



## Influence of bile acid derivates on morphine analgesic effect in mice

### Uticaj derivata žučne kiseline na analgetski efekat morfina kod miševa

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#### Abstract

**Background/Aim.** It is known that bile acids improve the absorption, bioavailability and pharmacodynamic characteristics of some drugs. Morphine analgesia is produced by activation of opioid receptors within the central nervous system (CNS) at both spinal and supraspinal levels. Since a morphine molecule contains 3 polar groups and therefore hard to transfer through the blood-brain barrier, the aim of the study was to examine the potential influence of bile acids derivates, namely sodium salt of monoketocholic acid (MKH-Na) and methyl ester of monoketocholic acid (MKH-Me), on analgesic effect of morphine. **Methods.** White male mice of NMRI-Haan strain, with body weight of 20–24 g, were used in this study. The analgesic effect of morphine (administered by subcutaneous and intramuscular route in a dose of 2 mg/kg), with and without pretreatment with MKH-Na (4 mg/kg) and MKH-Me (4 mg/kg) was estimated by the hot plate method. **Results.** Administration of MKH-Me prior to subcutaneous administration of morphine increased the morphine analgesic effect but the increase was not statistically significant. At the same time administration of MKH-Na did not affect morphine analgesic effect. The analgesic effect of morphine increased when administered intramuscularly 20 min after MKH-Me administration. When compared with the group of animals treated only with morphine, a statistically significant increase in analgesic effect was detected 10, 30, 40 and 50 min after morphine administration ( $p < 0.05$ ). Pretreatment with MKH-Na did not affect morphine analgesic effect. **Conclusion.** According to the results of this study it can be presumed that after intramuscular morphine administration methyl ester of monoketocholic acid increases morphine transport into the central nervous system and consequently the analgesic effect, as well. Further research on bile acids-morphine interaction both *in vitro* and *in vivo* is necessary to completely elucidate the mechanism of this interaction and increase in the morphine analgesic effect.

#### Key words:

morphine; mice; bile acids and salts; blood-brain barrier.

#### Apstrakt

**Uvod/Cilj.** Poznato je da žučne kiseline poboljšavaju apsorpciju, povećavaju biološku raspoloživost i poboljšavaju farmakodinamske osobine nekih lekova. Budući da je analgezija izazvana morfinom posledica aktivacije opioidnih receptora u centralnom nervnom sistemu na spinalnom i supraspinalnom nivou, i da molekul morfina sadrži tri polarne grupe zbog čega teško prolazi kroz hematoencefalnu barijeru, cilj studije bio je da se ispita potencijalni uticaj derivata žučnih kiselina, natrijumove soli monoketoholne kiseline (MKH-Na) i metil estera monoketoholne kiseline (MKH-Me), na analgetski efekat morfina. **Metode.** Studija je sprovedena na belim miševima NMRI-Haan soja, muškog pola, telesne mase 20–24 g. Analgetski efekat morfina (primenjenog supkutano i intramuskularno u dozi 2 mg/kg) sa i bez pretretmana MKH-Na (4 mg/kg) i MKH-Me (4 mg/kg) procenjivan je metodom vrele ploče. **Rezultati.** Primena MKH-Me pre supkutane primene morfina pojačala je analgetski efekat morfina bez statistički značajne razlike. Primena MKH-Na nije uticala na analgetski efekat morfina primenjenog supkutano. Analgetski efekat intramuskularno primenjenog morfina, 20 minuta nakon primene MKH-Me, bio je pojačan. U poređenju sa grupom životinja kod kojih je primenjen samo morfin, statistički značajna razlika u analgetskom efektu zabeležena je 10, 30, 40 i 50 minuta nakon njegove primene ( $p < 0,05$ ). Pretretman sa MKH-Na nije uticao na analgetski efekat morfina primenjenog intramuskularno. **Zaključak.** Na osnovu rezultata studije može se pretpostaviti da nakon intramuskularne primene morfina metil estar monoketoholne kiseline povećava transport morfina u centralni nervni sistem i posledično dovodi do pojačanja analgetskog efekta morfina. Dalja istraživanja interakcija žučnih kiselina i morfina *in vitro* i *in vivo* neophodna su da bi se u potpunosti rasvetlio mehanizam interakcije, a time i mehanizam pojačanja analgetskog efekta morfina.

#### Ključne reči:

morfin; miševi; žučne kiseline i soli; krvno-moždana barijera.

## Introduction

The application of bile acids in human medicine dates back to the thirties of the 20th century. It has been shown that bile acids improve the absorption, bioavailability and pharmacodynamic characteristics of some drugs<sup>1, 2</sup>. Bile salts form micelles, which increase the permeability of the mucosal membrane by overcoming resistance at the aqueous diffusion layer<sup>3</sup>. They are also capable of enhancing drug delivery by interacting with membrane lipids and proteins that affect membrane fluidity<sup>4</sup>. The effects of bile acids on biological membranes are similar to those of detergents and they appear to have the potential to aid intestinal, buccal, transdermal, ocular, rectal and pulmonary absorption<sup>1</sup>.

Morphine is the prototypical opioid analgesic. It interacts with  $\mu$  and  $\kappa$  opioid receptors to exert its analgesic effect<sup>5</sup>. Analgesia is produced by activation of opioid receptors within the central nervous system (CNS) at both spinal and supraspinal levels<sup>6</sup>. Morphine is a weak base that is relatively water soluble and poorly lipid soluble. After oral administration it is predominantly absorbed in the proximal small bowel and has a poor and variable bioavailability (20–40%)<sup>7</sup>. In humans, following absorption, 90% of drug is metabolized, principally by glucuronidation in the liver, to form morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Morphine is widely distributed within the body. Excretion of morphine occurs predominantly in urine in the form of morphine glucuronides with unchanged morphine representing between 2% and 10% of the total, independently of the dose. Some 70–80% of an administered dose is excreted within 48 h of administration and most of this appears within the first 24 h<sup>8</sup>.

Since morphine analgesia is produced by activation of opioid receptors within the CNS at both spinal and supraspinal levels, the concentration of morphine at any point in time at active sites in the CNS will depend on the systematic and the CNS disposition of the drug. Alteration at either of these locations may influence morphine CNS concentrations and thus the degree of antinociception.

Having in mind characteristics of bile acids and their derivatives as well as the characteristics of morphine, the aim of this research was to determine the influence of bile acids derivatives, namely sodium salt of monoketocholic acid (MKH-Na) and methyl ester of monoketocholic acid (MKH-Me), on analgesic effect of morphine detected by antinociceptive hot plate method.

## Methods

White male mice of NMRI-Haan strain, with body weight of 20–24 g, were used in this study. Each control and experimental group was formed of 6 animals. Animals had free access to food and water, and subjected to 12-h light and dark cycles, at a room temperature of 22°C. Laboratory animals were under human care in accordance with the criteria given in the 'Guide for the Care and Use of Laboratory Animals'<sup>9</sup>. The study was approved by the Ethics Committee on Laboratory Animal Welfare of the University of Novi Sad, approval No. IV-2011-05.

For the experiment we used: morphine from Sigma-Aldrich; MKH-Na and MKH-Me – synthesized at the Department of Chemistry, Faculty of Sciences, University of Novi Sad, according to the procedure by Miljkovic et al.<sup>10</sup>; hot/cold plate – Ugo Basile (kept at  $53 \pm 1^\circ\text{C}$ ); chronometer.

The study groups included the following: C1 – the control group without treatment ( $n = 6$ ); C2 – the control group given morphine intramuscularly (*im*) at 2.0 mg/kg ( $n = 6$ ); C3 – the control group given morphine subcutaneously (*sc*) at 2.0 mg/kg ( $n = 6$ ); C4 – the control group given MKH-Na subcutaneously (*sc*) at 4.0 mg/kg ( $n = 6$ ); C5 – the control group given MKH-Me subcutaneously (*sc*) at 4.0 mg/kg ( $n = 6$ ); E1 – the experimental group given MKH-Na *sc* 4.0 mg/kg 20 min before morphine *im* at 2.0 mg/kg ( $n = 6$ ); E2 – the experimental group given MKH-Me *sc* 4.0 mg/kg 20 min before morphine *im* at 2.0 mg/kg ( $n = 6$ ); E3 – the experimental group given MKH-Na *sc* 4.0 mg/kg 20 min before morphine *sc* at 2.0 mg/kg ( $n = 6$ ); E4 – the experimental group given MKH-Me *sc* 4.0 mg/kg 20 min before morphine *sc* at 2.0 mg/kg ( $n = 6$ ).

The doses and time interval of administration of bile acid derivatives were determined according to previously published studies in which they were determined to be optimal for the analgesic model used in this study<sup>11–13</sup>.

The analgesic effect of morphine was estimated by the hot plate method<sup>14</sup>. Mice were gently restrained and placed on the hot plate surface ( $53 \pm 1^\circ\text{C}$ ). The latency for paw withdrawal from the heated surface was manually recorded with a chronometer. Only the clear withdrawal of the either rear paw was taken into account, discarding the nonspecific generalized struggle observed in some cases. Three measures at 10-min intervals were taken at the beginning of the study or before the treatment (groups of animals other than the control one) and their means were considered as basal (control) latencies. The mean basal values were sought around 11–13 s and animals showing any latency equal to or higher than 20 s were rejected. A maximal latency value (cut-off) of 30 s was determined.

The latency for paw withdrawal from the heated surface (reaction time in seconds) was recorded as follows: control (basal) reaction time for each animal in all the study groups; the study group C1 – experimental reaction time was recorded at 10, 20, 30, 40, 50, 60, 80, 100 and 120 min after CRT measurement; study groups C2, C3, E1, E2, E3 and E4 – ERT was recorded 10, 20, 30, 40, 50, 60, 80, 100 and 120 min after morphine administration; study groups C4 and C5 – reaction time measurement started 20 min after MKH-Na and MKH-Me administration (control reaction time). The experimental reaction time was recorded at 10, 20, 30, 40, 50, 60, 80, 100 and 120 min from this time point.

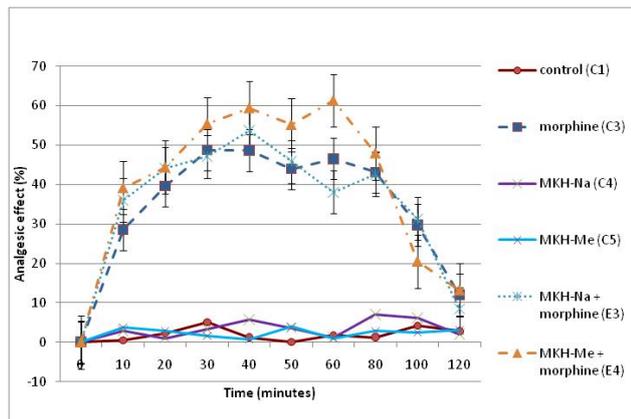
The morphine dose for both routes of administration was 2.0 mg/kg. The analgesic effect determined in seconds was expressed as a percentage of prolongation of measured experimental reaction time (ERT) compared to control reaction time (CRT) according to the equation:

$$\text{Analgesic effect (\%)} = \frac{\text{ERT} - \text{CRT}}{\text{CRT}}$$

Statistical analysis of collected data was performed by MedCalc 9.2.0.1. The statistical significance was determined using Student's *t*-test. A *p*-value of 0.05 was considered statistically significant.

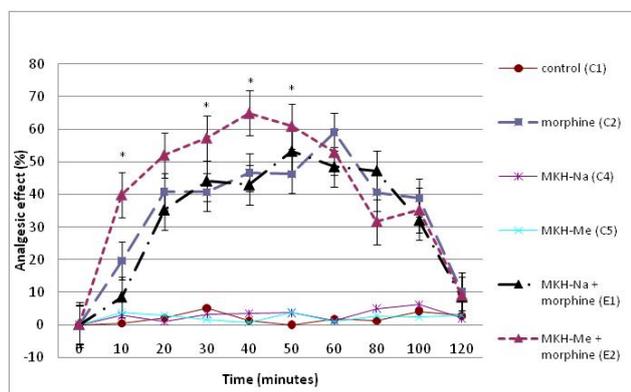
## Results

Figure 1 shows that MKH-Me administration prior to subcutaneous administration of morphine increased the morphine analgesic effect, but this increase was not statistically significant. At the same time MKH-Na administration did not affect morphine analgesic effect.



**Fig. 1 – Analgesic effect after subcutaneous administration of morphine, morphine + sodium salt of monoketochoic acid (MKH-Na) and morphine + methyl ester of monoketochoic acid (MKH-Me) before morphine administration and 10, 20, 30, 40, 50, 60, 80, 100 and 120 minutes after morphine administration for the groups C3, E3, E4 and at the corresponding time points for the groups C1, C4 and C5.**

Figure 2 shows the increased analgesic effect of morphine when morphine was administered intramuscularly 20 min after MKH-Me administration. When compared with the



**Fig. 2 – Analgesic effect after intramuscular administration of morphine, morphine + sodium salt of monoketochoic acid (MKH-Na) and morphine + methyl ester of monoketochoic acid (MKH-Me) before morphine administration and 10, 20, 30, 40, 50, 60, 80, 100 and 120 min after morphine administration for the groups C2, E1, E2 and at the corresponding time points for the groups C1, C4 and C5. (\**p* < 0.05).**

group of animals treated only with morphine, a statistically significant increase in analgesic effect was detected 10, 30, 40 and 50 minutes after morphine administration (*p* < 0.05). Pretreatment with MKH-Na did not affect morphine analgesic effect after its intramuscular administration.

## Discussion

Since it is known that morphine molecule containing 3 polar groups hardly passes through the blood-brain barrier (BBB), the potential influence of bile acids derivatives on blood-brain barrier permeability and the influence on analgesic effect of morphine was examined.

In this experiment hydrosoluble sodium salt of monoketochoic acid did not affect morphine analgesic effect regardless the route of morphine administration. Similar findings were obtained in the study of Vasovic et al.<sup>13</sup>, in which no significant interaction of sodium salt of monoketochoic acid and tramadol was detected regarding the antinociceptive effect in healthy mice.

This finding is in accordance with some authors suggesting that liposoluble derivatives of bile acids are more successful in promoting drug transport through biological barriers<sup>1, 15</sup>. The results published by Posa et al.<sup>12, 16</sup>, who studied lidocaine and bile acid derivatives interactions, also support this assertion. However the potential of hydrosoluble salts of bile acids to promote drug transport was also verified<sup>11, 17, 18</sup>.

In our study methylester of monoketochoic acid (liposoluble), given subcutaneously 20 minutes before subcutaneous morphine administration, enhanced morphine analgesic effect although the increase in analgesic effect was not statistically significant when compared to experimental group treated only with morphine. On the other hand methylester statistically significantly increased the analgesic effect of morphine when morphine was administered intramuscularly. This increase in analgesic effect in the period from 10 to 50 min after morphine administration was statistically significant compared to animals treated only with morphine except at the time point of 20 min after morphine administration.

According to Kuhajda et al.<sup>19</sup>, this could be attributed to the formation of two different types of the molecular aggregates between bile acids and morphine. Molecular aggregate of morphine and one molecule of 12-monoketochoic acid (12-MKC) in which morphine binds to the side of the steroid skeleton of 12-MKC, results in a molecular aggregate that is more hydrophobic than the morphine itself what accelerates the ingress of morphine to the lipid phase of the membrane and increases its analgesic effect. The additional increase in analgesic effect results from the formation of the hydrophilic aggregate of the two bile acid molecules with one molecule of morphine in the intracellular space. Since hydrophilic aggregate is less lipophilic than morphine itself the morphine flux directed towards the extracellular space is reduced.

Another possible mechanism to which the increase in analgesic effect could be attributed is the inhibition of P-glycoprotein (P-gp) mediated morphine efflux from the CNS. P-gp, located in brain capillary endothelial cell membranes, may function as a component of the blood-brain barrier<sup>20, 21</sup>.

Morphine may be a substrate for a P-gp, as demonstrated *in vitro* and in knockout mice studies<sup>22, 23</sup>. Disruption of P-gp in brain capillary endothelial cells would in theory result in enhanced accumulation of morphine in the brain. Since it is known that bile acids are also substrates for the P-gp the possible mechanism could involve competition for the P-gp transport system between bile acids and morphine<sup>24, 25</sup>.

One fact in this study remains to be further studied. Although the increase in analgesic effect was detected after subcutaneous administration of methyl ester of monoketocholic acid it was not statistically significant. At the same time a detected increase in analgesic effect was statistically significant after intramuscular administration of methyl ester of monoketocholic acid. Whether this occurred due to higher standard deviation of the measured values in the experimental group treated subcutaneously or there exist some differences in pharmacokinetics related to different routes of administration, it remains to be further studied. According to most of the studies there is no significant difference in absorption of morphine after intramuscular and subcutaneous administration<sup>26, 27</sup>. However some studies suggest there exists a difference in plasma levels 15 min after administration, with higher levels detected after subcutaneous administration. It is possible that due to intense liver metabolism better part of subcutaneously administered dose of morphine is metabolised to morphine-6-glucuronide which possesses a weaker analgesic effect than morphine<sup>28, 29</sup>, or morphine-3-glucuronide which is without analgesic effect at all<sup>30, 31</sup>, and even has the potential of antagonising morphine analgesic effect<sup>32, 33</sup>. In that manner smaller amount of morphine would be available for possible interaction with bile acids at the level of blood-brain barrier. Consequently lower amount of

morphine would be present in CNS resulting in weaker analgesic effect. On the other hand, some sources state that the peak analgesia of morphine occurs within 50–90 min following subcutaneous administration and 30–60 min after intramuscular injection without explaining what is the background of this difference<sup>34</sup>. If this is maybe a consequence of slower or weaker absorption of morphine after subcutaneous administration remains to be further studied.

### Conclusion

According to the results of this study it can be presumed that after intramuscular morphine administration methyl ester of monoketocholic acid increases morphine transport into the central nervous system and the analgesic effect, as well. Further research on bile acids-morphine interaction both *in vitro* and *in vivo* is necessary to completely elucidate the mechanism of this interaction and increase in the morphine analgesic effect.

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