Salmon Calcitonin Reduces Oxaliplatin-Induced Cold and Mechanical Allodynia in Rats

Manahito Aoki, Asami Mori, Tsutomu Nakahara, Kenji Sakamoto,* and Kunio Ishii

Department of Molecular Pharmacology, Kitasato University School of Pharmaceutical Sciences; 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan. Received October 9, 2012; accepted November 13, 2012

Oxaliplatin is commonly used anti-cancer drugs, but it frequently causes peripheral neuropathic pain. Recently, we reported that elcatonin, a synthetic analog of eel calcitonin, attenuated the oxaliplatin- and paclitaxel-induced cold and mechanical allodynia in rats. In the present study, we determined whether salmon calcitonin also had anti-allodynic effects on oxaliplatin-induced neuropathy in rats. The rats were treated with a single dose of oxaliplatin (6 mg/kg, intraperitoneally (i.p.)). Oxaliplatin resulted in cold and mechanical allodynia. We assessed the anti-allodynic effects of subcutaneously administered salmon calcitonin (20U/kg/d) by cold stimulation (8°C) directly to the hind paw of the rats and by using the von Frey test. Salmon calcitonin almost completely reversed the effects of both cold and mechanical allodynia. These results suggest that salmon calcitonin is also useful for treatment of oxaliplatin-induced neuropathy clinically.

Key words oxaliplatin; salmon calcitonin; peripheral neuropathy

Platinum-based drugs is commonly administered to patients with various types of cancers. Oxaliplatin is commonly used for the treatment of advanced metastatic colorectal cancer, and is known to induce a specific acute and chronic form of neuropathy. In particular, cold allodynia of the hands, feet, perioral region, and throat appears soon after its administration. Indeed, neurotoxicity as a result of oxaliplatin is often the reason for treatment discontinuation, dose reduction, or hospitalization, rather than tumor progression.

Calcitonin is a hormone that occurs naturally in the human body, and inhibits bone resorption through the direct inhibition of osteoclastic activity. It is effective against severe pain associated with vertebral fracture, postosteoporotic fracture, Paget’s disease, and lumbago. In addition, calcitonin has been reported to relieve pain that is not related to osteoporosis, such as that of diabetic neuropathy, reflex sympathetic dystrophy, and migraine, and formalin-induced hyperalgesia and allodynia. Recently, we reported that elcatonin, a synthetic analog of eel calcitonin, attenuated oxaliplatin- and paclitaxel-induced cold and mechanical allodynia in rats. Although blockade of the cellular signaling related to transient receptor potential ankyrin-1 (TRPA1) and melastatin-8 (TRPM8) is involved in the anti-allodynic action of elcatonin, it has been still unclear whether stimulation of calcitonin receptors is involved in the underlying mechanisms.

Salmon calcitonin is similar to human calcitonin hormone in many ways, and more potent than human calcitonin. Therefore, salmon calcitonin is also widely used to treat postmenopausal osteoporosis. In the present study, to determine whether salmon calcitonin would also show the same effects as elcatonin, and to get a suggestion about the role of calcitonin receptors in the anti-allodynic action of calcitonins, we tested the effect of salmon calcitonin on cold and mechanical allodynia induced by administration of oxaliplatin.

MATERIALS AND METHODS

Animals All experimental procedures used in this study conformed to Procedures for animal experiments which were approved by the Committee for Animal Experiments at Kitasato University and the Guiding Principles for the Care and Use of Laboratory Animals, which has been approved by the Japanese Pharmacological Society. Male Sprague-Dawley rats weighing 150–250 g (Japan SLC, Hamamatsu, Japan) were housed 4–6 per cage in a room under controlled temperature (22±2°C), humidity (55±5%), and a 12-h light/dark cycle, and were allowed free access to regular rat chow and tap water.

Oxaliplatin-Induced Allodynia Models Oxaliplatin (Elplat®, Yakult Co., Ltd., Tokyo, Japan) was dissolved in 5% glucose solution. The vehicle-treated rats were injected with 5% glucose solution (control group). Rats were treated with a single, intraperitoneal dose of oxaliplatin (6 mg/kg) or its vehicle (Day 1 only). Salmon calcitonin (Sigma, St. Louis, MO, U.S.A.) was dissolved in 0.9% benzyl alcohol, 1% gelatin in saline (pH 4.0), and was administered in the dorsal subcutaneous tissue (20 U/kg) on 5 successive days, starting 2 d after the oxaliplatin administration (Days 3, 4, 5, 6, and 7). The doses of salmon calcitonin was chosen based on a previous report.

Cold Stimulation Cold allodynia was assessed by the system that we developed for assessing cold allodynia in rats, whereby we modified existing equipment to allow cold stimulation to be applied directly to the hind paw of rats. The truncated aluminum cone that provides cold stimulation to the hind paw of rats was maintained at 8°C. Each rat was placed in a clear plastic box (20×17×13 cm) with a wire mesh floor and was allowed to habituate until it was more or less still. The tip of the cold plate was placed in contact with the rat’s hind paw until it withdrew it. The time taken for the rat to withdraw its hind paw from the cold plate (“withdrawal latency”) was used as the nociceptive measure. A cut off period of 15 s was observed to avoid damage to the paw. Ten trials (5 trials per each hind paw) were carried out at 1-min intervals. The mean withdrawal latency of the 10 trials was used for data analysis. To determine the effect of salmon calcitonin on cold and mechanical allodynia induced by oxaliplatin, these tests were performed before oxaliplatin administration (Pre), 2 d after oxaliplatin administration (Pre-Day 3; before salmon calcitonin administration), 6 h after salmon calcitonin administration (Day 3), and on Days 5, 7, and 10.

von Frey Test Mechanical allodynia was measured in rats...
using the von Frey test. Rat was placed in a clear plastic box (20×17×13 cm) with a wire mesh floor and was allowed to habituate until it was more or less still. The von Frey filament for 4-g force (Touch Test Sensory Evaluator Set, Stoelting, Wood Dale, IL, U.S.A.) was applied to the midplantar skin of both hind paws (5 trials per each hind paw, i.e. 10 trials per rat). Each application was held for 6 s. The number of withdrawal responses to the stimulation was counted.

Statistical Analysis The data represent the means±S.E.M. At first, we performed repeated-measures analysis of variance (ANOVA). However, the interaction between time and treatment was statistically significant. Therefore, we performed one-way ANOVA followed by Tukey–Kramer test among all of the measuring points to make multiple comparisons. Differences were considered to be statistically significant when $p<0.05$.

RESULTS

Figure 1 shows the effect of salmon calcitonin on oxaliplatin-induced cold allodynia in rats. Prior to the first drug administration, each treatment group had equivalent hind paw withdrawal latencies to the cold stimulation. On Day 3, rats treated with oxaliplatin had significantly shorter withdrawal latencies than the vehicle-injected control group ($p<0.01$). However, the administration of salmon calcitonin following the development of cold allodynia significantly increased the withdrawal latencies back to initial levels from Day 7 after salmon calcitonin administration to Day 10 ($p<0.01$).

Figure 2 shows the effect of salmon calcitonin on oxaliplatin-induced mechanical allodynia. Prior to the first drug administration, each treatment group had an equivalent number of withdrawal responses using the von Frey test. The rats treated with oxaliplatin tended to have a higher number of withdrawal responses than the control group and the salmon calcitonin-injected group. Although salmon calcitonin itself had no significant effect in von Frey test, the administration of salmon calcitonin following the development of mechanical allodynia tended to increase the number of withdrawal responses back to initial levels from 6 h after salmon calcitonin administration to Day 10. However, this effect is not statistically significant.

Subcutaneous administration of the vehicle slightly increased the withdrawal latencies to the cold stimulation and reduced the number of withdrawal responses in von Frey test, suggesting that benzyl alcohol and/or gelatin may have weak anti-allodynic effects.

DISCUSSION

This study demonstrated that salmon calcitonin almost completely reversed cold allodynia, and tended to reduce mechanical allodynia, in the oxaliplatin-treated rats. The anti-allodynic effects of salmon calcitonin are very similar to those of elcatonin, suggesting that not only elcatonin but also salmon calcitonin is useful for a therapeutic drug for oxaliplatin-induced neuropathic disorder.

Although the plasma half-life of calcitons are almost 50–80 min, anti-allodynic effects of salmon calcitonin and elcatonin maintained for 3 d after the cessation of treatment. This fact suggests that the changes of gene expression are involved in the anti-allodynic effects of calcitonins. The most possible candidate whose gene expression is modified by calcitonins is a thermosensitive channel family. It has been reported that oxaliplatin increases mRNA level of TRPA1 and TRPM8, and thermosensitive channels such as TRPA1 and TRPM8 are involved in the mechanism of neuropathy by oxaliplatin. We recently reported that blockade of the cellular signaling related to TRPA1 and TRPM8 is involved in the anti-allodynic effect of elcatonin. Therefore, it is possible...
that restoration of the expression level of these thermosensitive channels is one of the mechanisms of the anti-allodynic effects of calcitonins.

Another candidate is voltage-dependent Na⁺ channel. Oxaliplatin and oxalate, which has been shown to be involved in oxaliplatin-induced cold hyperalgesia and mechanical alldynia,20 have been found to activate the Na⁺ current,21 suggesting that oxaliplatin-induced cold alldynia may be caused by hyperactivation of these channels. Recently, mexiletine22 and lidocaine,15,23 both of which are voltage-dependent Na⁺ channel blockers, were reported to have an anti-allodynic effect in the oxaliplatin-injected rats. It has been reported that administration of elcatonin ameliorates enhanced arterial contractility24 and repeated administration of elcatonin is attributable to normalization of the sodium channels expression in chronic constriction injury neuropathic rats.25 Thus, restoration of the expression level of the sodium channels may be one of the mechanisms of the anti-allodynic effects of calcitonins.

Oxaliplatin has been shown to gradually reduce peripheral blood flow, and the prostaglandin E₂ analog limaprost has been shown to restore mechanical alldynia and increase peripheral blood flow.26 Elcatonin has been reported to improve regional blood flow,27 suggesting that improvement of regional blood flow may be involved in the anti-allodynic effects of calcitonins.

Because the two structurally different calcitonins have the similar effects, it is possible that stimulation of calcitonin receptors is involved in the anti-allodynic effects of calcitonins. Unfortunately, suitable antagonists of calcitonin receptor for in vivo study are not available at the present. If such antagonists become available, we need to clarify the effects of these drugs on the anti-allodynic effects of calcitonins, to confirm that stimulation of calcitonin receptors are involved in the underlying mechanisms. Recently, calcitonin receptors have been reported to express in peripheral nerve tissues such as Schwann cells, not in dorsal root ganglion cells.25 Therefore, we assume that neurotrophic factors derived from such cells as Schwann cells may modify the gene expression in dorsal root ganglion cells.25 Thus, restoration of the expression level of the sodium channels may be one of the mechanisms of the anti-allodynic effects of calcitonins.

In conclusion, the present study demonstrated for the first time that salmon calcitonin reversed the effects of both cold and mechanical alldynia induced by oxaliplatin in rats. The anti-allodynic effects of salmon calcitonin were similar to those of elcatonin. These results suggest that calcitonins are useful for prevention of oxaliplatin-induced peripheral neuropathy.

REFERENCES


