Effect of Multipotent Mesenchymal Stromal Cells Secretome on Imiquimod-Induced Psoriasis in Rats

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Abstract

Background: Multipotent mesenchymal stromal cells secretome contains a range of anti-inflammatory factors and can be used for the treatment of psoriasis.

Material and Methods: The study included 16 male Wistar rats weighing from 214 to 256 g, which were divided into 2 subgroups: 8 rats — positive control, treated with fluocinolone acetonide ("Sinaflan" 0.025% ointment, MPZ, Russia), 8 rats - an experimental group that received a cream with a secret bone marrow MSC. Psoriasiform inflammation was caused by daily application of 50 μl of 1% imiquimod suspension (Xi'an Leader Biochemical Engineering Co., Ltd, China) on the skin of the back from the inner and outer surfaces in olive oil with DMSO in a ratio of 9:1 for 4 days. After the development of severe inflammation, treatment was started on both sides: on the one hand, each animal received a baby cream (negative control), on the other hand, fluocinolone or cream with the addition of MSC secretome in the corresponding group, application was carried out 1 time per day for 3 days. On the 8th day of the experiment, the animals were euthanized and skin fragments were collected for histological examination.

Results and Discussion: On the day 4 of the imiquimod use, all animals showed the development of inflammation, with the appearance of coarse lamellae, excoriations, evenly on both sides in the application of the inducer of inflammation. The relief of inflammation as a result of daily single application of cream, cream with MSC secretion and fluocinolone was observed in the experimental group and in the positive control from the 2nd day of use, in the negative control on the 3rd day of the cream application the inflammation decreased slightly.

Conclusion: The efficacy of topical administration of MSC secretome in relieving psoriasiform inflammation in Wistar rats is comparable to that of glucocorticoid with high activity (class 3 according to the European classification of Miller & Munro) fluorocinolone acetate, both by clinical signs and by morphometric indicators.

Key words: Inflammation, Secretome, Multipotent Mesenchymal Stromal Cells, Imiquimod, Psoriasis.

Background

Psoriasis is a systemic disease with an unclear etiology and a well-studied pathogenesis. Preferably, the skin and joints are affected due to immuno-inflammatory processes, where T-cell responses, especially Th17-mediated, which are known [1], play a key role in the pathogenesis of psoriasis. Traditional systemic therapy for psoriasis includes methotrexate, cyclosporine, acetretin, less commonly azathioprine, lefluenamide, sulfasalazine, and other immunosuppressants [2]. In recent years, antipsoriatic therapy has undergone changes, a large number of genetically engineered biological agents have appeared: anti-TNF-α - etanercept, infliximab and adalimumab, anti-IL12/IL23 p40 - ustekinumab, anti-phosphodiesterase-4 - apremilast, anti-IL-17A - secukinumab, ixekizumab [3, 4]. All of them are aimed at blocking key mediators of immune inflammation after systemic administration. This approach is not selective and is also accompanied by undesirable side effects in the form of the development of general immunosuppression with an increase in infectious diseases.

In topical preparations, the progress is much less, the main drugs, as before, remain glucocorticoids with known side effects, vitamin D analogues - calcipotriol (calcipotriene), retinoic acid derivative - tazarotene, birch tar, which have narrower indications [5, 6].

Thus, the problem of development new drugs for the treatment of psoriasis remains an actual task. Among promising candidates, we consider the secretome of multipotent mesenchymal stromal cells (MSCs). The anti-inflammatory and immunosuppressive effects of MSCs are known by inhibiting the activity of cytotoxic T-lymphocytes, dendritic cells, macrophages, stimulating regulatory T lymphocytes, with corresponding shifts in cytokine production [7, 8], including a decrease in Th17

differentiation and increased differentiation and activity of regulatory T-lymphocytes [9].

Preclinical research at the organismic [10-16], cellular [17] and subcellular levels [18, 19] with pathology modeling are a necessary for creating new drugs. The most popular vectors are the reduction of oxidative stress [20-23], inflammation [24, 25] and apoptosis cascades [26-29]. For the development of new drugs and therapies for any disease, it is desirable to have model laboratory animals of various kinds. Until recently, there were no relevant models of psoriasis at all: a xenotransplantation of psoriatic skin fragments from patients to mice with severe combined immunodeficiency mice (SCID) was used [30]. Despite the development of individual psoriasis-like skin changes in mice with various immunodeficiencies, these models were also not successful due to their insensitivity to standard anti-psychotic therapy [31]. This led to the appearance of genetically modified mouse strains. Mice with an epidermis-specific deletion of the inhibitor of nuclear factor κB kinase 2 develop a T cell–independent psoriasis-like skin disease.

The authors conclude that the critical function of nuclear factor κB kinase 2-mediated nuclear factor κB activity in keratinocytes is to regulate mechanisms that maintain the immune homeostasis of the skin [32], with overexpression of cytokines IL-6, TGF-α, IL-1α, IFN-γ, BMP-6, etc [33]. However, the features of these lines and the specific conditions of detention limit their availability to many researchers. Therefore, the development of a model of imiquimod-induced psoriasiform inflammation should be considered a significant step forward in the study of psoriasis and increasing the efficiency of developing new drugs for its treatment [34].

Imiquimod is a chemical TLR-7/8 agonist [35] and has been developed for the treatment of genital warts caused by human papillomavirus, senile keratosis and superficial basal cell carcinoma of the skin. One of the detected side effects was the development of a psoriasiform skin inflammation in the application area of a cream when applying 5% imiquimod cream [36-39]. However, until recently, reproducible models of imiquimod-induced psoriasis were described in mice, both genetically modified, and later on the BALB/c and C57BL/6 lines.

Recently, we have developed a model of imiquimod-induced psoriasiform inflammation in Wistar rats [40], which was used in the present study, the purpose of which was to study the effectiveness of the bone marrow MSCs secretome topical administration in arresting imiquimod-induced psoriasiform inflammation.

Materials and Methods

The study was conducted in accordance with the ethical standards, the declaration approved by the ethics committee.

Mixing cream with secret MSC

MSCs were obtained by washing out the bone marrow from the diaphysis of the femoral and tibial bones of Wistar rats, followed by rubbing it through a nylon membrane with pores of 100 μm, erythrocyte lysis with a buffer containing ammonium chloride, and cultivating people and laying themselves on DMEM medium (Paneco, Russia) 10% ETS (Biosera, France), antibiotics penicillin/streptomycin (Paneco, Russia) per 100 mm culture plates (Costar, USA) at 37°C in a humid atmosphere with 5% CO2. After the formation of 90% of the MSC monolayer of the 4th-5th passage, the medium on the plates was changed to 10 ml of DMEM/F12 without serum and antibiotics. Cell cultivation was continued for 3 days, after which the supernatant was collected, dried on a rotavapor R-300 rotary evaporator (Buchi, Switzerland) at 42°C. The dry residue was dissolved in 1/10 of the initial volume of distilled water and added to 9/10 of the initial volume of an indifferent cream (“Detskiy”, Avanta, Russia), mixing thoroughly. Storage of the cream before use and during the experiment was carried out at + 4°C.

Modeling and treatment of psoriasiform inflammation

The study included 16 male Wistar rats weighing from 214 to 256 g, which were divided into 2 subgroups: 8 rats — positive control, treated with fluocinolone acetonide (“Sinaflan” 0.025% ointment, MPZ, Russia), 8 rats - an experimental group that received a cream with a secret bone marrow MSC. Negative control as a cream treatment without the addition of MSC secretome, was performed in the same rats, on the contralateral side. Psoriasiform inflammation was caused by daily application of 50 μl of 1% imiquimod suspension (Xi'an Leader Biochemical Engineering Co., Ltd, China) to the ears from the inner and outer surfaces in olive oil with DMSO in a ratio of 9:1 for 4 days.

After the development of severe inflammation, treatment was started on both sides: on the one hand, each animal received a baby cream (negative control), on the other hand, fluocinolone or cream with the addition of MSC secretome in the corresponding group, application was carried out 1 time per day for 3 days. On the 8th day of the experiment, the animals were euthanized and skin fragments were collected for histological examination. Skin fragments were fixed with 4% formaldehyde, then stained with hematoxylin and eosin. Morphometric studies were performed on an AxioScope A1 (Zeiss) microscope in the Fiji [41] software package. Statistical processing was

performed in MS Excel 2013 with the Dunnet criterion.

Results and Discussion

On the day 4 of the imiquimod use, all animals showed the development of inflammation, with the appearance of coarse lamellae, excoriations, evenly on both sides in the application of the inducer of inflammation. The relief of inflammation as a result of daily single application of cream, cream with MSC secretion and fluocinolone was observed in the experimental group and in the positive control from the 2nd day of use, in the negative control on the 3rd day of the cream application the inflammation decreased slightly.

The results of the histological study of autopsy material are presented in Figures 1-2.

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The results of the histological study of autopsy material are presented in Figures 1-2.

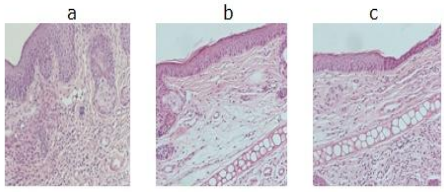

Figure-1: Acanthosis severity: a - negative control, b - positive control (fluocinolone), c - experimental group (MSC secretome)

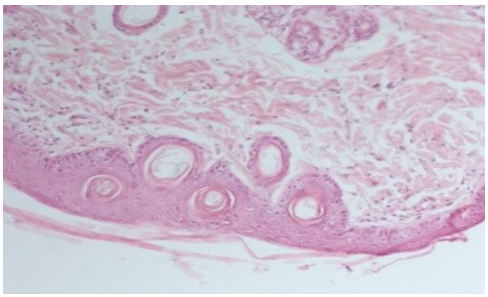

Figure- 2: Hypertrophy of the capillaries in the dermis under the influence of imiquimod.

As shown A.L. van der Fits et al. [31], imiquimod-induced dermatitis was partially dependent on the presence of T-cells, while the development of the disease was almost completely blocked in mice deficient in IL-23 or IL-17 receptor, which demonstrates the key role of the IL-23/IL -17 axis. These lesions showed increased epidermal proliferation, abnormal differentiation, epidermal accumulation of neutrophils in microabscesses, neoangiogenesis and infiltrates consisting of CD4+ T cells, CD11c+ dendritic cells and plasmacytoid dendritic cells.

Imiquimod induced epidermal expression of IL-23, IL-17A and IL-17F, as well as an increase in Th17 spleen cells. As can be seen, the changes in BALB/c and C57BL/6 mice in this work and the identified skin changes in Wistar rats are very similar, which confirms the eligibility of using the latter in modeling psoriasiform inflammation under the influence of imiquimod [42].

Morphometric indicators are presented below on the fig. 3-5.

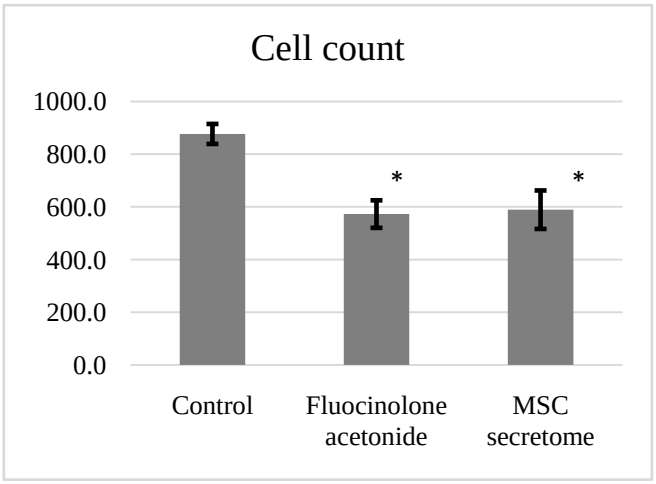

Figure -3: Total cellularity of skin. * - p <0.05.

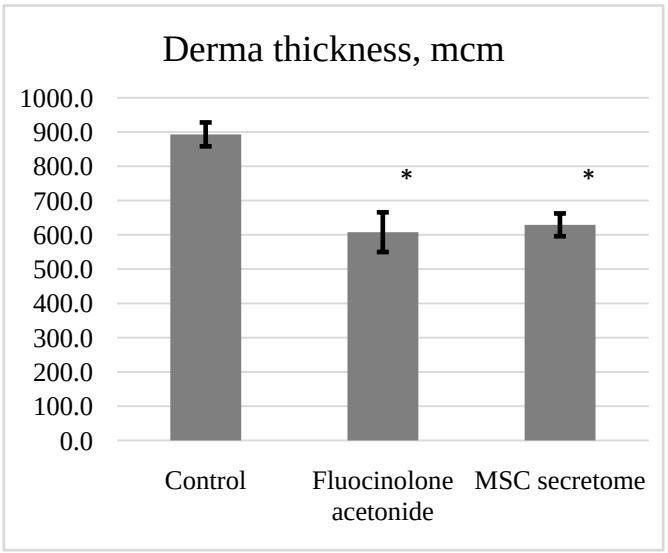

Figure- 4: Derma thickness. * - p <0.05.

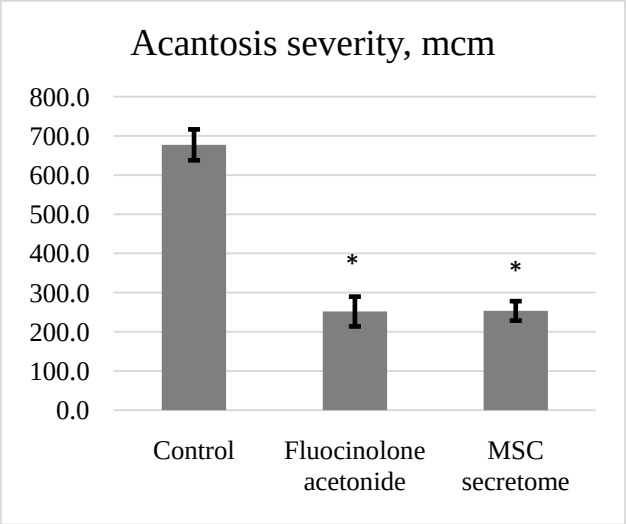

Figure -5: Acanthosis severity. * - p <0.05.

The efficacy of topical administration of MSC secretome in relieving psoriasiform inflammation in Wistar rats is comparable to that of glucocorticoid with high activity (class 3 according to the European classification of Miller & Munro) fluorocinolone acetate, both by clinical signs and by morphometric indicators. So, the total cellularity of the specimens in the negative control was 876.9±77.5 (M±sd), in the positive control – 572.8±75.4, and in the experimental group – 589.4±105.4. The thickness of the dermis in microns in the control is 893.0±71.1, with the use of fluocinolone acetate – 607.8±83.7, and in the case of using the secretome of MSC – 629.1±48.0. Also, under the influence of factors produced by MSCs, the following characteristic for psoriatic skin lesions decreased statistically significantly: acanthosis – 253.6±35.9 μm, against 252.0±54.8 μm under the influence of fluocinolone acetate and 677.1±80.6 microns in the negative control.

Thus, the anti-inflammatory and immunosuppressive activity of the complex of humoral factors constituting the secret of MSCs provided relief for acute psoriasiform skin inflammation and showed potential for further in-depth experimental research in the treatment of Th17-dependent inflammation.

The obtained experimental data require further research in the direction of establishing cellular targets for the components of the MSC secretome in psoriasiform inflammation induced by imiquimod, since Th17 are not the only participants in this process. In [43], direct evidence is presented for the ability of the innate immune system to "control" psoriasis through TLR7 (agonist receptor 7) imiquimod. Induction of pronounced type 1 interferon activity in the lesion focus and the discovery of a large number of precursors of dendritic cells initiating psoriatic skin lesions suggest that the precursors of dendritic cells may be targets for a TLR7 imiquimod agonist for psoriasis. Whether precursors of dendritic cells represent key cellular mediators of innate immune responses that cause pathogenic events leading to psoriasis will need to be determined in future studies.

There are numerous works suggested that MSCs inhibit the proliferation of human and animal T cells stimulated with polyclonal mitogens, allogeneic cells, soluble antigens, anti-CD3 and anti-CD38 antibodies [44-46], which requires further experiments to clarify how biologically active factors presented in the secretome of MSCs suppress imiquimod-induced skin inflammation.

The efficacy of topical administration of MSC secretome in relieving psoriasiform inflammation in Wistar rats is comparable to that of glucocorticoid with high activity (class 3 according to the European classification of Miller & Munro) fluorocinolone acetate, both by clinical signs and by morphometric indicators. So, the total cellularity of the specimens in the negative control was 876.9±77.5 (M±sd), in the positive control – 572.8±75.4, and in the experimental group – 589.4±105.4. The thickness of the dermis in microns in the control is 893.0±71.1, with the use of fluocinolone acetate ¬– 607.8±83.7, and in the case of using the secretome of MSC – 629.1±48.0. Also, under the influence of factors produced by MSCs, the following characteristic for psoriatic skin lesions decreased statistically significantly: acanthosis – 253.6±35.9 μm, against 252.0±54.8 μm under the influence of fluocinolone acetate and 677.1±80.6 microns in the negative control.

Conclusion

Thus, the anti-inflammatory and immunosuppressive activity of the complex of humoral factors constituting the secret of MSCs provided relief for acute psoriasiform skin inflammation and showed potential for further in-depth experimental research in the treatment of Th17-dependent inflammation.

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