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DNA vaccine-encoded glycoprotein B of HSV-1 fails to protect chronic morphine-treated mice against HSV-1 challenge

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Abstract

The use of morphine has been demonstrated to increase susceptibility to infections. Herpes simplex virus type 1 (HSV-1) is a highly successful pathogen among immunocompromised individuals. In the present study, due to the importance of HSV vaccination in morphine abusers, the effects of chronic morphine exposure on the host response to a HSV-1 gB DNA-based vaccine have been investigated. The study is addressing an important aspect of vaccine development among the susceptible (immunocompromised) hosts. BALB/c mice were exposed to morphine over 11 days. They were then vaccinated with DNA vaccine or KOS strain as a live vaccine. The findings showed that the morphine-treated animals failed to respond to DNA vaccination evaluated by the anti-HSV gB antibody titer, delayed type hypersensitivity (DTH) and lethal HSV-1 challenge. Under the same conditions, the KOS vaccine showed a reduced

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Ab titer and DTH response in morphine-treated mice, but could protect mice against the lethal challenge and was safe for vaccination of morphine-treated animals.

Keywords: Morphine; Herpes simplex virus; DNA vaccine

Résumé

L'utilisation de la morphine a été démontrée à la susceptibilité d'augmentation aux infections. Virus de simplex d'herpès que le type 1 est un microbe pathogène fortement réussi parmi immunocompromised des individus. Dans la présente étude due à l'importance de la vaccination de HSV dans des trompeurs de morphine les effets de l'exposition chronique de morphine sur la réponse de centre serveur à un vaccin HSV-1 gB ADN-basé par ont été étudiés. L'étude adresse un aspect important du développement vaccinique parmi (immunocompromised) les centres serveurs susceptibles. Des souris de BALB/c ont été exposées à la morphine plus de 11 jours. Elles ont été alors vaccinés avec le vaccin d'ADN ou la contrainte de KOS comme vaccin de phase. Les résultats ont prouvé que les animaux morphine-traités n'ont pas répondu à la vaccination d'ADN évaluée par le titre d'anticorps de gigaoctet d'anti-HSV, le type retardé l'hypersensibilité (DTH) et le défi HSV-1 mortel. À la même condition, le vaccin de KOS a montré un titre réduit d'ab et la réponse de DTH dans les souris morphine-traitées, mais a pu protéger des souris contre le défi mortel et était sûr pour la vaccination des animaux traités par morphine.

Mots Clés: Morphine; Virus de simplex d'herpès; Vaccin d'AND

1. Introduction

Clinical observations of increased susceptibility to infectious diseases among opioid users have long suggested that opioid use alters immune status [1–3]. In animal models, in vivo administration of morphine has been demonstrated to suppress specific immune responses. Morphine has been shown to increase susceptibility to microbial infection [4,5]. Regarding the higher susceptibility of drug abusers to infectious diseases and the importance of vaccination of this population [6], it is of interest to evaluate the immunologic response of opiate addicts to different types of vaccine. There are several potential advantages to the use of plasmid DNAs as vaccines in addition to the ease of their manipulation and preparation. DNA vaccines have the potential ability to stimulate both cell-mediated and humoral immunity. On the other hand, DNA vaccines have perceived safety advantages over the use of live virus [7–9]. In spite of these known advantages, the potency of DNA immunization in morphine addicts remains to be investigated.

Herpes simplex virus type 1 (HSV-1) causes a wide range of diseases such as orolabial infections, pharyngitis and keratoconjunctivitis in humans [10]. In newborn and immunocompromised individuals, this infection may be severe and cause fatal encephalitis [11–13]. The risk of HSV infection as an opportunistic agent in

immunocompromised individuals has stimulated great efforts to achieve an effective vaccine for HSV-1 infections [14].

In the present study, the DNA construct encoding HSV-1 glycoprotein B (gB-1) as a suitable candidate for vaccination was used to immunize morphine-treated BALB/c mice and the results were compared with those immunized with the non-virulent KOS strain of HSV-1. This study examines the ability of gB DNA vaccine to induce immunity in morphine-addicted mice and compares with that induced by the KOS strain live vaccine in this animal model.

2. Materials and methods

2.1. Cell and virus

Vero cell line was used for propagation of viruses. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Wild HSV-1 was isolated from a cold sore lesion of a patient. The virus was confirmed as HSV-1 with an HSV-1 specific monoclonal antibody [15]. The neurovirulence of the virus was proved by injection of the virus into mice and isolation of the virus from brains of dead mice. Wild HSV-1 and KOS strains were grown on Vero cells, titered and stored at −70 °C.

2.2. Mice

Six- to eight-week-old female BALB/c mice were obtained from the Razi Institute (Karaj, IRI). Mice were given free access to food and water, housed for 1 week before the experiment, and maintained in a light/dark cycle with lights on from 0600 to 1800 h. All experiments were done according to the Animal Care and Use Protocol of Tarbiat Modares University.

2.3. DNA vaccine

Plasmid DNA encoding HSV-1 gB was constructed by insertion of the gB-1 gene into pcDNA3 under the control of the CMV promoter as described [16].

2.4. Chronic morphine treatment

Morphine sulfate (TEMAD.IRI) dissolved in normal saline at a concentration of 50 mg/kg was injected subcutaneously into each mouse every day for 11 days before injection of vaccines and during the experimentation [17]. Chronic morphine dependency was confirmed with the withdrawal of $\frac{1}{5}$ mice through intraperitoneal injection of 5 mg/kg naloxone. Symptoms of withdrawal such as diarrhea and jumping were observed. Withdrawn mice were removed from the experiment. Control groups received normal saline with a similar regimen.

2.5. Immunization

On the 11th day of morphine administration, BALB/c mice were injected intramuscularly with 100 μg of DNA or intradermally with 10^5 pfu live KOS (the groups are called gB-Mor and KOS-Mor, respectively). Phosphate-buffered saline (PBS) was injected to the third group of morphine-treated mice as a negative control of vaccines (PBS-Mor group). The vaccination was repeated twice at intervals of 7 days in DNA-immunized groups. Saline-treated groups were injected with the same protocol (gB-Sal, KOS-Sal and PBS-Sal groups).

2.6. Antibody assay

Blood samples were collected 21 days after the primary inoculation by tail bleeding. Serial twofold dilutions of heat inactivated sera were prepared and mixed with equal volumes of DMEM containing 100 $TCID_{50}$ of virus for 1 h at 37 °C. The mixtures were inoculated into the Vero cells cultured in 96-well plates (NUNC, Denmark). The formation of CPE was followed for 3 days. The highest dilution of each serum that neutralized HSV-1(KOS) was taken as the serum titer.

2.7. Delayed type hypersensitivity (DTH) assay

Virus suspension containing 10^5 pfu KOS strain was UV-inactivated and injected into the right footpad of each mouse in 100 μl volumes. Vero cell extract was injected into the left footpad as a negative control. The footpad thickness was measured with a dial caliper (Germany) after 48 h and the results were expressed as the mean percentage increase (the mean increase of five animals) in the footpad thickness. The results were calculated according to the following formula: [(thickness of right footpad challenged with inactive KOS)−(thickness of left footpad challenged with Vero extract)] × 100/(thickness of left footpad challenged with Vero extract).

2.8. Intraperitoneal challenge

One month after starting the vaccination, mice were challenged with 4 MLD_{50} (1×10^5 pfu) of wild virus, which is the minimum dose that causes 100% mortality in unvaccinated mice. The mortality rate was followed for 2 weeks.

2.9. Statistical analysis

Antibody titer and DTH response were analyzed by one-way ANOVA test. Student's *t*-test was used for animal weight. Kaplan–Meier analysis and the log rank test were used for survival rate. Values of $P < 0.05$ were considered to be significant.

3. Results

3.1. Chronic morphine treatment reduces weight gain

The body weight was measured at days 0 and 28 in chronic morphine-dependent mice ($n = 15$). Mice treated chronically with morphine displayed a reduced weight gain ($3.7(\pm 0.62)$ g) compared to that of saline-treated groups ($9.9(\pm 0.86)$ g; $P < 0.0001$).

3.2. Antibody titer decreases significantly in morphine-treated mice immunized with gB-1 DNA vaccine

To determine the effect of gB DNA or live virus (KOS) immunization on induction of humoral response in morphine-treated and saline-treated mice, neutralizing antibody (Ab) titer was measured 21 days after immunization. Among the KOS and gB-1 vaccinated groups, the gB-Mor group showed significantly less Ab titer (gB-Sal $P = 0.003$, KOS-Sal $P = 0.0001$ and KOS-Mor $P = 0.008$). None of the PBS control groups showed an Ab titer. Table 1 shows the mean neutralizing antibody titer in each group.

3.3. Morphine-treated mice fail to induce DTH response

To determine antigen-specific, cell-mediated responses in vivo, the DTH reaction was measured 25 days after the primary inoculation. While a significant DTH response was induced by gB or KOS in saline-treated mice compared with the PBS

Table 1
Antibody response in virus- and DNA-immunized mice[a]

Immunization	No. of responder	Neutralization titer ($\log_{10}$)
gB	5/5[b]	1.35 ± 0.3[c]
gB + Mor	5/5	0.87 ± 0.16
KOS	5/5	1.65 ± 0.21[d]
KOS + Mor	5/5	1.23 ± 0.16[e]
PBS or PBS + Mor	0/5	$\leqslant 0.15$

[a]BALB/c mice immunized with 100 μg DNA, with 10^5 pfu live HSV-1 (KOS), or PBS. The vaccination was repeated two times at 7 days intervals in DNA immunized groups. Means of serum neutralizing antibody titers ($n = 5$) were measured using individual serum collected after 21 days of the post primary inoculation.

[b]Minimal titer of antibody response is equivalent to half dilution of serum that neutralized 100 $TCID_{50}$ of HSV-1.

[c,d]Significantly different values obtained from saline-treated gB-1 or KOS immunized mice in comparison with morphine-treated gB-1 immunized mice (gB-1 $P = 0.003$ and KOS $P = 0.0001$).

[e]Significantly different values obtained from morphine-treated KOS immunized mice in comparison with morphine-treated gB-1 mice ($P = 0.008$).

Table 2
Development of DTH in DNA or HSV immunized mice[a]

Immunization	Mean ± SD
gB	21.80 ± 3.29[b]
gB + Mor	7.03 ± 2.40[c]
KOS	33.28 ± 8.37[d]
KOS + Mor	11.39 ± 4.55[e]
PBS	3.30 ± 2.40
PBS + Mor	2.80 ± 2.20

[a]Each group consisting of five mice was immunized with DNA, 10^5 pfu HSV-1 (KOS), or PBS-as negative control. For DTH assay each mouse was injected with either 10^5 pfu of UV-inactivated HSV-1 (titered before inactivation) in right footpad or Vero extract on the left footpad.

[b,d]Significantly different values obtained from saline-treated gB-1 or KOS immunized mice in comparison with PBS ($P = 0.0001$).

[c,e]Not significantly different values obtained from morphine-treated gB-1 or KOS immunized mice in comparison with PBS ($P > 0.05$).

immunized groups ($P < 0.0001$), none of these scheduled vaccines could induce the DTH reaction in morphine-treated mice ($P > 0.05$) (Table 2).

3.4. Chronic morphine-dependence decreases protective immunity against HSV-1 challenge

To determine the effect of morphine-dependence on protective immunity induced by gB-1 DNA or KOS, mice were challenged with 4 MLD_{50} of wild HSV-1 and the survival rate was recorded for 14 days. Fig. 1 shows the survival rate of vaccinated mice after HSV-1 challenge. A significant decrease in resistance to virus challenge was recorded in the gB-Mor group compared with the gB-Sal or KOS-Sal group (gB-Sal $P = 0.044$ and KOS-Sal $P = 0.005$) (Table 3). Although the KOS-Mor vaccinated group in comparison with the gB-Sal or KOS-Sal vaccinated mice showed less protection against HSV-1 challenge, but the difference was not statistically significant ($P > 0.05$) (Table 3).

4. Discussion

Long-term abuse of morphine modulates functions of the immune system and increases susceptibility to infections [18]. Although there is a report of enhancement of protectivity against HSV-1 in morphine-treated mice [19], recent studies have shown that morphine increases HSV infections in BALB/c mice [20,21] and humans [22,23]. It seems to be dependent mainly on the species of animal, dosage, route of challenge and timing of opioid administration. Among the various types of vaccines designed for prevention of infection, live vaccines have been suggested to be more successful in induction of effective immunity, but they carry greater risk for immunocompromised individuals [7]. DNA vaccines may well be safer for

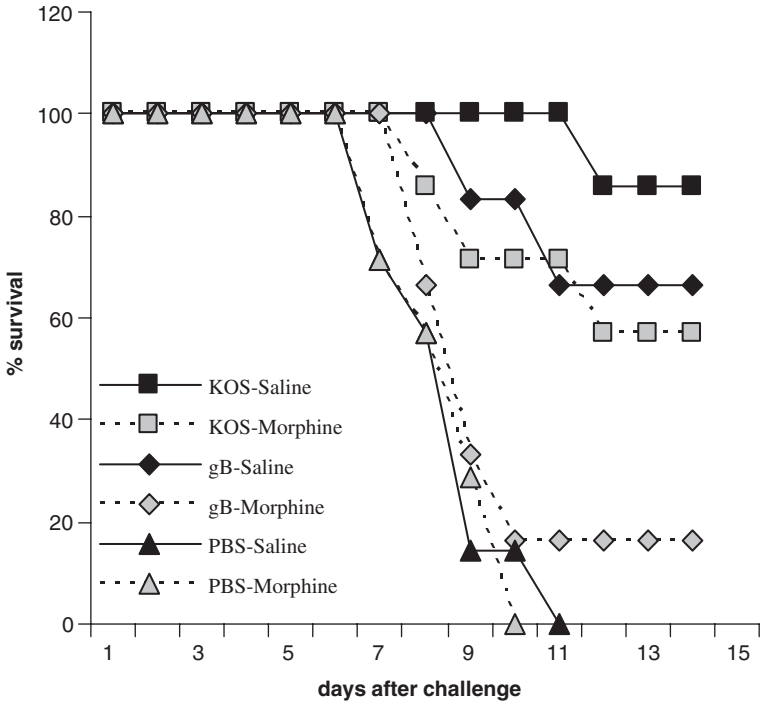

Fig. 1. Survival of chronic morphine-treated mice immunized with HSV-1 (KOS), gB-1 DNA or PBS (KOS-Morphine, gB-Morphine, PBS-Morphine) and saline-treated mice (KOS-Saline, gB-Saline, PBS-Saline) followed by lethal challenge with wild type HSV-1. One month after beginning of immunization, mice were challenged with 4 MLD_{50} of wild HSV-1 and survival rate was recorded daily for 2 weeks.

immunocompromised subjects than attenuated viral infection. In the present study, gB-based DNA vaccine was compared to live attenuated HSV-1 vaccine to protect long-term morphine-treated mice against the wild-type HSV-1. To achieve the best results for each vaccine, the optimum dose of injection that has been reported to be more effective was used [24,25]. The daily dose of morphine for addiction of mice was also chosen on the basis of previous data [17]. Chronic morphine-dependence was confirmed as described in Materials and methods. The data revealed that vaccination of chronic morphine-treated mice with gB-1 DNA vaccine could not produce significant humoral or cellular immune responses, and failed to protect mice against the lethal challenge with wild-type HSV-1. In the KOS-Mor group, in spite of reduced DTH and Ab response, they could resist the HSV-1 challenge. These findings can be explained through the knowledge of the immune cells involved in induction of protective immunity by DNA and live attenuated vaccines. B-cell proliferation and antibody production are suppressed in morphine-administrated mice [26]. This suppressive effect decreases or inhibits the humoral response, as the present data showed a decrease of neutralizing antibody in morphine-treated animals. The previous data have suggested that, for an effective role of DNA

Table 3
Resistance to acute HSV challenge in virus- and gB DNA-immunized mice[a]

Immunization	No. of mice survived/ no. of mice challenged	Average time of death ± SD (days)	Percent of survival (%)
gB	4/6	12.50 ± 0.9[b**]	66.67
gB + Mor	1/6	9.25 ± 0.91[e]	16.67
KOS	6/7	13.71 ± 0.26[c***]	85.71
KOS + Mor	4/7	12.00 ± 0.99[d*]	57.14
PBS	0/6	8.07 ± 0.48	0.0
PBS + Mor	0/6	7.92 ± 0.48	0.0

$^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.0001$ compared to saline- or morphine-treated PBS immunized mice.

[a]These data show means of survival rate for each group challenged with acute HSV-1.

[b,c]Significantly different values obtained from saline-treated gB-1 or KOS immunized mice in comparison with morphine-treated gB-1 immunized mice (gB-1 $P = 0.044$ and KOS $P = 0.005$).

[d]Not significantly different values obtained from morphine-treated KOS immunized mice in comparison with saline-treated gB-1 or KOS immunized mice ($P>0.05$).

[e]Not significantly different values obtained from morphine-treated gB immunized mice in comparison with saline- or morphine-treated PBS immunized mice ($P>0.05$).

vaccines, they need to have functional activity of many components of the immune system, including T CD4+, T CD8+, B cells and T γ/δ cells [24]. Manikan et al. have shown that the mechanism of protection by the gB DNA vaccine would appear to mediate by the function of CD4+ T cells and with a type 1 cytokine profile [25]. In the other report, CD4+ T cell knock-out mice failed to produce a suitable response to the DNA vaccine [24]. Morphine partially suppresses T cell activity [3] and alters the T_{H1} phenotype to T_{H2} [27]. The DTH reaction, representing antigen-specific CD4+ T cells and macrophage activity, is suppressed in morphine-administered mice [28]. In our experiment, morphine caused no response to the DTH assay, which implies the suppression of CD4+ T cell activity by morphine. In the case of the KOS vaccine, it has been shown that in T and B cell knock-out mice, KOS vaccination induces mortality before the wild-type HSV-1 virus challenge, and it is not safe for immunization [24,29]. However, our study has shown that it was safe for vaccination of morphine-treated mice and no mortality was recorded before wild HSV-1 challenge. Previous reports have shown that the lack of CD4+ T cells did not impair KOS protectivity. The authors concluded that when KOS is used for vaccination, CD4+ T cells do not have a critical role in induction of protective immunity against HSV-1 [24]. Furthermore, there are some reports suggesting that KOS or DNA vaccine in CD8+ T cell knock-out mice could not protect them against HSV-1 lethal challenge [24,30]. They concluded that an effective DNA vaccine needs the function of many immune cells including CD8+ and CD4+ T cells while, a live attenuated HSV-1 vaccine like KOS can confer protection at least in the absence of functional CD4+ T cells. Carpenter and Carr have suggested that morphine suppresses only 25–30% of CD8+ T cells [17]. Thus, in the present study it seems that the partial activity of CD8+ T cells has been enough to confer KOS

protection. Whereas, in order to induce effective immunity, besides the CD8+ T cells, DNA vaccines need CD4+ T cells (the cells that are impaired almost completely in production of T_{H1} cytokines and proliferation functions in morphine-dependent animals).

In conclusion, the present data suggest that the gB-1 DNA vaccine with the protocol of immunization described here is not able to protect morphine-treated mice against acute virus challenge, at least at the high doses of morphine administration. This finding confirms the recent data for a chimeric DNA vaccine that could partially overcome the immunosuppressive effect of morphine but it failed in the prevention of tumorgenicity with a high dose of morphine administration [31]. This information is new in the field of genetic immunization for infectious diseases such as AIDS that are more prevalent among drug abusers. Including T_{H1} cytokine genes on DNA constructs, production of chimeric DNA vaccines and employment of other DNA immunization protocols may improve the vaccine potency and will be investigated in future studies.

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