TOPICAL IMMUNOMODULATORY THERAPY IN CLINICAL AND EXPERIMENTAL DERMATOLOGY

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Summary

Topical immunomodulatory therapy is of great interest in the treatment of both the inflammatory, but also autoimmune or tumoral dermatological diseases. Understanding the mechanisms of action of these molecules is essential in order to achieve an optimal treatment outcome of many dermatological diseases. This paper presents the main classes of topical immunomodulatory substances, both steroid and non-steroidal, underlying their mechanism of action, adverse reactions, approved and also off-label indications. Corticosteroids have revolutionized topical therapy with an important anti-inflammatory and immunosuppressive role, but with significant side effects in long-term use. This is why there has been a need for substances with similar therapeutic results but without the disadvantage of these side effects. The non-steroidal topical immunomodulatory molecules presented in this article are calcineurin inhibitors, imiquimod, sinecatechins, interferon and diphenciprone.

Key words: *topical immunomodulators, cortico-steroids, calcineurin inhibitors, imiquimod, sinecatechins, interferon, diphenciprone.*

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Introduction

Topical immunomodulators are molecules that alter the immune response when applied to the skin in the sense of suppressing or stimulating the immune cascade. These are classified as steroid and non-steroidal. Non-steroidal immunomodulators are represented by calcineurin inhibitors, imiquimod, sinecatechins, interferon and diphenciprone whereas steroidal immunomodulators are represented by cortico-steroids.

1. Topical corticosteroids

Since 1952 [1], when the topical hydrocortisone was first used in the 1-2% concentration, topical corticosteroids are the main category of drugs used in dermatology, being prescribed in most acute, subacute and chronic inflammatory dermatoses. From a pharmacological point of view, topical corticosteroids are glucocorticoids with a certain chemical formula, having the cortisol structure in common, with certain modifications that cause an anti-inflammatory effect. These changes are decisive for the various properties related to solubility, absorption, lipophilic state and molecular mechanisms.

Mechanism of action

Topical corticosteroids act by modifying the functions of epidermal, dermal and leukocyte immunocompetent cells. The results are the synthesis of lipocortin and the inhibition of interleukin 1 formation. [2] Lipocortin is a glycoprotein that inhibits phospholipase A2, decreasing the release of arachidonic acid, thus reducing the formation of prostaglandins, prostacyclin and pro-inflammatory leukotrienes. All these mechanisms determine the anti-inflammatory, anti-mitogenic and immunosuppressive properties of corticosteroids.

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Classification

Topical corticosteroids are categorized by potency in 4 groups and 7 classes based on the vasoconstriction test and clinical trials. The vehicle greatly influences the effectiveness of the products. Generally, ointments increase corticoid potency, as they have occlusive effect and determine intense hydration of the stratum corneum with increased absorption of the substance. [3]

Corticosteroids have an anti-inflammatory effect by decreasing the number and vascular wall adhesion of the main cells involved in inflammation (monocytes/macrophages, granulocytes). They also decrease the number of NK cells, Langerhans cells and the production of T-lymphocytes and IL-2, with low expression of ELAM-1 and ICAM-1 in the endothelial cell thus minimizing vascular permeability. By inhibiting the nuclear factor kB and activator protein 1, it stops the activation of proinflammatory genes. [5]

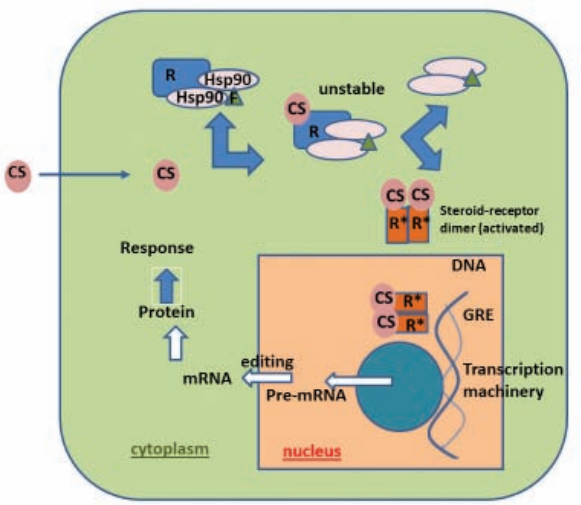

Figure 1. Image model of the interaction of a corticosteroid (CS) and its receptor (R). The intracellular receptor is linked to stabilizing proteins represented by Heat-shock protein 90 (Hsp-90) and others including FKBP5 noted as F in the picture. Once the corticosteroids are bound, the receptor complex becomes unstable, releasing Hsp 90 and associated molecules, being now able to dimerize, entering into the nucleus and binding to GRE (glucocorticoid response element) and regulating transcription by RNA polymerase II and transcription factors. The mRNA that results is edited and exported into the cytoplasm for the synthesis of proteins that determine corticosteroid response. An alternative way besides interacting with GRE is linking to other transcription factors, like NF-kB in the nucleus of the cell.

The vasoconstrictor effect may be due to the inhibition of vasodilator molecules like histamine and bradykinin. [6] The antiproliferative effect, very useful in psoriasis, is responsible for the atrophy and fibrosis side effects of these drugs.

The side effects of topically used corticosteroids can be both systemic and local. Systemic side effects occur by hypothalamic-pituitary-adrenal axis inhibition and are represented by hyperglycaemia, diabetes mellitus and mineralocorticoid-like effects (hypocalcaemia and oedema) as a result of hydrocortisone usage in particular.

The local adverse effects are: hypopigmentation (triamcinolone) and atrophy, acne, glaucoma, cataracts, facial hypertrophy, infections, perioral dermatitis, purpura, tachyphylaxis, especially in case of psoriatic patients. Non-fluorinated compounds most frequently cause allergic reactions. [7]

The main dermatological diseases that are responsive to cortisone treatment are dermatitis (atopic, seborrheic, prurigo, etc.), papulo-squamous diseases (psoriasis, lichen plan), pigmentary diseases (vitiligo), vesicular-bullous diseases (bullous pemphigoid, pemphigus foliaceus), autoimmune diseases (lupus erythematosus, dermatomyositis, morphoea). [8]

2. **Topical calcineurin inhibitors**

Topical calcineurin inhibitors are local immunomodulatory and anti-inflammatory agents that have the advantage of not showing the local adverse effects of corticosteroids. Ciclosporin A is the first calcineurin inhibitor that appeared, due to the necessity of using immunosuppressive agents in renal transplantation. Thus, the positive effect was observed in patients who had both psoriasis, atopic dermatitis and other dermatological diseases. Because of the large molecular mass, ciclosporin cannot be used topically. Subsequently, a new compound called tacrolimus with immunosuppressive properties of 10-100 times greater appeared. [9]

Calcineurin inhibitors approved for the treatment of atopic dermatitis in adults and children over 2 years of age and used in other

Table 1. **Classification of topical corticosteroids. Reproduced after [3], [4]**

I. Superpotent Class 1 clobetazol propionate cream, ointment, gel, lotion, foam, shampoo, spray 0.05%, fluadrenolide tape 4mcg/cm2 halobetasol propionate ointment and cream 0.05%
II. High potency Class 2 amcinonide ointment 0.1%, betamethasone dipropionate ointment and gel 0.05%, desoximetasone ointment and cream 0.25% and gel 0,05% diflorasone diacetate ointment 0.05% fluocinonide cream, ointment or gel, solution 0.05% halcinonide ointment,cream, solution 0.1% mometasone furoate ointment 0.1% triamcinolone acetonid ointment 0.5% clobetasole propionate scalp application solution 0.05% Class 3 amcinonide cream and lotion 0.1% betamethasone dipropionate cream and lotion 0.05%, betamethasone valerate ointment 0.1%, diflorasone diacetate cream 0.05%, triamcinolone acetonide ointment 0.1% and cream 0.5% fluticasone propionate ointment 0.0005%
III. Moderate potency Class 4 betamethasone valerate foam 0.12% desoximetasone cream 0.05%, fluocinolone acetonide ointment 0.025%, fluadrenolide ointment 0.025% fludroxycortide ointment 0.05%, hydrocortisone valerate ointment 0.2%, triamcinolone acetonide ointment and cream 0.1% or spray 0.2% Class 5 betamethasone dipropionate lotion 0.05 %, betamethasone valerate cream and lotion 0.1%, fluocinolone acetonide cream 0.025% or oil and shampoo 0.01% hydrocortisone butyrate cream, ointment and lotion 0.1%, hydrocortisone valerate cream 0.2%, hydrocortisone probutate cream 0.1% triamcinolone acetonide lotion 0.1% and ointment 0.025% clocortolone pivalate cream 0.1% fluticasone propionate cream and lotion 0.05% fluadrenolide cream and lotion 0.05% prednicarbate ointment and cream 0.1%
IV. Low potency Class 6 alclometasone dipropionate ointment and cream 0.05% triamcinolone acetonide cream 0.1% and lotion 0.025% betamethasone valerate lotion 0.05%, desonide cream 0.05%, fluocinolone acetonide solution and cream 0.01% Class 7 dexamethasone sodium phosphate cream 0.1%, hydrocortisone acetate cream 1%, methylprednisolone acetate cream 0.25%

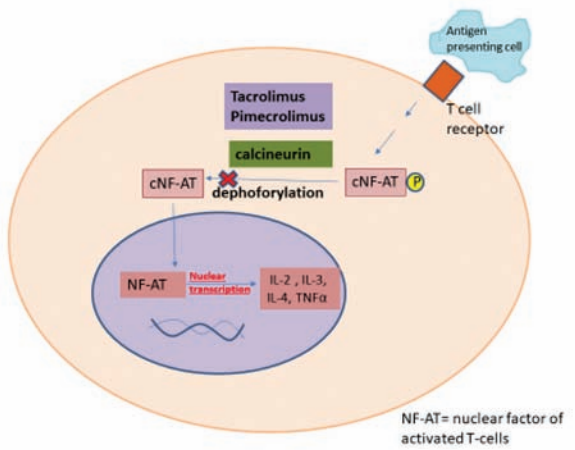

Figure 2. The mechanism of action of calcineurin inhibitors

responsive steroid dermatoses are represented by tacrolimus ointment 0.1% and 0.03% and pimecrolimus cream 1%.

Tacrolimus is produced by a soil bacterium called *Streptomyces tsukubaensis*, originating in the Tsukuba region of Japan. Pimecrolimus is produced by the fermentation of *Streptomyces hygroscopicus var. ascomycetous*.

Mechanism of action

In the process of T lymphocyte activation, by binding specific receptors to antigen presenting cells, there is an increase in calcium influx into the cytoplasm that binds to calmodulin with activation of calcineurin.

Calcineurin is a phosphatase that dephosphorylates the cytoplasmic subunit of the activated T cell nuclear factor. The dephosphorylated subunit then translocates into the nucleus where it promotes specific cytokine synthesis (IL-2, IL-3, IL-4, TNFα).

Pimecrolimus is indicated in moderate cases of atopic dermatitis but also as a maintenance treatment for the prevention of relapses, while tacrolimus is indicated in moderate to severe atopic dermatitis, being also used as a first-line drug instead of topical corticosteroids [10]. Pimecrolimus is especially recommended in intertriginous and periocular areas, because in these areas the use of topical corticosteroids is relatively contraindicated [11]. The risk of malignancies development following treatment has not been demonstrated. [12]

For the initial intensive treatment is used the 0,1% tacrolimus ointment in two applications per day until the clinical remission of the lesions (not more than 14 days), whereas in the maintenance treatment should be applied once a day, two days a week with approximately 2-3 days spacing between applications. Pimecrolimus is recommended to be applied twice daily until the eruption disappears (up to 6 weeks) with intermittent administration.

Local adverse reactions are represented by erythema, skin irritation, burning sensation and pruritus. Frequently, alcohol intolerance also occurs, and conditions such as folliculitis, acne and herpes infection may appear. Since tacrolimus has a macrolide structure, its administration is contraindicated in macrolide hypersensitivity. Tacrolimus may determine allergic contact dermatitis and a rosacea-like granulomatous reaction on the face as stated by some reports.

For patients with vitiligo, topical tacrolimus application may have promising results, but only small studies and case studies are reported in the literature, so standardized trials are required in several patients. [13]

Also, in the case of seborrheic dermatitis, 0.1% tacrolimus ointment applied for a short period of time successfully replaces corticosteroids, but further studies are needed to control efficacy and safety for this common condition. [14]

In the case of rosacea patients, treatment with tacrolimus reduces erythema but has no effect on papulo-pustules. [15]

Preliminary studies have shown that the use of 0.075% tacrolimus ointment reduces erythema, pruritus and symptomatology in patients with steroid induced rosacea when topical steroids and aggravating factors of rosacea are eliminated, such as caffeine, spices, alcohol. [16]

In lichen sclerosus, cases of complete remissions were recorded at 1 year after a 1% tacrolimus ointment daily application, but more studies are needed to validate this use. [17]

3. **Imiquimod**

Imiquimod is an imidazoquinoline, along with sotirimod and resiquimod, being the first of a new class of immune response modulators (MRIs) with antiviral and antitumor effects demonstrated through clinical trials.

Mechanism of action

The imiquimod molecule activates both innate immunity and cellular immune response by activating TLR 7 (Toll like receptors 7). TLR receptors constitute a family of pathogen recognition receptors expressed on several types of innate immune cells, including neutrophils, macrophages and dendritic cells, dermal endothelial cells and mucosal epithelial cells and represent the primary sensors of the innate immune system. Along with TLR coupling to their ligands begins the production of various pro-inflammatory cytokines, chemokines and effector molecules, depending on the type of activated cells. Imidazo-quinolines are the first drugs known to activate TLR receptors 7. [18-21]

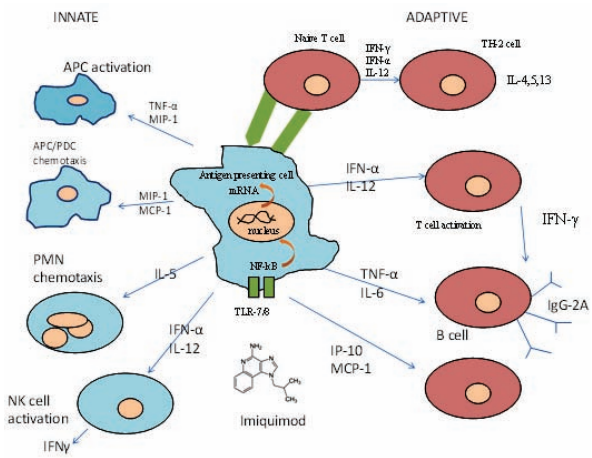

Figure 3. The mechanism of action of imiquimod

Imiquimod stimulates the innate immunity by producing various cytokines such as IFNα, TNFα, IL-1, IL-6, IL-8, IL-10, and by increasing the activity of NK cells and secreting macrophages who produce as well nitric oxide and cytokines. Additionally, adaptive immunity is also indirectly stimulated, INFα regulating cytotoxic T lymphocyte activity, in addition to antiviral and antiproliferative effects. The maturation of antigen presenting cells (APC) is promoted with migration into lymph nodes and specific immune response organization. The result is the elimination of the viral infected cell in a selective way by switching the immune response to cytotoxic Th-1 mediation (IL-12, TNF-α) in spite of the Th-2 humoral mediation (IL-4 and IL-5 inhibition). [22-24]

Rare systemic side effects of imiquimod are represented by headaches, flu-like symptoms or fatigue although only a small amount of the drug is absorbed systemically. At the site of imiquimod application can appear erythema, oedema, scaling, ulceration, infection, hypopigmentation or hyperpigmentation.

Indications

Although initially imiquimod was only approved for external genital warts therapy, it was subsequently approved by the FDA for both actinic keratosis and superficial basal cell carcinoma. Imiquimod stimulates the immune system for the elimination of condylomas and spontaneous regression of warts suggests the presence of a cell-mediated active response. Warts removal rates are 50-61.8%, the recurrence rate at the 3-month follow-up visit is 9-14% and side effects are related to local depigmentation or vitiligo-like lesions. [25-27]

Imiquimod can also be used in superficial basal cell carcinomas in cases where surgical excision, electrotherapy or diathermic cleansing cannot be performed. Promising results were also reported in cases of Bowen disease, molluscum contagiosum, keloid, malignant lentigo, cutaneous T-cell lymphoma, cutaneous melanoma metastases and cutaneous leishmaniasis. [3]

Imiquimod has been used to treat haemangiomas in children in paediatric practice. A complete 10-week remission result was reported in a 4-month-old child with haemangioma being treated with 5% imiquimod. Also, remarkable results have been reported in a study of 10 haemangiomas of the infant treated with 5% imiquimod for 16 weeks, in which 4 patients had complete remissions. [28]

Promising results were obtained in the case of tibia-localized Bowen disease, difficult to treat surgically, with histological remission in 93% of cases [29] and also in Bowen disease with other locations such as perianal [30], infraumbilical [31], genital [32]. Interesting results have also been reported for keloid scars in the auricular lobe. [33]

Imiquimod is also utilized in experimental mice models for psoriasis. This widely used model demonstrates the anti-psoriasis effect of newly tested substances or improved formulas of already authorized molecules. Topical application of 5% imiquimod cream causes lesions

Table 2. **Imiquimod posology**

External genital condyloma	One application / day, 3 days / week, 16 weeks
Superficial basal cell carcinoma	One application / day, 5 days / week, 6 weeks
Actinic keratosis	One application / day, 3 days / week, 4 weeks with one month evaluation.
Imiquimod cream should be left to act locally for at least 8 hours.	

characteristic of plaque psoriasis with specific IL-17/IL-23 axis dependent histopathological changes like epidermal hyperproliferation, neoangiogenesis, neutrophilic microabcess formation and infiltration with CD4 positive T cells and dendritic cells. [34] Thus, the corticosteroids mechanism of action was confirmed and further investigated and along with betamethasone testing, evidence of the inhibitory effect of Th-17 and Th-1 lymphocyte-dependent cytokines was made. [35] Also, recent in vitro and in vivo studies using the imiquimod mouse model of psoriasis demonstrate the superior anti-psoriasis effect of tacrolimus in a tocopheryl polyethylene glycol 1000 Succinate (TGPS) - based micro-emulsion. [36]

4. **Sinecatechins**

Sinecatechins are a standardized green tea leaf extract of *Camelia sinensis*, a species of the *Theaceae* family, mainly composed of tea polyphenols, especially catechins (over 85%). The most important catechin in the ointment is epigallocatechin-3-gallate, having the highest biological activity. [37-43]

Mechanism of action

Sinecatechins have an antiviral, antioxidant and immunomodulatory role. The antiviral mechanism involves the direct selective destruction of infected koilocytes. By inhibiting the synthesis of IL-10 and EGF occurs the inhibition of the koilocytes proliferation along with the activation of the p53 and pRb tumor suppressor genes that determines cell apoptosis. [44-47]

The immunomodulatory mechanism involves the stimulation of cellular immunity, with cytokine synthesis, such as *IL1 beta*, *TNF alpha*, *IFN gamma*, and activation of T-lymphocytes, macrophages and antigen presenting cells. Along with *TNF beta* inhibition occurs the cancellation of immune response suppression mechanism. By favoring local inflammation appears erythema, pruritus and so-called burning sensation. [44-47]

The antioxidant mechanism involves the activation of antioxidant enzymes (glutathione, catalase, superoxide dismutase) with free radicals neutralization and inhibition of prooxidative enzymes (COX 1,2-LOX 5), tumoral cell proliferation, matrix enzymes (MMP 2,7,9) and angiogenesis [44-47]. The recommended dose is 0.5 cm of ointment for all warts, applied 3 times a day.

Adverse reactions are local reactions such as erythema, oedema, pruritus and pain at the application site.

Indications

The 15% sinecatechin ointment is indicated since 2015 in the American CDC guide for the treatment of external anogenital warts, and the 10% concentration is a grade A recommendation in the European guidelines for the treatment of external genital warts since 2012.

Cases of complete remission of facial warts resistant to other treatments are reported after 20 days of treatment with 10% sinecatechin ointment. [47]

Also recalcitrant molluscum contagiosum infections have been successfully treated with sinecatechins. [48]

A case of vulvar carcinoma in situ was reported in a patient who refused surgical intervention with complete remission after sinecatechin treatment. [49]

The anti-psoriasis effects of epigallocatechin-3-gallate were tested on the imiquimod model of psoriasis induction in mice. The results obtained showed reduction of inflammatory lymphocyte infiltrate and decrease in IL-17A, IL-17F, IL-22,

Table 3. **Off-label indications of interferon**

Tumor proliferation	Inflammatory diseases	Infections
Basal cell carcinoma (α)	Atopic dermatitis (β)	Common warts (α,β,γ)
Actinic keratosis (α)	Keloid (α,γ)	Veruciform epidermodisplasia (α)
Squamous carcinoma (α)	Behçet's disease (α)	Herpes zoster (α)
Buschke-Lowenstein giant condyloma (α)	Sclerodermia (α)	Herpes simplex (α)
T-cell lymphoma (α, β, γ)	Scleromixedem (α)	Acral necrolytic erythema (with hepatitis C) (α)
Granulomatosis (α, β)		Leishmaniasis (β)
Infantile Hemangioma (α)		Leprosy (β)
Angiomas (α)		*Mycobacterium avium infection complex (γ)*

IL-23 levels, with possible promising uses in topical anti-psoriasis therapy. [50]

5. **Interferons**

Interferons represent a family of glycoproteins secreted by most eukaryotic cells in response to a variety of viral, bacterial and tumoral stimuli. There are three antigenic forms of interferon: alpha (leukocytic), beta (fibroblastic), gamma (immune). In dermatology, it is of particular importance IFN-α 2a and IFN-α 2b that have an immunomodulatory, antiviral and antiproliferative role. Interferons can be administered both intralesional and parenteral and are metabolized by kidneys. [3]

Mechanism of action

Once the interferon molecule is bound to specific target cell receptors, JAK-STAT signalling pathway is activated, with dual phosphorylation, STAT dimerization, and translocation into the nucleus where gene transcription is induced.

The immunomodulatory effect of interferon occurs by activating MHC I and II, increasing the number of NK cells and inhibiting the production of cytokine Th-2 (IL-4 and IL-5).

Side effects

Besides flu-like symptoms and cutaneous reactions at the site of injections, interferon can determine psoriasis flares, neurologic and psychiatric effects, cardiovascular effects, rhabdomyolysis, gastro-intestinal effects and bone marrow suppression. [3]

Indications

The indications endorsed by the FDA for interferon are: acuminate condylomas (IFN-α 2b), malignant melanoma (IFN-α 2b), AIDS associated Kaposi's sarcoma (IFN-α 2b and IFN-α 2a) and chronic granulomatosis (IFN gamma). Off-label indications are tumoral proliferation, inflam-matory diseases and infections.

Human leukocyte alpha interferon (2 x 10^6 IU/g) in hydrophilic cream may be considered an effective treatment for the first episodes of genital herpes in man. [51] It also produces superior results compared to the 0.5% podophyllotoxin cream in the alternative treatment of genital warts in men. [52]

6. **Diphencyprone**

Diphencyprone is a potent immunostimulatory contact sensitizer that has been widely used since 1980 in the *treatment* of alopecia areata and common warts.

Mechanism of action

The mechanism of action in alopecia is based on the "theory of antigenic competition" which implies that the immune response to certain antigens is inhibited by another immune response to a more potent antigen. In the untreated disease, peribulbar infiltrate is predominantly composed of CD4 + T cells with a CD4 / CD8 ratio of 4: 1. Following treatment, this ratio becomes 1: 1, with an increase in the number of CD8 + peribulbar T cells. In addition, abnormal HLA - A, B, C and DR expression decreases in the follicle epithelium.

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Conflict of interest
NONE DECLARED

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