Zirconium oxide nanoparticles: Protocolicidal effect against hydatid cyst protoscoleces with interleukins evaluation in mice

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Abstract

The current study investigated the effect of zirconium oxide nanoparticles NPs on the levels of interleukins in mice inoculated with protoscoleces of Echinococcus granulosus, after exposure to ZrO₂ NPs at the doses, 5, 10, 50, and 100 µg/ml for 30 minutes, and different periods of infection, on the other hand, control groups inoculated with unexposed protoscoleces to the nanoparticles. Following the infection, blood serum was obtained from animals to determine interleukin levels using sandwich ELISA technique three, four, and five months following the infection. The outcome of the study demonstrated a significant decrease in IL-5, IL-12 levels, and a significant increase in IFN-γ levels in treated mice compared with control groups. The lowest value of IL-5 was 11.164 ng/ml at the dose of 100 µg/ml compared to the control group 47.446 ng/ml, four months after infestation, while the lowest value for IL-12 was 12.824 ng/ml, at 100 µg/ml, compared to 112.485 ng/mL in the control group, five months to infection. IFN-γ was significantly increased in the treated mice, 207.39 ng/ml, at 100 µg/ml, compared to 110.07 ng/mL in the control group, five months after injury. Based on the research outcome, ZrO₂ nanoparticles could be exploited as a consequential titer for assessing the immune situation and, consequently as a dynamic curative of hydatidosis.

Keywords

Hydatidosis
Interleukin-12
Interferon-γ

Introduction

Cystic echinococcosis is a zoonotic disease induced by Echinococcus granulosus larval stage infection. The disease causes health problems worldwide and causes economic losses estimated at 3$ billion annually (1,2). Hydatid cysts develop in different host organs, such as the liver 70%, lungs 30%, and even in the heart, brain, bones, spleen and kidneys, but at a lower rate, leading to death (3-5). Iraq is one of the countries affected by this endemic disease, transmitted to humans by eating food contaminated with E. granulosus eggs or by contact with infected dogs (6,7). The diagnosis of this disease is still challenging. Many cases show symptoms after years due to the absence of clear pathological signs. Symptoms implicate abdomen discomfort, nausea, obstruction of the bile ducts, in the chest, breathing difficulties, and neurological signs due to seizures as a result of brain injury (8,9). Some of the most common methods for detecting hydatid cysts in humans are X-ray, ultrasound, computed tomography, and magnetic resonance imaging (10-12). These methods are characterized by an inability to differentiate cysts from abscesses. Therefore, early diagnosis must be confirmed through other high-quality tests, such as serological techniques, such as immunoelectrophoresis, double diffusion test, and direct immunofluorescence assay, as new sensitive techniques (13,14). The accidental rupture of cysts or rupture during surgery is a serious medical problem due to the ability of the protoscoleces to develop into new cysts. Thus, many chemicals were used as protoscolicidal pre cyst removal (15,16). Infection with
Echinococcus granulosus prompts a humoral and cellular response in the host, increasing in serum antibodies as an essential titer for diagnosing the disease and Th1 and Th2 cytokines (17). The interaction between the host and the parasite is significantly impacted by host immunity. The parasite secretes substances that impact immunological cells, releasing antibodies, activating T cells, and inducing an inflammatory immune response. Antibodies are essential to combat the parasites, protect the host, and prevent disease. E. granulosus induces a biphasic response, represented by Th2 response implicates cytokines, as well as Th1 cytokines, while Th17 cells are potential pro-inflammatory cells contribute the secretion of IL-22 and IL-21 (18, 19). Roles of cytokines in host immunity differ with different species of parasites, their size, location within the host, and their metabolic products. Heavy infection with parasites stimulates Th2 cytokines (20). Most studies on mice and humans confirmed the dominance of Th1 cytokine response, characterized by the IFN-γ release produced by dendritic cells with IL-12. It was found that both have an essential role in eliminating parasites during early infection. The parasite can influence the host's immune response by secretory excretory secretions, resulting in a Th2 response and the parasite's survival, associated with the cytokines IL-4, IL-5, and IL-10, depending on the parasite's receptor ability, which leads to chronic infection (21-23). Cytokines represent one of the pharmacological agents capable of improving the immune response to ABZ. In an experimental study, one of the mixture of ABZ with IFN-γ and IL-12 had prophylactic effects of 100% and 97%, respectively against echinococcosis, indicating the importance of cytokine therapy in preventing disease (24). Patients with cystic echinococcosis who did not react to treatment exhibited Th2 type response, while patients who responded to treatment revealed a Th1 type 1 response. Researchers explored interleukin -10 as a quantitative signal of the parasite's escape from the host's immune response (25). Nanoparticles have immunomodulatory properties that help enhance weak conjugated antigens' antigenic properties. They act as adjuvants, antigenic properties differ according to their size and surface charge, and the size of nanoparticles determines whether the loaded nanoparticles induce Th1 cells with interferon-γ and Th2 cells with IL-4, IL-10 when recognized by the immune system. Several studies confirm that nanostructured vaccine formulations provide certain advantages against pathogens in the induced immune response, as they generally work to stabilize and increase the supply of antigens or act as modulators of the immune system (26-30). Other research applied to mice treated with a group of antihelminthic drugs Oxfendazole, Praziqantel, and Albendazole after infection with hydatid cysts showed an increase in the level of IFN-γ in the hepatic and splenic inflammatory cells in the treated groups, these results confirmed that effective treatment reduces IL-10 in human infections leading to in situ immune activation by increasing L-2 IFN-γ. It was confirmed that elimination of the parasite will restore the host cell response (31,32).

The current study targeted investigating levels of interleukins, as a considerable indicator of immune response, in mice inoculated with protoscoleces of hydatid cysts exposed to ZrO2 nanoparticles as an alternate protoscolicidal therapy during surgical operations.

Materials and methods

Ethical approve

The study was accomplished in accordance with the declaration of Helsinki, and the protocol was approved by the Ethics committee of College of Veterinary Medicine at University of Mosul in ethical approval code UM.VET.2022.083 in 19/10/2022.

Animals

Seventy-five male albino Scottish mice, 3-4 weeks old, were used and reared in the animal house of The College of Veterinary Medicine, University of Mosul. Mice were inoculated with 2000 protoscoleces viable and exposed to different concentrations of ZrO2, 5,10,50, and 100 µg/ml for 30 minutes. Control groups were administered with 2000 vital unexposed protoscoleces. After 3, 4, and 5 months of infection, mice were anesthetized, blood was obtained from the ophthalmic Venous Plexus, according to Waynforth (33), and serum was obtained according to authenticated methods kept in the freezer at -20 centigrade till use.

Cytokine assays

Sandwich ELISA technique, ELISA kit from Bioassay Technology Laboratory (BT Lab), China, was used to detect interleukins in the blood serum of infected animals by using a 96-hole cutlet. Serum samples, all reagents, and standard solutions were prepared at room temperature. The standard protein for each interleukin was prepared, five numbered Eppendorf tubes were used, and 120 µl of the standard diluent was added to each tube. The number of strips required for measurement was determined. The strips were inserted in the places designated for use. 50 µl of standard solutions and 40 µl of samples were added to the holes. 10 µl of diluted serum was added to the samples in the pit, then 50 µl of streptavidin HRP to the samples and the standard. The plate was covered with sealer, incubated posteriorly for 60 minutes at 37°C, and washed five times with at least 0.35µl of the washing solution for a minute each. 50 µl of substrate solution A and 50 µl of substrate solution B were added to each well. After that the plate was incubated for ten minutes at 37 °C. Subsequently, 50 µl of the stop solution was added to each hole, so the blue color turned yellowish immediately. The optical density was determined immediately using a microplate reader set at a wavelength of 450 nanometers within 10 minutes of adding the stop solution.
Statistical analysis

Data were analyzed by complete random design and analysis of variance. Degrees of differences were estimated by Ducan’s multiple range test. Statistical analysis Software version nine was used (34).

Results

The current study showed significant differences (P≤0.01) in the level of cytokines IL-5, IL-12, and IFN-γ (P≤0.05) in mice administrated with protoscoleces treated with zirconium nanoparticles compared to the control group (Table 1). Table 2 demonstrates substantial variations in the level of cytokines (P≤0.01), represented by a decrease a in the level of IL-5 in the treated collection, 11.164 ng /ml at 100 µg/ml, four months postinfection, compared to untreated ones, 75.171 ng /ml, five months next infection. Table 3 detected a significant decrease in IL-12 level, 12.824 ng /ml at 100 µg/ml throughout infection, compared with the control collection of 112.485 ng/ml after the fifth month of infection. Table 4 detected substantial variations represented by an increase in the level of IFN-γ in the treated collection, 207.39 ng /ml, five months after infection, compared to the control group, 110 ng /ml, three months post infection.

Discussion

The outcome of the current research showed a significant rise in the interferon-gamma level and a decrease in the level of interleukins IL-5 and IL-12. Cytokine therapy is one of the effective ways to prevent the formation of hydatid cysts and to treat cases in which surgeries cannot be performed. TH1 cytokines are essential in initiating protective immune responses against pathogens comprising bacteria, viruses, and parasites. IFN-γ IL-12 stimulates increased macrophages and NK cells as antigen-presenting cells to destroy pathogens (35).

Table 1: Level of cytokines IL-5, IL-12, and IFN-γ in experimental mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Degree of freedom</th>
<th>Duration</th>
<th>Handling</th>
<th>Duration × handling</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-5</td>
<td>Summation of square medium square</td>
<td>1370.290**</td>
<td>9066.392**</td>
<td>4397.281**</td>
<td>3325.80991</td>
</tr>
<tr>
<td></td>
<td>F amount</td>
<td>685.1452**</td>
<td>2266.5981**</td>
<td>549.66020**</td>
<td>55.43017</td>
</tr>
<tr>
<td>IL-12</td>
<td>Summation of square medium square</td>
<td>22.48057</td>
<td>60063.527**</td>
<td>5006.26708**</td>
<td>1684.03633</td>
</tr>
<tr>
<td></td>
<td>F amount</td>
<td>11.24029</td>
<td>15015.88185**</td>
<td>625.78338**</td>
<td>28.06727</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Summation of square medium square</td>
<td>1580.56506</td>
<td>5331.61120</td>
<td>92223.2389*</td>
<td>300620.479</td>
</tr>
<tr>
<td></td>
<td>F amount</td>
<td>790.2825</td>
<td>1332.902</td>
<td>11527.904*</td>
<td>0.001</td>
</tr>
</tbody>
</table>

** Considerable significance P≤0.01. *Considerable significance P≤0.05.

Table 2: Level of IL-5 in experimental mice

<table>
<thead>
<tr>
<th>Duration</th>
<th>Three months</th>
<th>Four months</th>
<th>Five months</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.507cb</td>
<td>47.446b</td>
<td>75.171a</td>
<td>56.041a</td>
</tr>
<tr>
<td>First group (5 µg/ml)</td>
<td>40.057bcd</td>
<td>42.383bc</td>
<td>37.833bcd</td>
<td>40.273b</td>
</tr>
<tr>
<td>Second group (10 µg/ml)</td>
<td>42.039bc</td>
<td>35.369cd</td>
<td>33.977f</td>
<td>37.128b</td>
</tr>
<tr>
<td>Third group (50 µg/ml)</td>
<td>35.325cd</td>
<td>29.611ed</td>
<td>38.276bcd</td>
<td>34.404b</td>
</tr>
<tr>
<td>Fourth group (100 µg/ml)</td>
<td>18.950fg</td>
<td>11.164g</td>
<td>22.619ef</td>
<td>17.733c</td>
</tr>
<tr>
<td>Medium</td>
<td>36.3756b</td>
<td>33.1946b</td>
<td>41.5752a</td>
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</tr>
</tbody>
</table>

*Similar letters indicate no significant difference and different letters indicate significant differences.

Table 3: Level of IL-12 in experimental mice

<table>
<thead>
<tr>
<th>Duration</th>
<th>Three months</th>
<th>Four months</th>
<th>Five months</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73.545c</td>
<td>96.242b</td>
<td>112.485a</td>
<td>94.090a</td>
</tr>
<tr>
<td>First group (5 µg/ml)</td>
<td>49.071d</td>
<td>36.880ef</td>
<td>43.359de</td>
<td>43.103b</td>
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<tr>
<td>Second group (10 µg/ml)</td>
<td>35.021f</td>
<td>32.024g</td>
<td>24.450h</td>
<td>30.498c</td>
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<td>Third group (50 µg/ml)</td>
<td>26.634gh</td>
<td>19.842hi</td>
<td>19.446hi</td>
<td>21.974d</td>
</tr>
<tr>
<td>Fourth group (100 µg/ml)</td>
<td>15.790j</td>
<td>13.208i</td>
<td>12.824i</td>
<td>13.940d</td>
</tr>
<tr>
<td>Medium</td>
<td>78.9415a</td>
<td>66.8475b</td>
<td>63.2238c</td>
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</tr>
</tbody>
</table>

Similar letters indicate no significant difference and different letters indicate significant differences.
Table 4: Level of IFN-γ in experimental mice

<table>
<thead>
<tr>
<th>Duration</th>
<th>Three months</th>
<th>Four months</th>
<th>Five months</th>
<th>Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>110.07ab</td>
<td>148.25ab</td>
<td>175.09ab</td>
<td>144.47a</td>
</tr>
<tr>
<td>First group</td>
<td>149.88ab</td>
<td>69.04b</td>
<td>101.18ab</td>
<td>106.7a</td>
</tr>
<tr>
<td>Second group</td>
<td>119.64ab</td>
<td>133.80ab</td>
<td>116.70ab</td>
<td>123.38a</td>
</tr>
<tr>
<td>Third group</td>
<td>122.77ab</td>
<td>111.75ab</td>
<td>202.40a</td>
<td>145.64a</td>
</tr>
<tr>
<td>Fourth group</td>
<td>126.09ab</td>
<td>160.30ab</td>
<td>207.39a</td>
<td>164.593a</td>
</tr>
<tr>
<td>Medium</td>
<td>125.69c</td>
<td>124.628b</td>
<td>160.552a</td>
<td></td>
</tr>
</tbody>
</table>

Similar letters indicate no significant difference and different letters indicate significant differences.

Recent research suggests that Th1 and Th2 reactions act together during infection with CE. Th2 response is connected with disease vulnerability (Active cyst) while preventive immunity is associated with Th1 response (Inactive cyst). The biological importance of the cellular response appears in some infectious diseases, where the responses of T-cells of the first type TH1 and the second type TH2 are associated with resistance and sensitivity, and immunological studies that have been applied to humans have indicated mixed immune responses are associated with infection, or susceptibility to disease in the case of active cysts, whereas TH1 responses are associated with protective immunity or resistance to disease in the case of inactive cysts, patients with cystic echinococcosis who did not respond well to chemotherapy usually showed a TH2 response, while patients who did not show TH1 response (36-38).

Antigen B (Ag B) affected the immune system response in rabbits immunized with AgB and full Freund's adjuvant. Three intramuscular booster shots were carried out again after two weeks following the immunization using incomplete Freund's adjuvant, and five rabbits were treated as the control group. IgG, IFN-γ, and IL-10 levels were determined using ELISA. IgG levels in the immunized rabbits were significantly higher 45.9 ng/ml than in the control group 16.6 ng/ml. The differences in Th1 (IFN-γ) and Th2 (IL-10) cytokine levels between the vaccinated and control groups were statistically significant, and the mean of IFN-γ values in the immunized rabbits 28.6 pg/ml were less than those in the control groups 83.6 pg/ml. The findings showed that AgB immunization-induced Th2 immune response in immunized rabbits, as evidenced by a large increase in total IgG and IL-10 and a significant decrease in IFN-γ concentration (39). Siracusano et al. (40) showed that IFN-γ improved the ability of macrophages to kill protoscoleces while IL-4, IL-10 reduced the activity. IFN-γ is the key to phagocytic cell function through NO production and inhibition of helminth growth and function. In addition to other pathogens, it thus plays a role in establishing protective immunity by TH1 during Echinococcus granulosus infestation, and the destructive role of nitric oxide associated with the production of TH1 response acting to reduce the vitality of the parasite. Elevated levels of IFN-γ and NO are found both in vitro and in vivo during human infection, while levels are not detectable upon reinfection.

Through studies in humans and mice, most researches addressed the dominance of the Th1 response, characterized by IFN-γ release. After being produced by DCS dendritic cells with IL-12, it was found that both are effective in eliminating the parasite at an early stage. Th2 and parasite survival associated explicitly with IL-5, IL-4, IL-10 and transitional growth factor (TGF) are generally associated with the receptive ability of the parasite and lead to chronic infection (40-42). It was also revealed that when the cyst dies naturally, is killed by chemotherapy, or is surgically removed, Th2 cell responses rapidly decline and Th1 cell response is dominant. This can be explained by the fact that Th1 lymphocytes contribute significantly to the inactive stages of the disease, with Th2 lymphocytes being more critical in the transitional and active stages (5,43). A recent study concurs with research concerned with pre-treatment IL-5 levels to monitor the change of cytokines in mice infected with various Echinococcus components, ELISA technique registered a shift in blood levels of IL-2, Interferon-gamma, TNF-alpha, IL-4, IL-5 and IL10 during 220-day infection compared to the group of animals that were not infected. Cytokine levels increased significantly at 260 days. IL-2 level reached a peak 80 days after infection, posteriorly quickly collapsed after 140 days post-injury, compared to uninfected series, a high amount of TNF-alpha was determined after 40 days; peaked 100 days after infection and dropped off swiftly after 140 days. After eighty days post-infection, IFN-gamma levels peaked and steadily started declining. Before 80 days, the levels of Interleukin -4, Interleukin -5, and Interleukin -10 stayed lower and significantly increased following a hundred days. At 100 days post-injury, the levels of IL-4 and IL-10 peaked, while IL-5 peaked at 140 days post-injury. The evidence pointed to vital host defense mechanism against metacestodes as the Th2 AMI (antibody-mediated immunity) is significant in the later stages of infection and has an essential role in the initial phases of infection (3).

**Conclusion**

According to the research, CE had each Th1 and Th2 cytokine profiles, with Th2 predominating throughout the active stage of the illness, and significantly decreasing in laboratory mice that responded to remediation with the
nanoparticles. The results demonstrated a considerable protoscolicidal activity of Zirconium oxide nanoparticles against hydatid cysts in mice by modulating the levels of interleukins through increasing INF-γ and decreasing IL-12 and IL-5, respectively. These nanoparticles can be used as a favorable alternative for hydatid cyst remedies to overcome the disadvantages of drug side effects.

Acknowledgment

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Conflict of interest

The authors profess that they have no conflict of interest.

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37. الخلاصة
38. تحرت الدراسة الحالية تأثير أوكسيد الزركونيوم النانوية في مستوى الالتهابات في الفئران المعالمة بالرؤيسات الأولية للمشكلة الحيوية، تعرضت أوكسيد الزركونيوم النانوية عند الجرعات 5، 10 و 100 ميكروغرام/مل، لمدة ثلاثين دقيقة، وفترات زمنية مختلفة من الأسابيع، من جهة أخرى جربت مجاميع السيطرة بالرؤيسات الأولية غير المعروفة للأماكن الأولية عند الجرعات 12، 33، 54 ميكروغرام/مل، لفترات زمنية مختلفة. ازداد مستوى إنترفيرون 5 وتراونمول/مل، بعد خمسة أشهر من الإصابة، الداء 증강의 경향이 관찰되었으나 면역.relevant results in a different systemic cytokine profile than single parasite infection. PLoS One. 2020;15(9):e0238909. DOI: 10.1371/journal.pone.0238909
39. نتائج الدراسة، من جهة أخرى، تم الحصول على مصل الدم من الحيوانات لتحديد مستوى الإنترليوكين 2 و 4 لوحدها و.compare to controls, which were exposed to the nanoparticles. After three, four, and five months from the infection, respectively, the treatment groups were treated with the eggs of Fasciola hepatica, while the control groups received no treatment. The results of the study showed that the nanoparticles of zirconium oxide can be used as a standard for the first time in the evaluation of the infection in the rat. London: Academic P
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