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

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Original Article

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 (κ،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

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Introduction

Cytokines are regulating proteins, which adjust the activity of their target cells, especially in the hematogenic system.[1,2] Cytokines play fundamental roles in the regulation of immune response and inflammation.[1,3,4] The production of cytokines by immune system cells may be stimulated by specific or non-specific stimulants.[3] Opium contains 8-17% morphine, 1-10% noscapine, 0.5-1.5% papaverine and 0.7-5% codeine[5] and is used as raw material in production of the above alkaloids.[5] Changes in cytokine levels have been proven in mice addicted to morphine and heroin (an active metabolite of morphine).[6] All three opioid receptors (K،δ،μ) are expressed on the surface of lymphocytes and mononuclear phagocytes.[7] 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).[8] Some other reports have shown morphine as a mutagen in T and B lymphocytes.[9,10]

In addition, morphine administration in animals leads to typical atrophy in spleen and liver and obvious decrease in lymphocytes in these organs.[10] Donahoe et al. reported a decrease in the ratio of $CD4^+$ T-helper to $CD8^+$ T-cytotoxic cells in heroine dependants.[11] Welters stated that the morphine distorts T lymphocyte responses to bacterial infections, reduces phagocytic function of macrophages and alters cytokine secretion.[12] 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.[13] In another study, It was reported that morphine induces TGF-β secretion in human peripheral blood mononuclear cells (PBMCs).[14] Singhal et al. reported that, morphine induces apoptosis in macrophages via inducing TGF-β secretion.[15] An in vivo study showed that the morphine restricts immunoglobulin A antigen dependent response and TGF-β production in intestinal lymphoid tissue.[16]

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.[17] Morphine controls the secretion of cytokines such as IFN-γ in human PBMCs, T lymphocytes and monocytes.[18] 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.[19] Svetlecic et al. showed that TGF-β, IL-13, and IL-4 levels increase significantly by papaverine administration.[20] As opium contains 20 types of alkaloids and 70 compounds,[5,21] 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 Pasture 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).[22] A serial dilution of 2.86×10^{-1}, 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[15] 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

(version 16, SPSS Inc., Chicago, IL, USA) and one-way ANOVA, followed by Bonferroni's post-hoc test was used to compare between different concentrations at each time point. For comparison between different time points repeated measure ANOVA with Bonferroni's post-hoc test was used. $P < 0.05$ was considered to be significant.

Results

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

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

IFN-γ levels were lower in 2.86×10^{-1} and 2.86×10^{-3} g/ml in 6 h incubation period and were higher in 2.86×10^{-1}, 2.86×10^{-3} and 2.86×10^{-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.

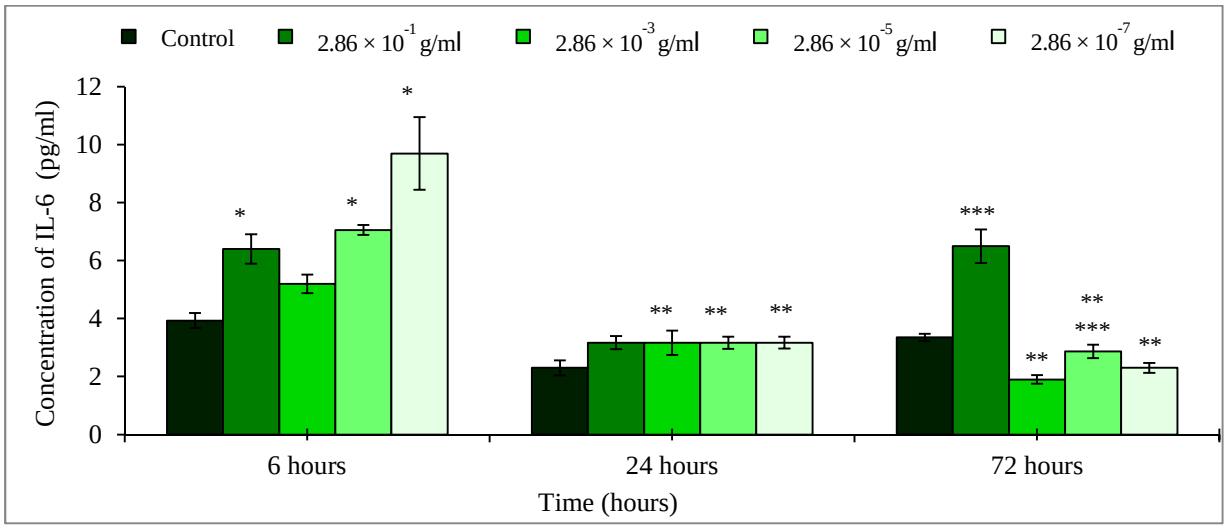

Figure 1. Effects of different concentrations of opium in 6, 24 and 72 hours incubation periods on IL-6 secretion by Jurkat cells

*P < 0.05 compared with control; **P < 0.05 compared with related concentration in 6 hours; ***P < 0.05 compared with related concentration in 24 hours

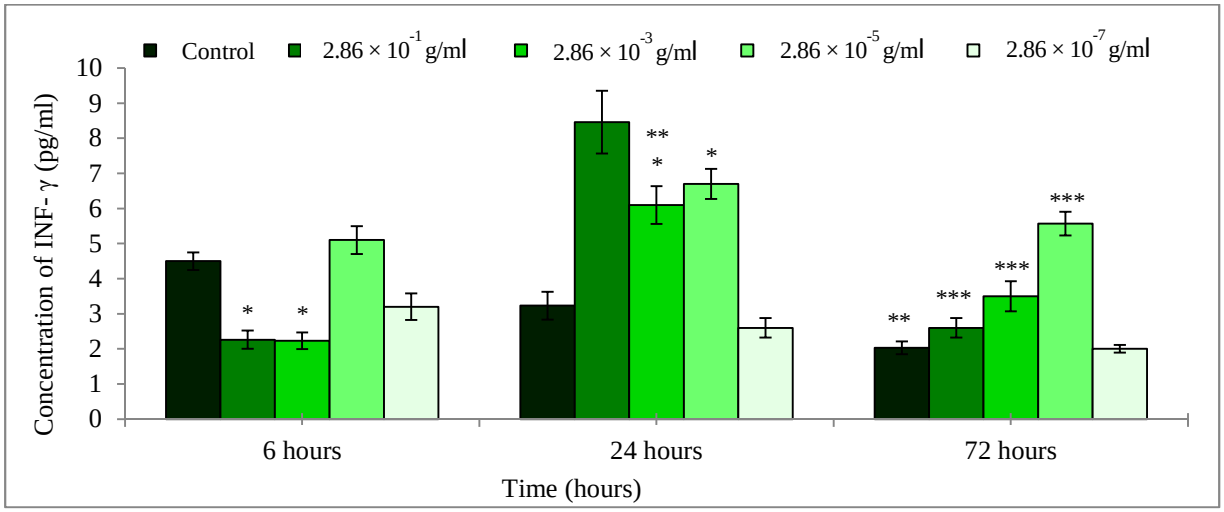

Figure 2. Effects of different concentrations of opium in 6, 24 and 72 hours incubation periods on IFN-γ secretion by Jurkat cells

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

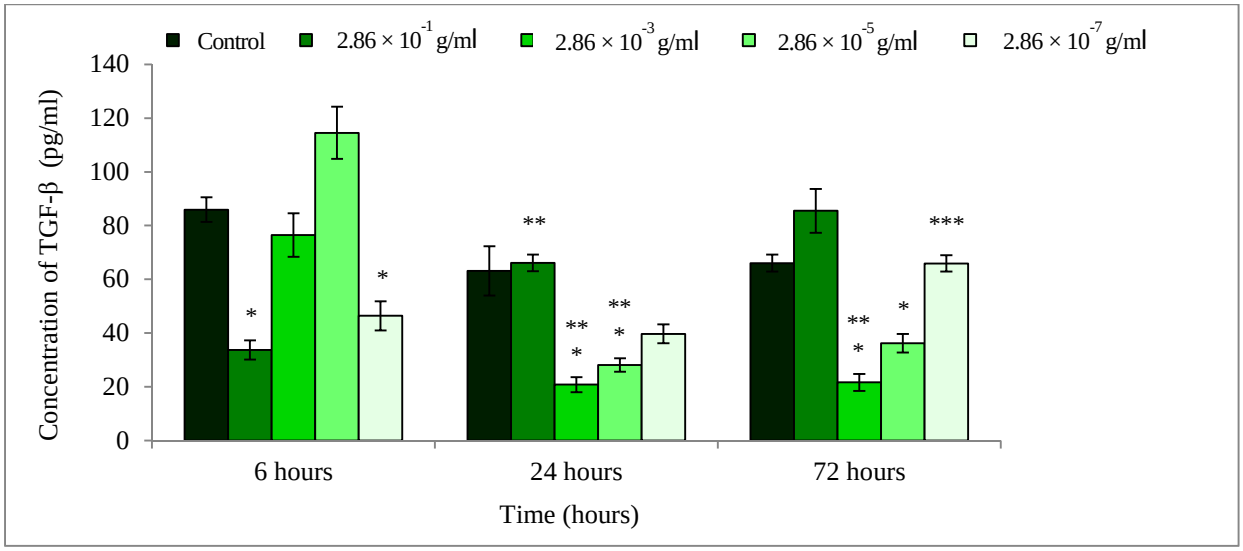

Figure 3. Effects of different concentrations of opium in 6, 24 and 72 h incubation periods on TGF-β beta secretion by Jurkat cells

*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 × 10^{-1} and 2.86 × 10^{-7} g/ml in 6 h and in concentrations of 2.86 × 10^{-3} and 2.86 × 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.[23] Previous studies have shown that chronic inflammation leads to several disorders such as cancers,[24,25] autoimmune diseases,[26] immunodeficiency diseases,[26] malfunction of the liver,[27] lungs,[28] and kidneys.[29]

Therefore, the addicted people would be vulnerable to such diseases. It has been shown that most opium derivatives especially morphine affect cytokine network.[13,29] For example, chronic morphine usage induces differentiation of Th_1 cells to Th_2 $CD4^+$, and $CD4^+$ T-cells to Th_2 through adenylyl cyclase pathway.[29] Morphine also reduces Th_1 cytokines (IL-2 and IFN-γ) and increases Th_2 cytokines (IL-4 and IL-5).[30] Peterson et al. reported that after 3 h of incubation with PBMCs, morphine causes decrease in IFN-γ secretion.[31]

Morphine also reduces IFN-γ secretion in thymus lymphocytes in in vivo.[17]

Our results showed that IFN-γ levels were significantly lower in doses of 2.86 × 10^{-1} and 2.86 × 10^{-3} g/ml in 6 h and higher in doses of 2.86 × 10^{-1}, 2.86 × 10^{-3} and 2.86 × 10^{-5} g/ml concentrations in 24 h incubation period. These results are in agreement with previous studies in which IFN-γ levels decreased in a short time.[17,31] 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.[32] 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[33] that is different with increase in secretion of IL-6 after exposure to concentrations of 2.86 × 10^{-1}, 2.86 × 10^{-5} and 2.86 × 10^{-7} g/ml of opium at 6 h.[32]

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.[15] Chao et al. too arrived at the conclusion that morphine induces the secretion of TGF-β in PBMCs.[14] 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.[13]

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.

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اثرات غلظت‌های مختلف تریاک بر ترشح سیتوکین‌های IL-۶، IFN-γ و TGF-β بر سلول‌های جورکت

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مقاله پژوهشی

چکیده

مقدمه: خطر ابتلا به بیماری‌های عفونی در افراد معتاد با تغییر پاسخ ایمنی ذاتی و اکتسابی افزایش پیدا می‌کند. هر سه نوع گیرنده اپیوییدی (K،δ،μ) بر سطح لنفوسیت‌ها و فاگوسیت‌های مونونوکلئار بیان می‌شوند. مطالعه حاضر با هدف بررسی اثر غلظت‌های مختلف تریاک بر ترشح برخی از سیتوکین‌های تولید شده توسط سلول‌های لنفوسیت طراحی شد.

روش‌ها: سلول‌های جورکت در زمان‌های ۶، ۲۴ و ۷۲ ساعت تحت تأثیر غلظت‌های مختلف تریاک قرار گرفتند. سپس میزان اینترلوکین ۶ (Interleukin-۶ یا IL-۶)، اینترفرون گاما (Interferon-γ یا IFN-γ) و فاکتور تبدیل رشد بتا (Transforming growth factor-β یا TGF-β) به وسیله تکنیک ELISA (Enzyme-linked immunosorbent assay) اندازه‌گیری گردید.

یافته‌ها: یافته‌ها نشان داد که میزان IL-۶ در ۶ ساعت در غلظت‌های مختلف تریاک افزایش یافت. میزان IFN-γ در مقایسه با گروه شاهد به طور معنی‌داری در ۶ ساعت کاهش و در ۲۴ ساعت افزایش یافت. از طرف دیگر، تریاک اثر مهاری را بر میزان ترشح TGF-β در ۶، ۲۴ و ۷۲ ساعت نشان داد.

نتیجه‌گیری: در مجموع نتایج مطالعه نشان داد که تریاک سبب القای ترشح سیتوکین‌های التهابی و مهار ترشح سیتوکین‌های ضد التهابی در سلول‌های جورکت می‌گردد. این نتایج شاید دلیل اثر منفی تریاک بر سیستم ایمنی است که به عفونت مزمن منتهی می‌شود و زمینه‌ای برای اختلالات بیشتر می‌باشد.

واژگان کلیدی: تریاک، سلول جورکت، IFN-γ، IL-۶، TGF-β

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