Transdermal delivery of anticholinergic bronchodilators

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Chapter 1

General introduction

1.0. Summary

This chapter gives a general introduction to obstructive airways diseases as well as to transdermal drug delivery. It provides background information on the characteristics of obstructive airways diseases, pulmonary function tests, mechanism of action of bronchodilators and therapy with bronchodilators. The second part, concerning transdermal drug delivery, describes the advantages and disadvantages of transdermal drug delivery, the anatomy and function of the skin, the permeation pathways through the skin, characteristics influencing skin permeation, determination of the characteristics of a permeant, the use of diffusion cells for measuring in vitro permeation, parameters in permeation experiments, and finally the performance of in vivo experiments.
1.1. Obstructive airways diseases

1.1.1. Characteristics of obstructive airways diseases

Obstructive airways diseases (CARA in Dutch for "Chronische Aspecifieke Respiratoire Aandoeningen") can be subdivided in asthma and chronic obstructive airways diseases (COPD: chronic bronchitis and lung emphysema). They are characterized by cough, expectoration of sputum and shortness of breath in combinations that can vary qualitatively and quantitatively from person to person and from time to time [1]. In asthma, there is widespread narrowing of the airways which is reversible and variable (usually attacks of breathlessness and wheeze) [2]. This narrowing is caused by an increased responsiveness of the trachea-bronchial tree to various stimuli, specific, such as allergens, histamine and acetylcholine, and non-specific, such as smoke and cold-air.

COPD, on the other hand, is characterized by chronic airways obstruction (with superimposed variation) and symptoms [2]. In chronic bronchitis the main feature is excessive mucus production with chronic or recurrent productive cough, which is manifest on most days of at least three month per year for at least two successive years [2]. Emphysema is a condition of the lungs characterized by abnormal, permanent enlargement of the air spaces distal to the terminal bronchioles. It is usually accompanied by destruction of the alveolar walls without obvious fibrosis, leading to the loss of elasticity of the lung parenchyma [2].

1.1.2. Pulmonary function tests

Pulmonary diseases can affect the volume of air that can be inhaled and exhaled. The spirometer is an instrument that is used to obtain information about the size of the patient’s lung and to evaluate the performance of the patient’s lungs, thorax, and respiratory muscles in moving air into and out the lungs. Different manoeuvres of inspiration and expiration into the spirometer can be used to measure lung volumes and capacities [3]. Below, a short description of the terms used in this thesis is given.

Vital capacity
The vital capacity is defined as the volume change at the mouth between the positions of full inspiration and complete expiration. The measurement described in this thesis is that of the inspiratory vital capacity (VC); it is performed in a relaxed manner, without undue haste or deliberately holding back, from a position of full expiration to full inspiration [3].

Forced expiratory volume in one second
Often, forced expiratory manoeuvres are used to amplify possible abnormalities in ventilation; the patient exhales into the spirometer as forcefully and completely as possible after maximal inspiration. From the resulting volume-time curve, the forced
expiratory volume in one second (FEV₁) can be measured. The FEV₁ is defined as the volume of air exhaled in one second from the start of the forced expiratory manoeuvre [3]. The FEV₁ is usually expressed as a percentage of the total volume of air exhaled and is reported as the FEV₁/VC ratio (Tiffeneau index) [3]. The patient’s ability is compared with predicted normal values for patients with similar physiological characteristics because lung volumes are dependent on age, race, gender, height and weight. The FEV₁ and VC are the most reproducible of the pulmonary function tests but need to be measured in the clinic with relatively sophisticated instrumentation and experienced personnel.

**Peak expiratory flow**

The peak expiratory flow (PEF) is the maximal flow during a forced expiratory manoeuvre starting from a position of full inspiration [3]. The PEF can easily be measured with various hand-held peak-flow meters and can be used at home to assess the effectiveness of bronchodilators. The changes in PEF generally parallel those of the FEV₁, however the PEF is a less reproducible measure than the FEV₁.

**Reversibility**

Spirometry is often used to determine the reversibility of airways diseases. Disease states may produce narrowing of the airways which can be reversed by therapy with bronchodilators. The response to bronchodilator drugs is usually assessed in terms of a change in FEV₁. Because the FEV₁ has low variability, it is considered to be the standard test to determine reversibility of the airways as well as bronchodilator efficacy. The observed response to a bronchodilator will depend upon its pharmacological class, the route of administration, the character of the disease, and in case of inhaled drugs on the inhalation technique and aerosol delivery system [3].

**Hyperresponsiveness**

Airway hyperresponsiveness or bronchial hyperresponsiveness is a common characteristic of asthma and COPD and is defined as an increased tendency of the airways to narrow to a variety of chemical, pharmacological or physical stimuli [4]. It can be mimicked in the laboratory by challenge tests with bronchoconstrictive stimuli, such as histamine or methacholine. The airway responses are mostly quantified by measuring the effects of the bronchoconstrictor on FEV₁. Each aerosol is inhaled by quiet tidal breathing at spontaneous frequency through the mouth for 2 min, using a nose clip. The first aerosol inhaled is the solvent and this is followed at 5 min intervals by doubling concentrations of histamine or methacholine from 0.03-32 mg/ml (or higher concentrations if necessary in research). The FEV₁ is measured before the test, and at 30 s and 90 s after each inhalation. The lowest recording will be used in the analysis. The test is stopped when the FEV₁ has fallen by 20% or more from baseline. The results are expressed as the concentration of methacholine/histamine causing a 20% fall in FEV₁ (PC₂₀) [4].

1.1.3. Use of bronchodilators
Airways disease, whether it is asthma, chronic bronchitis or emphysema, is inherently accompanied by airways obstruction. Anticholinergics and β₂-agonists are used as bronchodilators to prevent or diminish airways obstruction.

Mechanisms of action

β₂-agonists stimulate β₂-adrenergic receptors present in the airways which results in bronchodilation and inhibition of mast cell degranulation. The effects are mediated through activation of the enzyme adenylyl cyclase which catalyses the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (c-AMP) [5-7]. Anticholinergic compounds produce bronchodilation by competitive inhibition of cholinergic receptors on bronchial smooth muscle. They antagonize the action of the neurotransmitter acetylcholine which is released from the vagal nerve and which will cause bronchoconstriction and mucus secretion due to stimulation of muscarinic receptors on target organs. Bronchoconstriction appears to be mediated by the nucleotide cyclic guanosine monophosphate (c-GMP). Blockade of the acetylcholine receptors with specific antagonists can diminish the bronchoconstrictory influence of the parasympathetic nervous system [5, 7, 8].

Therapy with bronchodilators

β₂-agonists may be administered by inhalation, orally or parenterally [7, 9]. Inhaled β₂-agonists, such as salbutamol and terbutaline, have a rapid onset of action and are effective for three to six hours [5, 10]. The advantage of inhalation therapy is the low incidence of side-effects, but the major disadvantage is the short duration of action.

Therefore, long-acting β₂-agonists have been developed to provide prolonged bronchodilation. Inhaled formoterol and salmeterol, have bronchodilator effects for up to 12 hours [5, 9-13]. For patients who cannot take inhalation therapy, orally administered β₂-agonists are available. Slow-release oral preparations, e.g. terbutaline, are given on a twice daily basis but side effects are greater than after inhalation. The prolonged bronchodilation of long-acting β₂-agonists results in less frequent dosing which improves patient compliance and may be useful in preventing nocturnal symptoms [5, 14-16]. Recently, several transdermal drug delivery systems of β₂-agonists have been developed and evaluated, but more clinical trials are needed to determine their bronchodilating effects [17-20].

Atropine, a tertiary amine, was used for many years in the treatment of airways obstruction, however it is systemically absorbed when delivered by inhalation, and has many unpleasant and potentially dangerous side effects [7, 21-23]. The only regularly used therapy with anticholinergics is the inhalation of ipratropium bromide (Atrovent®). Since the drug is a quaternary ammonium compound, it does not cross the blood-brain barrier and therefore does not produce central side effects. [23, 24]. However, because of the relatively short duration of action (4 to 6 hours), it must be given four times a day [25].

A new long-acting anticholinergic bronchodilator, Ba 679 Br, has recently been developed. It is structurally related to ipratropium bromide and after inhalation the
bronchodilator effect persisted for 10 to 15 hours [26]. Transdermal administration of the tertiary amine scopolamine, has been tested in a small group of patients having diverse types of lung diseases [27]. However, further clinical trials are required because this study was not placebo-controlled.

1.2. Transdermal drug delivery

1.2.1. Transdermal drug delivery: Advantages and disadvantages

Recently, there has been an increased interest in the drug administration via the skin for both local therapeutic effect on diseased skin (topical delivery) as well as for systemic delivery (transdermal delivery) of drugs [28].

Systemic drug delivery through the skin may have several advantages over conventional drug therapy [29-33]. It circumvents variables that may influence gastro-intestinal absorption, such as the drastic changes in pH along the gastro-intestinal tract, food-intake, intestinal motility. It might avoid systemic first-pass metabolism as it circumvents the liver, thereby increasing the bioavailability. Transdermal delivery may produce sustained, constant and controlled levels of drug in the plasma, thereby improving patient compliance since frequent intake of the drug is no longer necessary. The transdermal route can also eliminate pulsed entry into the systemic circulation, which might often cause undesirable side effects. Finally, therapy can easily be terminated by simple removal of the dosage form in problematic cases.

Transdermal therapy, however, also has its disadvantages [30, 32, 34]. The excellent barrier properties of the skin may prevent the entry of drug molecules (wanted and unwanted) from the external environment. Compounds may activate allergic responses and the drug may be metabolized by microflora on the skin’s surface or by enzymes in the skin [35-37]. Another disadvantage is the variability in skin permeability. Therefore, transdermal therapeutic systems have been developed to control the delivery of the drug and minimize intersubject variation [20, 31, 32].

1.2.2. The skin: Anatomy and function

The skin of an average adult body covers a surface area of approximately 2 m² and receives about one-third of the blood circulating through the body [31, 33]. The skin separates the vital organs from the external environment, protects against physical, chemical, microbial and radiological attack, acts as a thermostat in maintaining body temperature and plays a role in the regulation of the blood pressure. It also serves as a food reserve and as a sensory organ transmitting external environmental information (e.g. pain, heat) [29, 38].
Microscopically, the skin is a multilayered organ, composed of many histological layers. It is generally subdivided into three layers: The epidermis, the dermis and the hypodermis [29, 31, 32, 38]. The epidermis can be subdivided into the stratum corneum and the remainder of the epidermis, the so-called viable epidermis.

**The hypodermis**
The hypodermis or subcutaneous fatty tissue merges with the overlying dermis. It supports the dermis and epidermis and serves as a fat storage area. This layer helps to regulate temperature, provides nutritional support and mechanic protection. It carries the principal blood vessels and nerves to the skin and may contain sensory pressure organs [29, 38].

**The dermis**
The dermis is a 3 to 5 mm thick layer and is composed of a matrix of connective tissue, which contains blood vessels, lymph vessels and nerves [29, 39]. The cutaneous blood supply has an essential function in the regulation of body temperature and provides nutrients and oxygen to the skin, while removing toxins and waste products. Capillaries reach to within 0.2 mm of the skin surface and provide sink conditions for most molecules penetrating the skin barrier. The blood supply thus keeps the dermal concentration of a permeated drug (permeant) very low, and the resulting concentration difference across the epidermis provides the
essential driving force for transdermal permeation [29, 39].

The viable epidermis

The viable epidermis is situated beneath the stratum corneum (the horny layer) and varies in thickness from 0.06 mm on the eyelids to 0.8 mm on the palms. It consists of various layers, characterized by different stages of differentiation. Going inwards, the permeant crosses the stratum lucidum, the stratum granulosum (or granular layer), the stratum spinosum (or spinous layer) and the stratum basale (or basal layer) [29, 38].

In the basal layer, mitosis of the cells constantly renews the epidermis and this proliferation compensates the loss of dead horny cells from the skin surface. As the cells produced by the basal layer move outward, they alter morphologically and histochemically, undergoing keratinization to form the outermost layer the stratum corneum [38, 39].

The stratum corneum

The outermost layer of the skin, the horny layer or stratum corneum, is approximately 10 µm thick when dry but swells to several times this thickness when fully hydrated [29, 38]. It contains 10 to 25 layers of parallel to the skin surface lying dead, keratinized cells, called corneocytes. It is flexible but relatively impermeable. The horny pads of the palms of the hand and foot-soles are adapted for weight bearing and friction [38]. Here, the stratum corneum is much thicker, at least 400 µm, with vertically stacked cells and it is more permeable to water and chemicals.

The stratum corneum has been identified as the principal barrier for penetration [29, 34]. The barrier nature of the horny layer depends critically on its constituents: 75-80% proteins, 5-15% lipids and 5-10% unidentified material on a dry weight basis. The protein fraction predominantly comprises alpha-keratin (70%), with some beta-keratin (10%) and the cell envelope (5%). The lipid constituents vary with body site (neutral lipids, sphingolipids, polar lipids, cholesterol). Phospholipids are largely absent, a unique feature for a mammalian membrane.

The architecture of the horny layer may be modeled as a wall-like structure [29, 34, 40]. In this model, the keratinized cells function as protein "bricks" embedded in a lipid "mortar" (Figure 1.1). The lipids are arranged in multiple bilayers, and it has been suggested that there is sufficient amphiphilic material in the lipid fraction, such as polar free fatty acids and cholesterol, to maintain a bilayer form. The precise molecular arrangement of the intercellular lipid is still being investigated.
1.2.3. Permeation pathways

A molecule may use two diffusional routes to penetrate normal intact human skin: The appendageal route and the epidermal route [29, 39, 40]. The appendageal route comprises transport via the sweat glands and the hair follicles with their associated sebaceous glands (Figure 1.2). These routes circumvent penetration through the stratum corneum and are therefore known as shunt routes. This route is considered to be of minor importance because of their relatively small area, approximately 0.1% of the total skin area. However, recent studies indicate that follicles may have a greater importance in percutaneous absorption than is generally assumed. The appendageal route may be more important for ions and large polar molecules which hardly permeate through the stratum corneum [29].

For drugs which mainly cross the intact horny layer, two potential micro routes of entry exists, the transcellular (or intracellular) and intercellular pathways (Figure 1.1). The principal pathway taken by a permeant is decided mainly by the partition coefficient (log K). Hydrophilic drugs partition preferentially into the intracellular domains, whereas lipophilic permeants (octanol/water log K > 2) traverse the stratum corneum via the intercellular route. Most permeants permeate the stratum corneum by both routes. However, the tortuous intercellular pathway is widely
considered to provide the principal route and major barrier to the permeation of most drugs [29].

1.2.4. Characteristics influencing skin permeation

Skin permeation is a complex process, with a variety of barriers to cross. Initially, a drug must first partition out of the dosing solution (vehicle) into the stratum corneum before diffusing across the viable epidermis and dermis from where most permeants are cleared by the circulation. For the majority of drugs, the main barrier is the stratum corneum. Fick’s first law can be applied to describe the diffusion processes in this layer [39]:

\[
J = K_p \cdot \Delta C = \frac{D \cdot K \cdot \Delta C}{L}
\]  

where:

- \(J\) = steady-state flux of the permeant through the stratum corneum (\(\mu g.cm^{-2}.s^{-1}\));
- \(K_p\) = permeability coefficient of the permeant in the stratum corneum (cm.s\(^{-1}\));
- \(\Delta C\) = concentration gradient of the permeant across the stratum corneum (\(\mu g.cm^{-3}\));
- \(D\) = diffusion coefficient of the permeant in the stratum corneum (cm\(^2\).s\(^{-1}\));
- \(K\) = apparent partition coefficient of the permeant between the stratum corneum and the vehicle;
- \(L\) = the length of the pathway through the stratum corneum (cm).

It is apparent from this equation that the flux is constant if the permeability coefficient and concentration difference are constant: The chemical and physical properties affecting these properties are discussed below.

The concentration gradient over the stratum corneum will depend primarily upon chemical characteristics of the permeant including solubility, lipophilicity, ionization and stability [39, 41]. To obtain high levels of permeant in the first layers of the stratum corneum, the permeant should have a high tendency to leave the vehicle and migrate into the skin, which is expressed in the value of the partition coefficient, \(K\), of the permeant. As the barrier within the stratum corneum is mainly lipoidal, a high lipid solubility is necessary for a maximal input of the permeant into the stratum corneum. Although for most drugs the stratum corneum is the main barrier, it should be noted that once a permeant has crossed the stratum corneum, it must partition into the underlying layers of the epidermis, dermis, and circulatory system. These tissues are more hydrophilic than the stratum corneum and can present a barrier to extremely hydrophobic permeants.
The magnitude of the partition coefficient, $K$, is affected by the composition of the vehicle, the chemical structure of the permeant and the charge of the permeant [39, 41]. For a given drug the partition coefficient may be increased by manipulating the composition of a vehicle in such a way that the permeant has a higher tendency to leave it. If the permeant is ionizable, the pH of the vehicle and the permeant’s ionization constant, $pK_a$, will determine the actual concentrations of ionized and non-ionized species and thus influence the partition coefficient. Generally, transport of ionized species occurs much less rapidly than transport of the non-ionized species [39].

The diffusion coefficient or diffusivity, $D$, is a rough measure of the ease with which a molecule can move about within a medium, in this case the stratum corneum [41]. It is dependent on molecular weight and volume, and the degree of interaction between the permeant and stratum corneum. The larger the molecule, the more difficult it is to move about, and the lower the diffusivity. Up to a molecular weight of at least 500 daltons, and perhaps 5,000 daltons, the molecular size plays no crucial role [39]. Non-specific and specific binding may occur in both the epidermis and dermis, reducing diffusivity and thereby decreasing skin permeability [39].

1.2.5. Determination of the characteristics of the permeant
The physicochemical properties of the permeant discussed above can be determined using literature, theoretical calculations, and experimental measurements [41]. The chemical structure, molecular weight, and pKa values are often available from literature.

The solubility of a permeant is best measured by allowing an excess permeant to equilibrate in the solvent while stirring at a constant temperature. After equilibration, a sample of the liquid is filtered, diluted with solvent, and analysed. The partition coefficient, $K$, can be calculated from solubility measurements, simply as the ratio of the solubility in one solvent to that in another solvent. Alternatively, they can be measured via liquid-liquid extraction. In this case, a fixed quantity of permeant is dissolved in one liquid, and this solution is then shaken with the other liquid at a constant temperature for at least 24 hours. The ratio of concentrations of permeant in the two liquids at equilibrium is the partition coefficient.

The diffusivity, $D$, can be determined in vitro by simply measuring the transdermal flux at early times until a steady-state flux is reached using diffusion cells (see section 1.2.6). A representative plot of the cumulative amount of drug crossing the skin against time is shown in Figure 1.3. The time before steady state is reached is characteristic for the diffusivity of the permeant in the membrane, and can be used to calculate the diffusivity [29, 41]. The lag time, $T_{lag}$, is the time obtained from extrapolation of the steady state portion of the graph to the intercept on the time axis, and is defined by the following equation [29, 41]:

$$T_{lag} = \frac{L^2}{6D}$$

where:

$L$ = thickness of the membrane (cm);
$D$ = diffusivity (cm$^2$.s$^{-1}$).

It should be kept in mind, however, that $L$ should represent the length of the pathway through the membrane, which most often does not correspond to the thickness of the membrane. Thus, in practice, this method for evaluating $D$ has several disadvantages as the exact length of pathway through the membrane is difficult to measure. It also may vary with the constituents of the vehicle. Additionally, lag times obtained from permeation experiments with human skin tend to be very variable and may include a component arising from interactions between the stratum corneum and the permeant.
The permeability coefficient of a permeant through a membrane, $K_p$, can be calculated from Fick’s first law as follows [29]:

$$K_p = \frac{J}{\Delta C} = \frac{\text{dm/dt}}{C_d \cdot A}$$  (1.3)

where:

- $\text{dm/dt}$ = the slope of the steady-state portion of the cumulative amount versus time plot as shown in Figure 1.3 ($\mu$g.s$^{-1}$);
- $A$ = skin area exposed to the dosing solution (cm$^2$);
- $C_d$ = the concentration of the permeant applied in the donor compartment ($\mu$g.cm$^{-3}$), which equals $\Delta C$ because of low concentrations in the receptor compartment.

Thus, if the donor concentration and the flux of the permeant are known, the permeability coefficient may be determined. The permeability coefficient is constant for a given permeant under a given set of experimental conditions and depends on the diffusion coefficient, the partition coefficient and the length of the pathway through the membrane.
1.2.6. Diffusion cells for measuring in vitro permeation

Permeation data of drugs such as lag time, permeability coefficient, diffusion coefficient, and flux, may be obtained from in vitro permeation experiments. The most common technique used to gather this type of data involves diffusion cells. Diffusion cells generally comprise two compartments, one containing the active component (donor vehicle) and the other containing a receptor solution, separated by a piece of excised skin or other membrane. Although many variations of diffusion cells exist, there are two basic designs: the static, or nonflowing cell, and the flow-through cell [42-45].

The Franz diffusion cell is one of the most widely used static designs for studying in vitro permeation. This cell has a static receptor solution reservoir with a side-arm sampling port (Figure 1.4). The skin is positioned between the two cell halves of a glass chamber. The two compartments are held together with a clamp. A thermal jacket is positioned around the receptor compartment and is heated with an external circulating bath. During the course of an experiment, small volumes are withdrawn from the stirred receptor solution for analysis and the receptor compartment is refilled with receptor solution to keep the volume of solution constant during the experiment.

In contrast to the sampling from static cells, the flow-through cells can provide automatic replacement of the receptor solution. As a result, the flow-through cell represents conditions more similar to those encountered in vivo because the entire contents of the receptor compartment are replaced on a continuous basis [42-45].

1.2.7. Parameters in permeation experiments

By using diffusion cells, the conditions for drug delivery should be controlled because drug permeation may vary with the skin or membrane used, and with the composition of the donor and receptor solution, respectively.

Membrane selection

Clearly, the most appropriate membrane for permeation studies is human skin [28, 45, 46]. However, in vitro permeation experiments with human skin are difficult to conduct due to the scarcity of human skin and controlling the gender, race, anatomical site, age and skin condition of the donor. Therefore, most in vitro permeation studies use animal skin.

A large number of different animal skins have been tested as possible models for human skin, such as the hairless mouse, rabbit, guinea pig, rat, pig, or shed snake skin. The criterion for selection should be the correlation between permeation rates using human skin and animal skin. Knowledge of the anatomical and histological properties of animal skin may be helpful to interpret the permeation data [28].

Another alternative for human skin is the use of synthetic membranes such as Silastic® (polydimethyl siloxane), cellulose acetate or polyurethane. Due to the complexities of skin, permeabilities cannot be predicted adequately from the
synthetic membrane data. Therefore, whenever possible human skin should be used in preference to animal skin or artificial membranes [29].

**Donor solution**

If the permeant is dissolved or dispersed in a vehicle, it must diffuse through that vehicle to the skin surface before it can be absorbed [39]. The vehicle can influence the release of the permeant from the vehicle and may interact with the stratum corneum. Factors that affect drug release include the physicochemical properties of the vehicle (e.g., viscosity) as well as the properties of the drug and vehicle together (e.g., solubility). The interaction of the vehicle with the stratum corneum may vary from simply occluding the skin to extracting lipid components from the stratum corneum [39].

To reduce the barrier function of the stratum corneum and to allow drugs to permeate more readily, penetration enhancers are often incorporated into a donor solution [29]. An ideal penetration enhancer reversibly reduces the barrier resistance of the stratum corneum without damaging the skin. The safest and most widely used penetration enhancer is water; increased hydration diminishes the resistance of the skin [29, 43]. Azone® (1-dodecylazacycloheptan-2-one, laurocapram) was the first molecule specifically designed as a skin-penetration enhancer; it enhances the skin transport of both hydrophilic and lipophilic drugs by reducing the order of the intercellular lipids [29]. Penetration enhancers, such as propylene glycol and ethanol, probably interact with the polar head groups of the lipid so as to modify hydrogen bonding and ionic forces [40]. If propylene glycol is used in combination with Azone®, its effect is most noticeable because it operates synergistically. It has also been shown that not only propylene glycol aids Azone® permeation, but Azone® also increases the permeation of propylene glycol [29, 34, 39, 40].

**Receptor solution**

The receptor solution used in diffusion cells should not only act as an acceptor for permeating drugs but should provide the water, biochemicals and ions needed for the skin membrane to function in the permeation experiment at the proper pH and osmotic strength [41, 46]. Often, this is physiological saline, Ringer’s solution, or some other physiologically relevant solution. Other important factors of the receptor solution are temperature, solubility and stirring [45, 46].

Control of the receptor solution temperature is important to minimize variations in experimental conditions. The temperature should be kept at normal physiological conditions, since temperature elevation may lead to increased hydration of the skin. It is known that a rise of 10°C in temperature can produce a 2- to 3-fold increase in permeation [42].

Solubility and stirring are important to allow the permeant to be taken up and transported away from the skin after it has passed through, avoiding a concentration build-up within or below the skin. Stirring of the receptor solution is also important to provide a homogeneous receptor solution [42, 45]. The concentration of the permeant in the receptor solution should remain low (less than 10%) compared with
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its solubility in the solution [46]. If the permeant is relatively insoluble, solubility enhancing components can be added to the receptor solution, however their effects on the skin must be considered.

Analysis of the drug in the receptor solution

Drugs permeating through the skin into the receptor solution can be quantified using different methods [47]. In this thesis, the radiometric assay and radioreceptor assay are used to analyse the permeated drug.

The radiometric assay is the most simple and widely used method to analyse permeants. Radioactive isotopes act as tracers for chemically similar non-radioactive compounds. The aqueous receptor solution (with radiolabelled drug) can be dissolved directly in a scintillation cocktail and after mixing the homogeneous sample can be counted [47]. However, radiometric assays cannot distinguish between parent compound and its decomposition products.

Radioreceptor assays are based on the ability of a drug to compete with a radiolabelled ligand for a specific receptor binding site [48]. This attachment is reversible, specific and saturable. The relationship between receptor, radioligand, and complex is shown in the following equation:

\[ R + L^* = RL^* \] (1.4)

where:

\[ R = \text{concentration of receptor; } \]
\[ L^* = \text{concentration of radioligand; } \]
\[ RL^* = \text{concentration of the complex between radioligand and receptor.} \]

The ratio of the dissociation and association rate constants is called the dissociation constant \( K_d \). It is inversely proportional to the affinity of the radioligand for the receptor [49]:

\[ K_d = \frac{[RL^*]}{[R] \cdot [L^*]} \] (1.5)

If an unlabelled ligand (L) is introduced into the system, we obtain an equation with two receptor complexes:

\[ R + L^* + L = RL^* + RL \] (1.6)
When a measured quantity of radioligand is added to a measured quantity of specific receptors, the radioligand binds to form a labelled complex. If an unlabelled ligand is introduced it will compete for the binding sites, displacing the radioligand. At equilibrium, the bound radioactivity remaining is a function of the amount of unlabelled ligand added. In this way, it is possible to determine unknown quantities of this unlabelled ligand by comparing the bound radioactivity displaced by an unknown sample with the amounts displaced by series of solutions containing known quantities of the unlabelled ligand [48, 49]. In principle, metabolites which possess a binding affinity to the receptor, may be codetermined by the radioreceptor assay.

1.2.8. In vivo experiments

Most skin permeation studies use animal in vitro models. However, as transdermal drug delivery to humans in clinical situations is often the final aim, the most desirable approach is to do human in vivo experiments [28]. Skin permeation in vivo is usually determined by the indirect method of measuring radioactivity in urine and plasma following topical application of the radiolabelled compound. In human studies, plasma concentrations in particular are extremely low following topical application and are almost always below the detection limit of assays that do not utilize radioactivity. Therefore, it is often necessary to use radiolabelled drugs. The clinical trials described in this thesis were performed to evaluate the effects of transdermal scopolamine on lung function parameters and on hyperresponsiveness to methacholine, as described in section 1.1.2. In order to determine the concentrations of scopolamine in urine and plasma of the patients, a highly sensitive radioreceptor method was needed because of the extremely low plasma concentrations. Since scopolamine is extensively metabolized to glucuronide and sulphate conjugates, we decided to quantitate scopolamine as well as the sum of scopolamine and its conjugated metabolites. The total amount of scopolamine (free plus conjugated) was determined after incubation of urine and plasma samples with glusulase, which is an enzyme preparation of a β-glucuronidase and arylsulfatase.

The developed procedure consisted of a semi-automated solid-phase extraction followed by analysis using radioreceptor assays. The principle of radioreceptor assays is described in section 1.2.7. Solid-phase extraction (SPE) was applied to effectively eliminate endogenous urine and plasma components as well as the interferences from the glusulase treatment. At the same time, SPE served as an excellent pre-concentration method [50].

1.3. References

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