

THE INFLUENCE OF MONTELUKAST ON MORPHINE-INDUCED PHYSICAL DEPENDENCE

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Abstract

This study investigates the influence of montelukast (MK), a cysteinyl-leukotriene 1 (CysLT₁) receptor antagonist, on morphine-induced physical dependence in rats. The study was carried out on four groups of 10 Wistar male rats each, as follows: group I (control) - saline, s.c.; group II - MK 10 mg/kg b.w. p.o./day for 10 days; group III - morphine s.c. (progressively increasing doses from 5 mg/kg b.w./day in 1st day to 90 mg/kg b.w. day in 10th day) and group IV - MK 10 mg/kg b.w. p.o. and morphine s.c. (as group III) 10 days. On the 11th day, the morphine and MK administration was stopped and all animals received naloxone i.p. 2 mg/kg b.w. and withdrawal syndrome was assessed for 30 minutes. MK association to morphine (group IV) significantly reduced the intensity of morphine-induced physical dependence and the withdrawal symptoms such as: grooming (8.3 ± 3.52 in morphine + MK group *versus* 12.4 ± 2.87 in morphine only, $p < 0.01$), compulsive mastication (10 ± 1.82 *versus* 12.7 ± 2.75 , $p < 0.05$), palpebral ptosis (2.1 ± 0.87 *versus* 3.5 ± 0.97 , $p < 0.01$), and decreased Gellert Holtzman score (88.5 ± 15 in morphine + MK group *versus* 119.3 ± 17.8 in morphine only group $p < 0.01$). The data show that cysteinyl-leukotrienes (CysLTs) are involved in morphine-induced dependence and that the MK administration during induction of morphine dependence, but not during withdrawal, alleviates the intensity of the withdrawal syndrome symptoms in rats.

Rezumat

Acest studiu a evaluat influența montelukastului (MK), un antagonist al receptorilor cisteinil-leucotriena 1 (CysLT₁), asupra dependenței fizice de morfină indusă experimental. Studiul a fost efectuat pe patru grupuri de câte zece șobolani Wistar adulți masculi: lotul I (control) a primit ser fiziologic s.c.; lotul al II-lea a primit MK 10 mg/kg corp p.o./zi, 10 zile; lotul al III-lea a primit morfină s.c. (în doză progresiv crescândă de la 5 mg/kg corp în prima zi la 90 mg/kg corp în ziua a 10-a) și grupul al IV-lea care a primit MK 10 mg/kg corp p.o. și morfina s.c. (la fel ca și lotul al III-lea) 10 zile. În a 11-a zi toate animalele au primit naloxon i.p. 2 mg/kg corp și apoi simptomele din cadrul sindromului de abinență au fost urmărite 30 de minute. Asocierea MK la morfină (lotul al IV-lea) reduce în mod semnificativ intensitatea dependenței fizice produsă de către morfină și unele simptome din cadrul sindromului de abinență cum ar fi: clăntănitul dinților ($8,3 \pm 3,52$ *versus* $12,4 \pm 2,87$, $p < 0,01$), masticăția compulsivă ($10 \pm 1,82$ *versus* $12,7 \pm 2,75$, $p = 0.013$), ptoza palpebrală ($2,1 \pm 0,87$ *versus* $3,5 \pm 0,97$, $p = 0,01$) și crește scorul Gellert Holtzman ($88,5 \pm 15$ la lotul cu morfină + MK față de $119,3 \pm 17,8$ la lotul care a primit numai morfină $p < 0.01$). Datele noastre arată că cisteinil-leucotrienele sunt implicate în dependența fizică la morfină și că administrarea MK în timpul inducției dependenței de morfină, dar nu și în timpul sindromului de abinență reduce intensitatea majorității simptomelor din timpul sindromului de abinență la morfina la șobolani.

Keywords: CysLTs, montelukast, rat, morphine, physical dependence

Introduction

Arachidonic acid and docosahexaenoic acid, the main polyunsaturated fatty acids in the brain are precursors for cerebral leukotriene production and together with their metabolites are involved in memory and behaviour [22]. It is known that neurons and glial cells can produce eicosanoids through all three major enzymatic routes (cyclo-oxygenase, lipoxygenase and epoxygenase route) [7]. The 5-lipoxygenase (LOX) pathway, the metabolic way for leukotrienes synthesis is present in the neurons of the cerebral structures involved in

opioid-induced dependence such as cortex, hippocampus, amygdala and others [4]. Moreover, the expression of the 5-LOX gene is correlated with the cellular synthesis of leukotrienes. Both types of CysLTs receptors CysLT₁ and CysLT₂ are present at the level of the neuronal membrane in different regions of the brain [10].

Although it is known that leukotriene production is altered in various brain diseases like Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson disease, cerebral tumours, seizures and others, recent data suggested also the possible involvement of CysLTs in opioid-induced dependence [18, 20, 21, 28].

This article aimed to assess the effect of a CysLT₁ receptor selective competitive antagonist, MK on morphine-induced physical dependence in rats.

Materials and Methods

This experiment was performed on adult Wistar male rats (n = 40) with the weight ranging from 180 to 220 g, that were obtained from Laboratory Animal Center of Cantacuzino Institute of Research, Bucharest, Romania. The animals were placed in groups of three in plexiglas cages with the floor covered with sawdust. The rats were exposed to a controlled environment with a 12 h light/dark cycle and a temperature of $20 \pm 1^\circ\text{C}$ before and throughout the experimental period. The animals had free access to food (standard lab rat chow) and tap water *ad libitum*. After a habituation period of one week, the rats were randomly divided into four groups (groups I - IV) of 10 rats each.

The group I was for control and was treated with saline dilution 1 mL/kg b.w./day s.c. for 10 days, 2 administrations per day at 7.00 h and 19.00 h; group II received MK 10 mg/kg b.w./day (aqueous suspension of carboxymethyl cellulose 0.1%) orally (p.o.) for 10 days; group III was given morphine s.c. (progressively increasing doses from 5 mg/kg b.w./day in the first day to 50 mg/kg b.w./day in the tenth day (the daily dose was divided into two equal administration at 7.00 h and 19.00 h) and group IV received MK 10 mg/kg b.w./day p.o. for 10 days and morphine s.c. (progressively increasing doses from 5 mg/kg b.w./day in the 1st day to 50 mg/kg b.w./day in the tenth day) (the daily dose was divided into two equal administration at 7.00 and 19.00 h). On the 11th day morphine and MK administration was stopped and all groups received naloxone 2 mg/kg b.w. i.p. Immediately after naloxone administration, we observed the main signs of morphine withdrawal syndrome using a video camera connected to a computer. The number of episodes of hyper-salivation, grooming, jumpings (escape attempts), compulsive mastication, teeth chattering, stretching and "wet dog shakes" (tremor) were counted under the same conditions for all groups for 25 minutes. Diarrhoea and palpebral ptosis were evaluated over periods of 5 minutes up to 25 minutes (0 – absent, 1 – present for 5 minute period, maximal score: 5 for 25 minutes). Locomotor behaviour was assessed by open - field test over periods of 5 minutes (0 for exploration of 1 to 3 squares, 1 for exploration of 4 to 6 squares, and 2 for exploration of 7 to 9 squares). Scores accumulated during periods of 5 minutes were added, resulting in a maximum score of 10 over a period 25 minutes.

Body weight was determined before and 25 minutes after naloxone administration and weight differences were calculated. For the assessment of

the intensity of physical dependence we used Gellert-Holtzman scale (modified) [8]. The statistical analysis was performed using analysis of variance (ANOVA) one way. Tukey test was used for multiple comparisons (software Stats Direct version 2.6). A value of $p < 0.05$ was considered significant.

All procedures were performed in accordance with EU Directive 2010/63/EU for animal experiments and with Recommendations of the Helsinki Declaration. The study protocol was approved by the Bioethics Committee of "Gr. T. Popa" University of Medicine and Pharmacy Iasi.

Montelukast sodium was purchased from Sigma Aldrich (Romania), morphine was purchased from Zentiva (Prague, Czech Republic) and naloxone hydrochloride from Fluka Chemie GmbH (Buchs, Switzerland).

Results and Discussion

Our data show that morphine administration in group III induced strong physical dependence and significantly influenced all the surveyed withdrawal symptoms as compared to control group: grooming (12.4 ± 2.87 versus 1 ± 1.05 in control group (saline group, $p < 0.001$), compulsive mastication (12.7 ± 2.75 versus 2.2 ± 1.39 , $p < 0.001$), palpebral ptosis (3.5 ± 0.97 versus 0.3 ± 0.48 , $p < 0.001$), hypersalivation (2.1 ± 0.73 versus 0.2 ± 0.42 , $p < 0.001$), diarrhoea (2 ± 1.24 versus 0 , $p < 0.001$), weight differences (23.5 ± 6.75 versus 0.1 ± 0.31 , $p < 0.001$), "wet dog shakes" (7.6 ± 1.57 versus 0.6 ± 0.51 , $p < 0.001$), teeth chattering (1.22 ± 0.44 versus 0.11 ± 0.33 , $p = 0.01$), stretching (1 ± 0.81 versus 0.1 ± 0.31 , $p < 0.01$), jumpings (3.9 ± 1.28 versus 0.2 ± 0.3 , $p < 0.01$) and locomotor behaviour (3 ± 0.47 versus 0.9 ± 0.73 , $p < 0.001$). Gellert-Holtzman score (119.3 ± 17.8 in morphine group versus 13.8 ± 5 in control group, $p < 0.001$) showed a very high difference between morphine group and control group and indicates a strong physical dependence induced by morphine.

The MK association to morphine in group IV significantly influenced the majority of the withdrawal symptoms as compared to the morphine only group: grooming (8.3 ± 3.52 versus 12.4 ± 2.87 in morphine only group, $p < 0.01$), compulsive mastication (10 ± 1.82 versus 12.7 ± 2.75 , $p < 0.05$), palpebral ptosis (2.1 ± 0.87 versus 3.5 ± 0.97 , $p < 0.01$) (Figure 1), hypersalivation (1.4 ± 0.51 versus 2.1 ± 0.73 , $p < 0.01$), weight differences (12.4 ± 2.17 versus 23.5 ± 6.75 , $p < 0.01$), diarrhoea (0.7 ± 0.82 versus 2 ± 1.24 , $p < 0.05$) (Figure. 2). There were no significant differences between the group receiving only morphine and the group that received morphine + MK regarding the "wet dog shakes", jumpings and locomotor behaviour (Figure 3).

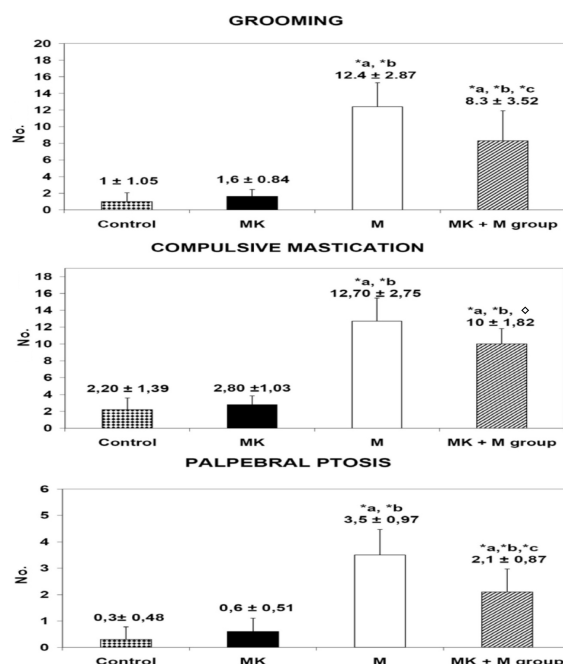


Figure 1.

Influence of MK association on some symptoms (grooming, compulsive mastication, palpebral ptosis) of withdrawal syndrome after morphine dependence

*a – $p < 0.001$ compared to control group, *b – $p < 0.001$ compared to MK only group, *c – $p < 0.01$ compared to morphine only group, ◇ – $p < 0.05$ compared to morphine only group, M = morphine, MK = montelukast

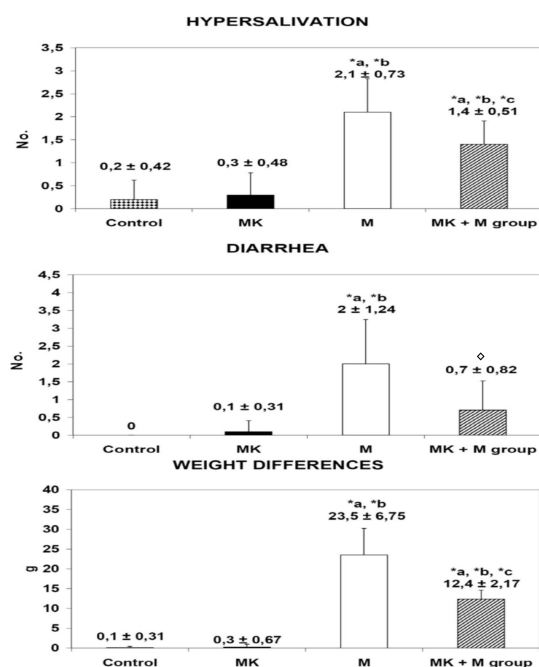


Figure 2.

The influence of MK association on some symptoms (hypersalivation, diarrhoea, weight differences) of withdrawal syndrome after morphine dependence

*a – $p < 0.01$ compared to control group, *b – $p < 0.01$ compared to MK only group, *c – $p < 0.01$ compared to group morphine only group, ◇ – $p < 0.05$ compared to group morphine only group, M = morphine, MK = montelukast

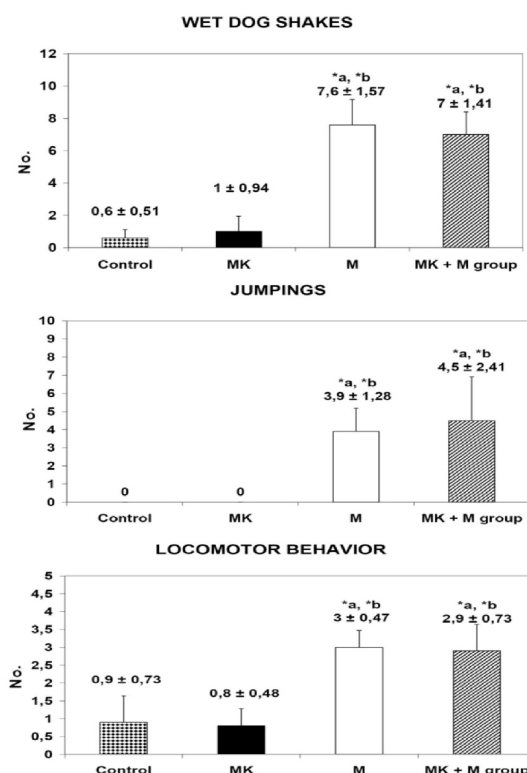


Figure 3.

The influence of MK association on some symptoms (“wet dog shakes”, jumpings, locomotor behaviour) of withdrawal syndrome after morphine dependence

*a – $p < 0.001$ compared to control group, *b – $p < 0.001$ compared to MK only group, M = morphine, MK = montelukast

Gellert-Holtzman score was (88.5 ± 15.10) in group IV *versus* 119.3 ± 17.8 in morphine only group ($p < 0.01$) (Figure 4). This score indicates a clear reduction in the intensity of physical dependence induced by morphine. There were not statistically significant differences regarding Gellert-Holtzman score between MK only group and the control group.

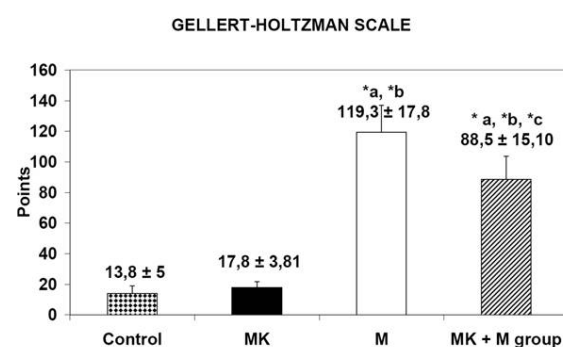


Figure 4.

Gellert-Holtzman scale

*a – $p < 0.001$ compared to control group, *b – $p < 0.001$ compared to MK only group, *c – $p < 0.01$ compared to group morphine only group, M = morphine, MK = montelukast

In brain, the greatest amount of leukotrienes is produced by the hypothalamus [16]. LTC₄ exhibits

hypothalamic and extra-hypothalamic actions such as involvement in learning, memory or aggressive behaviour [1]. Morphine induces a strong dependence, both psychical and physical. The experimental model used in this study is useful to investigate especially the influences on physical dependence intensity. In morphine-induced dependence, dopamine, endocannabinoid system, glutamate and GABA are considered to be key neurotransmitters, but other substances like nitric oxide (NO), P substance, nuclear factor kappa B (NFkB), tumour necrosis factor (TNF)-alpha are also involved [14, 17, 19, 23, 24, 26].

The CysLT₁ receptor competitive antagonist (MK) reaches efficient concentrations into the brain [20]. There are at least few possible mechanisms by which CysLT₁ receptors blockade by MK reduced the morphine- induced physical dependence. Different authors showed that CysLTs can be synthesized and are available in the *nucleus accumbens*, *dorsal striatum*, periductal grey substance, ventral tegmental area and mesolimbic system [16], cerebral areas involved in two essential circuits of dopamine-containing neurons [25]. These neurons form the nigrostriatal system and the mesocorticolimbic circuit are involved in the control of motor behaviour, and in rewarding aspects [9]. The changes of dopamine concentrations in the *nucleus accumbens* and other brain areas is an important part of physical dependence mechanism. The inclusion of arachidonic acid into brain membranes is facilitated by dopamine receptor agonists, data that sustains the eicosanoids - dopamine connection [3, 11]. In humans norepinephrine levels are significantly increased during the first days of morphine withdrawal syndrome and the increased norepinephrine concentrations are associated with enhanced leukotrienes production up to two folds [13]. Moreover, the higher levels of glucocorticoids during the withdrawal enhance 5-LOX expression in the brain *in vivo* and in neural cultures *in vitro* [15].

It is known that leukotrienes are involved in glutamate action in brain and that CysLT₁ receptor blockade reduces the activity of some cerebral glutamatergic synapses [5]. Morphine stimulates presynaptic release of glutamate both directly and indirectly through stimulation of dopamine synthesis in different regions of the brain.

Decreased neurogenesis which is considered to be a new risk factor for drug-taking behaviour is also associated with 5-LOX pathway products [6]. Recent data show that MK significantly enhances neural stem and progenitor cell proliferation and exhibits neuroprotective effects through decreasing the expression of pro-inflammatory mediators (TNF-alpha, IL1-beta, IL6) [27]. Furthermore, CysLT₁ receptor antagonists like pranlukast inhibit

NF-kappa B synthesis and this way interferes with the effect of morphine on the limbic system and reward system [12]. Our data are in accordance with other data regarding the involvement of leukotrienes and other eicosanoids in morphine dependence [2, 20].

Conclusions

To conclude, our data show that CysLTs are involved in morphine-induced physical dependence. The MK administration during morphine experimentally induced physical dependence in rats alleviates the intensity of morphine dependence. As a result, the intensity of symptoms during the withdrawal syndrome is reduced. Because MK was administered only during the induction of physical dependence, but not during abstinence syndrome, we believe that the blocking of CysLT₁ receptors is involved in reducing the development of physical dependence of morphine.

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