Protoscolex metabolites of Coenurus cerebralis as antigenic-produced humoral immune response in sheep

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Abstract

The purpose of the current experiment was to test the immunization against Coenurus cerebralis in sheep. Sixteen animals (6 months old, from 1 October 2020 to 30 March 2021 in Najaf city) were recruited to perform the experiment, in which eight of them were injected twice at 21-day interval using the cellular metabolic antigen of C. cerebralis protoscolex cultivated and then emulsified by complete Freund's adjuvant (CFA). The shots were injected intramuscularly at a dose of 1 ml (15 mg of antigenic protein determined in a separate experiment). The second group of eight sheep served as controls (injected intramuscularly with 1 ml sterile saline only at the days of injections). Blood samples were collected from all animals at day-0 (before injection) and at day 10, 18, and 24 after the first injection, and at day 10, 16, 26, 40, 48, 53, 61, 80, 85, and 89 after the second injection. Serum activity was studied by indirect enzyme-linked immunosorbent assay (iELISA). The findings, by iELISA, revealed that the cellular antigen of C. cerebralis protoscolices is an active stimulator of antibody response. Day-10 (after the first injection) showed significantly (P<0.05) 3.4 to 9.9 time-higher antibody levels compared to those from day-0. This elevation in the titer of antibodies was increased after receiving the second dose showing 6.3 to 12 time-higher antibody presence even at the final days of blood collection compared to those from day-0. No changes were noticed in the sera of the control animals. The obtained data allow us to conclude that metabolites synthesized by cultivation are active immunogenic components that activate the humoral part of the immune system manifested by the increases in the antibody titers. This gives a solid ground for future work regarding alternative methods of discovering immunization techniques against cestodes.

Keywords: Antibody, Coenurus cerebralis, ELISA, Protoscolex, Sheep

Introduction

Taenia multiceps (Multiceps multiceps) is a taeniid cestode which infests the small intestine of dogs, coyotes, foxes, and jackals as final host during its adult phase (1). C. cerebralis larval stage is commonly located in the nervous systems of small ruminants such as sheep, goats and large ruminants such as buffaloes, cattle, camels, and equids leading to cerebral coenurosis marked by neurological abnormalities (2,3). Non-cerebral coenurosis has also been identified in other parts of the body such as in the muscular tissues and visceral organs of the affected intermediate hosts (4,5). Small ruminants have a very high infestation risk with C. cerebralis (6). In small ruminants, coenurosis is linked to disease burden, fatality and major economic losses (7). Reports of human infection have been identified in Egypt, Canada, France, and the United States. Infection occurs by the fecal-oral path, which involves contaminated food and/or water with the infective stage (eggs) (6).

Clinical signs, epidemiological occurrences, histological defects and morphological characteristics of C. cerebralis
have generally been used to diagnose taeniid tapeworms (8). The important aspects for morphometric recognition are hook number, size, and shape (9); nevertheless, these parameters are subject to errors particularly in the situation of sterile, young or degraded coenurior also in the maturity phase. Molecular tests have been extensively utilized in research relating to population biology, epidemiological and phylogenetic studies as well as in distinguishing the identity of cysts of different taeniids (10).

Small ruminant production is regarded as a critical component of long-term economic growth in the developing countries. Nevertheless, these infections have been identified as a significant serious obstacle for the livestock farming industries leading to stagnant economic growth (11). The purpose of the current experiment was to test the immunization against Coenurus cerebralis in sheep.

Materials and methods

Samples and experimental design

Sixteen sheep (6 months old) (From 1 October 2020 to 30 March 2021 in Najaf city) were recruited to perform the experiment, in which eight of them were injected twice at 21-day interval using the cellular metabolic antigen of C. cerebralis protoscoleces. The second group of eight sheep served as controls (12). Blood samples were collected from all animals at day-0 (before injection) and at day 10, 18, and 24 after the first injection, and at day 10, 16, 26, 40, 48, 53, 61, 80, 85, and 89 after the second injection.

Production of protoscolex metabolites

Metabolites for experimental antigenicity were obtained by cultivating C. cerebralis protoscoleces in a Heraeus CO2 based incubator at 37°C using automated control of the gases (CO2 - 5%, O2 - 95%) at 70% humidity in RPMI-1640 medium. The antigens were then emulsified by CFA. The metabolites synthesized in the process of cultivation of C. cerebralis protoscoleces are active immunogens that activate the humoral component of immune system, which is manifested by synthesis of specific antibodies and gives grounds to speak about an alternative method of obtaining immune drugs from cestodes.

Experiment

The shots were injected intramuscularly at a dose of 1 ml (15 mg of antigenic protein determined in a separate experiment which discussed in production of protoscolex metabolites) in the treatment groups. The control sheep were injected intramuscularly with 1 ml sterile saline only at the days of injections.

IELISA

Serum activity was studied by iELISA. After determining the optimal antigen concentration, solid phase sensitization was performed using the cellular antigen. The conjugate in the reaction was antibodies based on peroxidase labeling prepared against sheep serum IgG (13). The reaction was evaluated using an automated colorimetical enzyme-immunoassay analyzer 340/ATC (STL-Labsistems, Austria), at 492 nm of a wavelength to measure the optical density (OD) (14).

Results

The findings, by iELISA, revealed that the cellular antigen of C. cerebralis protoscoleces is an active stimulator of antibody response. Day-10 (after the first injection) showed significantly (P<0.05) 1.6 to 2.1 time-higher antibody levels compared to those from day-0 (Table 1).

This elevation in the titer of antibodies was increased after receiving the second dose showing 0.151 to 0.291 time-higher antibody presences even at the final days of blood collection compared to those from day-0. No changes were noticed in the sera of the control animals (Table 2).

Table 1: Antibody titers in sheep injected with cellular antigens of C. cerebralis protoscoleces

<table>
<thead>
<tr>
<th>Days of study</th>
<th>Optical density in IER* in experimental animals</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>10**</td>
<td>2.107 ± 0.035</td>
<td>1.822 ± 0.045</td>
</tr>
<tr>
<td>18</td>
<td>2.211 ± 0.046</td>
<td>2.011 ± 0.054</td>
</tr>
<tr>
<td>24</td>
<td>2.111 ± 0.056</td>
<td>2.321 ± 0.056</td>
</tr>
<tr>
<td>31/10***</td>
<td>2.211 ± 0.051</td>
<td>2.377 ± 0.051</td>
</tr>
<tr>
<td>37/16</td>
<td>2.111 ± 0.046</td>
<td>1.711 ± 0.046</td>
</tr>
<tr>
<td>47/26</td>
<td>2.110 ± 0.054</td>
<td>2.011 ± 0.054</td>
</tr>
<tr>
<td>61/40</td>
<td>2.326 ± 0.056</td>
<td>2.222 ± 0.056</td>
</tr>
<tr>
<td>69/48</td>
<td>2.439 ± 0.056</td>
<td>2.441 ± 0.056</td>
</tr>
<tr>
<td>74/53</td>
<td>2.654 ± 0.056</td>
<td>2.352 ± 0.056</td>
</tr>
<tr>
<td>82/61</td>
<td>2.591 ± 0.056</td>
<td>2.524 ± 0.056</td>
</tr>
<tr>
<td>101/80</td>
<td>2.422 ± 0.056</td>
<td>2.655 ± 0.056</td>
</tr>
<tr>
<td>106/85</td>
<td>2.510 ± 0.056</td>
<td>2.811 ± 0.056</td>
</tr>
</tbody>
</table>

* IER: Immunoenzymatic reaction (P≤ 0.001). ** First Injection. *** Second injection.
Recent studies have identified signals in the research of helminth parasites, but also identified a range of species-specific gene targets that could be used to establish new coenurosis treatments and controls. Exactly, this agrees with our findings in which metabolites of the cells belong to the *C. cerebralis* protoscolex have led to the production of immune responses represented by the increases in the circulating antibodies.

The identification of helminth-secreted extracellular vesicles (EVs) has proposed a new model in the research of host-parasite communication. EVs are generated by a wide range of cell types and organisms, such as parasites. EVs are a cell-to-cell exchange system that happens at homeostasis by the movement of genetic information, proteins, lipids, and signals. Some studies have identified those vesicles as immunogenic materials to produce protection against certain parasites (21-26).

**Conclusion**

The obtained data allow us to conclude that metabolites synthesized by cultivation are active immunogenic components that activate the humoral part of the immune system manifested by the increases in the antibody titers. This gives a solid ground for future work regarding alternative methods of discovering immunization techniques against cestodes.

**Acknowledgments**

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**Conflict of interests**

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**References**

1. Varcasia A, Tamponi C, Tosciiri G, Pipia AP, Dore F, Schuster RK, Kandil OM, Manunta ML, Scala A. Is the red fox (*Vulpes vulpes*) a...


لاستجابة الأجسام المضادة. أظهر اليوم 10 (بعد الحقن الأول) مستويات أضداد أعلى بشكل ملحوظ (أ< 0.05) 3.4 إلى 9.9 مرة مقارنة بتلك الموجودة في اليوم صفر. لوحظت زيادة هذا الارتفاع في عيار الأجسام المضادة بعد تلقي الجرعة الثانية مما أظهر وجود أجسام مضادة أعلى من 6.3 إلى 12 مرة حتى في الأيام الأخيرة من جمع الدم مقارنة بتلك الموجودة في اليوم 0. لم يلاحظ أي تغييرات في مصل حيوانات السيطرة. تسمح لنا البيانات التي تم الحصول عليها بالاستنتاج أن المستضدات التي تم تحضيرها عن طريق الزراعة هي مكونات مناعية نشطة والتي تشتمل الجزء الخلقي من الجهاز المناعي الذي يتألف في الزيادة في عيار الأجسام المضادة. وهذا يعطي أرصدية صبة للعمل المستقبلي فيما يتعلق بالطرق البديلة للاكتشاف تكنولوجيا تحسين ضد الديدان الشريطية.