SUMMARY

Estrogen HRT has been widely used for treatment of the postmenopausal symptoms to improve the quality of life of women and to prevent osteoporosis as well as reducing the risk of coronary heart disease. Recently, also a stimulation of wound healing has been described. But, because of the negative outcome of the heart estrogen/progestin replacement study (HERS) and women's health initiative (WHI) studies, and the increased risk of breast and endometrial cancers, today, estrogen replacement therapy is recommended only for symptomatic women and for a period as short as possible. Extensive hepatic estradiol metabolism of oral estrogens may be the cause for this adverse effect on the cardiovascular outcome. To minimize these adverse effects of oral estrogen HRT the transdermal route of administration may be an important consideration. Moreover, the use of testosterone (precursor for local estrogen formation) as adjuvant therapy to the estrogen HRT was recommended by recent studies to reduce the dose of estrogen. Therefore, we compared the perfused pig forelimb, split skin of porcine and human origin, human separated epidermis, and commercially reconstructed human epidermis, as well as an airway model for testing percutaneous absorption and metabolism of sex-steroid hormones, estradiol and testosterone, to identify the models of high reproducibility and predictability for the in vivo situations.

Since both steroids show low cutaneous uptake, firstly very sensitive assay methods had to be established. For the estimation of the testosterone and hydrocortisone concentrations, a liquid scintillation counting method was used with a sufficient LOD. The quenching effect of BSA in the acceptor medium was overcome by obtaining the calibration curves of substances in identical solvents. The ethanol extraction rate of cotton wool was about 72.5 ± 2.7. Liquid scintillation counting, however, was not valid for studying the metabolism of E2 because it is non specific for the metabolites. Therefore, for the determination of the E2 metabolism in the skin, a commercially available RIA had to be adapted which then allowed to determine the tissue and acceptor medium concentrations of E1, E2 and even of their conjugated metabolites. The sensitivity of RIA methods was 3.75 and 10.0 pg /tube for E2 and E1, respectively. Validation of the RIA and LSC methods were performed using spiked E2 and E1 levels in the acceptor medium. Although, estrogen extraction with ethyl acetate yielded the highest E2 and E1 recovery, ether estrogen extraction was used, because of its ability to extract only E2 and E1. Also, the time and the amount of enzyme required for obtaining complete hydrolysis of estrogen conjugates were studied in the first experiments. Total estrogen recovery from the Franz cell experiment was 88.3 to 100.5 %.

In the first experiments studying testosterone permeation, the static and the dynamic experimental set up which are used in this investigation were compared for obtaining the best setup, but the difference between both set ups failed to reach statistical significance. Then the effect of BSA addition on the permeation of substances was investigated in both E2 and testosterone experiments. Testosterone permeation was significantly (twofold) increased, whereas E2 permeation was non significantly increased. Also, the type of transferring tube in case of the dynamic set up was studied to determine accurately if there is an adsorbed substance if rubber tubes were used for transferring the permeated substances. The solubility of testosterone, one of the most important physicochemical aspects which can affect the cutaneous testosterone permeation, was a problem because of the difficulty to dissolve testosterone in water based donor media. Therefore, ethanol had to be introduced. Testosterone is well dissolved in ≥ 60% ethanol-water solutions. However, this high concentration of ethanol is toxic to the skin. Testosterone is also soluble in oils e.g miglyol. Oil donor vehicles, however, prevented almost completely any testosterone permeation. Therefore, an ethanol-miglyol mixture had to be used and the amount of ethanol was thoroughly balanced versus the skin toxic effects and a compromise had to be made. Ethanol content in 10/90 (v/v) ethanol/miglyol donor vehicle will act as permeation enhancer and induces less toxicity. Testosterone permeation from 10/90 ethanol-miglyol exceeded that from 100% and 60% ethanol solutions. Since we used ethanol in the donor vehicle, we had to carry MTT-test to evaluate the viability of reconstructed epidermis. The results showed that 10/90 E/M is less toxic for the skin than 60% and 100% ethanol. Moreover, MTT-test results showed also that the acceptor medium PBS plus 5 % BSA is more toxic than PBS alone. Lactate and LDH levels were also determined and confirmed the results of the MTT test. Histological examinations of the reconstructed epidermis after the experiments, however, revealed that the donor vehicle has no major effect on the histology of reconstructed tissue, if PBS or PBS plus 5%BSA served as acceptor media.

The significant differences in testosterone permeation between fresh and cryopreserved split porcine skin, between the abdominal and back skin as well as between isolated human epidermis and cryopreserved human split skin were not observed during our study. Testosterone permeation through different batches of reconstructed human epidermis varied clearly less than through different animals and human donators. Reconstructed human epidermis showed the highest flux and permeation with the shortest lag time. Flux of split skin of animal and human origin was rather close. With split porcine skin, the lag time was almost one hour.

In order to evaluate our operating protocol, we compared testosterone permeation through the same reconstructed epidermis to hydrocortisone absorption as a more hydrophilic control. Also, two water-based formulations, O/W and W/O emulsion, were compared to the 10/90 ethanol/miglyol solution. The presented results showed slightly higher permeation of hydrocortisone than testosterone. The testosterone permeation from 10/90 E/M was lower than O/W and W/O emulsions. However, in contrast to testosterone, hydrocortisone permeation using 10/90 E/M was higher than that of both O/W and W/O emulsions. The difference in permeation between 10/90 E/M and emulsions was significant. The presented results of an identical experiment with pig skin using the same formulations confirmed this result however the permeation of both testosterone and hydrocortisone through pig skin was clearly less. Testosterone and hydrocortisone permeation through the alveolar model exceeded uptake by split porcine skin about fivefold, and uptake by reconstructed epidermis 2-(testosterone) to 3fold (hydrocortisone). Using the EpiAirway model, hydrocortisone exceeded testosterone permeation about twofold.

Determination of estrogen uptake by the reconstructed epidermis showed the influence of application vehicle on E2 uptake and metabolism. Differences in the estrogen concentrations in the acceptor fluid depending on the vehicle form were obvious for both, native E2 and its metabolites. The estrogen uptake by reconstructed human epidermis was 41.7 % using the gel and 15.25% using identical dose as 0.1% ethanolic solution. Using gel, 42.6 % of the totally absorbed amount was found to be metabolized. E1 was the dominating metabolite. The E2/E1 ratio was 0.96 after applying the gel and clearly higher after ethanolic solution. Less than 0.2% permeated into the acceptor medium from ethanolic solution, whereas 28.78% permeated into the acceptor medium from the gel.

The presented data also showed that, estrogen permeation through split pig skin was clearly less as compared to reconstructed epidermis following the application of sisare gel and slightly reduced following the ethanolic solution. With split pig skin also the influence of ethanol on E2 metabolism was less pronounced. E2 tissue levels following the application of ethanolic solution were clearly lower than E2 levels following the application of the gel which is in contrast with reconstructed epidermis.

E2 uptake after the application of an E2-TTS to perfusion model for 6 h was 42.85% of the incorporated dose. The E2 concentration as well as those of E2 conjugates increased considerably in the perfusion medium and in the tissue. While E2 already increased within the first hour in the medium, E2-conjugates were first elevated after 2 h. There was even a lag

time of about 3 h until E1 and E1 conjugate concentrations raised. In skin and muscle of forelimbs beneath the application side, the concentration of E2 and its metabolites exceeded those of the control leg about 1000fold. Moreover, 30 % of the penetrated amount of E2 in the skin is subjected to metabolization, 11 % of the estrogens were identified as conjugated E2, 19 % as E1, and 0.5 % as conjugated E1.