Chapter I

General Background

Introduction

Dermatological preparation is a conventional dosage form intended for local effects. However, the preparation can also provide systemic drug delivery through percutaneous absorption. Two consequen tial steps are generally involved in transdermal or topical drug delivery, i.e. the release of drug from the formulation and the subsequent percutaneous absorption (Shima et al, 1981).

Either one of the two steps can be the rate-limiting process in the overall skin absorption, depending on the physicochemical properties of the drug and the vehicle. Wu et al (1992) demonstrated that difference in release rate of the drug from the formulation can affect its topical bioavailability and may lead to difference in therapeutic effectiveness. On the other hand, difference in the ability to penetrate various layers of skin can also lead to difference in bioavailability (Bhattachar et al, 1992). Scheuplein and Bland (1971) reported that the optimum skin permeability for percutaneous absorption depended on the adequate solubility of the drug in the formulation as well as its partition coefficient. In general, percutaneous absorption of the drug can occur through one of the following three pathways (Inagi et al, 1981):

1. Transcellular route
2. Intercellular route
3. Transappendageal route

The first two pathways may be collectively called the transepidermal route and constitute the major component of the overall skin permeation since they are major parts of the skin barrier. On the other hand, transappendageal route involves passage of the drug through hair follicle and sweat glands, leading to a rapid penetration of drug. However, this pathway constitutes less than 0.1% of the total surface area and may be significant only in the initial period of skin penetration before the steady state flux has been established (Chien , 1992).

Evaluation of topical products applied to the skin is a difficult task. If the products are intended to produce systemic effect after skin application, determination of the drug concentration in the systemic circulation may provide the most accurate method for evaluation of their *in vivo* permeation (Feldman and Maibach, 1967). However, this method is very cost- and time-consuming and it could be extremely difficult to determine the very low level of the transdermally absorbed drug in the plasma. Evaluation of their pharmacological action or clinical effectiveness such as the skin blanching effect produced by topical corticosteroid preparations can also be of value. Nevertheless, measurements of pharmacological activity can be highly subjective unless quantitative parameters are available(Hiramatsu et al , 1990) . Assessment of clinical effectiveness of topical products in patients, although providing direct measurement of their therapeutic merits, is very difficult to control and may be even more expensive than the plasma drug analysis (Radermacher et al , 1991).

Alternative approaches to the *in vivo* plasma drug analysis and pharmacological/clinical efficacy assessment have been the development of

appropriate *in vitro* techniques using various types of diffusion cells and membranes which can mimic the *in vivo* release and permeation. Excised human or animal skins have been utilized for this purpose. In theory, human skin is the best membrane for the *in vitro* permeation study (Barry ,1985). However, its major limiting factors are difficulties in obtaining the specimens. Handling of the excised human skin also poses another problem. In addition, different specimens from various sites of the same body vary greatly with respect to thickness and skin composition (Maibach,1976). Age, sex and race also contribute considerably to the variation of the skin properties (Schalla et al ,1979). To circumvent the above problems associated with the human skin, several animal skins have been employed. They are more readily available, less expensive and provide many characteristics similar to the human skin. For example, skin specimens from rat, pig, monkey and snake have been used in the *in vitro* permeation studies of many drugs (Bartek et al,1972). The primary purpose of using human and animal skins in the *in vitro* study is to establish a possible predictive correlation with the *in vivo* drug permeation through the skin (Schalla et al ,1982).

Although there exists a number of similarities, there is as yet no animal skin that completely mimics the penetration characteristics of the human skin (Gummer, 1988). Excised animal skins also demonstrate variable properties, depending on the method of preparation and species used. They are usually more permeable than the human skin, partly because of the greater number of hair follicles. For example, rat and pig skins were reported to be more permeable to many drugs than the human cadaver skin as reported by Bronaugh et al (1986). Recently, the use of shed snake skin has received greater attention as a useful alternative to the human skins (Itoh et al ,1990a). It is a non-living tissue, consisting of pure stratum corneum

with no hair follicles. The permeability of several compounds through the shed snake skin was found to be similar to, but often slightly less than, that through the human skin (Itoh et al, 1990). Shed snake skin has been used in the permeation study of several drugs such as 5-fluorouracil , ibuprofen , hydrocortisone , indomethacin ,ketoprofen , naproxen , diclofenac (Itoh, Xia, Magavi et al, 1990; Itoh, Magavi,Cassady et al, 1990; Itoh, Wasinger, Turnen et al, 1992; Bhatt et al, 1991; Bhattacher et al, 1992).

Variation in the *in vitro* data as a result of skin usage, be it animal or human skin samples, is the most serious concern in data interpretation and correlation analysis. Alternatively, the use of artificial membranes can also be used in the *in vitro* diffusion study in place of the excised skin specimens. They are convenient to handle, easy for use and give little variation (Chowdary et al, 1991).

Currently, there are many topical preparations commercially available in the market with numerous indications for use. Evaluation of their *in vivo* performance or topical bioavailability is therefore a formidable task due primarily 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 nonsteroidal anti-inflammatory drug (NSAID). Its main therapeutic indications are for the treatment of surface and deep skin inflammation as well as the inflammation of joints and muscles. Diclofenac is one of the NSAIDs widely used for these purposes. Riess et al(1986) have shown that topically applied NSAID like diclofenac can penetrate into muscles and joints. They also have shown that this led to the systemic absorption of about 6 percent of the dose and was also associated with serum drug concentrations well below the range at which side effects usually occur.

หอสมุดกลาง สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

The same authors also found that the topical application of diclofenac over an inflamed joint resulted in the synovial fluid and synovial tissue drug concentrations greater than the plasma drug concentration.

Interest has thus been placed on the topical bioavailability evaluation of the NSAIDs, with a particular attention on topical products containing diclofenac. Diclofenac diethylammonium was chosen as the model drug in this study due to its 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 systematically evaluated for their *in vivo* performance nor there are any established *in vitro* techniques that can correlate with the *in vivo* percutaneous absorption.

As previously stated, the *in vivo* investigation in animals or humans is preferrable to the *in vitro* methods, with the most reliable method for the *in vivo* bioavailability assessment 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, 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 organs of these topical NSAID 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 found in the skin, especially in the stratum corneum, should reflect the amount of drug at the sites of action which, in this case, are relatively close to the skin surface. Analysis of drug remaining in the skin at various time points may also give some rough indication of the rate of percutaneous absorption. As a result, an *in vivo* evaluation technique based on the skin stripping approach has been developed in this study and tested for correlation with the *in vitro* diffusion of drug using various membranes. This new approach was easy to perform, rapid, less expensive and also gave less variation in the results than the more complicate analysis of drug in the plasma. The technique also avoided the ethical and compliance problems associated with the blood withdrawal process needed in the plasma drug analysis.

Objectives:

1. To develop a simple technique for topical bioavailability evaluation of diclofenac gel products based on an *in vivo* skin stripping approach.
2. To study the correlation of this *in vivo* technique with various *in vitro* tests including the *in vitro* release and permeation studies using different membranes.
3. To compare the topical bioavailability of commercial diclofenac gel products employing the above techniques.