Experimental Parasitology 189 (2018) 66–71

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Naltrexone; as an efficient adjuvant in induction of Th1 immunity and protection against *Fasciola hepatica* infection

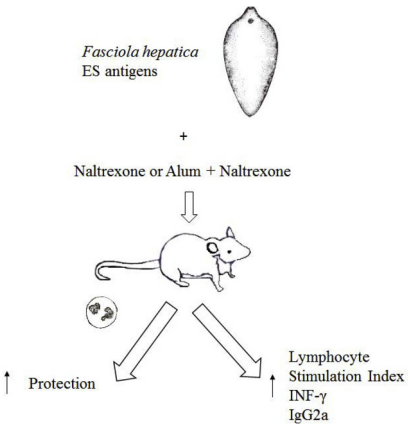
Hakim Azizi [a], Hadi Mirzaeei [b], Ali Akbar Nasiri [c], Ali Bazi [d], Aliyar Mirzapour [e], Mehrdad Khatami [f], Kareem Hatam Nahavandi [g], Ako Azimi [h], Hajar Yaghoobi [i, *]

[a] Department of Medical Parasitology, School of Medicine, Zabol University of Medical Sciences, Zabol, Iran
[b] Department of Medical Genetics, School of Medicine, Zabol University of Medical Sciences, Zabol, Iran
[c] Department of Anesthesiology, Zabol University of Medical Sciences, Zabol, Iran
[d] Clinical Research Development Unit, Zabol University of Medical Sciences, Zabol, Iran
[e] Department of Medical Parasitology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[f] School of Medicine, Bam University of Medical Sciences, Bam, Iran
[g] Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
[h] Maragheh University of Medical Sciences, Department of Basic Sciences, Maragheh, Iran
[i] Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

HIGHLIGHTS

- Naltrexone adjuvant promotes IFN-γ and Th1 immune response against fasciolosis.
- Naltrexone renders higher stimulation index of lymphocytes in immunized mice.
- Naltrexone-adjuvanted vaccine leads to protection against fasciolosis in mouse.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Available online 27 April 2018

Keywords:
Naltrexone
Vaccination
Fasciolosis
Fasciola hepatica
Alum

ABSTRACT

Toxic effects of available therapeutics are major drawbacks for conventional management approaches in parasitic infections. Vaccines have provided a promising opportunity to obviate such unwanted complications. In present study, we examined immune augmenting capacities of an emerging adjuvant, Naltrexone, against *Fasciola hepatica* infection in BALB/c mice. Seventy BALB/c mice were divided into five experimental groups (14 mice per group) including 1- control (received PBS), 2- vaccine (immunized with *F. hepatica* E/S antigens), 3- Alum-vaccine (immunized with Alum adjuvant and E/S antigens), 4- NLT-vaccine (immunized with NLT adjuvant and E/S antigens), and 5- Alum-NLT-vaccine (immunized with mixed Alum-NLT adjuvant and E/S antigens). Lymphocyte stimulation index was assessed by MTT

* Corresponding author. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran.
E-mail address: hajar.yaghoobi64@gmail.com (H. Yaghoobi).

https://doi.org/10.1016/j.exppara.2018.04.015

assay. Production of IFN-γ, IL-4, IgG2a and IgG1 was assessed by ELISA method. Results showed that NLT, either alone or in combination with alum, can induce immune response toward production of IFN-γ and IgG2a as representatives of Th1 immune response. Also, using this adjuvant in immunization experiment was associated with significantly high proliferative response of splenocytes/lymphocytes. Utilization of mixed Alum-NLT adjuvant revealed the highest protection rate (73.8%) in challenge test of mice infected with *F. hepatica*. These findings suggest the potential role of NLT as an effective adjuvant in induction of protective cellular and Th1 immune responses against fasciolosis.

1. Introduction

Fasciolosis is a zoonotic infection caused by *Fasciola hepatica* and *Fasciola gigantica* trematodes. Fasciolosis is an infection primarily affecting livestock with financial and nutritional importance. Due to the close contact, humans can also be infected by *Fasciola* species. According to the world health organization report, there are currently 2.4 million affected humans across the globe with 180 million at the risk of the infection (Meemon and Sobhon, 2015). Therapeutic approaches against fasciolosis have encountered increasing drug resistance (Rojas-Caraballo et al., 2014). Because of this, prevention of fasciolosis is a priority for saving funds in both health care system and food industry. In this regard, vaccination policies provide a promising strategy.

Efficiency of vaccines in promoting a potent immune response against infections is dependent on a number of features such as immunogenicity of the antigenic determinants, adjuvant properties, and administration routes (Chantree et al., 2013). Among these, the most important may be the capability of adjuvant compartment to properly modulate immune reactivity. Currently, there is a shortage in the number of approved adjuvants in clinical application mainly due to safety concerns. Alum has been used as an adjuvant for more than seven decades and is currently in used and approved by Food and Drug Administration (FDA) agency. This adjuvant, however, acts in favor of T-helper 2 (Th2) and humoral immune responses and is considered as a desirable adjuvant against extracellular pathogens (Jazani et al., 2010, 2011). Nevertheless, application of alum in vaccines against intracellular organisms has been less promising (Harandi et al., 2010). Therefore, it is advisable to provide alternative adjuvants with ability to augment the cellular immunity against intracellular organisms.

Vaccination experiments against fasciolosis have been performed using different antigenic and adjuvant determinants. However, exploiting conventional adjuvants such as Freund's and alum has been associated with non-protective Th2 immune responses (Sansri et al., 2015; Changklungmoa et al., 2013). Naltrexone (NLT) is an opioid antagonist currently used by drug addicted (Woody, 2014). This prescription drug has a good safety history and is approved by the FDA for using in humans. In previous studies, the role of opioid antagonists including NLT has been noted as a proficient adjuvant in induction of both cellular and humoral immunities against multiple infections. Specially, NLT has been highlighted as an appropriate adjuvant against parasitic infections such as *Toxoplasma* and *Plasmodium* species (Khorshidvand et al., 2016; Tappeh et al., 2013; Shahabi et al., 2014). Nevertheless, there was no study on the potential role of NLT as an adjuvant in vaccination against fasciolosis. In present study, excretory-secretory (E/S) antigens derived from *F. hepatica* were used to immunize BALB/c mice along with NLT adjuvant.

2. Materials and methods

2.1. Animals

Seventy female BALB/c mice aged 6–8 weeks were prepared from Razi Vaccine and Serum Research Institute, Karaj, Iran. They were kept according to the ethical requirements and animal care instructions announced by Ethical Committee of Zabol University of Medical Sciences. These mice were assigned to one of five immunization groups in our study (14 mice per group). Five selected mice per each group were used for determination of lymphocyte stimulation index, as well as cytokines, and antibody measurements. Seven mice per group were further subjected to the challenge test. Two mice per groups that were weaker than others were spared for being used in experiments.

2.2. Preparation of metacercariae

F. hepatica eggs were obtained from bile ducts and gallbladder of naturally infected sheep that were killed with standard procedure at an abattoir. Before harvesting the eggs, *F. hepatica* parasites were characterized based on morphological features (i.e. shorter length, smaller ventral sucker, and larger cephalic cone) to avoid misidentification as *F. gigantica*. The eggs were then stimulated to shed miracidia by dark incubation in 0.85% normal saline for two weeks, and subsequently illuminated for 2 h. The hatched miracidia were then transferred to *Lymnaea truncatula* with two miracidia per *L.truncatula* snail. After 45 days of the infection, cercariae began to shed from the snails. These were then collected using floating cellophane papers. Cercariae then immediately started to transform to metacercariae which were stored at 4 °C until use.

2.3. Obtaining excretory and secretory (E/S) antigens

For this purpose, adult *F. hepatica* parasites were gathered from gallbladder of naturally infected sheep. After collection, the organisms were rinsed in 0.85% normal saline six times to take out remnants of bile ducts and surrounding tissues. These were then cultured in RPMI1640 medium containing antibiotics (penicillin and streptomycin) at 37 °C. For obtaining E/S antigens, the medium was periodically centrifuged every 6 h (15,000 g, 30 min, 37 °C) for 24 h (Kueakhai et al., 2013). E/S antigens enriched in supernatant medium were collected. The supernatants were detected by Lowry's method (Bio-Rad) to determine the protein concentration. These antigens were stored at −80 °C until use.

2.4. Immunization

Mice were randomly assigned to one of five immunization groups (14 per group). These groups included: 1- Control (non-