APPLICATION OF A NEW HUMAN SKIN ORGANOCULTURE MODEL TO STUDY CUTANEOUS METABOLISM OF [4, ^{14}C]-TESTOSTERONE

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BACKGROUND

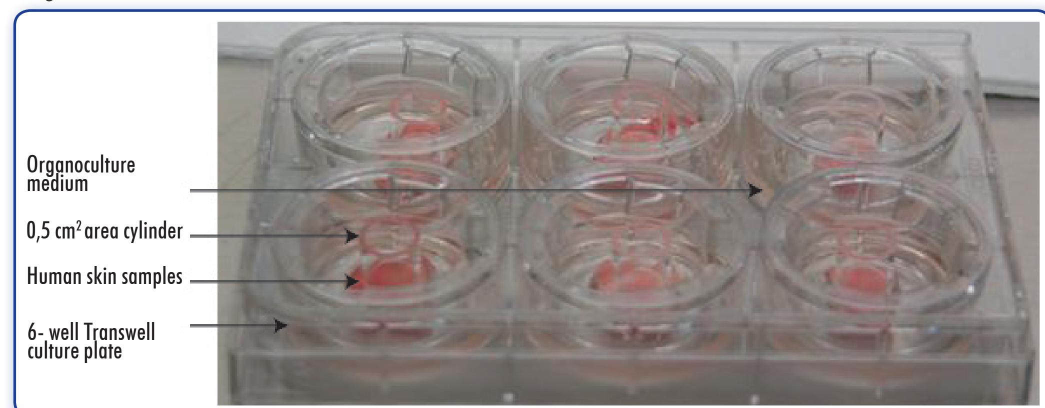
Dermal metabolism is recognized as an important consideration in evaluating the local exposure of topically applied pharmaceutical products. Different models have been used to study the dermal metabolism of specific chemicals such as Franz cell, skin homogenates and isolated keratinocytes. The objective of this work has been to validate the use of a new human skin organoculture model to study skin metabolism using [4, ^{14}C]-Testosterone as a reference molecule.

METHODS

In Vitro Incubation

Fresh human skin samples from two different donors (one man and one woman) were seeded in Transwell inserts in 6-well plate. Receive chambers were filled with 1,5 mL of organoculture medium (Skin Long Term Culture Medium). Skin samples were treated with 10 μL of [4, ^{14}C]- Testosterone solution (5 mM ~ 250 μCi/ mL) and kept in cell culture incubator set at 37°C, 5% CO_2 and saturated hygrometry under gentle shaking for 4 and 16 hours.

Figure 1 SKIN ORGANOCULTURE MODEL



Extraction of [4, ^{14}C]-Testosterone

Organoculture medium samples were extracted using Waters Oasis HLB cartridges. Epidermis and dermis were separated by scratching with a scalpel and were extracted in methanol after crushing. Organoculture medium, epidermis and dermis extracts were analyzed by HPLC with radioactive detection.

In vitro skin metabolite profiles were compared with *in vitro* liver metabolite profiles obtained in a pool of human liver microsomes (5 minutes incubation) and human cryopreserved hepatocytes (1 hour incubation).

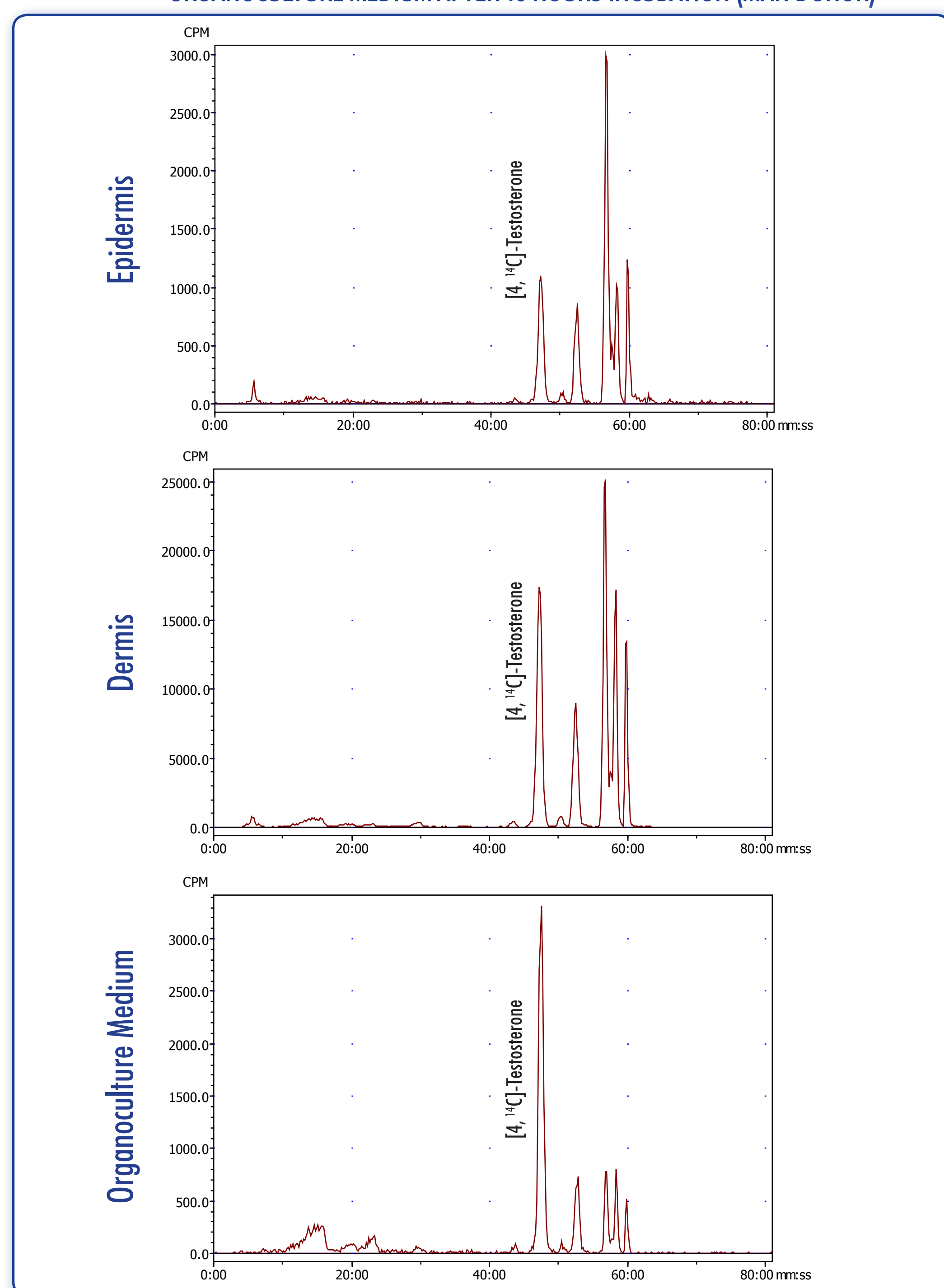
HPLC conditions were:

Column: Lichrospher 100 RP18, 250*4,6 mm, 5 μm
Guard column: Lichrospher 100, RP18, 4*4 mm, 5 μm
Mobile phases: A: water, B: Acetonitrile
Flow rate: 1 mL/ min
Gradient: 0 min 25%B; 50 min 50%B; 55 min 90%B; 70 min 90%B; 71 min 25%B; 81 min 25%B

RESULTS

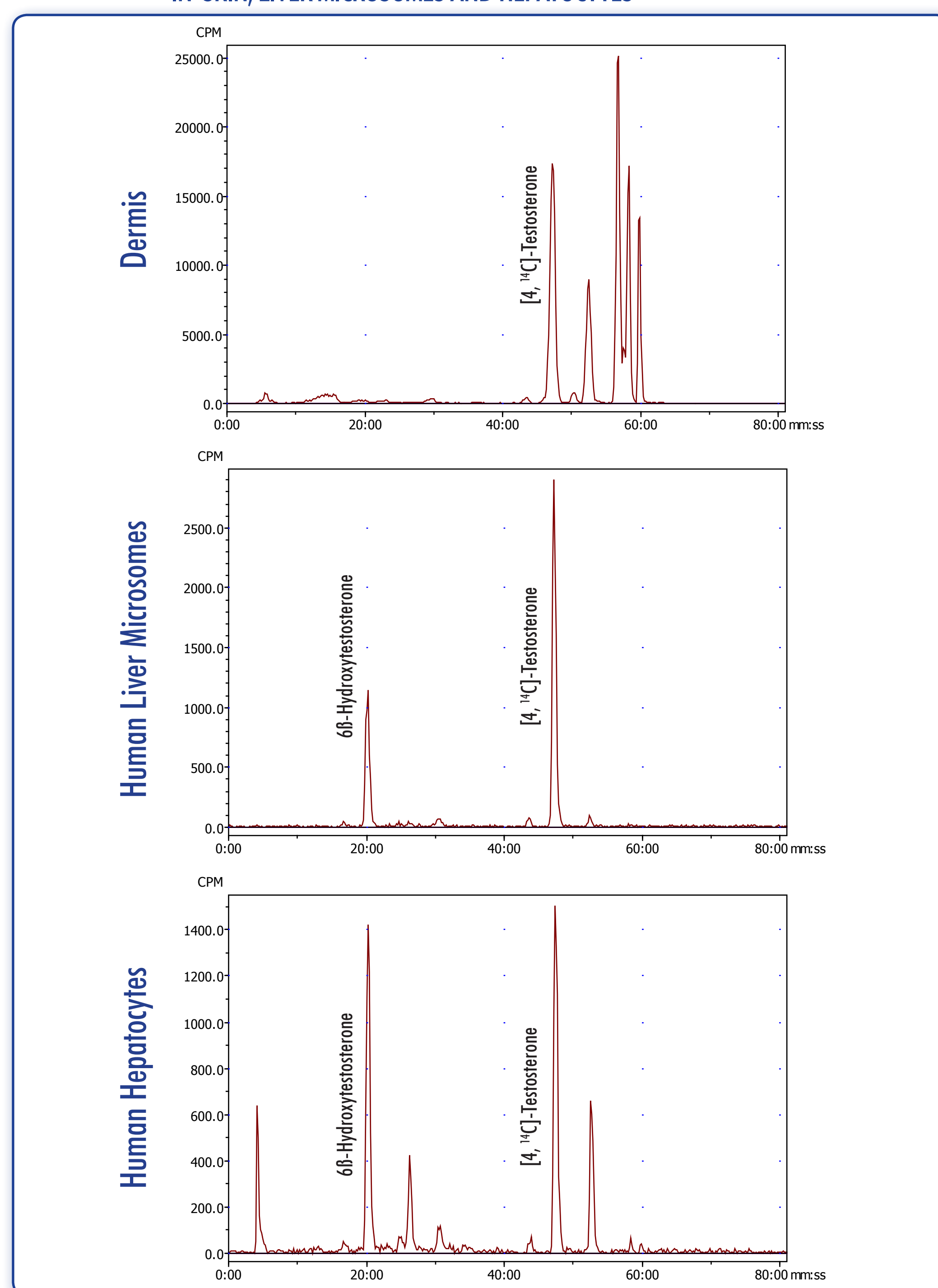
1. SKIN METABOLISM

Figure 2 [4, ^{14}C]-TESTOSTERONE METABOLITE PROFILES IN EPIDERMIS, DERMIS AND ORGANOCULTURE MEDIUM AFTER 16 HOURS INCUBATION (MAN DONOR)



2. COMPARISON SKIN AND LIVER METABOLISM

Figure 3 METABOLITE PROFILES OF [4, ^{14}C]-TESTOSTERONE OBTAINED IN SKIN, LIVER MICROSOMES AND HEPATOCYTES



Skin metabolite profiles obtained after 4 hours were qualitatively similar to those obtained after 16 hours. Moreover, no sex related difference was observed. Skin metabolism is qualitatively different from liver metabolism (microsomes and hepatocytes).

DISCUSSION

The results obtained with this new model showed that fresh excised human skin was able to metabolize efficiently [4, ^{14}C]-Testosterone. Several metabolites were observed in organoculture medium, epidermis and dermis with no sex related difference. The results also showed that skin metabolism of [4, ^{14}C]- Testosterone was clearly different from liver metabolism. Indeed, the main metabolites of [4, ^{14}C]-Testosterone obtained in the skin resulted from reduction reactions whereas the main metabolites obtained in the liver resulted from oxidation reactions.

CONCLUSION

The new human skin organoculture model is an easy-to-handle, simple, rapid and suitable tool to study in vitro cutaneous metabolism of radiolabeled compounds.

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