Interaction between Analgesic Effect of Nano and Conventional size of Zinc Oxide and Opioidergic System Activity in Animal Model of Acute Pain

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ABSTRACT

Introduction: Today Nano-medicine tries to produce new drugs to reduce the dosage and side effects of their conventional forms. According to the interaction between zinc and opioidergic system activity, this study has investigated the effect of new kind of zinc supplement, nano zinc oxide (nZnO), in compared to its conventional form (cZnO), in presence and absence of opioidergic system activity on acute pain.

Methods: Adult male Wistar rats (weighting 200±20gr) divided into groups: control (receiving saline %0.9), nZnO (1, 5, 10, 20 mg/kg), cZnO (1, 5, 10, 20 mg/kg), naloxone 1mg/kg, morphine 6 mg/kg, and co- injected groups of morphine and/or naloxone with nZnO (5mg/kg) and/or cZnO 10 mg/kg. Hot plate assay was used to evaluation of nociception and post injected latencies were recorded every 30 min for 90 min after I.P. injections of drugs. In co-injected groups latency time recorded after 60 minutes.

Results: Data indicated that both of ZnO supplements reduced latency time in dose and time dependent on the effect of nZnO was higher than cZnO. Also these components could improve anti-nociception effect of morphine and naloxone could not change the effect of these supplements.

Discussion: It seems that nZnO has more efficacy than its conventional form to showing analgesic effect that probably is related to the physicochemical properties of nZnO. Also may be these supplements have interaction with opioideric system in body.

1. Introduction

Nanotechnology by producing new materials that, are attractive from many aspects, especially when entering to biological systems, introduced as a leader technology in overall the world (Colvine, 2003). The same properties that lead to the technical advantages of nanotechnology also lead to unique biological effects (Dreher, 2004). These new materials have longer halftime and higher ability in living systems to interaction with cellular components and can open a new way to the treatment of human diseases and disorders (Murthy, 2007; Dreher, 2004). Nanomedicine is a new branch of nanotechnology that use nanoparticles for therapeutic targets such as drug delivery specially into central nervous system , Imaging and diagnostic, treatment of tissue damages and etc (Cho& Borgens, 2012; Chandra, Barick& Bahadur, 2011;Kreuter, 2005).

The nZnO is a metal oxide nanoparticle that, rather than its technological application, has attracted the attention of many researchers in biology and medicine (Wang, 2008; Wang, 2004). The nZnO has been em-

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ployed as a tissue regeneration scaffold for CNS neurons, it tolerates and conducts an electrical charge, which has been shown to guide and encourage neuronal growth (Wang, 2008; Ansari et al., 2011), also uses in drug delivery systems(Chandra, Barick & Bahadur, 2011). The nZnO can release zinc ions (Zn2+) in the aqueous state (Yank& Xie, 2006) and some studies have shown that zinc as a most prevalent trace element in animals body is capable to influence pain sensation when administered exogenously (Hmbidge at al., 2010; Liu, Wakkor& Tracey, 1999; Larson& Kitto, 1997; Matsunami & Kirishi, 2011). Systemic injections of ZnCl2 have transitional anti nociceptive effects in the writhing assay (Matsunami& Kirishi, 2011). Also intraperitoneal (I.P.) administration of ZnCl2 suppresses thermal hyperalgesia or neuropathic pain in rats (Liu, Wakkor& Tracey, 1999), while intra-thecal (I.T.) administration of a Zinc chelator causes thermal hyperalgesia (Larson& Kitto, 1997). A clinical study has shown that the low plasma zinc concentrations associated with tongue pain, a type of neuropathic pain (Yoshida et al., 2010). Even though nZnO can releases zinc (Zn2+) (Yank& Xie 2006) but there is no evidence about the effects of nano size of ZnO or even conventional form of zinc oxide (cZnO as traditional zinc supplement in diets) on nociception.

On the other hand from many years ago opiates have been used for treatment every kind of pain especially chronic and sever pains (Mao et al., 2002). The chronic exposure of opiates especially in chronic pains leads to dramatic behavioral and neural changes known as tolerance and physical dependence, which limits the usefulness of opioids (Mao et al., 2002). Finding the joinable drugs that reduce side effects of main analgesic drugs like morphine and increase their efficacy is a key to optimizing the management of pain with opiates (Cameron et al., 2006; Sasaki, 2002; Sevostianova et al., 2003).

Some studies have reported that there is interaction between availability of Zinc and opioidregic system activity (Darsun et al., 1995). In addition the concentration of zinc in the cerebrospinal fluid of ex-heroin addicts is significantly lower than healthy peoples (Potkins, 1982) and dietary zinc deficiency in mice decreases the anti-nociceptive effect of morphine (Darsun et al., 1995) while intra-thecal injection of zinc reduced development of acute tolerance induced by morphine (Larson, Kovacs & Spartz, 2000). The aim of this study as the first investigation is to compare the effect of nZnO and cZnO on acute pain and then interaction between ZnO supplements and opioidergic system on nociception.

2. Methods

2.1. Animals and Drugs

Adult male Wistar rats weighting 200±20 gr were obtained from Animal house of Medical Science of Joundi Shapoor in Ahvaz and kept in room with a 12:12 h light/dark cycle and 20-24 ˚C temperature. All animals had freely access to food and water, just in during the test time and all tests down between9-13 in light phase. In every experiment were used 6-8 animals and each animal used just for once. All procedures were carried out in accordance with institutional guidelines for animal care and use.

Suspensions in the study such as nZnO (particle size<70nm, lolitec Co Germany) and cZnO (Merk Co Germany) prepared by sonication for 16 minutes in saline %0.9 by ultrasonic bath. Before of injection, suspensions shacked for 1 minute to reduce aggregation. Morphine sulphate (Temad Co Iran) and Naloxone hydrochloride (Sigma Co Germany) just dissolved in %0.9 saline before injections.

2.2. Experimental Design and Treatment

To test the effect of zinc supplements on acute pain, animals groups received nZnO (0,1,5,10,20 mg/kg) and cZnO (0,1,5,10,20 mg/kg) I.P. separately and post injected latencies were recorded every 30 min for 90 min after injections (Cameron et al ., 2006). In examining the effect of opioidergic system activity on analgesic effect of nZnO and cZnO, we used morphine sulphate (6mg/kg) and naloxone hydrochloride (1mg/kg) according to their ability to influence oipioid receptors activity in pain tests (Tamaddonfard, Erfanparast & Khalilzade., 2012; Sevostianova et al,. 2003; Boyete-Davis, Thompson& Fuchs., 2008) and co-injected with saline or analgesic doses of nZnO and cZnO, then hot-plate test examined. All drugs injected I.P. in volume of 1ml/kg and control group just received 0.9% saline.

2.3. Hot Plate Assay

Rats were placed on a hot plate maintained at 53±0.5 ºC and the latency to lick the hind paw was recorded as pain index. A 60 sec cut-off point was used to prevent tissue damage if no response occurred (Cameron et al., 2006).

2.4. Statistical Analysis

Data were expressed as the mean ±S.E.M. and for multiple groups were analyzed by (ANOVA) with the tukey post hoc test by using Instate v.3 software and student t-test was used to comparison between two groups. In all cases $p<0.05$ was considered significant.

3. Results

3.1. The Effect of cZnO (1, 5, 10, 20 mg/kg) on Latency Time to Pain Stimulus

Figure 1 shows analgesic effect of cZnO in a limit dose. After 90 minutes, just cZnO 10mg/kg significantly increased latency time to pain stimulus in compared to control group and in compared to cZnO 1mg/kg at the same time [F(4,29)= 4.26, P=0.05], while there is no any significant differences between another doses at the same times in compared to each other or control group [at 0 minutes: F(4,29) =0.083, Not Significant (NS), at 30 minutes: F(4,29)=1.32, NS, at 60 minutes: F(4,29) =2.58, NS] . Also analgesic effect of cZnO 10mg/kg improved by going time and this dose could show maximum analgesic effect, so we selected it for following experiments.

3.2. The Effect of nZnO (1, 5, 10, 20 mg/kg) on Latency Time to Pain Stimulus

Figure 2 shows analgesic effect of nZnO in dose dependent manner. After 30 minutes, nZnO 20mg/kg increased latency time in compared to control group [at 30 minutes F(4:25)=3.41, P=0.05] and after 60 minutes all

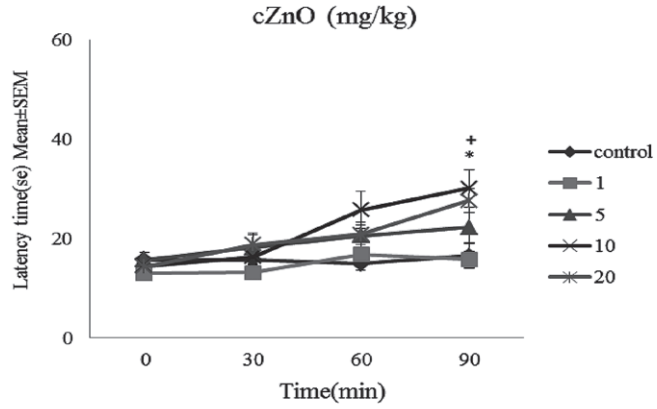

Figure 1. The effect of cZnO (1, 5, 10, 20 mg/kg) on latency time to pain stimulus in hot plate test: each line show the mean ± SEM for one dose in four times (every 30 min from 0 up to 90). *P<0.05 show significant differences between cZnO 10mg/kg and control group and +P<0.05 show significant difference between cZnO 10 mg/kg and cZnO 1mg/kg at the time of 90 minutes.

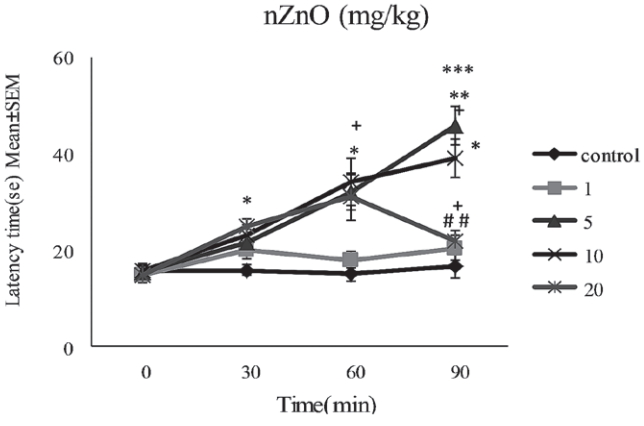

Figure 2. The effect of nZnO (1,5,10,20 mg/kg) on latency time to pain stimulus in hot plate test: each line show the mean ± SEM for one dose in four time (every 30 min from 0 up to 90). In every time *P<0.05, **P<0.01, ***P<0.001 show significant differences between treatment groups and control at the same time. +P<0.05show differences between nZnO 10 mg/kg and nZnO 1mg/kg after 60 and 90 minutes, and ##P<. 0.01 show significant difference between nZnO 5mg/kg and 1mg/kg after 90 minutes.

of nZnO 5, 10 and 20 mg/kg reduced pain in compared to control group [at 60 minutes F(4:25)=5.23, P=0.05] also nZnO 10mg/kg reduced pain in compared to nZnO 1mg/kg (P<0.05). At the time of 90 min both of nZno 5 and 10 mg/kg could increase analgesia in compared to control group [at 90 minutes F (4:25) =9.17, P=0.001] also in compared to nZnO 1mg/kg (P<0.01, P<0.05). By going time every effective doses could improve its analgesic effect and just nZnO 20mg/kg after 90 minutes reduced its effect. According to these results nZnO 5mg/kg can induce maximum analgesia effect so we selected it for following tests [at 0 min F(4,25)= 0.11, NS].

3.3. Comparison between Equal Doses of nZnO and cZnO in the Same Latency Time to Pain Stimulus

Table 1 shows that there were no any difference in analgesia effects of all treatment groups before receiving drugs while 30 minutes after injection, doses of 1mg/kg and 10 mg/kg show significant differences [at 1mg/kg F=1.43, P=0.01, at 10 mg/kg F=2.27, P=0.05] also after 60 and 90 minutes nZnO 5mg/kg significantly reduced pain in compared to its equal dose [for dose of 5mg/kg at 60 minutes F=1.61, P=0.05, at 90 minutes F=1.20, P=0.01]. In equal doses at the other times there were no differences.

3.4. The Effect of Morphine and Naloxone as an Agonist and Antagonist of Opioidergic System on Latency Time to Pain Stimulus

Figure 3 shows that morphine 6 mg/kg could reduce latency time to pain stimulus 60 minutes after injection in compared to control group at the same time [at 60 minutes F(2:15)= 5.80, P=0.05] while naloxone 1mg/kg couldn't change latency time in compared to control group or morphine 6mg/kg in any time [at 0 min

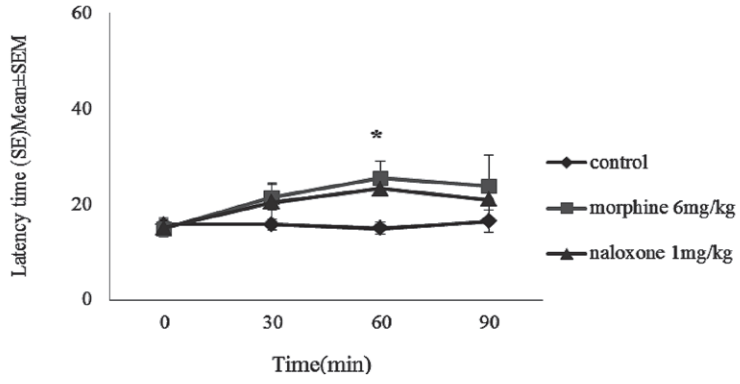

Figure 3. The effect of morphine (6mg/kg) and naloxone (1mg/kg) on latency times to pain stimulus: each line show the mean ± SEM for every drugs in four times (every 30 min from 0 up to 90). Control group received saline/ saline, other groups received morphine or naloxone / saline. *P<0.05 show significant difference between morphine 6mg/kg and control group at the same time.

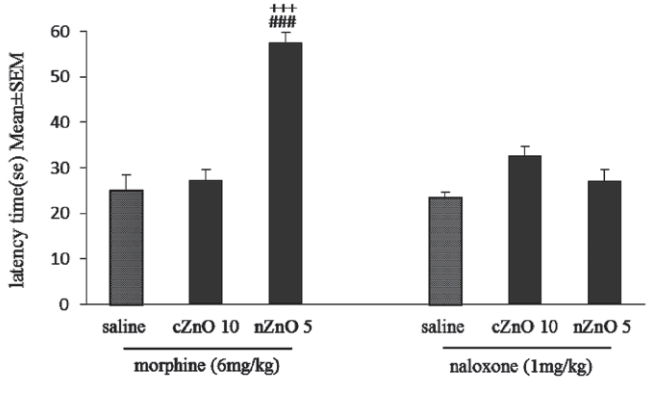

Figure 4. The effect of morphine (6mg/kg) and naloxone (1mg/kg) on analgesic effect of cZnO and nZnO: each bar show the mean ± SEM. +++P<0,001show significant difference in compared to saline/morphine 6mg/kg control group, ###P<0.001 show significant difference in compared to cZnO10/morphine 6mg/kg.

Table 1. Comparison between analgesic effect of equal doses of nZnO and cZnO in the same times. Student t -test was used to comparison between equal doses at the equal times.

Groups	Time			
cZnO(mg/kg) vs nZnO (mg/kg)	0	30	60	90
1vs 1	NS	** P<0.01	NS	NS
5 vs 5	NS	NS	* P<0.05	**P<0.01
10 vs 10	NS	*P<0.05	NS	NS
20 vs 20	NS	NS	NS	NS

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F(2:15)=0.02, NS, at 30 minutes F(2:15), NS, at 90 minutes F(2:15), NS] . We selected this time to evaluate the effect of opioidergic system activity on analgesic effect of cZnO and nZnO.

3.5. Interaction between Opioidergic System Activity and Analgesic Effect of cZnO and nZnO

Figure 4 show that morphine co-injected with cZnO 10 mg/kg hasn't any effect on latency time in compared to morphine/saline control group, while co–injection of morphine with nZnO 5mg/kg significantly increased latency time in compared to its control group also in compared to cZnO10mg/kg/morphine [F(2:15)=25.77, P=0.001]. This indicates that co-administration of nZnO with morphine can synergize anti nociceptive effect of morphine also in compared to nZnO 5mg/kg can induce analgesic effect in lower time actually opioid receptor activity could improve analgesic effect of nZnO too.

In addition Co- injection of naloxone with cZnO 10mg/kg or nZnO 5mg/kg couldn't change latency time 60 minutes in compared to naloxone/saline control group [F(2:15)=5.64 , NS]. According to these results the block of opioid receptors could prevent the analgesic effect of cZnO or nZnO.

4. Discussion

In this study we used hot plate test as an animal model to induce acute pain and evaluated the effect of our components on this kind of pain (Kesmati, Barfinejad & Fathimoghadam., 2007; Larson, Gozariu & Cadden 2001). Results showed that cZnO at the dose of 10mg/kg could induce maximum analgesic effect at the highest time in compared to the control group at the same time (figure1) also all doses of nZnO 5, 10 and 20 mg/kg could reduce pain and maximum analgesic effect achieved at the dose of 5mg/kg after 90 minutes, while by increasing dose anti nociceptive effect of nZnO decreased (figure2). Both of cZnO and nZnO at the best analgesic doses reduced pain by going time, this may be related to zinc oxide pharmacokinetic (Wang, 2004; Dawei, Zhisheng & Augua, 2010) and its ability to release zinc, or the effect of zinc on its post synaptic receptors (Yank& Xie, 2006; Takeda et al., 2004; Hambidge et al., 2010). Some evidences indicated that the release of zinc from ZnO particles is lower than other inorganic or organic zinc supplements (Edwards& Baker, 1999) and time may be is a factors for ZnO activity that allows to it releases zinc.

In post synaptic space zinc acts on many channels and receptors and is a non-competitive inhibitor for N-methyl-D-aspartate (NMDA) glutamate receptors that can attenuate glutamate ability to influence this receptor. NMDA receptor is one of the major receptors in pain processes and many studies have shown competitive or noncompetitive inhibitors of it can reduce pain sensation (Westbrook & Mayer, 1987; Nozaki et al., 2011; Mony et al., 2009). So may be in our study zinc by blocking NMDA receptors induced its analgesic effect. On the other hand Zinc reduces the release of glutamate by increasing GABA as an inhibitory neurotransmitter (Wang et al., 2001; Takeda et al., 2004; Takeda et al., 2004) and this can be another possible way that induced analgesic effect of nZnO or cZnO.

Analgesic effect of nZnO in compared to cZnO achieved in lower dose (Table1). Nanoparticles are more reactiveness than their conventional form and there are more available reactive site on the surface of these particles (Cho& Borgens, 2012) also nanoparticles can penetrate more easily through every barrier in body and interact with many cell components in organs (Jullerat& Jeannevet, 2008; Dursan et al., 1995; Ansari et al., 2011; Chandra, Barick& Bahadur, 2011) these allow them to show potentiated effects which can't observe in their conventional form (Kool, Ortiz& van Gestel, 2011). From Table 1, we can find that there were no significant difference between highest doses of nZnO and cZnO, in compared to each other; this may be related to the level of zinc concentration and saturation effect produced by

them (Teisseyre, Merick& Mozrzyma, 2007). Based on our result we have learned after 90 minutes nZnO 20mg/kg significantly reduced latency time to level of nZnO 1mg/kg and control groups at the same time (Figure2). Rather than saturation effect this may be related to the nanoparticles tendency to aggregate, by increasing dose or time, that can influence their efficacy (Singh et al., 2006; Sonavane, Tomoda& Makino, 2008).

Previous studies have announced that systemic injection of naloxone 1mg/kg could block opioid receptors completely in pain tests (Tamaddonfard, Erfanparast& Khalilzade, 2012). In our study blocking of opioid receptors by I.P. injection of naloxone 1mg/kg, could prevent analgesic effect of cZnO 10mg/kg or nZnO 5mg/kg (Figure 3) while co-administration of morphine with nZnO 5mg/kg has synergism effect and significantly potentiate anti nociceptive effect induced by morphine (Figure4). The ability of nZnO to potentiate analgesic effect of morphine probably is due to nanoparticles properties and their ability to influence many neurotransmitter systems in compared to their conventional particles (Hagens , 2007; Murthy, 2007; Cho& Brogens, 2012).

There are common and uncommon mechanisms that can paly role in opioidergic system activity and antinociceptive mechanisms activated by zinc supplements (Dursan, 1995; Potkins, 1982; Larson, Kovacs& Spartz, 2000; Mao, 1999).

The evidence exists for the interaction between the mu opioid receptors and NMDA receptors in pain related processes (Mao, 1999). NMDA receptor antagonists have been known to cause potentiation of the opioid anti nociception when given in combination with opiates (Cameron et al., 2006; Mao, 1999). Cameron et al 2006 showed that co-administration of MK801¬ as NMDA receptors antagonist with morphine enhance acute morphine analgesia in hot plate test also disrupts the development of morphine analgesic tolerance in adult male mice (Cameron et al., 2006). Maybe this effect is due to the inhibitory action of zinc (released from nZnO) on NMDA receptors (Westbrook& Mayer, 1987). Morphine can potentiate GABAA receptors activity and zinc respectively decrease and increase the release of glutamate and GABA, these alternative ways can help to reduce the pain (Sasaki, 2002; Takeda et al., 2004; Wang et al., 2001).

In conclusion both of conventional ZnO and nano ZnO could be effective to reduce acute pain and the effect of nZnO was higher than cZnO. Analgesic mechanism applied by nZnO could influence anti nociceptive mechanism activated by opioidergic system activity. We suggest that nZnO can use as a joint drug to increase morphine efficacy in treatment of acute pain but more investigation need for information on the safety of this manufactured nanoparticle, also we need to engage the other pain experiment to validate our finding.

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