https://doi.org/10.31925/farmacia.2023.6.9 **ORIGINAL ARTICLE**

THE IMMUNE STATUS OF HEROIN ADDICTS DURING TREATMENT WITH METHADONE

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Abstract

Literature data reveal generally a negative impact of drugs of abuse on the immune system. However, at this level, a variety of changes have been reported among heroin addicts, indicating both decreased and increased immune functions. There are only limited and highly variable reports concerning the impacts of heroin addiction on the functions of the immune system. Thus, the aim of current study was to dynamically investigate the impact of opiates on the immune response in heroin addicted patients during the first six months of methadone substitution therapy. A broad panel of immune parameters assessing effects of opioids on both innate and adaptive immunity were investigated. The study revealed that heroin disrupts the immune response, but only certain aspects indicate immunosuppression, such as elevated sCD25 levels, low levels of serum cytokines, and poor granulocyte response through activation *via* PKC. The switch to oral methadone from i.v. heroin initially determines a partial restoration of the immune defence capacity.

Rezumat

Datele din literatură dezvăluie în general un impact negativ al substanțelor de abuz asupra sistemului imunitar, dar au fost raportate o varietate de modificări ale sistemului imunitar, indicând atât scăderea, cât și creșterea funcțiilor imunitare la dependenții de heroină. Cu toate acestea, există doar raportări limitate și foarte variabile cu privire la impactul dependenței de heroină asupra funcțiilor sistemului imunitar. Astfel, scopul prezentului studiu a fost de a investiga dinamic impactul opiaceelor asupra răspunsului imun la pacienții dependenți de heroină, în primele șase luni de terapie de substituție cu metadonă. A fost investigat un panou larg de parametri imunitari care a evaluat efectele opioidelor asupra imunității înnăscute și adaptative. Studiul a arătat că heroina perturbă răspunsul imunitar, dar numai unele aspecte indică imunosupresie (niveluri crescute de sCD25, niveluri scăzute de citokine serice, răspuns slab al granulocitelor la activarea prin PKC). Trecerea de la administrarea injectabilă de heroină la administrarea orală de metadonă determină la început o restabilire parțială a capacității de apărare imună.

Keywords: immune status, heroin addicts, methadone, opioids, lymphocytes, cytokines, lymphocyte proliferation

Introduction

Literature data, beginning from 1975 with a paper of Lefkowitz *et al*. [1], presented a negative impact of drugs of abuse on the immune system. Drug induced immune-suppression may lead to increased incidence of chronic bacterial and viral infections and decrease anticancer immune defence, which further arise the economic and social costs associated with drug addiction. With the onset of the global AIDS epidemic, research on the impact of addictive drugs on immunity became even more critical as epidemiologic data indicated that a third of AIDS patients used intravenous drugs, primarily heroin. Therefore, the US National Institute on Drug Abuse launched a funding program to investigate how opioids affect the immune system [2, 3]. Human immunodeficiency virus (HIV) causes AIDS, which leads to an immune system collapse that leaves a person extremely vulnerable to opportunistic microorganisms [4, 5] and abuse-related drugs including opiates have been proposed as potential co-factors that accelerate the course of the disease [6, 7].

As the research in this direction has progressed, a variety of changes in the immune system have been observed, indicating both decreased and increased of the immune functioning in heroin addicts. Although, there are only limited and highly variable reports concerning the impacts of heroin addiction on the functions of the immune system.

Opiates appear to affect the immune responses directly through opioid receptors expressed by immune cells and indirectly through receptors on neural cells [8]. A decrease in the proliferative response of T lymphocytes to a wide range of concentrations of mitogenic phytohemagglutinin (PHA) stimuli and the modulation of the surface markers on T cells in heroin-addicted subjects has been reported [9].

It has been demonstrated that chronic heroin use results in measurable suppression of some components of the human cellular immune system. The alteration of peripheral blood T- and B-lymphocyte proliferative responses were determined during different periods of withdrawal in heroin addicts. Therefore, a significant decrease in the response of T-lymphocytes to PHA-stimulation and secretion of IL-2 were observed during these withdrawal periods. Moreover, the IL-4 production was suppressed and the IL-10 production was significantly increased [10].

It has been revealed that the immune system abnormalities in heroin addicted patients can be restored to almost normal values by controlled treatment with methadone. Therefore, it has been shown that PHA-lymphocytes proliferation was lower in untreated heroin addicts than in controls, while it was normal in methadone treated patients. An altered Th1/Th2 balance, characterized by reduced IL-4, IFN-gamma and TNF-alpha, but normal IL-2 levels, was present in untreated heroin addicts, while the Th1/Th2 balance was well conserved in the methadone treated patients [11].

Several lines of evidence showed that methadone can either improve, impair, or have no effect on the immune function. Preclinical data indicate that methadone exerts an *in vitro* inhibitory effect on the proliferation of rat splenic lymphocytes [12]. Recent findings suggest that methadone maintenance treatment influences the immune system functions of opioid-dependent patients and may also induce long-term systemic inflammation. Therefore, the increase in the production of IL-1β, IL-6 and IL-8 was observed in methadone-treated patients compared to the healthy control group. The correlations between plasma TNF-α and IL-6 levels and the methadone dosage were noticed, while the IL-1β level was significantly correlated with the duration of methadone maintenance treatment [13].

Significantly, both *in vitro* and *in vivo* research has revealed that methadone may dysregulate the immune responses of NK cells [14], human T-lymphocytes [15, 16], and mononuclear phagocytes [17, 18]. Low concentration of methadone has shown to exert an *in vitro* immunomodulatory action on T lymphocytes from healthy subjects and from heroin addicts [19, 20].

Despite all this knowledge, whether peripheral immune cells and their secreted cytokines interact with the central nervous system (CNS) to modulate synaptic structure or addictive behaviour remains largely unknown [21].

For these reasons, in the current study, we investigated the impact of opiates on the immune response in humans, taking as a model the heroin addicted patients, whether or not they were under methadone substitution treatment, following complex studies on characterization of various groups of drugs users during methadone substitution therapy [22, 23]. The addict patients included in the study were dynamically investigated during the methadone substitution therapy. Thus, this model allows highlighting the impact of opioids on the immune response based on the changes induced by stopping i.v. heroin and switch to oral methadone, an opioid with long duration of action (which determines maintenance of the opioid receptors on immune cells permanently saturated by achieving stable plasma levels). A broad panel of immune parameters allowing the assessment of the effects of opioids on both innate and adaptive immunity were determined.

Materials and Methods

Chemicals

Methadone hydrochloride, Biocol, RPMI1640 cell culture medium, foetal bovine serum, antibiotic-antimycotic solution were obtained from Promega Corporation. Phytohemagglutinin M (PHA) and pokeweed mitogen (PWM) were purchased from Sigma-Aldrich. Tritium-labelled uridine was kindly provided by the Institute of Physics and Nuclear Engineering "Horia Hulubei", Măgurele, Romania.

Human subjects

We studied a group of long-term heroin addicts (n = 21), who volunteered at the Centre for Evaluation and Treatment of Drug Addicts for Young People "St. Stelian" Bucharest, Romania, for methadone substitution treatment. The patients in the study group, aged 19 - 35, were free of drugs affecting the immune system and had no history of neuropsychiatric disorders. Age-matched normal healthy subjects (n = 21) formed the control group. The patients were tested before methadone therapy at onset, 1 month and 6 months later. The study was approved by the Ethical Committees of the Centre and was carried out in accordance with the Declaration of Helsinki. Subjects participating in this study gave written informed consent.

Biological samples

Mononuclear cells were isolated from peripheral blood collected on lithic heparin by centrifugation in density gradient (Biocol, Promega, d = 1.077) [24]. Cells were counted in a Burker-Turk chamber. Cellular viability, scored by the Trypan blue exclusion test, exceeded 98%. Mononuclear cells were cultured for 72 h in the presence of methadone and lectinic mitogens specific for T-lymphocytes (phytohemagglutinin, PHA) or B lymphocytes (poke weed mitogen, PWM).

Peripheral distribution of lymphocyte populations

The peripheral distribution of lymphocyte populations and subpopulations was performed by flow cytometry

using MultiTEST IMK kit and MultiSET software according to the manufacturer (Becton Dickinson, San Jose, CA, USA).

The serum level of soluble form of the IL-2 receptor (sCD25)

The serum level of soluble form of the IL-2 receptor (sCD25) was determined by an ELISA quantitative immunoassay technique enzyme-sandwich type according to the kits manufacturer's instructions (EuroClone, Milan, Italy and R&D Systems Minneapolis, MN, USA).

Pro- and anti-inflammatory cytokine serum profile

The pro- and anti-inflammatory cytokine serum profile has been determined by multiplex array using Luminex xMAP multiplexing technology and Beadlyte kit (Upstate, Lake Placid, NY, USA). The following cytokines were measured: IL-1beta, IL-2, IL-4, IL-6, IL-8, IL-12, TNF-alpha, IFN-gamma, and GM-CSF. Briefly, the mononuclear cells were isolated by centrifugation in density gradient. The cells (3×10^6/mL) were cultured for 18 hours to allow the adherence of monocytes, non-adherent cells were removed, and the adherent cells were treated with methadone. After 5 hours of exposure to methadone, lipopolysaccharides (LPS, 2 μg/mL) were added and the incubation continued for another 19 h. The concentration of pro- and anti-inflammatory cytokines in the culture supernatants of LPS activated monocytes was performed according to the instruction of the kit manufacturer.

The proliferation/activation of lymphocytes

The proliferation/activation capacity of peripheral lymphocytes was assessed as uridine uptake *via* the alternative nucleotide biosynthesis pathway, reflecting RNA synthesis [25]. Uridine incorporation by isolated mononuclear cells, non-stimulated or *ex vivo* activated with lectin mitogens (10 μg/mL PHA or 2.5 μg/mL PWM), was measured by the tritium-labelled uridine incorporation test [26]. Briefly, triplicate test samples (200 μL final volume), containing mononuclear cell suspension (0.2×10^6 cells), were incubated in the absence or the presence of mitogens, in 96 well plates, for 72 h at 37°C, in 5% CO_2 atmosphere. Six hours prior to harvesting, cell cultures were labelled with 1 μCi tritium labelled uridine (21 Ci/mmol). Cells, harvested on glass filters, were measured for radioactivity in scintillation liquid, using a Canberra-Packard beta-counter (PerkinElmer Life and Analytical Science, Boston, MA, USA). Results were expressed as pulses/min (ppm).

The respiratory burst of peripheral granulocytes

The respiratory burst of granulocytes was evaluated by flow cytometry as generation of intracellular reactive oxygen species (in this case, hydrogen peroxide) in heparinized peripheral blood system. Both resting and stimulated respiratory burst was assessed, using the following stimuli: opsonized bacteria (*E. coli*), N-formylmethionyl-leucyl-phenylalanine (fMet-Leu-Phe, fMLP), a granulocyte chemotactic bacterial peptide, and phorbol myristate acetate (PMA), an activator of protein kinase C (PKC).

The detection was performed by flow cytometry using BurstTest kit (ORPENGEN Pharma, Heidelberg, Germany) and dihydrorodamine 123 (DHR 123) as detection agent of intracellular hydrogen peroxide. The labelling of cells with propidium iodide, which binds to DNA allows excluding the dead cells and the cell aggregates.

Briefly, 100 μL of heparinized whole blood are activated with the stimulus, or with buffer (control sample) and incubated at 37°C for 10 min. After adding the substrate (DHR 123), the samples are incubated for another 10 min at 37°C in the dark. The red blood cells are lysated with the kit buffer at room temperature for 20 min. The samples were centrifuged for 5 min at 250 g at 4°C, after which the cell pellet was washed and treated with propidium iodide on ice for 10 min. The samples were analysed within 30 minutes afterwards on a FACSCalibur flow cytometer (Becton Dickinson, Erembodegem, Belgium) using CellQuest software. In the histogram number of events - FL-2 (corresponding to DHR123) is defined as area (M1) characterized by week FL-2 (non-activated cells) and other zone (M2) characterized by high FL-2 (activated cells). The percentage of responsive cells (the M2 area) and the intensity of cellular response in the M2 area (mean fluorescence channel - the geometric mean) are determined. The results were statistically processed as mean ± standard deviation of the mean.

Statistical analysis

Experimental data were expressed as mean ± standard error of the mean. The comparison between the group of addict patients and healthy subjects, and between different subgroups of patients was done with t test for unequal variance or t test for paired samples. Correlations between immune parameters were determined following the Pearson correlation test.

Results and Discussion

Peripheral distribution of the mononuclear cells

We determined the number of peripheral blood mononuclear cells (PBMC), lymphocytes (L) and monocytes (Mo) in peripheral blood in patients undergoing methadone maintenance therapy (TD) before starting the treatment and after one and six months of therapy (T0, T1, T6). Healthy subjects of the same age group constitute the control group (N) in order to determine the normal range. The results presented in Figure 1 indicate that before starting the therapy with methadone, heroin addict patients (HA) showed increased numbers of PBMC compared with normal values ($p_2 = 0.048$), and this anomaly increases strongly after a month of methadone therapy ($p_2 = 0.004$). Heroin use is associated with increased number of Mo ($p_2 = 0.016$), which further increased for the HA group after one month of therapy with methadone ($p_2 = 0.003$).

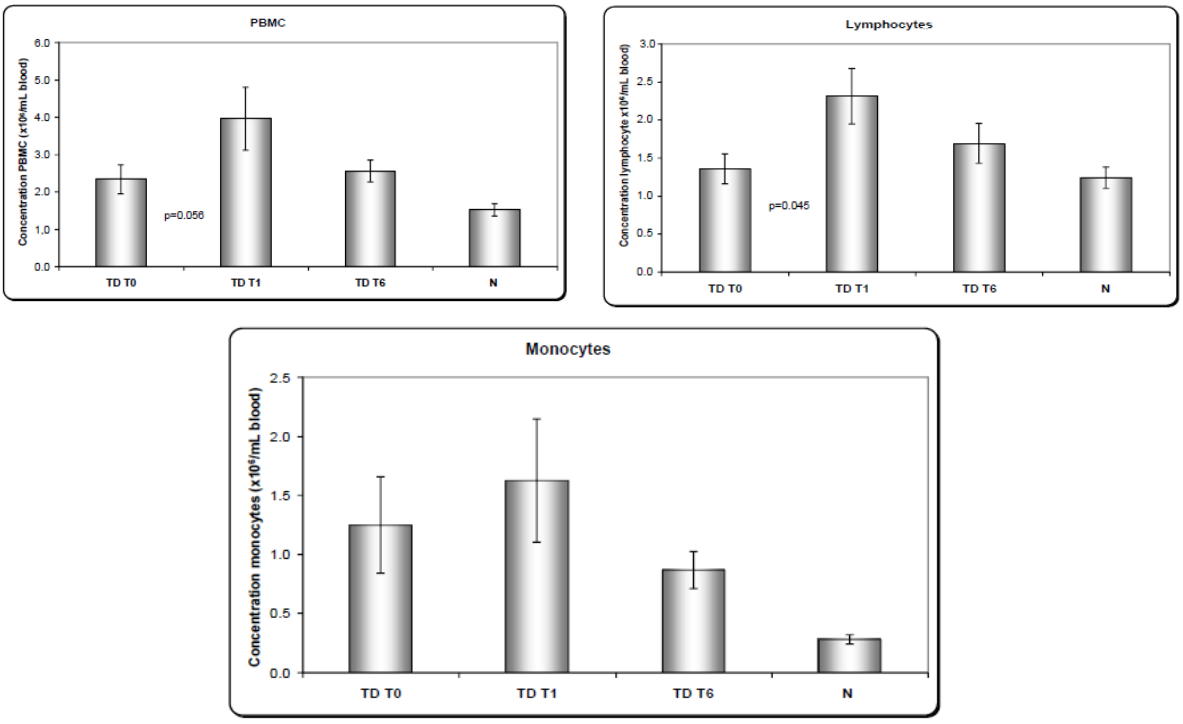

Figure 1.
Evolution of peripheral blood mononuclear cells of TD patients before therapy (TD T0), after 1 month (TD T1) and after 6 months of therapy (TD T6), N – healthy subjects

Although peripheral lymphocyte count is relatively normal before the therapy with methadone, it increases significantly above the normal values after one month of treatment ($p_2 = 0.025$). After 6 months of therapy with methadone, the number of PBMC, Mo and L returned to the values recorded before the initiation of therapy.

Peripheral distribution of lymphocyte populations and subpopulations

We have also assessed the percentage distribution of populations of T lymphocytes in peripheral blood before and after one and six months of methadone therapy (TD T0, TD T1 and TD T6) comparing with a control group (N).

Within a month, methadone therapy induces rapid changes in the distribution of peripheral B lymphocytes and NK cells (Figure 2).

Data showed that the percentage of B lymphocytes ($p_2 = 0.00002$) decreased to the lower limit of normal, accompanied by an increase of the proportion of NK cells ($p_2 = 0.013$), which is normalized. Consequently, the ratio B/NK, initially low, increased ($p_2 = 0.008$) and normalized (from 1.9 ± 0.3 to 3.4 ± 0.7, compared to the normal value of 3.2 ± 0.9). The percentage of B lymphocytes was unbalanced at 6 months (T1-T6: $p_2 = 0.036$, TD T6-N: $p_2 = 0.034$).

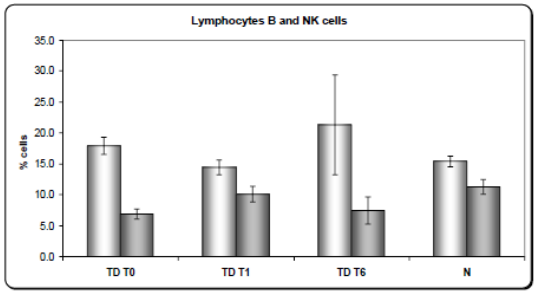

Figure 2.
Evolution of the percentage of B and NK lymphocytes in peripheral blood in TD patients during treatment with methadone
TD T0 – before therapy, TD T1 – 1 month of therapy and TD T6 – 6 months of therapy, N – healthy subjects

The profile of the pro- and anti-inflammatory serum cytokines

Cytokines are recognized as being responsible for the body inflammatory response process aiming at removing the inflammatory stimulus or responding to harmful tissue damage [27].

The investigation of the profile of the pro- and anti-inflammatory serum cytokines showed that heroin use is associated with low serum concentration of cytokines in TD patients, compared with clinically healthy subjects, except for IL-10, Th2 cytokine with anti-inflammatory activity (Figure 3).

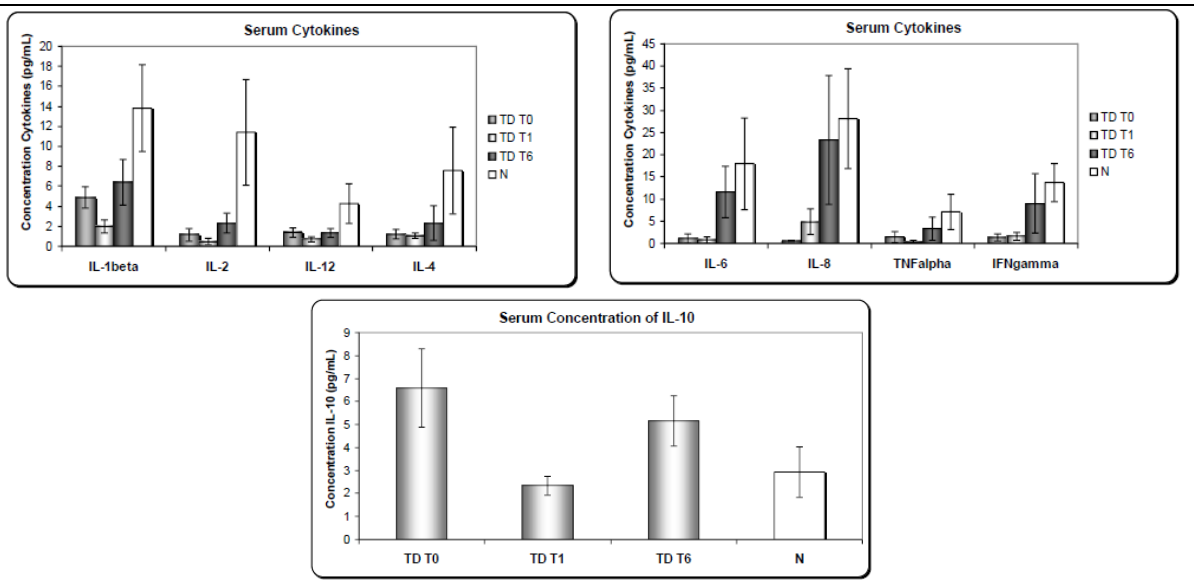

Figure 3.

Serum Th1/Th2 cytokine profile in TD patients after 1 and 6 months of therapy with methadone

TD T0 – before therapy, TD T1 – 1 month of therapy, TD T6 – 6 months of therapy, N – healthy subjects

After one month of methadone therapy, we observed a trend towards normalization by increasing the serum concentration of IL-6 and by decreasing the level of IL-10. The other investigated cytokines continued to present lower values compared to the normal ones. The results showed that IL-1beta, IL-2 and, to a lesser extent, IL-12 registered a decrease at 1 month, thus emphasizing the difference from normal, but the levels returned to baseline after 6 months of therapy. The results inversely correlate with the increase of peripheral lymphocytes and monocytes at 1 month of therapy that returned to baseline after 6 months of therapy. IL-6, IL-8, TNF-alpha and IFN-gamma tend to normalize after 6 months of therapy. IL-10 exhibited elevated initial values compared with normal, however they decreased significantly at one month of therapy ($p_2 = 0.028$), and increased back to baseline after 6 months ($p_2 = 0.015$).

Heroin addict patients present lower than normal serum concentrations of pro- and anti-inflammatory cytokines. The exception is IL-10 which is statistically higher than the normal average. It is possible that IL-10, Th2 type cytokine with suppressor activity, to inhibit the secretion of other cytokines. After a month of therapy with methadone, IL-10 decreased and normalized. In parallel, IL-6 (a pro-inflammatory cytokine) normalizes as well. It appears that IL-6 is more sensitive than the other cytokines investigated at IL-10 level, although the correlation study did not reveal a direct connection between the two cytokines. After 6 months of therapy with methadone, a trend toward normalization of IL-6, TNF-alpha and IFN-gamma the levels was observed. This increase and normalization do not seem to be regulated *via* IL-10, since this suppressor cytokine rises above normal at 6 months of therapy with methadone. IL-1beta, IL-2 and IL-4 showed a reduction at 1 month of therapy with methadone, despite recorded low baseline values before therapy. At 6 months, these cytokines returned to baseline, which is lower than normal. Further studies on correlations between cytokines and especially the ratio of pro- and anti-inflammatory cytokines are needed to identify the activity profile of serum cytokines. IL-8, chemotactic cytokine for granulocytes, had low baseline, but tended to normalize within 6 months of therapy with methadone.

Serum level of the soluble form of IL-2 receptor (sCD25)

We investigated the evolution of sCD25 serum levels during 6 months of therapy with methadone in 11 TD patients. The data in Figure 4 showed that TD patients have elevated sCD25 compared with clinically healthy subjects ($p_2 = 0.006$), and these values tend to increase further during the treatment with methadone (TD-N: $p_2 < 0.006$).

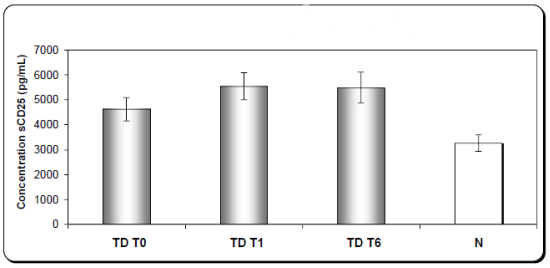

Figure 4.

Serum levels of soluble form of IL-2 receptor (sCD25) in TD patients after 1 and 6 months of methadone therapy

TD T0 – before therapy, TD T1 – 1 month of therapy, TD T1 - six months of therapy, N – healthy subjects

The sCD25 values remain high in drug-addicted patients during 6 months of methadone therapy; this may be due to proteolytic cleavage of the transmembrane form of CD25 (mCD25). sCD25 can inhibit cellular immune responses by competing for IL-2 binding to mCD25. The data obtained showed a mechanism for regulating the immune response where the associated T cell activation acts in an autocrine manner to limit the proliferation of these cells.

The activation/proliferation capacity of peripheral lymphocytes

We investigated the ability of polyclonal activation/ proliferation of peripheral lymphocytes. The experimental results (Figure 5) showed that there are not statistically significant differences between addict patients and clinically healthy subjects. However, we noted that the basal response of the mononuclear cells in addict patients seems to be moderately increased compared to normal values ($p_1 = 0.073$).

This finding must be verified by the other experimental methods, such as the assessment of the expression of the early (CD69) or late activation marker (CD25, HLA-DR) by lymphocytes.

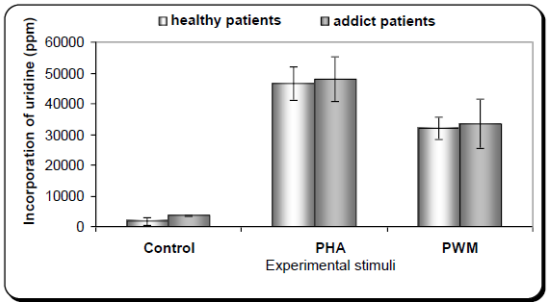

Figure 5.
The activation/proliferation capacity of lymphocytes from the addict patients and healthy subjects (activated *ex vivo* with PHA or PWM)

Respiratory burst of peripheral granulocytes

We determined the respiratory burst of granulocytes in patients undergoing methadone maintenance therapy before starting the treatment and after one and six months of therapy (T0, T1, T6). Results (Figure 6) showed increased proportion of cells responsive to *ex vivo* stimulation with *E. coli* for heroin users compared with control group ($p_2 = 0.002$).

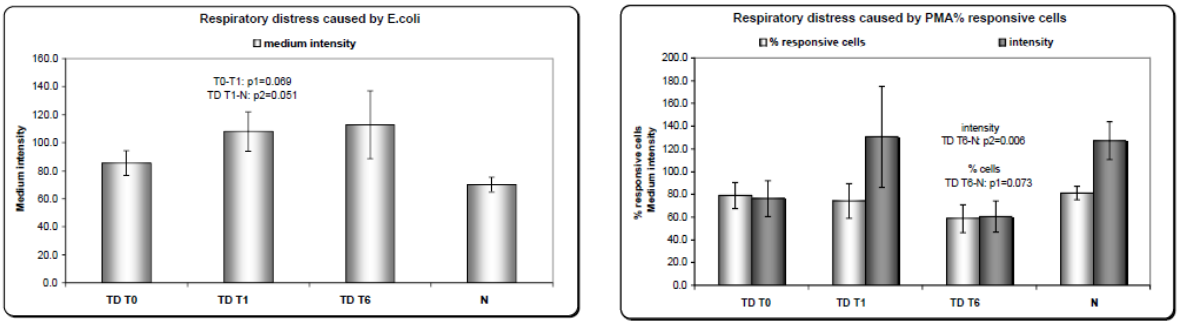

Figure 6.
Evolution of intracellular antimicrobial activity of peripheral PMN of TD patients within 6 months of methadone therapy
TD T0 – before therapy, TD T1 – 1 month of therapy, TD T6 – 6 months of therapy, N – healthy subjects

After 1 month of methadone therapy (TD T1, Figure 6), an increased oxidative response of PMN activated experimental with *E. coli* ($p_2 = 0.005$) is recorded, above the average normal values ($p_2 = 0.015$). During methadone therapy, the percentage of cells responsive to PMA remains lower than normal ($p_1 < 0.058$), but the initial low intensity of the cell response to PMA ($p_2 = 0.001$) tends to normalize after a month of therapy. The *ex vivo* reactivity of PMN to the bacterial fMLP did not change during one month of therapy with methadone.

After 6 months of methadone therapy (TD T6, Figure 6), the intensity of the respiratory burst induced by *E. coli* remains high. Meanwhile, the percentage of cells responsive to PMA and, in particular, the intensity of cellular response induced by the PKC activator, evolves towards lower values compared to normal, practically returning to the values recorded before the institution of methadone therapy.

Heroin consumption increases the percentage of granulocytes responsive to *E. coli*. Cellular response intensity rises above the normal after one month of therapy with methadone and remains high even after 6 months of replacement therapy. According to these results, methadone does not cause nonspecific immune suppression of granulocyte, especially since the granulocyte chemotactic cytokine, IL-8, tends to normalize. Addict patients have low proportions of granulocytes responsive to activation *via* PKC and switching from heroin to methadone does not change this anomaly. However, if the initial intensity of respiratory burst induced *via* PKC was reduced compared to normal, cell functionality tends to normalize after a month of replacement therapy, but returns to low levels after 6 months of methadone

consumption. These findings are consistent with another study showing a depressed T cells response to phyto-hemagglutinin (PHA) in peripheral blood of heroin addicts [9].

No clear correlations were found between the administered dose of methadone and the direction of evolution of the immune response during the methadone treatment.

The presented experimental data showed the disruptions of the immune response in chronic heroin users, namely increased number of peripheral monocytes, increased proportions of B lymphocytes, accompanied by low percentage of NK cells. Previous studies reported the morphine suppression of NK cell activity in rat, mice and humans [2]. The decreased concentrations of serum cytokines, the deviation of serum cytokines profile to Th2 (IL-10 associated), the high levels of sCD25, and a poor response of granulocytes to the activation *via* PKC were also registered.

The transition from heroin consumption to therapeutic methadone administration has also impacted the immune system. The increase in the number of peripheral monocytes is enhanced, and there is an abnormal increase in the number of peripheral lymphocytes. Meanwhile, the proportions of B lymphocytes and NK cells tend to normalize, but the balance between them is disrupted in favour of NK cells. The IL-10 anti-inflammatory cytokine and the IL-6 pro-inflammatory cytokine tended to normalize, the serum levels of sCD25 remained higher than normal, and the peripheral granulocytes begun to respond normally to the PKC activation and the oxidative response of granulocytes to bacterial stimuli was intensified.

After 6 months of therapy with methadone, the return of the majority of immune parameters to the values registered for the heroin users is observed. However, the data highlights some positive immunological aspects, such as the normalization trend in the number of peripheral lymphocytes and monocytes and the increased microbicide activity of the peripheral granulocytes.

It is not excluded that methadone therapy does not fully exercise a direct effect on the immune system, but also through other neuroimmune networks such as HPA axis (Hypothalamic-Pituitary-Adrenal Axis = hypothalamus-pituitary-adrenal axis).

The fact that some of the investigated immune parameters tend to change after a month of therapy with methadone, but after 6 months of treatment return to the values recorded before the replacement therapy, suggests that certain components of the immune system respond to chronic methadone consumption as a drug acting on the μ opioid receptor type (MOR).

Our findings are consistent with another study that investigated the immune system function of heroin addicted patients who received methadone or buprenorphine maintenance treatment for six months showing that immune system abnormalities in heroin addicted patients could be restored to almost normal values by rational treatment with methadone and buprenorphine [11]. However, further studies involving long term investigation are needed as Chan YY *et al*. revealed increased pro-inflammatory cytokines levels correlated with the duration of methadone maintenance treatment causing subsequent neuronal inflammation and damage [13].

Studies have shown that opioids can interact differently within immune cells and have varying effects on the immune system, but the results are not always consistent. Short term administration of methadone seems to have a positive impact on the immune system. Comparatively, long-term administration has a negative impact [28].

Conclusions

The obtained results showed that heroin disrupts the immune response, but only certain aspects indicate immunosuppression, such as elevated sCD25 levels, low levels of serum cytokines, and poor granulocyte response to activation *via* PKC. The shift to the therapeutic consumption of methadone initially results in a partial restoration of the immune defence capacity. However, over time, the immune system responds to methadone as if it were a persistent drug bombarding the opioid receptors in both the nervous system and the immune cells.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Lefkowitz SS, Chiang CY, Effects of certain abused drugs on hemolysin forming cells. *Life Sci.*, 1975; 17(12): 1763-1768.
2. Eisenstein TK, The Role of Opioid Receptors in Immune System Function. *Front Immunol.*, 2019; 10: 2904.
3. Friedman H, Newton C, Klein TW, Microbial infections, immunomodulation, and drugs of abuse. *Clin Microbiol Rev.*, 2003; 16(2): 209-219.
4. Haverkos HW, Curran JW, The current outbreak of Kaposi's sarcoma and opportunistic infections. *CA Cancer J Clin.*, 1982; 32(6): 330-339.
5. Mansell PW, Acquired immune deficiency syndrome, leading to opportunistic infections, Kaposi's sarcoma, and other malignancies. *Crit Rev Clin Lab Sci.*, 1984; 20(3): 191-204.
6. Donahoe RM, Falek A, Neuroimmunomodulation by opiates and other drugs of abuse: relationship to HIV infection and AIDS. *Adv Biochem Psychopharmacol.*, 1988; 44: 145-158.

7. Friedman H, Drugs of abuse as possible co-factors in AIDS progression: summary of panel discussion. *Adv Exp Med Biol.*, 1996; 402: 225-228.
8. McCarthy L, Wetzel M, Sliker JK, Eisenstein TK, Rogers TJ, Opioids, opioid receptors, and the immune response. *Drug Alcohol Depend.*, 2001; 62(2): 111-123.
9. Govitrapong P, Suttitum T, Kotchabhakdi N, Uneklabh T, Alterations of immune functions in heroin addicts and heroin withdrawal subjects. *J Pharmacol Exp Ther.*, 1998; 286(2): 883-889.
10. Zaki NG, Osman A, Moustafa H, Saad AH, Alterations of immune functions in heroin addicts. *Egypt J Immunol.*, 2006; 13(1): 153-171.
11. Sacerdote P, Franchi S, Gerra G, Leccese V, Panerai AE, Somaini L, Buprenorphine and methadone maintenance treatment of heroin addicts preserves immune function. *Brain Behav Immun.*, 2008; 22(4): 606-613.
12. Bârcă M, Manda G, Ciobanu AM, Bălălău C, Lupuleasa D, Burcea-Dragomiroiu GTA, Pop AL, Popa DE, Baconi DL, Immunomodulatory effects of methadone following methotrexate therapy in a rat model of arthritis. *Farmacia*, 2017; 65(3): 423-428.
13. Chan YY, Yang SN, Lin JC, Chang JL, Lin JG, Lo WY, Inflammatory response in heroin addicts undergoing methadone maintenance treatment. *Psychiatry Res.*, 2015; 226(1): 230-234.
14. Klimas NG, Blaney NT, Morgan RO, Chitwood D, Milles K, Lee H, Fletcher MA, Immune function and anti-HTLV-I/II status in anti-HIV-1-negative intravenous drug users receiving methadone. *Am J Med.*, 1991; 90(2): 163-170.
15. Börner C, Lanciotti S, Koch T, Höllt V, Kraus J, μ opioid receptor agonist-selective regulation of interleukin-4 in T lymphocytes. *J Neuroimmunol.*, 2013; 263(1-2): 35-42.
16. Kafami L, Etesami I, Felfeli M, Enayati N, Ghiaghi R, Aminian A, Dehpour A, Methadone diminishes neuroinflammation and disease severity in EAE through modulating T cell function. *J Neuroimmunol.*, 2013; 255(1-2): 39-44.
17. Li Y, Wang X, Tian S, Guo CJ, Douglas SD, Ho ZW, Methadone enhances human immunodeficiency virus infection of human immune cells. *J Infect Dis.*, 2002; 185(1): 118-122.
18. Wang MR, Wu DD, Luo F, Zhong CJ, Wang X, Zhu N, Wu YJ, Hu HT, Feng Y, Wang X, Xiong HR, Hou W, Methadone Inhibits Viral Restriction Factors and Facilitates HIV Infection in Macrophages. *Front Immunol.*, 2020; 11: 1253.
19. Ciobanu AM, Neagoe I, Baconi D, Bârcă M, Bălălău D, Bălănescu AR, Manda G, Particular inhibitory action of low concentrations of methadone on peripheral T lymphocytes. *Farmacia*, 2011; 59(5): 603-610.
20. Parlar A, Annaç E, Arslan SO, Çam SA, Pretreatment with glabridin prevents carrageenan-induced inflammation: The roles for cytokines and oxidative stress production. *Farmacia*, 2021; 69(1): 135-141.
21. Zhu Y, Yan P, Wang R, Lai J, Tang H, Xiao X, Yu R, Bao X, Zhu F, Wang K, Lu Y, Dang J, Zhu C, Zhang R, Dang W, Zhang B, Fu Q, Zhang Q, Kang C, Chen Y, Chen X, Liang Q, Wang K, Opioid-induced fragile-like regulatory T cells contribute to withdrawal. *Cell*, 2023; 186(3): 591-606.e23.
22. Ciucă Anghel DM, Vlăsceanu A, Gheroghe T, Ciobanu AM, Stan M, Nițescu V, Floroiu D, Tsatsakis A, Baconi D. Characterization of various drugs users groups of drugs users. Highlights on legal highs users. *Farmacia*, 2023; 71(1): 18-28.
23. Duff C, The place and time of drugs. *Int J Drug Policy*, 2014; 25(3): 633-639.
24. Boyum A, Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest.*, 1968; 97: 77-89.
25. Fletcher MA, Klimas N, Morgan R, Gjerset G, Lymphocyte proliferation in Manual of Clinical Laboratory Immunology, 4th ed. - Rose NR, de Macario EC, Fahey JL, Freedman H, Penn GM Eds., American Society for Microbiology, Washington DC, USA, 1992; 213.
26. Manda G, Neagu M, Livescu A, Constantin C, Codreanu C, Radulescu A, Imbalance of peripheral B lymphocytes and NK cells in rheumatoid arthritis. *J Cell Mol Med.*, 2003; 7(1): 79-88.
27. González-Lugo OE, Escobar-García DM, Pozos-Guillén AdJ, Peña PP, Luis AC, Sosa-Macías M, Aseff IL, Rangel-López A, Vértiz-Hernández AA, Analgesic ED50 diclofenac-eugenol combination effect on proinflammatory cytokines – IL-1β, IL-6, TNFα AND MAPKs – in rat muscle. *Farmacia*, 2022; 70(4): 583-588.
28. Liang X, Liu R, Chen C, Ji F, Li T, Opioid System Modulates the Immune Function: A Review. *Transl Perioper Pain Med.*, 2016; 1(1): 5-13.