7. Summary

Comparative study of physiological skin parameters affecting percutaneous penetration in different mammalian species

Skin physiology significantly influences percutaneous penetration and varies among mammalian species. The two major routes that substances pass through the skin are the follicular and the intercellular pathways. Both routes were investigated in animals.

Hair follicle morphology and distribution were analysed using cyanoacrylate skin surface biopsies (CSSB). This method was established in animals for the first time. Hair follicles were numerically assessed and examined in eight species. The infundibulum of the follicle forms the reservoir for topically applied particles penetrating into the follicle; this anatomic structure was measured. Tremendous differences exist among the eight species. Despite a higher follicular density, monkeys, rats and mice possess low follicular volumes. The highest volume was calculated in dogs, followed by Guinea pigs. Thus, the hair follicles of these two species exhibited a higher reservoir capacity. Comparing follicular surfaces, hair follicles of dogs and rabbits are most important regarding percutaneous penetration. The lowest values were calculated in monkeys and pigs. CSSB analysis of hair follicles revealed objective parameters characterizing hair follicle morphology in mammalian species for the first time. The follicular volume is a novel parameter describing reservoir capacity of hair follicles in animal species. It was proven that follicular surface and skin permeability are directly linked. For the first time, a comprehensive study involving numerous species and individuals demonstrated that individual hair follicle morphology is a key point for the understanding of skin permeability and follicular penetration.

The four major components of the intercellular stratum corneum lipids: cholesterol, ceramides, free fatty acids and cholesterolester were analysed. The ratios of the four major lipid classes regarding skin permeability were examined. Four animal species were investigated: pigs and for the first time dogs, cats and rabbits.

The stratum corneum was enzymatically separated and the lipids were extracted and analysed using HPTLC. In pigs, the two major components of stratum corneum lipids were ceramides and cholesterol. These results, as well as the total amount of extracted lipid are consistent with the literature. In contrast, the stratum corneum of dogs, rabbits, and particularly cats, revealed more cholesterolester.

Thus, in all densely haired species, the lipid composition was never dominated by ceramides. A characteristic species-dependent pattern of ceramides, representing the composition of the different ceramide classes, was proven to exist in stratum corneum lipid matrix. A further approach for understanding the relevance of the stratum corneum lipid composition is represented by the cholesterol-ceramide ratio that has never before been calculated in animal species. A low ratio correlates with poor barrier function of the skin with rabbits possessing the lowest ratio followed by cats. Values of dogs and pigs were similar. These results are compatible with skin permeability data of these species. Thus, a correlation of lipid composition and skin permeability as demonstrated in human beings has been established in the four species.

This study provides fundamental knowledge for the understanding of percutaneous penetration of animal species. The measurement of species-specific follicle parameters allows the calculation of the follicular reservoir capacity and resorption for substances applied to the skin and more accurately estimates follicular penetration. This knowledge is of particular relevance in the processing of topical drugs. The development of individually adapted drugs in veterinary medicine can be optimized if morphological differences among the species are considered. Lower dosages of topical drugs might be needed in species with small follicular surfaces. The composition of stratum corneum lipid matrix may no longer be assumed as constant among the mammalian species. In fact, intercellular lipid content and composition differ among animal species. Varying proportions of the major components and characteristic patterns of ceramides lead to the conclusion that the barrier function of the stratum corneum is differs among species. This fact may also explain differences in intensity and duration of pharmacological effects as well as adverse effects existing among topical drugs. A possible correlation between the pathogenesis of certain dermatological disorders and the ascertained differences in skin morphology must be excluded by further investigation.

Finally, the data in this work contributes to the evidence-based recommendation that laboratory animals such as mice, rats, guinea pigs and rabbits are not appropriate models for human skin research. Due to a lack of consistent hair follicle anatomy, cutaneous permeability is expected to greatly differ from human skin. In rabbits, differences in stratum corneum lipid content and composition were noted when compared to human skin. In contrast, the skin of porcine pinna highly resembles human skin. The porcine pinna appears to be the most appropriate pragmatic experimental paradigam for the exploration of human skin pharmacology.