Effects of Different Concentrations of Opium on the Secretion of Interleukin-6, Interferon-γ and Transforming Growth Factor Beta Cytokines from Jurkat Cells

Gholamreza Asadikaram PhD, Somayeh Igder MSc, Zahra Jamali MSc, Nader Shahrokhi PhD, Hamid Najafipour PhD, Mostafa Shokoohi MSc, Abdollah Jafarzadeh PhD, Mohammad Kazemi-Arababadi PhD

Abstract

Background: The risk of infectious, autoimmune and immunodeficiency diseases and cancers rise in opioid addicts due to changes in innate and acquired immune responses. Three types of opioid receptors (K-δ-μ) are expressed on the surface of lymphocytes and mononuclear phagocytes. The present study was designed to examine the effects of different concentrations of opium on the secretion of some cytokines produced by lymphocyte cells.

Methods: Jurkat cells were exposed to different concentrations of opium for periods of 6, 24 and 72 h in cell culture medium. The amount of interleukin-6 (IL-6), interferon-γ (IFN-γ) and transforming growth factor-β (TGF-β) were then measured using enzyme-linked immunosorbent assay (ELISA) method.

Findings: The results showed that opium increases the secretion of IL-6 in different concentration of opium in 6 h. The amount of IFN-γ decreased in 6 h and increased in 24 h significantly compared with control. On the other hand, opium had an inhibitory effect on the TGF-β secretion in 6, 24 and 72 h.

Conclusion: Overall, the study showed that opium stimulates pro-inflammatory and suppressed anti-inflammatory cytokine secretion in Jurkat cells. This may account for the negative effect of opium on the immune system leading to chronic inflammation and a base for many disorders in opium addicts.

Keywords: Opium, Jurkat cell, Interferon-γ, Interleukin-6, Transforming growth factor beta

Citation: Asadikaram Gh, Igder S, Jamali Z, Shahrokhi N, Najafipour H, Shokoohi M, et al. Effects of Different Concentrations of Opium on the Secretion of Interleukin-6, Interferon-γ and Transforming Growth Factor Beta Cytokines from Jurkat Cells. Addict Health 2015; 7(1-2): 47-53.

Received: 11.08.2014 Accepted: 23.10.2014
Introduction

Cytokines are regulating proteins, which adjust the activity of their target cells, especially in the hematogenic system. Cytokines play fundamental roles in the regulation of immune response and inflammation. The production of cytokines by immune system cells may be stimulated by specific or non-specific stimulants. Opium contains 8-17% morphine, 1-10% noscapine, 0.5-1.5% papaverine and 0.7-5% codeine and is used as raw material in the production of the above alkaloids. Changes in cytokine levels have been proven in mice addicted to morphine and heroin (an active metabolite of morphine). All three opioid receptors (K-δ-µ) are expressed on the surface of lymphocytes and mononuclear phagocytes. Most opium derivatives affect the function of the cytokine network. One example is the effect of heroin on the secretion of cytokines such as interleukin (IL)-2, IL-10, IL-5, IL-4, and interferon-γ (IFN-γ). Some other reports have shown morphine as a mutagen in T and B lymphocytes.

In addition, morphine administration in animals leads to typical atrophy in spleen and liver and obvious decrease in lymphocytes in these organs. Donahoe et al. reported a decrease in the ratio of CD4+ T-helper to CD8+ T-cytotoxic cells in heroine dependants. Welters stated that the morphine distorts T lymphocyte responses to bacterial infections, reduces phagocytic function of macrophages and alters cytokine secretion. In a study on the effect of opium on transforming growth factor beta (TGF-β) secretion, it was concluded that TGF-β decreased in male addicted and increased in female addicted rats. In another study, it was reported that morphine induces TGF-β secretion in human peripheral blood mononuclear cells (PBMCs). Singhal et al. reported that, morphine induces apoptosis in macrophages via inducing TGF-β secretion. An in vivo study showed that the morphine restricts immunoglobulin A antigen dependent response and TGF-β production in intestinal lymphoid tissue.

Fecho et al. also reported that heroin in the presence of concanavalin A stimulation restrains T lymphocyte production, reduces IFN-γ secretion, reduces the cytotoxic activity of natural killer cells, and reduces the ratio of active immune cells to CD8+ cells. Morphine controls the secretion of cytokines such as IFN-γ in human PBMCs, T lymphocytes and monocytes. In low doses, morphine has pro-inflammatory effects and in higher doses it has anti-inflammatory effects along with a reduction in the production of IL-6, IL-1, and TNF-α through µ-opioid receptors. Svetlecic et al. showed that TGF-β, IL-13, and IL-4 levels increase significantly by papaverine administration. As opium contains 20 types of alkaloids and 70 compounds that may have effects different from its constituents, we decided to study the effects of opium, in the form used by opium addicts, on the secretion of some cytokines by Jurkat cells.

Methods

Jurkat cells (purchased from Pasteur Institute of Iran) were cultured in RPMI1640 culture medium (Invitrogen Co. Germany) supplemented with 10% (V/V) heat-inactivated fetal bovine serum, (Invitrogen Co. Germany), 50 U/ml penicillin (Sigma Co. USA) and 50 mg/ml streptomycin (Sigma Co. USA).

Opium was dedicated by anti-drug section of Kerman Police, Iran. Based on their information the origin of opium was Helmand in Afghanistan. Analysis of this opium by GC-mass spectrometry showed; Alkaloids more than 30.0% (from which morphine 16%, codeine 5.5%, thebaine 4.4%, papaverine 3.2% were the most abundant constituents) and the rest consisted of non-alkaloid organic and non-organic substances from which 13.5% was water (moisture). A serial dilution of 2.86 × 10^{-3}, 2.86 × 10^{-5}, 2.86 × 10^{-7} g/ml opium was prepared in RPMI1640 medium. These calculations were based on effective concentrations of morphine on Jurkat cell line and the assumption that opium contains 16% morphine. Cells were grown in 48-well plate and then were exposed to the culture medium alone or different concentrations of opium in periods of 6, 24 and 72 h.

Cytokines were measured at the end of opium exposure period in the supernatant of Jurkat cells culture media by enzyme-linked immunosorbent assay (ELISA) technique as instructed by kit manufacturer (E-bioscience). Data were acquired from three repetitions for each concentration. Results are presented as mean ± standard errors. Data analysis was performed by SPSS.
Results

Effects of opium on IL-6, IFN-γ and TGF-β production

The secretion of IL-6 after exposure to $2.86 \times 10^{-1}$, $2.86 \times 10^{-5}$ and $2.86 \times 10^{-7}$ g/ml opium was significantly higher at 6 h compared with control ($P < 0.01$) (Figure 1).

IFN-γ levels were lower in $2.86 \times 10^{-1}$ and $2.86 \times 10^{-3}$ g/ml in 6 h incubation period and were higher in $2.86 \times 10^{-1}$, $2.86 \times 10^{-3}$ and $2.86 \times 10^{-5}$ g/ml concentrations in 24 h incubation period compared to control ($P < 0.02$) (Figure 2). Significant differences were also found between different concentrations in each period as illustrated in figure 2.
Figure 3. Effects of different concentrations of opium in 6, 24 and 72 h incubation periods on TGF-β beta secretion by Jurkat cells

\*P < 0.05 compared with control group; **P < 0.05 compared with 6 h; ***P < 0.05 compared with 24 h

TGF-β levels decreased significantly compared with control in concentration of $2.86 \times 10^{-1}$ and $2.86 \times 10^{-7}$ g/ml in 6 h and in concentrations of $2.86 \times 10^{-3}$ and $2.86 \times 10^{-5}$ g/ml in 24 and 72 h incubation periods ($P < 0.04$) (Figure 3). Significant differences were also found between different concentrations in each time period (Figure 3).

**Discussion**

The main finding of this study was an increase in pro-inflammatory cytokines (IL-6 and IFN-γ) and decreased in anti-inflammatory cytokine (TGF-β) production by Jurkat cells exposed to opium (Figures 1-3). From these findings, we may conclude that opium suppresses immune system. Studies carried out, in vivo and in vitro, on the effects of opioids on cell and humeral immune system have shown that the total number of T lymphocytes or the percentage of activated T lymphocytes in peripheral blood of opioid dependent subjects decrease significantly. Previous studies have shown that chronic inflammation leads to several disorders such as cancers, autoimmune diseases, immunodeficiency diseases, malfunction of the liver, lungs and kidneys.

Therefore, the addicted people would be vulnerable to such diseases. It has been shown that most opium derivatives especially morphine affect cytokine network. For example, chronic morphine usage induces differentiation of Th1 cells to Th2 CD4+, and CD4+ T-cells to Th2 through adenylyl cyclase pathway. Morphine also reduces Th1 cytokines (IL-2 and IFN-γ) and increases Th2 cytokines (IL-4 and IL-5). Peterson et al. reported that after 3 h of incubation with PBMCs, morphine causes decrease in IFN-γ secretion. Morphine also reduces IFN-γ secretion in thymus lymphocytes in vivo.

Our results showed that IFN-γ levels were significantly lower in doses of $2.86 \times 10^{-1}$ and $2.86 \times 10^{-7}$ g/ml at 6 h. However, increase in this cytokine observed in longer times in the present study remains to be explained.

Interestingly, some studies have shown that morphine has inflammatory effects in low doses and anti-inflammatory effects (less IL-6, IL-1 and TNF-α production) in higher doses. In another study, Chao et al. showed that low doses of morphine did not have any effects on the production of inflammatory cytokines such as IL-6 and TNF-α in PBMCs that is different with increase in secretion of IL-6 after exposure to concentrations of $2.86 \times 10^{-1}$, $2.86 \times 10^{-5}$ and $2.86 \times 10^{-7}$ g/ml of opium at 6 h.

Singhal et al. have shown that morphine in $10^{-4}$, $10^{-6}$, $10^{-8}$ molar concentrations induce apoptosis.
and increase in TGF-β secretions in mouse macrophages in J774A cell line. Chao et al. too arrived at the conclusion that morphine induces the secretion of TGF-β in PBMCs. These results are compatible with our findings in a previous study in which we tested the effects of opium on serum TGF-β secretion in male and female rats. It was shown that TGF-β secretion decreased in male and increased in female addicted rats.

The fact that the Jurkat cells used in the present study were obtained from a boy justifies the findings of the present study. As morphine is the main alkaloid of opium it may be concluded that the current results are in agreement with previous studies that had shown the inflammatory effects of the low levels of the morphine on the immune systems. There is a possibility that opium derivatives other than morphine may affect the signaling pathway of the inflammatory and anti-inflammatory cytokines, which would neutralize the pure effects of morphine on this cell line. The results of this study may be ascribed to the outcome of the effects of different compounds in opium.

**Conclusion**

Overall, the results of the present study showed that opium increased secretion of pro-inflammatory and decreased secretion of anti-inflammatory cytokines from Jurkat cells in culture medium (Figures 1-3). This function of opium verifies the effects of opiates in facilitating the process of chronic inflammation which can lead to a group of disorders. Considering the fact that in vitro and in vivo studies on opium are limited, justification of all results is difficult, and in future studies it is necessary to examine the mechanisms of action of opium leading to increase and decrease in the secretion of different cytokines.

**Conflict of Interests**

The Authors have no conflict of interest.

**Acknowledgements**

This work was supported by grants from Rafsanjan University of Medical Sciences and is the product of MSc thesis of Somayeh Igder. The authors thankfully acknowledge for support of the dissertation grant.

**References**

1. Arababadi MK, Mosavi R, Khorramdelazad H, Yaghini N, Zarandi ER, Araste M, et al. Cytokine patterns after therapy with Avonex(R), Rebif(R), Betaferon(R) and CinnoVex in relapsing-remitting multiple sclerosis in Iranian patients. Biomark Med 2010; 4(5): 755-9.
2. Kazemi AM. Interleukin-4 gene polymorphisms in type 2 diabetic patients with nephropathy. Iran J Kidney Dis 2010; 4(4): 302-6.
3. Arababadi MK, Pourfathollah AA, Jafarzadeh A, Hassanshahi G, Daneshmandi S, Shamsizadeh A, et al. Non-association of IL-12 +1188 and IFN-gamma +874 polymorphisms with cytokines serum level in occult HBV infected patients. Saudi J Gastroenterol 2011; 17(1): 30-5.
4. Hassanshahi G, Arababadi MK, Khorramdelazad H, Yaghini N, Zarandi ER. Assessment of CXCL12 (SDF-1alpha) polymorphisms and its serum level in posttransfusion occult HBV-infected patients in Southeastern Iran. Arch Med Res 2010; 41(5): 338-42.
5. Archer S, Bidlack J, Mulholland GK. Opium alkaloids and affinity labels. NIDA Res Monogr 1990; 96: 21-34.
6. Pacifici R, di CS, Bacosi A, Pichini S, Zuccaro P. Pharmacokinetics and cytokine production in heroin and morphine-treated mice. Int J Immunopharmacol 2000; 22(8): 603-14.
7. Martucci C, Franchi S, Lattuada D, Panerai AE, Sacerdote P. Differential involvement of RelB in morphine-induced modulation of chemotaxis, NO, and cytokine production in murine macrophages and lymphocytes. J Leukoc Biol 2007; 81(1): 344-54.
8. Kuang YM, Zhu YC, Kuang Y, Sun Y, Hua C, He WY. Changes of the immune cells, cytokines and growth hormone in teenager drug addicts. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2007; 23(9): 821-3. [In Chinese].
9. Bryant HU, Bernton EW, Kenner JR, Holaday JW. Role of adrenal cortical activation in the immunosuppressive effects of chronic morphine treatment. Endocrinology 1991; 126(6): 3253-8.
10. Arora PK, Frde E, Petitto J, Waggie K, Skolnick P. Morphine-induced immune alterations in vivo. Cell Immunol 1990; 126(2): 343-53.
11. Donahoe RM, Bueso-Ramos C, Donahoe F, Madden JJ, Falek A, Nicholson JK, et al. Mechanistic implications of the findings that opiates and other drugs of abuse moderate T-cell surface receptors and antigenic markers. Ann N Y Acad Sci 1987; 496: 711-21.
12. Welters I. Opioids and immunosuppression. Clinical relevance? Anaessthesist 2003; 52(5): 51-6.
Effects of Opium on the Secretion of IL-6, IFN-γ and TGF-β

Asadikaram et al.

442-52. [In German].

13. Asadikaram G, Asilbanha M, Sayadi A, Jafarzadeh A, Hassanshahi G. Impact of opium on the serum levels of TGF-beta in diabetic, addicted and addicted-diabetic rats. Iran J Immunol 2010; 7(3): 186-92.

14. Chao CC, Hu S, Molitor TW, Zhou Y, Murtaugh MP, Tsang M, et al. Morphine potentiates transforming growth factor-beta release from human peripheral blood mononuclear cell cultures. J Pharmacol Exp Ther 1992; 262(1): 19-24.

15. Singhal PC, Kapasi AA, Franki N, Reddy K. Morphine-induced macrophage apoptosis: the role of transforming growth factor-beta. Immunology 2000; 100(1): 57-62.

16. Peng X, Cebra JJ, Adler MW, Meissler JJ, Jr., Cowan A, Feng P, et al. Morphine inhibits mucosal antibody responses and TGF-beta mRNA in gut-associated lymphoid tissue following oral cholera toxin in mice. J Immunol 2001; 167(7): 3677-81.

17. Fecho K, Nelson CJ, Lysle DT. Phenotypic and functional assessments of immune status in the rat spleen following acute heroin treatment. Immunopharmacology 2000; 46(3): 193-207.

18. Ye L, Wang X, Metzger DS, Riedel E, Montaner LJ, Ho W. Upregulation of SOCS-3 and PIAS-3 impairs IL-12-mediated interferon-gamma response in CD56 T cells in HCV-infected heroin users. PLoS One 2010; 5(3): e9602.

19. Roy S, Wang J, Kelschenbach J, Koodie L, Martin J. Modulation of immune function by morphine: implications for susceptibility to infection. J Neuroimmune Pharmacol 2006; 1(1): 77-89.

20. Svetlecic J, Molteni A, Herndon B. Bronchiolitis obliterans induced by intratracheal papaverine: a novel animal model. Lung 2004; 182(2): 119-34.

21. Buchbauer G, Nikiforov A, Remberg B. Headspace constituents of opium. Planta Med 1994; 60(2): 181-3.

22. Najafipour H, Joukar S, Malekpour-Afsar R, Mirzaeipour F, Nasri HR. Passive opium smoking does not have beneficial effect on plasma lipids and cardiovascular indices in hypercholesterolemic rabbits with ischemic and non-ischemic hearts. J Ethnopharmacol 2010; 127(2): 257-63.

23. Wang J, Barke RA, Charboneau R, Roy S. Morphine impairs host innate immune response and increases susceptibility to Streptococcus pneumoniae lung infection. J Immunol 2005; 174(1): 426-34.

24. O’Callaghan DS, O’Donnell D, O’Connell F, O’Byrne KJ. The role of inflammation in the pathogenesis of non-small cell lung cancer. J Thorac Oncol 2010; 5(12): 2024-36.

25. Munoz LE, Janko C, Schulze C, Schorn C, Sarter K, Schett G, et al. Autoimmunity and chronic inflammation - two clearance-related steps in the etiopathogenesis of SLE. Autoimmun Rev 2010; 10(1): 38-42.

26. Villa A, Notarangelo LD, Roifman CM. Omenn syndrome: inflammation in leaky severe combined immunodeficiency. J Allergy Clin Immunol 2008; 122(6): 1082-6.

27. Mollica MP, Lionetti L, Putti R, Cavaliere G, Gaita M, Barletta A. From chronic overfeeding to hepatic injury: role of endoplasmic reticulum stress and inflammation. Nutr Metab Cardiovasc Dis 2011; 21(3): 222-30.

28. De Dooy JJ, Mahieu LM, Van Bever HP. The role of inflammation in the development of chronic lung disease in neonates. Eur J Pediatr 2001; 160(8): 457-63.

29. Azarang A, Mahmoodi M, Rajabalian S, Shekari MA, Nosratabadi J, Rezaei N. T-helper 1 and 2 serum cytokine assay in chronic opioid addicts. Eur Cytokine Netw 2007; 18(4): 210-4.

30. Roy S, Wang J, Charboneau R, Loh HH, Barke RA. Morphine induces CD4+ T cell IL-4 expression through an adenylyl cyclase mechanism independent of the protein kinase a pathway. J Immunol 2005; 175(10): 6361-7.

31. Peterson PK, Sharp B, Gekker G, Brummitt C, Keane WF. Opioid-mediated suppression of interferon-gamma production by cultured peripheral blood mononuclear cells. J Clin Invest 1987; 80(3): 824-31.

32. Finley MJ, Happel CM, Kaminsky DE, Rogers TJ. Opioid and nociceptin receptors regulate cytokine and cytokine receptor expression. Cell Immunol 2008; 252(1-2): 146-54.

33. Chao CC, Molitor TW, Close K, Hu S, Peterson PK. Morphine inhibits the release of tumor necrosis factor in human peripheral blood mononuclear cell cultures. Int J Immunopharmacol 1993; 15(3): 447-53.
TGF-β and IFN-γ Effects on the Secretion of IL-6, IFN-γ and TGF-β by heroin-exposed cells

Asadikaram et al.

چکیده

مقدمه: خطر ابتلا به بیماری‌های عفونی در افراد مبتلا به نگش گیاه نواری افزایش یافته‌است. هر سه نوع گیاه‌های اپوپیپیدی بر سطح لنفوسیت‌ها و فاکتور نیکالین بیان می‌شوند. مطالعه حاضر با هدف بررسی اثرات مختلف استفاده از سیتوکین‌های تولید شده توسط سیتوکین‌های لنفوسیتی طراحی شد.

روش‌ها: سلول‌های جورکت در زمان‌های 6، 24 و 48 ساعت تحت تأثیر غلظت‌های مختلف تریاک قرار گرفتند. سپس میزان اینترلاکین 6 (TGF-β) (IFN-γ) و انترلکین 6 (IL-6) از سلول‌ها به وسیله تست ELISA انجام گردید.

یافته‌ها: سلول‌های جورکت در زمان‌های 6، 24 و 48 ساعت تحت تأثیر غلظت‌های مختلف تریاک قرار گرفتند. سپس میزان اینترلاکین 6 (TGF-β) (IFN-γ) و انترلکین 6 (IL-6) از سلول‌های جورکت به وسیله تست ELISA انجام گردید.

نتایج گیری: در مجموع نتایج مطالعه نشان داد که تریاک سبب تغییر نسبی سبک ترشح سیتوکین‌های الکترى و مهار ترشح سیتوکین‌های ضرری در سلول‌های جورکت می‌گردد. این نتایج نشان داد که تریاک سبکی می‌کند که عفونت رخ‌دهانه را به تغییرات ایجاد می‌کند.

واژگان کلیدی: تریاک، سلول جورکت، IL-6, IFN-γ, TGF-β

ارجاع: اسکرو کامپوس رضا، ایکدر، سالیم، جمال زهر، مهران شریفزاده، نجفی نور، مهدی، شکوهی، محسن، جمالی، نورمحمد، کاظمی، شهابندازی، محمد، اثرات غلظتهای مختلف تریاک بر ترشح سیتوکین‌های الکتری و SLC بر سلول‌های جورکت. مجله ایمنی و سلامت 1394؛ 7 (2)-34-52.

تاریخ دریافت: 93/5/20

تاریخ پذیرش: 93/8/1

Email: gh_asadi@kmu.ac.ir

Addict Health, Winter and Spring 2015; Vol 7, No 1-2

http://ahj.kmu.ac.ir, 4 April