Antinociceptive effects of ONO-9902, an enkephalinase inhibitor, after visceral stress condition in rats

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Purpose: The present study was designed to examine the antinociceptive effects of orally administered ONO-9902, an enkephalinase inhibitor, on both somatic and visceral pain after visceral stress conditions.

Methods: Twenty six male rats were examined. Tail-flick (TF) and colorectal distension (CD) tests were used to determine somatic and visceral antinociceptive effects, respectively. Measurements were performed in rats under immediate post-stress conditions (group ST; $n = 14$) and in rats not under stress conditions (group NST; $n = 12$). In the stressed group, the same device, CD, for visceral antinociceptive effects was used for visceral stress and was applied with an intracolonic pressure of 60 mmHg for 20 min after drug administration. The TF latency and CD threshold were measured before and at 30, 40, 50, 60 and 90 min after administration of ONO-9902 300 mg $\cdot$ kg $^{-1}$ or distilled water.

Results: Orally administered ONO-9902 did not produce any changes in the % maximum possible effect (%MPE) in either TF or CD tests in the unstressed group. In the stressed group, %MPE in the CD test increased 18% and 31% at 30 and 40 min, respectively, after oral administration of ONO-9902 compared with the control group ($P < 0.05$). However, %MPE to TF test did not alter even after the CD-induced stress condition.

Conclusion: These results suggest that ONO-9902 may have analgesic effects on visceral pain but not on somatic pain under immediate post-stress conditions.

Objectif: Étudier l'activité antinociceptive du ONO-9902, un inhibiteur de l'encéphalinase, administré oralement pour la douleur somatique et la douleur viscérale dans des conditions de stress viscéral.

Méthodes: Vingt-six rat mâles ont été étudiés. Les tests d'oscillation de la queue (tail flick: TF) et de la distension colorectale (DC) étaient utilisés pour déterminer l'effet antinociceptif somatique et viscéral. Les mesures étaient effectuées sur des rats immédiatement après le stress (groupe ST; $n = 14$) et sans le stress (groupe NST; $n = 12$). Dans le groupe stress, le même dispositif, DC, de recherche des effets antinociceptifs servait à créer un stress viscéral par application d'une pression colique de 60 mmHg 20 min générée après l'administration du produit. La latence de TF et le seuil de DC étaient mesurés 30, 40, 50, 60 et 90 min après l'administration de ONO-9902 300 mg $\cdot$ kg $^{-1}$ ou d'eau distillée.

Résultats: L'administration orale de ONO-9902 n'a pas produit de changement dans le % de l'effet maximum possible (%EMP) pour les test TF ou DC dans le groupe non stressé. Dans le groupe stressé, le %EMP du test DC a augmenté respectivement de 18% et de 31% à 30 et 40 min après l'administration orale de ONO-9902 comparativement au groupe contrôle ($P < 0,05$). Cependant, le %EMP du test TF n'a pas changé même après les conditions de stress induites par DC.

Conclusion: Ces résultats suggèrent que l'ONO-9902 peut avoir des effets analgésiques sur la douleur viscérale mais non sur la douleur somatique dans des conditions immédiates d'après stress.

Key words

ANALGESIA: measurement, stress;
ANALGESICS: enkephalinase inhibitor, ONO-9902;
PAIN: visceral, somatic.

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Endogenous opioids such as methionine-enkephalin may play an important role in regulating the processing of nociceptive information. These agents are mainly inactivated by the metalloendopeptidase, enkephalinase.¹ Inhibition of enkephalinase is an area of great interest for the development of new analgesics, because

enkephalinase inhibitors (EIs) increase the levels of endogenous enkephalins in the central nervous system.^{2,3} The antinociceptive effects of EIs in the normal physiological state is controversial.⁴⁻⁸ Administration by oral, intrathecal or intracerebral routes, EIs demonstrated a dose-dependent antinociceptive action in the rat.⁴⁻⁶ In contrast, EIs had no antinociceptive effects in hot-plate licking and tail-flick tests.^{7,8} These different results may be due to the different intrinsic enkephalin concentration in the experimental conditions. On the other hand, it has been demonstrated that enkephalin stores in the central nervous system were released by stimuli which evoke nociceptive events,⁹ suggesting more effective antinociception by EIs in conditions in which the increase of enkephalin level is induced. Recent studies have shown that surgery and pregnancy induced analgesia was enhanced by EIs.^{10,11} However, there is little information regarding the antinociceptive effects of EIs under the conditions of visceral stress. The purpose of this study was to evaluate the antinociceptive effect of orally administered ONO-9902, an EI, on both somatic and visceral stimuli under the presence and absence of visceral stress conditions.

Methods

The protocols for this experiment were approved by the Animal Research and Use Committee of Shimane Medical University. Male Sprague-Dawley rats weighing 300–400 g were used. Data from 26 animals were included in data analysis. Rats were assigned to one of two groups, stress group (group ST; $n = 14$) and non-stress group (group NST; $n = 12$). In group ST, an intracolonic pressure of 60 mmHg for 20 min was applied by using the colorectal distension (CD) device, for measuring visceral antinociceptive effects, to achieve visceral stress. According to previous studies, a CD pressure of 60 mmHg is sufficient to produce noxious behavioural responses in animals.^{12,13}

Animals were maintained on a 12 hr light-dark schedule and were housed individually with free access to food and water. Animals were tested in a clear plastic chamber. To reduce the influence of handling on nociceptive responses, all animals were handled and trained in the test situation for at least five days before the experiments.

The Tail Flick (TF) test was performed to measure the response to noxious somatic stimulation. The time between stimulus onset and withdrawal of the tail from the heat source (a 100-W projector lamp) focused on a distal segment of the tail (approximately 5 cm from the tip) was defined as the response latency. The apparatus (model DS20, Ugo Basile, Comerio-Varese, Italy) was calibrated to give an average baseline latency of about

four seconds. A cut-off latency of 10 sec was used to prevent tissue damage.

The CD system, modified from Ness's methods,¹² was used to assess visceral nociception and to achieve visceral stress. This test involves inflation of an 8 cm, flexible latex balloon consisting of two parts, stimulating and sensing balloons. The pressure within each part was monitored continuously via in-line pressure transducers and recorded (Rectigraph, Sanei, Tokyo, Japan). The balloon was inserted intraanally into the descending colon and rectum under light halothane anaesthesia. The CD was used after recovery from anaesthesia. For the nociceptive test, pressure within the stimulating balloon was steadily increased at a rate of 2.5 mmHg·sec⁻¹ beginning at 0 mmHg and continuing until the abdominal musculature contracted and a repeated, rapid increase of the pressure (spike like wave) in the sensing balloon was detected. The minimal pressure at which abdominal contractions were triggered was defined as the threshold for visceral nociception. A maximum pressure of 60 mmHg was determined the cut-off distension pressure.

On the day of the experiments, a CD balloon was inserted during light halothane anaesthesia, and the rat was allowed to recover for at least for 20 min before determination of baseline values for both TF and CD tests. After determination of the baseline values, ONO-9902 (gift from Ono Pharmaceutical Co. Ltd.) 300 mg·kg⁻¹, dissolved in 4 ml of distilled water, or 4 ml distilled water for the control study were administered *po*. In group ST, visceral stress with a CD of 60 mmHg was applied 10 to 30 min after ONO-9902 or distilled water administration. The TF and CD tests were repeated at 30, 40, 50, 60 and 90 min after drug administration. The tests were performed in each rat at the same time points with two-minute intervals between each test.

The TF latency and CD threshold were converted to percent maximum possible effect (MPE): %MPE = (post drug value – baseline value)/(cut-off value – baseline value) × 100%. The %MPE is presented as mean ± SEM. Changes in %MPE were analyzed using repeated measure ANOVA followed by Bonferroni multiple comparison for post-hoc test. A *P* value <0.05 was considered to be statistically significant.

Results

In group NST, there were no differences in the baseline values of TF latency (control; 4.7sec, ONO-9902; 4.4 sec) and CD threshold (control; 19.2 mmHg, ONO-9902; 20.0 mmHg). ONO-9902 *po* did not produce any change in %MPE in either TF or CD tests in the non stress state (Figure 1A and B).

In group ST, there were no differences in the baseline

values of TF latency (control; 4.9 sec, ONO-9902; 4.6 sec) and CD threshold (control; 18.6 mmHg, ONO-9902; 18.9 mmHg). The %MPE in CD test increased to $18\% \pm 7$ (mean $\pm$ SEM) and $31\% \pm 5$ at 30 and 40 min ($P < 0.05$), respectively, after oral administration of ONO-9902 while %MPE in the CD test was not altered in the control group after exposure to CD for 20 min (Figure 2B). The peak effect was observed 40 min after administration and gradually returned to baseline 60 min after administration. In contrast, ONO-9902 *po* did not demonstrate any change in %MPE in the TF test even under visceral immediate post-stress conditions (Figure 2A).

Discussion

These results indicate that an EI, ONO-9902, produces analgesia to visceral but not to somatic pain under immediate post-stress conditions. However, it does not show any analgesic effect in the physiologically normal unstressed state.

The antinociceptive effects of EIs in previous reports are summarized in the Table. The results were variable and depended on the particular drug, dose, pretreatment and nociceptive test. Systemically or intrathecally administered EIs showed a dose-dependent antinociceptive action in the rat.⁴⁻⁶ On the other hand, acetorphan showed antinociceptive effects assessed using the jumping response to hot plate and the phenylbenzoquinone-induced writhing tests but not in the licking response to hot plate and tail withdrawal tests.⁷ Thiorphan showed selective antinociceptive effects in jumping response to hot plate and phenylbenzoquinone-induced writhing tests but not in licking response to hot plate and TF tests.⁸ The different antinociceptive effects of TF and CD tests are, in part, due to the different characteristics of somatic and visceral pain. Afferent innervation of pelvic viscera which contributes to the response of CD test originates in the lumbosacral dorsal root ganglia and is carried by several peripheral nerves. Some neuropeptides, including the enkephalins and dynorphins in such nerves and ganglia, may act as transmitters in afferent pathways to the pelvic viscera.¹⁴ One explanation for the present results is that CD stress induces an increase of enkephalin concentration, which may be insufficient to produce apparent antinociception in such an opioid-rich pathway, and ONO-9902 potentiates the inhibitory action of enkephalin to the CD test. Second, the dissociated effect might have resulted from the different pathways involved in the reflex, i.e., supraspinal or spinal reflex. Costentin *et al.* suggested that supraspinal responses might be more prone to inhibition by endogenous opioids than are spinal responses.⁸ Moreover, some tests, such as CD, which involve complex organized

FIGURE 1 Time-course effects on percent maximum possible effect (%MPE) in the tail flick (TF) test (A) and colorectal distension (CD) test (B) after oral administration of distilled water (control) ($n = 6$) or ONO-9902 ($n = 6$) in group NST group. Data are presented as means $\pm$ SEM.

FIGURE 2 Time-course effects on percent maximum possible effect (%MPE) in the tail flick (TF) test (A) and colorectal distension (CD) test (B) after oral administration of distilled water (control) ($n = 7$) or ONO-9902 ($n = 7$) in group ST. Data are presented as means $\pm$ SEM. *Significantly different from the control group. $P = 0.0188$ at 30 min and $P = 0.0006$ at 40 min.

behaviour might be more amenable to modification by endogenous opioids.¹⁰

In the present study, CD was applied to induce visceral stress. Acute intestinal distension with a balloon catheter induces neuronal c-fos-like expression which is induced by noxious stimuli in secondary neurons of the sensory pathway.¹⁵ In addition, noxious stimuli such as mechanical, thermal, chemical and electrical stimulation increase the enkephalin concentration in the spinal cord^{6,9,16} and the CD modified the cutaneous nociceptive transmission.^{17,18} Pain or stressful stimulation can produce analgesia which may be mediated by increased endogenous opioid.¹⁹ Recently, it has been reported that an EI enhanced the stress or pregnancy induced analge-

TABLE Antinociceptive effects of enkephalinase inhibitors

Drug	Pre-treatment	Admin route	Nociceptive test					Reference
			HP jump (sup/som)	HP lick (sup/som)	Writhing (sup/visc)	TF (sp/som)	CD (sup/visc)	
SCH 34826	—	po	+	?	+	?	?	4
SCH32615	—	intrathecal	+	?	?	+	?	5
SCH32615	—	intracerebral	+	?	?	+	?	6
Acetorphan	—	intracerebral	+	—	+	—	?	7
Thiorphan	—	intracerebral	+	—	+	—	?	8
SCH32615	Surgery	sc	+	+	?	?	?	10
SCH32615	Pregnancy	sc	+	+	?	?	?	11
ONO-9902	CD stress	po	?	?	+	—	+	Present

HP = hot plate; TF = tail flick; CD = colorectal distension; sup = supraspinal; som = somatic; visc = visceral.

The characteristics of nociceptive tests are shown in parentheses.

+: effective, —: not effective, ?: not performed

sia.^{10,11} Considering those findings and the visceral antinociception during the CD stress in the present study, it can be speculated that CD induced stress in animals and enkephalin activity was elevated during the visceral stress by CD although the enkephalin level during CD stress could not be measured. The present results suggest that EIs could enhance the effects of increased enkephalin under visceral stress conditions caused by CD and may then induce the antinociception. Those observations also suggest that the differential antinociceptive effects of EIs may depend on the experimental conditions, especially on the intrinsic enkephalin concentration.

Methodological influence on the CD thresholds may have occurred in the stress group because the same experimental method is used to both produce the visceral stress and measure visceral antinociceptive effects. However, we conclude that ONO-9902 produced antinociceptive effects on visceral but not somatic stimulus under immediate post-stress conditions because saline showed neither visceral nor somatic antinociception under the same condition.

There are few clinical reports of EIs. Meynadier reported that intrathecally administered thiorphan and bestatin, an aminopeptidase M inhibitor, elicited reproducible and long-lasting analgesia in cancer patients.²⁰ An antinociceptive effect of thiorphan *iv* was also found in patients submitted to a painful myelographic test.²¹ In contrast, thiorphan *iv* did not modify the threshold of either the nociceptive reflex or the subjective pain sensation elicited by electrical stimulation in volunteers.²² In animal studies, including the present, EIs may provide clinical use as an analgesic when the response to painful stimuli is modulated by endogenous enkephalins. This suggests its possible use in patients suffering from intolerable pain of visceral origin including

cancer-related pain which may influence the enkephalin level.

It is concluded that ONO-9902 exerts antinociceptive effects on visceral pain and not on somatic pain under immediate post-stress conditions.

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