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Effect of Thiamine on Morphine Analgesia and Tolerance in Rats

Arzuhan Çetindağ Çiltaş[1a*], Ayşegül Öztürk[1b]

[1]*Sivas Cumhuriyet University, Vocational School of Healthcare Services, Department of Physiotheraphy, Sivas, TURKEY*

**Corresponding author*

Research Article

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ABSTRACT

The latest research has demonstrated that inflammation, oxidative stress, and apoptosis play a major role in morphine analgesia and tolerance development. This search goal is to examine the possible role of thiamine use on oxidative stress, inflammation, and apoptosis in the development of morphine analgesia and morphine tolerance in rats.

Methods: Thirty-six male Wistar rats were used in this study. The rats were severed into six groups: saline, 100 mg/kg thiamine, 5 mg/kg morphine, thiamine + morphine, morphine tolerance, and thiamine + morphine tolerance. The resulting analgesic effect was measured by hot plate and tail movement analgesia tests. TAS and TOS, inflammation parameters, and apoptosis protein levels of the dorsal root ganglion tissues sample were measured using an ELISA kit.

Results: When thiamine was given alone, it did not show an anti-nociceptive effect ($p>0.05$). In addition, thiamine enhanced the analgesic effect of morphine ($p < 0.05$) and also significantly reduced tolerance to morphine ($p < 0.05$). However, it reduced TOS when administered with a single dose of morphine and tolerance induction ($p < 0.05$). In addition, thiamine reduced apoptosis protein levels after tolerance development ($p < 0.05$).

Conclusion: Consequently, these results may attain by reducing TOS, inflammation, and apoptosis.

Keywords: Thiamine, Morphine Analgesia, Morphine Tolerance, Total Antioxidant Status (TAS), Total Oxidant Status (TOS), Apoptosis, İnflammation

Tiaminin Ratlarda Morfin Analjezisi ve Toleransı Üzerine Etkisi

Süreç

Geliş: 03/08/2022
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ÖZ

Özet: Son araştırmalar inflamasyon, oksidatif stres ve apoptozun morfin analjezisi ve tolerans gelişiminde önemli bir rol oynadığını göstermiştir. Bu araştırmanın amacı, sıçanlarda morfin analjezisi ve morfin toleransının gelişiminde oksidatif stres, inflamasyon ve apoptoz üzerinde tiamin kullanımının olası rolünü incelemektir.

Yöntemler: Bu çalışmada 36 adet erkek Wistar rat kullanıldı. Sıçanlar salin, 100 mg/kg tiamin, 5 mg/kg morfin, tiamin+morfin, morfin toleransı ve tiamin+morfin toleransı olmak üzere altı gruba ayrıldı. Ortaya çıkan analjezik etki, hot plate ve tail flick analjezi testleri ile ölçüldü. Dorsal kök ganglion doku örneğinin TAS ve TOS, inflamasyon parametreleri ve apoptoz protein seviyeleri bir ELISA kiti kullanılarak ölçüldü.

Bulgular: Tiamin tek başına verildiğinde antinosiseptif etki göstermedi ($p>0,05$). Ek olarak, tiamin morfinin analjezik etkisini arttırdı ($p < 0.05$) ve ayrıca morfine toleransı önemli ölçüde azalttı ($p < 0.05$). Ancak, tek doz morfin ve tolerans indüksiyonu ile uygulandığında TOS'u azalttı ($p < 0.05$). Ek olarak, tiamin, tolerans gelişiminden sonra apoptosis protein seviyelerini azaltmıştır ($p < 0.05$).

Sonuç: Sonuç olarak, bu sonuçlara TOS, inflamasyon ve apoptozu azaltarak ulaşılabilir.

Anahtar Kelimeler: Tiamin, Morfin Analjezisi, Morfin Toleransı, Toplam Antioksidan Durumu (TAS), Toplam Oksidan Durumu (TOS), Apoptoz, İnflamasyon

[1a] *acetindag@cumhuriyet.edu.tr* *https://orcid.org/ 0000-0002-5420-3546* [1b] *fztaysegul@yahoo.com* *https://orcid.org/ 0000-0001-8130-7968*

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Introduction

Morphine is an analgesic, which is often applied the relieve violent and chronic pain today. If the antinociceptive properties of morphine develop tolerance, the duration of analgesic action decreases. However, despite many studies, the mechanism for developing opioid tolerance is unclear[1,2].

Research on morphine tolerance has generally focused on the effect of neurons in this development. However, it was recently reported that glial cell activation also plays a role in morphine tolerance[3–5]. As a result of the activation of glial cells, many proinflammatory cytokines are secreted[6]. Prolonged use of morphine induces the activation of microglia, which are glial cells in the central nervous system[7,8]. Microglial cells cause pain by releasing many proinflammatory cytokines, such as interleukin-1 (IL-1β). In this case, morphine binds to toll-like receptor 4 (TLR4) and releases proinflammatory cytokines[9]. In addition, IL-1β, which is the most critical factor in inflammation processes, is produced by a nod-like receptor protein 3 (NLRP3) as pro-inflammatory cytokine interleukin-1β (Pro-IL-1β) and then secreted as IL-1β[10]. These cytokines are involved in increasing the hyperactivity of dorsal root ganglia (DRG), which causes morphine to reduce analgesic efficacy and sensitivity[11]. In addition, long-term morphine treatment leads to oxidative stress (OS) in various cells[12,13]. Oxidative stress on some cellular mechanisms resulted in neuronal apoptosis in DRG[12,14].

Thiamine (TIA) is an essential water-soluble vitamin critical for carbohydrates, amino acid catabolism, and gluconeogenesis. It also acts as a cofactor of enzymes Cut-off time in almost the entire organism[15,16]. The fact that thiamine is involved in both energy pathways and defense mechanisms developed against oxidative stress suggests that it will help diagnose and treat many diseases in the field of health[17]. In the absence of thiamine, apoptosis, and neurodegeneration occur in cells. The reduction in thiamine phosphate and thiamine-dependent enzymes multiplies oxidative stress and leads to neurodegeneration. But the effects of thiamine on the development of morphine analgesia and morphine tolerance are still unknown.

This search aims to examine the possible role of thiamine use in rats on apoptosis inflammation pathways, and oxidative stress in the development of morphine analgesia and morphine tolerance.

Methods

Animals

Wistar Albino (230-250 grams for each group; n=6) rats were used in the study. The rats were provided by the Animal Center Laboratory of Sivas Cumhuriyet University. At a steady temperature (22 ± 3°C), the ad libitum was kept in standard conditions with a standard diet and water in a 12 h light-dark period. The experiment took place between 09:00 and 17:00. The study protocol was approved by Sivas Cumhuriyet University's Animal Ethics Committee. (Approval No: 65202830-050.04.04-448), experimental processes were initiated.

Drugs

Thiamine (B1) (Solgar) and morphine sulfate (Sivas Cumhuriyet University Hospital, Turkey) were dissolved in a saline solution. Thiamine and morphine were freshly prepared and injected into the animals during the experimental days. Before the analgesia tests, morphine (5 mg/kg) and thiamine (100 mg/kg) was given subcutaneously (s.c.) and intraperitoneally (i.p.), respectively.

Experimental protocol

Analgesic effects of thiamine and morphine were utilized at 30-minute intervals (30, 60, and 90 minutes) using tailf-lick and hot-plate antinociception tests. The animals were divided into six groups: Saline, thiamine (TIA), 5 mg/kg of morphine (M), TIA+ M, morphine tolerance (MT), and TIA+MT. Thiamine and saline were given to animals as intraperitoneal and morphine subcutane in the specified doses (volume of administration, 1 ml/kg). After analgesic tests were carried out, the animals were sacrificed by decapitation. Dorsal root ganglion (DRG) (T12-L5 levels) were collected from animals evaluated (Figure 1).

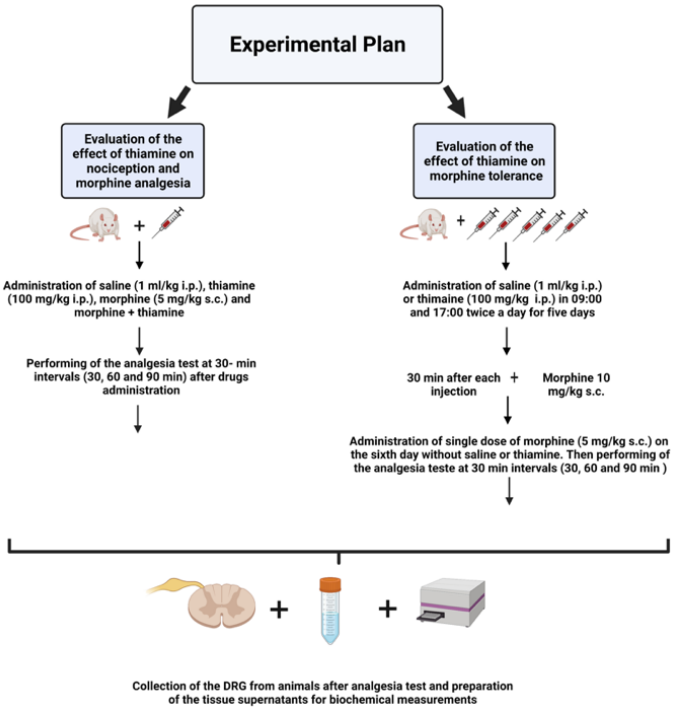

Figure 1. The study's experimental design.

Antinociception tests

Tail-Flick test

Thermal nociception was measured using a common tail-flick device (May TF 0703 Tail-flick Unit, Commat, Turkey). After administering saline or drugs, the radiant heat source was focused at a distance of 3 cm on the perpendicular portion of the tail. Following the administration of saline or drugs, tail-flick latencies (TFL) were measured. Cut-off times were established 15 seconds to prevent tissue injury[1,2].

Hot Plate test

It is thought that the antinociceptive reaction in hot plates occurs under the influence of central and peripheral mechanisms. The rats were placed individually on a hot plate (May AHP 0603 Analgesic Hot-plate, Commat, Turkey) at 54 ± 3 ° C. The delay up to the first claw licking or splash reaction to avoid heat was recorded as a pain threshold indicator. Cut-off time is set to 30 seconds to prevent damage to the paws[1,2].

Morphine tolerance induction

The procedure used to induce morphine tolerance was defined in earlier investigations 18. To create morphine tolerance, animals were randomly chosen and administered 10 mg/kg of morphine s.c. twice daily (at 9:00 and 17:00) for five days18-20. In addition, morphine (10 mg/kg) was given 30 minutes after every thiamine administration to determine the effects of thiamine (100 mg/kg) on morphine tolerance. Analgesic morphine dosage recommendation (5 mg/kg). It was administered on day six without saline or thiamine, and tail-flick and hot-plate tests were measured at 30-minute intervals (30, 60, and 90 minutes) to assess tolerance.

DRG tissue homogenate preparation

DRG tissue samples in the cold phosphate buffer saline solution of the animals were homogenized using a mechanical homogenizer (Analytic Jena Speed Mill Plus, Jena, Germany) and centrifuged for 10 minutes at a temperature of 4 ºC at 4000 rpm. Then supernatants were obtained and stored at -80 °C until biochemical analysis. A Bradford protein test kit (Merck, Germany) was used to determine the total protein levels in the samples[21].

Total antioxidant status (TAS) measurement

TAS concentrations at tissue level were determined by an automated test method previously developed by Erel based on monitoring the reaction rate of free radicals by measuring the absorption of colored dianidil radicals during free radical reactions that begin with hydroxyl radical production in a Fenton reaction. Antioxidants in tissue samples should suppress coloring in proportion to their concentration[22]. The results were expressed as micromolar Trollox equivalents per gram tissue protein (µmol Trollox Eq /g protein).

Total oxidant status (TOS) measurement

Tissue TOS concentrations were measured by Erel's automated test method [23]. Since iron ion is oxidized to iron ion when sufficient amounts of oxidant are present in the environment, the technique allows measuring TOS levels by measuring the tissue level of iron ions using xylenol oranges. Hydrogen peroxide was used for calibration of the analysis 23. The test results were expressed as the equivalent of micromolar hydrogen peroxide per gram tissue protein (µmol H2O2 Eq /g protein).

Measurement of NLRP-3, pro-IL-1β, IL-1β, Caspase-1, Caspase-3 and Caspase-9

DRG supernatants NLRP-3, pro-IL-1β, IL-1β, Caspase-1, Caspase-3, and Capase-9, were measured using rat ELISA commercial kits (Shanghai Sunred Biological Technology, Shanghai, China).

The operational protocols were by the manufacturer's instructions. In short, standard and tissue samples were added to a plate and incubated for 60 minutes at 37 ° C. After washing, dyeing solutions were added and incubated for 15 minutes at 37 ° C. The stop solution was added and read at 450 nm. Standard curves were used to calculate all kits. The coefficients of variation in and between the plates were less than 10%.

Analgesic tests data analysis

To calculate the maximum percentage of antinociceptive action (MPE), tail movements and hot plate delays (in seconds) were converted into an antinociceptive activity percentage with the following equation: % MPE = [(post-drug delay - primary delay) / (cutting value - Basic delay)] × 100 [18].

Statistical analysis

The results are given as SEM (standard error of average) ± average. Antinociceptive effect was measured, and average MPE was calculated. Variance analysis (One-Way Anova) and posthoc Tukey test were used in the analysis of the data. The significance value was regarded as $p<0.05$.

Results

Effect of Thiamine on Nociception, Morphine Analgesia, and Morphine Tolerance

Tail flick and hot plate tests were used for 90 minutes with a 30-minute interval to assess the analgesic effect of thiamine. Although there was an increase in antinociceptive tests in the 30th, 60th, and 90th minutes compared to the thiamine saline group, this increase was not significant (Figure 2A, 2B, $p>0.05$). This data showed that thiamine alone does not have a significant analgesic effect. However, in tail-flick and hot-plate tests, the administration of thiamine together with morphine boosted the antinociceptive impact of morphine ($p<0.05$; Figure 2A, 2B). In addition, the maximum increased effect of thiamine on morphine was demonstrated in the tail-flick test in the 60th minute and the hot-plate test in the 30th minute.

Morphine analgesia tests showed their peak in the 60th minute. However, in both tests, the MPE was statistically higher in the morphine-given group than in the morphine tolerance group. In both the tail-flick and hot-plate tests, the administration of thiamine to morphine-tolerant rats dramatically reduced their morphine tolerance compared to morphine-tolerant rats. ($p<0.05$) (Figure 2A, 2B).

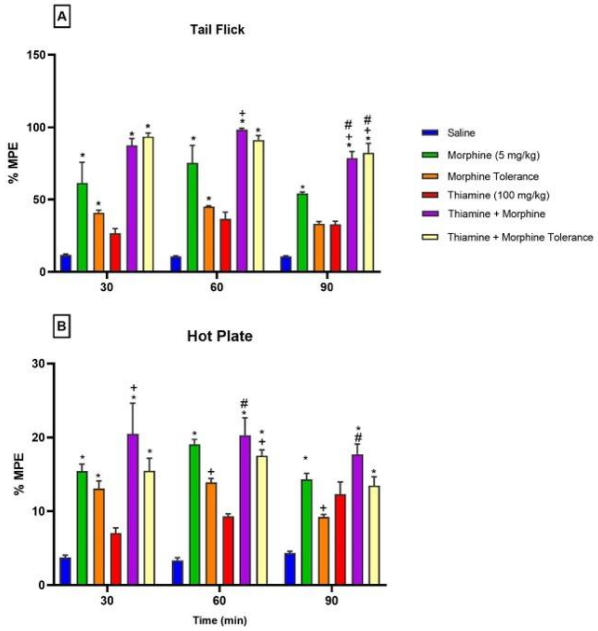

Figure 2. Thiamine's effect on nociception, morphine analgesia, and tolerance to morphine. In the tail flick test (A); In the hot plate test (B. The percent MPE (n = 6) values are expressed as means + SEM. *p < 0.05, compared to the saline group, +p<0.05, compared to the morphine group and #p<0.05, compared to the morphine tolerance group.

Effect of thiamine on TAS and TOS parameters in morphine analgesia and tolerance in DRG

TAS and TOS levels in dorsal root ganglia are shown in Figure 3A and Figure 3B. Compared to the saline group, the morphine tolerance group's TAS levels drastically decreased ($p< 0.05$) (Figure 3A). However, the application and combination of thiamine did not influence the development of

morphine and morphine tolerance in DRG (p>0.05) (Figure 3A).

Morphine alone enhanced TOS levels in DRG compared to saline. However, the morphine tolerance group enhanced TOS levels compared to saline and morphine groups. However, the application of thiamine with morphine reduced TOS levels compared to both the morphine and morphine tolerance group (p<0.05) (Figure 3B).

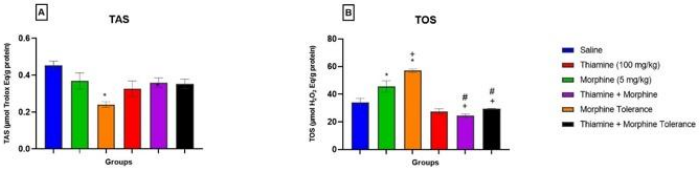

Figure 3. In morphine analgesia and tolerance in DRG, the effect of thiamine on total antioxidant status (TAS) (A) and total oxidant status (TOS) (B) levels was investigated. The percent MPE values are expressed as means + SEM. *p < 0.05, compared to the saline group, +p<0.05, compared to the morphine group and #p<0.05, compared to the morphine tolerance group.

Effect of thiamine on apoptosis in morphine analgesia and tolerance in DRG

The levels of caspase-9 and caspase-3 in the dorsal root ganglia are shown in Figure 4A and Figure 4B. The morphine tolerance group increased caspase-9 and caspase-3 compared to saline. In addition, the combination of thiamine with morphine did not alter caspase-9 and caspase-3 levels compared with both the morphine and morphine tolerance group. However, the thiamine + morphine tolerance combination reduced caspase-9 and caspase-3 levels, compared to the morphine tolerance group (p<0.05) (Figure 4A, 4B).

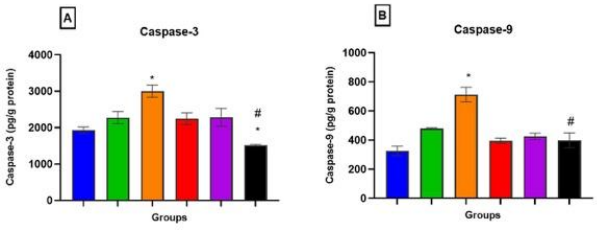

Figure 4. In morphine analgesia and tolerance in DRG, the effect of thiamine on apoptosis caspase-3 (A), caspase-9 (B) was investigated. The percent MPE values are expressed as means + SEM. *p < 0.05, compared to the saline group, +p<0.05, compared to the morphine group and #p<0.05, compared to the morphine tolerance group.

Effect of thiamine on inflammation in morphine analgesia and tolerance in DRG

To better understand the effect of inflammation in the development of morphine analgesia and morphine tolerance of thiamine, we determined caspase-1, IL-1β, NLRP-3, and pro-IL-1β levels in dorsal root ganglia with ELİSA kits (Figure 5A, Figure 5B, Figure 5C, and Figure 5D). The application of a single dose of morphine increased caspase-1 levels in DRG compared to the saline group. (Figure 5A). Single-dose morphine administration and morphine tolerance enhanced pro IL-1β levels in DRG compared to saline, as shown in Figure 5C (p<0.05). In addition, the morphine tolerance group was found to statistically increase the level of pro-IL-1β compared to the morphine group (p<0.05). The combination of thiamine+morphine and thiamine+morphine tolerance reduced pro IL-1β levels statistically (p<0.05) (Figure 5B). Il-1β levels increased statistically significantly compared to the saline group of the morphine group alone (p<0.05). Furthermore, morphine tolerance increased IL-1β levels (p<0.05) (Figure 5C). As shown in Figure 5C, compared to the saline, the combination of thiamine +morphine and thiamin+morphine tolerance reduced IL-1β levels in DRG (p<0.05). As shown in Figure 5D, single-dose morphine and morphine tolerance increased NLRP-3 levels compared to saline (p<0.05). In addition, the morphine tolerance group increased NLRP-3 levels in DRG compared to morphine (p<0.05) (Figure 5D).

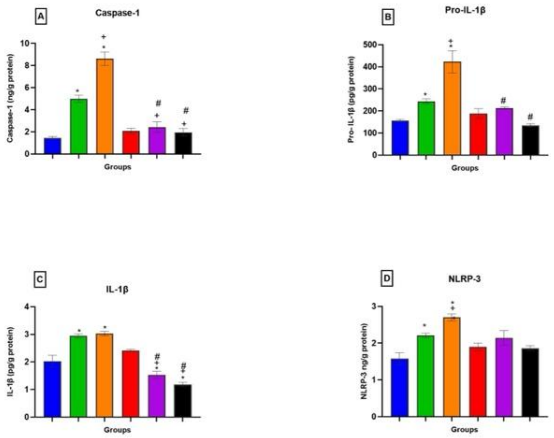

Figure 5. In morphine analgesia and tolerance in DRG, the effect of thiamine on inflammation parameters caspase-1 (A), Pro- IL 1 β (B), IL-1 β (C) and NLRP-3 (D) was investigated. The percent MPE values are expressed as means + SEM. *p < 0.05, compared to the saline group, +p<0.05, compared to the

morphine group and #p<0.05, compared to the morphine tolerance group.

Discussion

Morphine is an opioid frequently used to treat severe and chronic pain[23]. Prolonged use of opioids causes analgesic effects and side effects such as respiratory depression, euphoria, sedation, and nausea[25,26]. Different analgesic approaches, such as low-dose morphine or a mix of auxiliary medications, are utilized to lessen these unfavorable effects of morphine [27]. In this study, thiamine in analgesia tests, also known as B1vitaminin, increased morphine analgesia and reduced analgesic tolerance when combined with morphine. According to our data, Thiamine's effects on morphine analgesia and tolerance building may be brought about by its ability to inhibit inflammatory and apoptotic pathways. However, our results showed that thiamine alone does not have an analgesic effect.

Numerous studies have demonstrated that both acute and ongoing morphine treatment can result in a considerable reduction in TAS levels in the mouse liver, as well as the rodent and human brain[28-30]. It has also been shown that chronic morphine therapy affects superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSHPx), that is, enzymes involved in endogenous antioxidant defenses [31,32]. In line with previous studies, our study has also found that morphine and morphine tolerance alone reduce DRG's antioxidant status (TAS). However, thiamine did not change tas levels combined with a single dose of morphine or in DRG. It may be suggested that morphine can suppress the antioxidant system, causing the development of tolerance. In addition, single doses of morphine and chronic morphine application have been shown to cause oxidative stress (TOS) in DRG. In addition, TOS levels in DRG increased more than the application of chronic morphine compared to a single dose and showed similar results from previous studies. This might be connected to the development of tolerance. However, thiamine reduced these morphine effects.

Vitamin B types effectively control inflammatory or neuropathic pain in different animal models and humans[33]. In some research in experimental animals, the combination of thiamine/pyridoxine/cyanocobalamin has shown an antinociceptive effect[34]. These vitamins have also been shown to increase the antinociceptive effect of nonsteroidal anti-inflammatory drugs [35]. Recently, riboflavin, another B complex vitamin, has been found to show anti-inflammatory effects. Riboflavin has been shown to reduce the synthesis of tumor necrosis factor (TNF) α, interleukin (IL)-1, and IL-6 from inflammatory cytokines caused by lipopolysaxkaritis (LPS)[36]. Thiamine (B1 vitamin), another vitamin B, which we also use in our study, is considered to have painkiller properties through antinociceptive, anti-inflammatory, and anti-neuropathic mechanisms. Our study found that the treatment of thiamine alone did not show antinociceptive, anti-inflammatory, or antiapoptotic effects. This may be due to the different doses of thiamine, the way it was given, and the experimental model.

Braga et al. thiamine has been shown to significantly reduce TNF-α concentrations in the DRG of animals treated with practicality. proinflammatory sickins (TNF- α, Il1-β, Il6) are excised in healthy spinal cord cells at low levels. Still, there is an increase in expression levels after peripheral nerve damage and inflammation [34]. The main source of this increase in the central nervous system (MSS) is activated glia. However, glial activation has been shown to inhibit inhibitors in developing hyperalgesia and allodin due to nerve damage and inflammation[37]. With acute and chronic administration of morphine, glial activation occurs. During this treatment process, the degree of glial activation increases. The administration of morphine and increased glial activation causes the release of more proinflammatory cytokines[38]. Increased proinflammatory cytokines then reduce the analgesic effect of morphine, causing the development of morphine tolerance[39]. In our study, morphine and morphine tolerance alone increased the levels of IL1-β, and pro-IL1-β. However, morphine tolerance increased inflammatory parameters in DRG (IL1-β, pro-IL1-β) more than a single dose of morphine. These results are in line with those of earlier research. In addition, the combination of thiamine, morphine, and morphine tolerance has alleviated these parameters. These findings showed us that thiamine reduces inflammation and exhibits antinociceptive activity with combined therapy.

Liu et al. have demonstrated that exposure to chronic morphine increases NLRP3, caspase-1, pro-IL1-β, and IL1-β, and these levels decrease with melatonin treatment. Chronic morphine exposure leads to excessive cellular ROS production and inflammatory activation of NLRP3 in microglia. Increased NLRP3 activation causes caspase-1 oscillation, and caspase-1 activation increases pro-IL1-β cytokine, causing an increase in IL1-β level. And this increase causes morphine analgesic tolerance and hyperalgesia. To reduce these side effects due to the use of morphine, thiamine can reduce ROS and, as a result, inhibit the activation of NLRP3 inflammation, suppressing the overactive IL-1β signal, which ultimately weakens the development of morphine analgesic tolerance[40]. Proinflammatory cytokines such as IL-1β, IL-6, and TNF-α released from glia in the central nervous system may be responsible for the development of central sensitization[41]. Increased spinal proinflammatory cytokines and glia activation not only induce central sensitization, but also affect the antinociceptive properties of morphine [42] . Raghavendra ve diğerleri, 2002). Suppress glial activation or inhibition of spinal proinflammatory cytokines reduces morphine tolerance and enhances the acute antinociceptive effect of morphine in neuropathic rats [43]. Moreover, beige-J mice, a species of mutant mouse with immunological deficiencies, have previously been shown to be resistant to morphine analgesia and have high IL-1 levels[44].

Various cellular mechanisms such as oxidative stress and inflammation in the initiation and spread of apoptosis directly trigger apoptosis, increasing the production of ROS. However, antioxidants and anti-inflammatories have been shown to reduce ROS production and increase its anti-apoptocytic effect[45]. Previous research has shown that prolonged exposure to morphine causes apoptosis cell death by activating mechanisms such as oxidative stress, inflammatory and endoplasmic reticulum (ER) stress in the dorsal horn regions of the spinal cord, which are critical to opioid analgesia [46,47]. Our findings found that morphine tolerance in DRG increased caspase-1, caspase-3, and caspase-9 levels. It was in line with earlier research in the literature[18,48,49]. Even though a single dose of morphine raised levels of inflammation and TOS, it did not change the caspase-3 and caspase-9 but caused an increase in caspase-1 levels, which are pyroptosis markers. This result may be that a single dose of morphine has a threshold value in the apoptosis process. On the other hand, using thiamine in combination with morphine tolerance reduced caspase-1, caspase-3, and caspase-9 levels in DRG. In the clinical management of pain, it can be concluded that thiamine has a therapeutic effect by maintaining a significant delay in the development of tolerance to morphine analgesia, suppression of inflammation, and apoptosis.

Conclusion

As a result, the data presented in this study suggest that thiamine is a useful adjuvant in the treatment of long-term opioids. For this medication to be utilized in conjunction with opioid drugs, more research is required.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgment

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