Article

Acute Morphine Administration Reduces Cell-Mediated Immunity and Induces Reactivation of Latent Herpes Simplex Virus Type 1 in BALB/c Mice

Shafi Mojadadi[1], Abbas Jamali[1, 2, 4], Behzad Khansarinejad[2, 3], Hoorieh Soleimanjahi[2] and Taravat Bamdad[2]

Acute morphine administration is known to alter the course of herpes simplex virus infection. In this study, the effect of acute morphine administration on the reactivation of latent herpes was investigated in a mouse model. Because of the important role of cytolytic T lymphocyte (CTL) activity in the inhibition of herpes simplex virus type 1 (HSV-1) reactivation, the effect of acute morphine administration on CTL responses was also evaluated. Furthermore, lymphocyte proliferation and IFN-γ production were evaluated for their roles in the induction of the CTL response. The findings showed that acute morphine administration significantly reduced CTL responses, lymphocyte proliferation, and IFN-γ production. Furthermore, acute morphine administration has been shown to reactivate latent HSV-1. Previous studies have shown that cellular immune responses have important roles in the inhibition of HSV reactivation. These findings suggest that suppression of a portion of the cellular immune response after acute morphine administration may constitute one part of the mechanism that induces HSV reactivation. *Cellular & Molecular Immunology*. 2009;6(2):111-116.

Key Words: acute morphine, herpes simplex virus-1 reactivation, cell-mediated immunity

Introduction

Herpes simplex virus type 1 (HSV-1) is an infectious agent that causes a variety of diseases worldwide (1). Natural infection with HSV activates both T and B lymphocytes and induces specific humoral and cellular immune responses (2). Following primary infection in the eye, the virus establishes latency in the trigeminal ganglia, from which it periodically reactivates and causes recurrent episodes of corneal inflammation, known as herpetic stromal keratitis (HSK) (3).

It has been proven that morphine alters the course of HSV-1 infection (4-6). Our previous work has shown that acute morphine administration increases mortality during HSV-1 infection in BALB/c mice (7). In humans, morphine administration induces reactivation of latent herpes simplex labialis (8). Recurrent HSV-1 ocular disease results from reactivation of the latent virus in the trigeminal ganglia, often following immunosuppression or exposure to a variety of psychological or physical stressors. For example, epinephrine iontophoresis, cyclophosphamide, and dexamethasone (Dex) administration, UV irradiation, and transient hyperthermia induce reactivation of the virus in animal models (9).

The association between stress and HSV-1 reactivation is well established in mice, but a specific mechanism by which stress facilitates HSV-1 reactivation has not been elucidated (10). Several factors, such as corticosteroids, IL-6, and reduction of cell-mediated immunity, especially $CD8^+$ T cells, have been shown to have important roles in HSV-1 reactivation (11). However, in this study we only evaluated the effect of acute morphine on cellular immune responses. Cellular immune responses, especially HSV-specific memory cytolytic T lymphocyte (CTL) activation, have critical roles in the control of HSV replication and reactivation (10). Suppression of specific cytokines, such as IFN-γ, has previously been shown to reduce HSV-specific memory CTL activation in the spleen (12, 13).

In vivo administration of morphine is known to affect immune responses by altering lymphocyte proliferation, antibody responses (14), and T lymphocyte-mediated cytoto-

[1]Neuroscience research center, Kerman University of Medical Sciences, Kerman, Iran;

[2]Department of Virology, Faculty of medical science, Tarbiat Modares University, Tehran, Iran;

[3]Dept of Microbiology, Arak University of Medical Sciences, Tehran, Iran;

[4]Correspondence to: Dr. Abbas Jamali, Dept of virology, School of medical sciences, Tarbiat Modares University, P.O. Box 14115-111, Tehran, Iran. Tel: +98-21-8801-1001 ext 4525, Fax: +98-21-8801-3030, E-mail: Jamali2004@gmail.com or jamali@modares.ac.ir

Received Sep 25, 2008. Accepted Mar 3, 2009.

xicity (15), influencing the delayed-type hypersensitivity reaction, and decreasing the production of cytokines, such as IFN-γ and IL-12 (16, 17). Based on morphine's similar effects on the immune system, the present study was conducted to determine whether acute morphine administration could induce the reactivation of latent herpes simplex virus 1 in a mouse model.

Materials and Methods

Cells and viruses

The 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 by IFA using an HSV-1 specific monoclonal antibody (18). The neurovirulence of the virus was confirmed by injection of the virus into mice and subsequent isolation of the virus from the brains of dead mice. Wild HSV-1 and KOS (non-virulent strain) were grown on Vero cells, titered, and stored at -70ºC.

Infection and treatment of mice

Six to eight-week-old female BALB/c mice were obtained from the Pasteur Institute of Iran (Karaj, Iran). Animals were handled in accordance with the Animal Care and Use Protocol of Tarbiat Modares University. The mice were anesthetized by an intraperitoneal injection of 100 mg/kg ketamine (Parke-Davis, Pontypool, UK) and 10 mg/kg xylazine (Bayer, Bury St, Edmunds, UK); each cornea was scratched 10 times with a 26 gauge needle. The corneas of mock-inoculated mice were treated with DMEM medium (Gibco BRL) containing fetal calf serum, and the corneas of the test group were treated with 3 μl medium containing 5×10^4 plaque forming units (pfu) of wild-type HSV-1; the mice were monitored to determine the success of the infection, as previously described (19). Thirty days after infection, when latency had been established (20), one group of mice received 75 mg/kg morphine subcutaneously (HSV-Morphine group), while the control mice received saline (HSV-Saline group). A third group of mice was hyperthermically stressed (HSV-Hyper group) by a published protocol (19). Briefly, mice were placed in 50 ml restraining tubes with 5 mm diameter holes drilled throughout each tube. The mice were gently placed into the water bath at 43°C. The mice were situated such that the water level did not exceed the neck region, so no physical effort was required by the animal to remain above the water level. However, the mice were constrained in the tubes, which limited their movement, suggesting some degree of restraint. Following a 10-min bath, the mice were removed, gently blotted with paper towels, and placed in a warm room (34°C) for 30 min to prevent hypothermia.

Isolation of virus from eye washings

Immediately before heat shock or acute morphine administration and three days after treatment, the mice were anesthetized and eye washings were collected and placed onto Vero cells for the isolation of the virus. These cultured cells were examined for signs of viral reactivation and cytopathic effects (CPEs) for up to 7 days. Then, cells which displayed CPE were analyzed by PCR to determine whether the HSV-1 genome was present.

Extraction of DNA from HSV-1 infected cells

The cells were harvested and subjected to freeze-thawing three times. Equal volumes of the harvested samples and lysis buffer [0.1 M Tris-Cl (pH 8.0), 0.01 M EDTA, and 1% sodium dodecyl sulfate containing 28.5 μl of proteinase K (20 mg/ml)] were incubated at 56°C for 4 h. The tubes containing the mixture were vortexed every 30 min. The DNA solution was extracted three times with phenol-chloroform and once more with chloroform, and then precipitated with ethanol overnight. After centrifugation, DNA was dissolved in 20 μl of double-distilled water (DDW) (21).

Polymerase chain reaction

The primer set used in this study (5'-TAC CCG AGC CGA TGA CTT AC-3' (forward) and 5'-GCG CTT GTC ATT ACC ACC GC-3' (reverse)) has been described by Mitchell et al. and amplifies a 130 bp fragment of the thymidine kinase (TK) gene of HSV-1 (22). Amplification reactions were performed in 25 μl volumes containing the extracted genomic DNA, 2.5 μl of 1× PCR buffer (10 mM Tris-Cl (pH 8.3), 50 mM KCl, 0.01% gelatin), 1.5 mM $MgCl_2$, 200 μM each deoxyribonucleotide triphosphate, 2 U Taq polymerase (MBI Fermentas, Hanover, MD), and 0.5 μM each primer. The amplification profile consisted of a single cycle of initial denaturation at 94°C for 4 min, followed by 34 cycles each consisting of denaturation at 94°C for 35 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. The amplified products were analyzed by gel electrophoresis on a 2% agarose gel containing 0.5 μg/ml ethidium bromide and were visualized by UV light.

CTL assay

Twelve hours after acute morphine administration, splenocyte single cell suspensions were prepared without *in vitro* stimulation. The WEHI-164 target cells were infected with 5 Multiplicity of Infection (MOI) HSV-1 (KOS) for 4 h and washed three times with the assay medium. CTL activity was measured by the lactate dehydrogenase (LDH) release assay in 96-well round-bottom plates (23). Target cells (2×10^4 cells/well) in a 100 μl volume were incubated with 100 μl of effector cells at various effector/target ratios (10 : 1 or 50 : 1) for 4 h in phenol red-free RPMI 1640 (GIBCO-BRL, UK) medium containing 3% FCS. After centrifugation, the supernatants (50 μl/well) were transferred to the 96-well flat-bottom plates, and lysis of target cells was determined by measuring LDH release using the LDH assay kit (Takara Company), according to the manufacturer's instructions. PBS buffer and a 0.1% Triton X-100 solution in PBS buffer were

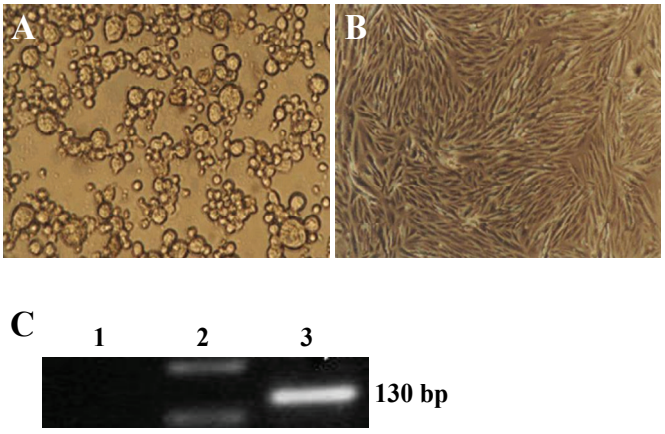

Figure 1. Acute morphine administration induces reactivation of the latent HSV-1. Cytopathic effect (CPE) of Vero cells caused by HSV-1 reactivation after inoculated with eye washings (A) or uninfected (B) for up to 7 days and observed with microscope. (C) Vero cells with or without CPEs were collected for PCR analysis. Lane 1, cell culture without CPE; Lane 2, DNA size marker; Lane 3, cell culture with CPEs (a 130 bp product of the HSV TK gene).

used as controls. The LDH-mediated conversion of the tetrazolium salt into the red formazan product was measured at 490 nm after incubation at room temperature for 30 min. The percentage of specific cytolysis was determined by the following formula: The specific cytolysis (%) = (optical density (OD) of experimental LDH release - OD of spontaneous LDH release of effector cells - OD of spontaneous LDH release from target cells)/(maximum LDH release of target cells - OD of spontaneous LDH release of target cells) × 100%. All determinations were performed in triplicate.

Lymphocyte proliferation assay

The lymphocyte proliferation rate was measured by using the MTT assay. Under sterile conditions, the spleens were removed and single cell suspensions were prepared in phenol red-free RPMI 1640 medium. RBCs were lysed by treatment with 0.75% NH_4Cl in Tris buffer (0.02%, pH 7.2). The concentration was adjusted to 1×10^6 cells/ml in phenol red-free RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, and 25 mM HEPES. One hundred microlitres of diluted cell suspensions were dispensed into 96-well flat-bottom culture plates. Mitogen phytohemagglutinin-A (PHA), at a final concentration of 5 μg/ml (positive control), or 5 MOI of the heat-inactivated KOS strain was added to each well, and the volume was adjusted to 0.2 ml. After incubating for 72 h at 37ºC in a humidified atmosphere containing 5% CO_2, cell proliferation was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (thiazolyl-blue) dye (MTT) assay (24). Briefly, 20 μl MTT was added to each well, and the plates were further incubated at 37°C for 4 h. Following the incubation, the supernatant from each well was aspirated carefully and formazan crystals were solubilized by adding 100 μl DMSO to each well. The absorbance of each well was then determined at a wavelength of 540 nm.

Table 1. Acute morphine administration induces HSV-1 reactivation in mice

Groups	n	Reactivated mice	Percent of reactivation
HSV-Hyper	9	3	33%
HSV-Morphine	9	2	22%
HSV-Saline	9	0	0%
Mock	9	0	0%

Immediately before heat shock or acute morphine administration and on day 3 after such treatment, mice were anesthetized and eye washings were taken and put onto Vero cells for the isolation of viruses. These cultured cells were examined for the signs of viral reactivation, CPEs, for up to 7 days. These results are summaries of two (for Mock group) to three (for HSV-Hyper, HSV-Morphine and HSV-Saline groups) experiments per treatment regimen.

IFN-γ assay

Twelve hours after acute morphine administration, spleens of individual mice were removed aseptically and homogenized in RPMI 1640 medium supplemented with 10% FCS and antibiotics. Red blood cells were osmotically lysed using ammonium chloride buffer (NH_4Cl 0.16 M, Tris 0.17 M). Cells were washed twice with RPMI 1640 medium and counted, and the viability was determined by trypan blue (0.4% w/v) exclusion. A total of 1×10^6 spleen cells (1×10^6 cells per well) were added to each well of a 24-well plate using RPMI 1640 medium supplemented with 10% FCS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 5×10^{-5} M 2-mercaptoethanol. Two wells were considered for each mouse. The cells were restimulated *in vitro* with 5 MOI of heat-inactivated virus. Plates were incubated at 37°C in 5% CO_2, and 48 h after stimulation, the supernatants were removed and kept at -70°C for evaluation of the secreted IFN-γ levels. The concentration of IFN-γ in the supernatants was estimated using a commercial ELISA kit (R&D systems).

Statistical analysis

CTL responses, lymphocyte proliferation, and IFN-γ production were analyzed by one-way ANOVA followed by tukey's test. Values of $p < 0.05$ were considered to be significant.

Results

Acute morphine administration induces reactivation of the latent HSV-1

To determine the effect of acute morphine on HSV-1 reactivation, three days after morphine treatment, mice were anesthetized and eye washings were collected and put onto Vero cells. The results showed that Vero cells inoculated with eye washings displayed CPEs (Figure 1A). The cytopathic Vero cells were analyzed by PCR to determine whether the HSV-1 genome was present. As shown in Figure 1B, a 130

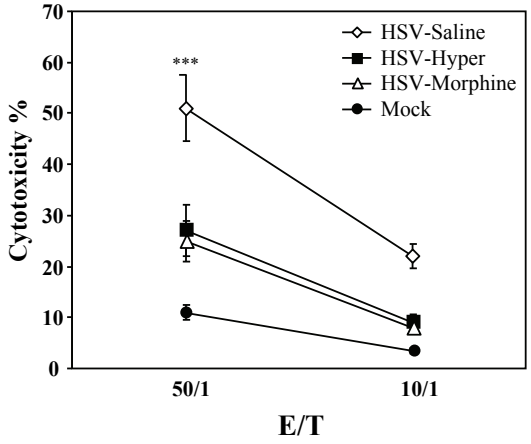

Figure 2. Acute morphine administration reduces CTL responses. Thirty days after HSV infection, mice (5 mice per group) were treated with morphine or exposed to hyperthermic stress. Twelve hours later, splenocyte single cell suspensions were prepared without *in vitro* stimulation. The WEHI-164 target cells were infected with 5 MOI HSV-1 (KOS) for 4 h and washed three times with assay medium. CTL activity was measured by LDH release assay in 96-well round-bottom plates. Values are the mean ± SEM of three experiments. ***$p < 0.0001$, compared to HSV-Hyper and HSV-Morphine groups.

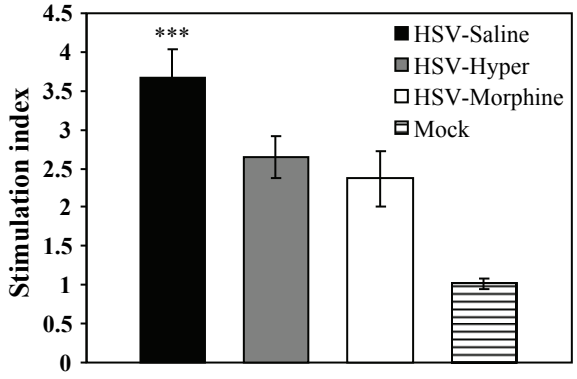

Figure 3. Acute morphine administration reduces lymphocyte proliferation. Thirty days after HSV infection, mice (5 mice per group) were treated with morphine or exposed to hypothermic stress. Twelve hours later, the spleens were removed and lymphocyte proliferation was evaluated by the MTT assay. Values are the mean ± SEM of three experiments. ***$p < 0.0001$, compared to HSV-Hyper and HSV-Morphine groups.

bp product from thymidine kinase (TK) gene of HSV-1 was obtained from cytopathic Vero cells, but not those without CPEs. The results were further analyzed and shown in Table 1. Both hyperthermic stress (33% of mice) and acute morphine administration (22% of mice) induce reactivation of HSV-1, and no reactivation of HSV was found in saline-treated or mock-infected groups.

Acute morphine administration reduces the specific CTL response against HSV-1

Twelve hours after acute morphine administration, the CTL response was examined using the LDH release assay. As shown in Figure 2, evidence of the CTL response was observed in all HSV-1 infected mice compared to the mock-infected mice ($p < 0.001$). However, the CTL response was significantly reduced in mice treated with the morphine or exposed to hyperthermic stress compared to saline-treated mice ($p \leq 0.0001$).

Acute morphine administration reduces lymphocyte proliferation

Since lymphocyte proliferative responses are generally considered a measure of cell-mediated immunity, HSV-1 antigen specific lymphocyte proliferation was evaluated using MTT assay. As shown in Figure 3, all HSV-1-infected mice have significantly higher lymphocyte proliferation rates compared to mock infected mice ($p < 0.05$). However, the lymphocyte proliferation response was significantly reduced in mice which treated with the morphine or exposed to hyperthermic stress compared to saline-treated mice ($p \leq 0.0001$).

Acute morphine administration reduces IFN-γ production

Twelve hours after acute morphine administration, the spleens were removed and IFN-γ levels were evaluated. As shown in Figure 4, all HSV-1-infected mice have significantly higher levels of IFN-γ production compared to mock-infected mice ($p < 0.001$). Furthermore, the results showed that IFN-γ production was significantly reduced in mice treated with morphine or exposed to hyperthermic stress, when compared to saline-treated mice ($p \leq 0.0001$).

Discussion

Previous studies have shown that morphine induces reactivation of the latent herpes simplex virus in humans, but the reactivation mechanism remains to be investigated in human or animal models.

In this paper, we showed that administration of a single dose of morphine after HSV-1 infection modulated some aspects of the cellular immune response and induced reactivation of latent HSV-1 in BALB/c mice. Opiates have been found to have many physiological and immunological effects that influence the pathogenesis of infectious diseases (17). In humans, it has been reported that morphine administration induces reactivation of latent herpes simplex labialis (8). In this paper we showed that acute morphine administration, similarly to hyperthermic stress, reduces CTL responses, lymphocyte proliferation, and IFN-γ production and induces reactivation of latent HSV-1 in a mouse model.

A previous study has shown that the maximum suppression of immune responses occurs 12-48 h after acute morphine administration (25). In this study, we use one single dose of 75 mg/kg morphine, based on previous studies that have shown this dose induces maximum immuno-suppression in mice (7). Richards et al. have shown that HSV reactivation induces cellular immunity 4 to 7 days after

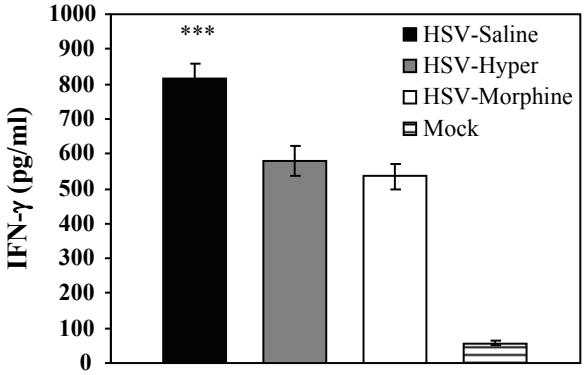

Figure 4. Acute morphine administration reduces IFN-γ production. Thirty days after HSV infection, mice (5 mice per group) were treated with morphine or exposed to hyperthermic stress. Twelve hours later, the spleens were removed and homogenized in RPMI 1640 medium. A total of 1×10^6 spleen cells (1×10^6 cells per well) were added to each well. Plates were incubated at 37°C in 5% CO_2, and 48 h after stimulation, the supernatants were removed for evaluation of the secreted IFN-γ levels. The concentration of IFN-γ in the supernatants was estimated using a commercial ELISA kit (5 mice per group). Values are the mean ± SEM of three experiments. ***$p < 0.0001$, compared to HSV-Hyper and HSV-Morphine groups.

reactivation (26). Therefore, in order to prevent interference between the primary immune response and secondary immunity induced during HSV reactivation, the effects of morphine administration on immune responses to HSV-1 reactivation were evaluated quickly (12 h) after the injection of morphine. So, this time and dose were selected to evaluate the effects of acute morphine administration on immune responses to HSV-1 reactivation in the nearest time after acute morphine administration with maximum of immunosuppression.

Reactivation of HSV-1 from latency may be closely tied to the effect of glucocorticoids on the immune response (26). It is now clear that opioids have a wide array of immunomodulatory effects on the innate and acquired immune systems through the hypothalamic-pituitary-adrenal axis by increasing glucocorticoid levels (13, 14). Glucocorticoids have previously been shown to moderately induce reactivation of latent HSV-1, both *in vitro* (27) and *in vivo* (28). Moreover, there is clinical precedence for herpes virus reactivation following systemic glucocorticoid administration (29, 30). Furthermore, blocking glucocorticoid production during hyperthermic stress has been shown to inhibit reactivation of HSV-1 (11).

This study has shown that both acute morphine administration and hyperthermic stress significantly reduce lymphocyte proliferative responses to HSV, and because the lymphocyte proliferative responses are generally related to cell-mediated immunity, it can be deduced that cell-mediated immune responses to the HSV-1 virus have been suppressed by both, acute morphine administration and hyperthermic stress.

There is a general agreement that morphine administration leads to suppression of several immune parameters (31). Thomas et al. have shown that opioids directly induce suppression of CTL activity when cultured with splenocytes (32). Furthermore, acute morphine administration suppresses IFN-γ production in rats (33). Another study has shown that morphine suppresses CTL activity and IFN-γ production in alloimmunized mice. The authors concluded that morphine exposure suppresses CTL activity though a pathway that may peripherally involve IFN-γ production (34).

In the present study, both acute morphine administration and hyperthermic stress exposure reduce CTL responses. Previous studies have shown that HSV-specific $CD8^+$ T cells are closely associated with latently-infected murine ganglia, and that these cells prevent HSV reactivation *in vitro* (10, 35). It has been reported that $CD8^+$ T cells do not prevent the reactivation of recombinant HSV, but do inhibit murine MHC class I in mice (36). These findings suggest that efficient inhibition of MHC class I by HSV is a key factor in its ability to reactivate in humans. $CD4^+$ T cells and IFN-γ have important roles in the induction of the CTL response to resultant lysis of infected cells and restriction of viral replication (37). IFN-γ can overcome HLA class I down-regulation and the resultant enhancement of the CTL response against HSV-infected cells (38). Hence, reducing IFN-γ production and lymphocyte proliferation through morphine treatment may be one of the mechanisms that induces HSV reactivation.

In conclusion, the results of this study have shown that administration of acute morphine induces reactivation of HSV-1 in a mouse model and may be caused by reduction of a parameter of cell-mediated immunity. However, future studies are needed to evaluated HSV-1 titer during reactivation and inducing clinically evident following acute morphine administration.

Furthermore, the mechanism by which stress facilitates HSV-1 reactivation has not yet been elucidated. Follow-up studies are needed to test other factors, such as IL-6 and other proinflammatory cytokines, that have important roles in HSV-1 reactivation during acute morphine administration.

Acknowledgement

This work was supported by the Neuroscience research center, Kerman University of Medical Sciences.

References

1. Roizman B, Knipe M. Herpes simplex virus and their replication, In: Fields virology, Lippicott Williams & Wilkins. 2001;2391-2510.
2. Whitly R, Roizman B. Herpes simplex virus infections. Lancet. 2001;357:1513-1518.
3. Hill TJ. Ocular pathogenicity of herpes simplex virus. Curr Eye Res. 1987;6:1-7.
4. Lioy DT, Sheridan PA, Hurley SD, et al. Acute morphine exposure potentiates the development of HSV-1-induced

encephalitis. J Neuroimmunol. 2006;172:9-17.
5. Sheridan PA, Moynihan JA. Modulation of the innate immune response to HSV-1 following acute administration of morphine: role of hypothalamo-pituitary-adrenal axis. J Neuroimmunol. 2005;15:8145-8152.
6. Alonzo NC, Carr DJ. Morphine reduces mortality in mice following ocular infection with HSV-1. Immunopharmacology. 1999;41:187-197.
7. Jamali A, Bamdad T, Soleimanjahi H, Pakdel FG, Arefian E. Acute morphine administration reduces white blood cells' capability to induce innate resistance against HSV-1 infection in BALB/c mice. Neuroimmunomodulation. 2007;14:16-23.
8. Davies PW, Vallejo MC, Shannon KT, Amortegui AJ, Ramanathan S. Oral herpes simplex reactivation after intrathecal morphine: a prospective randomized trial in an obstetric population. Anesth Analg. 2005;100:1472-1476.
9. Sainz B, Loutsch JM, Marquart ME, Hill JM. Stress-associated immunomodulation and herpes simplex virus infections. Med Hypotheses. 2001;56:348-356.
10. Freeman ML, Sheridan BS, Bonneau RH, Hendricks RL. Psychological stress compromises $CD8^+$ T cell control of latent herpes simplex virus type 1 infections. J Immunol. 2007;17: 9322-9328.
11. Noisakran S, Halford WP, Veress L, Carr DJJ. Role of the hypothalamic pituitary adrenal axis and IL-6 in stress-induced reactivation of latent herpes simplex virus type 1. J Immunol. 1998;160:5441-5447.
12. Bonneau RH. Stress-induced effects on integral immune components involved in herpes simplex virus (HSV)-specific memory cytotoxic T lymphocyte activation. Brain Behav Immun. 1996;10:139-145.
13. Brenner GJ, Moynihan JA. Stressor-induced alterations in immune response and viral clearance following infection with herpes simplex virus-type 1 in BALB/c and C57BL/6 mice. Brain Behav Immun. 1997;11:9-20.
14. McCarthy L, Wetzel M, Sliker JK, Eisenstein TK, Rogers TJ. Opioids, opioid receptors, and the immune response. Drug Alcohol Depend. 2001;62:111-123.
15. Carpenter GW, Carr DJ. Pretreatmant with β-funaltrexamince blocks morphine-mediated suppression of CTL activity in alloimmunized mice. Immunopharmacology. 1995;291:129-140.
16. Molitor TW, Moilla A, Risdahl JM, Murtaugh MP, Chao CC, Peterson PK. Chronic morphine administration impairs cell-mediated immune responses in swine. J Pharmacol Exp Ther. 1991;260:581-586.
17. Friedman H, Newton C, Klein TW. Microbial infections, immunomodulation, and drugs of abuse. Clin Microbial Rev. 2003;16:209-219.
18. Soleimanjahi H, Roostaee MH, Rasaee MJ, Mahboodi F, Bamdad T. Immunogenecity and efficacy of baculovirus-derived glycoprotein D of herpes simplex virus type-1 in mice. Arch Razi Ins. 2003;55:19-28.
19. Sawtell NM, Thompson RL. Rapid *in vivo* reactivation of herpes simplex virus type 1 in latently infected murine ganglionic neurons after transient hyperthermia. J Virol. 1992; 66:2157-2169.
20. Chen SH, Yao HW, Huang WY, et al. Efficient reactivation of latent herpes simplex virus from mouse central nervous system tissues. J Virol. 2006;80:12387-12392.
21. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning. Cold Spring Harbor Laboratory Press. 2001;6.3-6.62.
22. Mitchell WJ, Gressens P, Martin RJ, DeSanto R. Herpes simplex virus type 1 DNA persistence, progressive disease and transgenic immediate early gene promoter activity in chronic corneal infections in mice. J Gen Virol. 1994;75:1201-1210.
23. Osorio Y, Ghiasi H. Comparison of adjuvant efficacy of herpes simplex virus type 1 recombinant viruses expressing TH1 and TH2 cytokine genes. J Virol. 2003;77:5774-5783.
24. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55-63.
25. Freier DO, Fuchs BA. A mechanism of action for morphine induced immunosuppression: corticosterone mediates morphine-induced suppression of natural killer cell activity. J Pharmacol Exp Ther. 1994;270:1127-1133.
26. Richards CM, Case R, Hirst TR, Hill TJ, Williams NA. Protection against recurrent ocular herpes simplex virus type 1 disease after therapeutic vaccination of latently infected mice. J Virol. 2003;77:6692-6699.
27. Halford WP, Gebhardt BM, Carr DJJ. Mechanisms of herpes simplex virus type 1 reactivation. J Virol. 1996;70:5441-5447.
28. Underwood GE, Weed SD. Recurrent cutaneous herpes simplex virus in hairless mice. Infect Immun. 1974;10:471-474.
29. Bruning AH, Samie MH. Recurrent herpes zoster and high dose inhaled steroids for asthma. S Afr Med J. 1994;84:873.
30. Shane SA, Wollman MW, Claassen D. Herpes simplex dissemination following glucocorticoids for upper airway obstruction in an adolescent girl. Pediatr Emerg Care. 1994;10: 160-170.
31. Eisenstein TK, Rahim RT, Feng P, Thingalaya NK, Meissler JJ. Effects of opioid tolerance and withdrawal on the immune system. J Neuroimmune Pharmacol. 2006;1:237-249.
32. Thomas PT, House RV, Bhargava HN. Direct cellular immunomodulation produced by diacetylmorphine (heroin) or methadone. Gen Pharmacol. 1995;26:123-130.
33. Gomez-Flores R, Suo JL, Weber RJ. Suppression of splenic macrophage functions following acute morphine action in action in the rat mesencephalon periaqueductal gray. Brain Behav Immun. 1999;13:212-224.
34. Scott M, Carr DJ. Morphine suppresses the alloantigen-driven CTL response in a dose-dependent and naltrexone reversible manner. J Pharmacol Exp Ther. 1996;278:980-988.
35. Khanna KM, Bonneau RH, Kinchington PR, Hendricks RL. Herpes simplex virus-specific memory $CD8^+$ T cells are selectively activated and retained in latently infected sensory ganglia. Immunity. 2003;18:593-603.
36. Orr MT, Mathis MA, Lagunoff M, Sacks JA, Wilson CB. CD8 T cell control of HSV reactivation from latency is abrogated by viral inhibition of MHC class I. Cell Host Microbe. 2007;2: 172-180.
37. Koelle DM, Corey L. Recent progress in herpes simplex virus immunobiology and vaccine research. Clin Microbiol Rev. 2003; 16:96-113.
38. Liu T, Khanna KM, Carriere BN, Hendricks RL. Gamma interferon can prevent herpes simplex virus type 1 reactivation from latency in sensory neurons. J Virol. 2001;75:11178-11184.