IJBCP International Journal of Basic & Clinical Pharmacology

Research Article

Effect of nalfurafine hydrochloride on the basal pressure of the sphincter of Oddi in anesthetized rabbits

Ryosuke Kobayashi, Kaoru Nakao*, Kiyoshi Yamada, Yohei Miyamoto, Hidenori Mochizuki

Toray Industries, Inc.
Pharmaceutical Research
Laboratories, 10-1, Tebiro
6-chome, Kamakura,
Kanagawa - 248-8555, Japan

Received: 09 February 2015
Accepted: 07 March 2015

*Correspondence to:
Kaoru Nakao,
Email: Kaoru_Nakao@nts.
toray.co.jp

ABSTRACT

Background: Opioid analgesics, which are classified as μ-opioid receptor agonists, are known to induce spasms or contraction of the sphincter of Oddi (SO), thereby inducing or exacerbating biliary diseases such as biliary obstruction, gallbladder dysfunction, cholelithiasis, pancreatitis, biliary dyskinesia, cholangitis, and cholecystitis. However, effects of κ-opioid receptor agonists on SO contraction have not been clarified. In the present study, we investigated the effect of nalfurafine hydrochloride (nalfurafine), (*E*)-*N*-[17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6β-yl]-3-(furan-3-yl)-*N*-methylprop-2-enamide monohydrochloride, a selective κ-opioid receptor agonist, on spontaneous contraction of rabbit SO.
Methods: SO contraction was measured using manometry in anesthetized rabbits. Rabbits were anesthetized with intravenous administration of 25 mg/kg sodium pentobarbital. An open tip catheter was inserted into the common bile duct toward the SO ampullae. Saline was perfused through the lumen of the open tip catheter at a constant rate of 6 ml/hr using a syringe pump. Nalfurafine, morphine, and pentazocine were intravenously (i.v.) administered and perfusion pressure was recorded.
Results: Morphine (0.3 mg/kg, i.v.) and pentazocine (3 mg/kg, i.v.) were found to increase SO perfusion pressure, suggesting that these opioid analgesics may cause SO contraction. In contrast, nalfurafine (0.2 μg/kg, i.v.) decreased the perfusion pressure, indicating that this κ-opioid receptor agonist suppresses SO contraction.
Conclusions: These findings suggest that nalfurafine is unlikely to induce or exacerbate biliary diseases and may be safely used in patients with these disorders.

Keywords: Nalfurafine hydrochloride, TRK-820, κ-opioid receptor, Sphincter of Oddi, Biliary disease

INTRODUCTION

Biliary disease is a generic term for digestive system diseases of the gallbladder, bile duct, pancreas, or pancreatic duct. The sphincter of Oddi (SO) is the smooth muscle of the choledochoduodenal junction that regulates the flow of bile and pancreatic juice into the duodenum and prevents duodenobiliary reflux.[1] It is thought that SO contraction leads to an increase in common bile duct pressure,[2] thereby inducing or exacerbating biliary diseases. In clinical situations, μ-opioid receptor agonists, such as morphine, buprenorphine, and pentazocine are known to induce SO spasms and exacerbate cholestatic and pancreatic disorders. However, the relationship between κ-opioid receptors and SO function has not yet been clarified.

The selective κ-opioid receptor agonist (*E*)-*N*-[17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorp hinan-6β-yl]-3-(furan-3-yl)-*N*-methylprop-2-enamide monohydrochloride, more commonly known as nalfurafine hydrochloride (nalfurafine), has been shown to be well-tolerated and effective in the treatment of pruritus, resistant to existing therapies or treatments in hemodialysis patients.[3-6] Nalfurafine was officially approved for clinical use in January 2009 by the Ministry of Health, Labour, and Welfare of Japan.[3,5] In this study, we investigated whether nalfurafine affected spontaneous SO contraction in anesthetized rabbits.

METHODS

Animals

Male New Zealand white rabbits (body weight: 2.0-2.5 kg; Japan SLC Inc., Shizuoka, Japan) were used. All rabbits were individually housed at room temperature (21-25°C), with 40-70% humidity and 12 hrs light/dark cycles (lights

on at 7:00 AM) for at least 1-week prior to experimentation. Rabbits were given free access to food and water *ad libitum*. All experiments were conducted with the approval of the Animal Experimentation Ethics Committee of Basic Research Laboratories, Toray Industries, Inc. (Tokyo, Japan).

Anesthesia

Rabbits were deprived of food 1-day before testing. On the day of testing, rabbits were anesthetized with intravenous (i.v.) administration of 25 mg/kg sodium pentobarbital to an auricular vein. Additional pentobarbital was administered to maintain a certain level of anesthesia throughout the experiment. Anesthetized rabbits were placed on a warm mat (42°C) in the supine position. Rabbits were intratracheally intubated with an endotracheal tube (16 Fr; Terumo, Tokyo, Japan), connected to a respirator (SN-480-5; Shinano, Tokyo, Japan). Ventilation was performed with a 4-6 ml/kg/ventilation volume range 40-60% ventilation flow and 45-55 rpm/min ventilation rate all of which were adjusted to maintain 7.34-7.45 blood pH, 35-45 mmHg PCO_2, and 80-105 mmHg PO_2 as measured by an I-STAT analyzer (Fuso Pharmaceutical Industries, Ltd., Osaka, Japan).

Cardiovascular monitoring

A polyethylene tube (PE50; Becton, Dickinson and Co., Franklin Lakes, NJ, USA) was inserted and fixed into a jugular vein for infusion of balanced salt solution (vehicle; Solita T-3, Ajinomoto Pharmaceuticals Co., Ltd., Tokyo, Japan) and test drug administration. Another tube (PE50), it was connected to the transducer (DX-300, Nihon Kohden, Tokyo, Japan), was inserted and fixed into a carotid artery for measurement of heart rate and blood pressure. Heart rate and blood pressure were measured using a PowerLab system (AD Instruments, Sydney, Australia) through a heart rate amplifier (AT-601G, Nihon Kohden, Tokyo, Japan) and blood pressure amplifier (AP-641G, Nihon Kohden, Tokyo, Japan), respectively.

Measurement of SO perfusion pressure

To monitor the motility of SO, an abdominal incision was made to expose the duodenum and the common bile duct. A small incision was made at the common bile duct, and a tube (PE50) was inserted toward the gallbladder to drain the bile. An open tip catheter, prepared by modifying a 5Fr ATOM feeding catheter (outer diameter, 1.7 mm), was also inserted into the common bile duct toward the SO ampullae. Saline was perfused through the lumen of the open tip catheter at a constant rate of 6 ml/hr (constant pressure perfusion) using a syringe pump (model 55-1111, Harvard Apparatus, MA, USA). Perfusion pressure was used as an indicator of SO contraction and was recorded using a PowerLab system (AD

Instruments) using the DX-300 transducer (Nihon Kohden) and blood pressure amplifier (AP-641G, Nihon Kohden). In addition, duodenum motility was monitored by a force transducer (F-12IS, Star Medical Inc., Tokyo, Japan) placed on the choledochoduodenal junction, bridge balance box (FB-01, Star Medical Inc., Tokyo, Japan), currier amplifier (AP-601G, Nihon Kohden), and a PowerLab system (AD Instruments).

Test drugs

Nalfurafine (Code No: TRK-820, Toray Industries, Inc.) was dissolved in 5% mannitol solution; nalfurafine was confirmed to be stable in this solution. Morphine hydrochloride hydrate (morphine; Takeda Pharmaceutical Co., Ltd., Osaka, Japan) and pentazocine hydrochloride (pentazocine; Pentagin injection 30, Daiichi Sankyo Co., Ltd., Tokyo, Japan) were diluted with saline. Nalfurafine, morphine, and pentazocine were i.v. administered at 1 ml/kg body weight. Sulfated cholecystokinin-octapeptide (CCK-8, Peptide Institute, Inc., Osaka, Japan) was dissolved in saline and i.v. administered at 0.1 ml/kg body weight.

Test schedule

As shown in Figure 1, after basal perfusion pressure stabilized, perfusion pressure was recorded for 3 mins. After the administration of CCK-8, the perfusion pressure of each rabbit recorded for 3 mins. Only rabbits showing more than 3 mmHg increase in maximum perfusion pressure for 3 mins after administration of CCK-8 compared with pre-CCK-8 values were used for further experiments. After 30 mins or longer, the perfusion pressure of CCK-8-responsive animals was recorded. Furthermore, the vehicle corresponding to each test drug was administered (60 sec i.v. infusion) and the perfusion pressure recorded for 3 mins. After 30 mins or longer, the perfusion pressure of each CCK-8 responsive animal was recorded for 3 mins, then test drugs were administered (60 sec i.v. infusion) and the perfusion pressure recorded for another 3 mins.
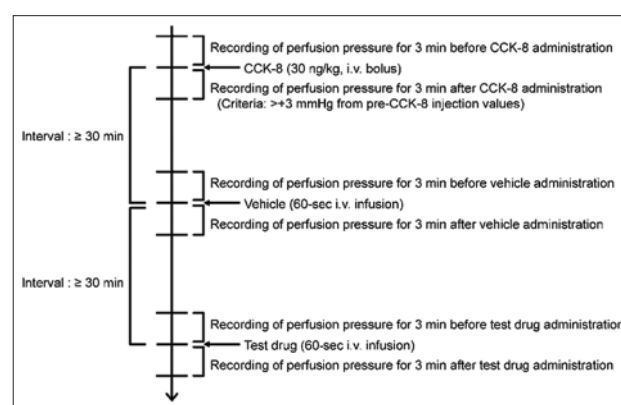

Figure 1: Testing schedule for sphincter of Oddi perfusion pressure recording.

Statistical analysis

All data were expressed as the mean±standard error of the means. Statistical analysis was performed using the percentage change in maximum perfusion pressure calculated by dividing post-dosing maximum perfusion pressure by pre-dosing maximum perfusion pressure. Comparison of vehicle-administration percentage change in maximum perfusion pressure with drug-injection values was performed by paired t-test. The significance level was set to p<0.05.

RESULTS

Effects of morphine and pentazocine on SO perfusion pressure

Morphine (0.3 mg/kg, i.v.) increased the perfusion pressure in 7/17 animals (41.2%). The percent change in maximum perfusion pressure in animals that responded to morphine (n=7) was 97.38±1.59% (average, −0.43 mmHg) with vehicle administration and 135.69±4.86% (average, +3.98 mmHg) with morphine (p<0.0001; Figure 2a). In addition, pentazocine (3 mg/kg, i.v.) increased the perfusion pressure in 7/11 animals (63.6%). The percentage change in maximum perfusion pressure in animals that responded to pentazocine (n=7) was 108.14±5.84% (average, +0.63 mmHg) with

vehicle administration and 160.96±13.05% (average, +6.49 mmHg) with pentazocine (p=0.0093; Figure 2b).

Effect of nalfurafine on SO perfusion pressure

Nalfurafine (0.2 μg/kg, i.v.) did not increase the perfusion pressure in rabbits (0/11 animals). Instead, the maximum perfusion pressure significantly decreased after its administration (Figure 3a and b). The percentage change in maximum perfusion pressure was 99.81±2.02% (average, +0.08 mmHg) with vehicle administration and 93.32±2.21% (average, −0.95 mmHg) with nalfurafine (p=0.0254, n=11).

DISCUSSION

SO regulates the flow of bile and pancreatic juice into the duodenum and prevents duodenobiliary reflux.[1] It is thought that SO contraction increases common bile duct pressure.[2] Therefore, it is suggested that an increase in SO contractions induces or exacerbates biliary diseases such as biliary obstruction, gallbladder dysfunction, cholelithiasis, pancreatitis, biliary dyskinesia, cholangitis, and cholecystitis. Moreover, Hastier et al. indicated that primary biliary cirrhosis is exacerbated by stenosis of SO.[7]

In clinical situations, μ-opioid receptor agonists such as morphine, buprenorphine, and pentazocine are known to induce SO spasms and exacerbate cholestatic and pancreatic disorders.[8] However, these opioid analgesics are often used for abdominal pain relief in biliary disease patients,[9] because they are the most effective pain relievers available to date. The selective κ-opioid receptor agonist nalfurafine has been shown to be well tolerated and effective for pruritus resistant to existing therapies or treatments in hemodialysis patients.[3-6] However, because effects of κ-opioid receptor agonists on SO contraction have not been clarified, we attempted to investigate whether nalfurafine would affect the spontaneous contraction of SO using anesthetized rabbits.

Morphine and pentazocine are typically administered at 10 and 60 mg/body/time i.v. and nalfurafine has been shown to have an antipruritic effect in hemodialysis patients at 5 μg/body/time i.v.[6] Therefore, in the present study, we used 0.2 μg/kg nalfurafine, 0.3 mg/kg morphine, and 3 mg/kg pentazocine (Table 1), so that the ratio of the injected dose of each drug was clinically relevant. Furthermore, because CCK-8 was reported to increase myoelectric activity of SO in anesthetized rabbits,[10] it was used for the selection of drug-responsive rabbits.
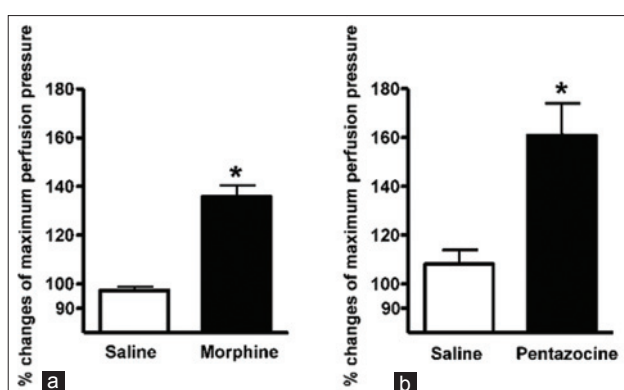

Figure 2: Effects of morphine (a) and pentazocine (b) on sphincter of Oddi perfusion pressure. Only rabbits showing more than 3 mmHg increase in maximum perfusion pressure 3 mins after administration of CCK-8 compared with pre-CCK-8 values were used for further experiments. Vehicle (saline) was administered to CCK-8-responsive rabbits, followed by intravenous (i.v.) morphine (0.3 mg/kg; a) or i.v., pentazocine (3 mg/kg; b). Each administration was performed at intervals of 30 mins or longer. The percent change in maximum perfusion pressure was calculated by dividing post-drug injection maximum perfusion pressure by the pre-drug values. Each column represents mean±standard error of the means (n=7, all CCK-8-responsive); *p<0.05 compared with vehicle.

In this study, 0.3 mg/kg, i.v. morphine and 3 mg/kg, i.v. pentazocine were found to increase the perfusion pressure, indicating SO contraction, similar to effects previously
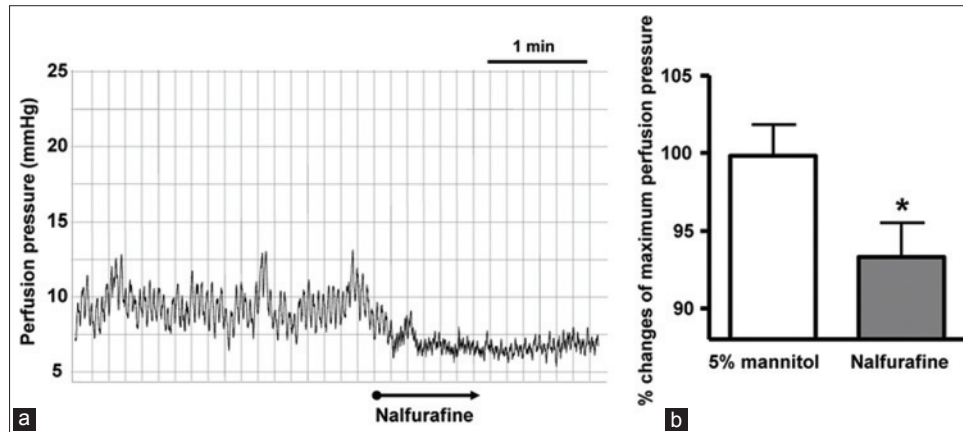
Figure 3: Effect of nalfurafine on sphincter of Oddi perfusion pressure. Only rabbits showing more than 3 mmHg increase in maximum perfusion pressure for 3 mins after administration of CCK-8 compared with pre-CCK-8 values were used for further experiments. Vehicle (5% mannitol) was administered to CCK-8-responsive rabbits, followed by intravenous (i.v.) nalfurafine (0.2 μg/kg). Each administration was performed at intervals of 30 mins or longer. Perfusion pressure recording before and after i.v., infusion of nalfurafine (0.2 μg/kg; a). The percentage change in maximum perfusion pressure was calculated by dividing the post-injection maximum perfusion pressure with the pre-dosing value (b). Each column represents mean±standard error of the means (n=11); *p<0.05 compared with vehicle.

Table 1: Test drug doses in the present study and in clinical use.

Test drug	Present study (i.v.)		Clinical dose (i.v.)	
	Dose	**Ratio**	**Dose**	**Ratio**
Nalfurafine	0.2 μg/kg	1	5 μg/body*	1
Morphine	0.3 mg/kg	1500	10 mg/body	2000
Pentazocine	3 mg/kg	15,000	60 mg/body	12,000

*Data from Wikström et al.[6]. i.v.: Intravenously

observed in humans.[2] Therefore, it is suggested that opioid-induced SO contraction in rabbits reflects that of human SO, although SO resists the flow of pancreatic juice into the duodenum in humans, it acts as a pump in rabbits.[1] On the contrary, 0.2 μg/kg, i.v. nalfurafine decreased the perfusion pressure, indicating a suppressive effect on SO contraction. Thus, nalfurafine is expected to have therapeutic potential for biliary diseases.

Morphine has been shown to mainly act as a μ-opioid receptor agonist *in-vitro*,[5,11] while pentazocine was shown to act as a mixed μ- and κ-opioid receptor partial agonist *in-vitro*.[11] In addition, other opioid analgesics, such as buprenorphine, fentanyl, and oxycodone, have agonistic activity on the μ-opioid receptor. Helm et al. reported that naloxone, a μ-opioid receptor antagonist, inhibits the increase in phasic wave frequency and amplitude elicited by morphine in a human SO manometric study, suggesting that SO contraction may be induced by activation of μ-opioid receptors.[2] In our previous study, it was clear that nalfurafine act as a potent and selective κ-opioid receptor agonist.[5,12] It has also been reported that activation of κ-opioid receptors have an effect opposite to that of μ-opioid receptors in a variety of pharmacological actions, including analgesia,

tolerance, reward, mesolimbic dopamine level, learning and memory processes, long-term potentiation, subjective effects, hippocampal epilepsy, bladder motility, and diuresis.[13] Thus, it is reasonable that the potent and selective κ-opioid receptor agonist nalfurafine was suppressive to SO contraction in the present study, unlike in μ-opioid receptor agonists, morphine, and pentazocine. In addition, because nalfurafine has been confirmed to have analgesic effects in various pain models,[14-21] it is expected to be safely used for relief of abdominal pain in biliary disease patients.

In conclusion, the present study has demonstrated that nalfurafine suppresses SO contraction in anesthetized rabbits, suggesting that nalfurafine, unlike μ-opioid receptor agonists, is unlikely to induce or exacerbate biliary diseases and that it may be safely used in patients with these disorders. Furthermore, considering that nalfurafine suppresses SO contractions, which induce or exacerbate biliary diseases, this drug may also have therapeutic potential for these disorders.

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