Original Paper

NeuroImmunoModulation

Neuroimmunomodulation 2007;14:16–23
DOI: 10.1159/000107284

Received: October 8, 2006
Accepted: February 2, 2007
Published online: August 15, 2007

Acute Morphine Administration Reduces White Blood Cells' Capability to Induce Innate Resistance against HSV-1 Infection in BALB/c Mice

Abbas Jamali[a] Taravat Bamdad[a] Hoorieh Soleimanjahi[a] Firouz Ghaderi Pakdel[b] Ehsan Arefian[a]

[a]Department of Virology, School of Medical Sciences, Tarbiat Modares University, Tehran, and
[b]Department of Physiology, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

Key Words
Acute morphine · Herpes simplex virus 1 · Innate resistance

Abstract
Objective: It has been reported that acute morphine administration modulates innate immune response to herpes simplex virus 1 (HSV-1) infection. In this study, the effect of acute morphine on innate resistance and its probable mechanisms in increasing the mortality rate during HSV-1 infection were investigated. ***Methods:*** Mice were infected with HSV-1 24 h prior to different doses of morphine or saline administration and the mortality rate was recorded. Spleen cells were obtained from morphine- or saline-treated mice, then natural killer (NK) cell activity and interferon-γ (IFN-γ) production were evaluated. The effect of morphine on white blood cells' capacity to induce protection against HSV-1 infection was evaluated by adoptive transfer of spleen cells to cyclophosphamide-treated mice that were previously infected with HSV-1. Furthermore, in a separate experiment, a different group of mice received corticosterone 24 h after HSV-1 infection. ***Results:*** Mortality rate in high-dose acute morphine-treated mice increased significantly compared to saline-treated mice ($p = 0.035$). NK cell cytotoxicity and IFN-γ mRNA levels also showed a significant reduction compared to those of control groups ($p < 0.001$ and $p = 0.014$, respectively). Corticosterone administration reduces innate resistance against HSV-1 infection compared to saline-treated mice ($p = 0.044$). Furthermore, adoptive transfer of normal but not morphine-treated spleen cells induces resistance against HSV infection in cyclophosphamide-injected mice ($p = 0.009$). ***Conclusions:*** The current study shows that acute morphine administration reduces white blood cells' capability to induce protection against HSV-1 infection via suppression of IFN-γ production and NK cells activity. This may be due to the increase in corticosteroids. Further studies are needed to test the effect of acute morphine on other immune cells.

Introduction

Herpes simplex virus type 1 (HSV-1) is a worldwide infectious agent causing a variety of diseases from sporadic benign infections to fatal disease in neonate and immunocompromised individuals [1]. Specific and nonspecific immune responses have been implicated in protecting against HSV-1 infections [2]. Like many other viral infections, innate immunity appears to have a significant role in restricting HSV replication and recovery from the infection [3]. Interferon-α (IFN-α), IFN-β and IFN-γ have been shown to be essential for HSV control [4–6]. Previous data showed that even in the absence of natural killer (NK) cells or specific immunity, IFNs can eliminate HSV-1 at the early stages of infection [4]. How-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1021–7401/07/0141–0016$23.50/0

Accessible online at:
www.karger.com/nim

Abbas Jamali, PhD, Taravat Bamdad, PhD
Department of Virology, School of Medical Sciences
Tarbiat Modares University, PO Box, 14115-111, Tehran (Iran)
Tel. +98 21 8801 1001, ext. 4525, Fax +98 21 8801 3030
E-Mail jamali@modares.ac.ir or bamdad_t@modares.ac.ir

ever, the role of NK cells in acute HSV-1 infection is still controversial and there are numerous contradictory reports [7–10]. Adoptive transfer studies have shown that NK cells are important for protection against HSV-1 [10]. In contrast, using NK-depleted mice, it was shown that NK cell function is not essential for resistance to HSV-1 [9]. It is considered that genetic background of animals and route of HSV-1 infection can alter NK cell capacity to confer innate resistance to HSV-1 infection. Other components of the innate immune system including dendritic cells as professional antigen-presenting cells and a source of interleukin-12 (IL-12) and IFN production, and macrophages as a source of IL-12 may play a role in resistance to HSV-1 infection [11, 12].

It is now clear that opioids have a wide array of immunomodulatory effects on the innate and acquired immune systems directly through opioid receptors of immune cells and/or indirectly through the hypothalamic-pituitary-adrenal axis by increasing glucocorticoids [13, 14]. Consistent with these findings, opioid administration has been associated with increased susceptibility of animals to microbial infection [15]. It has been proven that morphine alters the course of HSV-1 infection [16, 17]. Furthermore, morphine reduces the activity of immune cells, including NK cells and macrophages, and decreases the production of cytokines like IFN-γ and IL-12 [18–20].

The modulation effect of morphine on the components of innate immunity may cause an increase in mortality rate during acute HSV-1 challenge. The current study has focused on the role of a single dose of morphine injection on the activity of white blood cells (WBCs), especially NK cells, and IFN-γ production in acute HSV-1 infection. Furthermore, innate resistance against lethal challenge of HSV-1 was investigated in acute morphine-treated mice. The role of glucocorticoids, which increase upon morphine administration, in the enhancement of HSV-1 mortality was also studied.

Materials and Methods

Cells and Viruses

Vero cell line was used for propagation of viruses. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum (FBS). K562, an NK-sensitive cell line [21], maintained in RPMI 1640 without phenol red, supplemented with 10% FBS, HEPES (20 mM), streptomycin (100 mg/ml) and penicillin (100 IU/ml) was used as target cell for the cytotoxicity assay. The cells were cultured in 96-well round-bottom plates (Nunc, Denmark) at a density of 4×10^5 cells/well. 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 [22]. The neurovirulence of the virus was proved by injection of the virus into mice and isolation of the virus from brains of dead mice. Briefly, the brains of dead mice were aseptically removed, homogenized and the extract was added on Vero cell monolayers. The isolated virus was confirmed as previously described. Wild HSV-1 and KOS (nonvirulent strain) were grown on Vero cells, titered and stored at –70°C.

Mice

Six- to eight-week-old female BALB/c mice were obtained from the Pasteur Institute (Karaj, Iran). Mice were housed for 1 week before the experiment, given free access to food and water, and maintained in a light/dark cycle with lights on from 6:00 to 18:00 h. All experiments were done according to the Animal Care and Use Protocol of Tarbiat Modares University.

Drugs

Morphine sulfate was purchased from TEMAD (Tehran, Iran) and dissolved in normal saline. The desired amount of morphine based on the weight of each mouse was subcutaneously injected at a total volume of 100 μl. Dry powder of cyclophosphamide (CY; ASTA Medica, Frankfurt, Germany) was dissolved in saline and intraperitoneally injected into mice at a concentration of 250 mg/kg.

HSV-1 Challenge

Considering the time of induction of maximum innate immunity, 24 h after HSV-1 infection was chosen for the evaluation of morphine effects on innate immunity. Mice (9 per group) were subcutaneously injected with different doses of morphine (10, 50 or 75 mg/kg) or with normal saline as control. All mice had been preinfected with 5×10^4 plaque-forming units (PFU) of wild HSV-1 24 h prior to morphine treatment. As a control of virus infection, a group of uninfected mice (9 per group) received 75 mg/kg morphine. The mortality rate was followed for 14 days. The dose of morphine which could induce a significant mortality comparing with controls was chosen for further experiments.

Spleen Cell Preparation

Under sterile conditions, spleens were removed and single-cell suspension was prepared in RPMI 1640 without phenol red (Gibco, UK). 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, counted and the viability was determined by trypan blue (0.4% w/v) exclusion.

NK Cytotoxicity Assay

Mice (5 per group) were given a single dose of 75 mg/kg of morphine or saline subcutaneously. Animals were sacrificed 24 h following morphine or saline administration and spleen cells were prepared as described in *Spleen Cell Preparation* for the NK cytotoxicity assay. This time point was selected based on the harvesting time of spleen cells from acute morphine- or saline-treated mice, which were transferred to CY-treated mice. To evaluate NK cells' cytotoxic activity, 50 μl of effector cells (spleen cells) were added to 50 μl of the target cells (K562 cells) at a ratio of 50/1 (target/effector) into 96-well round-bottom plates. The plates were centrifuged at 150 *g* for 5 min at 4°C and then incubated at 37°C and 5% CO_2. Effector and target cells were incubated alone

in the same conditions at a final volume of 100 μl. After 4 h of incubation, 10 μl of MTT (5 mg/ml PBS) was added to each well of the plate and incubated for 3 h [23]. MTT was reduced by living cells resulting in the formation of forazen crystals. To dissolve the crystals, a volume of 150 μl DMSO was added to each well, shaken for 15 min and then incubated at 37°C for 2 h. The optical density was measured at 570 nm. Using the following formula, the cytotoxic activity was calculated: 100 × [1 – (OD effector + target cells) – OD effector cells]/OD target cells. The cytotoxicity was determined for each sample as the mean of triplicate samples ± standard error.

IFN-γ Assay

Mice were intraperitoneally infected with 1×10^5 PFU of the HSV-1 KOS strain. The KOS strain was used to prevent HSV mortality during the experiment. After 24 h, the mice received a single dose of 75 mg/kg of morphine subcutaneously. The control group was injected with normal saline under the same conditions. All mice were sacrificed 3 days after infection and spleen cells for IFN-γ assay were prepared as described in *Spleen Cell Preparation*. A total number of 5×10^6 spleen cells were plated on each well of 24-well plates using RPMI 1640 supplemented with 10% FBS, 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 (multiplicity of infection) of heat-inactivated virus. Plates were incubated at 37°C in 5% CO_2 and 48 h after stimulation the cells were subjected to RNA extraction. Total RNA was isolated from harvested cells and subjected to RT-PCR. PCR amplification was performed with 2 sets of specific primers for IFN-γ and β2m. The IFN-γ mRNA expression was measured semiquantitatively by the band densitometry ratio of IFN-γ to β2m as described elsewhere [24].

HSV-1 Minimal Lethal Dose in CY-Treated Mice

Female BALB/c mice were treated with CY (n = 40) or with normal saline (n = 10) as a negative control. The CY-treated mice were divided into 4 groups (10 per group) 24 h after injection and inoculated with different doses of HSV-1. The mortality rate was followed for 14 days.

Adoptive Transfer

Considering the time of maximum suppression of NK cell activity in acute morphine-treated mice (12–48 h) [25], 24 h after morphine administration was chosen as the best time for adoptive transfer of spleen cells that had been obtained from acute morphine-treated mice to CY-treated mice and for studying its effect on innate immunity. Mice were given a single dose of 75 mg/kg of morphine or saline subcutaneously. Spleens were aseptically removed 24 h later and single-cell suspension was prepared in culture media (see *Spleen Cell Preparation*). A total number of 1×10^8 spleen cells of morphine- or saline-treated mice were intravenously injected to the mice 24 h after inoculation of 5×10^3 PFU of HSV-1 (minimal lethal dose of HSV in CY-treated mice). All mice had been treated with CY 24 h prior to HSV-1 infection. The negative control groups (9 per group) received HSV-1 or CY only [10].

HSV-1 Infection of Corticosterone-Treated Mice

In a separate experiment, different groups of normal mice were infected with 5×10^4 PFU of HSV-1. After 24 h, corticosterone (Sigma) was dissolved in ethanol (1.5–2.0 ml) and added to 450 ml of water for a final concentration of 200 μg/ml and used as the drinking water in corticosterone-treated mice. A group of animals received water containing vehicle (1.5–2.0 ml of ethanol) only. The treatment continued for the duration of the experiment [16].

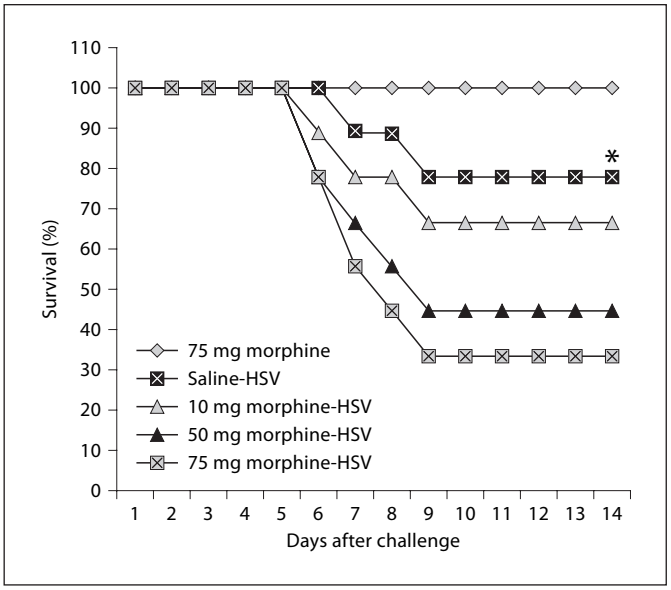


Fig. 1. Innate resistance against HSV-1 challenge is reduced in acute morphine-treated mice. Mice (9 per group) were exposed to different doses of morphine or saline 24 h after HSV challenge. Survival rate was recorded daily for 2 weeks. * $p = 0.035$ compared to the high-dose acute morphine-treated group (75 mg morphine-HSV).

Statistical Analysis

Natural cytotoxicity and IFN-γ production were analyzed by Student's t test. Kaplan-Meier analysis and the log-rank test were used for survival rate. $p < 0.05$ was considered significant.

Results

Acute Morphine Treatment Increases Sensitivity to HSV-1 Infection

The effect of acute morphine administration on HSV-1 infection in BALB/c mice was determined (fig. 1). Mice treated with a single dose of 75 mg/kg of morphine were significantly more susceptible to HSV-1 lethal challenge compared to those in the saline-treated group ($p = 0.035$). Although administration of lower doses of morphine could also reduce the survival rate, the data were not significant when compared to those of the saline-treated group. No death was recorded in the group of mice taking 75 mg/kg morphine alone (table 1).

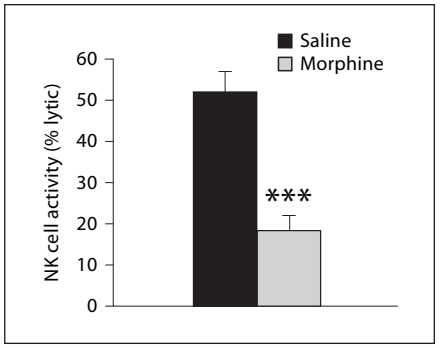


Fig. 2. Effect of acute morphine administration on NK cell activity. Mice (5 per group) were treated with morphine (75 mg/kg) or saline and NK cell activity was evaluated with MTT assay after 24 h. The result suggested that acute morphine-treated mice showed significant reduction in NK cell activity compared to saline-treated mice. *** $p < 0.001$.

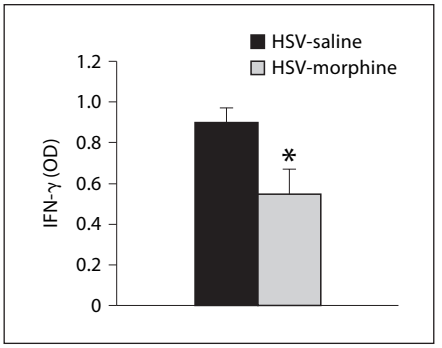


Fig. 3. Effect of acute morphine on mRNA IFN-γ production. Mice (5 per group) 24 h after HSV-1 infection were treated with morphine (75 mg/kg) or saline. Three days after HSV-1 infection, splenocytes were cultured at 5×10^6 cells/ml with 5 PFU/cell of heat-inactivated HSV-1 for 48 h. The result showed that acute morphine administration significantly reduces mRNA IFN-γ production compared to saline-treated group. * $p = 0.014$.

Table 1. Resistance to acute HSV challenge in acute morphine- or saline-treated mice

Groups	Mice survived/ mice challenged	Average time to death, days	Survival %
HSV-saline	7/9	12.66 ± 0.8	77.8
HSV-10 mg mor	6/9	11.44 ± 1.22[a]	66.7
HSV-50 mg mor	4/9	9.36 ± 1.32[a]	44.4
HSV-75 mg mor	3/9	8.78 ± 1.26[b]	33.3
PBS-75 mg mor[c]	9/9	ND	100

These data show the means for survival rate of each group challenged with acute HSV-1. Mice were exposed to different doses of morphine or saline 24 h after HSV-1 challenge and survival rate was recorded for 2 weeks. mor = Morphine; ND = not detected.

[a] Although mice treated with lower dose of morphine (10 and 50 mg/kg) showed increase in mortality rate compared to saline-treated mice, no significantly different values were obtained from these groups compared to saline-treated mice.

[b] Mice that were treated with high dose of acute morphine (75 mg/kg) showed significant decrease in innate resistance against HSV-1 challenge compared to saline-treated mice; $p = 0.035$.

[c] No mortality was recorded in mice that were treated with morphine (75 mg/kg) alone.

Acute Morphine Treatment Suppresses NK Cell Cytotoxic Activity

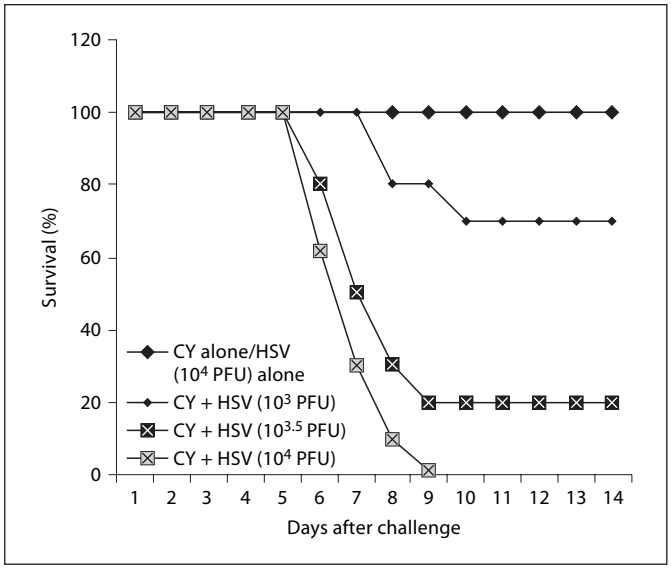
To determine the effect of acute morphine injection on NK cell cytotoxic activity, the splenic cells obtained from morphine- or saline-administered mice were subjected to MTT assay. The result showed that 24 h after administration of acute morphine, cytotoxic activity significantly decreased in splenic NK cells compared to the NK cell activity of the saline-treated group ($p < 0.001$; fig. 2).

IFN-γ Production Reduced in Acute Morphine-Treated Mice

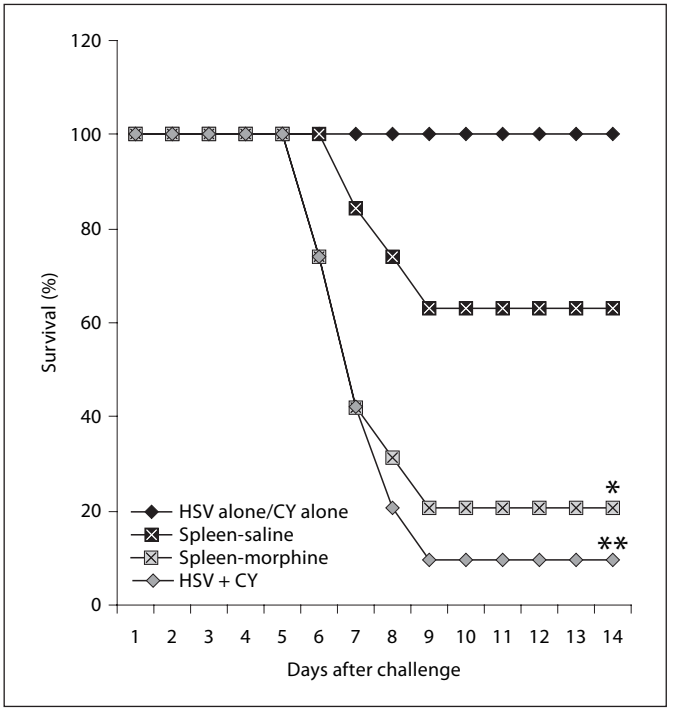
Spleen cells from morphine- and saline-treated HSV-1-infected mice were collected 3 days after infection. The cultured cells were restimulated with heat-inactivated HSV-1 and supernatants were harvested after 48 h. IFN-γ was assayed by multiplex RT-PCR. Administration of a single dose of morphine 24 h after infection with HSV-1 significantly suppressed the production of IFN-γ ($p = 0.014$) compared to that of the saline-treated control group (fig. 3).

The Effect of Different Doses of HSV-1 on Mortality Rate of CY-Treated Mice

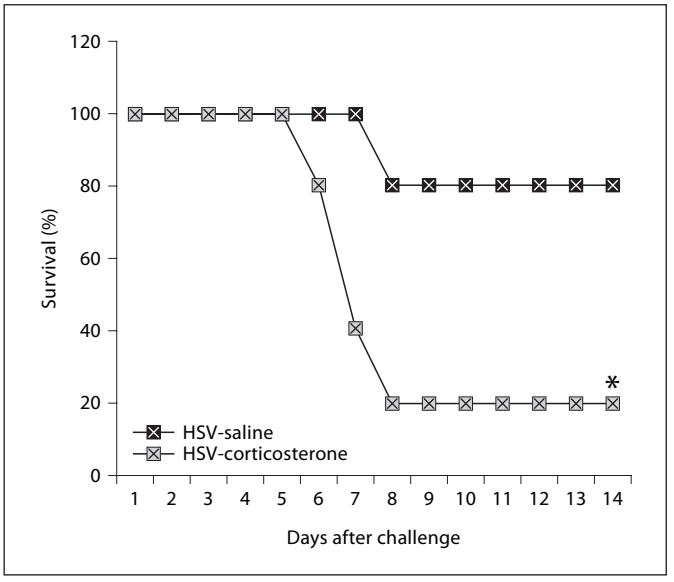
To determine the suitable dose of HSV-1 to be used for the challenge of CY-treated mice during the adoptive transfer experiment, different doses of HSV-1 were injected to the CY-treated animals. The results showed that the mortality rate was markedly increased in CY-treated mice that were infected with HSV-1, while no death was observed in groups with HSV-1 or CY alone. The titer of 5×10^3 PFU of HSV-1 which caused 80% mortality in CY-treated mice was chosen as suitable dose for evaluation of the effect of adoptive transfer of spleen cells to restore innate resistance against HSV-1 challenge in CY-treated animals (fig. 4).



4



5



6

Fig. 4. Effect of CY on innate resistance against HSV-1 challenge. Mice (10 per group) were exposed to CY (250 mg/kg) or saline 24 h before infection with different doses of HSV-1. Survival rate was recorded daily for 2 weeks. The titer of 5×10^3 PFU of HSV-1 which caused 80% mortality in CY treated mice was chosen as suitable dose for the evaluation of the effect of adoptive transfer of spleen cells to restore innate resistance against HSV-1 challenge in CY-treated animals.

Fig. 5. Effect of acute morphine treatment on the resistance to HSV-1 challenge induced by adoptive transfer of spleen cells to CY-treated mice. Mice (9 per group) were infected with HSV 24 h after treatment with CY. Spleen cells that were obtained from acute morphine- or saline-treated mice, were transferred to CY-treated mice 24 h after HSV-1 infection. Survival rate was recorded daily for 2 weeks. * $p = 0.037$; ** $p = 0.009$ compared to the normal spleen cells adoptive transfer group.

Fig. 6. Effect of corticosterone treatment on innate resistance against HSV-1 challenge. Mice (5 per group) were exposed to corticosterone or vehicle 24 h after HSV infection ($1 \times 10^{4.5}$ PFU). Survival rate was recorded daily for 2 weeks. The results suggested that corticosterone-treated mice showed significant reduction in innate resistance against HSV-1 challenge compared to vehicle-treated mice. * $p = 0.044$.

Passive Transfer of Morphine-Impaired Spleen Cells Fails to Protect CY-Treated Mice from HSV-1 Mortality

The effect of acute morphine administration on the in vivo ability of splenic cells to induce protectivity against HSV-1 in immunosuppressive mice was evaluated by adoptive transfer of spleen cells obtained from morphine- or saline-treated mice. Figure 5 shows the mortality rate of different groups following HSV-1 infection. Transfer of normal spleen cells to the CY-treated mice could significantly protect them against lethal HSV-1 infection compared with the control group which received RPMI 1640 medium instead of spleen cells ($p = 0.009$) and the other group which received spleen cells that were obtained from acute morphine-treated mice ($p = 0.037$). The spleen cells of morphine-treated mice could not pro-

Table 2. Effect of acute morphine on innate resistance that was induced by adoptive transfer of spleen cells to CY-treated mice

Groups	Mice survived/ mice challenged	Average time to death, days	Survival %
Spleen cells-saline	6/9	12 ± 0.95[a]	66.7
Spleen cells-mor	2/9	8.66 ± 0.99[b, *]	22.2
HSV + CY	1/9	8 ± 0.77**	11.1
HSV alone[c]	9/9	ND	100
CY alone[c]	9/9	ND	100

These data show the means for survival rate of each group challenged with acute HSV-1. Mice were exposed to CY and challenged with HSV-1 after 24 h. Spleen cells obtained from morphine- or saline-treated mice were transferred to CY-treated mice 24 h after HSV-1 challenge and survival rate was recorded for 2 weeks. mor = Morphine; ND = not detected.

[a] Significantly different values were obtained from CY-treated mice receiving normal spleen cells compared to CY-treated mice receiving morphine-treated spleen cells or RPMI medium. * $p = 0.037$; ** $p = 0.009$ compared to the normal spleen cells adoptive transfer group.

[b] No significantly different values were obtained from CY-treated mice that received morphine-treated spleen cells compared to the RPMI medium transfer group ($p > 0.05$).

[c] No mortality was recorded in mice that were treated with CY or HSV-1 alone.

tect mice against HSV infection when compared with the control group. No death was recorded in groups with virus or CY alone (table 2).

Corticosterone Treatment Significantly Enhances HSV-1 Infection

To determine the effect of glucocorticoid increase following acute morphine administration on innate immunity against HSV-1 infection, in separate experiments mice artificially received corticosterone 24 h after HSV-1 infection. The result showed that corticosterone treatment significantly reduces the innate resistance against HSV-1 ($p = 0.044$) compared to the control group (fig. 6).

Discussion

Opiates have been found to have many physiological and immunological effects that influence the pathogenesis of infectious diseases [15]. In this paper, we showed that the administration of a single dose of morphine after HSV-1 infection modulated the innate resistance against HSV-1. A recent study has shown that acute morphine changes innate immune response to HSV infections in BALB/c mice [18].

The present study indicates that the administration of a single high dose of morphine (75 mg/kg) in HSV-1-infected BALB/c mice significantly increases the mortality rate of HSV-1 infection. The role of the dosage of morphine on the rate of immunosuppression is not clear. Some reports have suggested that although low doses of morphine have immunosuppressive effects in mice, higher doses of morphine induce higher rates of immunosuppressive and physiological adverse effects such as the rise of increasing permeability of blood-brain barrier [25, 26].

The MTT assay showed decreased natural cytotoxicity in morphine-treated mice. The reduction of NK cell activity can be considered as one of the possible mechanisms of enhanced mortality in our test groups. A recent study strongly suggests that opioid-induced increase in nucleus accumbens D1 receptor activation inhibits splenic NK activity via neuropeptide Y released from the sympathetic nervous system [19].

As the other mechanism of mortality induced by morphine, the role of IFN-γ was evaluated. In innate immunity, IFN-γ has a critical role in inducing resistance against HSV-1 infection [4, 5]. The central role of IFN-γ in controlling HSV-1 infection has been demonstrated using gene knockout mice. IFN-γ gene knockout mice succumbed to viral-mediated encephalitis following intravitreal inoculation with HSV-1 [27]. Acute morphine administration has been shown to increase plasma catecholamines by activating central sympathetic outflow [28]. Catecholamine secretion as a result of sympathetic nervous system activation has been found to suppress mitogen-induced IFN-γ production [29].

In this study, we showed that single-dose administration of morphine 24 h after infection with HSV significantly suppressed the production of IFN-γ. This result confirmed a recent study by Sheridan et al. [18] showing that administration of a single dose of morphine 4 h prior to infection with HSV-1 reduced IFN-γ production. CY transiently reduces peripheral WBC counts by 90% in mice. Previously published data demonstrated that pretreatment of mice with CY resulted in increasing susceptibility to HSV-1 infection. The ability of HSV-1 resistance was returned by the transfer of normal spleen cells into CY-treated mice on the second day after CY treatment. The report suggested that NK cells have the main role in this induced resistance [10]. Our results suggested that adoptive transfer of spleen cells obtained from acute morphine-treated mice failed to protect immunosup-

pressive mice in vivo (CY-treated mice). So, to our knowledge, these are the first data to clearly declare that acute morphine can increase mortality by reduction of WBCs' capability to induce protection against HSV infection. Although Halford et al. [9] hypothesized that WBCs but not NK cells may have the capacity to induce innate resistance to HSV-1, it is not clear which WBCs of the innate immune system limit HSV-1 infection by IFN production and other antiviral activity.

Corticosteroids are immunosuppressive hormones that induce apoptosis of NK cells and suppress IFN-γ production [30, 31]. Acute morphine has been shown to increase corticosteroid production [18]. To study the possibility of glucocorticoid-mediated reduced innate resistance against HSV-1 in morphine-treated mice, in a parallel experiment we artificially elevated the circulating level of corticosteron in a group of mice 24 h after HSV inoculation. This time is exactly the time of morphine treatment. Our study has shown that in corticosterone-treated mice mortality significantly increased after HSV-1 infection, which is similar to the previous study which showed that corticosterone administration prior to HSV infection enhances mortality [16].

Pathogenesis and death are end points that occur 5–10 days after HSV-1 inoculation and the cumulative results of the interaction of the hundreds of variables that dictate the efficiency of viral replication and viral spread. Innate immune responses have the most important role in restriction of HSV-1 replication and spread to central nervous system. Acute morphine administration directly through opioid receptors of immune cells and/or indirectly through the rise of corticosteroids induces modulation effect on components of innate immunity.

In conclusion, the results of the present study suggest that the changes in the innate immune response by acute morphine administration reduce WBCs' capability to induce protection against HSV-1 infection. The increase in corticosteroids by morphine may be considered as a result of the modulation of these immune factors. Although morphine suppresses IFN-γ production and NK cell cytotoxicity against HSV-1 infection, further studies are needed to test the reduced potential of IFN-γ-producing cells such as dendritic cells or macrophages that may play a critical role in innate resistance against HSV-1 infection by acute morphine administration. In addition, the role of other components of the innate immune system, like macrophages as a source of IL-12, needs to be investigated.

Acknowledgment

We thank Prof. Zuhair M. Hassan for his helpful discussions.

References

1 Roizman B, Knipe M: Herpes simplex virus and their replication; in Knipe DM, Howley PM, et al (eds): Fields Virology. Philadelphia, Lippincott Williams and Wilkins, 2001, pp 2391–2510.

2 Whitley R, Roizman B: Herpes simplex virus infections. Lancet 2001;357:1513–1518.

3 Koelle DM, Corey L: Recent progress in herpes simplex virus immunobiology and vaccine research. Clin Microbiol Rev 2003;16:96–113.

4 Vollstedt S, Arnold S, Schwerdel C, Franchini M, Alber G, Di Santo JP, et al: Interplay between α/β and γ interferons with B, T, and natural killer cells in the defense against herpes simplex virus type 1. J Virol 2004;78:3846–3850.

5 Carr DJJ: Increased levels of IFN-γ in the trigeminal ganglion correlate with protection against HSV-1-induced encephalitis following subcutaneous administration with androstenediol. J Neuroimmunol 1998;89:160–167.

6 Pinto AJ, Morahan PS, Brinton M, Stewart D, Gavin E: Comparative therapeutic efficacy of recombinant interferons-α, -β, and -γ against alphatogavirus, bunyavirus, flavivirus, and herpesvirus infections. J Interferon Res 1990;10:293–298.

7 Adler H, Beland JL, DelPan NC, Kobzik L, Sobel RA, Rimm IJ: In the absence of T cells, natural killer cells protect from mortality due to HSV-1 encephalitis. J Neuroimmunol 1999;93:208–213.

8 Tanigawa M, Bigger JE, Kanter MY, Atherton SS: Natural killer cells prevent direct anterior-to-posterior spread of herpes simplex virus type 1 in the eye. Invest Ophthalmol Vis Sci 2000;41:132–137.

9 Halford WP, Maender JL, Gebhardt BM: Re-evaluating the role of natural killer cells in innate resistance to herpes simplex virus type 1. Virol J 2005;2:56.

10 Rager-Zisman B, Quan PC, Rosner M, Moller JR, Bloom BR: Role of NK cells in protection of mice against herpes simplex virus-1 infection. J Immunol 1987;138:884–888.

11 Al-Khatib A, Campbell IL, Carr DJJ: Resistance to ocular herpes simplex virus type 1 infection in IL-12 transgenic mice. J Neuroimmunol 2002;132:41–48.

12 Clark GJ, Angel N, Kato M, López JA, MacDonald K, Vuckovic S, Hart DNJ: The role of dendritic cells in the innate immune system. Microbes Infect 2000;2:257–272.

13 Vallejo R, de Leon-Casasola O, Benyamin R: Opioid therapy and Immunosuppression. Am J Ther 2004;11:354–356.

14 Mc Carthy L, Wetzel M, Sliker JK, Eisenstein TK, Rogers TJ: Opioids, Opioid receptors, and the immune response. Drug Alcohol Depend 2001;62:111–123.

15 Friedman H, Newton C, Klein TW: Microbial infection, immunodulation, and drugs of abuse. Clin Microbial Rev 2003;16:209–219.

16 Alonzo N, Carr D: Morphine reduces mortality in mice following ocular infection with HSV-1. Immunopharmacology 1999;41:187–197.

17 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.
18 Sheridan P, Moynihan J: Modulation of the innate immune response to HSV-1 following acute administration of morphine: role of hypothalamo-pituitary-adrenal axis. J Neuroimmunol 2005;158:145–152.
19 Saurer TB, Ijames SG, Lysle DT: Neuropeptide Y Y1 receptors mediate morphine-induced reductions of natural killer cell activity. J Neuroimmunol 2006;177:18–26.
20 Gomez-Flores R, Suo JL, Weber RJ: Suppression of splenic macrophage functions following acute morphine action in the rat mesencephalon periaqueductal gray. Brain Behav Immun 1999;13:212–224.
21 Hassan ZM, Yaraee R, Zare N, Ghazanfari T, Sarraf Nejad AH, Nazori B: Immunomodulatory affect of R10 fraction of garlic extract on natural killer activity. Int Immunopharmacol 2003;47:1483–1489.
22 Bamdad T, Roostaee MH, Sadeghizadeh M, Mahboudi F, Kazemnejad A, Soleimanjahi H: Immunogenicity and protective effect of a DNA construct encoding certain neutralizing epitopes of herpes simplex virus type-1 glycoprotein B. Folia Biol (Praha) 2005;51:109–113.
23 Ribeiro-Dias F, Barbuto JAM, Tsujita M, Jancar S: Discrimination between NK and LAK cytotoxic activities of murine spleen cells by MTT assay: differential inhibition by PGE and EDTA. J Immunol Methods 2000;241:121–129.
24 Arefian E, Bamdad T, Soleimanjahi H, Sabahi F, Akhoond MR, Parsania M, Ghaemi A: Evaluation of γ-interferon kinetics in HSV-1 infected mice in different days post infection (in vivo) and post re-stimulation (in vitro). Comp Immunol Microbiol Infect Dis 2007;30:1–9.
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 Oishi R, Baba M, Nishibori M, Itoh Y, Saeki K: Involvement of central histaminergic and cholinergic systems in the morphine-induced increase in blood-brain barrier permeability to sodium fluorescein in mice. Naunyn Schmiedebergs Arch Pharmacol 1989;339:159–165.
27 Geiger KD, Nash TC, Sawyer S, Krahl T, Patstone G, Reed JC, et al: Interferon-γ protects against herpes simplex virus type 1-mediated neuronal death. Virology 1997;238:189–197.
28 Appel NM, Kiritsy-Roy JA, Van Loon GR: Mu receptors at discrete hypothalamic and brainstem sites mediate opioid peptide-induced increases in central sympathetic outflow. Brain Res 1986;378:8–20.
29 Andrade-Mena CE: Inhibition of γ interferon synthesis by catecholamines. J Neuroimmunol 1997;76:10–14.
30 Migliorati G, Nicoletti I, D'Adamio F, Spreca A, Pagliacci C, Riccardi C: Dexamethasone induces apoptosis in mouse natural killer cells and cytotoxic T lymphocytes. Immunology 1994;81:21–26.
31 Munck A, Guyre PM: Glucocorticoids and immune function; in Ader R, Felten D, Cohen N (eds): Psychoneuroimmunology, ed 2. San Diego, Academic Press, 1991, pp 447–474.