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ORIGINAL RESEARCH

Intranasal Administration of the Combination of Dextro-Ketamine and Dexmedetomidine for Treatment of Diabetic Neuropathic Pain in Rats

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Introduction: Diabetes mellitus (DM) has become a public health problem, which is associated with high morbidity and mortality, due to the chronic complications, such as diabetic neuropathy. Current recommendations for the treatment of neuropathic pain achieve a reduction of 30% in only 30% of cases. Therefore, it is necessary to identify new therapeutic approaches to improve the quality of life of diabetic patients.

Methods: This work evaluated the antinociceptive effect of intranasal administration of the combination of dextro-ketamine (keta), a non-competitive glutamatergic receptor antagonist, and dexmedetomidine (DEX), a selective alpha2-adrenergic agonist, in rats with neuropathic pain induced by streptozotocin-DM.

Results: The thermal hyperalgesia and mechanical allodynia observed in DM model are reduced with the intranasal administration of the combination of keta and DEX (200 + 0.10 μg/kg) after 3 days of treatment. The antinociceptive action could be due to reduction of Ca^{2+} influx with lower glutamate release and reduced excitability through the activation of alpha2-adrenergic receptors by DEX and reduction of NMDA receptor activation by glutamate with lower excitability due to the antagonism produced by keta. DM induced increased expression of glial fibrillary acid protein (GFAP) and tumor necrosis factor-alpha (TNF-alpha) detected by immunohistochemistry, indicating greater astrocyte activity and intense inflammatory response. Intranasal administration for 10 days of the combination of low doses of keta and DEX promoted an intense decrease in the expression of both GFAP and TNF-alpha, indicating lower activation of astrocytes in the spinal cord and reduced production and release of TNF-alpha, favoring the reduction of inflammation.

Conclusion: Intranasal administration of low doses of keta with DEX could be a new therapeutic approach to reduce neuropathic pain and consequently improve the quality of life of diabetic patients.

Keywords: diabetes, neuropathic pain, dextro-ketamine, dexmedetomidine

Introduction

Diabetes (DM) has multiple causes, but obesity and insulin resistance play an important role that results in glycotoxicity and lipotoxicity. Intense lipolysis, increased production of pro-inflammatory cytokines, leptin and decreased adiponectin contribute to the establishment of chronic complications consequent of DM.[1] Retinopathy, nephropathy, cardiomyopathy and neuropathy are long-term complications of DM, contributing to reduced quality of life and increased number of deaths. The incidence of neuropathy, in diabetic patients is approximately 20%, and chronic complication is of particular

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importance when the mortality rate is 27% in ten years.[2] Diabetic peripheral neuropathy (DN) is one of the main complications that arises with the chronic evolution of DM, characterized by the progressive degeneration of axons and nerve fibers due to chronic exposure to hyperglycemia. DN affects about 50% of all diagnosed cases and is common with distal and symmetrical polyneuropathy.[3] Paresthesia, allodynia and hyperalgesia are frequent symptoms, and the progression of the disease can develop motor nerve dysfunction, with consequent distal weakness and loss of proprioceptive sensitivity. DN is also associated with peripheral vasculopathy, which is due to inflammation in the microvasculature,[4,5] with increased cytokines, including interleukin-1beta (IL-1B), interleukin-6 (IL-6), and tumor necrosis factor (TNF-alpha).

Neuropathic pain is a result of significant plastic alteration after peripheral nerve injury that facilitates pain transmission with a reduction in the activation of interneurons and, consequently, a lower release of inhibitory neurotransmitters such as gamma-aminobutyric acid (GABA), glycine, enkephalin, cannabinoids and adenosine. In addition, there is an intense release of excitatory neurotransmitters, including glutamate, which is neurotoxic. Thus occurs increased pain transmission and less activation of inhibitory mechanisms.[6] The immune-inflammatory system also contributes to the condition of neuropathic pain, as it is associated with the activation of microglia in the dorsal horn of the spinal cord, which in turn increases the production of pro-inflammatory cytokines, responsible for increasing the spontaneous activity of the nociceptor.[7]

Treatment of diabetic neuropathic pain includes the anticonvulsants gabapentin and pregabalin, carbamazepine or lidocaine patch, which do not provide therapeutic success to all patients.[8] Alternative treatments include tricyclic antidepressants, opioids and cannabinoids.

Treatment of neuropathic pain is limited to analgesia, but complete pain relief is unlikely. Improvement in quality of life and sleep should be encouraged from the time of diagnosis.[2] Since neuroinflammation plays an important role in DM-induced neuropathic pain, it was important to evaluate the effects of dextro-ketamine (Keta) that, in addition to promoting analgesia, also reduces neuroinflammation and dexmedetomidine (DEX), which decreases the release of inflammatory cytokines. In this work, the combination of Keta and DEX, which could interfere with the involvement of cytokines in central pain sensitization, was evaluated in DM-induced neuropathic pain in rats.

Materials and Methods

Animals

The Animal Ethics Committee of the Universidade Federal do Rio de Janeiro approved the protocols used (license number 037/23). Male Wistar rats (n = 18, 240–280 g) were kept in accordance with Brazilian Guide of Production, Maintenance and Utilization of Animals for Teaching or Scientific Research Activities (1st edition, 2016) approved by the National Council for Control of Animal Experimentation. Rats were fed with standard chow and water ad libitum and housed under controlled conditions (22–24°C; 12/12 h dark/light cycle).

Induction of DM

To induce DM, intravenous administration of streptozotocin (STZ, Sigma Aldrich, Germany) dissolved in a solution with 3% monosodium citrate (NaH2(C3H5O(COO)3, Sigma Aldrich, Germany), pH 4.5 was performed in the caudal vein of rats. Before and after 14 days of administration of 60 mg/kg STZ, blood samples were collected from animals fasted for 8–10 h to measure glucose (Accu-Chek®, ROCHE, Germany). Animals with blood glucose higher than 250 mg/dL were considered diabetic and randomly assigned to two experimental groups for treatment with intranasal administration of vehicle (saline) or combination of dextro-ketamine (Keta) and dexmedetomidine (DEX).

Experimental Protocol and Pain Behavioral Tests

Twenty-eight days after STZ injection, diabetic rats were randomly divided into two groups (6 animals/group) and treated with either vehicle (saline) or the association of dextro-ketamine hydrochloride (Keta) and dexmedetomidine hydrochloride (DEX). The combination of Keta and DEX consisted of low doses with no analgesic effect when used isolated. The selected 200 and 0.1 μg/kg for intranasal Keta and DEX corresponded to half dose in which reduced pain in rats[9] and

to one-tenth used to treat pain in patients, respectively.[10,11] The substances were administered by nasal instillation of Keta (Cristália Produtos Químicos Farmacêuticos Ltda, SP, Brazil) and DEX (Cristália Produtos Químicos Farmacêuticos Ltda, SP, Brazil) in the left and right nostrils, respectively, during 10 days. Six non-diabetic littermates also received intranasal vehicle for the same duration.

The behavior of the animal in response to hyperalgesia resulting from DN was evaluated through the paw removal test consequent to thermal stimulation. After the acclimatization period, radiant heat was applied to the plantar part of the animal's hind leg until it makes the movement of removing the paw. A plantar analgesia meter device (IITC-336, IITC Life Science Inc., USA) measured the time between the start of the stimulus and the withdrawal of the paw, which was the control withdrawal latency. Maximum exposure time to light source (cutoff) was determined as three times the control value to avoid tissue damage. Latency of paw removal was determined before and after treatment with the combination of Keta and DEX.

To evaluate the onset of mechanical allodynia, changes in tactile sensitivity in response to a mechanical stimulus were detected using a digital analgesimeter device (EFF-301, Insight, Brazil). Before and after treatment with combination, increasing force (in gram) was applied to the surface of the animal's hind leg to determine the paw withdrawal at which the animal responds to the stimulus.

Immunohistochemistry

Spinal cords from diabetic animals were removed after perfusion of 4% phosphate-buffered paraformaldehyde (PFA) solution at 10 mL/min, through the insertion of a cannula into the ascending aorta. The cord was cut into 3 segments (L4, L5 and L6) and embedded in OCT compound and sectioned in 10-μm cross-sections in a cryostat (model CM1850; Leica, Wetzlar, Germany). Slides were prepared for incubation of secondary antibodies such as glial fibrillary acid protein (GFAP, anti-rabbit polyclonal Abcam ab7260) and TNF-alpha (anti-mouse monoclonal Abcam ab1793) for reading under a confocal microscope (LSM 510, Zeiss) and quantifying in the ImageJ program as described elsewhere.[12]

Non-Invasive Blood Pressure Measurement

To assess the effects of the association of Keta and DEX to hemodynamics, systolic and diastolic pressures and heart rate were measured using a tail plethysmograph (Coda Monitor, Kent Scientific Corporation, Connecticut, USA). Animals were kept under a radiant heat source (37°C) and in the proximal portion of the tail, the cuff was placed to measure blood pressure before and after the STZ injection and at the end of the intranasal treatment with the combination of Keta and DEX.

Statistical Analysis

GraphPad Prism software, version 6.0 (GraphPad Software Incorporated, San Diego, USA) was used to analyze all data from behavioral, hemodynamics and immunohistochemistry. Data expressed as mean ± standard error and $p < 0.05$ were considered statistically significant. Analysis of Variance (One Way – ANOVA) followed by the Dunnett test (post hoc) was used to compare data from different experimental groups.

Results

Induction of DM-Induced Neuropathic Pain

Body weight and plasma glucose level were measured in the animals after intravenous injection of a single dose of 60 mg/kg STZ. There was no change in body weight in the different experimental groups, with 272.4 ± 9.5; 248.0 ± 9.3; 236.3 ± 11.4 g before, 4 weeks after DM induction and after intranasal administration of the combination of Keta and DEX, respectively (Figure 1A), indicating the absence of weight gain in diabetic animals. Blood glucose level increased from 105.9 ± 2.9 to 434.5 ± 18.0 mg/dL after 4 weeks of DM induction and maintained at 452.0 ± 71.4 mg/dL after treatment with the combination. Thus, the establishment of DM was confirmed due to the toxic action of STZ on the pancreas, but there was no interference of the combination of Keta and DEX in the metabolic disorder. DM-induced neuropathic pain was observed since the occurrence of thermal hyperalgesia and mechanical allodynia in rats after 2

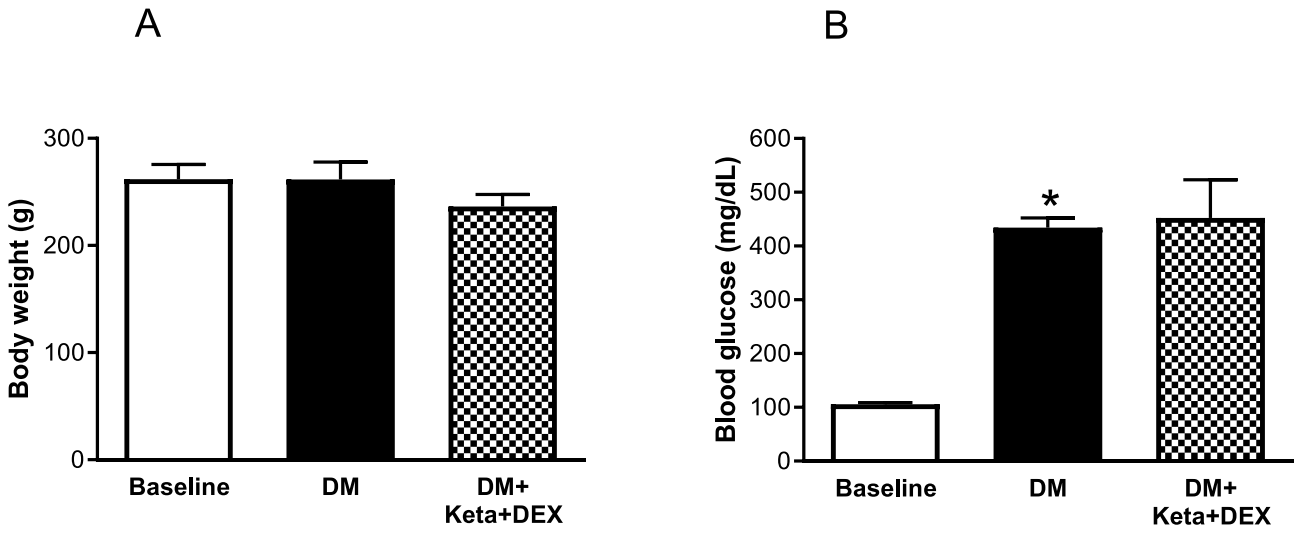

Figure 1 Weight gain (**A**) and blood glucose level (**B**) in animals before (baseline, BL) and after STZ injection (DM) for a period of 4 weeks and followed by treatment with the combination Keta + DEX. Data expressed as mean ± E.P.M. (n= 25). *p<0.05.

weeks of DM induction by intravenous STZ injection. Thermal hyperalgesia was characterized by a reduction in paw withdrawal latency from 23.1 ± 1.3 (BL) to 18.6 ± 1.2 and 13.5 ± 0.8 s, 1 and 4 weeks after DM induction. While mechanical allodynia was detected by lowering the paw removal threshold from 49.0 ± 2.7 (BL) to 47.9 ± 2.5 and 35.6 ± 2.0 g (Figure 1B). These characteristics remained for more than 4 weeks, indicating the establishment of diabetic neuropathic pain. Therefore, the protocol used to evaluate the effect of the intranasal administration of the combination consisted of the determination of hyperalgesia and allodynia 4 weeks after the administration of STZ (DM induction).

Improvement of Hyperalgesia and Allodynia

In order to select the doses to use in the combination of Keta and DEX, the effect of 0.10 and 0.20 mg/kg of dextro-ketamine alone and 0.05 and 0.10 µg/kg of dexmedetomidine was initially evaluated. After 5 or 90 min of intranasal administration, both Keta and DEX did not show significant antinociceptive activity when administered alone. The paw removal threshold of diabetic animals was 32.1 ± 1.5 g (baseline, BL), which remained at 40.2 ± 4.9 g after 7 days of administration of Keta, 0.2 mg/kg (Figure 2). Similarly, the reduced threshold caused by DM remained after the last intranasal administration of DEX because it remained at 35.2 ± 9.8 g, indicating the maintenance of mechanical

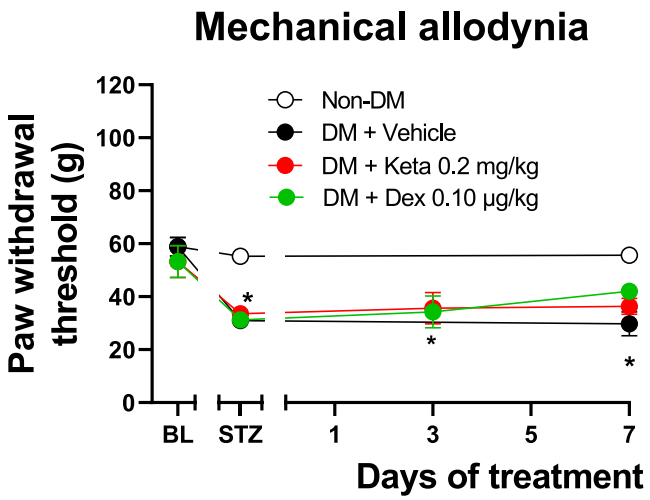

Figure 2 Intranasal administration of dextro-ketamine (Keta) and dexmedetomidine (DEX) in DM-induced mechanical allodynia. Threshold was determined before DM induction (BL), after 4 weeks of DM induction (STZ), and after 3 and 7 days of intranasal administration. Data expressed as mean ± S.E.M (n= 5). *p <0.05 compared to BL.

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allodynia. Animals treated with Keta and DEX alone did not show significant threshold changes at 5 or 90 min after nasal instillation, throughout the treatment of 7 days. Thus, in the next step, the intranasal administration of the combination of ineffective doses of Keta (0.1 or 0.2 mg/kg) and DEX (0.10 μg/kg) in thermal hyperalgesia and mechanical allodynia induced by DM was evaluated.

The onset of DM-induced neuropathic pain was determined by the reduction of paw withdrawal latency from 16.0 ± 1.0 to 9.8 ± 0.3 s 4 weeks after STZ administration, as shown in Figure 3A. This characteristic associated with diabetic neuropathic pain was reversed only when the combination of Keta and DEX was administered intranasal at doses of 0.2 mg/kg + 0.1 μg/kg. The latency of paw withdrawal induced by the thermal stimulus was recovered to 13.5 ± 0.9; 15.9 ± 0.9; 15.3 ± 0.9; 17.1 ± 1.2 s after 3, 7, and 10 days of treatment (Figure 3A).

Mechanical allodynia was observed in diabetic animals, with a reduction in the paw withdrawal threshold from 61.2 ± 3.7 to 35.4 ± 3.0 g, 4 weeks after DM induction with STZ (Figure 3B). Therefore, there was a significant reduction in the paw removal threshold due to mechanical stimulation in diabetic animals, confirming the presence of neuropathic pain. However, the intranasal administration of the combination of Keta and DEX at doses of 0.2 mg/kg + 0.1 μg/kg reduced allodynia after 1 day of treatment. The threshold was 45.3 ± 3.6; 42.4 ± 3.4; 48.9 ± 3.5; 53.2 ± 3.9 g after 1, 3, 7 and 10 days of treatment with the combination (Figure 3B).

No Hemodynamic Alteration Induced by Combination of Dextro-Ketamine and Dexmedetomidine

Since ketamine has a stimulatory effect on sympathetic activity[13] and DEX increases blood pressure in a dose-dependent manner,[14] it was investigated the interference of the combination on systolic (SBP) and diastolic (DBP) pressure in diabetic animals. SBP and DBP were determined in the animals before DM induction, using a tail plethysmograph, whose measurements were 112.4 ± 7.9 and 79.3 ± 8.0 mmHg, respectively. Diabetic animals did not show changes in those parameters after 4 weeks of DM induction and not after 10 or 20 min of intranasal administration of the Keta and DEX (0.2 mg/kg + 0.1 μg/kg) (Figure 4).

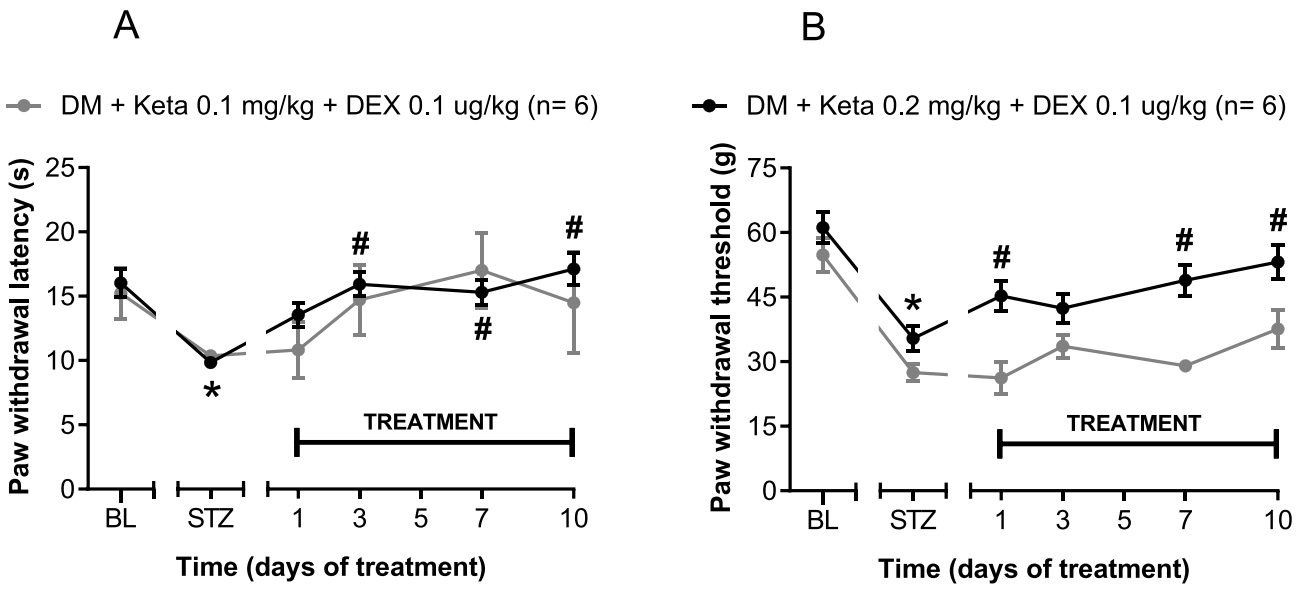

Figure 3 Antinociceptive effect observed after intranasal administration of the combination of dextro-ketamine (Keta) and dexmedetomidine (DEX) in DM-induced thermal hyperalgesia and mechanical allodynia. Latency (**A**) and threshold (**B**) were determined before DM induction (BL), after 4 weeks of DM induction (STZ), and after 1, 3, 7, and 10 days of combination intranasal administration. Data expressed as mean ± S.E.M (n= 17). *p <0.05 compared to BL; #p<0.05 compared to STZ.

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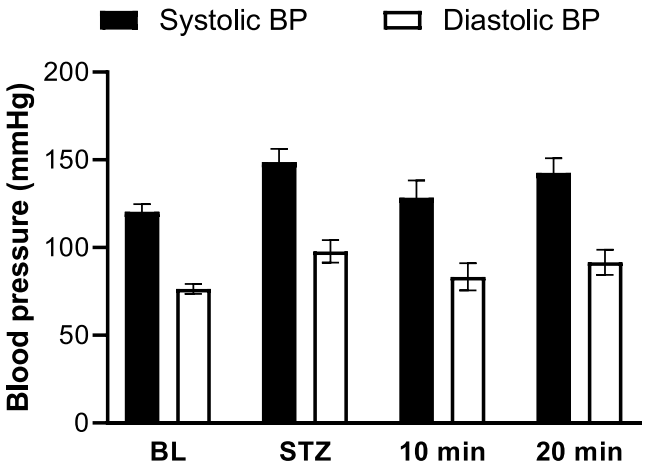

Figure 4 Hemodynamic parameters observed in diabetic animals treated with the combination of ketamine (Keta) and dexmedetomidine (DEX) at 200 μg/kg + 0.1 μg/kg. Systolic (SBP) and diastolic (DBP) blood pressure were measured before (baseline - BL) and after DM induction (STZ) and 10 and 20 min after intranasal administration of the association. Data expressed as mean ± SEM.

Reduction of Inflammation in Spinal Cord of Diabetic Rats

In order to verify the influence of the intranasal administration of the combination of Keta and DEX (0.2 mg/kg + 0.1 μg/kg) on DN-induced neuroinflammation, the condition of activation of glial cells was evaluated which in turn, causes an increase in the production of cytokines. The astrocyte activation profile was investigated by identifying the presence of glial fibrillar acid

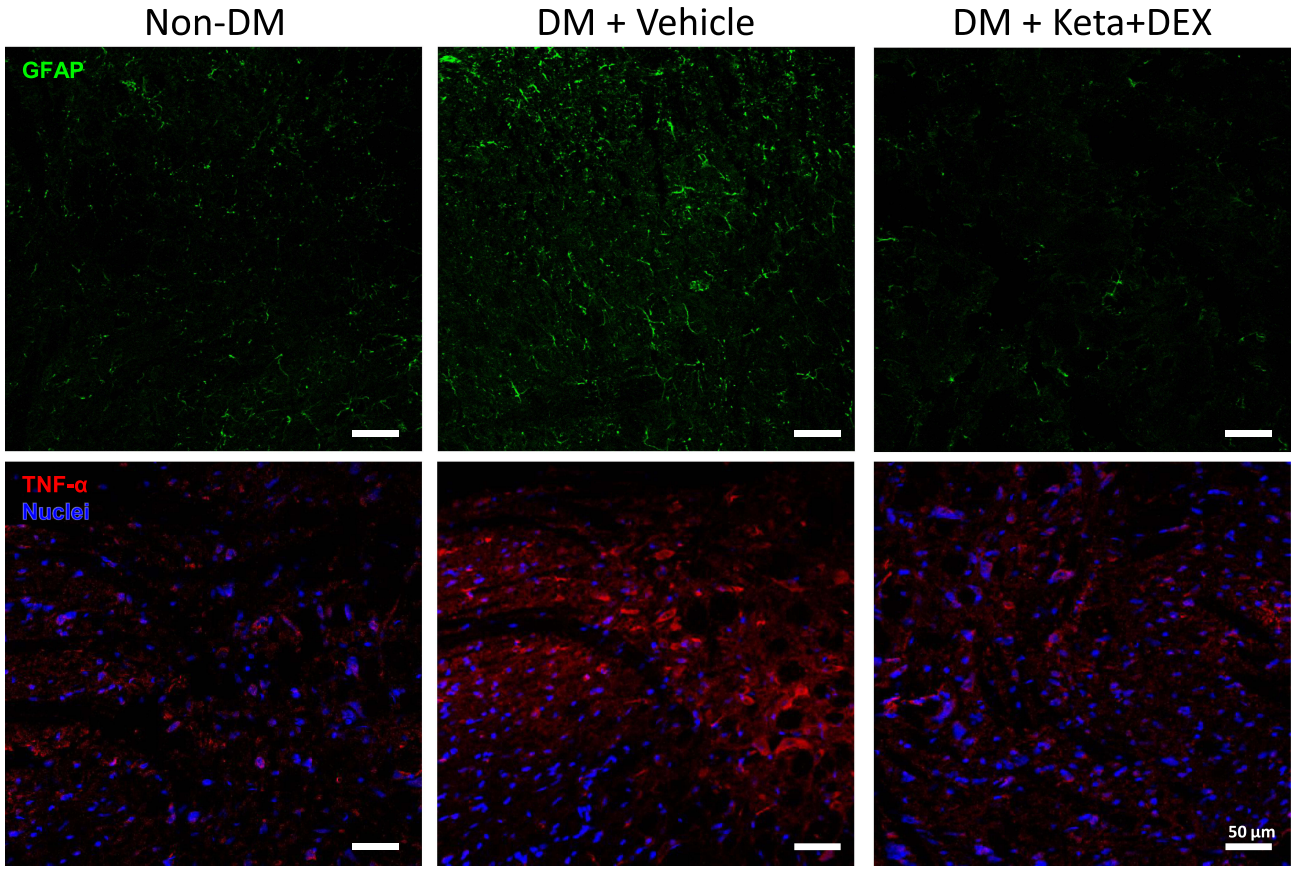

Figure 5 Representative immunofluorescence images of GFAP and TNF-alpha expression. Each panel consists of an image of the dorsal horn of the spinal cord, from a non-diabetic animal (Non-DM), a diabetic treated with saline (DM) or a diabetic treated with the combination of dextro-ketamine (Keta) and dexmedetomidine (DEX) at 0.2 mg/kg + 0.1 μg/kg.

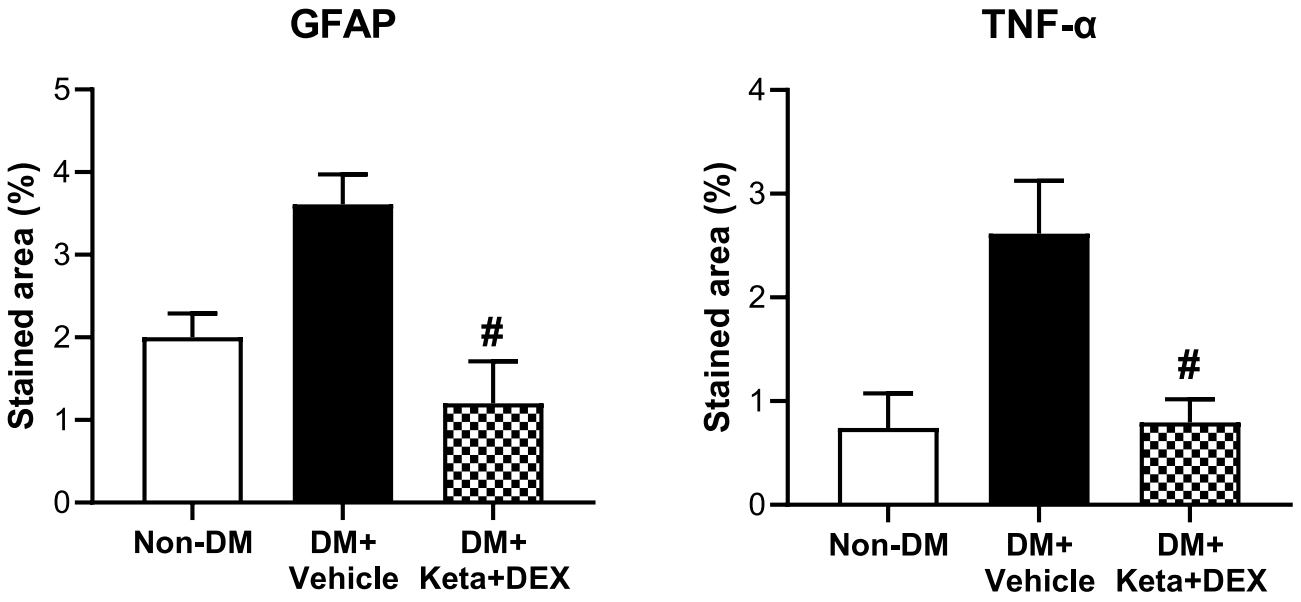

Figure 6 Expression of GFAP and TNF-alpha in the spinal cord of diabetic animals treated or not with the combination of Keta and DEX (0.2 mg/kg + 0.1 μg/kg). Quantification of the percentage of GFAP and TNF-alpha staining. Data represent the average ± SEM of 5 animals per group. # P <0.05 compared to the diabetic group.

protein (GFAP) and the inflammatory component through the higher expression of TNF-alpha in tissue from diabetic animals treated with vehicle or with combination (Figure 5).

Higher GFAP immunohistochemical staining (%) was detected in spinal cord from diabetic animals treated with vehicle (saline) when compared to the group of non-diabetic animals. However, animals treated with the combination of Keta and DEX showed lower GFAP labeling, with a reduction in expression of about 56% (Figure 5). Since astrocyte activation is associated to greater cytokine release in the spinal cord, TNF-alpha labeling was investigated in the different experimental groups (Figure 5). There was greater TNF-alpha staining in the spinal cord from diabetic animals treated with vehicle, confirming the activation of astrocytes and consequent greater local inflammation, which reversed in diabetic animals treated with the combination of Keta and DEX (0.2 mg/kg + 0.1 μg/kg). The increased GFAP and TNF-alpha staining in dorsal horn from diabetic rats reversed from 3.61 ± 0.36 to 1.2 ± 0.50% and 2.62 ± 0.51 to 0.80 ± 0.22%, respectively, after treatment with combination (Figure 6).

Discussion

DN is the main comorbidity of DM, which symptoms result mainly from peripheral alterations. Neuropathic pain is a consequence of peripheral sensitization, triggered by neuronal injury or inflammation. The pathogenesis of neuropathic pain can be evaluated in different animal models including mechanical nerve injury (sciatic nerve constriction, spinal nerve ligation); use of chemotherapy and DM.[15] The great challenge is to define an ideal therapeutic regimen to improve the quality of life of patients suffering from neuropathic pain. Currently, as there is a larger population of obese people, it is important to identify alternative therapy for the growing number of patients with neuropathic pain induced by DM.[2] Preclinical investigation uses tests for evaluation of thermal hyperalgesia and mechanical allodynia in diabetic animals. After 4 weeks of DM induction, the diabetic animals showed an increase in the latency of paw withdrawal (s) to the thermal stimulus and a lower load threshold (g), reaffirming the onset of neuropathic pain, with hyperalgesia and allodynia. Injury and inflammation increase depolarization of nerve fiber membranes[16] and peripheral sensitization.[17] Long term of this condition, it progresses to spinal sensitization with glutamate receptor phosphorylation (NMDA), greater Ca^{2+} influx and greater postsynaptic excitation. In addition, there is an increase in the expression of Ca^{2+} channels, whose exacerbation of Ca2+ influx causes greater glutamate release in the presynaptic region, leading to excessive activation of NMDA receptors.[18] Thus, the activity of NMDA receptors is considered a pharmacological target for analgesic action by the inhibition produced by gabapentinoids.[19]

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Dextro-ketamine is a non-competitive NMDA receptor antagonist leading to lower activation of glutamatergic neurons. In chronic pain, the analgesic action of ketamine is clinically important when there is contraindication to therapeutic use with opioids.[20] Hepatic metabolism to active products including 6-hydroxynorketamine and 5.6-dehydronorketamine, favors an increase in the duration of the analgesic effect of ketamine,[21] which is dependent on the route of administration used. When used through intranasal instillation, the duration is 60 min.[22] The nasal cavity has about 3–5% absorption surface area that allows rapid access of substances to the CNS. DEX, a potent selective alpha2-adrenergic agonist, is used clinically to produce sedation and analgesia. Due to its short half-life of 2 h, it has a rapid recovery effect making it the ideal premedication in pediatric patients.[23] Intranasal administration of DEX has a bioavailability of 81.8%.[24]

The intranasal route for the administration of dextro-ketamine and DEX is widely used to produce sedation, especially in pediatric patients, because it is less invasive and can replace the intravenous route.[25] There is still no evidence to demonstrate the efficacy of the combination of dextro-ketamine and DEX in cases of chronic pain. Thus, there was an interest in evaluating the effect of intranasal administration of the combination of dextro-ketamine and DEX in a model of neuropathic pain resulting from DM. This confirmed hypothesis would be of great clinical interest, since drugs prescribed for the treatment of neuropathic pain, such as pregabalin, duloxetine and tapentadol, do not benefit all patients, justified by the inadequate reach of the substance to the site of action.[26]

Hyperalgesia and allodynia observed in the STZ-induced DM model in rats reversed with the intranasal administration of the combination of Keta and DEX. The observation of the recovery time of the effect after the end of the treatment was not included in the experimental protocol, which would be of interest due to the existence of active metabolites of ketamine. The improvement of these parameters could be a consequence of: 1. Reduction of Ca^{2+} influx in nociceptive afferent fibers, with less glutamate release and less excitability, due to the activation of alpha2-adrenergic receptors by DEX;[27] 2. Reduced activation of NMDA receptors by glutamate with lower excitability in the brain and spinal cord, due to antagonism produced by ketamine.[28]

After a peripheral neuronal injury induced by DM, proliferation of microglia in the dorsal horn of the spinal cord immediately occurs, aggravated by the increased expression of macrophage colony-stimulating factor (CSF1) in the dorsal root ganglion, resulting from the increased secretion of IL-1ß by macrophages at the site.[29] Preemptive blockade of microglia can reduce allodynia and hyperalgesia, but if the intervention is performed after the injury, no important result is observed. The lack of improvement is probably due to the role of astrocytes in the development of chronic pain. Both microglia and astrocytes have a reciprocal interaction of activation, and while microglia have a more important function in the early phases, astrocytes also act in the late phase.[30]

In chronic pain, the reactive state of astrocytes is characterized by morphological, molecular and functional alterations, and the identification of this excessive activity is obtained through the determination of the increase in GFAP in the dorsal horn of the spinal cord, using the immunohistochemistry technique. Increased GFAP expression has already been demonstrated in animal models of rodents and primates with diabetic neuropathic pain,[31] which was also observed in the STZ-induced DM model presented in this work. These cells in reactive mode, in the dorsal horn of spinal cord, lead to the sensitization of nociceptive neurons through pro-inflammatory mediators, such as cytokines and chemokines, being determinant in the development and maintenance of chronic pain. Active astrocytes and microglia produce TNF-alpha. The knowledge of the function of astrocytes in the condition of chronic pain allows the development of new therapeutic approaches.[32] Thus, the administration of substances directed to act on a new pharmacological target, such as astrocytes, could regulate the release of inflammatory mediators, reducing neuroinflammation. In addition, the possible inhibition of TNF-alpha could also reduce glial activity and consequently minimize hyperalgesia.[33]

It has been previously demonstrated that the administration of repeated doses of ketamine decreased pain scores in patients with chronic pain, whose improvement was accompanied by a reduction in numerous inflammatory markers, including TNF-alpha.[34] Similarly, the administration of DEX in animals with diabetic neuropathic pain also decreased the level of TNF-alpha, associated with a decrease in the rate of microglia in a reactive state.[27] In the present study, DM induced an increase in the expression of GFAP and TNF-alpha in the dorsal horn of the spinal cord detected by immunohistochemistry, indicating higher astrocyte activity and intense inflammatory response. Intranasal administration for 10 days, of the combination of low doses of Keta and DEX in diabetic animals, promoted an intense decrease in the

expression of both GFAP and TNF-alpha. These results suggest that the combination prevented the greater activation of astrocytes in the spinal cord and reduced the production and release of TNF-alpha, favoring a state of lower inflammation and probably reflecting the positive results found in the pattern of hyperalgesia and allodynia. An important clinical benefit of the combination of Keta and DEX in the treatment of chronic pain would be the possibility of reducing the inflammatory component and reducing the prescription of opioids. Currently, the main clinical indication for intranasal administration of DEX (2 ug/kg) or esketamine (1 mg/kg) is preoperative sedation in pediatric patients.[24] Satisfactory sedation and improved patient cooperation were also achieved with administration of DEX (1 ug/kg) combined with esketamine (0.5 mg/kg).

The great advantage of our hypothesis would be the use of low doses in the combination of Keta and DEX (0.2 mg/kg and 0.1 ug/kg), which could reduce the volume to be administered and, consequently, reduce the probable side effects of mucosal irritation, coughing and sneezing. Evidence of the beneficial effect of the combination of the treatment of neuropathic pain could be a stimulus to the innovation and development of a nasal spray formulation, whose particles could increase absorption and shorten latency.[35]

In recent decades, there have been attempts at a new approach to treat diabetic neuropathic pain. The investigation of new selective Na channels (Nav 1.7 and 1.8) and TRPA1 blockers had he expectation to reduce adverse effects.[36] Continuous systemic administration of the local anesthetic, bupivacaine has been shown to inhibit the activation of microglia, in the dorsal horn of the spinal cord from mice with STZ-induced DM.[37] Inhibition of the GABA transporter, which reduces its uptake and metabolism could be effective for the treatment of neuropathic pain, but as these receptors are widely expressed in the CNS they could produce several side effects.[38] None of these attempts have been successful for clinical use in adult patients.

In conclusion, the intranasal administration of the combination of low doses of dextro-ketamine and dexmedetomidine could be a new therapeutic approach to reduce neuropathic pain and consequently improve the quality of life of diabetic patients. However, further research is required before using intranasal administration of the combination in diabetic patients. The length and frequency of therapy and the duration of pain relief after treatment disruption should be established. Advent of adverse effects after longer period of clinical use is still unknown.

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Disclosure

The authors declare no conflict of interest.

References

1. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. *Int J Physiol Pathophysiol Pharmacol*. 2019;11(3):45–63.
2. Rosenberger DC, Blechschmidt V, Timmerman H, Wolff A, Treede RD. Challenges of neuropathic pain: focus on diabetic neuropathy. *J Neural Transm*. 2020;127(4):589–624. doi:10.1007/s00702-020-02145-7
3. Feldman EL, Callaghan BC, Pop-Busui R, et al. Diabetic neuropathy. *Nat Rev Dis Primers*. 2019;5(1):42. doi:10.1038/s41572-019-0097-9
4. Feldman EL, Nave KA, Jensen TS, Bennett DLH. New Horizons in Diabetic Neuropathy: mechanisms, Bioenergetics, and Pain. *Neuron*. 2017;93(6):1296–1313. doi:10.1016/j.neuron.2017.02.005
5. Calcutt NA. Diabetic neuropathy and neuropathic pain: a (con)fusion of pathogenic mechanisms? *Pain*. 2020;161(Supplement 1):S65–S86. doi:10.1097/j.pain.0000000000001922
6. Volcheck MM, Sm G, Fleming KC, Mohabbat AB, Luedtke CA. Central sensitization, chronic pain, and other symptoms: better understanding, better management. *Cleve Clin J Med*. 2023;90(4):245–254. doi:10.3949/ccjm.90a.22019
7. Lima CKF, Silva RM, Lacerda RB, et al. LASSBio-1135: a Dual TRPV1 Antagonist and Anti-TNF-Alpha Compound Orally Effective in Models of Inflammatory and Neuropathic Pain. *PLoS One*. 2014;9(6):e99510. doi:10.1371/journal.pone.0099510
8. Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. *Lancet Neurol*. 2010;9(8):807–819. doi:10.1016/S1474-4422(10)70143-5
9. Claudino R, Nones C, Araya E, Chichorro J. Analgesic Effects of Intranasal Ketamine in Rat Models of Facial Pain. *J Oral Facial Pain Headache*. 2018;32(3):238–346. doi:10.11607/ofph.1973

10. Uusalo P, Seppänen SM, Järvisalo MJ. Feasibility of Intranasal Dexmedetomidine in Treatment of Postoperative Restlessness, Agitation, and Pain in Geriatric Orthopedic Patients. *Drugs Aging*. 2021;38(5):441–450. doi:10.1007/s40266-021-00846-6
11. Seppänen SM, Kuuskoski R, Mäkelä KT, Saari TI, Uusalo P. Intranasal Dexmedetomidine Reduces Postoperative Opioid Requirement in Patients Undergoing Total Knee Arthroplasty Under General Anesthesia. *J Arthroplasty*. 2021;36(3):978–985.e1. doi:10.1016/j.arth.2020.09.032
12. Vieira MC, Monte FBM, Eduardo Dematte B, et al. Antinociceptive Effect of Lodenafil Carbonate in Rodent Models of Inflammatory Pain and Spinal Nerve Ligation-Induced Neuropathic Pain. *J Pain Res*. 2021;14:857–866. doi:10.2147/JPR.S295265
13. Zanos P, Moaddel R, Morris PJ, et al. Ketamine and Ketamine Metabolite Pharmacology: insights into Therapeutic Mechanisms. *Pharmacol Rev*. 2018;70(3):621–660. doi:10.1124/pr.117.015198
14. Weerink MAS, Struys MMRF, Hannivoort LN, Barends CRM, Absalom AR, Colin P. Clinical Pharmacokinetics and Pharmacodynamics of Dexmedetomidine. *Clin Pharmacokinet*. 2017;56(8):893–913. doi:10.1007/s40262-017-0507-7
15. Colloca L, Ludman T, Bouhassira D, et al. Neuropathic pain. *Nat Rev Dis Primers*. 2017;3(1):17002. doi:10.1038/nrdp.2017.2
16. Mickle AD, Shepherd AJ, Mohapatra DP. Sensory TRP Channels. *Progress in Molecular Biology and Translational Science Elsevier*. 2015;73–118.
17. Ren K, Dubner R. Interactions between the immune and nervous systems in pain. *Nat Med*. 2010;16(11):1267–1276. doi:10.1038/nm.2234
18. Bhandari R, Sharma A, Kuhad A. Novel Nanotechnological Approaches for Targeting Dorsal Root Ganglion (DRG) in Mitigating Diabetic Neuropathic Pain (DNP). *Front Endocrinol*. 2022;12:790747. doi:10.3389/fendo.2021.790747
19. Chen J, Li L, Chen SR, et al. The α2δ-1-NMDA Receptor Complex Is Critically Involved in Neuropathic Pain Development and Gabapentin Therapeutic Actions. *Cell Rep*. 2018;22(9):2307–2321. doi:10.1016/j.celrep.2018.02.021
20. Balzer N, McLeod SL, Walsh C, Grewal K. Low-dose Ketamine for Acute Pain Control in the Emergency Department: a Systematic Review and Meta-analysis. *Acad Emerg Med*. 2021;28(4):444–454. doi:10.1111/acem.14159
21. Zanos P, Gould TD. Mechanisms of ketamine action as an antidepressant. *Mol Psychiatry*. 2018;23(4):801–811. doi:10.1038/mp.2017.255
22. Carr DB, Goudas LC, Denman WT, Brookoff D, Lavin PT, Staats PS. Safety and efficacy of intranasal ketamine in a mixed population with chronic pain. *Pain*. 2004;110(3):762–764. doi:10.1016/j.pain.2004.04.014
23. Jun JH, Kim KN, Kim JY, Song SM. The effects of intranasal dexmedetomidine premedication in children: a systematic review and meta-analysis. *Can J Anesth*. 2017;64(9):947–961. doi:10.1007/s12630-017-0917-x
24. Anttila M, Penttilä J, Helminen A, Vuorilehto L, Scheinin H. Bioavailability of dexmedetomidine after extravascular doses in healthy subjects. *Br J Clin Pharmacol*. 2003;56(6):691–693. doi:10.1046/j.1365-2125.2003.01944.x
25. Heath A, Rios JD, Pullenayegum E, et al. The intranasal dexmedetomidine plus ketamine for procedural sedation in children, adaptive randomized controlled non-inferiority multicenter trial (Ketodex): a statistical analysis plan. *Trials*. 2021;22(1):15. doi:10.1186/s13063-020-04946-3
26. Kuthati Y, Navakanth Rao V, Busa P, Tummala S, Davuluri Venkata Naga G, Wong CS. Scope and Applications of Nanomedicines for the Management of Neuropathic Pain. *Mol Pharm*. 2020;17(4):1015–1027. doi:10.1021/acs.molpharmaceut.9b01027
27. Lu Y, Lin B, Zhong J. The Therapeutic Effect of Dexmedetomidine on Rat Diabetic Neuropathy Pain and the Mechanism. *Biol Pharm Bull*. 2017;40(9):1432–1438. doi:10.1248/bpb.b17-00224
28. Kreutzwiser D, Tawfic QA. Expanding Role of NMDA Receptor Antagonists in the Management of Pain. *CNS Drugs*. 2019;33(4):347–374. doi:10.1007/s40263-019-00618-2
29. Tsuda M. Microglia in the spinal cord and neuropathic pain. *J Diabetes Investig*. 2016;7(1):17–26. doi:10.1111/jdi.12379
30. Lu HJ, Gao YJ. Astrocytes in Chronic Pain: cellular and Molecular Mechanisms. *Neurosci Bull*. 2023;39(3):425–439. doi:10.1007/s12264-022-00961-3
31. Kiguchi N, Ding H, Peters CM, et al. Altered expression of glial markers, chemokines, and opioid receptors in the spinal cord of type 2 diabetic monkeys. *Biochim Biophys Acta Mol Basis Dis*. 2017;1863(1):274–283. doi:10.1016/j.bbadis.2016.10.007
32. Ji RR, Nackley A, Huh Y, Terrando N, Maixner W. Neuroinflammation and Central Sensitization in Chronic and Widespread Pain. *Anesthesiology*. 2018;129(2):343–366. doi:10.1097/ALN.0000000000002130
33. Uusalo P, Lehtinen M, Löyttyniemi E, Manner T, Scheinin M, Saari TI. Premedication with intranasal dexmedetomidine decreases barbiturate requirement in pediatric patients sedated for magnetic resonance imaging: a retrospective study. *BMC Anesthesiol*. 2019;19(1):22. doi:10.1186/s12871-019-0690-1
34. Zhou Y, Wang C, Lan X, Li H, Chao Z, Ning Y. Plasma inflammatory cytokines and treatment-resistant depression with comorbid pain: improvement by ketamine. *J Neuroinflammation*. 2021;18(1):200. doi:10.1186/s12974-021-02245-5
35. Lu X, Tang L, Lan H, Li C, Lin H. A Comparison of Intranasal Dexmedetomidine, Esketamine or a Dexmedetomidine-Esketamine Combination for Induction of Anaesthesia in Children: a Randomized Controlled Double-Blind Trial. *Front Pharmacol*. 2021;12:808930. doi:10.3389/fphar.2021.808930
36. Chiaretti A, Barone G, Rigante D, et al. Intranasal lidocaine and midazolam for procedural sedation in children. *Arch Dis Child*. 2011;96(2):160–163. doi:10.1136/adc.2010.188433
37. Finnerup NB, Attal N, Haroutounian S, et al. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol*. 2015;14(2):162–173. doi:10.1016/S1474-4422(14)70251-0
38. Breitinger U, Breitinger HG. Excitatory and inhibitory neuronal signaling in inflammatory and diabetic neuropathic pain. *Mol Med*. 2023;29(1):53. doi:10.1186/s10020-023-00647-0