

Effect of morphine and a low dose of ketamine on the T cells of patients with refractory cancer pain *in vitro*

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Received December 20, 2018; Accepted July 18, 2019

DOI: 10.3892/ol.2019.10750

Abstract. The combination of morphine and ketamine is considered safe and efficacious in many patients. However, a considerable number of immunomodulatory effects have been reported to be produced by both morphine and ketamine. The aim of the present study was to assess the direct effect of morphine and a low dose of ketamine on the T cells of patients with refractory cancer pain *in vitro*. Venous blood was obtained from patients with refractory cancer pain and peripheral blood mononuclear cells were isolated using the Ficoll-Hypaque density gradient method. Anti-CD3 beads were used to isolate T cells by positive selection. Subsequently, the T cells were treated with vehicle, 200 ng/ml of morphine or 200 ng/ml of morphine + 100 ng/ml ketamine for 24 h, following which the cells were stimulated with anti-CD3 and anti-CD28. Flow cytometric analysis of CD3⁺ T cells, and interleukin (IL)-2 and interferon (IFN)- γ in the supernatant, reverse transcription-quantitative PCR analysis for the detection of IL-2 and IFN- γ and western blotting for the detection of p65 nuclear factor (NF)- κ B were performed. *In vitro*, the CD4⁺ and CD8⁺ T cell counts, CD4⁺/CD8⁺ ratio, secretion of IL-2 and IFN- γ in the supernatant, mRNA expression levels of IL-2 and IFN- γ and expression of p65 NF- κ B were significantly decreased following treatment with morphine and morphine + ketamine, compared with results in the control group (all $P < 0.05$). However, there was no significant difference between treatment with morphine and that with morphine + ketamine. Treatment with morphine + ketamine *in vitro* decreased the immune functions of patients with refractory cancer pain, although the effect of treatment with morphine and a low

dose of ketamine did not differ significantly from that with morphine treatment alone.

Introduction

Pain associated with cancer is a serious problem for patients, significantly affecting their quality of life and is difficult to treat. A considerable proportion of patients suffer from refractory cancer pain. Refractory pain is associated with the growth and metastasis of tumors (1,2), therefore, pain relief may be beneficial for improving the patient's quality of life while also supplementing cancer therapy. The World Health Organization has proposed that a three-step analgesic ladder is effective for treating cancer pain (3). For neuropathic pain in advanced cancer, the first-line therapeutic agent is typically an opioid, such as morphine (4-7), however, pain may not be fully relieved for many patients. In these cases, the combination of pharmacological agents may be necessary to relieve the refractory pain. The combination of morphine and ketamine has been considered safe and efficacious in numerous patients (8-13). Clinically, patients with refractory cancer pain report improved pain relief with fewer side effects when morphine and a low dose of ketamine were used compared with morphine alone.

A number of patients experience cancer pain driven by nociception and neuropathy (14) and neuropathic cancer pain driven by nociception is considered to be resistant to opioids (15-17). For neuropathic pain, peripheral and central sensitization are the primary causes. Ketamine is an N-methyl-D aspartate (NMDA) antagonist and is also a non-opioid anesthetic agent. The primary clinical application of ketamine is for short diagnostic or surgical procedures (17). NMDA receptors are activated by the excitatory neurotransmitter glutamate in the spinal dorsal horn, which results in sensitization of the central nervous system (18-21). Therefore, attenuating this sensitization may underlie the analgesic effects of ketamine (19-21). Similarly, NMDA antagonism may be involved in the ketamine-mediated attenuation of acquired opioid tolerance (20-22).

However, in laboratory animals and humans, morphine has been reported to produce numerous immunomodulatory effects *in vivo* and *in vitro*. As a potent immunomodulator, morphine has inhibitory and stimulatory effects on immune

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Key words: morphine, ketamine, T cells, cancer pain, interleukin-2, interferon- γ , nuclear factor- κ B

function, including altering the expression of CD4⁺ and CD8⁺ T cells, reducing the activity of natural killer cells (23-25), altering the balance between Th1 and Th2 cells (26,27), increasing the production of interleukin (IL)-12 by peritoneal macrophages (28), increasing the production of IL-6 and tumor necrosis factor when induced by lipopolysaccharides, and increasing the activity of nuclear factor- κ B (NF- κ B) in macrophages and peripheral blood mononuclear cells (PBMCs) (29,30). In particular, in activated T cells, morphine has been shown to enhance the expression of IL-4 and suppress the expression of interferon (IFN)- γ and IL-2 (31-35). For patients with cancer and an impaired immune system, the immunomodulatory effects of morphine may cause unwanted side effects during pain treatment. However, there are few reports regarding the effects of morphine on T cells in patients who suffer from refractory cancer pain. Similarly, there are few reports regarding the effect of ketamine on immune cells, particularly in T cells. Therefore, the aim of the present study was to assess the direct effect of morphine and a low dose of ketamine on the T cells of patients with refractory cancer pain *in vitro*.

Materials and methods

T cell culture, T cell activation and reagents. The entire protocol (SDTHEC201504001) was approved by the Ethics Committee of Shandong Cancer Hospital and Institute (Jinan, China; Chairperson Dr Jinming Yu) and conducted according to the principles of the Helsinki Declaration. Informed, written consent was obtained from all patients with cancer pain, and the patients were involved in the study for >3 months.

Blood was withdrawn following venipuncture. Peripheral blood mononuclear cells (PBMCs) were isolated using the Ficoll-Hypaque density gradient method according to the manufacturer's protocol (Cedarlane). The T cells were isolated by positive selection using anti-CD3 beads (Miltenyi Biotec, Inc.) according to the manufacturer's protocol, and confirmed by fluorescence-associated cell sorting (85% purity). A final concentration of 1×10^5 cells/ml were plated into 96-well plates, and the T cells were cultivated at 37°C and 5% CO₂ in RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 mg/ml streptomycin (all from Gibco; Thermo Fisher Scientific, Inc.).

The T cells were treated with either vehicle (normal saline), 200 ng/ml of morphine (Humanwell Healthcare (Group) Co., Ltd.), or 200 ng/ml of morphine + 100 ng/ml ketamine (pure hydrochloride salt without preservatives, Sigma-Aldrich; Merck KGaA) for 24 h at 37°C. By determining the plasma concentration of morphine and ketamine in patients with refractory cancer pain in the clinic, and referring to other human and animal experiments using morphine and ketamine, a morphine concentration of 200 ng/ml and ketamine concentration of 100 ng/ml were established (36-38). These concentrations are approximately equal to the doses of morphine and ketamine commonly used in patients with refractory cancer pain in the clinic. Subsequently, the cells were stimulated with anti-CD3 and anti-CD28 (R&D Systems, Inc.; mouse-anti-human, 5 mg/ml) for 24 h at 37°C. The groups were designated as the control group, morphine group and morphine + ketamine

group. To activate T cells, it is necessary for the antigen-specific T cell receptor and a second co-receptor, such as CD28, to be sufficiently occupied (39). In the present study, anti-CD3 and anti-CD28 stimulation were used to investigate the effects of morphine and ketamine on the T cells.

Flow cytometric analysis of CD3⁺ T cells. Following experimental treatment, the T cells (1×10^5 /ml) were harvested by centrifugation for 5 min at 300 x g room temperature, washed in phosphate-buffered saline and subsequently incubated with trichromatism monoclonal antibody reagent [CD4 fluorescein isothiocyanate/CD8 phycoerythrin/CD3 PerCP (BD Biosciences), 0.5 mg/ml] according to the manufacturer's protocol. Subsequently, the quantity and percentage of CD3/CD4/CD8T cells were measured using a flow cytometer (FACSCalibur; BD Biosciences).

Supernatant cytokine protein analysis. Cytokine-specific ELISA kits (R&D Systems, Inc.) were used to measure the protein concentrations of IL-2 and IFN- γ according to the manufacturer's protocol. The experiments were repeated three times.

Reverse transcription-quantitative PCR analysis. Total RNA was extracted from the T cells by lysis using guanidinium isothiocyanate and phenol acid extraction. A total of 1 μ g RNA, 0.5 μ l RNase H minus (Promega Corporation) and 1 μ l Moloney murine leukemia virus reverse transcriptase were used for cDNA synthesis with the following thermocycling conditions: 95°C 5 min; 95°C 15 sec, 60°C 35 sec, 40 cycles; 72°C 5 min; 4°C terminal. For each PCR, 2 μ l cDNA was used and the primers were obtained from BD Biosciences. The primer sequences were as follows: IL-2 forward, 5'-GAATGGAATTAATAATTACAAGAATCCCC-3' and reverse, 5'-TGTTTCAGATCCCCTTTAGTTCCAG-3'; and IFN- γ forward, 5'-TCG GTAAGTGAAGTGAATGTCCA-3' and reverse, 5'-TCCTTTTTCGCTTCCCTGTTTT-3'. The Light Cycler-Fast Start DNA Master SYBR Green I kit (Roche Diagnostics) was used for the RT-qPCR analysis of IL-2, and the Revert Aid™ First Strand cDNA Synthesis kit (Roche Diagnostics) was used for IFN- γ , according to the manufacturer protocols. The quantitative analysis of original template was performed by the change of fluorescence of the amplification product.

Western blot analysis for activated p65 NF- κ B. The T cells were lysed with RIPA protein lysis buffer (100 μ g/ml Nonidet P-40, 150 mol/l NaCl and 50 mmol/l Tris-HCl supplemented with a protease inhibitor mixture). Following one freeze/thaw cycle, the lysates were centrifuged for 15 min at 12,000 g (2-8°C). A BCA protein concentration kit was used to determine protein concentration. Following electrophoresis using 10% SDS-PAGE, the resolved protein samples were transferred onto an ECL nitrocellulose membrane (EMD Millipore) by electroblotting. 5% (w/v) of skimmed milk powder was used for blocking for 1 h at room temperature. Subsequently, the nitrocellulose membranes were incubated overnight at 4°C with primary antibody [rabbit anti-NF- κ B p65 antibody (cat. no., ab16502); dilution, 1:2,000; Abcam/rabbit anti- β -actin (cat. no., ab8227); dilution, 1:2,000; Abcam], and then incubated with the

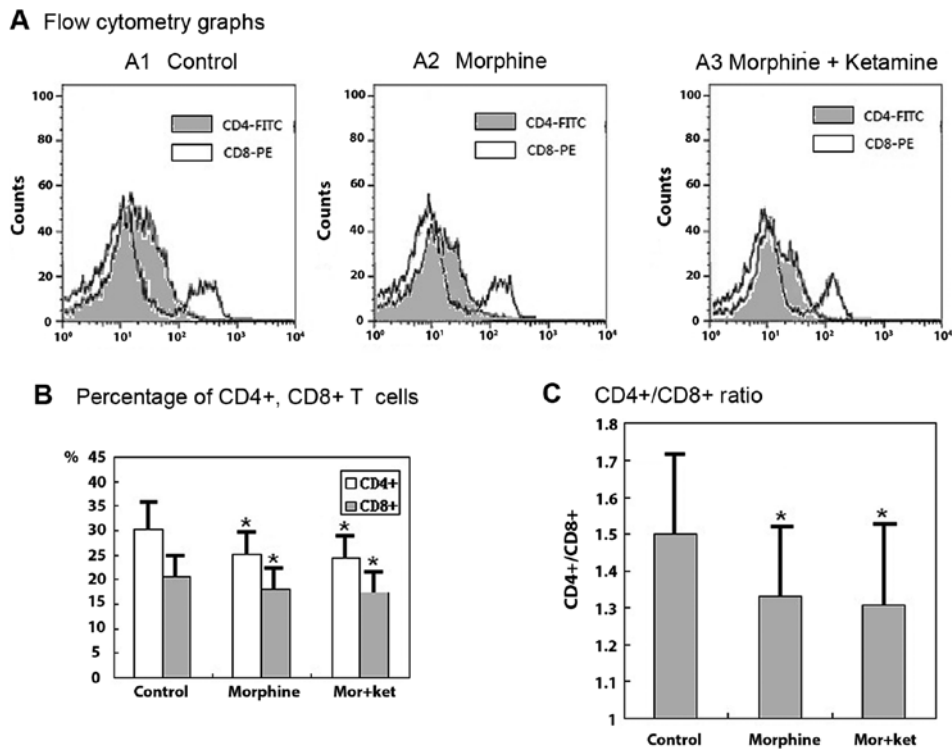


Figure 1. Effects of morphine and low-dose ketamine on the quantity and percentage of CD3⁺/CD4⁺/CD8⁺T cells from patients with refractory cancer pain. Flow cytometry was performed to examine cells *in vitro*. (A) Flow cytometry graphs of the (A1) control group, (A2) morphine group and (A3) morphine + ketamine group. (B) Percentages of CD4⁺ and CD8⁺ T cells. (C) Ratios of CD4⁺/CD8⁺ T cells. *P<0.05, vs. Control. Mor + ket, morphine + ketamine.

secondary goat anti-Rabbit horseradish peroxidase antibody (cat. no., ab205718; 1:2,000; Abcam) at room temperature for 2 h. Protein concentration was determined by ECL western blotting detection kit (cat. no., ab65623; Abcam). Image J version 1.8.0 (National Institutes of Health) was used for quantification of blots.

Statistical analysis. All variables were assessed in triplicate and all experiments were repeated at least three times. All data are expressed as the mean \pm standard deviation. Statistical comparisons between experimental groups were performed, where appropriate, with SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). Statistical significance was analyzed using a paired t-test or one-way ANOVA followed by a non-parametric Student-Newman-Keuls test for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

Flow cytometric analysis of CD3⁺ T cells. The counts of T cells expressing CD4 and CD8 were significantly decreased when the cells were treated with morphine and when treated with morphine + ketamine. The decrease in the count of CD4⁺ T cells was larger than that of CD8⁺ T cells, therefore, the ratio of CD4⁺/CD8⁺ was also significantly decreased by morphine and morphine + ketamine treatments (Fig. 1A-C; all P<0.05). However, the decreases in the CD4⁺ and CD8⁺ T cell counts and the CD4⁺/CD8⁺ ratio did not differ significantly when the cells were treated with morphine compared with those when the cells were treated with morphine + ketamine.

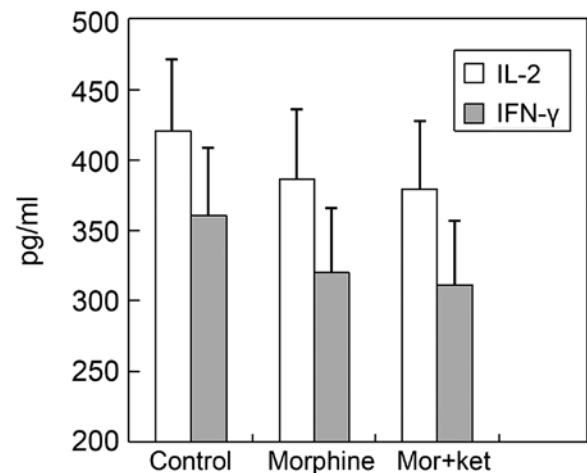


Figure 2. Supernatant protein concentrations of IL-2 and IFN-γ of T cells from patients with refractory cancer pain treated with morphine and low-dose ketamine *in vitro*. IL-2, interleukin-2; IFN-γ, interferon-γ; Mor + ket, morphine + ketamine.

Supernatant cytokine protein analysis and RT-qPCR analysis. There were no significant differences in the concentrations of IL-2 and IFN-γ in the supernatants of the morphine group and the morphine + ketamine group compared with those in the control group (Fig. 2). However, the mRNA expression levels of IL-2 and IFN-γ were significantly decreased in the morphine group and the morphine + ketamine group compared with those in the control group (Fig. 3; P<0.05). The results of the morphine group did not differ significantly from those of the morphine + ketamine group.

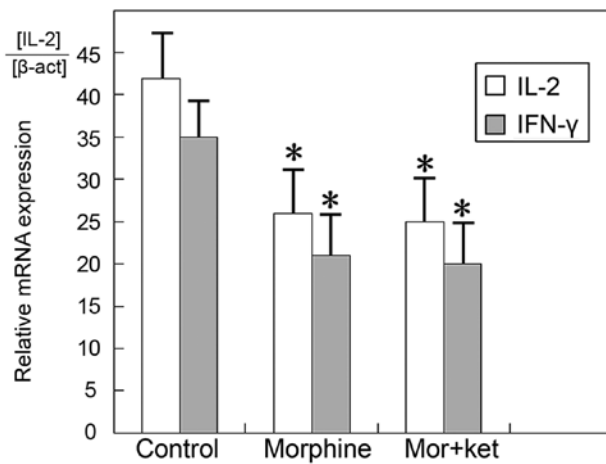


Figure 3. Reverse transcription-quantitative polymerase chain reaction analysis of IL-2 and IFN- γ of T cells from patients with refractory cancer pain treated with morphine and low-dose ketamine *in vitro*. * $P<0.05$, vs. Control. IL-2, interleukin-2; IFN- γ , interferon- γ ; Mor + ket, morphine + ketamine.

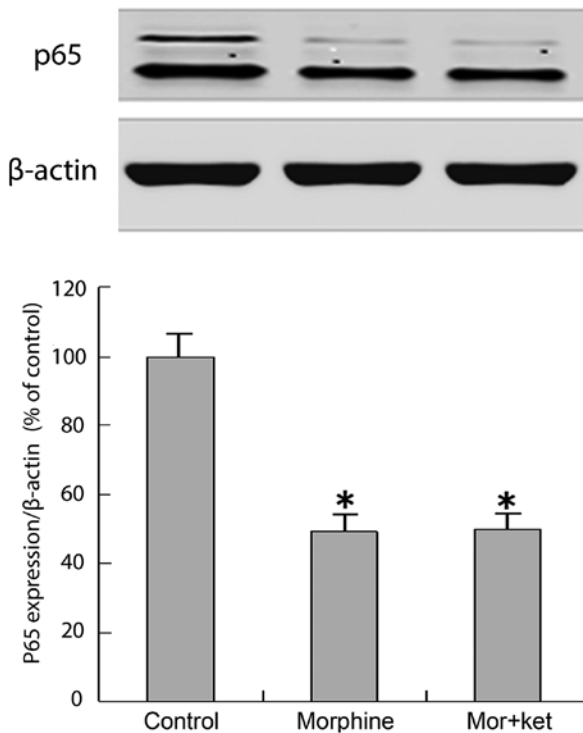


Figure 4. Western immunoblot analysis of activated p65 nuclear factor- κ B of T cells from patients with refractory cancer pain treated with morphine and low-dose ketamine *in vitro*. * $P<0.05$, vs. Control. Mor + ket, morphine + ketamine.

Western blot analysis of activated p65 NF- κ B. The morphine and morphine + ketamine treatments decreased the quantity of activated p65 NF- κ B entering the nuclei of T cells compared with that in the control group (Fig. 4; $P<0.05$). The results in the morphine group did not differ significantly from those in the morphine + ketamine group.

Discussion

Patients with refractory cancer pain suffer from pain and consequent stress on a daily basis. Those individuals experiencing

severe pain, stress or who become addicted to opioids are generally immunosuppressed resulting from activation of the neuroendocrine-immune network and the potent effect of neuroactive ligands on cells of the immune system (40-45). Opioids have been demonstrated to modulate immune responses, and opioid receptors are expressed on immune cells (41). *In vivo* and *in vitro*, the therapeutic and chronic use of morphine affects the physiological function of the immune system, including innate and adaptive immunity (46,47).

T cells and the various subpopulations serve important roles in cell-mediated immunity. The ratios of the subpopulations of T cells are stably maintained to ensure an effective immune response. In the present study, CD4⁺ and CD8⁺ T cells, and the ratio of CD4⁺/CD8⁺ T cells were all decreased by morphine, which suggested an attenuation of the immune response.

IL-2 is a pivotal cytokine for T cell function, and a previous study demonstrated that chronic treatment with morphine can inhibit the mRNA expression and secretion of IL-2 (48). Similarly, in the present study on patients with refractory cancer pain, the mRNA expression levels of IL-2 and IL-2 were down-regulated by morphine treatment. A previous study showed that the expression of IL-2 was regulated almost entirely at the transcriptional level and that the essential elements lie within 300 bp upstream of the start codon (49), which can be bound by several inducible transcription factors including NF- κ B. It has been hypothesized that the production of IL-2 is inhibited with chronic morphine treatment at the transcriptional level and through epigenetic mechanisms (31,34,35,50,51).

As a cytokine modulating all phases of immune processes, IFN- γ is produced in large quantities in activated T cells (52) and serves an important role in host resistance to infection (53). Previous studies have demonstrated that chronic morphine treatment inhibited IFN- γ protein synthesis (32,54), similar to the results of the present study. Furthermore, the results of the present study demonstrated that the mRNA expression levels of IFN- γ were downregulated by morphine, suggesting that morphine modulated IFN- γ by altering the gene expression and protein synthesis of IFN- γ . It is hypothesized that transcription of the IFN- γ gene is coordinated by the transcription factor NF- κ B (55-57).

As a central regulator of the immune response (58), NF- κ B regulates the transcription of various genes, including a number of cytokines and genes encoding immunoreceptors (59,60), and it is sequestered in the cytoplasm in the majority of cell types (61). NF- κ B is also vital for regulating the activation, proliferation and differentiation of T cells (62). Roy *et al* (30) demonstrated that morphine prevented the binding of NF- κ B to its consensus DNA sequence in macrophages and activated T cells of patients who had received chronic morphine treatment.

In vivo, the pathways regulating the immunomodulatory effects of opioids transduced to the immune effector cells primarily include the following: i) Indirectly via the central nervous system, for example, by activation of the hypothalamic-pituitary-adrenal axis (63,64); ii) directly via atypical opioid receptors (25); and iii) directly via the classic μ - (preferential), δ - and κ -opioid receptor subtypes present on immune cells (65,66) that belong to the G_i protein-coupled receptor superfamily (67). Additionally, it has been shown that the activation of NF- κ B was inhibited by elevated cAMP in T cells at the molecular level (68-70). Therefore, morphine

may bind to μ -opioid receptors and activate G_i protein-coupled receptors, resulting in the intracellular accumulation of cAMP, and thus leading to decreased NF- κ B activation and the inhibition of IL-2 and IFN- γ transcription.

Ketamine is often administered epidurally or intravenously in combination with an opioid for the treatment of chronic cancer pain (71,72). For the clinical treatment of refractory cancer pain, ketamine is often used as an adjuvant analgesic; as ketamine alone is not used for refractory cancer pain, a group treated with ketamine only was not included in the present study. However, ketamine also affects immune cells. It has been suggested that ketamine inhibits the production and function of dendritic cells (73). Treatment with ketamine alone has also been shown to increase the ratios of Th1/Th2 and IFN- γ /IL-4 (74). However, in the present study, ketamine did not induce significant effects in any of the measurements, including CD4⁺ T cells, CD8⁺ T cells, the CD4⁺/CD8⁺ ratio, concentrations of IL-2 and IFN- γ in the supernatant, mRNA expression levels of IL-2 and IFN- γ and the activation of NF- κ B. A possible explanation for the lack of effect of ketamine was the dose used, which may have been too low. In clinical use, the dose of ketamine used is variable. The epidural administration of ketamine is typically ~0.4 mg/ml (~1.5 mM) (75-80), whereas intrathecal administration is typically <25 mg/ml (~93 mM) (81-83). Another study demonstrated that the infusion dose of ketamine for cancer-associated treatment was 0.084-0.6 mg/kg/h (20). In the present study, a dose of 100 ng/ml of ketamine was considered to be ineffective.

In conclusion, *in vitro* treatment with morphine alone and in conjunction with ketamine inhibited the immune functions of patients with refractory cancer pain. Treatment decreased the counts of CD4⁺ and CD8⁺ T cells, CD4⁺/CD8⁺ ratio, concentrations of IL-2 and IFN- γ in the supernatant, mRNA expression levels of IL-2 and IFN- γ and the activation of NF- κ B, but the effect of morphine in conjunction with ketamine did not differ significantly from the effect of morphine alone. The present study may provide evidence for clinicians to offer more relief of pain and less suppression of immune function to patients with refractory cancer pain.

Acknowledgements

Not applicable.

Funding

The present study was supported by a grant from the Natural Science Foundation (Shandong, China; grant no. ZR2011HM039).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

NBZ, KGW, ZJF designed the study and completed the organization and writing. NBZ conducted the experiments and analyzed the data.

Ethics approval and consent to participate

The entire protocol (SDTHEC201504001) was approved by the Ethics Committee of Shandong Cancer Hospital and Institute (Jinan, China; chairperson Dr Jinming Yu) and was conducted according to the principles of the Helsinki Declaration. Informed, written consent was obtained from patients with cancer pain.

Patient consent for publication

The patients consented to publication.

Competing interests

The authors declare that they have no competing interests.

References

1. Page GG, Ben-Eliyahu S, Yirmiya R and Liebeskind JC: Morphine attenuates surgery-induced enhancement of metastatic colonization in rats. *Pain* 54: 21-28, 1993.
2. Sasamura T, Nakamura S, Iida Y, Fuji H, Murata J, Saiki I, Nojima H and Kuraishi Y: Morphine analgesia suppresses tumor growth and metastasis in a mouse model of cancer pain produced by orthotopic tumor inoculation. *Eur J Pharmacol* 441: 185-191, 2002.
3. Zech DF, Grond S, Lynch J, Hertel D and Lehmann KA: Validation of World Health Organization guidelines for cancer pain relief: A 10-year prospective study. *Pain* 63: 65-76, 1995.
4. Collins JJ, Grier HE, Kinney HC and Berde CB: Control of severe pain in children with terminal malignancy. *J Pediatr* 126: 653-657, 1995.
5. Hurwitz CA, Duncan J and Wolfe J: Caring for the child with cancer at the close of life: 'There are people who make it and I'm hoping I'm one of them.' *JAMA* 292: 2141-2149, 2004.
6. Jalmell L, Kreicbergs U, Onelöv E, Steineck G and Henter JI: Symptoms affecting children with malignancies during the last month of life: A nationwide follow-up. *Pediatrics* 117: 1314-1320, 2006.
7. Wolfe J, Grier HE, Klar N, Levin SB, Ellenbogen JM, Salem-Schatz S, Emanuel EJ and Weeks JC: Symptoms and suffering at the end of life in children with cancer. *N Engl J Med* 342: 326-333, 2000.
8. Finkel JC, Pestieau SR and Quezada ZM: Ketamine as an adjuvant for treatment of cancer pain in children and adolescents. *J Pain* 8: 515-521, 2007.
9. Mercadante S, Lodi F, Sapio M, Calligara M and Serretta R: Longterm ketamine subcutaneous continuous infusion in neuropathic cancer pain. *J Pain Symptom Manage* 10: 564-568, 1995.
10. Elsewaisy O, Slon B and Monagle J: Analgesic effect of subanesthetic intravenous ketamine in refractory neuropathic pain: A case report. *Pain Med* 11: 946-950, 2010.
11. Webster LR and Walker MJ: Safety and efficacy of prolonged outpatient ketamine infusions for neuropathic pain. *Am J Ther* 13: 300-305, 2006.
12. Visser E and Schug SA: The role of ketamine in pain management. *Biomed Pharmacother* 60: 341-348, 2006.
13. Subramaniam K, Subramaniam B and Steinbrook RA: Ketamine as adjuvant analgesic to opioids: A quantitative and qualitative systematic review. *Anesth Analg* 99: 482-495, 2004.
14. Grond S, Radbruch L, Meuser T, Sabatowski R, Loick G and Lehmann KA: Assessment and treatment of neuropathic cancer pain following WHO guidelines. *Pain* 79: 15-20, 1999.
15. Cherny NI, Thaler HT, Friedlander-Klar H, Lapin J, Foley KM, Houde R and Portenoy RK: Opioid responsiveness of cancer pain syndromes caused by neuropathic or nociceptive mechanisms: A combined analysis of controlled, single-dose studies. *Neurology* 44: 857-861, 1994.
16. Martin LA and Hagen NA: Neuropathic pain in cancer patients: Mechanisms, syndromes, and clinical controversies. *J Pain Symptom Manage* 14: 99-117, 1997.

17. Enarson MC, Hays H and Woodroffe MA: Clinical experience with oral ketamine. *J Pain Symptom Manage* 17: 384-386, 1999.
18. Coggeshall RE and Carlton SM: Receptor localization in the mammalian dorsal horn and primary afferent neurons. *Brain Res Brain Res Rev* 24: 28-66, 1997.
19. Persson J, Axelsson G, Hallin RG and Gustafsson LL: Beneficial effects of ketamine in a chronic pain state with allodynia, possibly due to central sensitization. *Pain* 60: 217-222, 1995.
20. Okon T: Ketamine: An introduction for the pain and palliative medicine physician. *Pain Physician* 10: 493-500, 2007.
21. Grande LA, O'Donnell BR, Fitzgibbon DR and Terman GW: Ultra-low dose ketamine and memantine treatment for pain in an opioid-tolerant oncology patient. *Anesth Analg* 107: 1380-1383, 2008.
22. Shimoyama N, Shimoyama M, Inturrisi CE and Elliott KJ: Ketamine attenuates and reverses morphine tolerance in rodents. *Anesthesiology* 85: 1357-1366, 1996.
23. Weber RJ and Pert A: The periaqueductal gray matter mediates opiate-induced immunosuppression. *Science* 245: 188-190, 1989.
24. Gavériaux-Ruff C, Matthes HW, Peluso J and Kieffer BL: Abolition of morphine-immunosuppression in mice lacking the mu-opioid receptor gene. *Proc Natl Acad Sci USA* 95: 6326-6330, 1998.
25. Roy S, Barke RA and Loh HH: MU-opioid receptor-knockout mice: Role of mu-opioid receptor in morphine mediated immune functions. *Brain Res Mol Brain Res* 61: 190-194, 1998.
26. Roy S, Charboneau RG and Barke RA: Morphine synergizes with lipopolysaccharide in a chronic endotoxemia model. *J Neuroimmunol* 95: 107-114, 1999.
27. Sacerdote P, Gaspari L and Panerai AE: The opioid antagonist naloxone induces a shift from type 2 to type 1 cytokine pattern in normal and skin-grafted mice. *Ann NY Acad Sci* 917: 755-763, 2000.
28. Peng X, Mosser DM, Adler MW, Rogers TJ, Meissler JJ Jr and Eisenstein TK: Morphine enhances interleukin-12 and the production of other pro-inflammatory cytokines in mouse peritoneal macrophages. *J Leukoc Biol* 68: 723-728, 2000.
29. Chao CC, Molitor TW, Close K, Hu S and Peterson PK: Morphine inhibits the release of tumor necrosis factor in human peripheral blood mononuclear cell cultures. *Int J Immunopharmacol* 15: 447-453, 1993.
30. Roy S, Cain KJ, Chapin RB, Charboneau RG and Barke RA: Morphine modulates NF kappa B activation in macrophages. *Biochem Biophys Res Commun* 245: 392-396, 1998.
31. Roy S, Chapin RB, Cain KJ, Charboneau RG, Ramakrishnan S and Barke RA: Morphine inhibits transcriptional activation of IL-2 in mouse thymocytes. *Cell Immunol* 179: 1-9, 1997.
32. Roy S, Balasubramanian S, Sumandeep S, Charboneau R, Wang J, Melnyk D, Beilman GJ, Vatsery R and Barke RA: Morphine directs T cells toward T(H2) differentiation. *Surgery* 130: 304-309, 2001.
33. Roy S, Wang J, Charboneau R, Loh HH and Barke RA: Morphine induces CD4+ T cell IL-4 expression through an adenylyl cyclase mechanism independent of the protein kinase A pathway. *J Immunol* 175: 6361-6367, 2005.
34. Wang J, Barke RA, Charboneau R, Loh HH and Roy S: Morphine negatively regulates interferon-gamma promoter activity in activated murine T cells through two distinct cyclic AMP-dependent pathways. *J Biol Chem* 278: 37622-37631, 2003.
35. Wang J, Barke RA and Roy S: Transcriptional and epigenetic regulation of interleukin-2 gene in activated T cells by morphine. *J Biol Chem* 282: 7164-7171, 2007.
36. Pacifici GM: Metabolism and pharmacokinetics of morphine in neonates: A review. *Clinics (Sao Paulo)* 71: 474-480, 2016.
37. Heiskanen T, Langel K, Gunnar T, Lillsunde P and Kalso EA: Opioid concentrations in oral fluid and plasma in cancer patients with pain. *J Pain Symptom Manage* 50: 524-532, 2015.
38. Khalili-Mahani N, Martini CH, Olofsen E, Dahan A and Niesters M: Effect of subanaesthetic ketamine on plasma and saliva cortisol secretion. *Br J Anaesth* 115: 68-75, 2015.
39. Zhang J, Salojin KV, Gao JX, Cameron MJ, Bergerot I and Delovitch TL: p38 mitogen-activated protein kinase mediates antigen integration of TCR/CD28 costimulation in primary murine T cells. *Immunol* 162: 3819-3829, 1999.
40. Rouveix B: Opiates and immune function: Consequences on infectious diseases with special reference to AIDS. *Therapie* 47: 503-512, 1992.
41. Sibinga NE and Goldstein A: Opioid peptides and opioid receptors in cells of the immune system. *Annu Rev Immunol* 6: 219-249, 1988.
42. Weigent DA and Blalock JE: Interactions between the neuroendocrine and immune systems: Common hormones and receptors. *Immunol Rev* 100: 79-108, 1987.
43. Nair MP, Schwartz SA, Polasani R, Hou J, Sweet A and Chadha KC: Immunoregulatory effects of morphine on human lymphocytes. *Clin Diagn Lab Immunol* 4: 127-132, 1997.
44. Kulkarni-Narla A, Walcheck B and Brown DR: Opioid receptors on bone marrow neutrophils modulate chemotaxis and CD11b/CD18 expression. *Eur J Pharmacol* 414: 289-294, 2001.
45. McCarthy L, Wetzel M, Sliker JK, Eisenstein TK and Rogers TJ: Opioids, opioid receptors, and the immune response. *Drug Alcohol Depend* 62: 111-123, 2001.
46. Roy S and Loh HH: Effects of opioids on the immune system. *Neurochem Res* 21: 1375-1386, 1996.
47. Bryant HU, Bernton EW and Holaday JW: Immunosuppressive effects of chronic morphine treatment in mice. *Life Sci* 41: 1731-1738, 1987.
48. Wang J, Charboneau R, Balasubramanian S, Barke RA, Loh HH and Roy S: Morphine modulates lymph node-derived T lymphocyte function: Role of caspase-3, -8 and nitric oxide. *J Leukocyte Biol* 70: 527-536, 2001.
49. Jain J, Loh C and Rao A: Transcriptional regulation of the IL-2 gene. *Curr Opin Immunol* 7: 333-342, 1995.
50. Wang JH, Barke RA and Roy S: Transcriptional and epigenetic regulation of interleukin-2 gene in activated T cells by morphine. *J Biol Chem* 282: 7164-7171, 2007.
51. Thomas PT, House RV and Bhargava HN: Direct cellular immunomodulation produced by diacetylmorphine (heroin) or methadone. *Gen Pharmacol* 26: 123-130, 1995.
52. Young HA and Hardy KJ: Interferon-gamma: Producer cells, activation stimuli, and molecular genetic regulation. *Pharmacol Ther* 45: 137-151, 1990.
53. Reuben JM, Lee BN, Paul M, Kline MW, Cron SG, Abramson S, Lewis D, Kozinetz CA and Shearer WT: Magnitude of IFN-gamma production in HIV-1-infected children is associated with virus suppression. *J Allergy Clin Immunol* 110: 255-261, 2002.
54. Beyers AD, van Rie A, Adams J, Fenhalls G, Gie R and Beyers N: Signals that regulate the host response to *Mycobacterium tuberculosis*. *Novartis Found Symp* 217: 145-159, 1998.
55. Sweetser MT, Hoey T, Sun YL, Weaver WM, Price GA and Wilson CB: The roles of nuclear factor of activated T cells and ying-yang 1 in activation-induced expression of the interferon-gamma promoter in T cells. *J Biol Chem* 273: 34775-34783, 1998.
56. Cippitelli M, Ye J, Viggiano V, Sica A, Ghosh P, Gulino A, Santoni A and Young HA: Retinoic acid-induced transcriptional modulation of the human interferon-gamma promoter. *J Biol Chem* 271: 26783-26793, 1996.
57. Kaminuma O, Elly C, Tanaka Y, Mori A, Liu YC, Altman A and Miyatake S: Vav-induced activation of the human IFN-gamma gene promoter is mediated by upregulation of AP-1 activity. *FEBS Lett* 514: 153-158, 2002.
58. Sen R and Baltimore D: Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* 46: 705-716, 1986.
59. Pahl HL: Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene* 18: 6853-6866, 1999.
60. Pannan BH and Robotham JL: The acute-phase response. *New Horiz* 3: 183-197, 1995.
61. Baeuerle PA and Baltimore D: Activation of DNA-binding activity in an apparently cytoplasmic precursor of the NF-kappa B transcription factor. *Cell* 53: 211-217, 1988.
62. Ghosh S, May MJ and Kopp EB: NF-kappa B and Rel proteins: Evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* 16: 225-260, 1998.
63. Cabot PJ, Carter L, Schafer M and Stein C: Methionine-enkephalin and Dynorphin A-release from immune cells and control of inflammatory pain. *Pain* 93: 207-212, 2001.
64. Roy S, Wang JH, Balasubramanian S, Sumandeep, Charboneau R, Barke R and Loh HH: Role of hypothalamic-pituitary axis in morphine-induced alteration in thymic cell distribution using mu-opioid receptor knockout mice. *J Neuroimmunol* 116: 147-155, 2001.
65. Kieffer BL: Recent advances in molecular recognition and signal transduction of active peptides: Receptors for opioid peptides. *Cell Mol Neurobiol* 15: 615-635, 1995.
66. Pol O and Puig MM: Expression of opioid receptors during peripheral inflammation. *Curr Top Med Chem* 4: 51-61, 2004.
67. Rogers TJ and Peterson PK: Opioid G protein-coupled receptors: Signals at the crossroads of inflammation. *Trends Immunol* 24: 116-121, 2003.

68. Haraguchi S, Good RA and Day NK: Immunosuppressive retroviral peptides: cAMP and cytokine patterns. *Immunol Today* 16: 595-603, 1995.
69. Jimenez JL, Punzon C, Navarro J, Munoz-Fernandez MA and Fresno MJ: Phosphodiesterase 4 inhibitors prevent cytokine secretion by T lymphocytes by inhibiting nuclear factor-kappaB and nuclear factor of activated T cells activation. *J Pharmacol Exp Ther* 299: 753-759, 2001.
70. Chen D and Rothenberg EV: Interleukin 2 transcription factors as molecular targets of cAMP inhibition: Delayed inhibition kinetics and combinatorial transcription roles. *J Exp Med* 179: 931-942, 1994.
71. Walker SM, Goudas LC, Cousins MJ and Carr DB: Combination spinal analgesic chemotherapy: A systematic review. *Anesth Analg* 95: 674-715, 2002.
72. Hocking G and Cousins MJ: Ketamine in chronic pain management: An evidence-based review. *Anesth Analg* 97: 1730-1739, 2003.
73. Ohta N, Ohashi Y and Fujino Y: Ketamine inhibits maturation of bone marrow-derived dendritic cells and priming of the Th1-type immune response. *Anesth Analg* 109: 793-800, 2009.
74. Gao M, Jin W, Qian Y, Ji L, Feng G and Sun J: Effect of N-methyl-D-aspartate receptor antagonist on T helper cell differentiation induced by phorbol-myristate-acetate and ionomycin. *Cytokine* 56: 458-465, 2011.
75. Chia YY, Liu K, Liu YC, Chang HC and Wong CS: Adding ketamine in a multimodal patient-controlled epidural regimen reduces postoperative pain and analgesic consumption. *Anesth Analg* 86: 1245-1249, 1998.
76. Tan PH, Kuo MC, Kao PF, Chia YY and Liu K: Patient-controlled epidural analgesia with morphine or morphine plus ketamine for postoperative pain relief. *Eur J Anaesthesiol* 16: 820-825, 1999.
77. Choudhuri AH, Dharmani P, Kumar N and Prakash A: Comparison of caudal epidural bupivacaine with bupivacaine plus tramadol and bupivacaine plus ketamine for postoperative analgesia in children. *Anaesth Intensive Care* 36: 174-179, 2008.
78. Gunes Y, Secen M, Ozcengiz D, Gündüz M, Balcioglu O and Isik G: Comparison of caudal ropivacaine, ropivacaine plus ketamine and ropivacaine plus tramadol administration for postoperative analgesia in children. *Paediatr Anaesth* 14: 557-563, 2004.
79. Ozbek H, Bilen A, Ozcengiz D, Gunes Y, Ozalevli M and Akman H: The comparison of caudal ketamine, alfentanil and ketamine plus alfentanil administration for postoperative analgesia in children. *Paediatr Anaesth* 12: 610-616, 2002.
80. Gunduz M, Ozalevli M, Ozbek H and Ozcengiz D: Comparison of caudal ketamine with lidocaine or tramadol administration for postoperative analgesia of hypospadias surgery in children. *Paediatr Anaesth* 16: 158-163, 2006.
81. Vranken JH, Troost D, Wegener JT, Kruis MR and van der Vegt MH: Neuropathological findings after continuous intrathecal administration of S(+)-ketamine for the management of neuropathic cancer pain. *Pain* 117: 231-235, 2005.
82. Sator-Katzenschlager S, Deusch E, Maier P, Spacek A and Kress HG: The long-term antinociceptive effect of intrathecal S(+)-ketamine in a patient with established morphine tolerance. *Anesth Analg* 93: 1032-1034, 2001.
83. Benrath J, Scharbert G, Gustorff B, Adams HA and Kress HG: Longterm intrathecal S(+)-ketamine in a patient with cancer-related neuropathic pain. *Br J Anaesth* 95: 247-279, 2005.



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