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Cholecystokinin-B Antagonist could Prevent and Reverse Repeated Morphine Injection-induced Antinociceptive Tolerance

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Abstract

Background: Opioids are a class of the most effective analgesics for treating many forms of acute and chronic pain. However, prolonged use of opioid causes analgesic tolerance, withdrawal syndrome, and paradoxical opioid-induced hyperalgesia, which attenuates benefits and hinder the effective use of opioids in human and in animals. Cholecystokinin (CCK) is an anti-opioid endogenous peptide and presumed to be involved in this tolerant-related phenomenon. In this study, we tested if combination with CCK-B receptor antagonist could ameliorate and/or treat the side effects after prolonged morphine treatment.

Materials and Methods: SD rats were subjective to morphine 4 mg/kg, s.c. twice daily for 5 days and developed morphine analgesic tolerance. The morphine-treated rats were allocated into four groups to respectively co-injected with LY225,910 (0.1, 0.5, or 1 mg/kg, s.c.), a potent CCK-B antagonist, or saline 1.0 ml 30 min before every morphine injection. Another two control groups are rats injected with either saline or LY225,910 (1 mg/kg, s.c.) alone for comparison. To test if LY225,910 could reverse opioid tolerance, the morphine-tolerant rats, which had been treated as abovementioned, were divided into three groups to receive single injection of saline, or high-dose LY225,910 (1 mg/kg or 5 mg/kg) on the 5th day. Tail-flick tests were measured every morning after each morphine injection, and "Maximal Possible Effects (MPE)" were calculated for analysis.

Results: Co-treatment with LY225,910 and morphine significantly attenuated morphine tolerance in a dose-dependent manner. Besides, the rats which showed

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Key Words:
Cholecystokinin, CCK antagonist, Morphine tolerance, Tail-flick test.

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antinociceptive tolerance to morphine could partially restore morphine analgesic effect by single injection of high-dose LY225,910 on the 5th day. However, LY225,910 alone did not change nociceptive threshold.

Conclusion: We concluded that LY225,910 could evidently prevent the development of morphine-induced antinociceptive tolerance and modestly reverse analgesic reduction after chronic morphine use in the rats. This finding suggests co-administration of CCK-B antagonist in patients with chronic morphine treatment may improve the morphine analgesic quality.

Conclusion:WeconcludedthatLY225,910couldevidentlypreventthedevelopmentofmorphine-inducedantinociceptivetoleranceandmodestlyreverseanalgesicreductionafterchronicmorphineuseintherats.Thisfindingsuggestsco-administrationofCCK-Bantagonistinpatientswithchronicmorphinetreatmentmayimprovethemorphineanalgesicquality.

Introduction

Morphine and related opioids are a class of the most effective analgesics for treating many forms of acute and chronic pain. Patients treated with morphine have large variations in dose requirement even though they suffered the similar pain states. Besides, utility of opioid analgesics is often hindered by development of analgesic tolerance that necessitates dose escalation regardless of the disease progression. It is now clear that the increased pain sensitivity after injury, such as hyperalgesia [1-5], can shared common mechanisms to those development of opioid tolerance or opioid-induced hyperalgesia [6,7].

In animal studies, repeated daily systemic injections of morphine to mice or rats produced significant rightward shifts in the antinociceptive effect of morphine in nociceptive assays [8,9]. Besides, chronic administration of morphine to nucleus accumbans induced marked tolerance to antinociception [10]. Moreover, sustained or repeated spinal administration of opioids or constant infusion of morphine resulted in abnormal pain states including thermal hyperalgesia and tactile hypersensitivity [11,12]. Large doses of intrathecal morphine have been associated with paradoxical hyperalgesia and hyperesthesias [13], and repeated injections of fentanyl at 15 minute intervals produced a significant hyperalgesia lasting up to 5 days afterward [14]. Both analgesic attenuation and nociceptive hypersensitivity following chronic or prolonged use of opioid have been implicatively attributed to actions of endogenous cholecystokinin release.

Cholecystokinin (CCK) play an important role in modulation of central opiate nociceptive mechanism and development of antinociceptive tolerance to morphine [15,16]. Systemic and spinal morphine resulted in an 89% increase in CCK levels in spinal cord perfusate and blood serum [17,18]. Prolonged exposure to morphine has resulted in an accelerated increase in CCK expression which in turn attenuated the antinociceptive effect of morphine, thus resulting in antinociceptive tolerance [17]. Microdialysis studies also revealed a naloxone-reversible marked increase in extracellular CCK in the frontal cortex of conscious rats after systemic morphine [19].

Therefore, numerous studies have demonstrated that co-administration of CCK antagonists with morphine prevented the development of antinociceptive tolerance [20-22]. Behavioral studies have demonstrated that CCK antiserum and CCK receptor antagonists reverse or prevent morphine tolerance, potentiate the antinociceptive actions of morphine, but did not potentiate morphine in naive rats [16,23-27]. Spinal or systemic CCK blocked antinociception mediated by endogenous opioids and exogenous morphine [28]. CCK antagonists elicited an enhancement of morphine-induced antinociception while producing no antinociceptive activity when given alone [20,28-32]. CCK-B antagonist, L365,260, inactive alone, significantly enhanced the antinociceptive effect of systemic or intrathecal morphine in rats and mice [33,34]. Recent evidences show that CCK-B but not CCK-A receptor antagonists may attenuate opiate dependence and withdrawal [35].

In this study, we conducted a proof-of-concept experiment to testify the possible effect of prevention or reversal of opioid-induced tolerance by co-administering a potent selective CCK-B antagonist, LY225910. Our purpose is to utilize this agent for further investigation of persistent opioid-induced hyperalgesia and responses of nociceptive neuronal activities in a future study.

Materials and Methods:

1. Preparation and EA stimulation

Studies were performed under the approval of the Animal Care and Use Committee and strictly followed the Guidelines for the Care and Use of Experimental Animals of Shin-Kong Memorial Hospital. Male Sprague-Dawley rats (250-350 g) were housed in groups of two to three at an environment of 22 °C with a 12-hr dark-light cycle and water and food pellets available ad libitum.

2. Induction of morphine tolerance

Tolerance to the antinociceptive effect of morphine was induced by using repeated subcutaneous boluses. Morphine was given twice daily, at 9 am and 4 pm, for successive 4 d and once at 9 am on the fifth day morning at the same dose of 4 mg/kg per bolus. Assessment of morphine antinociception was conducted daily using a tail-flick test at 30 min after morphine injection in the morning.

3. Behavioral tests

The routine tail-flick test was made with baseline latencies of 3-5 sec and a cutoff time of 10 sec to assess the antinociceptive effects of morphine. The percentage of maximal possible antinociceptive effect (%MPAE) was calculated by comparing the test latency before [baseline (BL)] and after a drug injection (TL) using the equation: %MPE = [(TL - BL)/(cutoff time- BL)] ´ 100%.

4. Experimental design (Table 1a and 1b)

Rats were randomly allocated into groups receiving morphine alone or morphine with selective CCK-B antagonist, 2-[2-(5-Bromo-1H-indol-3-yl)ethyl]-3-[-(1-methylethoxy- phenyl)-4-(3H)-quinazolinone, LY-225910 (Tocris-Cookson inc, UK), of 3 different doses: 0.1 mg/kg, 0.5 mg/kg and 1 mg/kg. LY225,910 was given subcutaneously 30 min before each morphine injection and this regimen was prepared before study so that the injection volume would be 0.1 ml/kg regardless of the group allocation. Another two control groups of the rats were injected with either saline alone or LY225,910 (1 mg/kg, s.c.) alone for comparison (Table 1a).

In another experiment (Table 1b), we examined the reversal effect of LY225,910 on the established morphine-tolerant rats. The repeated morphine injected rats (the same as the abovementioned) were divided

to receive subcutaneous injection of either saline, LY225,910 (at dose of 1 mg/kg or 5 mg/kg) 30 min before the last morphine injection on the fifth day. One sham control groups were included by injections of normal saline for 4 successive days, and a final injection of morphine at a dose of 4 mg/kg sc in the normal saline group on the 5th day morning.

6. Statitical analysis

Data obtained from the tail-flick test were first calculated to yield mean %MPE. The data for both tail-flick were analyzed by using two-way ANOVA to detect overall differences among treatment groups. When significant main effects were observed, the Dunnett post hoc test were performed to determine sources of differences. A value of $p < 0.05$ was considered statistically significant.

Results

It was found that morphine antinociceptive tolerance developed rapidly after subcutaneous repeated injections at the dose of 4 mg/kg twice daily for 4 days, and the antinociceptive effect significantly dropped to less than 20 % compared to the baseline on the fifth day (Fig 1 and 2). Co-administration of CCK-B antagonist LY225,910 with morphine injections could dose-dependently reduced antinociceptive tolerance, and the effect became evident along time (Fig 1). For the highest dose of LY225910 (1mg/kg) in Fig. 1, tail-flick threshold preserved by 50% in comparison with the baseline level on the fifth day. Note-worthily, repeated injections of LY225,910 alone did not change the nociceptive threshold and the rats could maintain a potent analgesic effect of morphine on the 5th day (Fig. 1).

We also found that LY225,910 may have reversal effect on morphine action in the established morphine-tolerant rats, however, much higher dose of LY225,910 is necessary to produce the reversal effect (Fig.2). LY225,910 co-administration at 1 mg/kg could prevent morphine tolerance (Fig. 1) but single-dose injection had no effect to reverse the antinociceptive tolerance (Fig. 2). A significant, modest reappearance of antinociceptive effect from 8.12% to 25.71% ($p < 0.05$) could be observed after a high-dose (5 mg/kg) injection.

Discussion

It has been well known the physiologically relevant interactions between endogenous CCK and opioid peptides. The present study further proved that the positive modulation of opioid responses by in-activation of CCK-B receptor by antagonist. We found that LY225,910, like other CCK-B antagonists, could prevent morphine tolerance and more importantly, reverse morphine antinociceptive depression.

Alterations of opioid receptors in ligand-receptor interaction following chronic morphine treatment have been suggested as a possible mechanism, including possible alterations in coupling of G-proteins to receptors, or in activities of adenylate cyclase and protein kinases [36-39]. Another mechanism is that activation of excitatory amino acid receptors such as the N-methyl, D-aspartate (NMDA) receptor implicated in the mechanisms of opioid tolerance, particularly m-opioid tolerance, and associated abnormal pain sensitivity [1,40,41]. As pain may be thought of as a ‘‘physiological antagonist of antinociception (or analgesia, clinically)’’ opioid-induced increased pain may manifest as ‘‘opioid tolerance’’ [4,42]. A knock-out study revealed that CCK-B receptor depleted mice exhibited not only spontaneous hyperalgesia to thermal nociception, but also a more severe withdrawal syndrome [43].

Apparently, the balance between endogenous

pronociceptive and antinociceptive systems attenuated the long-term therapeutic goals of morphine and possibly resulted in opioid tolerance and paradoxical opioid-induced hyperalgesia [15,17,26,44,45]. Ossipov and colleagues [46] had termed CCK as an ''endogenous pronociceptive" or "anti-opioid" agent. They summarized from several anatomic studies linking CCK to nociception and addressed that: (1) CCK immunoreactivity is present in the PAG, raphe nuclei, and medullary reticular formation; (2) the distributions of CCK overlap with those of endogenous opioid peptides and opioid receptors in the CNS, implicating complementary roles in nociceptive modulation; (3) CCK is detected in the spinal cord superficial laminae and is derived from descending projections and interneurons, but is not found in peripheral nerves (primary afferent terminals or spinal dorsal root ganglia).

Ossipov et al [46] further explained why CCK functioning as an endogenous pronociceptive agent. First, inrathecal and intra-celebroventricular administration of CCK causes enhanced dorsal horn neuron activity and signs of behavioral hyperalgesia; second, systemic morphine increased expression of CCK in cerebrospinal fluid; third, microinjection of CCK into the RVM enhances sensitivity to normally innocuous mechanical stimulation, thermal stimulation, visceral tests of nociception, and blocks the antinociceptive effect of systemic morphine, possible through mechanism that opposing morphine-mediated excitation of OFF cells versus selectively activating ON cells [47]. Therefore, Ossipov and colleagues' [47] extensive investigations concluded that ''… prolonged exposure to opioids induces neuroplastic changes resulting in enhanced ability of CCK to excite spinopetal facilitatory pathways arising from theRVM.'' Although the specific mechanism of CCK's action is yet unknown, it may ''counteract opioid-induced inhibition of depolarization-induced calcium influx into primary afferent neurons by eliciting mobilization of calcium from intracellular stores, thus maintaining nociceptive neurotransmitter release.'' [45,46]

Conclusion

We concluded that CCK-B antagonist could prevent and treat morphine tolerance-induced hyperalgesia in rats. This finding suggests co-administration of CCK-B antagonist in patients with chronic morphine treatment may improve the morphine analgesic quality.

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Legends

Table 1. Study Protocols

A. Co-administration of morphine and CCK-B antagonist

Group	Drug	Day 1		Day 2		Day 3		Day 4		Day 5
		am	pm	am	pm	am	pm	am	pm	am
Con	NS 1 ml, sc	+	+	+	+	+	+	+	+	M
LY1-Con	LY(1 ml, sc)	+	+	+	+	+	+	+	+	M
Mor	M (4 mg/kg, sc)	M	M	M	M	M	M	M	M	M
LY0.1	LY(0.1 mg/kg, sc)+M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M
LY0.5	LY(0.5 mg/kg, sc)+M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M
LY1	LY(1 mg/kg, sc) +M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M	LY+M
Tail-flick	Behavior test	+		+		+		+		+

B. Post-treatment with CCK-B antagonist for morphine-induced withdrawal

Group	Drug	Day 1		Day 2		Day 3		Day 4		Day 5
		am	pm	am	pm	am	pm	am	pm	am
Con	NS 1 ml, sc	+	+	+	+	+	+	+	+	M
Mor	M (4 mg/kg, sc)	M	M	M	M	M	M	M	M	M
L1.0_post	M+LY(1.0 mg/kg, sc)	M	M	M	M	M	M	M	M	LY+M
L5.0_post	M+LY(5.0 mg/kg, sc)	M	M	M	M	M	M	M	M	LY+M
Tail-flick	Behavior test	+		+		+		+		+

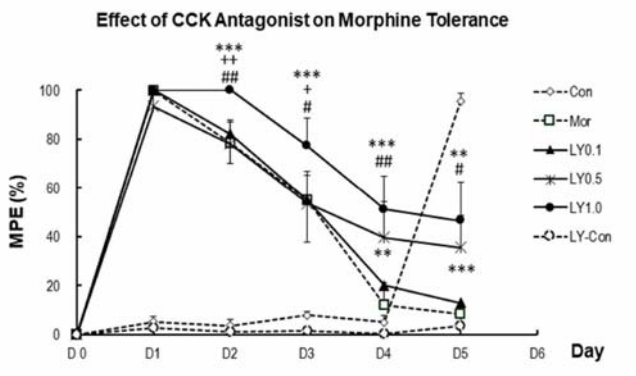

Figure 1. Co-administration with CCK-B antagonist, LY225,910 reduced anti-nociceptive tolerance induced by repeated daily morphine injections. The rat numbers respectively for the groups of the Con, Mor, LY0.1, LY0.5, LY1.0, LY1.0-Con are 7, 8, 8, 6, 6, 6. Abbreviations *: p< 0.05, **: P< 0.01, ***: P< 0.001 for the groups vs Con; +: p< 0.05, ++: P< 0.01 for the groups vs Mor; #: p< 0.05, ## P< 0.01, for the groups vs LY0.5.

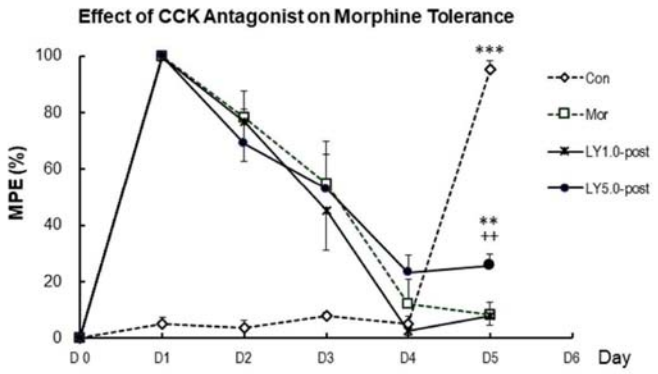

Figure 2. High-dose LY225,910 reversed prolonged morphine-induced hypoalgesia. The rat numbers respectively for the groups of the Con, Mor, LY1.0-post, LY5.0-post, are 7, 8, 5, 5. Abbreviations **: P< 0.01, ***: P< 0.001 for the group vs Mor; ++: P< 0.01 for the group vs LY1.0-post.

膽囊收縮素乙型受體拮抗劑可以預防及逆轉反覆注射嗎啡所誘發之疼痛耐受現象

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背景： 類鴉片製劑是目前臨床上治療急性或慢性疼痛最有效的止痛藥劑。然而，長期使用類鴉片藥物會產生止痛耐受性、戒斷症候、或矛盾地類鴉片誘發疼痛過度等副作用，減弱或阻礙了臨床上的使用。膽囊收縮素 Cholecystokinin (CCK) 是一種反鴉片內源性胜 物質，內認為會參與此耐受性的現象。在本驗證性的研究中，我們重複測試了合併使用乙型膽囊收縮素 (CCK-B) 受體拮抗劑及嗎啡，是否可以改善甚至治療長期嗎啡使用後產生的這類副作用。

材料及方法： SD 大鼠接受嗎啡皮下注射，一天兩次，每次 4 毫克／公斤，連續五天一直到第五天早上，如此會誘發大鼠產生嗎啡止痛耐受性。這些嗎啡鼠分成四組，分別在嗎啡注射前 30 分鐘，接受生理食鹽水 (1 毫升)、或三種不同劑量的強力選擇性 CCK-B 拮抗劑 LY225,910 (0.1, 0.5, or 1 毫克／公斤) 的皮下注射。另外兩組對照組大鼠，分別單獨皮下注射生理鹽水或 LY225,910 (1 毫克／公斤) 作為比較。另一實驗測試 LY225,910 是否可以治療或逆轉已形成的類鴉片耐受性：對前述方式已經產生嗎啡止痛耐受的大鼠，分成三組，在第五天最後一次嗎啡注射前 30 分鐘，分別接受食鹽水、高劑量 LY225,910 (1 毫克／公斤或 5 毫克／公斤) 的單次皮下注射，並比較行為反應。所有大鼠均在每天早上嗎啡注射後，以閃尾試驗測試疼痛閾值改變，最後計算「最大可能效應」(maximal possible effect, MPE) 做為統計的分析。

結果：合併使用 LY225,910 和嗎啡可以明顯抑制嗎啡止痛耐受性，且效果與 LY225,910 的劑量相關。此外，已經產生嗎啡耐受性的大鼠，也可以在第五天利用高劑量的 LY225,910 逆轉或恢復部分的嗎啡止痛效果。然而，LY225,910 本身並不會對疼痛閾值產生改變。

結論：我們認為 CCK-B 拮抗劑可以明顯預防嗎啡反覆注射的止痛耐受性及反轉慢性嗎啡使用後減弱的止痛效果。未來兩者合併，應可以在臨床上幫助慢性疼痛病人長期使用嗎啡的治療品質。

關鍵字： 膽囊收縮素、乙型膽囊收縮素 (CCK-B) 受體拮抗劑、嗎啡耐受性，閃尾試驗