学 位 論 文 の 要 旨

氏名　　榊原　学

学　位　論　文　名　Effects of Intrathecal κ-Opioid Receptor Agonist on Morphine-Induced Itch and Antinociception in Mice

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論 文 内 容 の 要 旨

INTRODUCTION

The mu-opioid receptor (MOR) agonist-induced itch is a serious adverse effect associated with pain treatment administered through systemic, spinal, and epidural routes. The opioid receptor antagonist naloxone can inhibit MOR agonist-induced itch, whereas antihistamine drugs are ineffective as antipruritics. Therefore, it is considered that MOR agonists induce itch mainly through MOR. However, opioid receptor antagonists are unavailable in the clinical setting because they attenuate the antinociceptive effects of MOR agonists. A recent study suggested that systemically administered kappa-opioid receptor (KOR) agonists alleviate intrathecal morphine-induced itch; moreover, systemic KOR agonists in combination with intrathecal morphine produce additive antinociceptive effects against a thermal stimulus in primates. In recent years, the orally administered KOR agonist TRK-820 has been applied to treat of itch in patients undergoing hemodialysis in a clinical setting. However, adverse effects, such as insomnia, induced by systemically administered KOR agonists may limit the usefulness of their antipruritic effects in patients. No report is available regarding the effects of the combination of intrathecally administered KOR and MOR agonists. We therefore investigated the effects of intrathecal TRK-820 on intrathecal morphine-induced itch, antinociception, and sedation.

MATERIALS AND METHODS

All experiments with animals in this study were approved by the Ethics Committee for

Animal Experimentation of Shimane University and they were handled according to our institutional guidelines. The studies were performed in male C57/ BL6 mice (22–27 g). Starting at least 2–3 days before testing, the mice were habituated each day under the same conditions of observation. On the testing day, the mice were individually placed in the observation cage to permit acclimation for approximately 30 min. After acclimation, the mice were administered one of the following treatments intrathecally: morphine (0.1, 0.3, or 1.0 nmol), the selective KOR agonist TRK-820 100 pmol, combination dose of morphine 0.3 nmol + TRK-820 (10, 30, or 100 pmol), and 5 μl of saline as the control. One hour after the intraperitoneal administration of the selective KOR antagonist nor-binaltorphimine (nor-BNI) 1.0 μmol, the effect of TRK-820 100 pmol on intrathecal morphine 0.3 nmol-induced scratching was also tested (n = 6 per group). Scratching behavior was videotaped for 60 min after intrathecal administration. The temporal and total numbers of scratches at various body sites by the hind paws during the first 60 min after intrathecal injection were counted. After observing the scratching behavior, sedation level was evaluated for 60 min by replaying the recorded videotape (n = 6 per group). The nociceptive threshold was determined as previously described by measuring the latency to withdraw the tail, which was immersed in heated water maintained at 48 ± 0.5°C. The mice were gently held in a soft towel, and the tips of their tails were immersed into heated water before and 5, 15, 30, 60, 90, 120, and 150 min after the intrathecal injection of the following agents: morphine (0.1, 0.3, or 1.0 nmol), TRK-820 (10, 30, or 100 pmol), combination dose of morphine 0.1 nmol + TRK-820 10 pmol, and 5 μl of saline as the control (n = 6 per group). If the mice did not remove their tails within 20 seconds (cut-off), the trial was finished to prevent tissue damage, and an upper limit of latency of 20 seconds was recorded.

RESULTS AND DISCUSSION

Intrathecal morphine at 0.3 and 1.0 nmol was associated with significantly higher numbers of scratches compared with that obtained in the saline group. Intrathecal TRK-820 100 pmol did not induce scratching. Intrathecal TRK-820 at doses of 10–100 pmol dose-dependently reduced the scratching induced by intrathecal morphine 0.3 nmol. On the contrary, the combination of intrathecal morphine 0.3 nmol + intrathecal TRK-820 100 pmol did not increase scratching compared with that in the saline group. Intraperitoneal nor-BNI completely inhibited the anti-scratching effect of intrathecal TRK-820 100 pmol. Intrathecal morphine dose-dependently increased the sedation score. The combination of morphine 0.3 nmol and TRK-820 did not alter the sedation score compared with that in the morphine 0.3 nmol group. Intrathecal morphine dose-dependently produced thermal antinociceptive effects. Intrathecal TRK-820 10 pmol did

not produce thermal antinociceptive effects, but TRK-820 30 and 100 pmol produced thermal antinociceptive effects. The combination dose of morphine 0.1 nmol + TRK-820 10 pmol produced significant thermal antinociceptive effects from 5 to 150 min after administration compared with that in the saline group and exerted significant thermal antinociceptive effects compared with the morphine 0.1 nmol group.

The present study uncovered two main findings. First, intrathecal TRK-820, a selective KOR agonist, dose-dependently attenuated intrathecal morphine-induced itch without increasing the sedation level. Second, intrathecal TRK-820 dose-dependently produced antinociceptive effects against a thermal stimulus, and intrathecal TRK-820 augmented intrathecal morphine-produced thermal antinociceptive effects compared with morphine alone.

As previously described, systemic administration of TKR-820, which has been applied in the treatment of itch in the clinical setting, induces side effects such as drowsiness and insomnia at high rates. Our results suggested that intrathecal TRK-820 100 pmol (corresponding to approximately 2 μg/kg) attenuated intrathecal morphine-induced itch without elevating the sedation level. This dosage is about two-fifths of the minimum TRK-820 dosage used for subcutaneous administration, which reduces morphine-induced itch in mice. This result suggests that intrathecal TRK-820 induces fewer adverse effects than those induced by systemic administration in human. It is well known that the systemic administration of morphine, which produces antinociceptive effects through a cerebrospinal pathway, induces respiratory depression and sedation. In contrast, even lower doses of intrathecal morphine exert more potent antinociceptive effects mainly through the spinal cord, but at higher doses, morphine spreads to the brain and causes adverse effects. Therefore, it was suggested that the highest morphine dose (1.0 nmol) caused sedation with a reduction in the number of scratches.

CONCLUSION

This study demonstrated that intrathecal TRK-820 reduces intrathecal morphine-evoked itch without elevating the sedation level. Furthermore, the combination of intrathecal morphine with intrathecal TRK-820 produced more potent antinociceptive effects against a thermal stimulus than those produced by morphine alone.

論文審査及び最終試験又は学力の確認の結果の要旨

甲・乙	氏名	榊原　学
学位論文名	Effects of Intrathecal κ-Opioid Receptor Agonist on Morphine-Induced Itch and Antinociception in Mice	
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論文審査の結果の要旨

モルヒネをはじめとする μ-Opioid Receptor (MOR) 作動薬の脊髄くも膜下腔投与は少量でも強力な鎮痛効果が得られる一方、副作用として約30～70% の患者に強烈な痒みを生じる。これまで動物実験において MOR 作動薬の脊髄くも膜下腔投与による痒みが κ-Opioid Receptor (KOR) 作動薬の全身投与で抑制されることが示唆されているが、詳細については不明な点が多い。そこで本研究では、マウスのくも膜下腔に MOR 作動薬と KOR 作動薬を併用することにより、モルヒネによる痒みの副作用が抑制できるのか、また鎮痛および鎮静における相互作用は認められるのかについて検討した。モルヒネを脊髄くも膜下腔投与したところ、生理食塩液投与の対照群に比べて、0.3～1.0 nmol 投与においてそう痒の指標である引っ掻き行動の有意な増加が認められた。そこで KOR 作動薬である TRK-820 (10～100 pmol) をモルヒネ 0.3 nmol と併用したところ、用量依存的に引っ掻き行動の抑制が認められた。また、KOR 作動薬 TRK-820 によるモルヒネ誘発そう痒作用の抑制効果は KOR 拮抗薬によりその効果が減弱したことから、KOR を介した作用であることが確認された。モルヒネと KOR 作動薬 TRK-820 の併用群では、モルヒネ単独群と比較して鎮静レベルに差は認められなかった一方で、鎮痛効果の増強が認められた。以上のことから、モルヒネ投与時におけるKOR 作動薬の少量脊髄くも膜下腔投与は、鎮静レベルを増悪させることなくモルヒネによる痒みの副作用を抑制し、かつ鎮痛効果を増強させることが明らかになった。これらの知見は、MOR 作動薬使用時におけるKOR 作動薬の鎮痛薬・鎮痒薬としての有用性を明らかにしたのみならず、モルヒネ系鎮痛薬の他の副作用軽減などにもつながる重要な研究であると考えられることから、博士(医学)の学位授与に値するものである。

最終試験又は学力の確認の結果の要旨

申請者は、モルヒネの脊髄くも膜下腔投与でみられる副作用の一つであるそう痒が、κ-Opioid Receptor (KOR) 作動薬の併用により効果的に抑制できることをマウスモデルで証明した。この併用は鎮静レベルには影響を及ぼすことなく、鎮痛作用において相乗的な効果をもたらすことも確認された。モルヒネ系鎮痛薬の臨床応用に関わる重要な知見であり関連知識も豊富であることから、学位授与に値すると判断した。（主査: 和田孝一郎）

申請者は、オピオイドの鎮痛作用を損なうことなく副作用として知られるそう痒を抑制させる方法として、κ-Opioid Receptor (KOR) 作動薬の併用効果に注目し、マウスの脊髄くも膜下腔に注射針を刺し、薬剤を投与するという高度な実験技術を用い、併用が有効であることを示した。公開審査における質疑応答も関連知識が豊富であることを彷彿させるものであり、学位授与に値するものと判断した。（副査 廣田 秋彦）

申請者は、臨床で問題となっているモルヒネの副作用であるそう痒の対応策として、κ-Opioid Receptor (KOR) 作動薬を併用することで痒みを抑制できることを証明した。また、モルヒネとKOR作動薬の併用は鎮静に影響を与えず鎮痛効果を増強することも明らかにした。この結果は今後の臨床応用につながる非常に有用な内容であるため、学位授与に値すると判断した。（副査 竹谷 健）

（備考）要旨は、それぞれ４００字程度とする。