Assessment of needle insertion pain with flexor reflex responses in anesthetized rats

Kazuo Okamoto, Nozomi Ami, and Hidehiko Oshima
Research and Development Center, Terumo Corporation

Abstract
Needle insertion pain is a serious problem for many patients, especially young patients with insulin–dependent diabetes mellitus. Although a reliable assessment method is essential for analyzing and reducing the pain caused by needle insertion, there are very few studies on the measurement of needle pain, because the mechanism of pain is complicated and its assessment is affected by the assessment methodologies. In this study, we assessed flexor reflex responses evoked by insertion of needles into the hind paws of anesthetized rats, and investigated the factors that affect needle pain. We studied the process of needle insertion into the skin using a high–speed microscope and load cell along with a constant insertion speed–controlled device. Needles of two sizes, 27 gauge (G) and 31 G (outer diameter: 0.40 and 0.25 mm, respectively), were inserted into the hind paws of anesthetized rats at two velocities. The resultant action potentials evoked by the flexor reflex response in the semitendinosus muscles were recorded by electromyography (EMG). The EMG magnitude evoked by 27 G needle insertion was greater than that with the 31 G needle, irrespective of insertion speed. Penetration force, which increased as the needle penetrated the plantar skin, might be indirectly related to EMG magnitude. Our method demonstrates that thicker–diameter needles evoke more intense flexor reflex responses, as assessed by EMG in anesthetized rats. This is the first study to assess needle insertion pain in animals, and this method could advance studies on reducing needle insertion pain in humans.

Keywords
Needle insertion; Pain; Flexor reflex; Electromyography; Rat

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INTRODUCTION

Needle insertion is a very common procedure for injection or diagnosis. Many drugs are injected into veins, muscles and the hypodermis, because some drugs, such as insulin and antibodies, are biological and cannot be taken orally. Fear of the pain caused by needles can compromise the efficacy of treatment by affecting patient compliance, especially in young patients with insulin–dependent diabetes mellitus.

Previous studies have shown that needle insertion pain depends on various factors: needle diameter and facet geometry, insertion angle and depth, injection velocity, and site of insertion in the body. Egekvist et al. investigated the pain evoked by standardized needle insertion into human skin, and reported the relationship between pain and the parameters of needle insertion (e.g. diameter, angle, speed). After their studies using various–sized needles (from 23 gauge (G) to 32G), Arendt–Nielsen et al. reported that the incidence of painful needle insertion was related to needle diameter. However, they could not identify definitive relationships between needle pain intensity and needle diameter. These results suggest the limitations of human studies in which needle insertion pain is scored on a visual analogue scale (VAS), since VAS scores are affected by subjective pain experiences and assessment methodologies.

The first step toward decreasing needle insertion pain would be identification of appropriate assessment methods for needle pain, preferably using animal experiments. Many animal models have been developed to assess pain reduction using different drugs and interventional devices. However, we were unable to find references in the literature to any animal models used to evaluate needle insertion pain.

Previous studies have shown that assessment of spinal flexor reflex withdrawal evoked by mechanical stimuli in anesthetized rats is a useful method for assessing nociceptive pain. Further, several studies have assessed vascular pain using electromyography (EMG) in anesthetized rats. Bladder distention pain has also been quantified by EMG in rodent models. This evidence suggests that assessment of the flexor reflexes evoked by nociceptive pain can also be used for measurement of needle insertion pain in anesthetized rats.

In our preliminary study, we demonstrated that the flexor reflex EMG recorded from the semitendinosus muscle was related to the intensity of needle insertion pain. Using high–speed microscopic observation, we compared the pain resulting from insertion of two different–sized needles (27 and 31 G) by measuring the penetration force and EMG generated.

METHODS

Animal preparation

All experimental procedures were performed according to the ethical guidelines of the International Association for the Study of Pain, and protocols were approved by the Committee on Animal Experiments of Terumo Corporation.

Male Sprague–Dawley rats (Charles River Laboratories Japan, Yokohama, Japan) weighing 270 – 320 g were housed under a 12-h light/dark cycle at constant temperature (23 ± 2 °C) and humidity (50 ± 10%). Tap water and standard laboratory chow were available ad libitum.

Measuring the flexor reflex

Rats were anesthetized with 2% isoflurane
Mylan Inc., Tokyo, Japan) and their body temperature was maintained using a heating pad. A bipolar electrode (TH–209, Unique Medical, Tokyo, Japan) was precisely inserted into the semitendinosus muscle with a slight incision of the skin of either of the hind limbs for EMG recording. Thereafter, isoflurane anesthesia was reduced to approximately until a suitable flexor reflex response was observed, as described below. To create consistent responses to needle insertion pain before the assessment, electrical stimuli were applied to the hind paw using a clip–type electrode (TH–207–123, Unique Medical). The noxious stimuli were induced with a constant current stimulation isolator (BSI–950 Biphasic Stimulus Isolator, Dagan Corporation, Minneapolis, MN, USA), triggered by pulse generation and analyzed with a signal processor (PowerLab, AD Instruments, Sydney, Australia). The level of anesthesia was controlled to evoke suitable EMG action potentials with the pain–inducing stimulus (2 ms, 5 mA, 40 Hz). Electrophysiology studies in anesthetized rats have shown that stimuli of this intensity are known to robustly activate C–fibers and/or Aδ–fibers.

Needle insertion

We observed the process of needle insertion into the rat skin with a high–speed microscope, and recorded penetration force and EMG. The measurements were simultaneously started by the press of a foot switch (Fig.1–A). We designed the needle insertion device to be controlled by a computer to ensure constant velocities of insertion and retraction (Fig.1–B). The electro–mechanical load cell (RX–1 / CPM–N, Aikoh Engineering, Osaka, Japan) was set on the insertion device, and a needle was attached to the tip of the cell for measurement of penetration force. The needle insertion and retraction process was recorded at the rate of 4,000 images/s with a

Fig.1 A recording and analysis system for the assessment of needle insertion pain.
The diagram shows a recording and analysis system for the assessment of needle insertion pain (A). A needle was inserted into the plantar surface of the rat hind paw, with the insertion device controlled by a computer (B). A needle was attached to the tip of the cell. The photograph shows the insertion device (C).

Fig.2 Shapes and sizes of 27 and 31 G needles.
Two different needle sizes, (A) 27 G (O.D.; 0.4 mm) and (B) 31 G (O.D.; 0.25 mm), were used for the assessment. Both needles are hypodermic injection needles with the same bevel shape and geometry.
high-speed microscope (VW–9000, Keyence, Osaka, Japan).

For assessment of needle insertion pain, we used two different diameter needles: 27 G (Fig.2–A, O.D.; 0.4 mm, Terumo, Tokyo, Japan) and 31 G (Fig.2–B, O.D.; 0.25 mm, Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Except for the diameter, both needles have nearly the same facet geometry and are commonly used for hypodermic injections.

The needles were inserted vertically into the plantar surface of the hind paw at constant velocities of 3 or 10 mm/s. The insertion depth was approximately 3 mm, which was indicated by a marker on the axis of the needles. The needles were retained in this position for 2 s, following which they were retracted. The insertion area was the glabrous plantar skin, which is usually used for behavioral tests. Insertions were separated by a minimal distance of approximately 3 mm inside a square of 5 × 10 mm. First, 27 G needles were inserted four times along one side of the square, following which 31 G needles were similarly inserted on the other side. An inter-stimulus interval of approximately 3 min was maintained between successive stimuli and new needles were used for each insertion. The entire process was then repeated on the opposite hind paw. Needles were inserted in each hind paw with stabilization of EMG recording condi-

**Fig. 3** Relationship between depth of insertion of the needle, as observed with a high-speed microscope, and penetration force.

(A): Typical high-speed microscope images of needle insertion into the hind paw. Steps (1) to (4) represent the process of 27 G needle insertion, details of which are described in the text. (B): Graph demonstrating penetration forces (mN) related to the needle positions in (A). The dotted line indicates the position of the needle tip.
tions in between. We used 8 rats for studying the effects of the two needle sizes and insertion speeds. During insertion, penetration force (mN) was measured at a point 3 mm from the tip of the needle. The point was determined using high-speed microscope images (Fig.3).

Assessment of the EMG during needle insertion

Using the EMG measurements, we evaluated the intensity of the flexor reflexes evoked by needle insertion. We calculated the area of the rectified signals of the EMG, which was integrated from the time of occurrence of action potentials to their cessation when the needle was retracted, as the EMG magnitude (µV·s). The EMG latency was measured as the time (s) interval between needle contact with the skin and the beginning of the EMG signals evoked.

Drug administration and EMG measurements

As mentioned above, rats were anesthetized with isoflurane, the amount of isoflurane administered being controlled to enable generation of a suitable EMG signal for evaluating local anesthetic block of the needle insertion pain. First, 27G needles were inserted four times at a velocity of 3 mm/s and the EMG magnitude was measured. Next, 20 µl of 2% lidocaine (AstraZeneca, Osaka, Japan) was injected subcutaneously in the hind paw with a 29G needle (Myjector, Terumo). After 5 min, 27G needles were inserted four more times and the resultant EMGs were recorded. We used 5 rats for performing this part of the study.

Statistical analysis

Results are expressed as mean ± SEM. Data analysis was performed using GraphPad Prism version 5.04 (GraphPad Software, San Diego, CA, US). The data were analyzed for statistical significance by the paired t-test (one-tailed). Values of p<0.05 were considered statistically significant.

RESULTS

Needle insertion process and penetration forces

We evaluated the relationship between the position of the needle during its insertion into the hind paw and the penetration force at that time. A high-speed microscope demonstrated the insertion positions of the needle (Fig.3–A), and the related forces generated during needle insertion into the skin up to a depth of 3 mm and its subsequent retraction after remaining in situ for 2 s were recorded (Fig.3–B). The dotted line in Fig.3–B indicates the position of the needle tip. When the tip of the needle was in contact with
the surface of the paw (Fig.3–B(1)), the penetration force was at the basal level. As the needle was inserted into the skin, the force increased sharply, showing the first peak when the edge of the bevel was at the skin surface (Fig.3–B(2)). On cessation of needle insertion at a depth of 3 mm, the force showed a second peak (Fig.3–B (3)). The penetration force gradually decreased when the needle position was maintained at the 3 mm depth, returning to baseline when the needle was removed from the skin (Fig.3–B(4)).

The penetration forces generated with the 27 and 31 G needles at the two needle insertion velocities were compared at the point when needle insertion was stopped. The force generated by the 27 G needle was statistically significantly higher than that with the 31 G needle at 3 mm/s (Fig.4–A, 339.0 ± 60.0 mN and 212.8 ± 15.8 mN, respectively, *p*=0.034). When the insertion velocity was increased to 10 mm/s, the difference between the penetration forces was lower, although it was not statistically significant (Fig.4–B, 234.4 ± 16.4 mN and 204.8 ± 16.3 mN, respectively, *p*=0.063). The force generated by the 31 G needle was less influenced by an increase in needle insertion velocity than that generated by 27 G needle insertion.

**EMG latency and magnitude with needle insertion**

We demonstrated the typical EMG and mechanical force generated when a 27 G needle was
inserted at a velocity of 3 or 10 mm/s. At 3 mm/s, EMG action potentials occurred during insertion of the needle tip up to near the edge of the bevel, becoming progressively stronger until cessation of needle insertion (Fig. 5–A). The action potential continued to be generated for a short interval after cessation of needle insertion, disappearing before the needle was retracted. The EMG at 10 mm/s showed that the latency period until occurrence of the action potential was shorter than that at 3 mm/s (Fig. 5–B). The duration of the action potential was also condensed as compared with that at an insertion velocity of 3 mm/s.

EMG latencies were compared between 27 and 31 G needle insertions at the two velocities. No differences were observed between the latencies of 27 and 31 G needles at a velocity of 3 mm/s (Fig. 6–A). However, as the insertion speed increased 3-fold, from 3 to 10 mm/s, both EMG latencies decreased inversely from about 0.6 to 0.2 s (Fig. 6–B).

EMG magnitudes were also compared between 27 and 31 G needle insertions. The EMG magnitude with 27 G needle insertion was significantly higher than with 31 G needle insertion at 3 mm/s (Fig. 7–A, 58.4 ± 19.8 and 21.8 ± 4.0, respectively, \( p=0.041 \)). At an insertion velocity of 10 mm/s as well, the EMG magnitude generated by the 27 G needle was significantly higher than that with a 31 G needle (Fig. 7–B, 42.0 ± 10.2 and 20.3 ± 10.2, respectively, \( p=0.0012 \)).
Effects of local anesthetic injection on EMG magnitude

We demonstrated the effect of lidocaine on the EMG magnitude evoked by the needle insertions (Fig.8). With injection of 20 µl of 2% lidocaine, the EMG magnitude generated by 27G needle insertion decreased significantly to nearly the basal level at 5 min after the injection, compared to that pre–treatment (p=0.042).

DISCUSSION

In the present study, we found that flexor reflex responses of the semitendinosus muscle of anesthetized rats were evoked by needle insertion into the plantar skin, and that the EMG magnitude was related to the diameter of the inserted needle at different insertion speeds. Using a high–speed microscope and a speed–controlled needle insertion device, we were able to study the relationship between the penetration force and the pain intensity with EMG as a real–time observation. Our results showed that the EMG magnitude with the 27G needle was higher than with the 31G needle at insertion velocities of both 3 and 10 mm/s. The only difference between the two needles was their diameter (the O.D. of the 27G needle was 1.6 times larger than that of the 31G needle; 0.4 mm vs. 0.25 mm), their insertion depth (3 mm) and velocity of insertion being identical. Further, local hypodermic administration of 2% lidocaine (20 µl) into the hind paw strongly inhibited the EMG magnitude. This result corresponds to the fact that lidocaine is commonly used to block needle pain 4,29,37. These evidences suggest that measurement of electrophysiological EMG magnitude enables reliable and objective assessment for quantifying needle insertion pain.

Flexor reflexes might be triggered by the activation of nociceptors in the hind paw skin that are pricked by the advancing needle. We investigated the relationship between the penetration force and the EMG magnitude on needle insertion into the hind paw. The penetration force generated with 27G needle insertion was higher than that with 31G needle insertion (339.0 ± 60.0 mN vs. 212.8 ± 15.8 mN at 3 mm/s), and was related to the intensity of EMG magnitude. These data indicate that the penetration force seems to evoke flexor reflexes by stimulation of mechanical nociceptive receptors in the rat’s skin. On the other hand, previous studies reported that reflex withdrawal of the legs of anesthetized rats by pinch or monofilament stimulation were evoked by forces of over 1000 mN 17–32. This evidence suggests that different mechanisms are responsible for the responses to stimulation of the surface of the skin versus penetration of the skin tissue.

When the needle was inserted into the skin of the rat’s hind paw, EMG action potentials were evoked until the tip of the needle reached a depth of 3 mm. The latency until the potentials were evoked was approximately 3–fold shorter with the faster insertion velocity (10 mm/s vs. 3 mm/s). Further, no differences were seen in latency between 27 and 31G needles at both insertion velocities. These evidences suggest that the distance traversed by the needle until the EMG was evoked was nearly the same at both the velocities.

Tissue injury resulting from needle insertion could directly activate free nerve endings (e.g., nociceptors) in the skin of the rat’s hind paw. Many previous studies reported that the skin, especially epidermis, dermis and/or hypodermis, contains the nociceptive nerve–endings of polymodal C– or Aδ–fibers 10,18,24,26,39,40. Tissue injury could also result in the release of noxious
substances, protons, neurotransmitters, or bradykinin in the damaged tissue. We expect that the nociceptive nerve activation evoked by needle penetration through the skin is transmitted to the spinal cord, following which protective flexor reflex responses of the muscles occur. Further studies are needed to understand the precise mechanisms of needle insertion pain.

From previous studies, needle insertion pain has been shown to depend on several parameters, including needle diameter, injection angle, insertion velocity and depth, and site of the body where the needle is being inserted. Egekvist et al. provided quantitative information on the occurrence, intensity and quality of pain related to the mechanical injury induced by needle insertion through the skin into the subcutaneous tissue in humans, and correlated the pain with mechanical parameters during insertion. Using different–sized needles and a needle insertion device, Arendt-Nielsen et al. showed that the frequency of painful needle insertion correlated with needle diameter. They tested the pain response to five different needle sizes (27, 28, 30, 31 and 32G) inserted into the human thigh at two insertion velocities (2 and 19 mm/s). They revealed that the 27G needle was significantly associated with a higher frequency of painful insertion than the 31G needle. However, needle diameter did not significantly affect pain intensity, as assessed by the VAS score.

Conversely, in this study we showed that the magnitude of the EMG of the flexor reflex response to insertion of a 27G needle was statistically significantly higher than that with a 31G needle. We chose these two needle sizes because 27G needles are popular for subcutaneous injections while 31G needles are regularly used for insulin injection; 31G needles are also less painful than 27G needles. However, needles even thinner than 31G have recently been developed, so we are now planning further studies to assess the pain associated with insertions using these needles.

The results of human experiments on pain are usually heavily affected by the individual’s psychological factors and the skill of practitioners. Hence, it might be difficult to objectively state the pain intensity, which is usually evaluated with VAS. Studies on anesthetized rats have the advantage of allowing assessment of the physiological response to needle insertion pain without psychological influences.

The flexor reflex response to various noxious stimuli, such as electrical and mechanical stimuli, is a well–known phenomenon in humans. Recent human studies have demonstrated the use of EMG in evaluating pain intensities or sensitivities. Further, various animal experiments have been performed using EMG induced by mechanical or electrical pain, as well as by vascular pain and the visceral pain of bladder distension.

The EMG magnitudes evoked by needle insertion into the plantar surface of the hind paws of anesthetized rats indicate that the rat flexor reflex response is useful to evaluate needle insertion pain. This assessment could contribute to research aiming to ease the clinical problems associated with the pain of injections.

CONCLUSION

We introduced a new method for assessment of needle insertion pain, which involves assessing the EMG magnitude generated by the flexor reflex response to standardized needle insertion. Our methods could help reveal the mechanism
of needle insertion pain, and might contribute to improved injection techniques.

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References


Address for correspondence: Kazuo Okamoto
Research and Development Center,
Terumo Corporation
1500 Inokuchi, Nakai-machi, Ashigarakami-gun,
Kanagawa 259-0151, Japan
Tel: +81-465-81-4129 / Fax: +81-465-81-4154
E-mail: Kazuo_Okamoto@terumo.co.jp

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