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Mineralocorticoid Receptor Antagonists—A New Sprinkle of Salt and Youth

Olivera Stojadinovic[1,2], Linsey E. Lindley[1], Ivan Jozic[1] and Marjana Tomic-Canic[1,3]

Skin atrophy and impaired cutaneous wound healing are the recognized side effects of topical glucocorticoid (GC) therapy. Although GCs have high affinity for the glucocorticoid receptor, they also bind and activate the mineralocorticoid receptor. In light of this, one can speculate that some of the GC-mediated side effects can be remedied by blocking activation of the mineralocorticoid receptor. Indeed, according to Nguyen et al., local inhibition of the mineralocorticoid receptor via antagonists (spironolactone, canrenoate, and eplerenone) rescues GC-induced delayed epithelialization and accelerates wound closure in diabetic animals by targeting epithelial sodium channels and stimulating keratinocyte proliferation. These findings suggest that the use of mineralocorticoid receptor antagonists coupled with GC therapy may be beneficial in overcoming at least some of the GC-mediated side effects.

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Interplay of the glucocorticoid receptor and mineralocorticoid receptor in skin and wound healing

In dermatology, we have long capitalized on the presence of receptors that mediate anti-inflammatory effects. For example, the effects of the glucocorticoid receptor (GR) may be seen in the use of topical and systemic steroids to treat a myriad of inflammatory skin disorders. However, the efficacy of topical glucocorticoid (GC) use does not come without unintended side effects, including skin atrophy and delayed wound healing. For these reasons, it has been the topic of intense scientific inquiry in an attempt to delineate the mechanisms underlying these sequelae of corticosteroid use. Promiscuous activation of cutaneous mineralocorticoid receptors (MR), due to high-affinity binding of excess cortisol, may be one potential driver. However, it has been shown recently that topical inhibition of the MR attenuates glucocorticoid-induced epidermal atrophy (Maubec et al., 2015). Nguyen et al. (2016) propose that cutaneous MR antagonism improves healing in pathological wounds treated with topical corticosteroids by promoting re-epithelialization. Although much is known about GR function in the skin, the importance of competition by activation of the MR and the implications thereof are just beginning to be recognized.

Both GR and MR belong to the steroid hormone nuclear-receptor superfamily of ligand-dependent transcription factors. GR is found in virtually every cutaneous compartment: epidermal and follicular keratinocytes, epithelial cells of eccrine and apocrine glands, sebocytes, melanocytes, immune cells within the epidermis and dermis, dermal fibroblasts, and smooth muscle cells. Cortisol produced systemically and locally, within the epidermal compartment, serves as the primary ligand for GR, thus potentiating its well-known downstream anti-inflammatory properties. Cortisol-bound GR homodimers mediate GC anti-inflammatory effects through a diverse array of mechanisms, including, but not limited to, transcriptional regulation that results in downstream blockade of prostaglandin production and physical interaction and inhibition

Clinical Implications

- Mineralocorticoid receptor antagonists have beneficial effects on corticosteroid-induced delayed wound closure.
- Regulation of the local skin corticosteroid production (11β-hydroxysteroid dehydrogenase type 1 and type 2) can affect mineralocorticoid receptor activation, thus affecting the development of skin atrophy and wound healing.
- Careful manipulation of mineralocorticoid receptor activation in skin may lead to novel approaches to improve elastin content and to reduce aging skin-associated atrophy.

of NF-κB, modulation of mRNA transcript stability, through membrane-associated receptors and secondary messengers (Rhen and Cidlowski, 2005; Stojadinovic et al., 2007, 2013; Vukelic et al., 2011).

In skin, GCs also bind MR with high affinity (Farman and Nguyen, 2016). However, MR are also expressed in the brain, heart, and in the epidermal compartment of skin: keratinocytes, sweat and sebaceous glands, and in the hair follicles. Importantly, the classically appreciated expression pattern of MR is in renal tubules, where it regulates sodium reabsorption (Farman and Nguyen, 2016). Attempts at defining a distinct physiological role for MR in skin were first made using a conditional mouse model in which targeted expression of MR was directed by the use of a keratinocyte specific promoter (K5-MR mice), which ultimately yielded a phenotype reminiscent of GC-induced epidermal atrophy (Sainte Marie et al., 2007). In contrast, more recent knockout mouse models have demonstrated that MR-KO embryos display epidermal hyperplasia (Boix et al., 2016).

The availability of active cortisol within the skin is controlled by its local synthesis and the interplay between two enzymes, 11β-hydroxysteroid dehydrogenase type 1 (HSD11B1) and type 2 (HSD11B2). Cortisol is produced locally in skin, and what's more, wounding triggers robust activation of cortisol synthesis (Vukelic et al., 2011). Activation of HSD11B1 results in cortisol production, whereas HSD11B2 functions to metabolize cortisol to its inactive form, cortisone. Moreover, the activity of MR and GR in the epidermis is determined by the availability of ligands, which is largely determined by the presence and activity of HSD11B1/2 and subsequent levels of active cortisol. While the DNA-binding domain of MR has high homology to that of GR, its ligand binding domain is capable of high-affinity binding of its native ligand aldosterone, as well as the GC ligand, cortisol. Thus, in states of cortisol excess, such as those experienced during topical application of

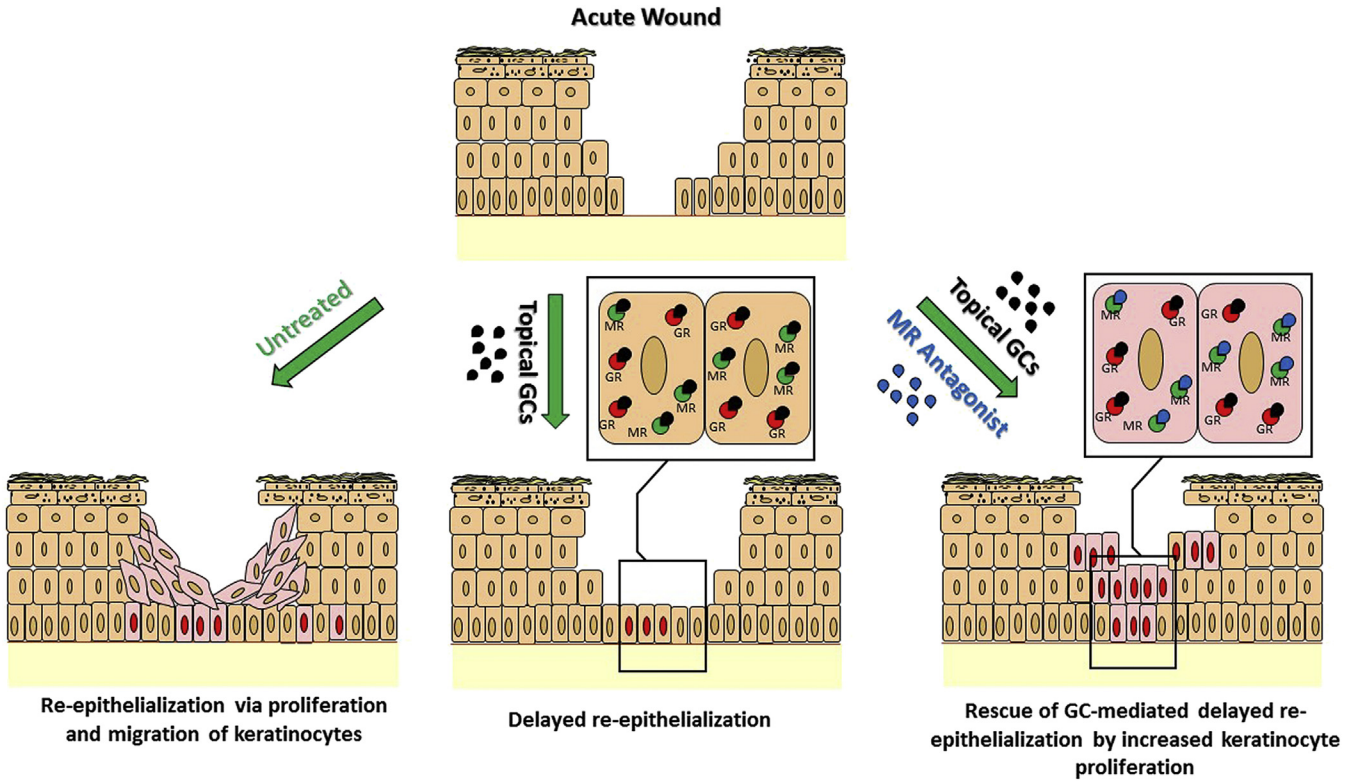

Figure 1. Antagonists of mineralocorticoid receptor rescue glucocorticoid-mediated inhibition of wound healing. After wounding, restoration of the epidermal barrier depends on several essential keratinocyte functions, including proliferation and migration (bottom right), which is inhibited by glucocorticoids (bottom center). Topical application of glucocorticoids leads to excess ligand that may occupy both GR and MR and trigger simultaneous signaling. Nguyen et al. (2016) show that antagonists of MR, when applied with topical glucocorticoids, may diminish this inhibition of healing by stimulating keratinocyte proliferation. GC, glucocorticoid; GR, glucocorticoid receptor; MR, mineralocorticoid receptor.

high potency corticosteroids, the MR may be occupied and activated by cortisol (Figure 1). This local regulatory mechanism of cutaneous GR production and GR/MR activation represents a double-edged sword in that, on the one hand, they can dampen pathologic inflammation through multiple mechanisms, and on the other, they can lead to skin atrophy, thereby leaving the skin more prone to injury while at the same time compromising wound healing. It is interesting that MR antagonists did not improve healing in the absence of topical GC, suggesting that endogenously produced cortisol during wound healing targets GR-mediated signals primarily, whereas the effects of MR may depend on the GR:MR ratio at any time and/or on modulation of HSD11B1/2 levels. Therefore, the differential expression of HSD11B1/2 may have implications for the activation of MR, highlighting the potential for novel therapeutic approaches, using local application of MR-specific antagonists.

The HSD11B1/2 switch: a cutaneous rheostat?

Several studies have found differential expression of both HSD11B1/2 and the MR in skin, especially in pathologic states prone to atrophy and poor healing such as in aging, UV radiation exposure, metabolic disorders, and chronic topical steroid use (Nagase et al., 2013; Skobowiat et al., 2013). In normal human skin, HSD11B1 activity predominates, whereas HSD11B2 levels and activity are both low, thus tipping the scales toward a higher level of active cortisol when the proper precursors have been synthesized or supplied exogenously (Kenouch et al., 1994; Nguyen et al., 2016). HSD11B1 levels are found to be increased further by both UVB exposure and skin aging, which perhaps not so ironically exhibit a similar phenotype to that of GC-treated skin, with atrophy and delayed wound healing (Tiganescu et al., 2013, 2015). Furthermore, inhibiting HSD11B1 activity using both genetic and topical therapeutic interventions in mouse studies augmented wound healing, while preventing cutaneous atrophy in aged mice (Maubec et al., 2015; Tiganescu et al., 2013, 2015). Mice lacking the expression of HSD11B1 were found to have reduced age-associated dermal atrophy, and the use of topical HSD11B1 inhibitors accelerated wound healing (Tiganescu et al., 2013, 2015).

Given the predominance of HSD11B1 in skin at baseline levels, and the natural increase in enzymatic HSD11B1 levels in response to common challenges such UV radiation exposure and chronological aging, it is tempting to speculate that MR occupancy by excess cortisol might be mediating the observed atrophy and poor healing. It is of interest that work in a mouse model of skin aging (UV irradiation in the context of the metabolic syndrome) demonstrated that inhibition of MR suppressed cutaneous aging (Nagase et al., 2013). Furthermore, Nguyen et al. (2016) provide the first mechanistic evidence that inhibition of MR activity inhibits the effects of excess GCs in skin. Topical MR inhibitors, spironolactone, its derivative canrenoate, or eplerenone rescued impaired wound closure and keratinocyte proliferation during wound healing in topically GC-treated mice as well as in diabetic mice (Nguyen et al., 2016), suggesting that MR antagonists may be beneficial for epidermal wound healing. Interestingly, genomic studies of human keratinocytes treated with GCs did not reveal MR to be a significantly regulated target (Stojadinovic et al., 2007), suggesting that there may be either species-specific differences or an additional level of cross-talk occurring among cell types and skin compartments. In addition, chronic wounds in humans are characterized by hyperproliferative, nonmigratory epidermis (Eming et al., 2014), and, thus, stimulation of proliferation may not be as beneficial for patients as for diabetic mice. Nguyen et al. (2016) demonstrated further that inhibition of the MR downstream target, epithelial sodium channel and a classical aldosterone-MR target (ENaC), rescues GC-mediated inhibition of healing using human skin explants. ENaC regulates sodium reabsorption that may lead to alterations in electrical fields, important for re-epithelialization. However, other studies have shown that ENaC activity is required for the ionic and electrical changes responsible for membrane depolarization. Such alterations induce cytoskeletal reorganization and regulate Rac-mediated lamellipodial cell crawling and Rho-mediated actin cable formation, the two methods by which cells migrate during wound healing (Chifflet et al., 2005; Del Mónaco et al., 2009; Justet et al., 2013). Hence, additional studies are required to elucidate the mechanism by which ENaC contributes to wound healing. Taken together, these data highlight a role for MR in the presence of excess cortisol and a potential for design of MR-targeted therapeutic approaches (Figure 1). Such therapeutics could be used to combat increased MR stimulation due to "physiologic" increases in local cutaneous cortisol production that are a consequence of increased levels of HSD11B1, as seen in aging, UV radiation damage, topical GC therapy, and metabolic imbalances.

Mineralocorticoid modulation—a possible "fountain of youth"?

Modulation of MR activity in the presence of excess endogenous or exogenous cortisol may be of benefit both clinically and cosmetically (Figure 1). Given the aforementioned studies, one can reason that targeted inhibition of HSD11B1 (a direct blockade of MR activation) or augmentation of HSD11B2 activity (through direct topical application) may have protective, antiaging effects and/or promote more efficient wound healing in humans treated with GCs. Of note, MR modulation with simultaneous treatment using the direct inhibitors spironolactone or eplerenone, in combination with MR-ligand aldosterone, has been shown to increase elastin deposition in human skin (Mitts et al., 2010). The effect of aldosterone in this study was shown to be mediated through an MR-independent, insulin-like growth factor receptor mechanism, and it was enhanced via direct inhibition of the MR receptor with spironolactone or eplerenone (Mitts et al., 2010). Interestingly, increased elastin deposition was observed with monotherapy treatment using either spironolactone or eplerenone in the absence of aldosterone. Thus, in addition to attenuating an aging, atrophic phenotype, inhibition of MR may also serve to increase the elasticity of skin, thereby improving overall skin quality.

Although current knowledge with respect to the role of MR signaling in

skin homeostasis, pathophysiology, and wound healing remains limited, data presented in Nguyen et al. (2016) provide new insights that underscore potential benefits of targeting the MR (Figure 1). However, whether such targeted intervention proves to be beneficial in patients harboring cutaneous injuries in the context of chronic diseases requiring systemic steroid treatment or in diabetes remains to be seen. Future studies will hopefully address this question.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Expanding the Mutation Spectrum of Ichthyosis with Confetti

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Ichthyosis with confetti is a rare, autosomal dominant disorder caused by frameshift mutations in *KRT10* or *KRT1* and characterized by the development of white, genetically revertant macules in red, diseased skin. All cases result from mutations affecting the tail domains of keratin-10 or keratin-1, and Suzuki et al. expand the mutation spectrum for ichthyosis with confetti caused by mutations in *KRT1*, showing that a polyarginine frameshift in the keratin-1 tail can also cause this disorder.

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Spontaneous correction of pathogenic mutations can occur rarely in somatic cells, leading to genetic reversion (Pasmooij et al., 2012). This "natural gene therapy" can easily be visualized in skin disorders, as populations of revertant cells give rise to areas of healthy-appearing wild-type epidermis, surrounded by adjacent diseased skin. Ichthyosis with confetti (IWC) is a rare disorder of keratinization that displays a dramatic example of genetic reversion. Although patients are born with features shared among other ichthyoses, including erythema, scaling, and palmoplantar keratoderma, thousands of confetti-like white spots appear over the body by late childhood or puberty. These increase in number and size over time, representing independent clones of keratinocytes that are genotypically wild type (Choate

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