

EXPERIMENTAL RESEARCH SHOWING THE BENEFICIAL EFFECT OF ORAL ZINC ADMINISTRATION IN OPIOID TOLERANCE

DIANA CIUBOTARIU¹, CRISTINA MIHAELA GHICIUC¹, RAOUL VASILE LUPUȘORU^{2*}, NELA BIBIRE³, LUMINIȚA AGOROEI⁴, CĂTĂLINA ELENA LUPUȘORU¹

“Grigore T. Popa” University of Medicine and Pharmacy, 16 Universității Street, code 700115, Iași, Romania

¹Faculty of Medicine, Department of Pharmacology

²Faculty of Medicine, Department of Pathophysiology

³Faculty of Pharmacy, Department of Analytical Chemistry

⁴Faculty of Pharmacy, Department of Toxicology

*corresponding author: rvlupusoru@yahoo.com

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Abstract

There are studies showing that zinc attenuates opioid-dependence. The aim of the present study was to assess the effect of oral zinc sulphate on opioid analgesia in tolerant rats. Analgesia was assessed in 3 rat groups: non-dependent (group A) and dependent (groups B and C), each with 3 subgroups. Dependence was induced with morphine in increasing doses (B: 5-day schedule, C: 10-day schedule; from 5 mg/kg bw - day 1 to 40 mg/kg bw - day 5, respectively 90 mg/kg bw - day 10). Subgroups 1 received saline, subgroups 2 and 3 received ZnSO₄, 14 days, 2 mg, respectively 4 mg/kg bw/day, orally, before inducing dependence in groups B and C. Basal and exposure latencies were assessed by the tail-flick test, before and 30 min after morphine administration: 5 mg/kg bw (group A, at the end of zinc treatment); 10 mg and 15 mg/kg bw (groups B and C, at the end of dependence schedule). Morphine induced strong analgesia in naïve and tolerant rats. In subgroup 1, the exposure latency increased vs. basal with 41% in group A (from 10.2 ± 0.4 s to 14.5 ± 0.4 s; p < 0.001) and with 19%, respectively 15% in groups B and C, where analgesia was reduced vs. non-dependent rats. Zinc did not influence pain perception. Zinc supplementation in patients on opioids for pain is worth being evaluated.

Rezumat

Sunt studii care demonstrează că zincul ameliorează dependența la opiacee. Scopul acestui studiu a fost evaluarea efectului sulfatului de zinc (*per os*) asupra analgeziei la opiacee la șobolani cu toleranță indusă. Au fost luate în studiu 3 loturi de animale: non-dependente (lot A) și cu dependență indusă (lot B și C), fiecare cu câte 3 sub-grupuri. Dependența s-a indus cu morfină în doze crescânde (B: schemă de 5 zile, C: schemă de 10 zile; doze de la 5 mg/kgc - ziua 1 la 40 mg/kgc - ziua 5 și 90 mg/kgc - ziua 10). Sub-grupurile 1 au primit ser fiziologic, iar 2 și 3 au primit ZnSO₄, oral, 14 zile, 2 mg, respectiv 4 mg/kgc/zi, anterior dependenței în cazul loturilor B și C. Latențele bazale și post-expunere au fost evaluate cu testul *tail-flick*, înainte și la 30 min după administrarea morfinei: 5mg/kgc (lot A, la finalul tratamentului cu zinc); 10 mg și 15 mg/kgc (loturi B și C, la finalul schemei de dependență). Morfina a indus analgezie puternică la animalele naive și tolerante. În subgrupul 1, latențele post-expunere au crescut vs. bazal cu 41% la lotul A (de la 10,2 ± 0,4 s la 14,5 ± 0,4 s; p < 0,001), cu 19%, respectiv 15% la loturile B și C, unde analgezia a fost redusă vs. animale naive. Zincul nu a influențat percepția dureroasă. Suplimentarea cu zinc la pacienții tratați cu opiacee pentru durere ar merita evaluată.

Keywords: zinc, morphine dependence, tail-flick test, tolerant rats

Introduction

There are evidences showing the alleviation of opioid dependence intensity by zinc-administration prior to dependence development in rats [3] and that zinc chelators increase morphine-withdrawal jumpings (with no influence on dependence) in mice [13]. Zinc ions properties of reducing opioids action at the receptors level may be an explanation for these findings, as zinc was shown to lower the affinity of several ligands to receptors [26, 28] and the receptors' density [18].

Zinc is a trace element with important roles as a cofactor of enzymes involved in nucleic acids

replication, protein synthesis, and antioxidant defence [11, 16, 19]. Zinc deficiency is a predisposing factor for malignant tumours [5] and infections [14], DNA damage and increased inflammatory status [1]. Cancer-related chronic pain is usually treated with opioids. Considering that Zn²⁺ reduces morphine-dependence and Zn²⁺ chelators enhance withdrawal, it can be inferred that zinc supplementation may reduce the risk of addiction in patients with chronic pain treated with opioids. This idea is sustained by the low levels of zinc evidenced in opioid users [7, 22, 23] and by the low toxicity of zinc [24, 27]. On the other hand, Zn²⁺, by inhibiting the action of opioids at receptors level, may lower

their analgesic efficacy, even though in opioid-free conditions Zn^{2+} induces well-documented analgesia [9, 12, 15, 17].

In order to determine if dietary zinc supplementation is beneficial in such persons, more experimental and clinical researches are needed. In this context, the aim of the present study was to evaluate whether the effect of reducing morphine dependence intensity of zinc is or is not accompanied by a decrease in morphine-analgesic efficiency in tolerant animals.

Materials and Methods

Animals. Adult Wistar male rats, purchased from “Cantacuzino” Institute Bucharest, were maintained in standard laboratory conditions. Water and standardized food (pellets) were provided *ad libitum*. The experiment was approved and conducted following the recommendations of the ‘Gr. T. Popa’ University Committee for Research and Ethical Issues Guidelines according to the ethical standards of the European Community.

Substances. Morphine sulphate (Morfină®, Zentiva®, Romania, ampoules of 1 mL with 2% morphine) was obtained on the basis of an authorization for research. Zinc sulphate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) used for oral administration was prepared as a sodium chloride 0.9% solution with 20 mg $\text{ZnSO}_4/100$ mL.

Morphine-dependence induction. Dependence was induced on a classical model after Caillé, 1999 [3], injecting morphine subcutaneously (s.c.) in escalating doses (two equal daily injections, at 7⁰⁰ and 19⁰⁰). In schedule-1 (short-term, group B), doses increased from 5 mg/kg bw (day 1) to 10 mg/kg bw (day 2) and then with 10 mg/kg bw/day, up to 40 mg/kg bw on day 5. In schedule-2 (long-term, group C), doses increased from 5 mg/kg bw (day 1) to 10 mg/kg bw (day 2) and then with 10 mg/kg bw/day, up to 90 mg/kg bw on day 10.

Pain-sensibility testing. Morphine-analgesia was assessed with tail-flick analgesiometer (Harvard Apparatus Ltd., UK). Intensity was set to 70%. Latency time is displayed with a precision of a tenth of a second. Rats were gently held by hand and their tail positioned on the apparatus. An infrared heat stimulus is positioned below the tail on a point of 3 cm rostral to the tail-tip. The cut-off time was set at 20 seconds to minimize tissue damage [25]. Latencies were determined 30 minutes before (basal) and 30 minutes after morphine (exposure) administration. Results are presented both as raw data (basal and exposure latency, seconds) and as latency-percentage increase (exposure vs. basal) following morphine administration. The cut-off basal latency is not standardized in tolerant rats; therefore, it was not

possible to use the maximal possible effect (%MPE).

Groups. Three groups, each with three sub-groups of 10 rats were used. In group A, morphine analgesia was assessed in non-dependent (naïve) conditions. In groups B (schedule 1) and C (schedule 2), dependence was induced (see above the schedules). In each group, subgroup 1 (control) received saline solution, and subgroups 2 (Zn 2) and 3 (Zn 4) received ZnSO_4 , 14 days, 2 mg, respectively and 4 mg/kg bw/day, orally, prior to tail-flick testing (group A) and to dependence induction (groups B and C). These zinc doses were previously shown to reduce naloxone-precipitated withdrawal. Morphine doses for tail-flick were: 5 mg/kg (group A – after 14 days of zinc in subgroups 2 and 3); 10mg/kg and 15mg/kg (group B and C, on the next day after ending the dependence schedule). For groups B and C, tail-flick tests for assessing morphine-tolerance (phenomena associated to dependence, induced on the classical model) were performed after an original schedule.

Data analysis and statistical procedure. Data are reported as the means $\pm$ SD. The statistical analyses and graphics were performed using SigmaPlot 11 software (SxST.it, Milan, Italy). Comparisons between groups were performed using a one-way analysis of variance (ANOVA) followed by the Fisher least significant difference (LSD) method for post hoc multiple comparisons test to determine the differences between groups.

Results and Discussion

Morphine determined well-expressed analgesia in naïve and tolerant rats, latency times increasing, basal vs. exposure: $p < 0.001$. For example, in control sub-groups not receiving zinc, latency time increased following 5mg/kg bw morphine administration by 41% in non-dependent rats: from 10.2 ± 0.4 s (basal) to 14.5 ± 0.4 s (exposure) (Figure 1).

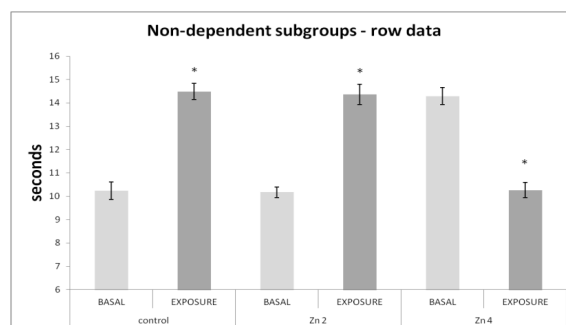


Figure 1.

Basal and exposure tail-flick times (seconds) in non-dependent rats following morphine (5 mg/kg bw), under normal (control) and zinc supplementation (Zn 2 and Zn 4) conditions. Statistical analysis: two-way ANOVA and a post hoc test: * $p < 0.001$ vs. basal.

In the 5 day-schedule dependence, 10 mg/kg bw of morphine determined a 20% increase: from 8.3 ± 0.2 s (basal) to 9.9 ± 0.4 s (exposure) (Figure 2).

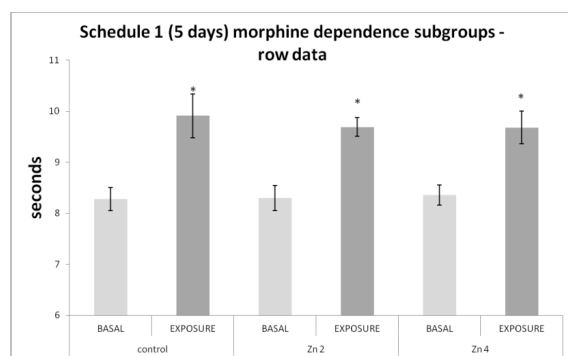


Figure 2.

Basal and exposure tail-flick times (seconds) in short-term schedule dependent rats, following morphine (10 mg/kg bw), under normal (control) and zinc supplementation (Zn 2 and Zn 4) conditions. Statistical analysis: two-way ANOVA and a post hoc test: * $p < 0.001$ vs. basal.

In the 10 day-schedule dependence, 15 mg/kg bw of morphine determined a 15% increase: from 7.6 ± 0.3 s (basal) to 8.8 ± 0.2 s (exposure) (Figure 3).

Analgesia was less evident in tolerant vs. naïve rats ($p < 0.001$) where both basal and exposure latency times were lower vs. non-dependent rats, in spite of using higher morphine doses in dependent groups.

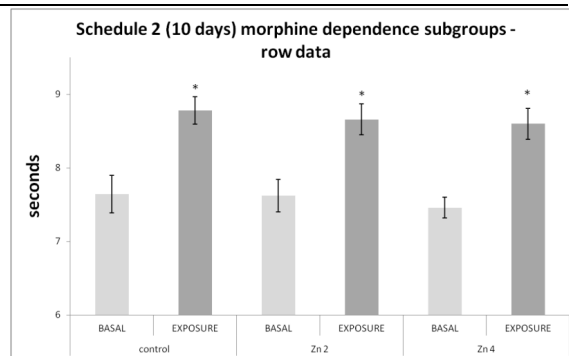


Figure 3.

Basal and exposure tail-flick times (seconds) in dependent rats (long-term schedule) following morphine (15 mg/kg bw), under normal (control) and zinc supplementation (Zn 2 and Zn 4) conditions. Statistical analysis: two-way ANOVA and a post hoc test: * $p < 0.001$ vs. basal.

None of the administered zinc oral schedules determined significant influence on the analgesic effect of used morphine doses in naïve or dependent animals. There are no significant differences between the analgesic effect of 10 mg/kg bw of morphine (s.c.) in the 5 day-schedule dependence and that of 15 mg/kg bw of morphine (s.c.) in the 10 day-schedule dependence, when evaluated as percentage increase (exposure vs. basal) (Figure 4).

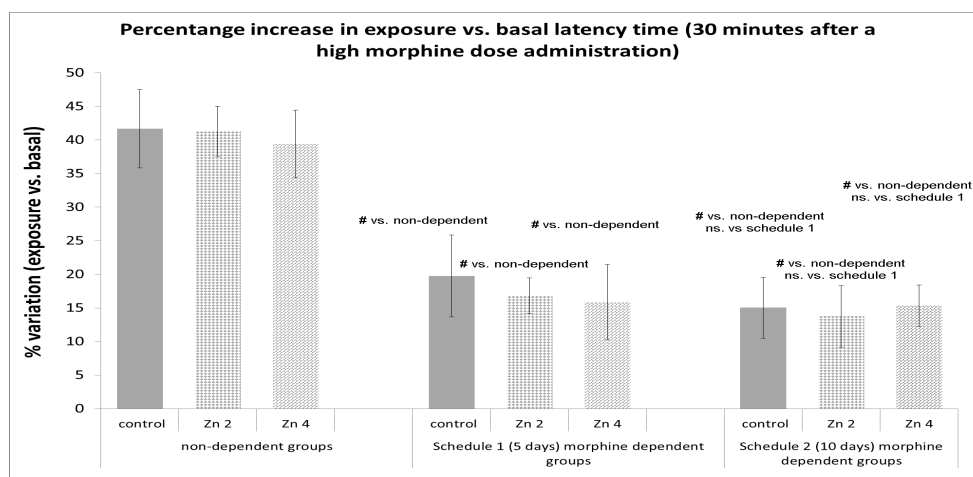


Figure 4.

Percentage increase (%) in exposure vs. basal tail-flick latencies. Statistical analysis: two-way ANOVA and a post hoc test: # $p < 0.001$ vs. non-dependent.

To our knowledge, there are just two studies investigating zinc ions or zinc deficit effects on opioid-dependence, and they showed concordant results: long-term zinc administration prior to dependence induction alleviates withdrawal [3], and zinc chelators potentiate it (especially jumping) [13]. These findings are probably related to the property of zinc ions to inhibit opioid-binding to receptors

[26, 28]. Particularly susceptible to this inhibition are the μ -receptors, while in case of δ -receptors, there have also been reported decreases in receptors' density following zinc treatment [18].

These studies allow the hypothesis that, in humans on opioids for chronic pain, dietary zinc salts supplementation may lower addiction risk. Clinical data evidencing reduced zinc levels in opioid-users

[7, 22, 23] and experimental data demonstrating decreased brain level of zinc opioid-treated animals [8] are also in the favour of this, and so is the low toxicity of oral zinc. Moreover, zinc deficiency is associated with depression-like symptoms [20, 21], which are known to induce drug-taking behaviour [10], so correcting zinc misbalances may reduce the craving for opioids by attenuating depression. If zinc inhibition of opioids receptor-binding explains, at least partially, opioid dependence reduction by zinc and withdrawal potentiating by zinc chelators, it nevertheless raises the problem of decreased analgesia to opioids in zinc supplementation-conditions. There are several animal studies showing that non-toxic zinc doses determine analgesia, but most of them considered opioid-free conditions [9, 12, 15, 17]. However, it is questionable if zinc pain-suppression is additive to opioid-analgesia; very few studies refer to zinc effect on pain perception in opioid-administration conditions. For instance, morphine-analgesia is inhibited by intrathecal Zn^{2+} , but intrathecal Zn^{2+} has no effect on acute tolerance to morphine-analgesia, while the divalent cations chelator $Na^+Ca^{2+}EDTA$ has no effect on either analgesia or tolerance [12]; morphine-analgesia is potentiated by zinc oxide nanoparticles (but not by the classical ZnO formulation [9]), and lowered by zinc deficiency [6]. Own studies [4] showed the lack of influence of intraperitoneal zinc chloride on the long-term opioid dependence schedule, but now we have shown the lack of effect of the oral zinc sulphate on both short- and long-time dependence, the oral route being more suitable for diet-supplementation and determining less side effects.

The present research shows that, in two models of morphine-dependence where oral zinc administration before dependence-induction was shown to reduce withdrawal, the acute antinociception of a high morphine dose is un-affected by two different zinc doses (2 and 4mg/kg daily, 2 weeks prior to dependence induction). These results are valuable when evaluating the opportunity of zinc supplementation in chronic pain opioid-treated patients, as they prove that, in animal models, dependence intensity reduction is not accompanied by decreased analgesic potency, though the mechanism suspected to be incriminated in dependence reduction (i.e. – morphine- to receptors binding reduction) is also involved in analgesia.

Conclusions

This experiment suggests a possible benefit of oral zinc administration in patients on opioid therapy for chronic pain.

Acknowledgements

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