Evaluation of Anti-inflammatory and Atrophogenic Effects of Glucocorticoids on Reconstructed Human Skin

Günther Weindl, Francesca Castello and Monika Schäfer-Korting

Institut für Pharmazie (Pharmakologie und Toxikologie), Freie Universität Berlin, Berlin, Germany

Summary — Topical glucocorticoids (GCs) are extensively used in the treatment of inflammatory skin diseases. However, their long-term use is often accompanied by severe and eventually irreversible adverse effects, with atrophy being the most important limitation. Currently, most non-clinical studies involve animal testing, so the results are not always representative of the situation in humans. The aim of this project was to establish an in vitro test protocol for the evaluation of the anti-inflammatory and atrophic potential of topically applied GCs in reconstructed human skin. Initial studies with fibroblasts and keratinocytes confirmed the anti-inflammatory and atrophogenic effects of GCs, as evidenced by decreased cytokine production and collagen mRNA expression. In non-pretreated reconstructed human skin (EpiDermFT™), the topical application of GCs for seven days strongly reduced the secretion of interleukin (IL)-6. GC-induced skin atrophy, known to appear only after prolonged treatment, was not detected by the analysis of epidermal thickness and collagen mRNA expression. However, reproducible epidermal inflammation was established for the first time in reconstructed human skin. Topical treatment with tumour necrosis factor (TNF) increased IL-6 release and strongly reduced epidermal thickness accompanied by severe parakeratosis. GC treatment of reconstructed human skin reduced IL-6 levels and completely resolved parakeratosis, leading to the normalisation of epidermal thickness. These induced inflammatory conditions mimic more closely the clinical situations in which GCs are used, and therefore appear to be more suitable for future investigations for the establishment of a human-based in vitro test protocol for evaluating wanted and unwanted GC effects.

Key words: glucocorticoids, inflammation, reconstructed human skin, skin atrophy.

Address for correspondence: Monika Schäfer-Korting, Freie Universität Berlin, Institut für Pharmazie (Pharmakologie und Toxikologie), Königin-Luise-Str. 2 & 4, D-14195 Berlin, Germany. E-mail: Monika.Schaefer-Korting@fu-berlin.de

Introduction

Glucocorticoids (GCs) remain the most effective drugs for the topical therapy of inflammatory skin diseases. However, the application of these drugs is often restricted by various adverse effects (1), of which skin atrophy is the most important. Keratinocytes and fibroblasts are known GC target cells in the skin (2, 3). Epidermal thinning (i.e. epidermal atrophy) is frequently observed after the long-term use of topical GCs, and this leads to an increase in permeability and an increase in trans-epidermal water loss, which generally indicates disrupted barrier function of the skin (4, 5). GCs induce antiproliferative effects in dermal fibroblasts by reducing the mitotic rate of the cells (3, 6), and significantly interfere with extracellular matrix (ECM) protein production. Thinning of the skin is also caused by a reduction in collagen mRNA expression and protein synthesis (7–10). GCs inhibit inflammatory cytokine production by keratinocytes, thus efficiently suppressing epidermal inflammation (3, 11–13). Furthermore, several matrix metalloproteinases (MMPs), which participate in degradation of the ECM, thereby helping keratinocytes to move over the underlying dermis, are repressed after the treatment of human skin (14) and keratinocytes with GCs (11).

Non-clinical test systems are important for the estimation of relative risk of skin atrophy induction by topically-applied GCs. Currently, GC-induced skin atrophy is usually assessed in basic and pharmaceutical research by using the hairless OFA hr/hr rat model (15). However, an evident limitation of many in vivo models is their limited predictivity of the situation in humans. The hairless rat seems to be more sensitive to GC-induced skin atrophy than humans, most likely as a result of increased drug absorption (2). In addition, in vivo testing is highly labour-intensive and is more time-consuming and compound-consuming — factors which are very critical in early drug development. Finally, animal experiments should be avoided wherever possible, for ethical reasons (2).

A first approach to the development of an in vitro test system was based on the inhibition of interleukin (IL)-1 synthesis in normal human keratinocytes and fibroblasts (13). The results were in close correlation with those from studies in human...