POTENTIAL IMPACT OF CERIUM DIOXIDE NANOPARTICLES (NANOCERIA) ON THE CONCENTRATION OF C-REACTIVE PROTEIN AND MIDDLE-MASS MOLECULES AFTER WOUND TREATMENT IN RATS

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Wound healing is a pivotal physiological process in which cooperation of many cells and their products is needed. Intentions for induced lesion restorment occur early at the inflammatory stage. Ending up to result in injured tissue repairment.

1. Introduction

Here we are going to focus on the level of two factors, C-reactive protein and Middle-mass molecules, that are considered as endogenous intoxication during the wound healing process.

C-reactive protein, hereinafter CRP, is an acute inflammatory protein that increases up to 1,000-fold at
sites of infection or inflammation. It is present as a homopentameric protein, called native CRP (nCRP), which can detach to produce five discrete monomers, called monomeric CRP (mCRP), at the site of inflammation and infection. CRP is mainly synthesized in the liver but also by lymphocytes, macrophages, smooth muscle cells, endothelial cells and adipocytes. When the tissue is injured, CRP level increases significantly in response to inflammation and infection (Fig. 1) [1].

CRP mainly activates the C1q molecule in the complement pathway resulting in the pathogen opsonization. It can also initiate cell-mediated pathways by the complement activation as well as attaching to IgG Fc receptors [2].

Once it attaches to Fc receptors, it induces pro-inflammatory cytokines releasement [3]. The ability of CRP to recognize self and foreign molecules according to their pattern recognition, makes it a unique complement activator as other activators can’t attain it due to their distinct antigenic epitopes recognition [3].

2. Literature review

As mentioned before, CRP is an inflammatory marker and during bacterial infection its level inclines [4].

Kingsley and Jones demonstrated that at the site of infection, CRP level increases in response to monocytic mediators like IL-1 and IL-6 and it has a stable decay rate [5].

In Mold et al. investigation on mice treated with 200 μg CRP, it was proven that CRP protects them against the infection caused by gram-positive Staphylococcus pneumoniae by attaching to their cell wall and leading complement pathway activation [6].

Middle-mass molecules (MMM) are considered as one of the suitable clinical indicator determining the pathological process development [7].

MMM is a type of combinations with the molecular mass up to 5,000Da and is divided into two major groups – average molecular mass substances and oligopeptides [8, 9].

When the metabolism alters to a reverse reaction, a big amount of metabolites and metabolic-waste products accumulate in blood remarkably high concentrations of a variety of biological substances, bacterial toxins, organs and tissues destruction products, protein and lipid hydroperoxides, etc. This pool of substances in blood is dispersed between the erythrocytes and plasma, and defines the concept of intoxication from the biochemistry point of view [10, 11].

Consequences of these substances accumulations in the blood cause tissue lysis and toxic product increment. Hence, MMM pool is the basic biochemical marker measuring the pathological protein metabolism level [7].

3. The aim and objectives of the study

The aim of the study is to measure the concentration of C-reactive protein and level of Middle-mass molecules in blood serum of rats during the wound healing procedure.

To achieve this aim, the following objectives were set:

1. Whether Nanoceria had any influence on the level of Middle-mass molecules in blood serum after the treatment?
2. Whether Nanoceria had any impact on the concentration of C-reactive protein in blood serum after the treatment?
3. Whether application of Nanoceria accelerated the rate of complete wound closure (re-epithelialization)?

4. Materials and Methods

4.1. Preparation of Nanoceria solution

The wound dressings were put together by electrospinning. The film comprising 0.05 % CeO2 (dis-
solved in 0.5% Carbopol) nanoparticles was elected as the optimal dressing for the in vivo study on full-thickness excisional wounds of rats. A peerless feature of these nanocrystals is that they can be applied multiple times: over weeks, cerium (IV) rich particles leisurely turn over to their initial cerium (III) content. In approximately all cases, the particles subsist colloidal-ly firm (e.g., non-aggregated) and could be applied multiple times. The in vivo study represents Nanoceria evidence in mouse tissues with no pathogenicity. Taken together, it is suggested that cerium oxide nanoparticles are well sustained in rats and are agglutinated into cellular tissues. The study illustrated that after 2 weeks, the wounds treated with the CeO2 nanoparticle-containing dressing attained a remarkable closure to nearly 100%. Our results delivered the evidence supporting the feasible applicability of CeO2 nanoparticle-containing wound dressing for the favored wound treatment as it hastens complete wound closure and diminishes wound area in comparison with non-treated animals.

4.2. Animal Model

The research was administered on 75 white laboratory male rats weighing 200–250 g, which were divided into three groups (25 rats in each group): intact group (without any wound), control group (wounded animals without any dressing application) and experimental group (wounded animals with Nanoceria application). Keeping animals and experiments were conducted according to ethical principles, adopted by Ukraine at the First National Congress on Bioethics, international agreements and national legislation in this area [12]. Before the experiment, the rats were retained in quarantine and marked. Before performing the full-thickness wound model, animals were anesthetized by sodium thiopental (BiochemieGmbH/Austria), at a dosage of 50 mg/kg. The animals of the experimental group were treated with “Nanoceria-Gel” which contains 0.05% CeO2 (dissolved in 0.5% Carbopol) nanoparticles for wound dressing. In the intact series wound healing happened without drug and only Carbopol while the control group was remained untreated. Before the experiment, epilation was carried out on the back area after anesthetizing rats and one full-thickness wounds of 1×1 cm² was formed in the skin of each mouse using a surgical scalpel and forceps. rats were treated with Nanoceria solution applied directly to the wound site once daily until healing.

The level of Middle-mass molecules was carried out by Gabrielyan with modification [13] method, based on the precipitation of macromolecular peptides and proteins of biological fluids using trichloroacetic acid and quantitatively in the centrifugation, obtained by the supernatant of medium-molecular peptides by absorption in a monochromic light stream at a wavelength of 254 nm. Serum and plasma of blood were collected and in the centrifuge tubes 1 ml of serum and 0.5 ml of trichloroacetic acid (100g/L), stirred and centrifuged for 30 min at 1500 rev./min. 0.5 ml of supernatant was taken and transferred to a test tube of 4.5 ml of distilled water. The contents of the test tube were mixed and a spectrophotometric measurement at λ 254 nm was performed against the distillate, expressed in the mind: unit × mg of protein-1.

Determination of CRP concentration was carried out by Turbidimetry. The concentration of C-reactive protein was determined by the kinetic method analysis at fixed time according to standard (reaction time – 120 s, delay time – 3 s) [14]. Quantitatively the concentration of C-reactive protein in the serum was evaluated by immunoturbidimetry using a CRP latex reagent. Latex particles, covered with specific antibodies to CRP, agglutinate when mixing with samples containing CRP. Agglutination causes a change of absorption at λ = 540 nm depending on the content of CRP in the sample. Concentration of C-reactive protein was determined by the concentration calibrator of the formula:

\[ C_{CRP} = \left( \frac{A_0 - A_1}{A_0 - A_1} \right) \times C_{caliber}. \]

where \( A_0 \) – absorption after 3 s, \( A_1 \) – absorption after 120 s, C – concentration.

The concentration of C-reactive protein in serum was expressed in mg×1.

4.3. Statistical analysis

The statistical analysis of the data was carried out by the “Statistica 8.0” software package. The type of in-group data distribution was verified via the Shapiro-Wilk test. As the data were distributed normally (p<0.05), two-way ANOVA was conducted to determine the significance of difference between means, with the Bonferroni post test. A difference between means was judged as statistically significant if p≤0.05. The mean and standard deviation (SD) were calculated for each group.

5. Result

The current study demonstrates the elevated level of endogenous intoxication markers (MMM) in blood serum in the control group of rats where Nanoceria was not applied on the wounds (Table 1). In contrast, where the treatment of the wounds was carried out by Nanoceria in the experimental group, the level of MMM decreased significantly in each day of the experiment till 20th day where the full re-epithelialization occurred.

We have shown an increase in the level of CRP on the 3rd, 6th, 9th and 14th days of the experiment in comparison with the control (up to 10 μg/ml) in blood serum of all the experimental groups (Table 2). Restoration of this indicator to normal values was observed in the groups of animals that received Nanoceria on the 20th day of the experiment, it correlated with full re-epithelialization (Fig. 2).

As shown in Fig. 2, the effect of Nanoceria on different days of the experiment is clearly captured. The significant difference between the experimental group of rats and the control group of rats is well distinguished. The complete wound closure for the experimental group occurred on 20±0.5 day and for the control group it was on 23±0.8.

Hence, Nanoceria application on full-thickness wound helps to accelerate the healing and to make wound area reduction faster.
Table 1

<table>
<thead>
<tr>
<th>Days of experiment</th>
<th>Intact group (without wound)</th>
<th>Control group (wounded rats without treatment)</th>
<th>Experimental group (wounded rats with Nanoceria treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd</td>
<td>0.12±0.02</td>
<td>0.96±0.08***</td>
<td>0.72±0.07**##</td>
</tr>
<tr>
<td>6th</td>
<td>0.12±0.02</td>
<td>1.05±0.08***</td>
<td>0.54±0.04***##</td>
</tr>
<tr>
<td>9th</td>
<td>0.12±0.02</td>
<td>0.71±0.06***</td>
<td>0.50±0.04***##</td>
</tr>
<tr>
<td>14th</td>
<td>0.12±0.02</td>
<td>0.58±0.06***</td>
<td>0.16±0.02##</td>
</tr>
<tr>
<td>20th</td>
<td>0.12±0.02</td>
<td>0.35±0.02**</td>
<td>0.11±0.02##</td>
</tr>
</tbody>
</table>

Note: * – p<0.05; ** – p<0.01; *** – p<0.001 compared to the intact group of animals; # – p<0.05; ## – p<0.01; ### – p<0.001 compared to the control group of animals

Table 2

<table>
<thead>
<tr>
<th>Days of experiment</th>
<th>Intact group (without wound)</th>
<th>Control group (wounded rats without treatment)</th>
<th>Experimental group (wounded rats with Nanoceria treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3rd</td>
<td>9.67±1.13</td>
<td>80.70±7.94***</td>
<td>90.51±10.24***</td>
</tr>
<tr>
<td>6th</td>
<td>9.67±1.13</td>
<td>92.50±10.06***</td>
<td>55.37±4.91***##</td>
</tr>
<tr>
<td>9th</td>
<td>9.67±1.13</td>
<td>40.00±3.64***</td>
<td>30.17±2.92***##</td>
</tr>
<tr>
<td>14th</td>
<td>9.67±1.13</td>
<td>33.71±2.66***</td>
<td>27.51±2.98 #</td>
</tr>
<tr>
<td>20th</td>
<td>9.67±1.13</td>
<td>18.07±1.77***</td>
<td>9.47±1.11 ##</td>
</tr>
</tbody>
</table>

Note: * – p<0.05; ** – p<0.01; *** – p<0.001 compared to the intact group of animals; # – p<0.05; ## – p<0.01; ### – p<0.001 compared to the control group of animals

Fig. 2. Wound area reduction. The appearance of the full-thickness surface in the control and experimental groups (Nanoceria) in different time (days) of the experiment: a – 3rd day; b – 6th day; c – 9th day; d – 14th day; e – 20th day
6. Discussion

CRP which is inflammatory protein displays an increased expression during the inflammatory process. The area of inflammation and host responses, where CRP acts as a mediator, is apoptosis, phagocytosis, complement pathway, no release and cytokine production [1].

The wound (full-thickness) progression is dependent on cooperation of a variety of different metabolic systems as well as contacting tissues, and for this mean biochemical homeostasis’ support is required too. Proteolytic enzymes play the key role in catalyzing proteins breakdown, and necrotized tissues are removed by these enzymes in the process of healing. The physiological balancing of synthesis and proteolysis is under the control protease inhibitors. These particular proteins inhibit unusual devastation of protein compounds [15].

A disturbance in the proteolysis system leads to endogenous intoxication, which causes the MMM level increment in return. This disturbance has other consequences, such as damaging of the native tissue by abnormal activation and on other hand, the insufficient activity of these enzymes leads to prolonged build-up of matrix components, hence delays wound healing [16, 17]. After evaluation of the results on the positive effect of Nanoceria on CRP and MMM pool for wound healing process, as well as confirmation of its Antioxidant, Antimicrobial, Growth factors restoration, ROS reduction, SOD restoration, Catalase reduction properties in our previous investigations, we consider Nanoceria as a powerful promising drug for further researches and investigations.

7. Conclusion

1. The level of MMM in the experimental group of rats decreased significantly in each day of the experiment till 20th day where the full re-epithelialization occurred compared to the control group of rats, where Nanoceria was not applied.

2. We have seen an increase in the level of CRP on each day of the experiment in both control and experimental groups, but the restoration of this indicator to the baseline level happened much faster in the experimental group (full re-epithelialization) on 20th day compared to the control group.

3. Also the complete wound closure for the experimental group occurred on 20±0.5 day and for the control group it was on 23±0.8. So, application of Nanoceria contributes to the rate of complete wound closure.

References

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СОЗОФІТИ ТА ОСЕЛИЩА ТЕРИТОРІЇ УРОЧИЩА ХОРТИЦЯ ПІД ОХОРОНОЮ БЕРНСЬКОЇ КОНВЕНЦІЇ

© С. Г. Охріменко, Є. Д. Ткач

Мета. Включення урочища Хортиця до складу території особливого природоохоронного значення – Смарагдового об’єкту, задля збереження його фітоценофонду. Для досягнення мети була проведена фітосоціологічна оцінка території щодо її відповідності вимогам Бернської конвенції.

Матеріали та методи. Вивчення фори та рослинності проводилося експедиційно-маршрутним методом, а також закладанням стаціонарних дослідних ділянок площею 100 м² та трансект за загальноприйнятими методиками.

Результати. В урочищі Хортиця представлені ландшафти характерні для Запорізької області, наскам-перед степові, з природною рослинністю у добре збереженому стані. На території урочища виявлені:
– всі сухі типи трав’янистих угруповань категорії Е1 за класифікацією EUNIS, описані для Запорізької області (Е1.11, Е1.2.1.1 – Е1.2.1.7);
– 20 типів оселищ, які охороняються резолюцією №4 Бернської конвенції (C 1. 226; C1.33; C1.3411; C 2.33; C2.34; C 3.4; C 3.51; D 5.2; E 1.1.1; E 1.2; E 3.4; E 5.4; G 1.11; G 1.3; G 1.7; Х 18).
– 3 види вищих судинних рослин (Aldrovanda vesiculosa L., Jurinea cyanoides DC., Serratula lycopifolia Beck.) занесені до додатку №1 резолюції №6 Бернської конвенції.

Отримані дані ввійшли до стандартних форм даних об’єктів претендентів Смарагдової мережі, були захищені на біогеографічному семінарі (м. Київ 6–8 вересня 2016 р.).

Висновки. Урочище Хортиця має високу фітосоціологічну цінність і потребує проведення заходів з до-слідження, охорони та збереження його біорізноманіття. Що підтверджується включенням у 2016 році урочища до складу об’єкту Смарагдової мережі України UA0000106 – «Каховське водосховище».

Ключові слова: флора, созофіти, оселище, раритетна компонента, урочище Хортиця, Бернская конвенція, Смарагдова мережа

1. Вступ
Пріоритетною екологічною проблемою нині є збереження фітоценофонду як функціональної, пере- дусім енергетичної основи біосфери. Темпи денату- радиції природних ландшафтів прискорюються, що зумовлює збіднення фіторізноманіття. Через скоро- діння площа, зайнятих природними екосистемами, знищуються первинні рослинні угруповання, змінюються структурно-функціональні характеристики екосистем, ландшафтів – втрачається біорізноманіт- ність у цілому [1].

Проблема збереження біорізноманітності визначена як пріоритетна для України на державному рівні. По-перше, методологія такого підходу закладе-на в Конституції України; по-друге, це є результатом підписання та ратифікації відповідних міжнародних угод; по-третє, збереження біорізноманітності включе- но до переліку семи приоритетів державної еко-номіки [2].

Важливою умовою збереження видового різ-номаніття України є ведення кадастрів біорізноманіт-тя, «Червоної книги України», складання списків ви- дів рослин та охорона цих видів, в тому числі тих, що потребують охорони не лише на державному рівні, а й на міжнародному [3].

Усвідомлення міжнародною спільнотою від-повідальності за збереження біорізноманіття призве-ло до розробки багатьох міжнародних угод (Конвен-