How does prednisolone work in pemphigus?

Jak działa prednizolon w pęcherzycy?

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Abstract

In this review the mechanism of action of glucocorticosteroids (GH) in pemphigus is discussed. In pemphigus, GH may work by: (i) inhibiting antibody synthesis; (ii) suppressing inflammation, especially eosinophilic spongiosis; and (iii) stopping acantholysis via a direct pharmacological effect on keratinocytes. The anti-acantholytic effects of methylprednisolone include upregulation of gene expression of adhesion molecules and protection of keratinocyte adhesion molecules from antibody-induced phosphorylation. Experimental drugs, such as lazaroids that are deprived of the mineralocorticoid and glucocorticoid effects of GH, should be tested for their ability to replace GH in the treatment of pemphigus.

Key words: *pemphigus, glucocorticosteroids, keratinocyte.*

Streszczenie

W tym doniesieniu poglądowym omówiono mechanizmy działania glukokortykosteroidów (GH) w pęcherzycy. W tej chorobie GH mogą: 1) hamować syntezę przeciwciał, 2) osłabiać zapalenie, przede wszystkim spongiozę neutrofilową, 3) zatrzymywać akantolizę przez bezpośrednie działanie farmakologiczne na keratynocyty. Działanie przeciwakantolityczne metyloprednizolonu obejmuje aktywację genów dla cząsteczek adhezyjnych i ochronę tych cząsteczek przed ich fosforylacją indukowaną przeciwciałami. Leki doświadczalne, takie jak lazaroidy, pozbawione działania na gospodarkę elektrolitową i cukrową, mogłyby być wypróbowane w pęcherzycy.

Słowa kluczowe: *pęcherzyca, glukokortykosteroidy, keratynocyt.*

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Purpose

Although it is possible to maintain pemphigus patients in remission using immunosuppressive drugs without glucocorticosteroid hormones (**GH**) [1, 2], initial treatment relies on the high dose of systemic GH [3-5]. I work on developing a non-steroidal treatment of pemphigus. The ultimate goal of my research is to eliminate the necessity of giving GH to the patients. I believe that alternative therapies fostering keratinocyte adhesion and/or specifically antagonizing the effects of pemphigus antibodies can be developed based on: (1) elucidation of the mechanism of action of pemphigus antibodies that cause keratinocyte to detach from each other (acantholysis); and/or (2) elucidation of the mechanism of anti-acantholytic effects of GH. These two approaches are independent. To proceed *via* the first approach, one needs to identify the type of an autoantibody that is chiefly responsible for acantholysis in each particular patient. Recent research demonstrates that pemphigus patients develop autoantibodies to several types of self-antigens which include both adhesion molecules, such as desmogleins 1 and 3, and cell-surface receptors that regulate function of adhesion molecules, such as acetylcholine receptors (see reference [6] for discussion). To proceed *via* the second approach, one needs to dissect the chief pharmacological effect of GH that mediates the efficacy of these drugs in pemphigus.

History

The first report of administration of GH to a pemphigus patient was published more than 60 years ago, in 1940 [7]. It was noticed that pemphigus is associated with changes in patients' blood chemistry suggestive of abnormal (deficient) function of the adrenal gland producing cortisone. Cortisone is a naturally

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occurring hormone that served as a prototype for development of all currently known synthetic GH drugs, including prednisone/prednisolone. In the index patient with pemphigus, adrenocortical extracts were used for treatment. The synthetic cortisone was introduced to the treatment of pemphigus some 10 years later [8]. Development of the notion that pemphigus is an autoimmune disease in the mid 1960s brought about corresponding changes in the teaching about the mechanism of therapeutic action of GH in pemphigus. Since then, it has been widely accepted that GH treat pemphigus owing to their immunosuppressive properties.

Controversy about direct effect of GH on keratinocytes

There are several lines of evidence that high doses of systemic GH control acantholysis *via* actions that may not to be limited to the immunosuppressive properties of these drugs but harbor direct anti-acantholytic effects on keratinocytes:

1) while the major decline in antibody titers occurs 3-4 weeks after GH administration [9], clinical lesions in pemphigus patients usually improve much more rapidly, within 24-48 hrs after initiation of a high dose, "pulse" therapy with methylprednisolone (**MP**), or dexamethasone, when the titer of pemphigus autoantibodies remains unchanged [10-12];
2) local administration of GH agents, such as a very potent topical corticosteroid, 0.05% clobetasol propionate cream, can alone control initially cutaneous lesions in mild cases of pemphigus vulgaris [13];
3) both MP [14] and hydrocortisone [15] have been independently shown to block acantholysis in skin organ cultures induced by diluted plasma from pemphigus patients. The anti-acantholytic dose of GH used in these experiments approximated the serum concentration of GH in a patient treated for an acute episode of pemphigus vulgaris [1]. Since in either experiment, antibody-producing cells were not added to the system, GH agents did not exhibit their anti-acantholytic effects by way of acting upon lymphocytes.

The skepticism about direct anti-acantholytic action of GH stems from the following experimental results. The first *in vitro* testing of the anti-acantholytic activity of GH by Shiltz *et al.* [16] showed that co-administration of a relatively high dose of pemphigus vulgaris IgG, 23 mg/ml, and 10 μM of hydrocortisone, or the same dose of triamcinolone acetonide, did not block acantholysis in the skin organ cultures. Likewise, the first *in vivo* testing of anti-acantholytic effects of GH by Anhalt *et al.* [17] showed that dexamethasone did not inhibit acantholysis induced in the skin of 1-2 d-old Balb/c mice injected with either 14.4 or 20 mg of pemphigus vulgaris IgG/gram of body weight/day together with or 24 hrs after dexamethasone, given at a total dose of either 10 or 20 mg/kg/day. The doses of dexamethasone used in these experiments greatly exceeded the maximum therapeutic dose of GH used in pemphigus, i.e., >4.5 g of prednisone per day. The authors concluded that the major therapeutic effect of GH was due to reduction of antibody synthesis rather than modification of the events that occur within the epidermis after antibody binding.

We hypothesized that the discrepancy between results of previously reported *in vitro* and *in vivo* experiments might be due to limitations of the mouse model used in previous studies [17, 18], and sought to develop an adequate model for *in vivo* testing the anti-acantholytic efficacy of GH drugs in pemphigus.

Development of a novel mouse model to test direct effects of GH in the skin

At the Society for Investigative Dermatology Meeting in Washington D.C., in 2001, we reported first results of our efforts to develop a novel animal model for testing the anti-acantholytic treatments of pemphigus [19]. Instead of 1-2 d-old, we used 3-5 d old Balb/c mice and found that while older animals might respond to anti-acantholytic treatments with GH, the untreated littermates of these pups did not always produce pemphigus-like lesions, thus complicating interpretations of the results. We speculated that rapidly developing hair follicles might reinforce epidermal integrity in older mice. Therefore, we sought to develop a more reliable animal model, and tested the strain of athymic mice that do not grow hair. When injected with 10 mg/g/d of pemphigus vulgaris IgG, these neonates developed pemphigus-like lesions. The 1-2 d-old pups died despite any anti-acantholytic treatments, whereas slightly older pups responded to experimental treatment with GH. Therefore, we selected older neonatal athymic nude mice weighing ~2 g as a model for testing anti-acantholytic drugs.

To increase accuracy of our evaluation of the anti-acantholytic efficacy of test drugs, we decided to assess the extent of acantholysis by actually measuring the length of intraepidermal split on the microphotograph of mouse skin, instead of scoring gross skin lesions using an arbitrary scale, as it was done in the past. Among various GH drugs, MP was selected because it represents an active form of prednisone - the most commonly used GH drug in the treatment of autoimmune pemphigus.

Using a novel animal model of experimental pemphigus, we demonstrated that all mice treated with MP survived injections of patients' IgG. Microscopically, the extent of acantholysis decreased from 77.5%± 2.3% in non-treated control mice to 22.5%± 4.3%, which is a statistically significant difference ($p<0.05$). MP did not prevent pemphigus IgG from binding to mouse epidermis, thus illustrating that its anti-acantholytic effect resulted from some putative pharmacological effect on keratinocytes which was strong enough to override the proacantholytic effect produced by the autoantibody.

Recent breakthrough in better understanding of the mechanism of direct GH action on keratinocytes in pemphigus

In a series of experiments reported by our research team at the Society for Investigative Dermatology Meeting in Los Angeles, CA, in 2002, we demonstrated that pemphigus vulgaris IgG and MP produce mutually opposite effects on keratinocyte adhesion molecules [20]. The complete report has been recently published [21]. To elucidate the mechanism responsible for a direct anti-acantholytic effect of MP, we compared effects of 8 hrs treatments of cultured keratinocytes with pemphigus vulgaris IgG (0.5 mg/ml), normal human IgG (0.5 mg/ml), and 0.25 mM MP on the rate of expression of genes regulating keratinocyte adhesion and some other vital functions. Changes in the gene expression were investigated by DNA microarray assay. This innovative technology allows in a single experiment to screen more than 10,000 human genes as possible candidate targets for pro-acantholytic action of pemphigus antibody and anti-acantholytic action of MP. Using DNA microarray assay, we found that compared to normal IgG, pemphigus IgG decreased transcription of 198 genes, and increased transcription of 31 genes. MP decreased transcription of 14 genes and increased transcription of 818 genes. MP upregulated transcription of the genes encoding adhesion molecules desmoglein 3, desmocollins, plakophilin, E-cadherin, P-cadherin, and catenin. In addition to adhesion molecules, pemphigus IgG and MP produced reciprocal effects on several other types of keratinocyte molecules that either have been reported to be involved in pemphigus, such as apoptosis inducers and proteases, or appeared to represent novel and unexpected candidates of being such. The latter group includes protein phosphatases, protease inhibitors, and lipocortins. In a series of immunoblotting experiments, we were able to confirm the results of DNA microarray assay. Both by immunoblotting and immunofluorescence, we found an upregulated synthesis of adhesion molecule proteins in keratinocytes treated with MP. Taken together, these experimental results clearly indicated that pemphigus is a rather complex disease in which the autoantibody induces deleterious effects that are mediated by changes in gene expression. This is in contrast to a previous explanation that in pemphigus autoantibody simply inactivates by steric hindrance the function of the molecule desmoglein 1 and/or 3 on the cell membrane of a keratinocyte. These results also offered a novel outlook on the mechanism of GH action in pemphigus, indicating that upregulated expression of the adhesion molecules in keratinocytes may be one of the most important therapeutic mechanisms.

Since previous work in the laboratory of Dr. Kitajima, pemphigus vulgaris IgG was found to alter an ability of desmoglein 3 to contribute to formation of desmosomes [22], we also tested ability of MP to abolish this effect, using experimental cell system provided by Dr. Kitajima. Using the DJM-1 cells that provide the most sensitive model for studying phosphorylation of adhesion molecules, we compared the effects of pemphigus vulgaris IgG and MP on the phosphorylation status of keratinocyte adhesion molecules. We found that due to pemphigus antibody binding to DJM-1 cells, the degree of phosporylation of desmoglein 3 as well as some other types of adhesion molecules increases several fold. In the presence of MP, the pemphigus vulgaris IgG-induced phosphorylation of adhesion molecules was suppressed.

Based on results of this study, we concluded that pemphigus vulgaris IgG induces acantholysis *via* a complex of intracellular genomic and nongenomic events some of which can be antagonized by MP at the transcriptional, translational or posttranslational levels.

Approaches to development of safer treatments of pemphigus with GH-like drugs

The above-discussed experimental results with MP have opened a door for novel approaches to the development of safer treatments of pemphigus patients. Perhaps, GH could be replaced by their analogues that lack glucocorticoid- and mineralocorticoid-like side effects. The prototype drugs already exist. They are called 21-aminosteroids or lazaroids. It has been demonstrated that lazaroids can duplicate some nongenomic effects of MP producing a neuroprotective action in acute neuronal injury [23].

The exact mechanisms of GH-mediated nongenomic effects remain unknown. There is increasing evidence for a rapid nongenomic action of steroids that is very different from the traditional view on the mechanism of

GH action on cells, where the GH effects are believed to be mediated exclusively through changes in the gene expression (reviewed in [24]). Recent research points to the cell membrane pumps and channels that mediate transmembrane ion exchange as specific receptors mediating nongenomic action of steroids in the epithelia. Hence, an overall biological role of the nongenomic response to steroids may be to increase electrolyte absorption and inhibit secretion in the epithelial tissues [25]. This suggests that the physiological effect of GH that is being exploited in the treatment of pemphigus is the inhibition of production of blister fluid by keratinocytes. It is worth noting that the origin of blister fluid in pemphigus is still unknown. One of the hypothetical sources are keratinocytes themselves that excessively secrete intercellular fluid in response to stimulation with autoantibodies.

To elucidate the mechanism of blister formation in pemphigus and develop safer treatments, we investigate pemphigus immunopharmacology. Since pemphigus vulgaris IgG has been experimentally shown to cause acantholysis without killing keratinocytes through activation of complement [26], the type of autoimmune reactions in pemphigus falls within the lines of a recently defined entity called "Inactivation/Activation of Biologically Active Molecules" [27]. Pemphigus antibody may either stimulate or inhibit cellular events controlled by some naturally occurring mediator, such as ing endocrine or a local hormone. According to this hypothesis, pemphigus IgG may cause acantholysis by stimulating certain "forbidden" functions of keratinocytes, such as secretion of a fluid. This will lead to skin blistering through an "active" pathway. A "passive" mechanism involves inhibition of the keratinocyte adhesive function by pemphigus IgG secondary to induction of apoptosis.

Conclusions

1. In pemphigus, GH may work by: (i) inhibiting antibody synthesis; (ii) suppressing inflammation, especially eosinophilic spongiosis; and by (iii) stopping acantholysis *via* a direct pharmacological effect on keratinocytes. The poorly understood requirement for extremely high doses of GH to control acantholysis in the acute stage of the disease hints that the anti-acantholytic action of GH may not be mediated by the immunosuppressive properties of these drugs.

2. Binding of pemphigus vulgaris IgG to keratinocytes evokes an array of biochemical changes inside the cells, leading to both genomic and nongenomic events interfering with the keratinocyte adhesive function. Although the exact mechanism *via* which GH counteract a pro-acantholytic effect of pemphigus IgG remains unknown, the anti-acantholytic effects of MP include upregulation of gene expression of adhesion molecules and protection of keratinocyte adhesion molecules from antibody-induced phosphorylation.

3. Better understanding of the immunopharmacology of pemphigus antibody action on keratinocytes will help identify novel drugs that can improve adhesion by correcting the altered cell function. Experimental drugs, such as lazaroids that are deprived of the mineralocorticoid and glucocorticoid effects, should be tested for their ability to replace GH in the treatment of pemphigus.

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