DERMAL ATROPHY IN VITRO MODELLING: EFFECTS OF GLUCOCORTICOIDS

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BACKGROUND

Topical glucocorticoids (GCs) represent the most important treatment for epicutaneous therapy of inflammatory skin diseases such as eczema, psoriasis, erythema and irradiation diseases (1-2). With more than 50 years clinical experience, GCs are one of the best investigated drugs in clinical use. Besides the desired anti-inflammatory effects GCs also induce several adverse effects such as skin atrophy, steroidal acne and striae formation (5). Among those, skin atrophy is the most frequently observed and due to its irreversibility it is a particularly serious condition after topical long-term GC therapy. Topical GCs are classified according to their anti-inflammatory potencies after Niedner into four classes: I) weak, II) moderate, III) strong and IV) very strong (Table I). More recently, in addition to classification on potency, the therapeutic index (TIX) has been introduced for topical GCs. The TIX indicates the ratio of beneficial (potency in atopic dermatitis, vasoconstriction) and adverse (atrophogenicity, pituitary adrenal axis suppression and allergic potential) effects of several GCs and thus integrates its advantages and limitations (3-4). Atrophy of the skin appears histopathologically with a flat dermal-epidermal junctions, reduced thickness of epidermis, decreased size of keratinocytes, reduced number of fibroblasts, rearrangement of the geometry of the dermal fibrous network and a decrease of dermal collagen.

SKIN ATROPHY IN VITRO : A MOLECULAR APPROACH

There is a double need within R&D of pharmaceutical industry: on one side to establish experimental in vitro models to screen and to predict the GCs potency (inducing side effects) after systemic treatment and on the other side in particular within dermopharmaceutical industry, the research of topically applied products to reduce, to counteract the effects of GCs treatments at skin level.
Thanks to the versatility and biological relevance of human FT-skin models it has been possible to recreate and mimicking the systemic like condition of GCs treatment and to apply a genomic approach to the induction of atrophy by monitoring relevant biomarkers of dermal compartment.

ATROPHY POTENCY SCREENING MODEL:

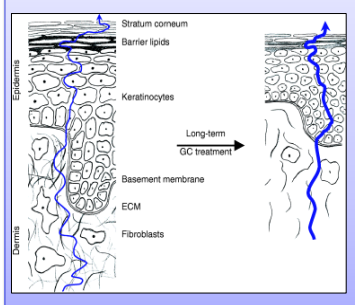
Treatment with HYDROCORTISONE has been performed as inducer of skin atrophy mimicking the epidermal and dermal modifications of atrophy characterized by a reduced epidermal thickness and turnover and by degradation of relevant biomarkers of dermal matrix.
The protocol should be applied to investigate the atrophy potency of new drug, for dose finding, to screen new targets and to reach a better understanding of the mechanism of action.

"REVERSIBLE" ATROPHY MODEL:

β-METHASONE VALERATE has been selected as a reference molecule inducer of skin atrophy. Tissues have been exposed to a systemic like treatment by introducing the GCs in the culture medium for different exposures.
The protocol should be applied to assess the efficacy of topically applied products in counteracting skin atrophy induction, to assess the degree of damage that ii is possible to induce and also a possible efficacy in reverting the induced damage.

EXPERIMENTAL DESIGNS

FT-SKIN	Phenion®, is constituted by epidermal keratinocytes and dermal fibroblasts grown in a specialized stable matrix to form a multilayered skin equivalent that resembles human skin.	
	ATROPHY POTENCY SCREENING MODEL	"REVERSIBLE" ATROPHY MODEL
ATROPHY MODELS "systemic like" exposure	The test item has been **dosed in the fresh culture medium every day for 7 days** to explore an experimental window consisting in the assessment of the efficacy of topically applied products in counteracting the atrophic process.	The test item has been **dosed in the fresh culture medium continuously for 3 and 6 days.** The 3 days followed by 3 and 5 days of recovery without GCs in order to reach a better prediction of atrophy inducing potency.
REFERENCE PRODUCTS	HYDROCORTISONE 10-100nM	β-METHASONE VALERATE 100nM
BIOMARKERS	gene expression (mRNA: COL1A1, ELN, HAS2) histo-morphological analysis by H&E at day 7	gene expression (mRNA: COL1A1, COL7A1, DCN, ELN)



Cutaneous effects of glucocorticoid (GC) treatment. The proliferation and the extracellular matrix (ECM) protein synthesis of keratinocytes and fibroblasts are suppressed by GCs. The intercellular lipid layers are also reduced by GCs, caused by the decreased synthesis of epidermal lipids.

The stratum corneum gets thinner, followed by an increased transepidermal water loss (blue arrow). The skin loses its barrier function, its tensile strength and elasticity caused by the water loss and the degraded ECM (1).

Tab. I Classification of glucocorticoids based on potency

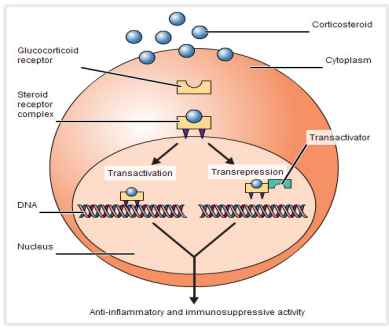
Class	Effects	Examples
I	weak	Hydrocortisone, hydrocortisone acetate, prednisolone
II	moderate	Dexamethasone, prednicarbate
III	strong	Betamethasone 17-valerate, mometasone furoate
IV	very strong	Clobetasol 17-propionate

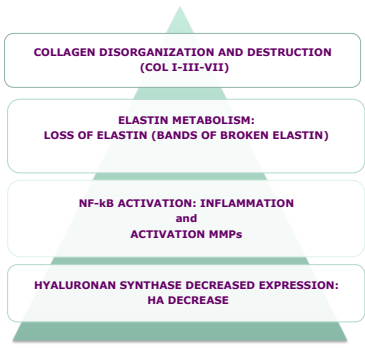
Tab. II Classification of glucocorticoids based on therapeutic indices and their potential to induce skin atrophy

TIX	Skin atrophy	Class	Glucocorticoid
1	1	I	Hydrocortisone
1,2	2	III	Betamethasone 17-valerate
1,5	2	IV	Clobetasol 17-propionate
2	1	II	Prednicarbate
2	1	II	Methylprednisolone aceponate
2	1	III	Mometasone furoate

MOLECULAR MECHANISM OF GCs

GCs mediate their effects via many genomic and non-genomic mechanisms. After diffusion into the cell GCs interact with specific receptor proteins in the target cell, glucocorticoid receptors (GR). The ligand-mediated activation of the GR induces conformational changes that lead to translocation of the receptor from the cytosol into the nucleus. In the nucleus GR interacts with glucocorticoid-response elements (GRE) and modulates gene expression either positively (transactivation) or negatively (transrepression).





ATROPHY POTENCY SCREENING MODEL

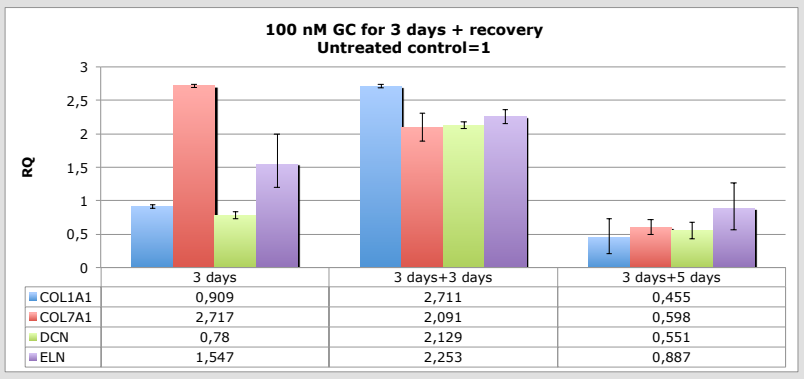
RQ results: HYDROCORTISONE – 7 days treatment		
	10 nM	100 nM
COL1A1	0,789	0,612
ELN	0,52	0,354
HAS2	0,998	0,697

GENE EXPRESSION ANALYSIS
The collagen synthesis in FT-skin was dose-dependently repressed by hydrocortisone. Therefore, this test system seems to be appropriate to determine the atrophogenic potentials of GCs.

HISTO-MORPHOLOGICAL ANALYSIS			
7 days treatment	STRATUM CORNEUM	VIABLE EPIDERMIS	DERMIS & DEJ
NEGATIVE CONTROL	Regular staining and thickness, compact lamellar structure is observed.	The 3 Kc layers (granular, spinous and basal) are visible and regular; the overall morphology and thickness are regular.	Dermal matrix morphology is characteristic of the FT-skin model: not homogeneous with several lack of matrix because of technical difficulty to include the full thickness skin. Fibroblasts are present in the upper dermis.
HYDROCORTISONE 10 nm	Reduced thickness	Reduced thickness where the 3 layers are no more observed; several necrotic zones detached from the DEJ.	Severe modification and damages to dermal matrix integrity; fibroblasts are visibles in the detached matrix.
HYDROCORTISONE 100 nm	Severe thickness reduction and modified morphology	Tissue necrosis and detachement from the DEJ.	Severe modification and damages to dermal matrix integrity; fibroblasts are visibles in the detached matrix.

Hydrocortisone induced a decrease in COL1A1, ELN and HAS2. It can cause severe dermal atrophy by suppression of collagen synthesis and accelerated collagen degradation: as far as the absolute RQ values of ELN gene is concerned a dose effect has also been observed.

"REVERSIBLE" ATROPHY MODEL



	3 days	3 days+3 days	3 days+5 days
COL1A1	0,909	2,711	0,455
COL7A1	2,717	2,091	0,598
DCN	0,78	2,129	0,551
ELN	1,547	2,253	0,887

After 3 days exposure to GC ELN and COL I gene espression were not significantly modified compared to control tissue, suggesting, at this time a not severe and reversible damage at dermal matrix level. This hypothesis is further supported by the COL VII significant over-expression: its role to form the anchoring fibrils that attaches the basament membrane to the underlying dermis is confirmed by the over expression observed as early defence mechanism to the atrophy induction.

This dose (100nM) of beta methasone valerate and this exposure (3 days) were thus selected as suitable to induce a reversible damage at demal level mimicking a reversible atrophy.

After the atrophy induction phase (3 days) a recovery of 3 and 5 days has been analyzed.
As a first defence reaction (3 days) all the selected gene signature for skin atrophy (structural genes: COL I, ELN, DCN and defence mechanism genes: COLVII) were up-regulated up to 3 days of recovery. After 5 days all the genes were down-regulated describing a severe modification.

This interesting experimental window (3 days + 3/5 days) has been opened to assess the efficacy of topically applied products during the recovery period in counteracting the atrophic process induction to evaluate a possible efficacy in restoring the skin after the induced damage.

CONCLUSION

It is known that the risk to develop skin atrophy increases with increasing concentrations of GC, duration of the therapy and **individual response.** In vitro models of the molecular and structural modifications occuring in atrophic skin represent an useful and ethical tool during product development and preclinical studies.In particular according to the new perspectives in Medicine they can be succefully applied for personalized medicine purpose.The 2 in vitro models developped have been successful in confirming **the atrophogenic potential of GCs.**
As expected the 2 reference products induced a repression of dermal markers allowing a validation of the model as fare as the mechanism of GC have been confirmed and they can be consodered as predictive of human response.
Hydrocortison in a dose-dependent way induced a decrease of COL1A1, ELN and HAS2 gene expression and β-METHASONE VALERATE down regulates the COLL I and decorin gene of after the 3 days of atrophy induction period: this effect has been demostarted to be reversible allowing to establish an experimental window in the recovery period where the efficacy of topically applied products shiuld be evaluated.
The two models are currently in progress: western blot will be performed to evaluate the protein levels at the dermal compartment adding more biomakers for the epithelial layers (proliferation, accelerated keratinocytes maturation and epidermal differentiation, barrier function biomarkers).

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