheless, we must be cautious in making casual extrapolations of the animal data to humans (Bouclier et al, 1989).

Piroxicam was chosen as the model drug in this study due to it popularity and proven clinical effectiveness in the relief of muscle pains and inflammations of joints and skins. Many topical products containing this compound have been available in Thailand for many years. With heavy promotion from various manufacturers, the television and other media, their use is expected to increase continuously. However, none of these commercial products have been systemically evaluated for their *in vivo* performance nor there are any established techniques that can accurately characterize the release and percutaneous absorption behavior of each product. Therefore, the objectives of this thesis were as followes:-

1. To evaluate different piroxicam gel formulations using various *in vitro* and *in vivo* models

2. To determine the most appropriate evaluation methods based on the correlation results

3. To develop and apply the *in vivo* skin stripping technique to evaluate piroxicam percutaneous absorption

4. To determine the topical bioavailability of piroxicam gel products according to the most appropriate methods.