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Common Trace Elements Alleviate Pain in an Experimental Mouse Model

Bogdan I. Tamba,[1,2]* Maria-Magdalena Leon,[1] and Tudor Petreus[3]

[1]Centre for the Study and Therapy of Pain, Gr. T. Popa University of Medicine and Pharmacy, Iasi, Romania
[2]A&B Pharm Corporation, Roman, Romania
[3]Department of Cell Biology, Gr. T. Popa University of Medicine and Pharmacy, Iasi, Romania

Trace elements represent a group of essential metals or metaloids necessary for life, present in minute amounts. Analgesic adjuvants can enhance the effect of other pain drugs or be used for pain control themselves. Previous studies on the effects of trace elements on nociception and their potential use as analgesic adjuvants have yielded conflicting results. In this study, we tested the hypothesis that three vital trace elements (Zn^{2+}, Mg^{2+}, Cu^{2+}) have direct antinociceptive effects. Groups of eight Swiss mice were intraperitoneally (i.p) injected with incremental concentrations of Zn^{2+} sulfate (0.5, 2.0 mg/kg), Zn^{2+} citrate (0.125, 0.5 mg/kg), Mg^{2+} chloride (37.5, 75, 150 mg/kg), Cu^{2+} chloride (0.5, 1.0, 2.0 mg/kg), and Cu^{2+} sulfate (0.5, 1.0 mg/kg) or saline (control). Evaluations were made by hot plate (HP) and tail flick (TF) tests for central antinociceptive effect, writhing test (WT) for visceral antinociceptive effect, and activity cage (AC) test for spontaneous behavior. Zn^{2+} induced pain inhibition in HP/TF tests (up to 17%) and WT (up to 25%), with no significant differences among the salts used. Mg^{2+} salts induced pain inhibition for all performed tests (up to 85% in WT). Cu^{2+} salts showed antinociceptive effects for HP/TF (up to 28.6%) and WT (57.28%). Only Mg^{2+} and Cu^{2+} salts have displayed significant effects in AC (Mg^{2+} anxiolytic/depressant effect; Cu^{2+} anxiolytic effect). We interpret these data to mean that all tested trace elements induced antinociceptive effects in central and visceral pain tests. Our data indicate the potential use of these cheap adjuvants in pain therapy.

Key words: zinc; magnesium; copper; pain; analgesic adjuvants

Trace elements represent a group of essential metals or metaloids necessary for life, present in minute amounts. Most of them (Fe, F, Mg, Si, Zn) are involved in various biochemical enzymatic and metabolic processes, and some have been investigated in relation to pain perception and control. Analgesic adjuvants are drugs not specifically designed for an analgesic effect but, when used, can enhance the effect of other analgesic drugs or improve pain control. Most popular analgesic adjuvants include antidepressants and anticonvulsants, whereas trace elements make the bottom of the list, despite being some of the most easily administered and cheapest available options.

Widespread and widely used in mammals, zinc (Zn^{2+}) is a virtually nontoxic trace element. Five to fifteen percent of the cerebral Zn^{2+} is found in the presynaptic vesicles of glutaminergic nerve terminations, having a possible role in synaptic transmission (Ketterman and Li, 2008; Kay and Toth, 2008; Pan et al., 2011). Mayer et al. (1989) showed that Zn^{2+} is released simultaneously with glutamate, exerting the effect of a noncompetitive N-methyl-D-aspartate (NMDA) antagonist, and Mayer and Vyklicky (1989) showed that Zn^{2+} does not prevent the NMDA binding site affinity or glycine binding on the NMDA receptor. Zn^{2+} also inhibits AMPA and intrathecal Zn^{2+} in mice and produces an antinociceptive effect but no change in the thermoalgezic tests (Bresink et al., 1996; Larson et al., 2000). Animal studies also showed that Zn^{2+} chelators induce hyperalgesia, that Zn^{2+} ions have antinociceptive roles in neuropathic pain, and that modulation of Zn^{2+} biological fraction induces analgesia (Liu et al., 1999; Larson et al., 2000; Rodriguez-Munoz et al., 2011). Zn^{2+} and Cu^{2+} also reduce pain and inflammation in patients (Honkanen et al., 1991; Lansdowne, 1996), and Kugelmas (2000) showed the utility of Zn^{2+} in muscle cramp pain.

Magnesium (Mg^{2+}), the fourth most abundant cation in the human body, is required for presynaptic delivery of acetylcholine and may induce similar effects with Ca^{2+} channel blockers. Mg^{2+} is able to block mechanic hyperalgesia in rats (Lee et al., 2011) and has an antinociceptive effect in neuropathic pain rat models

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*Correspondence to: Bogdan I. Tamba, Centre for the Study and Therapy of Pain, Gr. T. Popa University of Medicine and Pharmacy, Iasi, Romania 700115. E-mail: bitamba@mail.umfiasi.ro

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(Xiao and Bennett, 1994). Begon et al. (2002) proved that intraperitoneal Mg^{2+} has a partially or fully reversible effect on mechanical hyperalgesia in diabetic rats and increased levels of Mg^{2+} in the cerebrospinal fluid and other brain regions. Koning et al. (1998) explain Mg^{2+} effect by interaction with the NMDA-linked Na/Ca channel, although other authors showed an NMDA-independent antinociceptive effect (Poleszak et al., 2008; Nikolaev et al., 2012).

Magnesium's effects in postoperative analgesia or habitual cephalalgia were investigated, with positive results (Mauskop et al., 1994; Lee et al., 2012). However, whereas Begon et al. (2000) advocate the Mg^{2+} passage through the blood–brain barrier (BBB), Takano et al. (2002) explain the antihyperalgesic effect of intrathecally administered Mg^{2+} by its not passing the BBB. Brill et al. (2002) later confirmed, in another clinical study, the effectiveness of i.v. Mg^{2+} in neuropathic pain. Still the data are at best contradictory, insofar as a previous peripheral neuropathy clinical study by Felsby et al. (1995) yielded negative results.

Cu^{2+} is an essential trace element that may participate too as a signaling molecule in the nervous system. Extracellular ionic Cu^{2+} is a powerful inhibitor for K channels (Ma et al., 2008), whereas Cu^{2+} deficit decreases dopamine levels in rat brain (W.R. Yu et al., 2008). Cu^{2+}-based preparations alone proved to be as efficient as morphine or displayed an improved analgesic effect when combined with nonsteroidal anti-inflammatory drugs in rats, possibly through an activation of Cu^{2+}-dependent opioid receptors (Okuyama et al., 1987). Popa and Lerche (2006) showed that Cu^{2+} acts as an Na channel blocker but expressed doubts over a possible therapeutic use. Some attempts to make a Cu^{2+}-based acetylsalicylic acid yielded limited results (Li et al., 1990). Later, Guilarte and Chen (2007) showed that the Cu^{2+} is an NMDA inhibitor, and Jones et al. (2007) presented a possible Cu^{2+}–serotonin toxic interaction in neurodegenerative diseases. In 2008, Ma et al. proved that Cu^{2+} is a powerful bradykinin and K channel inhibitor, with both playing an important role in pain transmission, and F. Yu et al. (2008) and Tamba et al. (2008) argued for a Cu^{2+}/Zn^{2+} superoxide dismutase role, linked to neuropathic and inflammatory pain mechanisms. This article's purpose is to evaluate the antinociceptive effects of these three vital trace elements (Zn^{2+}, Mg^{2+}, Cu^{2+}) conditioned as various salt forms in different concentrations, in a mouse model.

MATERIALS AND METHODS

All animal experimental procedures employed in the present study were strictly in accordance with the European Community guidelines regarding ethics and were approved by our animal care and use committee. The animal breeding facility of the Central Drug Testing Laboratory, ''Gr. T. Popa'' University of Medicine and Pharmacy, supplied adult male Swiss mice with an average weight of 35 ± 2 g. The animals were housed in a temperature-controlled room (21°C ± 2°C) with a 12 hr/12 hr light/dark cycle, four mice per cage, and were allowed to acclimate for at least 24 hr before use, with free access to food and water (Zimmermann, 1983; Nolen, 2011). All reagents were purchased from Sigma Aldrich Gmbh and included Zn^{2+} sulfate, Zn^{2+} citrate, Mg^{2+} chloride, Cu^{2+} chloride, and Cu^{2+} sulfate. To eliminate possible interference from the sulfate group when tested, another salt of the same trace element was also investigated. Different groups of eight mice were intraperitoneally injected with one of the following formulations: 0.9% saline for the control group (0.3 ml), Zn^{2+} sulfate (0.5 and 2.0 mg/kg), Zn^{2+} citrate (0.125 and 0.5 mg/kg), Mg^{2+} chloride (37.5, 75, and 150 mg/kg), Cu^{2+} chloride (0.5, 1.0, and 2.0 mg/kg), and Cu^{2+} sulfate (0.5 and 1.0 mg/kg), all salts solved in 0.3 ml saline/mouse.

Nociception Studies

The antinociceptive effect of the tested substances was evaluated by hot plate test (HP test) and tail flick test (TF test), behavioral tests that quantify the thermal nociception. For the TF latency test, animals were placed inside restraining cages at least 5 min before the measurement. Constant heat intensity was applied to the dorsum of the lower one-third of the animal's tail, and, when the animal flicked its tail in response to the noxious thermal stimulus, both the heat source and the timer were stopped automatically. A maximum TF latency of 15 sec was permitted to minimize tissue damage to the mouse's tail. The test was performed at 15, 30, and 45 min and 1 hr after the administration of substances or saline (control).

The HP test was performed on mice using an Ugo Basile HP device. Temperature was set at 54°C ± 0.5°C. A chronometer measured the latency observed from the time when the mouse was placed on the heated surface until the first overt behavioral sign of nociception, such as 1) the mouse licking a hind paw, 2) vocalization, or 3) an escape response. Analgesic measurements were performed at baseline and 15, 30, and 45 min and 1 hr after administration of the drug or 0.9% saline (control group). A cutoff time of 30 sec was used for the HP test. Treatments that produced a significant increase in the nociceptive thresholds were considered to be antinociceptive (Mungiu et al., 2002).

The antinociceptive effect on visceral pain of the tested substances was also evaluated by a peripheral mechanism (writhing test to acetic acid). The abdominal stretch, or writhing assay, was performed by injecting 0.1 ml of 1.0% acetic acid intraperitoneally in manually restrained mice. Immediately after injection, animals were placed in a large glass cylinder. The number of abdominal stretches occurring in successive 5-min intervals was counted beginning 5 min after acetic acid, for a 30-min period after intraperitoneal injection of diluted acetic acid. The tested substances were administered 5 min prior to acetic acid intraperitoneal injection. Values are reported as the mean (±SEM) for each treatment, with groups composed of eight mice. Treatments that produced a significant decrease in the number of abdominal stretches were considered to be antinociceptive. The mice were kept under observation for 72 hr and then sacrificed.

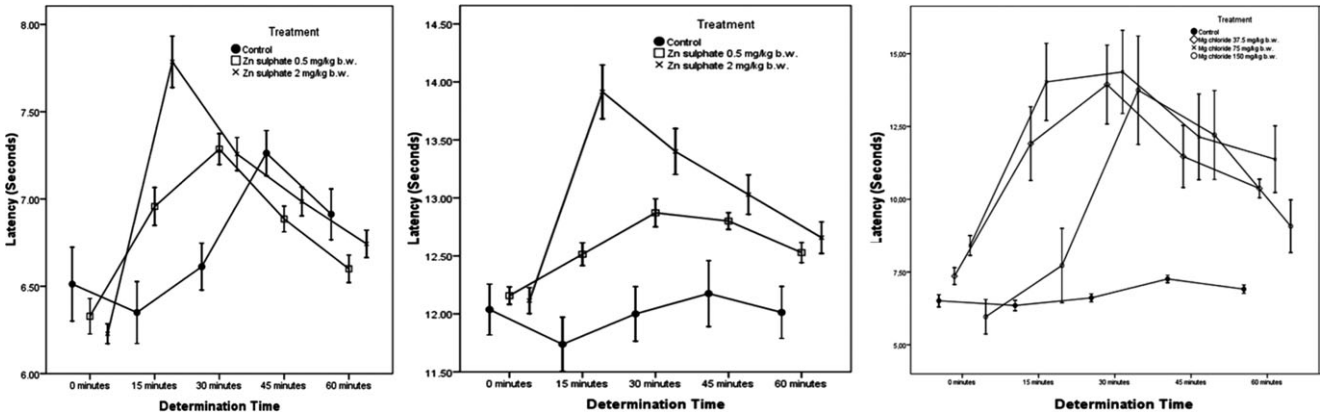

Fig. 1. **Left**: Latencies for TF test, after intraperitoneal administration of Zn^{2+} sulfate in different concentrations (0.5, 2.0 mg/kg). **Center**: Latencies for HP test, after intraperitoneal injection of Zn^{2+} sulfate in different concentrations (0.5, 2.0 mg/kg). **Right**: Latencies for TF test, after intraperitoneal injection of Mg^{2+} chloride at different concentrations (37.5, 75, 150 mg/kg).

Spontaneous behavior was evaluated by activity cage test. This test is performed to record spontaneous co-ordinated activity in individual mice and variation of this activity over time. Mice were allowed to acclimate to the testing room the night before testing. Animals were weighed and tested between 9:00 and 11:00 AM. Horizontal and vertical locomotor activity was monitored for 2 min with the Ugo Basile Activity Cage System. Horizontal and vertical activity was defined as the total number of beam interruptions throughout a 2-min observation period. The test was performed 15 min following the administration of the tested substances.

Statistical Analysis

SPSS 16.0 for Windows from the IBM SPSS Data Collection was used. Data are expressed as the mean ± SD for each measurement time. Differences between treatment groups were evaluated by one-way ANOVA for comparison at each time point, followed by Bonferroni post hoc tests. $P < 0.05$ was considered a significant difference for all tests. Pain inhibition with various stimuli (Wu et al., 2003) was also calculated according to the formula

$$\% \text{ Inhibition} = [(Tx - T0)/(Tm - T0)] \times 100,$$

where T0 is the latency before the drug administration (baseline), Tx is the latency for various consecutive time intervals, and Tm is the maximum time allowed (cutoff time) to avoid any possible lesions to the test animal.

RESULTS

Measurements performed during the TF test show that Zn^{2+} sulfate induces an increase in TF latency at 15 and 30 min from intraperitoneal injection, which indicated a significant antinociceptive effect. The TF latency increase and therefore the analgesic effect also seemed to be dose dependent at 15 min.

For the 0.5 mg/kg Zn^{2+} sulfate dose, the TF latency was significantly increased at 15 and 30 min, with a peak at 30 min (7.29 ± 0.23 sec). In the 45th and 60th minutes after the administration, TF latency dipped under the control values, but with no statistical significance. For a 2 mg/kg Zn^{2+} sulfate dose, TF latency showed the same profile (Fig. 1).

TABLE I. Average Pain Inhibition Values for Tested Substances in TF and HP Assays After Administration of Zn^{2+} Sulfate 0.5 and 2.0 mg/kg; Mg^{2+} Chloride 37.5 and 150 mg/kg; and Cu^{2+} Chloride 0.5, 1.0, and 2.0 mg/kg

Drug	0 Min	15 Min	30 Min	45 Min	60 Min
Zn^{2+} sulfate, 0.5, TF	0.00	7.26	11.03	6.40	3.11
Zn^{2+} sulfate, 0.5, HP	0.00	2.00	4.01	3.60	2.08
Zn^{2+} sulfate, 2.0, TF	0.00	17.79	11.74	8.63	5.85
Zn^{2+} sulfate, 2.0, HP	0.00	10.08	7.20	5.11	3.03
Mg^{2+} chloride, 37.5, TF	0.00	49.79	65.57	50.12	38.26
Mg^{2+} chloride, 37.5, HP	0.00	23.18	31.88	24.88	5.75
Mg^{2+} chloride, 75, TF	0.00	57.31	58.15	34.79	34.18
Mg^{2+} chloride, 75, HP	0.00	29.60	20.41	16.88	17.20
Mg^{2+} chloride, 150, TF	0.00	17.67	72.68	71.39	45.40
Mg^{2+} chloride, 150, HP	0.00	30.24	20.54	23.13	13.74
Cu^{2+} chloride, 0.5, TF	0.00	4.96	1.90	−0.81	−2.57
Cu^{2+} chloride, 0.5, HP	0.00	3.17	1.97	−0.01	−1.84
Cu^{2+} chloride, 1.0, TF	0.00	9.58	6.88	4.49	1.81
Cu^{2+} chloride, 1.0, HP	0.00	8.59	6.80	5.10	2.92
Cu^{2+} chloride, 2.0, TF	0.00	18.97	28.60	23.62	18.00
Cu^{2+} chloride, 2.0, HP	0.00	15.52	23.73	19.59	13.44

Pain inhibition calculations showed that Zn^{2+} sulfate induces a dose-dependent analgesic effect, lowering the TF pain by up to 17.78%. A dose of 0.5 mg/kg reduced the TF pain by 11.03% at 30 min, whereas the 2.0 mg/kg dose induces a maximal inhibition of 17.78% at 15 min. The maximum dose effect is recorded at 15 min after administration (Table I).

The HP test (HP) following intraperitoneal administration of Zn^{2+} sulfate showed increased and dose-dependent latencies at 15, 30, 45, and 60 min (Fig. 1) Statistically significant effects were recorded at 15 and 30 min for a 0.5 mg/kg dose, with a peak at 30 min (12.87 ± 0.32 sec); at 45 and 60 min, the

latency was increased but with no statistical significance. The same effect was recorded for all intervals for a 2.0 mg/kg dose, with a peak at 15 min (13.91 ± 0.62 sec).

HP latency conversion to a percentage of the maximum possible effect showed that Zn^{2+} sulfate induces an antinociceptive effect, reducing the pain by 10.08%. A dose of 0.5 mg/kg reduced the HP pain by 4% at 30 min, whereas the 2.0 mg/kg dose induces a maximal inhibition of 10.08% at 15 min. The maximum dose-dependent effect is recorded at 15 min from injection (Table I).

Acetic acid-induced writhing test results show that Zn^{2+} sulfate induced a statistically significant nociceptive effect, illustrated by the decrease of the total number of writhings at 10, 20, and 30 min for the 0.5 mg/kg dose and at 5, 10, 15, 20, and 25 min for the 2.0 mg/kg dose; the maximum inhibition for visceral pain was recorded at 10–15 min after Zn^{2+} sulfate administration. Our results showed that pain inhibition was more significant at the 2.0 mg/kg dose (25.71% at 10 min) than at the 0.5 mg/kg dose (17% at 10 min; Table II). The activity cage test after the administration a dose of 1.0 mg/kg showed no significant changes during evaluation of the spontaneous behavior (see Fig. 3). Similar data on all test evaluations were obtained for the Zn^{2+} citrate doses of 0.125 and 0.5 mg/kg, respectively (see Supp. Info.).

TABLE II. Average Pain Inhibition Values for Tested Substances in the Writhing Test After Administration of Zn^{2+} Sulfate 0.5 and 2.0 mg/kg; Mg^{2+} Chloride 150 mg/kg; and Cu^{2+} Chloride 0.5, 1.0, and 2.0 mg/kg

Drug	5 Min	10 Min	15 Min	20 Min	25 Min	30 Min
Zn sulfate 0.5 mg/b.w.	13.61	17.01	7.52	16.30	4.15	14.29
Zn sulfate 2 mg/b.w.	18.27	25.71	20.52	25.02	17.33	0.00
Mg chloride 150 mg/b.w.	86.03	83.75	84.71	89.54	86.82	85.71
Cu chloride 0.5 mg/b.w.	21.37	26.29	30.45	35.48	43.69	48.21
Cu chloride 1 mg/b.w.	22.92	40.80	39.62	41.58	43.69	46.43
Cu chloride 2 mg/b.w.	22.40	40.80	38.10	57.28	54.47	42.86

Measurements performed during the TF test show that Mg^{2+} chloride induced a significant TF latency at 15 and 30 min after intraperitoneal administration, indicating a significant antinociceptive effect. In the 45th and 60th minutes, TF latency values dropped under control values, with no statistical significance. TF latency seems to be dose dependent: for 37.5 and 75 mg/kg Mg^{2+} chloride doses, TF latency was significantly increased for all intervals, with a peak at 30 min (at 13.94 ± 4.29 sec). Increasing the dose to 150 mg/kg changed only the evolution of the antinociceptive effect but not its intensity (Fig. 1). After TF latency conversion to a percentage of the maximum possible effect, Mg^{2+} chloride induced a dose-dependent analgesic effect by lowering the TF pain by 72.68% (Table I).

The HP test (HP) after intraperitoneal administration of Mg^{2+} chloride showed an antinociceptive effect for all doses and all recorded intervals, but only some doses/intervals were statistically significant. The response intensity is not dose dependent. Significant latencies were recorded for the low dose (37.5 mg/kg) at 15 and 30 min (including a peak of 15.75 ± 5.32); for medium and high doses, the effect was faster and the peak latency recorded at 15 min was of 14.16 ± 6.50 for the 75 mg/kg dose and 15.03 ± 5.46 for the 150 mg/kg dose (Fig. 2).

HP latency conversion to a percentage of the maximum possible effect showed that Mg^{2+} chloride induced an antinociceptive effect, lowering the pain by 31.89%. The maximum dose-dependent effect was recorded at 15 min after administration of the low dose and at only 15 min for the medium and higher doses (Table I). Acetic acid-induced writhing test results showed that Mg^{2+} chloride induces a statistically significant nociceptive effect, by decreasing the total number of writhings at 15–20 min for the 150 mg/kg dose (Table II). The activity cage test with a dose of 150 mg/kg showed significant changes during evaluation of the spontaneous behavior, recording an important sedation effect together with an anxiolytic action (Fig. 3).

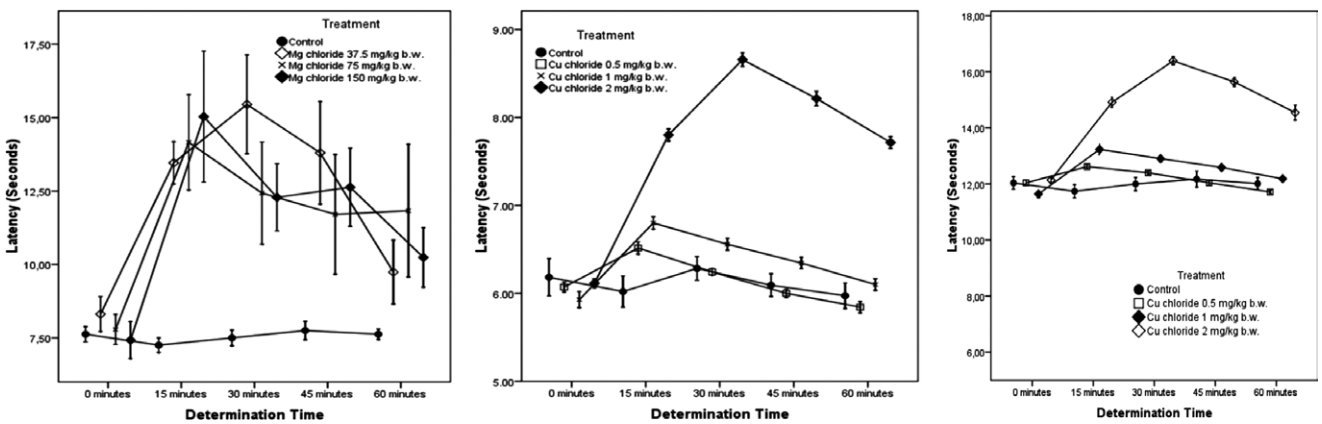

Fig. 2. **Left**: Latencies for HP test, after intraperitoneal injection of Mg^{2+} chloride at different concentrations (37.5, 75, 150 mg/kg). **Center**: Latencies for TF test, after intraperitoneal injection of Cu^{2+} chloride at different concentrations (0.5, 1.0, 2.0 mg/kg). **Right**: Latencies for HP test, after intraperitoneal administration of Cu^{2+} chloride at different concentrations (0.5, 1.0, 2.0 mg/kg).

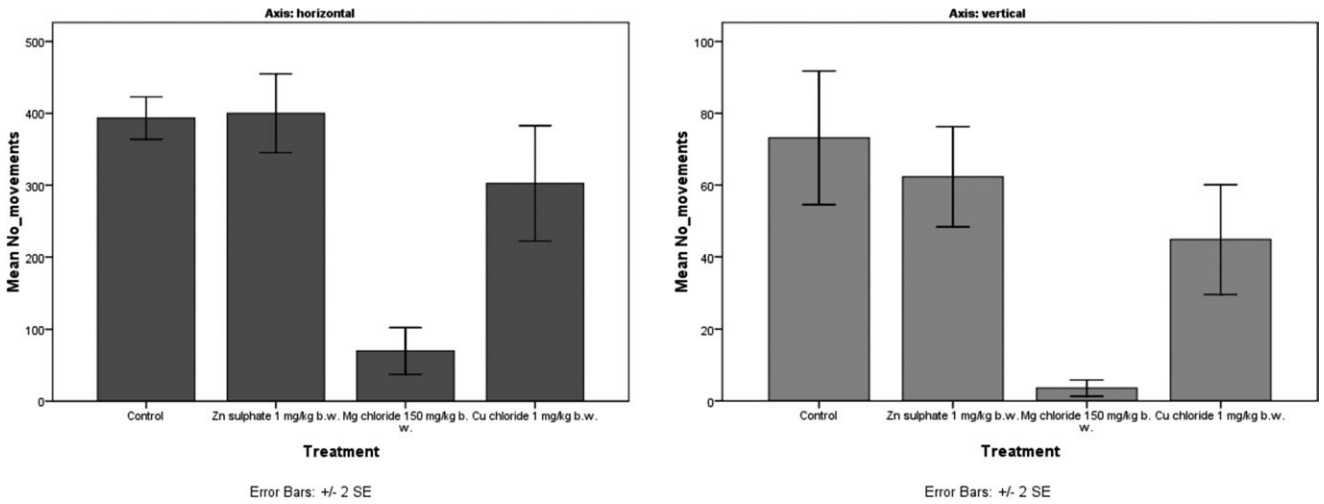

Fig. 3. Number of spontaneous movements per time interval in activity cage test, on both axes, after administration of 0.9% saline for the control group, Zn^{2+} sulfate 1 mg/kg, Mg^{2+} chloride 150 mg/kg, and Cu^{2+} chloride 1.0 mg/kg.

Measurements performed during the TF test showed that Cu^{2+} chloride induced a significant TF latency at 15, 30, 45, and 60 min after intraperitoneal administration, indicating a significant antinociceptive effect. TF latency seems to be significantly dose dependent only at 15 min for 0.5 mg/kg and 1 mg/kg Cu^{2+} chloride dose. The most important analgesic effect was recorded for the 2 mg/kg Cu^{2+} chloride dose, with a maximum effect at 30 min and a mean TF latency of 8.66 ± 0.21 (Fig. 2). After TF latency conversion to a percentage of the maximum possible effect, Cu^{2+} chloride induces a dose-dependent analgesic effect by lowering the TF pain by 28.60% (2 mg/kg dose, at 30 min; Table I).

The HP test (HP) following intraperitoneal administration of Cu^{2+} chloride showed antinociceptive effects for all doses and all recorded intervals, but only some doses/intervals were statistically significant. The response intensity is dose dependent. Significant latencies were recorded for the low dose (0.5 mg/kg) at 15 min (including a peak of 12.61 ± 0.19); at 30 min, the latency values dipped under control values. For the medium dose (1 mg/kg), significant latencies were recorded at 15 min (with a peak of 13.23 ± 0.51) and at 30 min. The higher dose (2 mg/kg) induced a maximum intensity effect at 30 min (with a peak of 16.39 ± 0.38; Fig. 2). HP latency conversion to a percentage of the maximum possible effect showed that Cu^{2+} chloride induces an antinociceptive effect, lowering the pain by 23.73% (Table I).

Acetic acid-induced writhing test results showed that Cu^{2+} chloride induced a statistically significant nociceptive effect, by decreasing the total number of writhings at 20–25 min for the 2 mg/kg dose (Table II). Activity cage test with a dose of 1 mg/kg Cu^{2+} chloride showed a slightly anxiolytic action (Fig. 3). Similar data for all test evaluations were obtained for the Cu^{2+} sulfate doses of 0.5 and 1.0 mg/kg (see Supp. Info.).

DISCUSSION

The antinociceptive effect of zinc in our evaluation processes was moderate, and we observed an inhibition of 4–17% in thermoalgesic tests and 17–25% in chemoalgesic tests. Our results showed no significant differences between the two Zn^{2+} salts used. Zn^{2+} sulfate, when explored by activity cage test, did now show any significant behavioral changes, but Zn^{2+}citrate had some positive results.

We have also shown that magnesium salts induced various degrees of antinociceptive action for all performed tests. In comparison with the HP test, which showed no dose-dependent effect (pain inhibition 30%), the TF test showed a pain inhibition of 58–72% at 30 min. The most suggestive values appear in the writhing test, in which the antinociceptive effect reaches 85%. Behavioral effects evaluated by activity cage test denote both a sedative and an anxiolytic action. Our results are consistent with previous studies (Begon et al., 2002), although the administration methods are different. Positive effects on the behavioral parameters argue for an Mg^{2+} chloride passage through the blood–brain barrier (BBB). There is a documented correlation between the antinociceptive and central nervous system-depressing actions (Fawcett et al., 1999; Durlach et al., 2000). From our results we conclude that Mg^{2+} chloride exerts an antinociceptive action at a dose of 150 mg/kg, with low results for TF test but with important effects on the HP test (inhibition values of 30% at 15 min, 56% at 30 min, and 22% at 60 min).

Our results regarding copper salts show potential antinociceptive effects during thermoalgesic tests and especially during chemoalgesic tests. It is difficult to explain the analgesic effect, although copper ion is involved in many molecular mechanisms of pain relief. Some research (W.R. Yu et al., 2008) has shown that

Cu^{2+} sulfate administration induces dopaminergic neuron lesions with antioxidant defense inhibition and apoptosis induction.

Zn^{2+} ion (in both salts used) showed moderate antinociceptive effects (4–25%), more evident on chemoalgesic test. Significant behavioral changes were recorded also following administration of Zn^{2+} sulfate and citrate. Magnesium (as chloride) induced an antinociceptive effect in analgesic tests (30–85%), with the highest values in writhing tests. It also induced sedative and anxiolytic actions. Copper chloride induced a dose-dependent antinociceptive effect (5% at minimal dose, 9.58% at medium dose, and 28% at maximal dose) in the TF test and of 3.17–23.7% in the HP test. Behavioral tests showed that Cu^{2+} chloride exerts an anxiolytic effect.

Zinc

Our research showed the Zn^{2+} effect in the periphery, where pain is transmitted by the A delta and C fibers. We hypothesize that, under our experimental conditions, Zn^{2+} influences glutamatergic receptors both at the peripheral and at the spinal levels (explaining why in most our experiments analgesia occurs after a latency of 10–15 min, the time required to cross the BBB and reach the synaptic space). How and where Zn^{2+} acts in the synaptic space is not precisely known, but several authors have provided additional info, although sometimes contradictory. Takeda et al. (2003) argue that zinc increases the presynaptic release of glutamate or GABA, with Smart et al. (2004) confirming these claims but advancing the hypothesis (not yet confirmed) that zinc acts as a direct neurotransmiter too, by postsynaptic signaling pathway modulation. Li and Clark (2000) presented further data showing a reduction of inflammatory pain (formalin model) from inhibition by zinc (zinc-protoporphyrin intrathecally) of hemoxygenase at the spinal level, but with no effect on peripheral thermal testing or hemoxygenase levels reported.

Another possible mechanism of action for zinc ions is interference with the potassium channels. Prost et al. (2004) showed that the zinc ion has the ability to open the K-ATP channel in pancreatic cells, acting on both sides of the membrane. Zinc also interferes with the nicotinic receptor ion channel, modulating nicotinic receptor function (Hsiao et al., 2008). On the other hand, in a recent study Taly et al. (2009) showed that Zn^{2+} acts as an alosteric modulator of the alpha–beta portion of the heteropentameric element (alpha 4–beta 2) of the nicotinic receptor. Of great importance to our study is that Taly et al. argue the modulator role of Zn^{2+} ions can both stimulate (alpha–alpha interface) and inhibit (beta–alpha interface) nicotinic izoreceptor alpha 4-beta 2 depending on the concentration of zinc used. This could, in our view, explain the sometimes similar and sometimes different results obtained by other authors regarding the involvement of zinc in pain, vs. our data (Hsiao et al., 2008; Taly et al., 2009). In fact, many authors consider the nicotinic receptor as a useful target for therapeutic agents in pain management (Meyer, 2006; Rowley et al., 2008). These hypothesis, however, require further exploration.

The Zn^{2+} sulfate dose that we used did not show significant changes in spontaneous behavior when assessed by the activity cage test. A literature review provided comparable data (Nozaki et al., 2011; Matsunami et al., 2011), except for Kroczka et al. (2001), who report an ''antidepressant-like'' effect in a forced swim test in rats, but without providing a dose that produced this effect or the zinc salt used.

Mg^{2+}

Our view is that one could explain the analgesic effect of Mg^{2+} by a peripheral mechanism, but the central behavior inhibitory actions of our tests lead to the conclusion that Mg^{2+} does cross the BBB. Hasanein et al. (2006) confirmed previous results regarding the antihyperalgesic effect of i.p. Mg^{2+} for mechanoalgezic tests in rats with diabetic neuropathy. However, the author proposes another mechanism for this effect: the normalization of glycemia rather than interference with the Na/Ca channel of the NMDA receptor or the lowering action on pronociceptive free radicals. As for the relationship between the antinociceptive effect and the CNS depressant activity, several authors have investigated this and confirmed it (Fawcett et al., 1999; Durlach et al., 2000). The current study showed a reduction in both horizontal and vertical mobility after the administration of Mg^{2+} chloride, in agreement with recent data obtained by Bindar et al. (2010).

Cu^{2+}

Our results show a potential analgesic effect of Cu^{2+} salts in both the thermoalgezic and the chemoalgezic tests. Although different from our experimental conditions, the literature data are consistent with our results. However, it seems difficult to explain clearly how Cu^{2+} ions induce analgesia. As with other bivalent trace elements, Cu^{2+} also is transported to specific molecules (Cu^{2+} [+]-ATPase) using guidance proteins (chaperone; Gonzales-Guerrero and Argüello, 2008). Cu^{2+} from the CNS cells is coupled in the enzymatic systems involved in the synthesis of catecholamines, opioid peptides, andf neuropeptides (Kim et al., 2008). A possible role of Cu^{2+} in analgesia was described by Jacka et al. (1983) from an inflammatory arthritis model in rats, which tested the antinociceptive effects of salicylate after Cu^{2+} pretreatment (mechanical pain tests). The data presented here and the literature data converge on Cu^{2+}'s certain involvement in analgesia, but unfortunately, because of various cellular and molecular actions, not always beneficial, high-selectivity pain drugs based on Cu^{2+} do not yet exist. In fact, we may never attain that, insofar as research by W.R. Yu et al. (2008) has shown that Cu^{2+} sulfate administration (striatal nuclei

injections in rats) damages the dopaminergic neurons, causing apoptosis.

We conclude that, after intraperitoneal injection, all tested trace elements induced various degrees of antinociceptive effects, demonstrated by thermoalgesic and chemoalgesic tests. These results continue our previous works (Tamba et al., 2008) and corroborate results from cited authors, supporting further efforts to identify molecular-level effects of these investigated salts and making trace elements an important nociception investigation target and possible inexpensive adjuvants for pain therapy (Gumilar et al., 2012).

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REFERENCES

Begon S, Pickering G, Eschalier A, Dubray C. 2002. Magnesium increases morphine analgesic effect in different experimental models of pain. Anesthesiology 96:627–632.

Bindar D., Tarţu L., Gărlea A., Niţă L., Merlimg V. 2010. Effects of Magnesiun Soft Matter Vesicles Carrier of the Behavioral Manifestations în Mice. Romanian. J Biophys 20:23–35.

Bresink I, Ebert B, Parsons CG, Mutschler E. 1996. Zinc changes AMPA receptor properties, results of binding studies and patch clamp recordings. Neuropharmacology 35:503–509.

Brill S, Sadgwick PM, Hamann W, di Vadi PP. 2002. Efficacy of intravenous magnesium in neuropathic pain. Br J Anaesth 89:711–714.

Durlach J, Bac P, Bara M, Guiet-Bara A. 2000. Physiopathology of symptomatic and latent forms of central nervous hyperexcitability due to magnesium deficiency, a current general scheme. Magnesium Res 13:293–302.

Fawcett WJ, Haxby EJ, Male DA. 1999. Magnesium, physiology and pharmacology. Br J Anaesth 83:302–320.

Felsby S, Nielsen J, Arendt-Nielsen L, Jensen TS. 1995. NMDA receptor blockade in chronic neuropathic pain, a comparison of ketamine and magnesium chloride. Pain 64:283–291.

Gonzales-Guerrero M, Argüello JM. 2008. Mechanism of Cu^+ transporting ATP-ases, soluble Cu^+ chaperones directly transfer Cu^+ to transmembrane transport sites. Proc Natl Acad Sci U S A 195:5992–5997.

Guilarte TR, Chen MK. 2007. Manganese inhibits NMDA receptor channel function, implications to psychiatric and cognitive effects. Neurotoxicology 28:1147–1152 [E-pub 26 June 2007].

Gumilar F, Agotegaray M, Bras C, Gandini NA, Minetti A, Quinzani O. 2012. Anti-nociceptive activity and toxicity evaluation of Cu(II)-fenoprofenate complexes in mice. Eur J Pharmacol 675:32–39.

Hasanein P, Parviz M, Keshavarz M, Javanmardi K, Mansoori M, Soltani N. 2006. Oral magnesium administration prevents thermal hyperalgesia induced by diabets in rats. Diabetes Res Clin Pract 75:17–22.

Honkanen V, Konttinen YT, Sorsa T, Hukkanen M, Kemppinen P, Santavirta S, Saari H, Westermarck T. 1991. Serum zinc, copper and selenium in rheumatoid arthritis. J Trace Elem Electrolytes Health Dis 5:261–263.

Hsiao B, Mihalak KB, Magleby KL, Luetje CW. 2008. Zinc potentiates neuronal nicotinic receptors by increasing burst duration. J Neurophysiol 99:999–1007.

Jacka T, et al. 1983. Copper salicylate as an anti-inflammatory and analgaesic agent in arthritic rats. Life Sci 32:1023.

Jones CE, Enderqood CK, Coulson EJ, Taylor PJ. 2007. Copper induced oxidation of serotonin, analysis of products and toxicity. J Neurochem 10:1035–1043.

Kay AR, Toth K. 2008. Is zinc a neuromodulator? Sci Signaling 1:re3.

Ketterman JK, Li YV. 2008. Presynaptic evidence for zinc release at the mossy fiber synapse of rat hippocampus. J Neurosci Res 86:422–434.

Kim BE, Nevitt T, Thiele DJ. 2008. Mechanism for copper acquisition, distribution and regulation. Nat Chem Biol 4:176–185.

Konig H, Wallner T, Marhofer P, Andel H, Horauf K, Mayer N. 1998. Magnesium sulfate reduses intra- and postoperative analgesic requirements. Anesth Analg 87:206–210.

Kroczka B, Branski P, Palucha A, Pilc A, Nowak G. 2001. Antidepressant-like properties of zinc in rodent forced swim test. Brain Res Bull 55:297–300.

Kugelmas M. 2000. Preliminary observation: oral zinc sulfate replacement is effective in treating muscle cramps in cirrhotic patients. J Am Coll Nutr 19:13–15.

Lansdown AB. 1996. Zinc in the healing wound. Lancet 347:706.

Larson AA, Giovengo SL, Shi Q, Velazquez RA, Kovacs KJ. 2000. Zinc in the extracellular area of the central nervous system is necessary for the development of kainic acid-induced persistent hyperalgesia in mice. Pain 86:177–184.

Lee AR, Yi HW, Chung IS, Ko JS, Ahn HJ, Gwak MS, Choi SJ. 2012. Magnesium added to bupivacaine prolongs the duration of analgesia after interscalene nerve block. Can J Anaesth 59:21–27.

Lee C, Song YK, Jeong HM, Park SN. 2011. The effects of magnesium sulfate infiltration on perioperative opioid consumption and opioid-induced hyperalgesia in patients undergoing robot-assisted laparoscopic prostatectomy with remifentanil-based anesthesia. Kor J Anesthesiol 61:244–250.

Li X, Clark JD. 2000. The role of heme oxigenase in neuropathic and incisional pain. Anesth Analg 90:677–682.

Li Y, Che J, Bao F. 1990. Effects of kuo-guan granule on plasma zinc, copper and erythrocyte GSH-Px (glutathione peroxidase) in patients with angina pectoris. Zhong Xi Yi Jie He Za Zhi 10:348–350.

Liu T, Walker JS, Tracey DJ. 1999. Zinc alleviates thermal hyperalgesia due to partial nerve injury. Neuroreport 10,1619–1623.

Ma Z, Wong KY, Horrigan FT. 2008. An extracellular Cu^{2+} binding site in the voltage sensor of BK and Shaker potassium channels. J Gen Physiol 131:483–502.

Matsunami M, Kirishi S, Okui T, Kawabata A. 2011. Chelating luminal zinc mimics hydrogen sulfide-evoked colonic pain in mice, possible involvement of T-type calcium channels. Neuroscience 181:257–264.

Mauskop A, Altura BT, Cracco RQ, Altura BM. 1994. Chronic daily headache—one disease or two? Diagnostic role of serum ionized magnesium. Int J Headache 14:24–28.

Mayer ML, Vyklicky L Jr. 1989. The action of zinc on synaptic transmission and neuronal excitability in cultures of mouse hippocampus. J Physiol 415:351–365.

Meyer MD. 2006. Neuronal nicotinic acetylcholine receptors as a target for the treatment of neuropathic pain. Drug Dev Res 67:355–359.

Mungiu OC, Ionescu DG, Jaba IM. 2002. Zinc and nociception, a pharmacological approach. Rev Med Chir Soc Med Nat Iasi 106:10–13.

Nikolaev MV, Magazanik LG, Tikhonov DB. 2012. Influence of external magnesium ions on the NMDA receptor channel block by different types of organic cations. Neuropharmacology 62:2078–2085.

Nolen RS. 2011. AVMA board approves panel on Euthanasia Report, updated guidelines cover more species and methods. J Am Vet Med Assoc 239:1269.

Nozaki C, Vergnano AM, Filliol D, Ouagazzal AM, Le Goff A, Carvalho S, Kieffer BL. 2011. Zinc alleviates pain through high-affinity binding to the NMDA receptor NR2A subunit. Nat Neurosci 14:1017–1022.

Okuyama S, Hashimoto S, Aihara H, Willingham WM, Sorenson JR. 1997. Copper complexes of non-steroidal antiinflammatory agents, an-

algesic activity and possible opioid receptor activation. Agents Actions 21:130–144.

Pan E, Zhang XA, Huang Z, Krezel A, Zhao M, Tinberg CE, McNamara JO. 2011. Vesicular zinc promotes presynaptic and inhibits postsynaptic long-term potentiation of mossy fiber-CA3 synapse. Neuron 71:1116–1126.

Poleszak E, Wlaz P, Wrobel A, Fidecka S, Nowak G. 2008. NMDA/glutamate mechanism of magnesium-induced anxiolytic-like behavior in mice. Pharmacol Rep 60:655–663.

Popa M, Lerche H. 2006. Cu^{2+}(1,10 phenantroline)3 is an open-channel blocker of the human skeletal muscle sodium channel. Br J Pharmacol 14:808–814.

Prost AL, Bloc A, Hussy N, Derand R, Vivadiu M. 2004. Zinc is both on intracellular and extracellular regulator of K^+-APT channel function. Retrieved fromhttp://jp.physoc.org/content/559/1/157.long.

Rodriguez-Munoz M, de la Torre-Madrid E, Sanchez-Blazquez P, Garzon J. 2011. NO-released zinc supports the simultaneous binding of Raf-1 and PKC gamma cysteine-rich domains to HINT1 protein at the mu-opioid receptor. Antiox Redox Signal 14:2413–2425.

Rowley TJ, Payappily J, Lu J, Flood P. 2008. The antinociceptive response to nicotinic agonists in a mouse model of postoperative pain. Anesth Analg 107:1952–1957.

Smart TG, Hosie AM, Miller PS. 2004. Zn^{2+} ions, modulatory of excitatory and inhibitory synaptic activity. Neuroscientist 10:432–442.

Takano Y, Soto E, Kaneko T, Sato I. 2002. Antihiperalgesic effects of intrathecally administred magnesium sulfate in rats. Pain 84:175–179.

Takeda A, Minumi A, Seki Y, Oku N. 2003. Differential effects of zinc on glutamatergic and GABA-ergic neurotransmiter systems in the hippocampus. J Neurosci Res 75:225–229.

Taly A, Corringer PJ, Guedin D, Lestage P, Changeux JP. 2009. Nicotinic receptors, allosteric transitions and therapeutic targets in the nervous system. Nat Rev Drug Discov 8:733–750.

Tamba B, Jaba IM, Şerban DN, Bohotin C, Mungiu OC. 2008. The influence of systematically administered divalent trace elements on the nociceptive process—new experimental data. J Clin Pharmacol 48:1135.

Xiao WH, Bennett GJ. 1994. Magnesium suppresses neuropathic pain responses in rats via a spinal site of action. Brain Res 666:168–172.

Yu F, Kamada H, Niizuma K, Endo H, Chan PH. 2008. Induction of mmp-9 expression and endothelial injury by oxidative stress after spinal cord injury. J Neurotrauma 25:184–195.

Yu WR, Jiang H, Wang J, Xie JX. 2008. Copper (Cu^{2+}) induces degeneration of dopaminergic neurons in the nigrostriatal system of rats. Neurosci Bull 24:73–78.

Wu T, Tian-Shan W, Fang-Zhou Y, Bao-Chang C. 2003. Analgesic and anti-inflammatory properţies of brucine and brucine-N-oxide extracted from seeds of Strychnos nuxvomica. J Ethnopharmacol 88:205–214.

Zimmermann M. 1983. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 16:109–110.