

CYTOKINES PROFILE IN EXPERIMENTAL CONTACT ALLERGIC DERMATITIS AND USE OF NANOENCAPSULATED AGENTS

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Background. Inflammation, oxidative and nitro-oxidative stress are the essentials of the pathogenesis of contact allergic dermatitis as well as cytokines imbalance.

Objective. The concentration of TNF- α , IL-1 β , IL-4 and IL-10 in blood serum of rats with nickel-induced contact allergic dermatitis was evaluated to determine whether it correlated with the use of free and nanoencapsulated preparations of betamethasone, superoxide dismutase (SOD) and potent highly selective inhibitor of iNOS (1400W).

Methods. To induce contact dermatitis (CD), 5 % nickel sulfate was used for 12 days. Experiments were performed on white inbred male rats, 180–220 g of body mass. All rats were divided into 10 groups (n=10). Group I – the control one; II – the animals with CD; III – the rats with CD treated with empty polymeric chitosan nanoparticles; groups IV–VI – the rats with CD treated with free SOD, 1400W and betamethasone; groups VII–IX – the rats administered with nanoencapsulated SOD, 1400W and betamethasone; X – CD + nano-composition of all agents.

Results. The statistically higher serum concentrations of TNF- α , IL-1 β and decrease of IL-4 and IL-10 in experimental contact dermatitis is proved in comparison with the healthy rats. Mono-treatment with betamethasone, SOD and 1400W is efficient, but the use of nanoparticles loaded with these preparations surpasses its effects. The use of the combination of all nanoencapsulated medicines is the most effective.

Conclusions. Chitosan nanoparticles loaded with topical anti-inflammatory glucocorticoid, and inhibitors of oxidative and nitro-oxidative stress is a promising method for treatment of allergic contact dermatitis and can be recommended for further research and use in clinics.

KEY WORDS: **contact nickel dermatitis; cytokines; nanoparticles; betamethasone; SOD; 1400W.**

Introduction

Contact dermatitis (CD) frequency has been rising recently [1, 2]. Its two types are differentiated: triggered by antigens as allergic contact dermatitis and by obligatory cutaneous irritants – irritant contact dermatitis (non-immunologic driven reaction) [3]. All types of dermatitis (atopic, psoriatic, allergic, etc.) are challenge for health care system because of chronic and relapsing character. It is forth reason for doctor's visiting among all skin disorders [4]. Today the first choice and the mainstay of dermatitis drug therapy are topical glucocorticoids (TGs): betamethasone, dexamethasone, fluocinolone, triamcinolone, etc. In spite of their high efficacy a lot of side effects (systemic and local) are common: skin atrophy, acneiform eruption, striae, and telangiectasia, as well as hypothalamic-pituitary-adrenal axis

suppression, glaucoma development, high risks of thrombosis and others [5, 6].

One more serious challenge for dermatitis treatment is transdermal delivery of active components. Skin is a prime barrier against a lot of environmental physical, chemical, and biological stressors (ultraviolet irradiation, bacteria, viruses, allergens, etc.). *Stratum corneum*, claudins and occludins of tight junctions are crucial in defense, but also impede the absorption of topical medicines [7]. Nanoencapsulated medications have been developed as a vehicle into the deeper skin layers and demonstrated its benefits [8–10]. Nanotechnology through the reduced particle size improves the absorption and concentration of the drug in the target tissue, its pharmacokinetic parameters and long-term release of the medication at the target site [11].

It is established at present that even regular use of TGs does not prevent the relapse of CD and does not provide the appropriate control of its severity [12]. Cytokines are crucial in pathogenesis of different types of dermatitis (allergic, atopic, etc.). Contact allergic dermatitis

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is mediated via T lymphocytes. Contacting the allergens epithelial cells may produce mediators, which intensify the cascade of immune response [13]. The combination of sensitized T cell plus antigen releases cytokines cascade and lead to inflammation [14].

The objective of the research is to study the cytokines profile in experimental allergic contact dermatitis and its correction with nanoencapsulated steroid anti-inflammatory agent, antioxidant and iNOS inhibitor.

Methods

Animals and experimental design. Experiments were performed on white inbred male rats 180–220 g of body mass. All rats were divided into 10 groups, 10 animals in the each one. group I – the control one involved the animals sensitized only with solid lanoline base; group II – the animals with contact dermatitis (CD) induced by sensitization with 5 % NiSO₄ dissolved in the base; group III – the rats with CD treated with empty polymeric chitosan nanoparticles; groups IV–VI involved the rats with CD treated with free SOD, 1400W and betamethasone accordingly; groups VII–IX – the rats, which were administered with nano-encapsulated SOD, 1400W and betamethasone; group X – CD + nano-composition of SOD, betamethasone and 1400W.

To induce the contact dermatitis the hair on the dorsal area, 4x4 cm in size, was removed and the cleaned skin area was administered with 4 g of solid lanoline composition containing 5 % nickel sulfate for 12 days. All correctors were used for one week after the development of CD twice a day.

We used Sigma-Aldrich (USA) betamethasone 17,21-dipropionate, PEGylated superoxide dismutase (SOD) and N-([3-(Aminomethyl) phenyl]methyl)ethanimidamide dihydrochloride (1400W)-potent highly selective inhibitor of iNOS. Chitosan nanoparticles loaded with above-mentioned preparations

were prepared according to the method described by Hussain Z., et al. [15]. Chitosan solution (25 mL, 0.2 % w/v, prepared in 1 % v/v acetic acid, pH 5.0) was incubated with Betamethasone solution (1 mg/mL in a 30:70 mixture of ethanol/water) or 1400W (1 mg/mL) or SOD solution (10 mg/mL) stirring for 30 minutes. Loaded nanoparticles were spontaneously formed by adding 10 mL of pentasodium tripolyphosphate solution (0.1 % w/v, in distilled water) dropwise with constant stirring at 700 rpm. The nanoencapsulated preparations were harvested by ultracentrifugation (28,000 rpm) for 30 minutes and subsequently resuspended. The size of nanoparticles was determined using the morphometric program Video-Test-5.0, Kappa ImageBase, and it was 40–100 nm.

All procedures for the animals were performed according to the rules and requirements of European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, Directive 2010/63/EU and were approved by local TSMU Ethic Committee. The rats were euthanized under the ketamine anesthesia on the 20th day of examination. Amounts of cytokines in blood serum were evaluated by ELISA test (RayBiotech Inc., Norcross, GA, USA). The concentrations of TNF-α, IL-1β, the Th2-specific cytokines IL-4 and IL-10 were analyzed.

Statistical analysis. The data are presented as mean±SE standard error. Statistical analysis was performed by the Statistica 10.0 (StatSoft Inc., USA) program. The distribution of indices was estimated using Shapiro-Wilk normality test. The significance of the results was determined by the Mann-Whitney U-test and ANOVA-test. A probability level of less than 0.05 was considered as statistically significant.

Results

It has been established that in CD, induced by nickel sulfate, blood serum concentration of

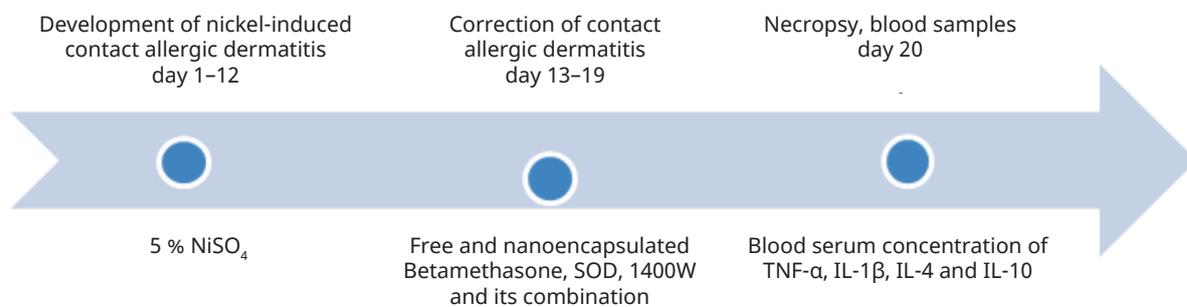


Fig. 1. Schematic diagram of experimental design.

pro-inflammatory cytokines TNF- α and IL-1 β significantly increased in 5.5 and 3.7 times compared to the intact rats (Table 1). Anti-

inflammatory Th2-specific cytokines IL-10 and IL-4 concentration was significantly lower, in 2.3 times and 2.4 times respectively.

Table 1. Blood serum concentration of cytokines in experimental contact nickel dermatitis, M \pm m, n=10

Groups	Intact rats	CD	CD + empty nanoparticles	CD + beta-methasone	CD + nano-beta-methasone	CD + SOD	CD + nano-SOD	CD + 1400W	CD + nano-1400W	CD + nano-combination of beta-methasone, SOD and 1400W
TNF- α , pg/ml	7.48 \pm 0.56	41.16 \pm 4.10*	40.54 \pm 3.98*	26.98 \pm 2.30 *,**	20.94 \pm 1.16 *,**, #1	35.48 \pm 1.95 *,**	30.22 \pm 2.02 *,**, #2	36.22 \pm 1.78 *,**	30.12 \pm 1.56 *,**, #3	12.88 \pm 1.28 *,**, γ^{123}
IL-1 β , pg/ml	10.46 \pm 0.88	37.98 \pm 2.42*	35.88 \pm 3.36*	24.77 \pm 1.75 *,**	19.35 \pm 1.28 *,**, #1	31,32 \pm 1.54*, **	26.45 \pm 1.54 *,**, #2	32.16 \pm 1.98 * *,**	26.66 \pm 1.38 *,**, #3	14.48 \pm 1.34 *,**, γ^{123}
IL-10, pg/ml	12.05 \pm 1.08	5.24 \pm 0.42*	5.15 \pm 0.42*	7.54 \pm 0.52 *,**	8.96 \pm 0.32 *,**, #1	6.88 \pm 0.50 *,**	7.88 \pm 0.64 *,**	6.32 \pm 0.44* *,**	8.48 \pm 0.46 *,**, #3	10.33 \pm 0.48 **, γ^{123}
IL-4, pg/ml	19.26 \pm 1.88	8.02 \pm 0.78*	7.68 \pm 0.66*	11.43 \pm 0.45 *,**	13.16 \pm 0.58 *,**	10.04 \pm 1.00* *,**	11.25 \pm 0.78 *,**	11.14 \pm 0.32 *,**	13.26 \pm 0.44 *,**, #3	15.68 \pm 0.77 **, γ^{123}

Notes: statistical significance $p < 0.05$ compared to: *intact rats; **CD rats; #1 – rats with CD treated with free form of beta-methasone, #2 – free form of SOD, #3 – free form of 1400W, γ^1 – nanoencapsulated betamethasone, γ^2 – nanoencapsulated SOD, γ^3 – nanoencapsulated 1400W.

The use of empty nanoparticles did not cause any significant changes in the indices. Use of each nanoencapsulated agent was more efficient to restore the cytokines imbalance in comparison with free form usage. But the most prominent effect was caused by the combination of all three nanoencapsulated correctors.

Betamethasone is a topical corticosteroid of high potency. Its free form caused positive changes in cytokines profile: the decrease in pro-inflammatory TNF- α and IL-1 β concentration by 34.5 and 34.8 %; and increase of anti-inflammatory IL-10 and IL-4 – by 43.9 and 42.5 % was evidenced. But nanoencapsulated TG was more effective and surpassed its free form efficacy.

TNF- α and IL-1 β rates were statistically significantly lower in this group in comparison with CD group by 49.1 and 49.05%; in comparison with its free form by 22.4 and 21.9 %.

Also the increase in anti-inflammatory interleukins IL-10 and IL-4 concentration was evidenced in group CD + nano-betamethasone: by 71.0 and 64.1 % in comparison with pathology; and by 18.8 % for IL-10 in comparison with free form of TG.

The same tendency was for free and nanoencapsulated forms of SOD. The use of free SOD caused decreased concentration of TNF- α and IL-1 β by 11.4 and 17.5 %; and increased rates of IL-10 and IL-4 by 31.3 and 25.2 % accordingly. Nanoencapsulated SOD surpassed the activity of its free form in decreasing of concentration of pro-inflammatory cytokines. The rate of TNF- α was statistically significantly lower by 17.2 %, IL-1 β – by 15.5 %. But there was a positive tendency only in correction of anti-inflammatory interleukins indices.

Highly selective inhibitor of iNOS was also quite efficient. The concentration of TNF- α decreased by 9.6 % in use of its free form; and by 26.8 and 29.8 % – of nanoencapsulated 1400W accordingly. The last rates were statistically significantly improved by 19.1 % for TNF- α and 17.0 % – for IL-1 β in comparison with CD + 1400W group. IL-4 concentration increased by 38.9 % in use of free 1400W; and by 65.3 % – of nanoencapsulated one in comparison with control pathology (the last index was higher by 19.0 % compared to CD + 1400W group). Value of IL-10 was statistically significantly different only in case of use of nano-form of 1400W by

61.8 % in the control group and by 34.2 % in CD + 1400W group.

The best results were evidenced in the group CD + nano-combination of betamethasone, SOD and 1400W. The combination of all nanoencapsulated agents was the most efficient in restoring of cytokines balance in comparison with mono-use of each corrector (table 1).

Discussion

Among a lot of allergens, nickel is one of the most common metals responsible for allergic CD. Its regular contacting for hypersensitive persons leads to a delayed-type hypersensitivity reaction and up to 20 % of the general population may be affected in Europe [16–18]. High frequency of positive nickel patch tests and high serum nickel concentration are also observed in people with intrinsic atopic dermatitis [19].

Pathogenesis of allergic contact dermatitis includes sensitization (or induction) and elicitation (or efferent) phases, which are innate (opposite to contact irritant dermatitis) [20]. It is established at present that cytokines are crucial in inflammation, including immune-involved dermatitis [21–23].

A lot of skin cells are able to produce cytokines [24] (Table 2). Dermal dendritic cells are crucial in the process of activating naive T cells; after contacting the allergen Langerhans cells start to synthesize IL-1β mRNA and to release the protein; then, keratinocytes are activated and release TNF-α and GM-CSF [25].

Table 2. Cytokines developed by epidermal cells

Epidermal cells	Cytokine (constitutive or inducible expression)
Keratinocytes	IL-1α, IL-1β, IL-1RA, IL-3 (mouse), IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18, TNF-α, G-CSF, GM-CSF, MCSF, Gro, MIP-2 (mouse), IP-10, RANTES, MCP-1, TGF-α, TGF-β
Langerhans cells	IL-1α, IL-1β, IL-6, IL-15, IL-18, TNF-α, Gro, MIP-2, MIP-1α, TGF-β
Melanocytes	IL-1α, IL-1β, IL-6, IL-7, IL-8, IL-10, IL-12, TNF-α, G-CSF, GM-CSF, MCSF, Gro, MIP-2 (mouse), RANTES, MCP-1, TGF-α, TGF-β

Skin hypersensitivity reactions are the result of imbalance between a variety of types of T-cell

responses and inflammatory mediators, including T-helper (Th) 2 cytokines and also T-helper 1 cells [26]. A Th1/Th2 hypothesis suggests that imbalance among these cells toward Th2 is the key to allergic reaction intensifying [22, 27]. The Th1 cells release of pro-inflammatory cytokines includes IL-1α, IL-1β, IL-8, IL-13, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF). They increase the vascular permeability and cause swelling and redness associated with inflammation and immunologic reactions in skin exposed to irritants, also affect the proliferation and differentiation of keratinocytes and mediators of cellular infiltration [3, 21, 22].

The key regulators are TNF-α and IL-1β. These pleiotropic cytokines are responsible for inflammation, apoptosis and necrosis of cells, phagocytic and cytotoxic activities [28].

IL-10 is an anti-inflammatory interleukin produced mainly by monocytes, T cells, macrophages, and dendritic cells. Mast cells can also produce IL-10, which limits the rate of leukocyte infiltration, inflammation, and skin disorders such as contact dermatitis [21]. IL-4 and IL-10 suppress Th1 immune response. IL-10 deficiency is crucial in maintaining of CD clinical signs [29].

The pro-inflammatory cytokines TNF-α and IL-1β and the Th1/Th2 cytokines IFNγ and IL-4 are involved in both the induction and elicitation of cutaneous immune response and are modulated by glucocorticoids, that is also proved by the results of our study. Glucocorticoids decrease TNF-α and IL-1β production and shift the Th1/Th2 ratio in a Th2 direction by augmenting production of IL-4 [16, 30]. Mechanisms of such imbalance are associated with activation of oxidative stress, which is realized by generation of reactive oxygen and nitric species (ROS and RNS) and depletion of antioxidant defense. ‘Vicious circle’ is running here: low antioxidant indices intensify the release of cytokines and inflammation processes that promotes the generation of ROS and RNS again.

Use of nano-encapsulated medications is emerging as potential therapeutics for a wide variety of diseases [22]. They have been successfully used for delivery of hydrophobic and hydrophilic small molecule drugs and biomacromolecules, nucleic acids of various sizes and structures. It is very important for skin diseases treatment to go through the *stratum corneum* to target tissues and subcellular compartments and nanoparticles are quite

successive for that. Nano-form of preparations, especially its combination was the most powerful to alleviate experimental contact dermatitis clinical signs and intensity of oxidative and nitro-oxidative stress [31, 32].

Our previous findings proved the importance of oxidative and nitro-oxidative stress activation in pathogenesis of CD. The last one is aggravated by cytokines. Nitric oxide synthase (NOS) is an enzyme that catalyzes the synthesis of NO and L-citrulline using oxygen and L-arginine as substrates. And its isoform iNOS is induced by the cytokines TNF- α , IL-1 [33].

So, modulation of NO-synthase (NOS) activity, use of antioxidants and free radical scavengers are important and prospect part of complex treatment for different types of CD (plus common recommendations for allergens avoiding, emollients and others) [2, 33, 35]. The perspective results of combined treatment with betamethasone-, SOD (superoxide dismutase) and 1400W (highly selective inhibitor of inducible nitric-oxide synthase, iNOS)-loaded nanoparticles were obtained. Such combination of the potent anti-inflammatory steroid agent, the powerful antioxidant and iNOS-inhibitor in nanoencapsulated form was more effective in experimental contact dermatitis than use of their free forms [31, 36].

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Conclusions

Experimental contact dermatitis induced by 5% nickel sulfate is accompanied by pathological cytokines imbalance. Mono-treatment with betamethasone, SOD (superoxide dismutase) and 1400W (highly selective inhibitor of inducible nitric-oxide synthase, iNOS) was efficient, but the use of nanoparticles loaded with these agents surpassed its effects. The use of combination of all nanoencapsulated medicines was the most effective.

Chitosan nanoparticles loaded with topical anti-inflammatory glucocorticoid, and inhibitors of oxidative and nitro-oxidative stress is a promising method for treatment of allergic contact dermatitis and can be recommended for further research and use in clinics.

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