Blocking of opioid receptors in experimental formaline-inactivated respiratory syncytial virus (FI-RSV) immunopathogenesis: from beneficial to harmful impacts

Article in Medical Microbiology and Immunology · December 2017
DOI: 10.1007/s00430-017-0531-0

CITATIONS
0

READS
56

13 authors, including:

Vahid Salimi
Tehran University of Medical Sciences
87 PUBLICATIONS 390 CITATIONS

See Profile

Alireza Tahamtan
Golestan University of Medical Sciences
29 PUBLICATIONS 79 CITATIONS

See Profile

Abbas Jamali
Pasteur Institute of Iran (IPI)
38 PUBLICATIONS 220 CITATIONS

See Profile

Shahram Shahabi
Hyland's Pharmaceutical Company - CA - USA
35 PUBLICATIONS 406 CITATIONS

See Profile

Some of the authors of this publication are also working on these related projects:

Investigation of the Cellular Immune Response to Recombinant Fragments of Filamentous Hemagglutinin and Pertactin of Bordetella pertussis in BALB/c Mice

Diagnosis of Candida Species Isolated from Patients with Vaginal Candidiasis and Healthy Individuals Based on Clinical Symptoms and Paraclinical Evidences
Blocking of opioid receptors in experimental formaline-inactivated respiratory syncytial virus (FI-RSV) immunopathogenesis: from beneficial to harmful impacts

Vahid Salimi1 · Habib Mirzaei1 · Ali Ramezani1 · Alireza Tahamtan1,2 · Abbas Jamali3 · Shahram Shahabi4 · Maryam Golaram5 · Bagher Minaei6 · Mohammad Javad Gharagozlou7 · Mahmood Mahmoodi8 · Louis Bont9 · Fazel Shokri5 · Talat Mokhtari-Azad1

Received: 30 July 2017 / Accepted: 9 December 2017 / Published online: 18 December 2017
© Springer-Verlag GmbH Germany, part of Springer Nature 2017

Abstract
Opioid system plays a significant role in pathophysiological processes, such as immune response and impacts on disease severity. Here, we investigated the effect of opioid system on the immunopathogenesis of respiratory syncytial virus (RSV) vaccine (FI-RSV)-mediated illness in a widely used mouse model. Female Balb/c mice were immunized at days 0 and 21 with FI-RSV (2 × 10⁶ pfu, i.m.) and challenged with RSV-A2 (3 × 10⁶ pfu, i.n.) at day 42. Nalmefene as a universal opioid receptors blocker administered at a dose of 1 mg/kg in combination with FI-RSV (FI-RSV + NL), and daily after live virus challenge (RSV + NL). Mice were sacrificed at day 5 after challenge and bronchoalveolar lavage (BAL) fluid and lungs were harvested to measure airway immune cells influx, T lymphocyte subtypes, cytokines/chemokines secretion, lung histopathology, and viral load. Administration of nalmefene in combination with FI-RSV (FI-RSV + NL-RSV) resulted in the reduction of the immune cells infiltration to the BAL fluid, the ratio of CD4/CD8 T lymphocyte, the level of IL-5, IL-10, MIP-1α, lung pathology, and restored weight loss after RSV infection. Blocking of opioid receptors during RSV infection in vaccinated mice (FI-RSV-RSV + NL) had no significant effects on RSV immunopathogenesis. Moreover, administration of nalmefene in combination with FI-RSV and blocking opioid receptors during RSV infection (FI-RSV + NL-RSV + NL) resulted in an increased influx of the immune cells to the BAL fluid, increases the level of IFN-γ, lung pathology, and weight loss in compared to control condition. Although nalmefene administration within FI-RSV vaccine decreases vaccine-enhanced infection during subsequent exposure to the virus, opioid receptor blocking during RSV infection aggravates the host inflammatory response to RSV infection. Thus, caution is required due to beneficial/harmful functions of opioid systems while targeting as potentially therapies.

Keywords Respiratory syncytial virus · Formalin-inactivated RSV · Immunopathogenesis · Opioids · Nalmefene

Abbreviations
RSV Respiratory syncytial virus
FI-RSV Formalin-inactivated RSV

T-helper
MORs µ-Opioid receptors
DORs δ-Opioid receptors

Talat Mokhtari-Azad
mokhtari@sina.tums.ac.ir

1 Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
2 Department of Microbiology, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran
3 Influenza and Other Respiratory Viruses Department, Pasteur Institute of Iran, Tehran, Iran
4 Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran
5 Department of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
6 Department of Anatomy, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
7 Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
8 Epidemiology and Biostatistics Department, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
9 Department of Paediatrics, Wilhelmina Children’s Hospital, University Medical Centre Utrecht, Utrecht, The Netherlands
Introduction

Respiratory syncytial virus (RSV) is the most leading cause of bronchiolitis and pneumonia in infants, children, and the elderly [1]. Globally, it is estimated that there are about 33.1 million episodes of RSV-associated acute lower respiratory tract infection per year in children younger than 5 years, with at least 3.2 million cases necessitating to hospitalization, and up to 118,200 deaths [2, 3]. Since infection with RSV does not convey persistent immunity, reinfection and disease occur throughout life [4]. Beyond the acute disease, RSV infection is also associated with long-term respiratory problems, such as recurrent wheezing, asthma, impaired lung function, and allergic sensitization [5]. The overwhelming burden of RSV infection each year is due to the lack of licensed vaccines and effective therapies [6].

RSV infection remains as a long-term public health challenge, and reducing the health burden of RSV has become a priority of the World Health Organization’s (WHO) BRaVe (Battle against Respiratory Viruses) initiative [7, 8]. The majority of studies have focused on the vaccine development, but it has been slower than expected [9]. The first candidate vaccine, a formalin-inactivated RSV (FI-RSV) vaccine, was tested in children in the 1960s, however, it was resulted in severe disease exacerbation upon natural infection, including two deaths [10]. The causes involved in the disastrous results of FI-RSV are still unclear, but this effect are mainly linked to dysregulation of the immune responses, such as T-helper (Th) cytokine pattern [11]. One of the necessities for designing a safe and effective vaccine is uncovering of the vaccine-enhanced disease mechanisms after FI-RSV vaccination and pathogenicity of virus in this unfavorable condition [12].

Opioids are a group of endogenous and exogenous compounds that function through the activation of opioid receptors, including µ-opioid receptors (MORs), δ-opioid receptors (DORs), and κ-opioid receptors (KORs) [13]. Activation of these receptors in nerve system exert analgesic effects, while opioid signaling in immune and immune-associated cells has immunomodulatory and anti-inflammatory activity [14]. Opioid receptors signaling plays a significant role in immune response and impacts on disease severity, such as viral infection [15]. We have previously shown that opioid signaling controls RSV replication in the airways and thereby modulate disease severity [16]. Importantly, it has been shown that opioid antagonists such as naltrexone and naloxone stimulate cellular immune responses which in turn shift immune responses toward Th1 pattern [15].

Although the effects of opioid system on primary RSV infection in our previous study are well demonstrated [16], the effects of this system on vaccine (FI-RSV)-mediated illness are still unclear. A better understanding of how opioid system (as a system interacted with the immune signaling) relate to disease progression following administration of the unfavorable (FI-RSV) vaccine is helpful to develop an effective therapies as well. In the present work, we used nalmefene as a universal opioid receptor blocker to determine the role of opioid system in unfavorable FI-RSV vaccine immunopathology in a well-established animal model of RSV infection. We also studied the ability of nalmefene to serve as an adjuvant to shift immune responses toward a protective Th1 pattern. Advantages of nalmefene relative to other opioid antagonists include longer half-life, greater oral bioavailability and no observed dose-dependent liver toxicity [17]. The results presented here provide evidence that targeting of opioid system is potentially attractive to develop effective therapies.

Materials and methods

Experimental design

Six- to seven-week-old female Balb/c mice, were obtained from the Institute Pasteur of Iran, Karaj, Iran. They were allocated in individual cages with food/water ad libitum under controlled condition in the animal house of School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. The current animal study was approved by the ethics committee of the Tehran University of Medical Sciences (No. 9591).

Mice were randomly assigned into controls (PBS–PBS, PBS-RSV, FI-RSV–FI-RSV) and experimental (FI-RSV + NL-RSV, FI-RSV-RSV + NL, FI-RSV + NL-RSV + NL) groups. The animals were immunized at days 0 and 21 with 0.05 ml of FI-RSV (2 × 10^6 pfu) intramuscularly (i.m.) and challenged with RSV-A2 (3 × 10^6 pfu) intranasally (i.n.) at day 42 (Fig. 1). The control groups received a similar volume of phosphate buffer saline (PBS). Nalmefene as a potent and universal classical opioid receptors antagonist (Sigma, The Netherlands) was dissolved in dimethyl sulfoxide and administered at a dose of 1 mg/kg accompanied by FI-RSV formulation (FI-RSV + NL). Also nalmefene was administered intraperitonealy (i.p.) 1 mg/kg in normal saline 0.9% daily after live virus challenge (RSV + NL).

Mice were sacrificed at day 5 after challenge (the peak day of viral load and immune cells influx into the lung)
using high dose of ketamine (i.p.) and bronchoalveolar lavage fluid (BALF) and lungs were harvested to measure airway immune cells influx, T-lymphocyte subtypes, cytokines/chemokines secretion, lung histopathology, and viral load. In this experiment, virus stock was propagated in HEp-2 cells as described previously by our groups [16], and FI-RSV was prepared by the method used for the original vaccine tested in the 1960s [18].

BAL cell analysis

BAL was performed through a catheter inserted into the trachea and by flushing the lungs with ice cold PBS as described previously [16]. The total number of cells present in the BALF and differential leukocyte counts were performed with Neubauer chambers and on smears stained with Wright-Giemsa dye, respectively.

T-lymphocyte flow cytometry

The CD4+ and CD8+ T-cell populations in the BALF were determined by flow cytometry. Briefly, an aliquot of 2 × 10⁵ BAL cells were washed twice with FACS buffer (1% BSA and 0.1% NaN₃ in PBS) and incubated with fluorochrome conjugated anti-mouse CD4 and CD8 immunoglobulins (Biolegend, USA) for 45 min at 4 °C in the dark. The cells were washed twice with FACS wash buffer and analyzed with a flow cytometer (Life technologies, USA). FlowJo® software (Tree Star, Inc., Ashland, OR, USA) was used to analyze the data.

Cytokines/chemokines assay

The concentrations of IFN-γ, MIP-1α, IL-10, and IL-5 in the BALF supernatant were measured with ELISA kits (PeproTech, USA) as described by the manufacturer. Concentrations of cytokines in the samples were calculated by interpolation from the standard curve.

Histological analysis

Lungs were removed and histology slides were prepared as described previously [16]. The slides were evaluated by light microscope and lung pathology, peribronchial and perivascular infiltration in the lungs were scored using standard criteria. The average of the sum of each reading was compared among groups.

Viral load assay

The virus titration in BALF supernatant was determined by plaque assay. Briefly, a tenfold serial dilution was prepared in DMEM medium and added to monolayer HEp-2 cells for 1 h at 37 °C. Following virus adsorption, cells were overlaid using 0.8% SeaKem ME Agarose (Lonza, USA) containing DMEM supplemented with 2.5% fetal calf serum (FCS), and incubated for 4–5 days at 37 °C. The methylcellulose overlay was aspirated and cells were fixed by 4% formaldehyde for overnight at room temperature. Cells were stained with 0.5% crystal violet in 20% ethanol and light microscope was employed to count RSV plaques.

Statistics

Statistical calculations and graph preparation were performed using GraphPad Prism v6.0 for Windows (GraphPad Software Inc., San Diego, CA, USA). The mean ± SEM is expressed in all data. The significance for each experiment was determined through Student’s t test and p values < 0.05 was considered statistically significant.
Results

Nalmefene administration in combination with FI-RSV vaccine (FI-RSV + NL-RSV)

The differences between RSV primary infection (PBS-RSV) and secondary infection in vaccinated mice (FI-RSV-RSV) were found in accordance with previous reports in Balb/c mice model. RSV primary infection induces immune response with a predominantly Th1 response and neutrophils are the predominant cell type while in FI-RSV there is a rapid and strong Th2-type system, which is associated with an eosinophilic influx into the BALF [4]. The data showed that administration of nalmefene in combination with FI-RSV (FI-RSV + NL-RSV) decreased immune cells influx to the BALF 5 days after live virus challenge compared to FI-RSV-RSV group (Fig. 2a). Differential analysis of the leukocytes in the BALF showed that nalmefene decreased eosinophil and monocyte infiltration, though only the monocyte count was statistically significant (p < 0.05) (Fig. 2d, f). Nalmefene administration in combination with FI-RSV decreased the ratio of CD4/CD8 T lymphocyte in the BALF 5 days after viral challenge (Fig. 3). As compared with FI-RSV-RSV group, blocking of opioid receptors by nalmefene during antigen presentation significantly decreased the level of MIP-1α, IL-10, and IL-5 production (p < 0.05) (Fig. 4). In agreement with the influx of immune cells, nalmefene administration decreased lung pathology following RSV infection (Fig. 5). Furthermore, administration of nalmefene in combination with FI-RSV restored weight loss after RSV infection (Fig. 6), and had no effect on viral replication compared to FI-RSV-RSV group (Fig. 7).

Nalmefene administration during RSV infection in vaccinated mice (FI-RSV-RSV + NL)

The data showed that blocking of opioid receptors via nalmefene during RSV infection in vaccinated mice (FI-RSV-RSV + NL) resulted in an increased influx of neutrophils and lymphocytes to the BALF 5 days after viral challenge compared with FI-RSV-RSV group, however, the differences were not statistically significant (Fig. 2c, e). Although the absolute number of lymphocytes was enhanced, but nalmefene decreased the ratio of CD4/CD8 T lymphocyte (Fig. 3). Nalmefene administration during RSV infection in FI-RSV mice group non-significantly decreased the production level of IL-5, IL-10, IFN-γ, and MIP-1α as measured in the BALF supernatant (Fig. 4). In this experiment, blocking of opioid receptors during RSV infection in vaccinated mice had no effect on lung pathology (Fig. 5), weight loss (Fig. 6), and also virus replication in compared to FI-RSV-RSV group (Fig. 7).

Nalmefene administration in combination with FI-RSV vaccine and during RSV infection (FI-RSV + NL-RSV + NL)

Administration of nalmefene in combination with FI-RSV vaccine and during RSV infection (FI-RSV + NL-RSV + NL) increased the total number of BALF cells 5 days after live virus infection compared with other challenged groups (Fig. 2a). Differential analysis of the leukocytes in the BALF showed an increased influx of neutrophils, eosinophils, and lymphocytes in FI-RSV + NL-RSV + NL group, though the lymphocytes and neutrophils count were statistically significant compared with FI-RSV-RSV and FI-RSV + NL-RSV groups, and the eosinophil count was statistically significant compared with FI-RSV + NL-RSV group (p < 0.05) (Fig. 2c, e, f). However, the ratio of CD4/CD8 T lymphocyte decreased significantly in compared to FI-RSV-RSV group (p < 0.05) (Fig. 3). In FI-RSV + NL-RSV + NL group the level of IFN-γ and MIP-1α production were enhanced compared to FI-RSV + NL-RSV and FI-RSV-RSV + NL groups (p < 0.05), and the level of IL-5 production was enhanced compared to FI-RSV + NL-RSV group (p < 0.05) (Fig. 4). Furthermore, our results did not show that nalmefene administration in combination with FI-RSV vaccine and during RSV infection impacted on lung pathology (Fig. 5), weight loss (Fig. 6), and virus replication (Fig. 7) compared with challenged groups.

Discussion

There is currently no specific vaccine and/or treatment for RSV infection. Although prevention strategy using a humanized monoclonal antibody has been developed, less than 3% of the high risk infants have access to this kind of prevention. The burden of disease disproportionately affects low-income countries, provides rationale for a safe and economical vaccine development in the target population aimed at preventing disease [12]. Since RSV infection occurs in a complicated immunopathogenesis fashion [19], a better understanding of the immune mechanisms operative in primary, secondary, and vaccine enhanced illness will be required to identify novel approaches to induce safe and long-lasting immunity to RSV infection that such a FI-RSV scenario never be repeated.

Opioid system have been found to have many physiological and immunological effects that influence the pathogenesis of infectious diseases since subpopulation of opioid receptors are found in many tissues with diverse density [15]. We have previously demonstrated that the
The functional variant OPRM1 A118G (Asn40Asp) in the OPRM1 gene is associated with clinical severity of RSV infection in infants, and that opioid receptors are implicated in the severity of disease in a Balb/c mice model of RSV infection [16]. Here, we investigated the role of opioid receptors in the immunopathogenesis of FI-RSV vaccine, and the ability of nalmefene as a new adjuvant to enhance the efficacy of RSV vaccine. As opioid signalling interacted with immune system and negatively controls the immune responses exploring the role of opioid system in RSV vaccine-enhanced infection may be beneficial to maintain immune homeostasis.

Fig. 2 BAL cell analysis. Female Balb/c mice (n=36/ six group) were intramuscularly immunized with FI-RSV or FI-RSV + NL at day 0 and boosted 3 weeks later. Mice were infected (day 42) intranasally with RSV-A2 (3×10^6 PFU) or PBS and injected daily (until day 5 after infection) with nalmefene (NL) at 1 mg/kg or PBS intraperitoneally. a Total BAL fluid cell count, b differential cell count, c absolute number of lymphocytes (d), monocytes (e), neutrophilic granulocytes (f) and, Eosinophil of BAL fluid cells were determined on day 5 after infection using light microscope. Bars represent mean ± SEM. A t test was used to compare differences between NL-treated and corresponding control groups (*p<0.05)
The fact that opioid antagonists induce immune responses toward a Th1 pattern makes them as a new adjuvant candidate in the induction of cellular immunity against intracellular parasites [20–23]. Our results showed that the administration of nalmefene as a universal opioid receptors blocker, when used in combination with the FI-RSV vaccine, decreased the vaccine enhanced infection. The decreased vaccine enhanced infection was associated with
the reduction of the immune cells influx to the BAL fluid, caused a diminished the lung lesions. Importantly, the nalmefone administration with FI-RSV vaccine inhibits the Th2 responses as measured through decreased the level of IL-5, IL-10, and MIP-1α. As mentioned above, the immunological causes of FI-RSV pathogenesis is mainly linked to inappropriate Th2-polarized responses and nalmefone in combination with FI-RSV can modulate Th cytokine patterns. These observations are partially in accordance with the results reported by previous studies suggesting that the administration of other opioid antagonists (naloxone and naltrexone) in the context of microbial vaccines can inhibit shifting immune responses toward inappropriate Th2-primed responses [20–23]. Nevertheless, it should be noted that the opioid antagonists represent distinct affinity for each opioid receptor subtype (MORs, DORs, and KORs), and that in contrast to naloxone and naltrexone, nalmefone has been shown to play a dual role (antagonist and agonist, or partial agonist) at KORs, which may exert various consequences in immune responses [24]. It is possible that short time administration of nalmefone in combination with vaccine and, therefore, the presence of this opioid antagonist in antigen presenting microenvironments, indirectly interferes with later immune signaling in previously activated immune cells and impacts on immune response pattern. One possible explanation for this finding is on the basis of following evidence; the endogenous opioid beta-endorphin (BE) plays a role in the Th1-Th2-type response switch [25]. Hence, it is possible that nalmefone inhibits shifting immune responses toward Th2-polarized responses, via antagonizing BE effects. However, more research is needed as future studies to fully determine the molecular mechanisms behind these observations.

Blocking of opioid receptors during RSV infection in FI-RSV mice group had no significant effect on RSV immunopathogenesis. These results are against our previous experiences during RSV primary infection, which showed that blocking of opioid receptors during primary RSV infection

Fig. 4 Cytokine/chemokine assay. Chemokines and cytokines concentrations were determined in BAL fluid supernatants, collected on day 5 after RSV infection, using enzyme-linked immunosorbent assay. Female Balb/c mice (n = 36/ six group) were intramuscularly immunized with FI-RSV or FI-RSV + NL at day 0 and boosted 3 weeks later. Mice were infected (day 42) intranasally with RSV-A2 (3 × 10⁶ PFU) or PBS and injected daily (until day 5 after infection) with nalmefone (NL) at 1 mg/kg or PBS intraperitonealy. a IFN-gama, b MIP-1 alfa, c IL-10, d IL-5. Bars represent mean ± SEM. A t test was used to compare differences between NL-treated and corresponding control groups (*p < 0.05)
Fig. 5 Histological analysis. Female Balb/c mice (n = 36/six group) were intramuscularly immunized with FI-RSV or FI-RSV + NL at day 0 and boosted 3 weeks later. Mice were infected (day 42) intranasally with RSV-A2 (3 x 10⁶ PFU) or PBS and injected daily (until day 5 after infection) with nalmefene (NL) at 1 mg/kg or PBS intraperitoneally. a Representative slides of hematoxylin and eosin-stained lungs were analyzed and scored on day 5 after infection. b Pathology scores percentage for each group are shown. Bars represent mean ± SEM. A t test was used to compare differences between NL-treated and corresponding control groups.
by nalmefene enhanced BAL cellular influx, and exaggerated lung pathology [16]. It seems that blocking of opioid receptors in primary infection and vaccine (FI-RSV)-mediated illness has different effect on behavior of later immune responses and pattern of cytokines. It was also shown that TLR9-induced signaling during FI-RSV immunization reduced vaccine-enhanced disease whereas immunostimulatory properties of TLR agonists enhanced disease severity when used during RSV infection [26].

We demonstrated that blocking of opioid receptors during RSV infection in vaccinated mice adjuvanted with nalmefene (FI-RSV + NL-RSV + NL) increased influx of immune cells to the BAL fluid, increased the level of IL-5, IFN-γ, and MIP-1α, and enhanced lung pathology. This finding is in agreement with our previous study [16]. One possible mechanism of nalmefene effects during RSV infection is due to the inhibition of anti-inflammatory and immunomodulatory action of endogenous opioids by blocking opioid receptors. This inhibition would enhance immune cells influx and inflammatory milieu through a direct effect on innate immune cells, such as monocytes, macrophages and dendritic cells [22]. As proposed by Kaneider et al., another possible mechanism is that nalmefene administration may increases the release of local pro-inflammatory neuropeptides, such as substance P (SP) that stimulates the maturation and migration of immune cells to the local draining lymph nodes and triggers inflammation [27]. Additionally, studies have shown that opioid agonists can transdeactivate chemokine receptors through the activation of opioid receptors, during a process, which is known as ‘Heterologous Desensitization’ [28]. In this way, it is possible that, as a potential mechanism, nalmefene administration during RSV infection in vaccinated mice, leads to antagonizing these effects and, therefore, causes to increased influx of immune cells. Furthermore, it has been demonstrated that opioid receptors blocking reduces regulatory T lymphocytes in Balb/c mice [29].

Investigations indicate that opioid antagonists function occurs in a dose- and time-dependent manner [16, 30]; for instance, in a study, administration of different doses of naloxone led to dose-dependent biphasic effects [30]. Hence, it seems likely that the different action of nalmefene in combination with vaccine (agonist like action) and during RSV infection (classic antagonist action) depends at the time of exposure to nalmefene. In combination with vaccine, nalmefene used only on the time of vaccine immunization, while during RSV infection it used daily up to 5 days after challenge. However, more research is needed to fully determine the molecular mechanisms underlying mysterious action of nalmefene. Also the role of other system such as cannabinoid and their receptors in these system will be interesting. As opioid and cannabinoid receptors mediate overlapping pharmacological responses, and interact directly
when coexpressed in the same cells, therefore, functional interactions between them during antigen presentation will be interesting to be studied new adjuvant discovery [31–33].

In conclusion, our results clearly indicate that: (1) nalmefene administration in combination with FI-RSV vaccine decreases the vaccine enhanced infection, (2) nalmefene administration as a new adjuvant candidate in unfavorable FI-RSV vaccine inhibits the shift of immune response toward Th2 pattern, (3) opioid receptor blocking during RSV infection aggravated the host inflammatory response to RSV infection. Thus, targeting of opioid system is potentially attractive to develop effective therapies. These findings may suggest the value of adopting a broad view when considering the desired pharmacology of an immune therapeutic based on the opioid receptors. The next step would be more manipulation of the opioid receptors to provide more extensive data, such as using selective and specific opioid receptors antagonists and agonists and revealing underlined mechanisms. Although there is limited literature regarding the adjuvancy of nalmefene, based on our findings using more recent vaccines would offer more benefits.

Acknowledgements This work was supported by Iran National Science Foundation (No.88000497).

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References


