Pharmacological Evaluation of Analgesic Effects of the Cholecystokinin₂ Receptor Antagonist Z-360 in Mouse Models of Formalin- and Cancer-Induced Pain

Koji YOSHINAGA,*^a Takayuki HORII,^a Hiroki HAMANO,^a Runa ETA,^a Tomoko OZAKI,^a Yuki ORIKAWA,^a Kazuyoshi YOSHII,^a Yoshihiro KAWABATA,^a Yuko HORI,^a Koichi SETO,^a Mineo TAKEI,^a and Yasushi KURAISHI^b

^a Central Research Laboratories, Zeria Pharmaceutical Co., Ltd.; 2512-1 Numagami, Oshikiri, Kumagaya, Saitama 360-0111, Japan; and ^b Department of Applied Pharmacology, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama; 2630 Sugitani, Toyama 930-0194, Japan.

Received October 9, 2009; accepted November 26, 2009; published online November 30, 2009

Z-360, a novel cholecystokinin₂ (CCK₂) receptor antagonist, has been developed as a therapeutic drug for pancreatic cancer and showed pain relief action in phase Ib/IIa clinical trial. This study was attempted to elucidate the analgesic efficacy of Z-360 in mice. Oral administration of Z-360 (30–300 mg/kg) showed a dose-dependent inhibitory effect on the late phase of nociceptive responses to formalin. YF476, another CCK₂ receptor antagonist, was without effects at 1 and 10 mg/kg. In contrast, the CCK₁ receptor antagonist devazepide inhibited the nociceptive responses to formalin. In a mouse model of cancer pain, significant anti-allodynic effect of Z-360 was observed after single and repeated oral administration of 100 and 300 mg/kg doses. Anti-allodynic effect was also observed after repeated administration of devazepide. Combined single treatment with morphine and Z-360 caused an increase inhibition of pain-related responses in the pain models produced by formalin and cancer. Although Z-360 has lower affinity for CCK₁ receptor than for CCK₂ receptor, Z-360 exhibited an inhibitory effect on sulfated CCK-8-induced gallbladder emptying, a CCK₁ receptor-mediated effect, at a dose of 100 mg/kg. These results suggest that Z-360 inhibits inflammatory and cancer pain probably through the blockade of CCK₁ receptors. Z-360 is expected to become a useful drug for the pancreatic cancer with analgesic effects as well as the prolongation of survival.

Key words formalin-induced pain; cancer pain; pancreatic cancer; cholecystokinin receptor; Z-360

It has been reported that pain occurs in 30% of patients with early cancer, 60 to 70% of patients with advanced cancer, and 75% or more of those with terminal cancer.¹⁾ Pain is one of the most prevalent conditions that diminish the quality of life. A three-step pain elimination ladder has been established by the World Health Organization as a worldwide standard therapeutic program for cancer pain, but there is still pain that remains beyond control with opioids and non-steroidal antiinflammatory drugs. Therefore, to achieve a breakthrough in combating cancer pain, energetic efforts have been made to develop novel analgesics with fewer adverse effects and new mechanisms of action.^{2–4)}

Z-360, calcium bis [(R)-(-)-3-[3-{5-cyclohexyl-1-(3,3-dimethyl-2-oxo-butyl)-2-oxo-2,3,4,5-tetrahydro-1H-benzo[b][1,4]diazepin-3-yl}ureido]benzoate], an orally active cholecystokinin₂ (CCK₂) receptor antagonist, is under development for the treatment of pancreatic cancer. In our previous studies, Z-360 inhibited pancreatic tumor growth and prolonged survival by combination with gemcitabine, a first-line drug for pancreatic cancer, in mouse models of pancreatic cancer xenograft.^{5,6)} In a phase Ib/IIa clinical trial in patients with advanced unresectable pancreatic cancer, pain relief was observed with Z-360 administration.⁷⁾ Pain is experienced by most patients with advanced pancreatic cancer and limits their daily activity. Z-360 might be useful in achieving pain relief for patients with pancreatic cancer. However, the analgesic action of Z-360 is not yet elucidated in animal models. In this study, we evaluated the antinociceptive and anti-allodynic effects of Z-360 in mouse models of formalin-induced pain and cancer pain, respectively.

MATERIALS AND METHODS

Animals Four to five-week-old male Crlj:CD1 (ICR) mice and five-week-old male C57BL/6N^{Cr}lCrlj (C57BL/6) ones and male Crl:CD (SD) rats were purchased from Charles River Laboratories Japan Inc. (Yokohama). They were kept under controlled temperature (23±3 °C) and humidity (55±20%). The room was lighted from 7:00 a.m. to 7:00 p.m. Food and water were freely available. Pain studies were carried out in accordance with guidelines outlined by the Committee for Research and Ethical Issues of International Association for the Study of Pain.⁸⁾ In addition, all experimental procedures were approved by the Institutional Animal Care and Use Committee of Zeria Pharmaceuticals Central Research Laboratories. Pain tests were carried out between 9:00 a.m. to 4:00 p.m.

Drugs Z-360 and YF476, (R)-1-[2,3-dihydro-2-oxo-1-pivaloylmethyl-5-(2-pyridyl)-1H-1,4-benzodiazepin-3-yl]-3-(3-methylaminophenyl)urea, were synthesized at the Central Research Laboratories of Zeria Pharmaceutical Co., Ltd. (Kumagaya). [¹⁴C]Z-360 (2.42 MBq/mg) was synthesized by Nemoto Science Co., Ltd. [¹⁴C]Sucrose (124 MBq/mmol) and [³H]H₂O (37 MBq/g) were purchased from NEN Life Science Products, Inc. Devazepide was purchased from Tocris Bioscience Inc. (St. Louis, MO, U.S.A.). Z-360 and devazepide were suspended in 0.5% (w/v) carboxymethyl cellulose sodium solution before use. Morphine hydrochloride was purchased from Daiichi-Sankyo Co., Ltd. (Tokyo) and diluted with physiological saline before use; weight of morphine refers to the salt.

* To whom correspondence should be addressed. e-mail: kouji-yoshinaga@zeria.co.jp

Formalin-Induced Pain Model Formalin-induced pain model in mice was carried out according to the method of Tjolsen *et al.*⁹⁾ The ICR mice were acclimated to the acrylic cages for 30 min before the formalin injection. Formalin (20 μ l of 5% (v/v) formalin solution in saline) was injected subcutaneously into the planter region of the right hind paw (intraplantar injection). Nociceptive response was quantified by the measuring the time spent in licking and biting the injected paw every 5 min with a stopwatch. The responses to formalin showed two phases; early (phase 1) and late (phase 2) phases of responses were recorded 0–5 and 10–40 min after formalin injection, respectively. The total time spent engaging in these responses was calculated in each phase. Z-360, YF476 or devazepide was administered orally 30 min prior to formalin injection. Morphine was administered subcutaneously 15 min prior to formalin injection.

Cancer Pain Model A mouse model of cancer pain was made according to the method of Sasamura *et al.*¹⁰⁾ B16/BL6 cells (Cell Resource Center for Biomedical Research, Tohoku University), melanoma cells derived from C57BL/6 mouse, were cultured in RPMI1640 medium containing 10% fetal bovine serum at 37 °C and a humidified atmosphere of 5% CO₂. The cells (2×10^5 cells/20 μ l) were injected into the planter region of the right hind paw of C57BL/6 mice. The paw withdrawal thresholds in response to probing with calibrated von Frey filaments were determined in the manner described by Chaplan *et al.*¹¹⁾ The B16/BL6 transplanted mice were individually placed in suspended cages with wire mesh bottoms and allowed to acclimate to their environment for at least 30 min. The hind paw was stimulated with a series of von Frey filaments applied to the planter surface. Stimulation was initiated with the 0.4 g filament and the 50% paw withdrawal threshold was determined by the non-parametric method of Dixon.¹²⁾ Z-360 was administered orally once a day from day 7 after transplantation and paw withdrawal threshold was measured 2 h after administration on day 7, 14 and 21. Regarding the combination test of Z-360 and morphine, animals were given single injections of these drugs on day 14 after transplantation; Z-360 was administered orally 60 min prior to the von Frey test and morphine was administered subcutaneously 15 min before. Devazepide was administered orally once a day from day 7 after transplantation and paw withdrawal threshold was measured 2 h after administration on day 14.

Sulfated Cholecystokinin-8 Induced Emptying of the Gallbladder Emptying of the gallbladder was induced by an intraperitoneal injection of sulfated cholecystokinin-8 (100 ng/kg) into fasted male ICR mice 15 min before the gallbladder isolation.¹³⁾ Z-360 was administered orally 60 min prior to sulfated cholecystokinin-8 injection. The gallbladder was removed under ether anesthesia and weighed.

Brain Uptake Index (BUI) of [¹⁴C]Z-360 or [¹⁴C]Sucrose The influx of [¹⁴C]Z-360 across the blood brain barrier (BBB) was determined by the brain uptake index (BUI) method reported previously.¹⁴⁾ An aliquot of 200 μ l Ringer's/*N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.4, 5 mM HEPES) was injected rapidly into the left common carotid artery. The injection solution contained [¹⁴C]Z-360 (92 kBq/ml) and [³H]H₂O (186 kBq/ml). In the BUI studies with [¹⁴C]sucrose, [¹⁴C]su-

crose (130 kBq/ml) and [³H]H₂O (194 kBq/ml) was dissolved in the injectate. Fifteen seconds after the carotid artery injection, the rats were decapitated. The radioactivity in the injection solution and the ipsilateral hemisphere to the injection were determined. The BUI value obtained as follows:

BUI (%)

$$= \frac{\text{amount of test drug in the brain}}{\text{amount of reference in the brain}} \div \frac{\text{amount of test drug in the injectate}}{\text{amount of reference in the injectate}}$$

In these experiments, [³H]H₂O was used as a reference compound.

Statistical Analysis Results are expressed as the mean $\pm$ standard error. Data were analyzed with SAS System Version 8.2 (SAS Institute Japan Ltd., Tokyo) using the *t*-test, Welch's test, Wilcoxon signed-rank test, parametric Williams test, parametric Dunnett's test, non-parametric Dunnett's test and non-parametric Tukey's test. Differences with a *p* value of less than 0.05 were considered statistically significant.

RESULTS

Effects of Z-360 in Formalin-Induced Pain Model

Oral administration of Z-360 showed 5.0, 23.2 and 37.5% inhibition of the phase 2 responses at doses of 30, 100 and 300 mg/kg, respectively, with significant effect at 300 mg/kg (Fig. 1A, parametric Williams test). Z-360 did not affect

Fig. 1. Effects of Z-360 on Formalin-Induced Pain in Mice

ICR mice were given an intraplantar injection of 5% (v/v) formalin and the licking and biting time was determined during the phase 1 (0–5 min) and phase 2 (10–40 min) periods. Z-360 (A and B, oral) and morphine (B, subcutaneous) were administered 30 and 15 min, respectively, before formalin injection. Values are represented as the mean $\pm$ standard error. *n* = 9–10 (A) and 12 (B). Data are analyzed with parametric Williams test (A) and non-parametric Tukey's test (B) for multiple comparisons. #*p* < 0.05, ##*p* < 0.01 as compared with vehicle.

the phase 1 responses. Z-360 (300 mg/kg) and morphine (2 mg/kg) administered alone inhibited the phase 2 responses by 47.3% and 46.0%, respectively, and the inhibition rate increased to 71.9% when both drugs were administered together (Fig. 1B, non-parametric Tukey's test).

Effects of CCK Receptor Antagonists in Formalin-Induced Pain Model There was a tendency toward a reduction in phase 2 response to formalin after administration of YF476 at an oral dose of 1 mg/kg, but the higher dose of 10 mg/kg did not affect the phase 2 response (Fig. 2A, parametric Dunnett's test). Oral administration of devazepide (0.1–10 mg/kg) showed a marked inhibition of phase 2 response, with significant effects at doses of 1 and 10 mg/kg (Fig. 2B, parametric Dunnett's test). The phase 1 responses were not affected by YF476 and devazepide at doses tested (Fig. 2).

Effects of Z-360 on the Mechanical Allodynia in Cancer Pain Model Transplantation of B16/BL6 caused allodynia, a significant and marked decrease in pain threshold for mechanical stimulation, in the region around the tumor mass from day 4 to at least day 21 after transplantation. Z-360 was administered orally once a day from day 7 after transplantation and paw withdrawal threshold was measured 2 h after administration on day 7, 14 and 21. On day 7, single administration of doses of 100 and 300 mg/kg produced slight but significant inhibition of allodynia (Fig. 3A, non-parametric Dunnett's test). Anti-allodynic effect was further enhanced by repeated administration of a dose of 100 mg/kg on day 14 and 21 (Fig. 3A, non-parametric Dunnett's test). The combination effect of single injections of Z-360 and morphine was examined on day 14 after transplantation. Although neither morphine (2.5 mg/kg) nor Z-360 (100 mg/kg) alone exhibited

a significant anti-allodynic effect, a combination of these drugs showed significant anti-allodynic effect (Fig. 3B, non-parametric Tukey's test).

The Effect of Devazepide in Cancer Pain Model In these series of experiments, repeated oral administration of Z-360 (100 mg/kg) produced a significant inhibition of cancer-induced allodynia again (Fig. 4, non-parametric Dunnett's test). Repeated oral administration of devazepide (1, 10 mg/kg) showed a dose-dependent anti-allodynic effect,

Fig. 2. Effects of CCK Receptor Antagonists on Formalin-Induced Pain in Mice

Pain-related responses to formalin were measured as described in the Fig. 1 legend. YF476 (A) and devazepide (B) were administered orally 30 min before injection of formalin. Values are represented as the mean $\pm$ standard error ($n=11-12$). Data are analyzed with parametric Dunnett's test for multiple comparisons (vs. vehicle, $*p<0.05$).

Fig. 3. Effect of Z-360 on the Mechanical Allodynia in Cancer Pain Model

B16/BL6 cells were injected into the plantar region of the hind paw of C57BL/6 mice. Paw withdrawal threshold for mechanical stimulation were determined with von Frey filaments. (A) Z-360 was administered orally once a day from day 7 after transplantation and the withdrawal threshold was measured 2 h after administration on day 7, 14 and 21. Values are represented as the mean $\pm$ standard error ($n=9-12$). Data are analyzed using non-parametric Dunnett's test (vs. vehicle, $*p<0.05$, $##p<0.01$, $###p<0.001$). (B) On day 14 after transplantation, morphine and Z-360 were administered at subcutaneous dose of 2.5 mg/kg and oral dose of 100 mg/kg, respectively. The withdrawal threshold were measured before and 15 and 60 min after morphine and Z-360, respectively. Values are represented as the mean $\pm$ standard error ($n=11-12$). Data are analyzed using non-parametric Tukey's test for multiple comparison (vs. vehicle, $*p<0.05$).

Fig. 4. Effects of Devazepide on Cancer Pain in Mice

B16/BL6 cell transplantation and the determination of paw withdrawal threshold were performed as described in Fig. 3 legend. Z-360 and devazepide were administered orally once a day from day 7 after transplantation and the withdrawal threshold was measured 2 h after administration on day 14. Values are represented as the mean $\pm$ standard error ($n=10-12$). Data are analyzed using non-parametric Dunnett's test (vs. vehicle, $*p<0.05$).

Fig. 5. Effect of Z-360 on Sulfated Cholecystokinin-8 Induced Emptying of the Gallbladder in Fasted Mice

Z-360 was administered orally 1 h before the sulfated cholecystokinin-8 injection. Sulfated cholecystokinin-8 (100 ng/kg) was administered intraperitoneally 15 min before the gallbladder isolation except the normal group. Gallbladder was removed and weighed. Values of gallbladder wet weight are represented as the mean $\pm$ standard error ($n=8$). Data are analyzed with *t*-test (normal vs. vehicle, *** $p<0.001$) or parametric Dunnett's test for multiple comparison (vs. vehicle, ### $p<0.001$).

Table 1. Brain Uptake Index (BUI) for [^{14} C]Z-360 and [^{14} C]Sucrose

	BUI (%)
[^{14} C]Z-360	10.17 $\pm$ 3.14
[^{14} C]Sucrose	7.98 $\pm$ 2.75

Mean $\pm$ standard error ($n=5$). An aliquot of 200 μ l Ringer's/HEPES buffer containing [^{14} C]Z-360 or [^{14} C]sucrose in the presence of [^{3} H]H₂O was injected rapidly into the left common carotid artery as indicated under Materials and Methods. Fifteen seconds after injection, the rats were decapitated, and the radioactivity of the injection solution and the ipsilateral hemisphere were determined.

with significant effect at 10 mg/kg (Fig. 4, non-parametric Dunnett's test).

The Effect of Z-360 on Sulfated Cholecystokinin-8 Induced Emptying of the Gallbladder An intraperitoneal injection of cholecystokinin-8 induced marked emptying of the gallbladder in fasted mice. It was dose-dependently reversed by oral administration of Z-360 (10–100 mg/kg), with significant effect at a dose of 100 mg/kg (Fig. 5, parametric Dunnett's test).

The Influx of [^{14} C]Z-360 to the Brain across the BBB By means of the *in vivo* carotid artery injection technique, the BBB influx for [^{14} C]Z-360 and [^{14} C]sucrose were determined. As shown in Table 1, the BUI of [^{14} C]Z-360 was obtained, *i.e.*, 10.17%, which was not significantly different from that of [^{14} C]sucrose, used as a vascular space marker (Table 1).¹⁵⁾

DISCUSSION

Rodent model of formalin-induced pain is frequently used to evaluate behavioral response to noxious stimulation because it closely resembles human responses to painful stimuli.^{16,17)} The nociceptive response to formalin shows two phases. The early phase (phase 1) seems to be caused predominantly by C-fiber activation due to the peripheral stimulus, while the late phase (phase 2) appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord.⁹⁾ In the present study, we found that single

administration of Z-360 (30–300 mg/kg) showed dose-dependent inhibitory effects on the phase 2 response, without effects on the phase 1 response. Thus, the results raise the possibility that the inhibitory effect of Z-360 is due to the modulation of peripheral and/or spinal processes of inflammatory pain but not due to the direct inhibition of nociceptive primary afferents.

Z-360 has potent CCK₂ receptor antagonist activity.^{5,6,18)} To determine the role of CCK₂ receptor blockade in the antinociceptive effect of Z-360, we evaluated whether another CCK₂ receptor antagonist YF476 would suppress phase 2 of formalin-induced nociceptive responses. YF476 has been shown to inhibit pentagastrin-stimulated gastric acid secretion, a CCK₂ receptor-mediated process, in dogs in dose-dependent manner with ED₅₀ value of 0.02 μ mol/kg (0.05 mg/kg).¹⁹⁾ Furthermore, YF476 showed the approximate 80% inhibition on pentagastrin-stimulated gastric acid secretion in rats at an intraduodenal dose of 1 mg/kg (unpublished observation, Yoshinaga *et al.*). With these findings taken into account, the results that YF476 did not produce a significant inhibition of phase 2 of formalin-induced pain at oral doses of 1 and 10 mg/kg argue against the involvement of CCK₂ receptor in the antinociception. Z-360 almost completely inhibits pentagastrin-stimulated gastric acid secretion in rats at an intraduodenal dose of 3 mg/kg.¹⁸⁾ In this study, Z-360 at an oral dose of 30 mg/kg did not inhibit phase 2 of formalin-induced pain. Thus, these results suggest that the antinociceptive effect of Z-360 is not primarily mediated by the blockade of CCK₂ receptor.

In vitro experiments revealed that Z-360 has affinity for both recombinant human CCK₂ and CCK₁ receptors with K_i of 0.47 and 316 nM, respectively.⁶⁾ Plasma concentrations of Z-360 are much higher than the K_i value for CCK₁ receptor; 1400–4000 nM are the concentrations 0.25–2 h after oral dose of 100 mg/kg.⁶⁾ Z-360 has no binding affinity (concentration that required 50% influence at $>10^{-6}$ M) for other nociception- and antinociception-related receptors, channels and enzymes. From these findings, we speculated that CCK₁ receptor antagonistic activity of Z-360 would be involved in the antinociceptive action, though affinity for the CCK₁ receptor is low. Z-360 exerted a dose-dependent inhibitory effect on cholecystokinin-8 induced gallbladder emptying, a CCK₁ receptor-mediated effect, at doses of 10–100 mg/kg, with a significant inhibition at 100 mg/kg. Devazepide, a CCK₁ receptor antagonist, showed the inhibition as well as Z-360 in this gallbladder emptying model. These results suggest that CCK₁ receptor blockade is conducive to the pharmacological action of Z-360 at oral doses of 100 mg/kg and over. Devazepide showed antinociceptive effects on formalin-induced pain after oral doses of 1 mg/kg and over, results suggesting that CCK₁ receptor blockade leads to the inhibition of formalin-induced pain. These results suggest that the antinociceptive effect of Z-360 is mediated *via* antagonistic action on CCK₁ receptor, rather than CCK₂ receptor.

To evaluate the effects of drugs for cancer pain and to develop more appropriate therapeutic modalities, it is important to use animal models of cancer pain produced by transplanting tumors.^{10,20–24)} Therefore, the analgesic effect of Z-360 was evaluated using a mouse model of cancer pain produced by orthotopic transplantation of B16/BL6 melanoma cells. It was found that Z-360 showed anti-allodynic effect at doses

of 100 mg/kg and over. The CCK₁ receptor antagonist devazepide (10 mg/kg) also had anti-allodynic effect in this model, suggesting that the suppressive effects of Z-360 (and also devazepide) are mediated at least by the blockade of CCK₁ receptor.

In this study, the influx rate of Z-360 was determined by the intracarotid artery injection technique and was an extremely low BUI value similar to that of sucrose used as a vascular space marker (Table 1).¹⁵⁾

Taken together these results indicated that the analgesic effects of Z-360 are mediated by CCK₁ receptor antagonistic action and may not be involved in central nerve system.

Several studies reported that CCK₂ receptor antagonists, L-365,260 and CI-988, which can penetrate into the brain, showed the potentiation of morphine analgesia.^{25–28)} Z-360 hardly penetrate into the brain and analgesic effects of Z-360 may be due to the blockade of CCK₁, rather than CCK₂, receptors. Therefore, the mechanism of the potentiation of morphine analgesia by Z-360 may differ from other CCK₂ receptor antagonists such as L-365,260 or CI-988.

The B16/BL6 cancer pain model is considered to be resistant to existing analgesics. Pain-related responses of this model are inhibited by relatively high doses of morphine, gabapentin and ET_A endothelin receptor antagonist,^{10,21,29)} but not respond to by nonsteroidal antiinflammatory drugs such as diclofenac or to other drugs used clinically, such as mexiletine, clonidine, suramin, and baclofen.²¹⁾ The notable feature of this cancer pain model is the intensity of mechanical allodynia. Allodynia was much higher in this cancer model than in the neuropathic model by herpes simplex virus type-1 infection in mice.²²⁾ Since mechanical allodynia exerts a profound influence on the quality of life of cancer patients, it may be a good index for the screening of cancer-pain killers.

In clinical study, Z-360 at oral doses of 120 mg and 240 mg showed safety and well-tolerated profiles, and pain relief action in patients with pancreatic cancer.⁷⁾ When Z-360 orally administered at a dose of 120 mg to human, the maximum plasma concentration of Z-360 was approximately 3000 nM. This plasma concentration of Z-360 was similar to that at oral administration of Z-360 at a dose of 100 mg/kg to mice.⁶⁾ These results suggest that Z-360 exert CCK₁ receptor antagonistic action in human. Therefore, analgesic effects of Z-360 at doses of 100 mg/kg and 300 mg/kg in these mice pain model are considered to associate with the effectiveness of Z-360 for cancer pain relief in patients with pancreatic cancer.

In conclusion, this study suggests that Z-360 has the analgesic effects on inflammatory and cancer pain and that the effect is mediated by blockade of CCK₁ receptor. Z-360 is expected to become a useful drug for the treatment of pancreatic cancer with analgesic effects as well as the prolonga-

tion of survival.

REFERENCES

- 1) Bruera E., Lawlor P., *Acta Anaesthesiol. Scand.*, **41**, 146–153 (1997).
- 2) Negus S. S., Vanderah T. W., Brandt M. R., Bilsky E. J., Becerra L., Borsook D., *J. Pharmacol. Exp. Ther.*, **319**, 507–514 (2006).
- 3) Mouedden M. E. I., Meert T. F., *Pharmacol. Biochem. Behav.*, **86**, 458–467 (2007).
- 4) Colvin L., Fallon M., *Eur. J. Cancer*, **44**, 1083–1090 (2008).
- 5) Grabowska A. M., Morris T. M., McKenzie A. J., Kumari R., Hamano H., Emori Y., Yoshinaga K., Watson S. A., *Regul. Pept.*, **146**, 46–57 (2008).
- 6) Kawasaki D., Emori Y., Eta R., Iino Y., Hamano H., Yoshinaga K., Tanaka T., Takei M., Watson S. A., *Cancer Chemother. Pharmacol.*, **61**, 883–892 (2008).
- 7) Meyer T., Calpin M., Palmer D., Valle J. W., Larvin M., Waters J., Coxon F., Borbath I., Peeters M., Nagano E., Kato H., *J. Clin. Oncol.*, **26**, May 20 suppl., 4636 (2008).
- 8) Zimmermann M., *Pain*, **16**, 109–110 (1983).
- 9) Tjolsen A., Berge O. G., Hunskaar S., Rosland J. H., Hole K., *Pain*, **51**, 5–17 (1992).
- 10) Sasamura T., Nakamura S., Iida Y., Fujii H., Murata J., Saiki I., Nojima H., Kuraishi Y., *Eur. J. Pharmacol.*, **441**, 185–191 (2002).
- 11) Chaplan S. R., Bach F. W., Pogrel J. W., Chung J. M., Yaksh T. L., *J. Neurosci. Methods*, **53**, 55–63 (1994).
- 12) Dixon W. J., *Annu. Rev. Pharmacol. Toxicol.*, **20**, 441–462 (1980).
- 13) Makovec F., Bani M., Cereda R., Chiste R., Pacini M. A., Revel L., Rovati L. C., *Pharmacol. Res. Commun.*, **19**, 41–51 (1987).
- 14) Oldendorf W. H., *Brain Res.*, **24**, 372–376 (1970).
- 15) Pardridge W. M., *Physiol. Rev.*, **63**, 1481–1535 (1983).
- 16) Dubuisson D., Dennis S. G., *Pain*, **4**, 161–174 (1977).
- 17) Abbott F. V., Ievirk R., Najafae R., Franklin K. B., *Pain*, **83**, 561–569 (1999).
- 18) Miura N., Yoneta T., Ukawa H., Fukuda Y., Eta R., Mera Y., Omata T., Kinomoto T., Kurimoto T., Ito Z., *Gastroenterology*, **120**, A311 (2001).
- 19) Takinami Y., Yuki H., Nishida A., Akuzawa S., Uchida A., Takemoto Y., Ohta M., Satoh M., Semple G., Miyake K., *Aliment. Pharmacol. Ther.*, **11**, 113–120 (1997).
- 20) Shimoyama M., Tanaka K., Hasue F., Shimoyama N., *Pain*, **99**, 167–174 (2002).
- 21) Kuraishi Y., Iida Y., Zhang H. W., Uehara S., Nojima H., Murata J., Saiki I., Takahata H., Ouchi H., *Biol. Pharm. Bull.*, **26**, 550–552 (2003).
- 22) Zhang H. W., Iida Y., Andoh T., Nojima H., Murata J., Saiki I., Kuraishi Y., *J. Pharmacol. Sci.*, **91**, 167–170 (2003).
- 23) Asai H., Ozaki N., Shinoda M., Nagamine K., Tohnai I., Ueda M., Sugiyama M., *Pain*, **117**, 19–29 (2005).
- 24) Andoh T., Sugiyama K., Fujita M., Iida Y., Nojima H., Saiki I., Kuraishi Y., *Biol. Pharm. Bull.*, **31**, 520–522 (2008).
- 25) Noble F., Blommaert A., Fournié-Zaluski M. C., Roques B. P., *Eur. J. Pharmacol.*, **273**, 145–151 (1995).
- 26) Singh L., Oles R. J., Field M. J., Atwal P., Woodruff G. N., Hunter J. C., *Br. J. Pharmacol.*, **118**, 1317–1325 (1996).
- 27) Vanderah T. W., Bernstein R. N., Yamamura H. I., Hruby V. J., Porreca F., *J. Pharmacol. Exp. Ther.*, **278**, 212–219 (1996).
- 28) Idänpään-Heikkilä J. I., Perrot S., Guilbaud G., Kayser V., *Eur. J. Pharmacol.*, **325**, 155–164 (1997).
- 29) Fujita M., Andoh T., Saiki I., Kuraishi Y., *J. Pharmacol. Sci.*, **106**, 257–263 (2008).