Magnesium and other bivalent cations influence upon sodium montelukast effect in experimental-induced thermoalgesia

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Abstract. We tested the influence of magnesium, zinc and copper upon the montelukast (MK, antagonist of cysteinyl leukotriene receptor type 1) effect in experimentally-induced thermoalgesia. We worked on 5 groups of 10 adults, each Wistar rats, that received: group I-control; group II: MK (10 mg/kg) unique administration; group III: MgCl2 (1 mM/kg/day) i.p., 3 days and MK (10 mg/kg) unique administration on the 3rd day; group IV: ZnCl2 (0.1 mM/kg/day), i.p., 3 days and MK (10 mg/kg) unique administration on the 3rd day; group V: copper acetate (0.05 mM/kg/day), i.p., 3 days and MK (10 mg/kg) unique administration on the 3rd day. We determined the thermoalgesic sensitivity (TS) using a tail flick analgesia meter, initially, 3 days after daily cation administration and 3 hours after MK administration. Our data show that MK has a statistically significant reduction of TS vs control group (3.76 ± 1.04 s vs 1.81 ± 0.98 s, p < 0.05). Copper and magnesium administration do not significantly change the MK effect to decrease TS. The co-administration of zinc and MK statistically significantly increased the TS of the group that received only MK (2.51 ± 0.21 s vs 3.76 ± 1.04 s, p < 0.05). Animals that received only cations (in the above mentioned doses) did not significantly change TS.

Key words: magnesium, zinc, copper, montelukast, leukotrienes, thermoalgesia, rat

Magnesium and other cations are involved in various normal and pathological processes in the nervous system. Pain is one of these processes influenced by cations.

Eicosanoids (including prostaglandins, leukotrienes, lipoxins, isoprostanes, etc.) are potent autacoid lipids involved in many homeostatic functions [1]. Cysteinyl leukotrienes (LTC4, LTD4, and LTE4) and LTB4 are a group of eicosanoids derived from arachidonic acid and other fatty acids through the 5-lipoxygenase pathway. Cysteinyl leukotrienes (CysLTs) exert their functions through 2 types of G protein-coupled receptors: Cys-LT receptor type 1 and Cys-LT receptor type 2 [2]. Leukotrienes play physiological roles but are also involved in important pathological processes, such as asthma [3], allergic rhinitis [4], anaphylactic shock [5], ischemia-related organ injury [4], cerebral ischemia [6], human tumor genesis [7], interstitial cystitis [8], inflammatory bowel disease [3], etc.

There are some data showing the involvement of leukotrienes in acute inflammatory and non-inflammatory pain. A significant increase in the production of CysLTs is observed after mechanical or thermal trauma in anesthetized rat [9]. The role of CysLTs in animal models of nociception has been reported. Also there are authors who indicate leukotrienes as signaling molecules in pain [10].
Montelukast (MK) is the most commonly prescribed selective Cys-LT receptor antagonist available worldwide. MK represents an important therapeutic advance in the management of bronchial asthma, exercise-induced asthma, allergic rhinitis, etc. [11].

This study investigates the influence of magnesium, zinc and copper on the effect of MK in experimental thermoalgesia.

Materials and methods

Adult, male Wistar rats (n = 50), weighing 180-200 g, were housed in groups of five in Plexiglas cages (65 x 40 x 30 cm) with the floor covered with sawdust. Animals were maintained in a controlled environment (12 h light/dark cycle with lights on at 07.00, temperature of 20 ± 1°C) before and throughout the experimental period. All experiments involving animals were approved by the University’s Committee for Bioethics and animal experimentation, according to international rules of experimental practice.

After habituation period of one week, the animals were randomly divided into 5 groups of 10 rats each. Group 1 was for control and treated with saline; group 2 received MK 10 mg/kg /day per os, one day; group 3 received MgCl₂ (1 mmol/kg/day) i.p., 3 days and MK (10 mg/kg)/day per os in the 3rd day, group 4 was administered ZnCl₂ (0.1 mmol/kg/day), i.p., 3 days and MK (10 mg/kg)/day per os in the 3rd day and to group 5 was given copper acetate (0.05 mmol/kg/day), i.p., 3 days and MK (10 mg/kg)/day per os in the 3rd day.

The tail-flick assay was performed initially, 72 hours after first administration of cations and to MK (10 mg/kg/day) in the 3rd day, group 4 was given copper acetate (0.05 mmol/kg/day), i.p., 3 days and MK (10 mg/kg)/day per os in the 3rd day.

The statistical analysis was performed using analysis of variance (ANOVA) one way followed by Tukey’s test (software StatPlus 2007). Differences were considered significant when p < 0.05.

Results and discussion

Our results regarding the influence of magnesium and other bivalent cations on the effect of MK in experimental thermoalgesia are shown in figure 1. MK (10 mg/kg/day) has a statistically significant antinociceptive effect as it increased the tail flick latency time (1.81 ± 0.98 s vs 3.76 ± 1.04 s, p < 0.01). The antinociceptive effect of MK shows the influence of leukotriene receptors in nociception.

MgCl₂ administration (1 mmol/kg) prolonged the tail flick latency time without being statistically significant (1.81 ± 0.98 s vs 2.26 ± 0.88 s vs control group, p = 0.051). Neither ZnCl₂ nor copper acetate administration significantly changed the tail flick latency time compared to the control group (2.33 ± 0.28 s and respectively 1.57 ± 0.59 s vs 1.81 ± 0.98 s). There was no important difference between the effects of magnesium or zinc on thermal nociception.

MgCl₂ or copper acetate administrations do not influence the MK effect of increasing tail flick latency time (3.76 ± 1.04 s vs 3.76 ± 0.91 s vs 3.76 ± 1.04 s). Administration of ZnCl₂ statistically significantly diminishes the MK effect to prolong tolerance to thermic stimuli (2.51 ± 0.21 s vs 3.76 ± 1.04 s, p < 0.05).

The plasmatic concentration of magnesium initially and at 1 h, 2 h, 4 h, 6 h, 12 h, 24 h and 72 h after first MgCl₂ administration is shown in figure 2. All plasmatic cation concentrations increased statistically significantly after MgCl₂, ZnCl₂ and respectively copper acetate administrations for 3 days versus initial levels.

Our results are close to literature data, which show that magnesium has moderate antinociceptive effects in animal and human pain [13, 14]. Experimental data show that magnesium sulphate administered after spinal cord injury decreases neuropatic pain [15] Magnesium sulphate reduces the propofol requirements from anesthesia during intravenous anesthesia [16, 17]. The co-administration of magnesium and fentanyl in epidural post-operative analgesia decreases the use of general anesthetic [18]. Magnesium acts both at a peripheral and at central nervous system levels. On the contrary, MK does not
pass the blood brain barrier so its action is based on blocking the peripheral CysLT receptors.

Magnesium, zinc and copper are important modulators of NMDA-receptor activity. There is general agreement that the N-methyl-D-aspartate receptor plays a significant role in thermal hyperalgesia. High concentrations of Mg$^{2+}$ inhibit the eicosanoid metabolism both at the level of arachidonic acid release and by direct inhibition of 5-lipoxygenase [19]. On the other hand, CysLTs formation is associated with the activation of NMDA receptors [20]. As demonstrated by in vitro studies, low Mg$^{2+}$ concentrations facilitate the opening of the Ca$^{2+}$ channel coupled with NMDA receptors [21].

Copper plays a role as a modulator of neuronal transmission in some synapses and acts directly on the NMDA receptor [22, 23]. Schlief et al. [24, 25] have shown that intense activation of the NMDA receptor by thermoalgesic stimuli is associated with copper release from neurons. Exogenous administration of copper enhances the effect of thermoalgesic stimuli. Even so, in our study copper acetate did not significantly statistically change the tail flick latency time.

Zinc is concentrated in the dorsal horn of the spinal cord and has been proposed to alter the excitability of primary afferent C fibers, structures believed to be important in nociceptive transmission. Ion channels gated by NMDA receptors are antagonized by zinc [26] at a site distinct from that of magnesium [27]. Zinc inhibits purified leukotriene A4 hydrolase and leukotriene biosynthesis. We consider that this contributes partially to zinc antinociceptive effect. IC$_{50}$ for zinc is 10 µM [19]. This concentration can be reached by administration of ZnCl$_2$ as in our study.

**Figure 1.** Tail flick latency time in groups which received montelukast and MgCl$_2$, ZnCl$_2$, copper acetate before administration of montelukast. *a* $p < 0.05$: MK, MK + MgCl$_2$, MK + ZnCl$_2$ and MK + Cu group vs control group; $p = 0.05$: MgCl$_2$, Cu acetate group vs control; *b* $p < 0.05$: MgCl$_2$, ZnCl$_2$ and Cu acetate group vs MK group; *c* $p < 0.05$: MK and MgCl$_2$ group vs MK + MgCl$_2$ group; *d* $p < 0.05$: MK group vs MK + ZnCl$_2$ group; *e* $p < 0.05$: Cu acetate group vs MK + Cu group.
In our opinion, ZnCl₂ decreases MK analgesic effect due to Zn²⁺ inhibitory action on MK receptor binding. The same mechanism is described in Zn²⁺ attenuation of morphine antinociception [28].

Our data are in agreement with Jain et al. [29] and Singh et al. [30] who suggested that leukotrienes play a role in paradigms of acute nociception. Zafirlukast, another CysLT receptor antagonist (2.5-20 mg/kg, p.o.) produced a significant and dose-dependent antinociceptive and antiinflammatory effect against acetic acid-induced chemonociception in mice and also attenuated the carrageenan-induced hyperalgesia. A selective suppression of the 5-LO pathway exhibits similar results with the CysLT receptor blockade. It is possible that CysLTs could be important factors in nociception.

Selective inhibition of 5-LO by zileuton determined the reduction of mechanically induced hyperalgesia in mice and rat. The same effect of zileuton was observed in radicular pain induced by herniated nucleus pulposus in rats [31].

The analgesic effect of MK (antagonist of cysteinyl leukotriene receptor type 1) is significant, even if this is not generally admitted. Zn significantly shifted this effect. This experiment indicates that animals that received only Mg, Zn or copper did not change TS significantly. It would be also of interest to perform other tests of nociception regarding the influence of divalent cations and leukotriene antagonists in pain.

**References**

3. Sharma JN, Mohammed LA. The role of leukotrienes in the pathophysiology of inflammatory disorders: is there a case for revisiting leukotrienes as therapeutic targets? *Inflammopharmacology* 2006; 14: 10-6.


