sign of the two studies might have caused this difference in incidence rates. Most of the laboratory tests observed as being abnormal were determined to have been unrelated to application of TM patches by investigators and some of those laboratory findings seemed to be pre-existing at baseline.

Seven deaths were observed during the study. Five were determined to have been unrelated to the application of TM patches: four were due to aggravation of primary cancers and the other one was caused by infection associated with a chemotherapy-induced decrease in neutrophil production. With respect to the remaining two deaths, which were due to primary stomach cancer and respiratory failure, the causal relationship with TM patch application was uncertain. However, these two deaths were presumably due to aggravation of underlying cancer or complications.

Measurements of serum fentanyl concentrations provided supporting evidence for the therapeutic effects of the fentanyl 12.5 μg/h TM patches. The mean trough serum fentanyl concentration on day 4 was 169.9 ± 103.4 pg/mL (n = 83). There were no significant differences in serum fentanyl levels on days 4, 7 and 10 when patients had received a 12.5 μg/h patch three times (*ad hoc* one-way ANOVA, $p = 0.31$). Although wide ranges of interindividual differences in fentanyl trough serum concentration were observed in the study, this finding has also been reported in patients using conventional reservoir-type fentanyl patches.[13,17] This finding could have been caused by administration of a uniform initial dose of fentanyl to all enrolled patients, without titration for each patient's bodyweight. These data on serum fentanyl concentrations resulting from repeated applications support previous findings that fentanyl does not accumulate and is readily metabolized by liver microsomal cytochrome P450 3A4.[19]

Conclusion

The new fentanyl TM patch formulation employed in this study achieved appropriate cancer pain control in patients switching from morphine and oxycodone. The TM patches demonstrated a similar safety profile to conventional TR formulations. Repeated application of the same patch dose resulted in similar trough concentrations of fentanyl, supporting the pharmacokinetic efficacy of the formulation.

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Differential Analgesic Effects of a Mu-Opioid Peptide, [Dmt¹]DALDA, and Morphine

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Key Words

[Dmt¹]DALDA · Morphine · Opioids · Analgesic effect

Abstract

Aims: H-Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt¹]DALDA), a highly selective μ-opioid peptide, is potently analgesic after systemic and intrathecal administration but is less potent given intracerebroventricularly. This study was performed to further characterize the analgesic effects of [Dmt¹]DALDA. ***Methods:*** We compared the effects of [Dmt¹]DALDA and morphine after systemic administration in two different acute pain tests, the tail flick test and the paw withdrawal test, and examined how antagonizing the spinal opioid actions would affect their analgesic effects. ***Results:*** [Dmt¹]-DALDA was markedly more potent in the tail flick test than in the hot plate test, while the potencies of morphine were similar in the two tests. Intrathecal naloxone completely blocked the effect of systemic [Dmt¹]DALDA in the tail flick test, while it only partially blocked the effect of morphine. At higher doses that produced analgesia in the hot plate test, the effect of [Dmt¹]DALDA in this test was only partially blocked by naloxone. ***Conclusion:*** Systemic [Dmt¹]DALDA has a unique analgesic property clearly different from that of morphine and it has a propensity to produce spinal analgesia.

Introduction

H-Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt¹]DALDA) is a dermorphin-derived tetrapeptide with extraordinary selectivity for the μ-opioid receptor [1]. It carries a net positive charge of 3+ at physiological pH. Despite its polar nature, [Dmt¹]DALDA is readily distributed to the CNS and produces long-lasting analgesic effects after systemic administration [2]. [Dmt¹]DALDA also produces very potent analgesic effects after intrathecal administration [3], but is much less potent after intracerebroventricular (i.c.v.) administration [2]. In this regard, it differs from the prototypical μ-agonist, morphine, which is equally potent after intrathecal and i.c.v. administration. Thus, [Dmt¹]-DALDA and morphine appear to have differential analgesic characteristics even though they are both μ-opioid agonists acting in the CNS. To further characterize the

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difference between the analgesic effects of [Dmt^{1}]DALDA and morphine, we compared the effects of the two compounds after systemic administration in two different acute pain tests, the tail flick test and the paw withdrawal test. The tail flick test has been widely used in evaluating analgesic effects of opioids. It utilizes a spinal reflex induced by a pain stimulus and, in theory, the CNS actions of analgesics being examined in this test either involve the spinal cord directly or involve descending analgesic pathways to the spinal cord [4]. On the other hand, the hot plate test involves both spinal and supraspinal pathways, and the site of action of analgesic agents in this assay system may be at any level of the pain pathway [4]. A difference in analgesic potency of an opioid agonist in the two tests may reflect its distinctive site or mode of action. To the best of our knowledge, there have been no reports on an opioid agonist that has differential effects in these two tests. In addition, to further investigate the differential effects of [Dmt^{1}]DALDA and morphine, it was examined how antagonizing their spinal opioid actions would affect analgesic activity.

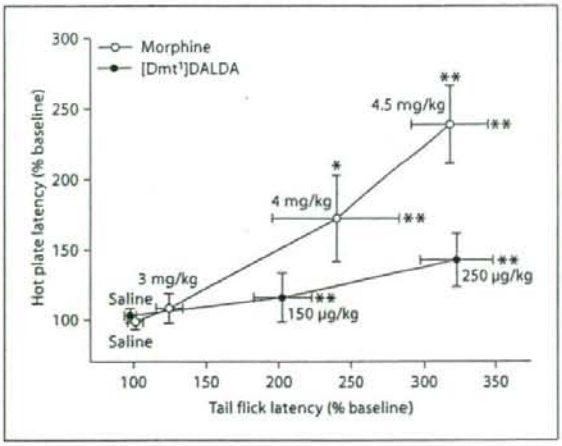

Fig. 1. Comparison of the analgesic effects of [Dmt^{1}]DALDA and morphine in the tail flick test versus the hot plate test. Tail flick and hot plate latencies were measured at 30 min after the administration of morphine or saline and 2 h after the administration of [Dmt^{1}]DALDA or saline. The number of animals was 6 or 7 for each group. Data are expressed as percent of baseline latency values. Tail flick latencies are plotted against hot plate latencies of each dose of morphine and [Dmt^{1}]DALDA. * Significantly different from saline control ($p < 0.05$). ** Significantly different from saline control ($p < 0.01$). [Dmt^{1}]DALDA at higher doses of 0.35 and 0.5 mg/kg produced a significant effect in the hot plate test (see text) but resulted in cut-off latency in all tested animals in the tail flick test (results not included in the graph).

Materials and Methods

Experiments were performed according to the guidelines of the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the United States National Institutes of Health, and were approved by the Institutional Animal Use Committee of the Chiba University Graduate School of Medicine.

Animals and Drugs

Male Sprague-Dawley rats (180–200 g) were used in the experiments. [Dmt^{1}]DALDA was synthesized as described elsewhere [1]. Morphine hydrochloride and naloxone hydrochloride were obtained from Takeda Pharmaceuticals, Osaka, Japan, and Sigma-Aldrich Co., St. Louis, Mo., USA, respectively.

Drug Administration

For subcutaneous (s.c.) administration, [Dmt^{1}]DALDA or morphine were dissolved in saline and were delivered in a volume of 0.1 ml/100 g rat weight. Subcutaneous administration was performed 2 h ([Dmt^{1}]DALDA) or 30 min (morphine) prior to pain testing. Spinal administration of naloxone or saline was carried out under light sevoflurane anesthesia by direct intrathecal injection at the level of L 2/3 via a 30-gauge needle connected to a polyethylene tube using a Hamilton syringe. Spinal injection was performed 10 min prior to pain testing in a volume of 10 µl.

Tail Flick and Hot Plate Tests

For the tail flick test, radiant heat was applied to the tail at 5 cm from the tip using a tail flick apparatus (IITC, Woodland Hills, Calif., USA). The time from the onset of the heat to the withdrawal of the tail (tail flick latency) was measured. The intensity of the radiant heat was adjusted so that baseline latencies would fall between 2.5 and 3.5 s. To avoid tissue damage, the heat stimulus was discontinued after 10 s (cut-off latency). For the hot plate test, rats were placed on a 52°C hot plate (Nissin Scientific Corp., Tokyo, Japan) and the latency to paw lick was recorded. A cut-off time was set at 30 s. Baseline latencies for both tests were obtained for each animal prior to the administration of the drug. In both tests, response latencies of each animal were determined at the time of peak effect of the drug administered (2 h for [Dmt^{1}]DALDA and 30 min for morphine).

Data Analysis

Response latencies in the tail flick and hot plate tests were expressed as percentage of baseline values. A one-way ANOVA was used for statistical analysis. A p value <0.05 was considered significant.

Results

Analgesic Effects in Acute Pain Tests

Morphine showed potent dose-dependent analgesic effects in both the tail flick test and the hot plate test (fig. 1). On the other hand, [Dmt^{1}]DALDA at doses of

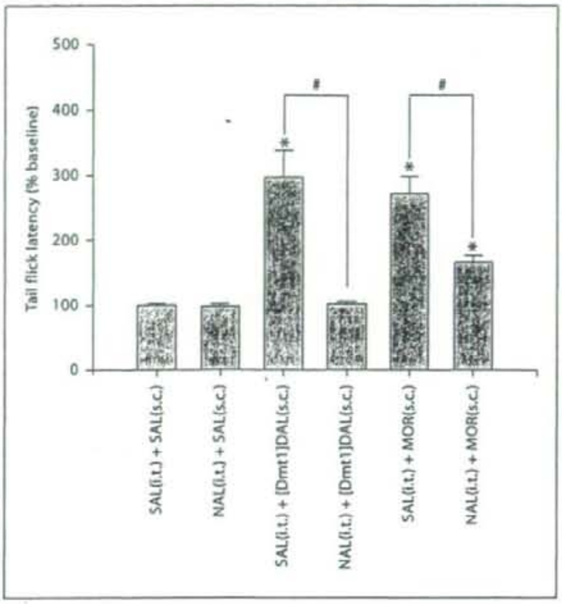

Fig. 2. Spinal naloxone (NAL) abolished the analgesic effects of s.c. [Dmt[1]]DALDA ([Dmt1]DAL) in the tail flick test but only partially blocked the effects of s.c. morphine (MOR). Tail flick testing was performed 2 h ([Dmt[1]]DALDA) or 30 min (morphine and saline) after s.c. administration of [Dmt[1]]DALDA (0.25 mg/kg), morphine (4.5 mg/kg) or saline (SAL). Intrathecal (i.t.) naloxone (20 μg) or saline were given 10 min prior to testing. The number of animals was 5 for each group. # Significantly different between groups ($p < 0.05$). * Significantly different from saline control ($p < 0.05$).

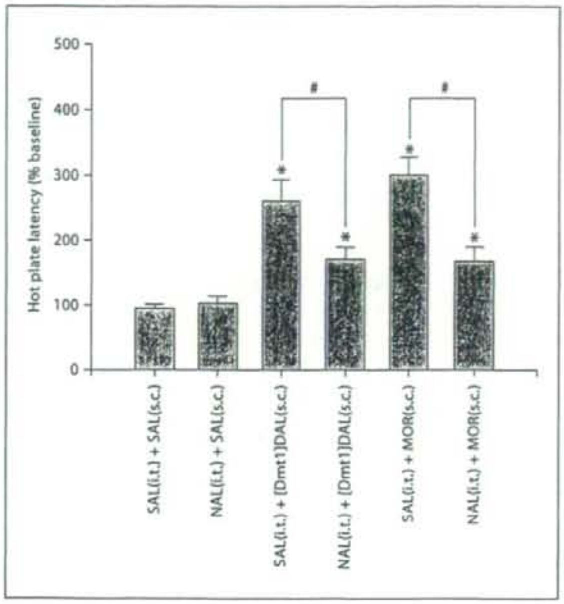

Fig. 3. Spinal naloxone (NAL) partially blocked the effects of both s.c. [Dmt[1]]DALDA ([Dmt1]DAL) and morphine (MOR) in the hot plate test. The hot plate test was performed 2 h ([Dmt[1]]DALDA) or 30 min (morphine and saline) after s.c. administration of [Dmt[1]]DALDA (0.5 mg/kg), morphine (4.5 mg/kg) or saline (SAL). Intrathecal (i.t.) naloxone (20 μg) or saline were given 10 min prior to testing. The number of animals was 5 or 6 for each group. # Significantly different between groups ($p < 0.05$). * Significantly different from saline control ($p < 0.05$).

0.15 and 0.25 mg/kg showed potent dose-dependent analgesic effects in the tail flick test, while no significant effect was seen in the hot plate test at these dose levels. The 0.25 mg/kg dose of [Dmt[1]]DALDA and the 4.5 mg/kg dose of morphine produced equivalent analgesic effects in the tail flick test, but only the latter dose of morphine elicited analgesia in the hot plate test (fig. 1). [Dmt[1]]DALDA at a higher dose of 0.35 mg/kg resulted in cut-off latency in the tail flick test in all animals tested, and at the same dose produced an analgesic effect (162.2 ± 12.2%, significantly different from saline control ($p < 0.05$)) in the hot plate test. Increasing the dose of this compound to 0.5 mg/kg resulted in a further increase in hot plate latency (250.8 ± 20.9%, significantly different from saline control ($p < 0.01$)). This was equivalent to the effect of morphine at a dose of 4.5 mg/kg in the hot plate test.

Effects of Spinal Naloxone

Twenty μg of naloxone given spinally 10 min prior to the tail flick test completely blocked the analgesic effect of s.c. [Dmt[1]]DALDA (0.25 mg/kg) (fig. 2). In contrast, the same dose of naloxone only partially blocked the analgesic effect of s.c. morphine at the dose of 4.5 mg/kg, a dose which is equipotent to a dose of 0.25 mg/kg [Dmt[1]]-DALDA in the tail flick test. In the hot plate test, 20 μg of spinal naloxone only partially blocked the effects of both morphine at 4.5 mg/kg and of [Dmt[1]]DALDA at 0.5 mg/kg (fig. 3). These doses of the two compounds produced equivalent effects in the hot plate test. No effect on [Dmt[1]]DALDA or morphine analgesia was observed in both pain tests by spinally injecting the same volume of saline (fig. 2, 3). Naloxone pretreatment itself did not affect tail flick or hot plate latency (fig. 2, 3).

Discussion

The tail flick test utilizes a spinal reflex induced by a pain stimulus, thus theoretically, the CNS actions produced by analgesic agents in this test directly involve the spinal cord or activate descending analgesic pathways to the spinal cord [4]. On the other hand, the hot plate test involves both spinal and supraspinal pathways, and the site of action of analgesic agents that affect this test may be at any level of the pain pathway [4]. Both the tail flick and hot plate tests are highly sensitive to opioids and have been widely utilized to demonstrate opioid analgesia in animal studies. The systemic administration of the prototypical μ-opioid agonist morphine has been shown to produce analgesia in both pain tests [5, 6]. In agreement with these earlier works, in the present study, we observed potent dose-dependent analgesic effects of morphine in the two tests at the same dose level. On the other hand, we found that systemic administration of [Dmt[1]]DALDA was markedly more effective in the tail flick test than in the hot plate test. Doses that produced potent analgesic effects in the tail flick test elicited no analgesic effect in the hot plate test. Furthermore, the opioid receptor antagonist naloxone given spinally completely abolished the analgesic effect of systemic [Dmt[1]]DALDA in the tail flick test, while the same dose of naloxone only partially blocked the analgesic effect of an equipotent dose of morphine. These results demonstrate that the characteristics of [Dmt[1]]DALDA's analgesic action are clearly different from those of morphine, despite the fact that both compounds are μ-opioids with CNS activity after systemic administration.

The results of the naloxone study utilizing the tail flick test suggest that [Dmt[1]]DALDA after systemic administration has a propensity to produce analgesia mainly in the spinal cord, while morphine produces both supraspinal and spinal analgesia. This finding is compatible with previous reports which demonstrated that [Dmt[1]]DALDA is highly potent in the tail flick test when given spinally [3], while much less potent when given i.c.v. [2]. The results suggest that [Dmt[1]]DALDA acts differently in the spinal cord and in the brain. This difference may be due to [Dmt[1]]DALDA's activation of other systems in the spinal cord that would potentiate the analgesic μ-opioid effect. In a previous study we showed that [Dmt[1]]DALDA inhibited norepinephrine reuptake, thereby increasing the effects of norepinephrine [3]. Norepinephrine has been shown to act synergistically with opioids to produce spinal analgesia [7, 8]. Furthermore, GTPγS-binding studies showed that [Dmt[1]]DALDA was a full agonist when the assay was performed with spinal cord membranes, whereas it showed partial agonism in the assay using brain membranes [9]. These findings indicate that [Dmt[1]]DALDA has differential actions on μ-receptors in the spinal cord versus the brain. This might be explained by differential distribution of μ-receptor variants in the spinal cord and in the brain. It has been shown that [Dmt[1]]DALDA differentially activated different μ-receptor variants as compared to the selective μ-agonist DAMGO [9].

We found that [Dmt[1]]DALDA at high doses, as compared to the low doses required in the tail flick test, produced an analgesic effect in the hot plate test which was only partially blocked by spinal naloxone. Thus, at these high doses, [Dmt[1]]DALDA can act similarly to morphine by blocking nociceptive pathways involved in the hot plate test and exerting analgesic action in the brain as well as in the spinal cord. However, lower doses of this compound that produced a potent effect in the tail flick test had no effect in the hot plate test. This greater potency in the tail flick test as compared to the hot plate test cannot be explained simply by the propensity of [Dmt[1]]DALDA to produce analgesic actions in the spinal cord as discussed above. Since the nociceptive impulse is transmitted through the spinal cord in both pain tests, if spinal μ-receptor activation uniformly suppresses nociceptive transmission, it would be anticipated that the potent spinal analgesia produced by [Dmt[1]]DALDA would result in analgesic effects in both tests. The differential effects of [Dmt[1]]DALDA in the two tests seems to suggest that the nociceptive transmissions are not uniform and that they may be differentially affected by drugs. Although both pain tests are based on thermal nociception, there is a difference in the pain stimuli between the two tests. In general, the hot plate test utilizes a lower intensity thermal stimulation that results in a longer latency for the animal to react as compared to the tail flick test. Such difference in stimulus intensity may activate different pathways in the spinal cord. We have previously shown that neuronal endothelin-1 has an effect in the tail flick test only when the stimulus intensity is markedly decreased [10]. Why [Dmt[1]]DALDA but not morphine appears to produce differential effects on nociceptive transmissions is unclear. It might involve non-uniformity within the spinal cord of [Dmt[1]]DALDA's potentiating effects on μ-opioid inhibition by the non-opioid mechanisms mentioned above, or possibly the activation of different μ-receptor variants distributed inhomogeneously among different nociceptive pathways within the spinal cord.

The propensity of [Dmt[1]]DALDA to produce spinal analgesia may have significance in the clinical setting. Firstly, [Dmt[1]]DALDA at analgesic doses may produce less supraspinal opioid side effects. Secondly, the potent spinal analgesia produced by [Dmt[1]]DALDA may be effective in pathological pain involving spinal mechanisms, in particular neuropathic pain. On the other hand, the mechanism that produces its relatively low effect in the hot plate test may result in relative ineffectiveness in clinical pain. Although morphine and other opioids are effective in neuropathic pain, high doses are frequently required that may be intolerable clinically. It remains to be shown whether [Dmt[1]]DALDA may be more effective than morphine in neuropathic pain due to its propensity to act in the spinal cord, and also due to its non-opioid actions such as norepinephrine reuptake inhibition and ROS scavenging actions [11].

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Conflict of Interest Disclosure

Patent applications have been filed by Cornell Research Foundation (CRF) for the technology (SS peptides) described in this article. Hazel H. Szeto and Peter W. Schiller are the inventors. CFR, on behalf of Cornell University, has licensed the technology for future research and development to a commercial enterprise in which CRF and Dr. Szeto have financial interest.

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Statistical Validation of the Relationships of Cancer Pain Relief With Various Factors Using Ordered Logistic Regression Analysis

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Objective: To clarify the relationships of cancer pain with various factors that prevent pain control statistically.

Methods: The participants were 71 terminal cancer patients admitted to the Department of Hematology/Oncology or Department of Gastroenterology/Hepatology, University Hospital, Kyoto Prefectural University of Medicine in whose pain control a pharmacist was involved as part of her clinical duties from January 2004 to November 2006. The effectiveness of pain control was evaluated using a 5-point verbal rating scale (0 = excellent, 1 = good, 2 = moderate, 3 = poor, and 4 = very poor) by interviewing the patients. As pain was rated using a graded scale and as many factors were involved in pain, analysis was performed using ordered logistic regression analysis. Moreover, prediction of an optimal model was performed by leave-one-out cross-validation to eliminate unnecessary variables. A program to perform leave-one-out cross-validation by ordered logistic regression analysis was prepared, independent variables used in the model were increased one by one, and calculation was performed in all combinations. Then, the optimal model was predicted by calculating the percent accuracy of predictions and Spearman rank correlation coefficient.

Results: Nausea [odds ratio (OR) = 1.948, $P = 0.0232$], sex (OR = 2.322, $P = 0.0030$), and bone metastasis (OR = 2.367, $P = 0.0017$) remained as variables significantly correlated with pain when the number of independent variables was 5, and sex (OR = 2.167, $P = 0.006$) and bone metastasis (OR = 2.093, $P = 0.005$) remained when the number of variables was 6.

Discussion: The statistical identification of factors preventing pain control is considered to contribute to the establishment of an evidence-based approach to cancer pain relief.

Key Words: cancer pain, pain relief, palliative care, ordered logistic regression analysis, sex differences

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Both pain treatment and palliative care have been areas farthest from evidence-based medicine (EBM), and many treatments in these fields have been performed on the basis of experience. EBM must also be established in pain relief by performing clinical studies on problems including pain assessment, effective use of drugs, and methods for the management of adverse effects.

Cancer pain is one of the major physical symptoms of cancer patients and the symptom that appears earliest among them, being reportedly observed in 30% to 40% of patients at the diagnosis of cancer and in 65% to 85% of patients with advanced cancer.[1–3] Cancer pain can be palliated by pain relief treatments of the World Health Organization in 85% to 95% of patients,[4–9] but pain control is still insufficient in some patients despite the administration of analgesics at sufficient doses. In the year 2002, the World Health Organization emphasized that "palliative care is an approach that improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems, physical, psychosocial, and spiritual.[10]" In the year 1996, the American Society of Clinical Oncology also declared in the mission statement, "it is the oncologists' responsibility to care for their patients in a continuum that extends from the moment of diagnosis throughout the course of the illness. In addition to appropriate anticancer treatment, this includes symptom control and psychosocial support during all phases of care, including those during the last phase of life.[11]"

Pain is nothing other than the report of "pain" by patients. However, pain is multifaceted, and Twycross[12] observed in his *Symptom Management in Advanced Cancer* that pain is easier to understand when it is regarded as total pain consisting of physical, psychologic, social, and spiritual pain.

We performed this study to clarify the relationships of cancer pain relief with various factors that prevent pain control. The actual procedure used was ordered logistic regression analysis, as pain was evaluated by a graded scale and as multiple factors involved in pain must be evaluated simultaneously.

PATIENTS AND METHODS

Study Term and Participants

The participants were 71 terminal cancer patients admitted to the Department of Hematology/Oncology or Department of Gastroenterology/Hepatology, University Hospital, Kyoto Prefectural University of Medicine from

January 2004 to November 2006 in whose pain control a pharmacist was involved as part of her clinical duties. This study was performed with approval of the Ethical Review Boards of Kyoto Prefectural University of Medicine and Osaka University. Patients with whom no communication was possible throughout the hospitalization period were excluded from the participants. Opioids were administered for the control of cancer pain Table 1 shows the characteristics of the 71 patients.

TABLE 1. Patients Characteristics and Extracted Factors That May Affect Pain When Pain State (After) was Evaluated (n=71)

	n (0/1)*	Mean ± SD	Range
Sex, female/male	24/47		
Age (y)		65 ± 10	35-84
Infection	33/38		
Previous chemotherapy	7/64		
Married	8/63		
Bone metastasis	45/26		
Outcome	47/24		
Physical symptoms			
Nausea	36/35		
Vomiting	47/24		
Constipation	33/38		
Appetite	67/4		
Dyspnea	48/23		
Cough	48/23		
Fever	29/42		
Psychiatric symptoms			
Depression	47/24		
Delirium	44/27		
Malaise	16/55		
Sleep disorders	46/25		
Confusion	43/28		
Hallucination	4526		
Sleepiness	39/32		
Concomitant medications			
NSAIDs	14/57		
Steroids	39/32		
Analgesic adjuvants	59/12		
Daily dosage of opioid			
Morphine IV mg		16.6 ± 81.6	0-670
Log (morphine IV mg)		−0.18 ± 1.51	−2-2.8
Fentanyl patch μg		3.13 ± 4.7	0-20
Log (fentanyl patch μg)		−0.55 ± 1.34	−2-1.4
Oxycodone p.o. mg		3.8 ± 9.16	0-40
Log (oxycodone p.o. mg)		−1.37 ± 1.29	−2-1.60
Liver function			
AST, U/L		200.1 ± 545	8-4410
ALT, U/L		102.3 ± 230	7-1856
ALP, U/L		903 ± 875	66-4690
γ-GT, U/L		187.6 ± 206.2	12-1018
Bilirubin, mg/dL		6.5 ± 10.5	0.21-38.6
Kidney function			
Serum creatinine, mg/dL		1.42 ± 1.43	0.28-8.54
Other laboratory test items			
Total protein, mg/dL		5.93 ± 1.29	3.3-11.3
Hemoglobin, g/dL		9.13 ± 2.13	3.1-13.3
Platelet, × 10³/μL		176 ± 119	3-468
Type of cancer			
Gastric	10		
Esophageal	10		
Pancreas	17		
Liver	11		
Gall bladder	4		
Cholangiocarcinoma	3		
Lymphoma	7		
Myeloma	2		
Others	7		

*The binary scales were female = 0 and male = 1 for sex, death = 0 and hospital change or discharge = 1 for outcome, and absent = 0 and present = 1 for others.

ALP indicates alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyltransferase; NSAIDs, nonsteroidal anti-inflammatory drugs; P.O., peros.

Evaluation of the State of Pain

Three kinds of the states of pain were evaluated by interviewing the patients using a 5-point verbal rating scale (VRS; 0 = excellent, 1 = good, 2 = moderate, 3 = poor, and 4 = very poor).

The dependent variable (Y = after) was defined as the state of pain just before hospital discharge or the last opportunity of communication. The dependent variable (Y = before) was defined as the state of pain on admission when pain control was poorest. The dependent variable (Y = before-after), improvement factor.

Statistical Analysis

Extraction of Variables

Variables were extracted in preparation for the analysis of multiple factors concerning pain control by regression analysis. The rating of pain on the VRS (Y = after) was 0 in 28 patients, 1 in 29, 2 in 11, 3 in 3, and 4 in none; the rating of pain on the VRS (Y = before) was 0 in 0 patients, 1 in 2, 2 in 12, 3 in 24, and 4 in 33; and improvement factor (Y = before-after) was 0 in 0 patients, 1 in 8, 2 in 31, 3 in 28, and 4 in 4 (Table 2).

The minimum number of patients necessary to form a category of the dependent variable was 4 in the ordered logistic regression analysis used in this study and fewer patients were rated as 3 or 4 (Y = after). Therefore, Y = after was recategorized into 3 levels: new level 0 indicates the original levels 0, new level 1 indicates the original levels 1, and new level 2 indicates the original levels 2, 3, and 4 (Table 3). Similarly, improvement factor (Y = before-after) was recategorized into 3 levels: new level 1 indicates the original levels 0 and 1, new level 2 indicates the original levels 2, and new level 3 indicates the original levels 3 and 4 (Table 4).

TABLE 2. The Rating of Pain on the VRS (After, Before) and Improvement Factor (n = 71)

VRS	After Male (Female)	Before (Male, Female)	Improvement Factor (Male, Female)
0	28 (14)	0	0
1	29 (20)	2 (1)	8 (7)
2	11 (10)	12 (9)	31 (23)
3	3 (3)	24 (12)	28 (15)
4	0	33 (25)	4 (2)

VRS indicates verbal rating scale.

Table 1 shows the independent variables (X) extracted from clinical records (retrospective survey). Binary scales were female = 0 and male = 1 for sex, death = 0 and hospital change/discharge = 1 for outcome, and no = 0 and yes = 1 for other factors.

Analytical Procedure

Ordered Logistic Regression Analysis (JMP 6: SAS Institute)

Ordered logistic regression analysis examines whether changes in variable Y (dependent variable) can be explained by changes in m variables ($X_1, X_2, \ldots, X_m$) (independent variables). For example, changes in the dependent variable Y are represented by the model: $Y = b_0 + b_1X_1 + b_2X_2 + \ldots + b_mX_m$ in multiple regression analysis and by the model: $\log\{Y/(1\text{-}Y)\} = b_0 + b_1X_1 + b_2X_2 + \ldots + b_mX_m$ in logistic regression analysis. In these models, b_0 is a constant member in the equation and $b_1, b_2, \ldots, b_m$, which represent the relationship between Y and X_i after elimination of the effects among the independent variables, are called partial regression coefficients. Regression analysis allows prediction concerning unknown data by constructing a model representing the phenomenon defined as the dependent variable on the basis of available data. Also, by evaluating the degree of the effect of each independent variable on the dependent variable according to the partial regression coefficient, it allows the analysis of factors (independent variables) that affect the dependent variable. In multiple regression analysis, dependent variables are continuous data, such as the concentration of a substance in blood, that are assumed to be linearly related to the independent variable. This concept of multiple regression can also be used to simultaneously examine the relationships among 2 or more categorical variables. However, the relationships among categorical variables are not simply linear, because the ranges of their values are finite. Therefore, generalized linear models are used. If quantitative variables are included in independent variables, and if the dependent variable is a categorical variable, logistic regression analysis can be performed by applying a generalized linear model through converting the probability that the value of a categorical variable falls in a particular category (p) into log {p/(1-p)}, which can take infinitely small to infinitely large values (logit conversion). A logistic regression analysis can be used for binary variables such as pain (present or absent). If there are 3 or more categorical variables in this study, ordered logistic regression analysis can be used. Ordered logistical regression analysis was performed in this study to efficiently use the variables (Y = after, before, or improvement factor) extracted by means of a 5-point scale and because a large number of factors (X in Table 1) are involved in pain.

TABLE 3. Dependent Variable (Y = After): Categorization of Data

Dependent Variable (Y)	VRS	No. Patients (71)
0	0	28
1	1	29
2	2, 3, 4	14

VRS indicates verbal rating scale.

TABLE 4. Dependent Variable (Y = Improvement Factor): Categorization of Data

Dependent Variable (Y)	Before-After	No. Patients (71)
1	0, 1	8
2	2	31
3	3, 4	32

Leave-one-out Cross-validation (LOOCV)

The ultimate objective of many multivariate analysis procedures is to construct a model for the prediction of related factors. In regression analysis, the relationships between the quantitative properties of samples of a variable (dependent variable) with those of 1 or more independent variables are established as a model. A common phenomenon that requires caution in all regression analysis procedures is that if the number of variables simultaneously considered in the model (m) is excessively large, results that "overfit" the data may be obtained to reduce the predictive ability of the model. Also, if the number of samples of each variable is insufficient, the reliability of the model is impaired. Usually, the necessary number of samples (n) is reported to be 5 to 10 times the number of variables (m). After the construction of a model by applying multivariate analysis, the appropriateness of the model must be confirmed by checking, for example, whether overfitting, which was described above, has occurred.

Overfitting is a loss in the applicability of the model to data other than training data (data used for the preparation of a model) owing to overadjustment of the estimated values of the parameter to training data in the learning process of a categorization model using many parameters. To avoid overfitting, parameters must be selected using cross-validation, according to the precision of categorization of the test data prepared for the measurement of the predictive ability using data other than the training data.

Cross-validation means dividing data into 2 parts, preparing a model using one of them as training data, and examining the predictive ability of the model using the other part as test data. It is a technique to evaluate the number of variables used and predictive ability of the model equation itself. The procedure of LOOCV used in this study was as follows. First, 1 sample is temporarily excluded from the training data and a model is constructed using the remaining training data. By employing this model, the dependent variable of the excluded sample is predicted, and the difference between the actual and predicted values of the dependent variable is recorded. Thereafter, the excluded sample is returned to the training data, another sample is excluded, a new model is constructed, and a new predicted value and its difference compared with the actual value are obtained. This process is repeated until all samples have been excluded once each. The optimal model can, thus, be selected from multiple models by comparison of the predictive ability.

Statistics and criteria used for this comparison are described as follows.

1. The percent accuracies of the model using test data by LOOCV.
2. Spearman rank correlation coefficients calculated according to the following expressions.

$$(R^2) = 1 - \frac{6\sum d^2}{n^3 - n}$$

d = the difference between each rank of corresponding values of x (= observed value) and y (= the predicted value), and n = the number of pairs of values.

RESULTS

Course of Analysis and Results

First, to accurately preserve the model, independent variables unnecessary for expressing the data as a model were excluded by calculating the percent accuracy. Independent variables were further screened by examining the multicolinearity, which occurs when there are correlations among independent variables and leads to the preparation of inappropriate models. As a result, 23 independent variables could be selected (Table 5) and the accuracy was 53/71.

Independent variables were further eliminated by estimating the optimal model on the basis of the evaluation of the predictive ability by LOOCV. A program to perform LOOCV by ordered logistic regression analysis was coded by us, and the optimal model was predicted by calculating the percent accuracy of the prediction results and Spearman rank correlation coefficients between the actual dependent variable and prediction results.

In the process of reducing independent variables one at a time from the 23, the calculation program stopped when all variables had been applied. Therefore, we divided the 23 independent variables into 4 categories consisting of 6, 6, 6, and 5 variables and further eliminated them. The 4 categories were established by combining independent variables considered to be most closely interrelated and distributing independent variables considered to affect pain control among these categories markedly.[12] For example, category A includes characteristics of patients (eg, sex), B includes physical factors (eg, nausea), C includes psychosocial factors (eg, depression), and D includes laboratory test values (eg, bilirubin). Eliminated independent variables varied according to how they were categorized and the results were unreliable. Therefore, we decided to extract necessary independent variables rather than eliminate unnecessary ones. The predictive ability was calculated first in all models in which only 1 variable was used and by adding 1 variable to the models in all possible combinations. As a result, independent variables that always remained (sex, bone metastasis, and nausea) could be determined when the number of independent variables used in the models was increased to 3-6. The results are shown below.

Procedure 1 [*Y* = After, Selection of Variables (*X*) When the Number of Variables was Increased to 5]

Among the combinations of 5 independent variables that gave the highest accuracy (41/71), Spearman rank correlation coefficient was highest (0.516) for the combination of sex, bone metastasis, nausea, sleep disorders, and cough. Ordered logistic regression analysis was performed using these independent variables. As a result, nausea, sex, and bone metastasis were significant (Table 6).

Spearman rank correlation coefficient was highest (0.538) for 8 combinations, all of which included sex, bone

TABLE 5. 23 Independent Variables that Remained After Elimination

Scale of Variable	Selected (23 Variables)	Eliminated (15 Variables)
Binary	Previous chemotherapy, sex, marriage, bone metastasis, outcome	Infection
	Physical symptoms: nausea, appetite, cough, fever	Physical symptoms: nausea, constipation, dyspnea
	Psychiatric symptoms: depression, sleep disorders, sleepiness	Psychiatric symptoms: delirium, malaise, confusion, and hallucination
	Concomitant medications: NSAIDs, steroids, analgesic adjuvants	
Continuous	Age	Opioid: daily dosage of morphine, fentanyl and oxycodone, log (daily dosage of morphine)
	Opioid: log (daily dosage of fentanyl and oxycodone)	Liver function: AST, ALP, γ-GT
	Liver function: ALT, bilirubin	Other laboratory test items: platelet
	Kidney function: Serum creatinine	
	Other laboratory test items: total protein, hemoglobin	

ALP indicates alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GT, γ-glutamyltransferase; NSAIDs, nonsteroidal anti-inflammatory drugs.

TABLE 6. Result of Procedure 1-1 (Y = After, Selected Variables When the Number of Variables was Increased to 5 on the Basis of the Percent Accuracy (Accuracy: 41/71, Spearman Rank Correlation Coefficient: 0.516)

Item	Estimated Value	Standard Error	χ^2Value	P	Odds Ratio	Confidence Interval of Odds Ratio	
						Lower 95%	Upper 95%
Sex	0.749	0.273	7.491	0.0061*	2.116	1.262	3.691
Bone metastasis	0.787	0.267	8.678	0.0032*	2.198	1.314	3.797
Nausea	0.553	0.259	4.561	0.0326*	1.738	1.054	2.947
Sleep disorders	0.297	0.262	1.284	0.2571	1.346	0.806	2.259
Cough	−0.123	0.273	0.204	0.6507	0.883	0.510	1.498

*$P < 0.05$.

metastasis, and nausea (total protein, fever, depression, cough, sleepiness, nonsteroidal anti-inflammatory drugs, and steroids were also included in some of them), and the accuracy was 39/71. As a result of ordered logistic regression analysis using these independent variables, nausea, sex, and bone metastasis were also significant (Table 7).

Procedure 2 [Y = After, Selection of Variables (X) When the Number of Variables was Increased to 6]

Spearman's rank correlation coefficient was highest (0.545) for the combination consisting of sex, bone metastasis, nausea, alkaline phosphatase, outcome, constipation, and appetite, and the accuracy was 39/71. Ordered logistic regression analysis was performed using these independent variables. As a result, sex and bone metastasis were significant (Table 8).

Procedure 3 (Y = Improvement Factor)

Improvement factor (Y = before-after) was also evaluated. Similarly to the process performed when the final state of pain (Y = after) was selected as the dependent variable, the percent accuracy and multicolinearity were examined and variables were eliminated by LOOCV. As a result, sex, nausea, steroids, and log (daily dosage of morphine) (accuracy, 40/71; Spearman rank correlation coefficient, 0.328) were selected when the number of variables was increased to 4. When ordered logistic regression analysis was performed using these variables as independent variables, only sex was significant (Table 9).

DISCUSSION

The result of ordered logistic regression analysis revealed cancer pain to be significantly correlated with nausea, sex, and bone metastasis. As shown in Tables 6 to 8, the odds ratio (OR) indicated that pain is exacerbated when the patient is male and has nausea and bone metastasis. The data distribution of these 3 independent variables was 47:24 (male:female) for sex, 35:36 (present:absent) for nausea, and 26:45 (present:absent) for bone metastasis, showing no marked disparity between the categories.

Bone metastasis was significantly correlated with cancer pain. There has been no previous study in which the relationship between bone metastasis and cancer pain was statistically analyzed, but bone metastasis is widely perceived in palliative medicine as the greatest factor that impairs the quality of life.[13–15] External irradiation is a standard pain control treatment for bone metastasis, but internal radiotherapy is also considered in patients with multiple bone metastases to reduce their burden.[16–19] Recently, a report that bisphosphonate is also effective for pain relief has attracted attention, but the evidence is

TABLE 7. Result of Procedure 1-2 (Y = After, Selected Variables When the Number of Variables was Increased to 5 on the Basis of Spearman Rank Correlation Coefficient (Accuracy: 39/71, Spearman Rank Correlation Coefficient: 0.538)

Item	Estimated Value	Standard Error	χ^2Value	P	Odds Ratio	Confidence Interval of Odds Ratio	
						Lower 95%	Upper 95%
Sex	0.842	0.283	8.805	0.0030*	2.322	1.367	4.144
Bone metastasis	0.861	0.275	9.769	0.0017*	2.367	1.392	4.187
Nausea	0.666	0.293	5.147	0.0232*	1.948	1.088	3.615
Total protein	−0.026	0.051	0.255	0.6129	0.974	0.886	1.081
Fever	0.107	0.318	0.112	0.7372	1.112	0.589	2.102
Depression	−0.232	0.301	0.595	0.4401	0.792	0.425	1.443
Cough	−0.125	0.329	0.145	0.7027	0.881	0.446	1.699
Sleepiness	0.076	0.283	0.072	0.7875	1.079	0.617	1.882
NSAIDs	0.012	0.322	0.001	0.9679	1.013	0.522	2.013
Steroids	0.040	0.256	0.024	0.8752	1.041	0.624	1.738

*$P < 0.05$.

NSAIDs indicates nonsteroidal anti-inflammatory drugs.

TABLE 8. Result of Procedure 2 (Y = After, Selected Variables When the Number of Variables was Increased to 6 on the Basis of Spearman Rank Correlation Coefficient (Accuracy: 39/71, Spearman Rank Correlation Coefficient: 0.545)

Item	Estimated Value	Standard Error	χ^2Value	P	Odds Ratio	Confidence Interval of Odds Ratio	
						Lower 95%	Upper 95%
ALP	0.0001	0.0002	0.347	0.555	1.000	0.999	1.000
Sex	0.7734	0.2848	7.372	0.006*	2.167	1.267	3.874
Outcome	−0.2769	0.2812	0.969	0.324	0.758	0.429	1.312
Constipation	0.2347	0.2593	0.819	0.365	1.264	0.760	2.110
Appetite loss	−0.0482	0.5401	0.007	0.928	0.952	0.308	2.833
Bone metastasis	0.7390	0.2670	7.660	0.005*	2.093	1.254	3.604
Nausea	0.4772	0.2640	3.266	0.070	1.611	0.971	2.733

*$P < 0.05$.

ALP indicates alkaline phosphatase.

still insufficient. Research on optimal palliative therapy for bone pain in which approaches including chemotherapy and radiotherapy are combined is anticipated.[20–22]

There have been a number of reports on sex differences in pain sensitivity.[23–50] Among their reports that there is a sex difference in the sensitivity of κ-opioid receptors, and that, because of their higher sensitivity, κ-opioids produce stronger analgesic effects in females.[27–30] On the other hand, many reports concluded that there is no sex difference, or females more frequently report pain, in many chronic pain types, including cancer pain.[31–50] In the study of the differences between women and men in their experience of cold-pressor pain, Keogh et al reported that women who concentrated on the emotional aspects of their pain may actually experience more pain as a result, possibly because the emotions associated with pain were negative.[41–43] Hau et al found that "testosterone reduced responsiveness to nociceptive stimuli in a wild bird.[44]" It was demonstrated that analgesic drugs such as morphine activated GIRK2. Thus, the male has higher threshold of tolerable pain because GIRK2 in male was present more than in female.[45,46] Moreover, there are many studies that proved the involvements of individual variations were larger than the sex differences in threshold of tolerable pain.[47–49] We know that women's pain threshold varies across the menstrual cycle.[50] Pain caused by cancer is multifaceted[12] and more, for example, is very different to that caused by physical injuries. However, as our study showed that cancer pain is exacerbated when the patient is male significantly for the first time, it may become a preface of the new discussions on sex differences in pain sensitivity in terminal cancer patients.

Unexpectedly, only nausea remained among many symptoms observed in terminal cancer patients. Patients having poorly controlled pain may more often report unpleasant symptoms such as "pain" and "sickness." Reports pain may also be related to the degree of patient satisfaction, that is, they may more often report pain when they are dissatisfied. Among previous studies, Meuser et al[51] discussed the importance of symptomatic relief in the treatment of cancer pain. This study indicated that the alleviation of nausea leads to better pain control. Multivariate analysis using nausea as the dependent variable and adding medication history including antiemetics and anticancer agents and opioids as independent variables is considered to be meaningful for further clarification of causative factors of nausea.

When independent variables were reduced one by one from the initial number of 23 in LOOCV, the calculation program stopped when all variables had been entered. As this program was designed to predict the optimal variable by changing the coefficient little by little, the number of variables that can be handled by the program may be surpassed if there are too many variables for the number of data, leading to a suspension of the calculation.

In the selection of independent variables by LOOCV for multivariate analysis using "after" as the dependent variable, sex, bone metastasis, nausea, alkaline phospha-

TABLE 9. Results of Procedure 3 (Y = Improvement Factor)

Item	Estimated Value	Standard Error	χ^2Value	P	Odds Ratio	Confidence Interval of Odds Ratio	
						Lower 95%	Upper 95%
Sex	−0.603	0.260	5.369	0.0204*	0.547	0.321	0.898
Nausea	−0.209	0.240	0.757	0.3840	0.811	0.500	1.300
Log (daily dosage of morphine)	−0.161	0.161	1.004	0.3162	0.850	0.614	1.160
Steroids	−0.206	0.237	0.757	0.3842	0.813	0.509	1.291

*$P < 0.05$.

(Accuracy: 40/71, Spearman rank correlation coefficient: 0.328).

tase, outcome, constipation, and appetite were included in the combinations that showed satisfactory results when the number of independent variables in the model was increased to 6. When ordered logistic regression analysis was performed using these 7 variables, nausea was not a significant variable. The inclusion of the 7 variables may have reduced the significance of nausea.

In multivariate analysis using the improvement factor as the dependent variable, Spearman rank correlation coefficient was low at 0.328. As the improvement factor was a variable prepared on the basis of a binary expression of subjective pain as "after" or "before," its use in objective evaluation may have been difficult. Selection of the dependent variable is difficult for the objective evaluation of subjective pain. To further improve correlation coefficients, other nonlinear regression analysis methods such as generalized additive models and logistic partial least squares analysis need to be evaluated.

As a result of this study, sex, bone metastasis, and nausea were factors that prevent pain relief. The effects of analgesics, which are reported to be affected by genetic factors, remain largely unclear, and our results do not explain everything. However, statistical identification of sex, bone metastasis, and nausea as factors that prevent pain control is considered to contribute to the establishment of EBM in pain relief and palliative care.

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