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Slow-releasing CO donor CORM-A1 modulates depression-like behavior in experimental model of chronic prostatitis/chronic pelvic pain syndrome

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Chronic pain conditions are often linked with depression, a common chronic mental health disorder that severely impairs quality of life. This association is particularly present in chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS). CP/CPPS is a prevalent urological disorder characterized by pelvic pain accompanied by neuropsychiatric comorbidities such as depression, for which underlying pathophysiological mechanisms remain unclear. Recently, anti-inflammatory and neuroprotective effects of carbon monoxide-releasing molecules (CORMs) have been demonstrated. The aim of this study was to investigate whether CO releaser, CORM-A1 could have beneficial effects on depressive-like behavior in an animal model of CP/CPPS. Adult male Wistar albino rats were divided into four groups (Sham-PBS, Sham-CORM, CP/CPPS-PBS and CP/CPPS-CORM), receiving intraprostatic injections of saline or λ-carrageenan, followed by daily treatments with PBS or CORM-A1 for seven days. Pain thresholds were measured by von Frey esthesiometer, while depression-like behaviors were assessed by the forced swimming test (FST). CP/CPPS rats exhibited mechanical hyperalgesia which has been significantly diminished by CORM-A1 administration. CORM-A1 treatment caused a significant decrease in floating time and a significant increase in swimming time in CP/CPPS rats compared to those treated with vehicle (CP/CPPS-PBS group). Correlation analysis showed a significant link between pain threshold and FST floating time as indicator of depressive-like behaviors. These findings suggest that CORM-A1 beneficially modulates pain perception and depressive-like behaviors in CP/CPPS rats.

Key words: depression, behavior, CP/CPPS, CORM-A1, pain

INTRODUCTION

Depression is not just a simple mood disorder, but a complex systemic disease affecting neuroendocrine, immune, and neurochemical systems (Chand et al., 2023). It presents chronic unhappiness, loss of interest, sleep disturbances, and suicidal thoughts, severely impacting quality of life (Liao et al., 2021; Tao et al., 2023). Research highlights pathophysiological mechanisms like neuroinflammation, oxidative stress, monoamine imbalances, and hypothalamic-pituitary-adrenal (HPA) axis dysfunction in depression etiology (Hassamal, 2023; Sălcudean et al., 2025). According to WHO (2023), around 280 million people globally live with depression, which affects both mental and physical health. When combined with chronic somatic diseases, depression tends to be more resistant to treatment and shows lower response rates (Costa et al., 2019; McIntyre et al., 2023).

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Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is an increasingly prevalent urological disease characterized by spontaneous pain in pelvic region, irritating obstructive urinary symptoms, sexual dysfunction. All these treatment-resistant symptoms trigger psychosomatic complaints and depression in patients. This suggests that CP/CPPS is a systemic syndrome associated with not only somatic, but also psychological effects. Namely, clinical and experimental studies show that the prevalence of depression and anxiety in individuals with CPPS is significantly higher than in the general population (Kwon & Chang, 2013; Evans-Durán, 2022) which negatively affect quality of life in these patients (Breser et al., 2017; Chen et al., 2020). Moreover, pain catastrophizing has been identified as an important psychological factor that further aggravates depressive symptoms by increasing the severity and impact of pain in these patients (Stamatiou et al., 2024).

Carbon monoxide-releasing molecules (CORMs) are novel compounds that provide controlled CO release. CO in low doses and under control conditions (Stucki & Stahl, 2020; Li et al., 2025) could have neuroprotective effects. Moreover, the effects of CO on the HPA axis suggest that it can play an important role in regulating the stress response (Mancuso et al., 2010), inflammatory response (Lin et al., 2019), redox status (Chaves et al., 2025). CORMs can suppress neuroinflammation by regulating cytokines production and exhibit antidepressant-like effects (Tao et al., 2023). These findings indicate that CORMs may be promising agents not only for neuroprotection, but also for the treatment of depression (Li et al., 2020; Zhao et al., 2024).

Current treatment options are largely inadequate in CP/CPPS, especially in the treatment of mental disorders associated with CP/CPPS. Therefore, there is constant need for novel drug developments and therapeutic strategies through tests in animal models of CP/CPPS. Reliable models of CP/CPPS could be developed by % λ-carrageenan administered intraprostatically (Mansour, 2024). It has been shown that it imitates prostate inflammation and (neuro)behavioral changes, including mechanical allodynia and depression-like behaviors (Šutulović et al., 2023). The role of CO-mediated signaling in development of depression in CP/CPPS has not been investigated yet, to the best of our knowledge. We hypothesized CORM-A1 could impact depression-like behavior in CP/CPPS model by altering pain perception.

Therefore, we opted herein to evaluate the effects of CORM-A1 on pain sensitivity and depression-like behaviors in an animal model of CP/CPPS. In our study, we measured scrotal pain threshold to determine mechanical pain sensitivity, applied the forced swim test (FST) to evaluate depression-like behaviors.

METHODS

Animals and Ethical Statement

Wistar Albino male rats weighing 250-300 g were used in the study (n=24). The animals were housed under standard laboratory conditions, including constant temperature (22±2°C; relative air humidity 50±5%; a 12-h light/dark cycle, light starting at 08:00 a.m.) and free access to water and food.

All procedures conducted in this study received approval from the relevant local authorities of Republic of Serbia and Ethics Committee of the Faculty of Medicine, University of Belgrade (Permit No. 323-07-01339/2017-05/3) and were performed in accordance with Directive 2010/63/Eu of the European Parliament.

Experimental Design

CP/CPPS has been surgically induced in rats by intraprostatic injection of 3% λ-carrageenan (forming CP/CPPS groups), while corresponding Sham rats received at the same time intraprostatic injection of 0.9% NaCl (forming Sham groups). Hereupon, rats from each of these groups have been treated with CORM-A1 (2 mg/kg/day, i.p.) or phosphate- buffered saline (PBS, 0.1 mL/kg/day, i.p,) as the solvent during the period of the seven days after surgery. Hence, i) Sham-PBS, ii) Sham-CORM, iii) CP/CPPS-PBS, iv) CP/CPPS-CORM groups were formed (n=6 per group). Rats were randomly assigned to these groups.

Mechanical pain threshold was evaluated using the an electronic von Frey (eVF) device two day before and seven days upon the surgery (Fig. 1).

Establishment of the Experimental CP/CPPS Model and Surgical Operation

Experimental CP/CPPS in rats was established following previously described protocols (Radhakrishnan & Nallu, 2009; Šutulović et al., 2019; 2021). Rats were anesthetized *via* intraperitoneal injection of sodium thiopental (40 mg/kg) and placed in the supine position on a heating pad. The lower abdomen and scrotum were shaved and disinfected with 70% ethanol applied three times, followed by a single application of povidone-iodine solution (10%) under aseptic con-

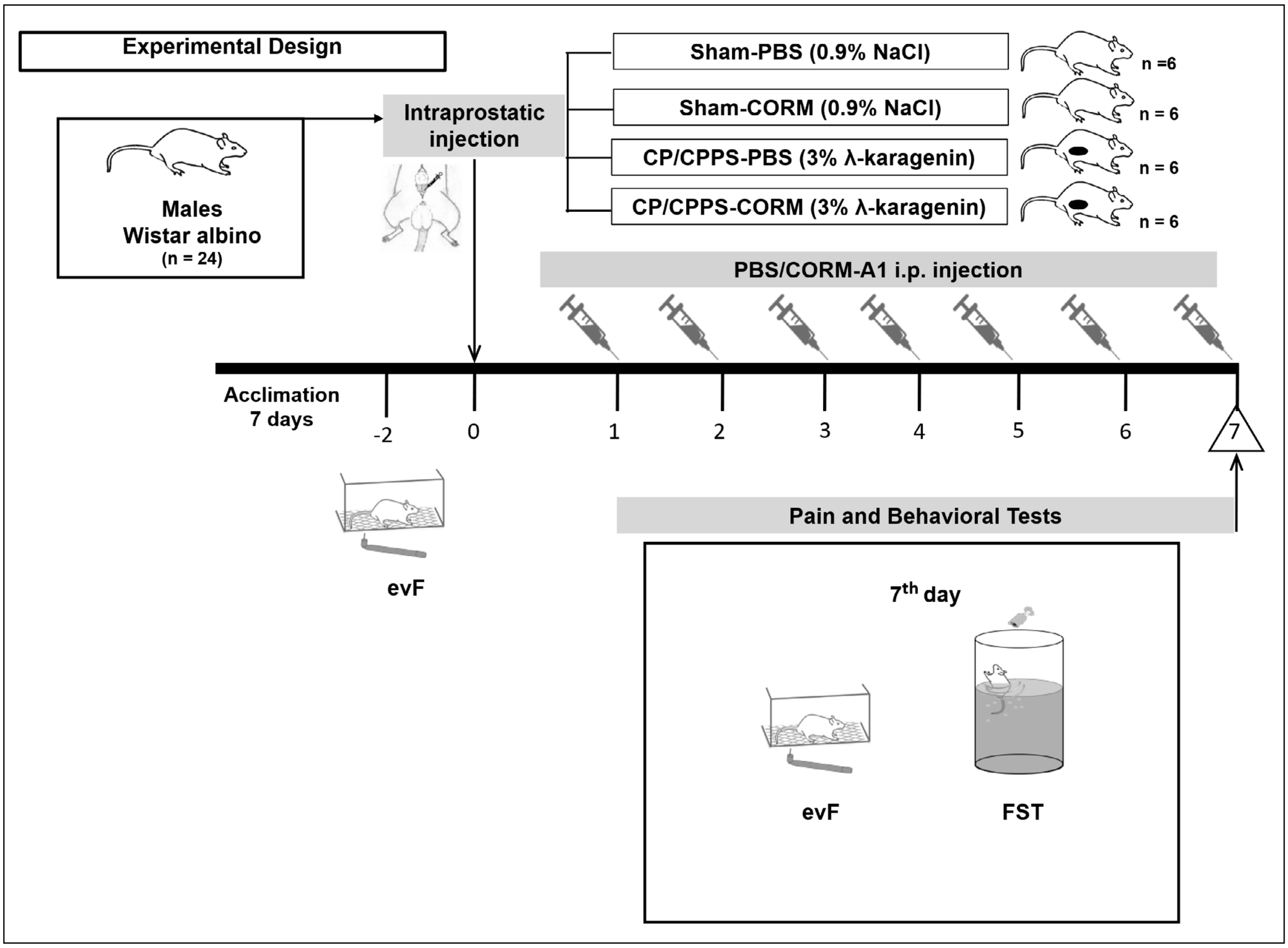

Fig. 1. Time course of experimental design. Rats (n=24) were randomly divided into: Sham-PBS (intraprostatic 0.9% NaCl injection and daily postoperative PBS administration, 0.1 mL/kg; n=6); Sham-CORM (intraprostatic 0.9% NaCl injection and daily postoperative CORM-A1 administration, 2 mg/kg; n=6); CP/CPPS-PBS (intraprostatic 3% λ-carrageenan injection and daily postoperative PBS administration, 0.1 mL/kg; n=6); CP/CPPS-CORM (intraprostatic 3% λ-carrageenan injection and daily postoperative CORM-A1 administration, 2 mg/kg; n=6). From the first to the seventh postoperative day, animals were given PBS or CORM-A1 at a dose of 2 mg/kg/day by intraperitoneal injection (i.p.), and mechanical pain sensitivity in scrotal region was evaluated by the eVF device two days before and seven days after surgery. Depression behaviors were assessed by the FST test.

ditions. To minimize postoperative pain, 2% lidocaine was administered subcutaneously at the incision site. A midsagittal incision of approximately 1–1.5 cm was made in the lower abdominal wall to expose the ventral lobes of the prostate, which were identified using anatomical landmarks, including the bladder and seminal vesicles.

In the CP/CPPS group, 25 µL of sterile 3% λ-carrageenan solution was injected into each ventral lobe using a 30-gauge Hamilton® syringe (total of 50 µL of solution per rat prostate). Sham animals received an equal volume of sterile 0.9% saline. The incision was then closed using absorbable 4-0 Polysorb™ sutures in a layered fashion. After recovery from anesthesia, animals were returned to their cages and monitored for signs of distress, bleeding, or infection.

Evaluation of Mechanical Pain Sensitivity: Scrotal Pain Threshold

To evaluate the development of experimental CP/CPPS and mechanical allodynia, scrotal pain threshold was measured on the 2nd days before intraprostatic procedure and 7th days after. Measurements were made with the eVF device. Animals were placed in Plexiglas chambers for 15–20 min before measurement and for 30 min during measurement. The eVF device was placed perpendicular to the scrotal skin and pressure gradually increased; maximum pressure was recorded when the withdrawal reflex was observed. At each time point, three consecutive measurements were obtained per animal, separated by at least 10 seconds, and the mean value was used as the pain threshold.

To minimize the risk of tissue injury, a cut-off force of 300 g was applied. Von Frey tests were conducted by trained experimenter blinded to group allocation to reduce bias.

Evaluation of Depression-Related Behaviors

Depression-related behaviors were assessed using the forced swimming test described by Porsolt et al. (1979). In this test, cylinders (50 cm height, 25 cm diameter) made of transparent Plexiglas (Elunit, Belgrade, Serbia) were used. The test was performed individually for each animal. The animals were carefully placed in cylinders containing water (35 cm depth) at 22±1°C. The behavior of the animals was video recorded for 5 min during the experiment (Logitech C210, Lausanne, Switzerland). Odor traces were removed with 70% ethanol upon draining cylinders between each animal test. After the test, the animals were dried appropriately and placed in their cages.

In the test, three behavioral categories were quantified: Struggling – defined as animal's climbing or scratching movements on the water surface with strong extremities and body movements; Swimming – defined as the animal moving horizontally on the water surface or dive with obvious extremities movements and Floating – described as the animal remaining immobile on the water surface, with nose above the surface and no extreme movements. The duration of each category was measured in seconds, and the relative distribution was calculated for each animal. To minimize bias, all video analyses were performed by a single investigator blinded to the treatment groups.

Chemicals

All chemicals (λ-carrageenan, PBS, CORM-A1) were purchased from Sigma Aldrich, St. Louis, MO, USA and were of analytical grade. λ-carrageenan was dissolved in sterile 0.9% saline to prepare a 3% solution. The solution was mixed at room temperature using a magnetic stirrer until being fully homogenous. The pH was measured and adjusted to 7.2–7.4 to match physiological conditions. Fresh solutions were prepared immediately before use to ensure stability and prevent degradation. CORM-A1 was dissolved in PBS at room temperature, pH being 7.4 and administered within 10 min of dissolution to prevent CO release from CORM-A1. Fresh solutions were prepared immediately before use at each time point in the experiment.

Statistics

The Shapiro-Wilk test was used to assess a normal distribution of data. Normally distributed data are presented as mean ± standard deviation (SD). Differences between groups in pain thresholds were assessed using one-way ANOVA and Tukey-Kramer LSD *post hoc* test, while intra-group comparisons were made using the t test for repeated measurements. Results of the FST were statistically assessed by one-way ANOVA test with Tukey-Kramer *post hoc* testing. Pearson coefficient was calculated to determine the association between pain threshold and floating time in rats with CP/CPPS.

RESULTS

Effect of CORM-A 1 on Scrotal Pain Threshold

Scrotal pain thresholds of all groups (Sham-PBS, Sham-CORM, CP/CPPS-PBS and CP/CPPS-CORM) were measured both two days (-2nd) before and seven days (7th) after surgery. There was no significant difference between the groups at baseline ($F_{(3,20)}$=0.27, p=0.84, Fig. 2). In the Sham groups (PBS and CORM), the pain threshold did not change before and after surgery (t(5)=0.94, p=0.39 and t(5)=0.61, p=0.56, respectively). On the contrary, in the CP/CPPS groups (with 3% λ-carrageenan injection), the pain threshold decreased significantly on the 7th day after surgery (for CP/CPPS-PBS -2nd *vs.* 7th day: t(5)=8.84, p<0.001 and for CP/CPPS-CORM -2nd *vs.* 7th day: t(5)=4.48, p=0.003). When the CP/CPPS groups were compared within themselves at 7th day, CORM-A1 administration significantly increased the pain threshold in the CP/CPPS-CORM group and provided lower pain levels than the CP/CPPS-PBS group ($F_{(3,20)}$=109.3, p<0.001, Fig. 2). This indicates that CORM-A1 has an analgesic effect. On the other hand, in the Sham groups, CORM-A1 administration did not cause a significant change in pain threshold (p=0.91, Fig. 2).

Effect of CORM-A 1 on Depression-Related Behavior

During the FST conducted on the 7th day after surgery, the distribution of three types of behaviors was observed: struggling, swimming and floating. Animals in both Sham groups (Sham-PBS and Sham-CORM) spent significantly longer time struggling compared to time during swimming and floating ($F_{(2,15)}$=258.67, p<0.001 for Sham-PBS and $F_{(2,15)}$=287.12, p<0.001 for Sham-CORM, Fig. 3). On the other hand, animals in both CPPS groups (CP/CPPS-PBS and CP/CPPS-CORM) spent significantly more time floating compared to swimming and strug-

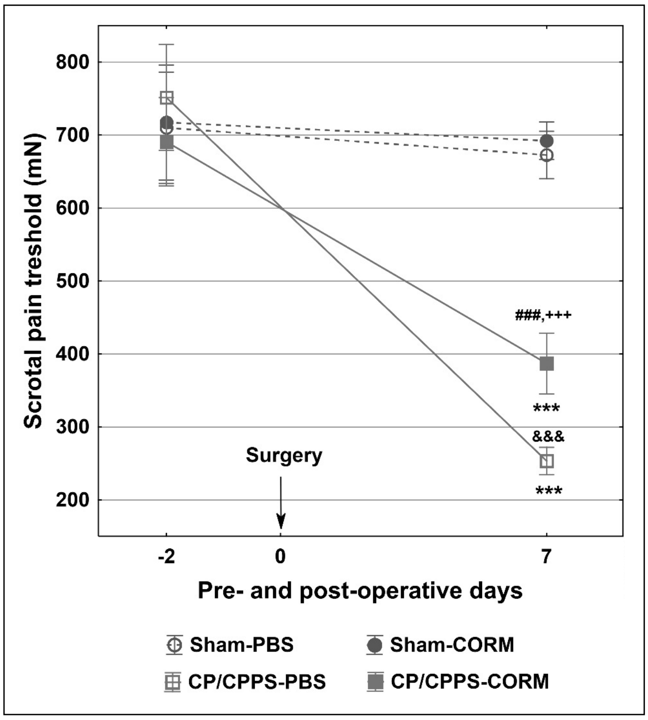

Fig. 2. Effect of CORM-A1 on scrotal pain threshold in animals with CP/CPPS. Animals underwent scrotal pain threshold testing with an eVF esthesiometer two days before and seven days after surgery. Groups were defined as follow (n=6 per group): Sham-PBS (intraprostatic 0.9% NaCl injection and daily postoperative PBS administration, 0.1 mL/kg); Sham-CORM (intraprostatic 0.9% NaCl injection and daily postoperative CORM-A1 administration, 2 mg/kg); CP/CPPS-PBS (intraprostatic 3% λ-carrageenan injection and daily postoperative PBS administration, 0.1 mL/kg); CP/CPPS-CORM (intraprostatic 3% λ-carrageenan injection and daily postoperative CORM-A1 administration, 2 mg/kg). Data are presented as mean ± standard deviation. Differences between groups were assessed using one-way ANOVA and Tukey-Kramer LSD *post hoc* test (&&&p<0.001 *vs.* Sham-PBS; +++p<0.001 *vs.* Sham-CORM; ###p<0.001 *vs.* CP/CPPS-PBS). Intra-group comparisons were made using the t test for repeated measurements (***p<0.001, *vs.* -1).

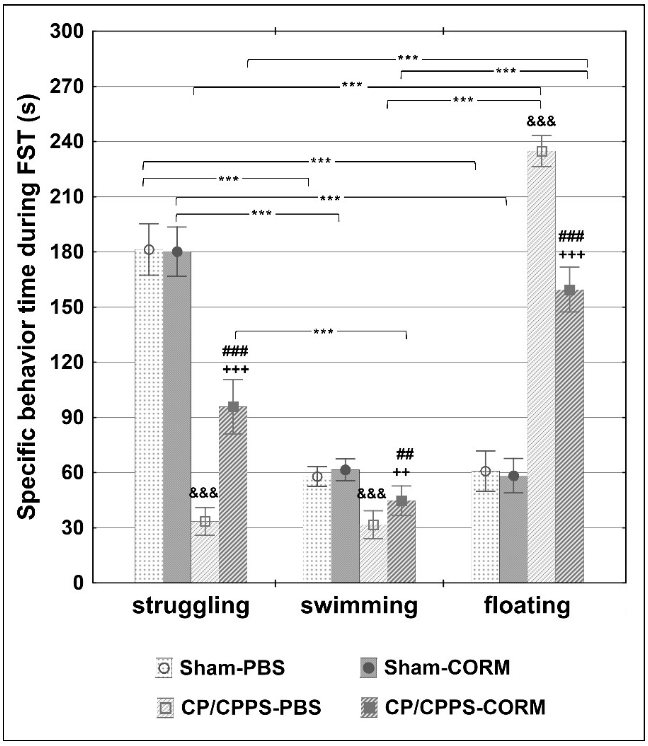

Fig. 3. Effect of CORM-A1 on the distribution and duration of struggling, swimming and floating during the forced swim test in animals with CP/CPPS. Depression-related behaviors were assessed using the forced swimming test on the 7th day upon surgery and model induction. Groups were defined as follow (n=6 per group): Sham-PBS (intraprostatic 0.9% NaCl injection and daily postoperative PBS administration, 0.1 mL/kg); Sham-CORM (intraprostatic 0.9% NaCl injection and daily postoperative CORM-A1 administration, 2 mg/kg); CP/CPPS-PBS (intraprostatic 3% λ-carrageenan injection and daily postoperative PBS administration, 0.1 mL/kg); CP/CPPS-CORM (intraprostatic 3% λ-carrageenan injection and daily postoperative CORM-A1 administration, 2 mg/kg). Values are expressed as mean ± standard deviation. Within-group differences in the distribution of struggling, swimming and floating were assessed using a one-way ANOVA test with Tukey-Kramer *post hoc* testing (***p<0.001). Statistical significance of differences in the distribution of struggling, swimming and floating between groups was determined using the same test (&&&p<0.001 *vs.* Sham-PBS; ++p<0.01, +++p<0.001 *vs.* Sham-CORM; ##p<0.01, ###p<0.001 *vs.* CPPS-PBS).

gling ($F_{(2,15)}$=1320.10, p<0.001 for CP/CPPS-PBS and $F_{(2,15)}$=137.67, p<0.001 for CP/CPPS-CORM, Fig. 3). Notably, CORM-A1 treatment led to a significant increase in struggling time ($F_{(3,20)}$=188.93, p<0.001) and swimming time ($F_{(3,20)}$=23.83, p<0.001) in the CP/CPPS-CORM group compared to respected control. Moreover, CORM-A1 treatment significantly decreased floating time in the CP/CPPS-CORM group (p<0.001) compared to the CP/CPPS-PBS group ($F_{(3,20)}$=407.20, p<0.001, Fig. 3).

Pain and Depression Correlation in CP/CPPS

Correlation analysis between scrotal pain threshold and floating time in the FST in rats with CP/CPPS showed a significant negative correlation between these indicators of pain perception and anxiety, respectively (Fig. 4). This was evident among both CP/CPPS-PBS group and CP/CPPS-CORM-A1 group.

DISCUSSION

Having in mind mechanisms, symptoms and comorbidities CP/CPPS is clearly a multidisciplinary disorder that is result of complex interactions between chronic inflammation on the periphery and the central nervous system. While current treatment approaches generally focus on relieving symptoms, the studies for novel

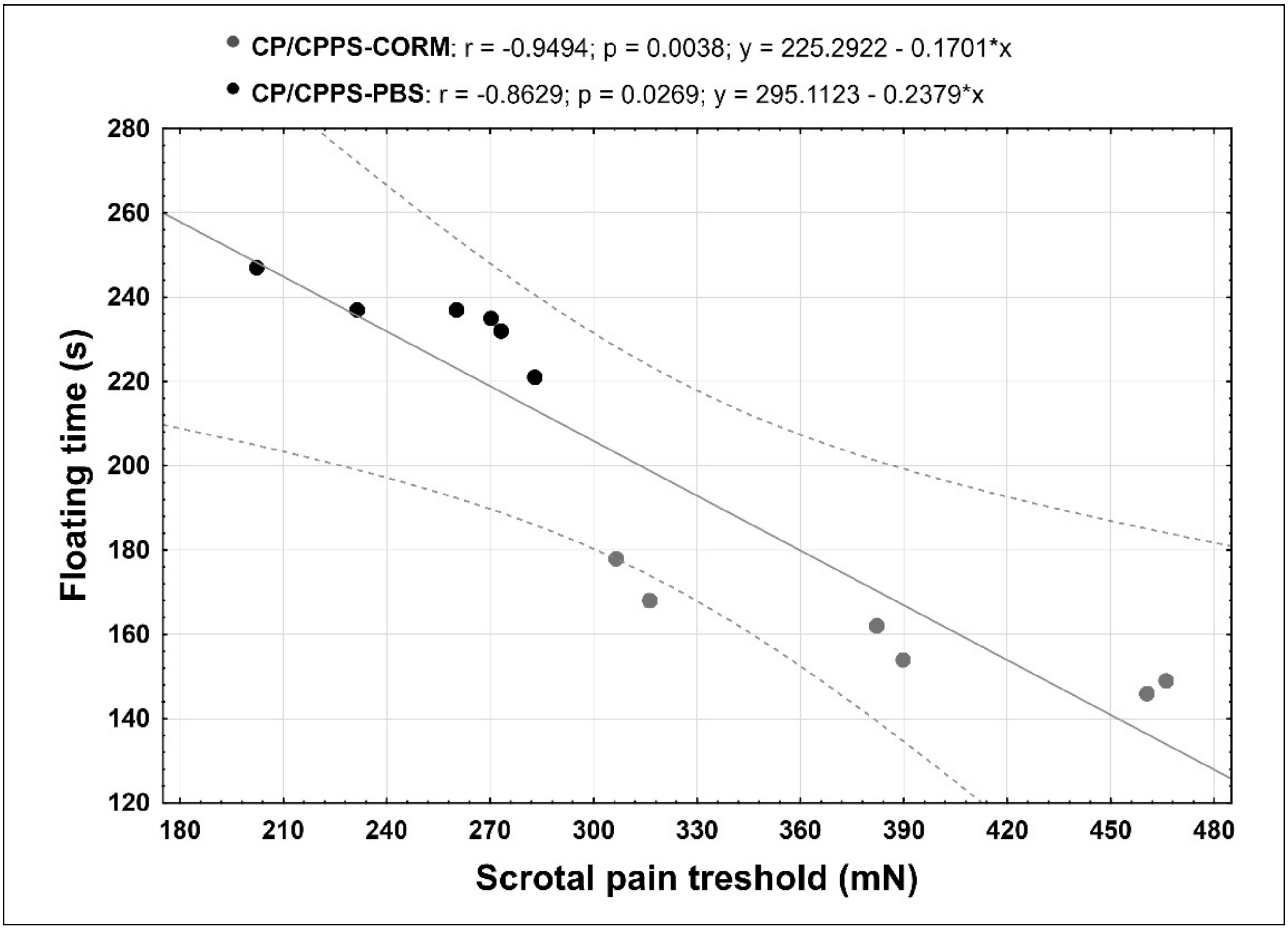

Fig. 4. Correlation between scrotal pain threshold and floating time in the FST in rats with CP/CPPS. A significant negative correlation was observed between scrotal pain threshold and floating time in both CP/CPPS-PBS and CP/CPPS-CORM groups, indicating that lower pain thresholds are associated with increased anxiety-like behavior. Pearson coefficient was calculated to determine the association between pain threshold and floating time in rats with CP/CPPS (CP/CPPS-PBS - intraprostatic 3% λ-carrageenan injection and daily postoperative PBS administration, 0.1 mL/kg) and CP/CPPS-CORM - intraprostatic 3% λ-carrageenan injection and daily postoperative CORM-A1 administration, 2 mg/kg).

therapeutic strategies that simultaneously target both peripheral and neuropsychiatric components of the disease is still ongoing.

Measurements made with the eVF test in our study revealed that the pain thresholds of the animals in the CP/CPPS model were significantly reduced to mechanical stimuli, thus developing hypersensitivity to pain. However, in rats treated with CORM-A1 in the CP/CPPS model (CP/CPPS-CORM group), the mechanical pain threshold in the scrotal region was significantly increased compared to the CP/CPPS-PBS group on the 7^{th} day.

The FST is considered an important tool for examining antidepressants efficacy, especially in preclinical studies for the evaluation of behavioral hopelessness, which is an indicator of depressive behaviors (Wang et al., 2022). Active swimming or escape attempts by the animal are generally considered to be indicative of motivation and a hopeful mood (Yankelevitch-Yahav et al., 2015). In the FST test, the primary indicator of depression-like behavior is the period in which the animal remains motionless, i.e., floating time. This motionlessness suggests that the animal has given up its attempts to escape and has entered a state of despair. In our current study, rats from Sham groups (Sham-PBS and Sham-CORM) exhibited longer struggling behaviors compared to swimming and floating activities. This result shows that these groups tend to struggle more and spend less time on floating activities. On the contrary, it was observed that CP/CPPS and CP/CPPS-CORM groups exhibited different patterns in terms of swimming and struggling times compared to Sham groups, indicating a significant increase in floating activities of these groups. Also, floating times in CP/CPPS groups were significantly higher compared to floating times in corresponding Sham groups. These results, hence, validated the depression-related behaviors profile seen in CP/CPPS disorder reported in the literature (Šutulović et al., 2023). Contrary, in the CP/CPPS-CORM group, it was observed that CORM-A1 treatment caused a significant decrease in floating time and a significant increase in swimming time compared to the CP/CPPS-PBS group. These findings suggest that CORM-A1 treatment may influence behavioral patterns in the CP/CPPS model and that the behavioral balance between struggling, swimming and floating may be modulated.

These findings suggest that exogenous carbon monoxide (CORM-A1) used in our study has shown to be a promising agent offering dual effects in the treatment of CP/CPPS by suppressing hyperalgesia (reducing effect on CP/CPPS-induced mechanical pain hypersensitivity) and significantly improving depression-like behaviors as its major brain-related comorbidity.

Many studies have reported a strong and reciprocal relationship between pain, pain catastrophizing thoughts, and depression in CP/CPPS patients (Tripp et al., 2004; Nickel et al., 2008; Huang et al., 2020). The "pain catastrophizing" way of thinking stands out as a critical factor in the maintenance of chronic pain and plays a critical role in shaping the clinical features of CP/CPPS (Shoskes et al., 2010; Keogh et al., 2010; Krsmanovic et al., 2014). This mental process includes cognitive distortions such as constantly replaying experienced pain events in the mind, perceiving pain as more severe than it actually is, and hopelessness about impending pain (Sullivan et al., 2001). Tripp et al. (2006) also demonstrated that such thoughts both increase pain intensity and significantly reduce patients' quality of life. Psychological stress levels in CP/CPPS patients positively correlated with the severity of symptoms (Bai et al., 2022). Also, age, urinary retention and brain-related comorbidities were associated with pain severity in CP/CPPS patients (Chen et al., 2020). Large cohort study showed that 5.34% of individuals diagnosed with CP/CPPS were diagnosed with depression during the three-year follow-up, while this rate was reported as 3.24% in the control group (Xia et al., 2011). Our previous study has shown that CP/CPPS can cause depression-like behaviors and learning-memory impairments in rat models. These effects were related to be associated with neuroinflammatory processes such as decreased hippocampal neurogenesis and astrocyte activation (Šutulović et al., 2023).

CP/CPPS has effects not only at the level of urinary system, but also at the central nervous system level (Zhang et al., 2025) and it could explain the coexistence of hyperalgesia and depression-like behavior even in herein used experimental model of CP/CPPS. Namely, results of our correlation analysis showed that the level of hyperalgesia correlated with level of depressive-like behavior in CP/CPPS groups, i.e., lower threshold correlated with higher floating time. CORM-A1 treatment exhibited dual effect increased pain threshold and decreased floating time in CP/CPPS animals compared to those treated with vehicle (PBS). Thus, CORM-A1 as exogenous CO donor could potentially disrupt the vicious cycle between pain and depression in this syndrome. Our results agree with recent study by Ge et al. (2025) who demonstrated significant functional changes in brain regions related to pain perception and emotional regulation in CP/CPPS patients. A number of studies have shown that CO has therapeutic potential in various neurological disorders such as spinal cord injury, Alzheimer's, Parkinson's diseases, and cerebral ischemia (Morroni et al., 2018; Zheng et al., 2019) *via* improving neuronal differentiation, prevent neuronal apoptosis, and stimulate endogenous neurogenesis (Choi et al., 2016; Almeida et al., 2016; Dreyer-Andersen et al., 2018). Moreover, studies in animal models using CORMs that carry controlled amounts of CO reveal that they regulate mitochondrial function, exhibit anti-inflammatory and antioxidant effects, and protect against neurodegeneration (Qiao et al., 2017; Wilson et al., 2017; Motterlini & Foresti, 2017).

Our findings are important for identifying novel pharmacological targets in CP/CPPS, having in mind that many studies have shown that traditional urological treatments do not sufficiently alleviate CP/CPPS symptoms (Anothaisintawee, et al., 2011; Polackwich & Shoskes, 2016; Anderson et al., 2018; Qin et al., 2024).

Exogenous CO has several neuroprotective effects *via* its involvement in redox status homeostasis and neurotransmitters balance. Some of these mechanisms could potentially explain herein observed antidepressant effects of CORM-A1. Increased ROS production and weakening of antioxidant defenses stand out as important mechanisms in development of CP/CPPS symptoms (Zhu et al., 2025). On the contrary, CORMs molecules reduce oxidative stress by effectively breaking down ROS and scavenging free radicals (Parfenova et al., 2018; Yuan et al., 2021). Also, the inducible HO-1 isoform responds to oxidative stress and inflammatory stimuli (Wu & Hsieh, 2022). CORM-3's suppression of IL-1β-induced neuroinflammation by increasing HO-1 expression stands out as an important mechanism in alleviating symptoms by contributing to the control of inflammatory processes (Lin et al., 2017). Dopaminergic system plays a role in the pain processing in chronic pain syndromes and the regulation of accompanying depressive and anxious symptoms (Zhao et al., 2022). Exogenous CO is known to increase extracellular dopamine levels and suppress dopamine reuptake (Taskiran et al., 2003). All these mechanisms mentioned herein are hypothetically responsible for the observed effects of CORM-A1 demonstrated in our current study. To elucidate more clearly related mechanisms, further studies are need.

Low-dose CO has been reported to have neuroprotective effects in Parkinson's disease, where the dopaminergic system is impaired (Gomperts et al., 2023). On the other hand, it has been demonstrated that dopamine stimulates HO-1 expression, and that removal of endogenous CO leads to changes in dopamine release (Schmidt et al., 1999; Ueno et al., 2020). These findings indicate that the HO/CO system may play a regulatory role in neuropsychiatric symptoms. Especillay, the fact that CO influences dopamine levels and circadian rhythms suggests the possible contribution of the dopamine/HO/CO axis in these processes (Bauer et al., 2024). Having this in mind, it can be suggested that the HO-1/CO system has therapeutic potential in reducing both pain and accompanying depression in CP/CPPS patients. The reduction of depression-related behaviors in the CP/CPPS CORM group in our current study corroborated these findings. The neuromodulator effects of CO may contribute to the alleviation of mental disorders associated with CP/CPPS by supporting dopamine balance at both peripheral and central levels.

Our current study also has some limitations. Dose-response studies and inactive CORM (iCORM) controls would provide additional mechanistic insights. CORM-A1 was originally characterized as a pharmacologically active CO-releasing molecule with effective *in vivo* activity (Motterlini et al., 2005). The 2 mg/kg dose regimen used in our study is consistent with doses widely applied in rodent literature, like those demonstrating protective and modulatory effects in an experimental autoimmune uveoretinitis model in rats (Fagone et al., 2015). Comprehensive review of CO-releasing molecules confirms that ≈2 mg/kg lies within the broadly accepted rodent range for *in vivo* CORM-A1 administration, while emphasizing that CO release is condition-dependent and proper controls are necessary (Yang et al., 2021). Therefore, the chosen dose of 2 mg/kg is within the widely accepted range of effective doses in rodent studies. The exact mechanisms of herein observed CORM-A1 effects should be further explored, having in mind its possible metabolic effects (Bilska-Wilkosz et al., 2022).

CONCLUSION

Results of our study shows that CORM-A1 significantly reduces depression-like behavior and alleviates hyperalgesia. Slow-releasing CO donor, CORM-A1, should be considered a promising candidate for the alleviation and treatment of chronic prostatitis and its associated neuropsychiatric comorbidities.

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