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Wang, Kelun; Luo, Yi; Asaki, Toshiyuki; Graven-Nielsen, Thomas; Cairns, Brian E.; Arendt-Nielsen, Thomas; Arendt-Nielsen, Lars

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ACID-INDUCED EXPERIMENTAL MUSCLE PAIN AND HYPERALGESIA WITH SINGLE AND REPEATED INFUSION IN HUMAN FOREARM

Kelun Wang[*1], Yi Luo[1], Toshiyuki Asaki[1], Thomas Graven-Nielsen[2], Brian E Cairns[1,2], Thomas Arendt-Nielsen[1], Lars Arendt-Nielsen[1,2]

[1]SMI, Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Denmark
[2]Center for Neuroplasticity and Pain (CNAP), SMI, Department of Health Science and Technology, Aalborg University

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Correspondence: Kelun Wang

SMI, Department of Health Science and Technology, The Faculty of Medicine, Aalborg University, Fredrik Bajers Vej 7D-3, 9220 Aalborg, Denmark.

Tel.: +45 9940 8745, Fax: +45 9815 4008

E-mail: kelun@hst.aau.dk

ABSTRACT

Background and purpose: Acid has long been thought to play an important role in the pain process. Animal study showed that repeated acid stimulation induced central sensitization. The purpose of the study is to investigate muscle pain and hyperalgesia evoked by intramuscular infusion of saline at different pH levels, and to compare the effect of a single versus repeated acid infusions.

Methods: Twenty healthy subjects received infusions of buffered saline (pH 5.0, 6.0, and 7.4) into the brachioradialis muscle in a randomized order. Twelve of the subjects received repeated infusions. The subjects rated the pain intensity on visual analogue scale (VAS). Thermal pain sensitivity, and pressure pain threshold (PPT) were assessed in both arm before, during, immediately after, one hour after, and one day after the infusion. A McGill Pain Questionnaire and pain mapping were completed after each infusion.

Results: The pH 5 solution caused significantly higher pain and larger areas than pH 6.0 or 7.4. The local PPTs were significantly decreased (hyperalgesia) during and immediately after infusion of all three solutions. No significant differences were detected between the first and second infusion.

Conclusions: The intensity of acid-induced muscle pain is pH-dependent. All three solutions induced pressure hyperalgesia at the infusion site. Repeated infusions did not induce increased pain or prolonged hyperalgesia as compared with a single injection. Human intramuscular acidic saline infusion could not produce chronic pain model.

Implications: The acid-induced pain model may reflect the early stage responses to tissue injury of clinical conditions. Repeated intramuscular acidic saline injection model of prolonged hyperalgesia in rodents could not be translated into a human for modelling chronic musculoskeletal pain.

Key words: Acid-induced pain; Hyperalgesia; Muscle pain; Experimental pain; Gender

difference

1. Introduction

Tissue acidosis has been observed as a regular phenomenon following inflammation, ischemia, arthritis, cancer, hematomas, and exercise [1, 2, 3]. Local tissue pH has been found to drop to 5.4 in purulent exudates, 4.7 in fracture-related hematomas, 5.0-4.0 in bone cancer, and 6.0 in patients with occlusive arterial diseases in the leg [3]. Considering the painful nature of all the conditions above, the high proton concentration might be a significant contributor to the associated pain [1, 4]. Previous studies also suggest that a strong reciprocal pain potentiating interaction exists between acidic pH and several inflammatory mediators and neurotransmitters, with low pH playing the dominant role [5, 6, 7]. The acid-sensing ion channels (ASICs) play an important role in the activation of nociceptors by low pH and thus may serve as potential targets for analgesic drug developing [3, 4, 6, 8, 9, 10].

Human and animal studies have shown that acid can induce both transient and sustained pain [3, 4, 11]. An acid-induced pain model in rats has been proven to be safe and without significant tissue damage by histological biopsy compared with other inflammation or tumour-induced pain models in rodents [11]. Using acid to produce pain in human skin and muscle has also proven safe [12, 13, 14]. Primary mechanical hyperalgesia was reported to be observed following the acid stimulation. In spite of the different methods adopted, the intensity of the acid-induced pain is pH-dependent [3, 7, 13, 14]. A previous study including intramuscular acidic stimulation reported that women experienced higher referred pain and exhibited a stronger correlation between local and referred pain than men [13].

Animal studies have reported that repeated intramuscular acidic stimulations induced spinal hyperexcitability with contralaterally spreading hyperalgesia [8, 11, 15, 16]. Local anaesthetics applied to the muscle previously injected with acidic saline could not inhibit the acid-induced contralateral spreading of hyperalgesia [11] indicating a central origin of the phenomenon. However, spreading of pain has not been found after repeated acid injection

into the masseter muscle in neither human [12] nor animal studies [17]. Since the results from human research within this field are limited, further studies should be conducted to elucidate the possible central mechanism of acid-induced muscle pain.

The aims of this human study were to investigate: 1) whether acid-induced muscle pain and 2) pressure hyperalgesia were pH dependent; 3) if spreading sensitization could be evoked by repeated versus single injection of acid stimulation; and 4) if there were gender differences in any of the parameters.

2. Methods

2.1 Subjects

Twenty healthy subjects (7 women, 24.3±3.1 years) participated in a three-session study with a single infusion in each session. Further, 12 of the 20 subjects (4 women, 24.1±2.8 years) participated in sessions with repeated infusions. None of the subjects had a history of pain or injuries or medical conditions that could interfere with normal somatosensory functioning. Women in the menstrual period were avoided. The study was approved by the local ethics committee (N 2011-0081) and conducted in accordance with the Declaration of Helsinki. All subjects gave written informed consent.

2.2 Experimental Protocol

The subjects participated in three sessions; each with a single infusion of buffered saline with one of three different pH levels (pH 5.0, 6.0 and 7.4). The infusions were conducted in random order and with a one-week interval between sessions. Further, 12 subjects received repeated infusions of either pH 5.0 or pH 7.4 solution with a one-day interval between infusions. In each session, neutral phosphate buffered saline (10 ml) was infused into the brachioradialis muscle over 20 min using a computer-controlled infusion pump.

Cold pain threshold (CPT), heat pain threshold (HPT), mechanical pain sensitivity (MPS), and pressure pain threshold (PPT) were assessed before, during, after, one hour after, and one day after the infusion. The pain intensity was rated by means of an electronic visual analogue scale (VAS). A McGill Pain Questionnaire and a pain map were completed after each infusion.

2.3 Acidic Infusions and Pain Assessment

The pH adjusted phosphate buffered saline (10 mL, Hospital Pharmacy of Aalborg University Hospital, Aalborg, Denmark) was randomly infused into the left brachioradialis muscle (2 cm from the superior border of cubital fossa) in a double-blinded manner with respect to the pH level. The infusion site was cleaned with alcohol and dried prior to the needle insertion. The needle (27 G, 19 mm, BD Microlance 3, Becton Dickinson, Ireland) was inserted into middle part of the brachioradialis muscle with a depth of 15 mm. The inserted needle was fixed to the skin using surgical tape and sterile cotton. A tube (200 cm, 1.5 ml, G30303M, Care Fusion, Switzerland) was connected to the needle from the syringe. The sterile buffers were infused at a constant rate of 30 ml/h for 20 minutes using a computer-controlled infusion pump (Asena CC MK-III, Alaris medical systems, USA). The needle and tube were removed immediately after completion of the infusion.

The subjects rated the induced pain intensity on an electronic VAS on which "0 cm" indicated "no pain" and "10 cm" represented "most pain imaginable". The VAS signal was sampled every 2 seconds from the beginning of the infusion until the pain intensity had returned to zero. The maximal pain (VAS peak) and the area under the curve (VAS area) were calculated. After the infusion, the subjects were asked to draw the pain areas on an arm drawing describe the quality of the pain on the McGill Pain Questionnaire (MPQ).

2.4 Assessment Sites

Two sites in the infusion side and two sites in the contralateral side were assessed (Fig. 1). CPT, HPT MPT, and PPT were assessed 1 cm from the infusion site (T2). All sensory assessments were also conducted at the infusion site (T1) at the same time points except during infusion. Further, the assessments were performed on the contralateral arm at the corresponding sites as controls (C2 and C1).

2.5 Cutaneous Thermal Pain Sensitivity

Cold pain threshold (CPT) and heat pain threshold (HPT) were measured (TSA 2001 II (CHEPS, Medoc, Israel) at T1 and C1 sites. The contact area of the thermode was 9 cm^2. The baseline temperature was 32 C° (centre of neutral range). The method of limits was used by applying ramp stimuli at a velocity of 1 C°/s. The cut-off temperatures were 0 C° and 55 C°. The volunteers were asked to press a button when the respective thermal sensations were perceived. The mean threshold temperature of three consecutive measurements was calculated.

2.6 Cutaneous Mechanical Pain Sensitivity

The cutaneous sensitivity was assessed using weight-calibrated pins (128 mN, custom made Aalborg University) at all assessment sites. The subjects rated the cutaneous mechanical pain sensitivity (MPS) on a 0-5-10 Numeric Rating Scale (NRS) on which "0" represented “no sensation", "5" represented "pain threshold", and "10" presented "worst pain imaginable". The mean of the three measurements was used in the statistical analysis.

2.7 Pressure Pain Sensitivity

A hand-held pressure algometer (Somedic AB, Sweden) was used to assess the PPTs. The

pressure was applied to all assessment sites at a constant rate of 30 kPa/s through a 1 cm^2 probe. The subjects were instructed to push a button immediately when they felt the pressure turning into pain. The PPTs were measured twice at each site. The interval between the two PPT trials was at least 40 sec and the mean of the two measurements was used in the statistical analysis.

2.9 Statistics

The normal distribution was checked for all data. The necessary logarithmic transformation was performed [18]. QST data were then analysed using a 3-way repeated measure analysis of variance (ANOVA) with gender as between-subject factor and testing site (T1, T2, C1, C2), pH levels (pH 5.0, 6.0, 7.4) and time (baseline, during, after, one hour after, and one day after) as within-subject factors. The VAS scores and pain areas of the different pH levels and of the single and repeated infusions were analysed by 2-way (pH level and gender) repeated measure of ANOVA. A Bonferroni test was employed for post-hoc comparisons in case of significant ANOVAs. All statistical calculations were performed using the Statistical Package for Social Sciences version 20 (SPSS, IBM). The significance level was set at $P<0.05$. The data are presented as mean and standard error of the mean (SEM).

3. Results

3.1 Acidic-evoked Pain

The VAS profiles of the three infusions with different pH levels are shown in Fig. 2A. Significant differences were detected in VAS peak (ANOVA: $P < 0.023$, Fig. 2B) and VAS area (ANOVA: $P < 0.012$, Fig. 2C). The infusion of the pH 5.0 solution caused higher VAS scores (Peak 4.65) than the pH 6.0 solution (Peak 3.37) ($P= 0.015$) and the 7.4 solution (1.68) ($P = 0.001$). In addition, the pH 7.4 solution caused the lowest VAS scores compared with the

pH 6.0 solution (P= 0.016).

The pain areas following the three different solutions are illustrated in Fig. 3. Infusion of the pH 5.0 solution evoked a larger area than infusion of pH 6.0 (P =0.036) and 7.4 (P =0.004), whereas the pH 7.4 solution evoked the smallest area compared with pH 6.0 and pH 5.0 solution (P< 0.021).

No gender differences were detected in VAS peak, VAS area, or drawing areas (P > 0.092).

3.2 Cutaneous Thermal and Mechanical Pain Sensitivity

The ANOVA of the CPT, HPT, or MPS among the different solutions at any of the tested sites were not significant (ANOVA: P > 0.073). Likewise, no significant change was detected before, during, immediately after, one hour after, and one day after the infusion (ANOVA: P > 0.103,) or between gender (ANOVA: P > 0.087, data not presented).

3.3 Pressure Pain Sensitivity

The ANOVA demonstrated that the PPTs were different over time (ANOVA: P < 0.017) but not between solutions pH levels (P > 0.092). Compared with baseline, relative PPTs decreased immediately after, 1 hour after, and 1 day after the infusion at T1 (p<0.017) and during infusion at the T2 site (p<0.030) (Fig. 4AB). No significant difference was detected in the contralateral sites (ANOVA: P > 0.105; Fig. 4CD) and no gender difference was found (ANOVA: P > 0.087).

3.4 Repeated Infusions

Eight out of 12 subjects received repeated infusion of pH 5.0 solution, and 4 of them received repeated infusion of pH 7.4 solution No significant difference in VAS scores was detected

between the two repeated sessions for neither pH 5.0 nor pH 7.4 ($P > 0.195$) (Fig. 5A). No difference was detected in CPT, HPT, MPS or PPT between the two repeated sessions for either pH 5.0 or pH 7.4 (ANOVA: $P > 0.106$, data not presented). Normalized PPT values after the 1st and 2nd infusion of pH 5.0 is shown in Fig. 5B.

4. Discussion

The intensity of the acid-induced muscle pain was pH dependent whereas the deep tissue pressure hyperalgesia was not pH dependent. Repeated acid stimulation did not induce more pain or prolonged pressure hyperalgesia as compared with a single injection. No gender differences were found.

4.1 Acid-induced Muscle Pain

In the present study, the single infusions of the pH solution into the human forearm muscle produced significantly higher pain intensities than the neutral buffer (pH 7.4) and the pH 6.0 buffer infusions, which is consistent with previous studies on acidic muscle pain models [13, 19,]. The study is the first to use infusion of buffered saline with different pH levels into the same group of human muscles. The results provide clear evidence that the acid-evoked pain was pH dependent. The thin myelinated Group III and unmyelinated Group IV nerve fibres in muscle are responsible for transmitting muscular nociceptive information and their endings are sensitive to inflammatory mediators including low pH stimulation [20, 21]. The decrease in tissue pH following muscle ischemia is believed to activate ASICs in muscle nociceptors, thus contributing to e.g. ischaemic muscle pain [8, 19, 22, 23]. Clinically it is known that local anaesthetics with pH levels as low as 5 occasionally produce transient pain upon injection [24, 25]. Thus acidic infusion could be used as a muscle pain model in both animal and human studies.

4.2 Factors affecting the pain evoked by acid infusions

Factors such as infusion volume and muscle size could affect acid-evoked pain intensity. In the present study, a total of 10 ml of buffered acid saline was infused into the brachioradialis muscle at a rate of 30ml/h. The average peak pain intensity of the evoked pain was 4.65 cm for the pH 5.0, 3.37 cm for the pH 6.0, and 1.68 pH 7.4 solution (Fig. 2). In previous animal and human studies, different volumes were used to induce pain. In studies examining male rats, acidic saline was injected in volumes of 20 μL into the rat masseter [26] and rat gastrocnemius muscle [8,] which successfully evoked pain and hyperalgesia. However, in human studies with injection of 0.5 ml unbuffered acid saline, approximately 3% of the total volume of the human masseter muscle which was comparable to the relative injection volume used in the rats, no significant pain was induced when compared with neutral saline injections [12]. Infusion of five times the volume (2.5 mL) of unbuffered acidic saline into the masseter muscle of human subjects induced pain levels similar to the results of single injection (0.5 ml) in previous study [27]. Thus, the results of the previous human studies did not provide evidence that the injection/infusion volume had a major impact on the acid-induced pain intensity. It seems other factors, such as infusion rate, and using buffered saline may play more important role in the acid-evoked pain intensity. However, the injection or infusion volume should be considered according to the muscle volume when using an acid infusion as pain model.

The infusion rate may play an important role in the acid-evoked pain. A previous study on acid-induced human skin pain indicated that raising the infusion rate leads to increasing pain by lowering the local pH more effectively and by increasing the tissue volume in which the proton concentration exceeds the threshold to excite nociceptors [28]. Infusion of isotonic pH 5.2 phosphate buffer into the flexor carpi radialis muscle produced pain correlated with

the flow rate of the infusion [19]. In a recent human study, acid saline (pH 3.3) was infused into the masseter muscle with a slow infusion rate of 15ml/h. The infusion evoked only mild pain and no mechanical allodynia or increased release of algesic substances assessed by microdialysis were detected [27]. In our previous study, acid saline (pH 5.2) was infused into the anterior tibialis muscle and the pain level was higher and PPTs were lower following an infusion rate of 40 mL/h compared to the infusion rate of 20 ml/h [13]. In the present study, the infusion rate of 30 mL/h was selected since the expected pain intensity was evoked by both pH 5.0 and pH 6.0 buffered saline during the pilot experiments when the different infusion rates of 10ml, 20 ml, 30ml, and 40 ml were tested.

In addition, using buffered saline instead of unbuffered saline might be the necessary to evoke pain. Previous human studies have suggested that acid-induced muscle pain may be more effectively produced by infusion of low pH (~5) phosphate buffers [13, 28] than by injections of unbuffered acidic saline [12, 27]. Recent human studies did not evoke the expected pain by means of unbuffered acid saline with pH 3.3 [12, 27]. This difference is possibly due to the ability of the muscle tissue to rapidly buffer pH changes after injections of acidic solutions. Compared with a buffered saline solution, an unbuffered saline solution could physiologically regain pH level more quickly because of the buffering capacity of the muscle tissue. Since ACIC3 channels generate sustained currents as long as the pH is acidic [29], the longer the pH in the muscle remains acidic and the longer the ASIC3 channels will be activated.

4.3 Mechanical Hyperalgesia

In the present healthy human study, PPT values at the infusion site (T1) and around the infused site (T2) were significantly decreased during the acidic infusion compared with baseline. However, no mechanical hyperalgesia was observed in the contralateral side

indicating that the acidic infusion caused local sensitisation without central mechanisms being involved. Further, no significant difference was detected in the three different pH solutions indicating that the local mechanical hyperalgesia was not pH-dependent but most likely a volume effect. Similar mechanical hyperalgesia was observed in the experimental muscle pain model conducted by injecting acidic buffer into the anterior tibialis muscle [13]. In contrast, in a recent human study acid-infusion into the masseter muscle did not evoke mechanical hyperalgesia in either the local or contralateral side [27]. It seems that only localised pain and short period local hyperalgesia were observed after infusion of acidic buffer in human studies.

4.4 Gender Differences

No sex-related differences in pain intensity, pain areas, or induced local pressure hyperalgesia were observed among the three different infusions in the present study. A previous study of intramuscular acidic stimulation reported that women experienced higher referred pain and exhibited a stronger correlation between local and referred pain than men [13]. In rats, expression of $ASIC_3$ receptors is greater in masseter muscle sensory afferent fibers in females compared with males [30]. It is unclear if a similar difference in ASIC channel expression occurs in humans or if the expression of ASIC channels by sensory afferent fibers varies depending on the muscle assessed. The present study only included a relatively small study sample. The non-significant findings may have resulted from inadequate statistical power. Future studies in humans may help to address whether there indeed sex-related differences in acid induced muscle pain.

4.5 Effect of Repeated Acid Infusions

In animal studies, repeated intramuscular injections of acidic saline produced a prolonged bilateral mechanical hyperalgesia lasting up to 30 days [11] providing the first insight into the molecular mechanisms underlying the development of chronic muscle hyperalgesia [8]. However, in the present human study, contralateral spreading of pain and hyperalgesia was not observed following repeated infusions of acidic saline. In line, repeated infusion of unbuffered acidic saline into human masseter muscle did not evoke any mechanical hyperalgesia in either the local or the contralateral side [12, 27]. Repeated infusions into the tibialis muscle induced short-lasting (20 minutes) local hyperalgesia without involving the contralateral side [13]. It is not clear why the repeated acidic infusion in humans did not reproduce any long-lasting and widespread hyperalgesia similar to those in animals. It should be noted that conflicting results were also found in a previous animal study where the mechanical allodynia could not be detected after two repeated injections of acidic saline into the masseter muscle [17]. The modality differences between acidic saline, buffered or unbuffered, flow rate, infusion volume, intervals between repeated infusions, different muscles, trigeminal region vs spinal region, and evoked pain intensity are likely to contribute to the controversial results. Another explanation might be the difference in the total amount of acid stimulation between the animal and human studies as a larger part of the muscle was actually stimulated in the animals; whereas only a small part of the muscle was affected in humans. Again, the relatively smaller sample size may also contribute to the negative result.

5. Conclusions

Infusions into the brachioradialis muscle induced pain that was pH-dependent and mechanical hyperalgesia that was pH independent. However, repeated intramuscular acidic saline injection model of prolonged hyperalgesia in rodents could not be translated into a

human for modelling chronic musculoskeletal pain.

6. Implications

The acid-induced pain model may reflect the early stage responses to tissue injury of clinical conditions. It was not possible to use this human intramuscular acidic saline infusion model to produce the type of prolonged local and widespread hyperalgesia that has previously been demonstrated to occur in animal models.

Ethical issues

None to declare

Conflict of Interest

None of the authors have potential conflicts of interest to be disclosed.

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Figure legends

Fig. 1. Infusion Sites and Testing Sites

T1: infusion site on the left brachioradialis muscle; T2: local testing site, 1 cm from infusion site. C1 and C2: testing sites on the contralateral brachioradialis muscle

Fig. 2. Visual Analogue Scale (VAS) Profile and Pain Area Under the Curve

Mean VAS scores after pH 5.0 (blue), pH 6.0 (red), or pH 7.4 (green) infusion of acidic buffered saline into the left brachioradialis muscle in healthy humans (Mean ± Standard Error, N=20). * indicates significant difference ($P < 0.05$).

Fig. 3. Pain Drawing Area

The pain distribution after pH 5.0, pH 6.0, or pH 7.4 infusion of acidic buffered saline into the left brachioradialis muscle in healthy humans (N=20). Blue and red lines represent men and women, respectively.

Fig. 4. Pressure Pain Threshold

Mean (± standard error of the mean, N=20) pressure pain thresholds relative (%) to baseline measures on the infused site T1 (A), local site T2 (B) and contralateral side C1 (C), C2 (D) by the infusion of pH 5.0 (blue), pH 6.0 (red), or pH 7.4 (green) buffered saline into the left brachioradialis muscle in healthy humans. * indicates significant difference ($P < 0.05$) compared with baseline.

Fig. 5. VAS scores and PPT Changes after Repeated Infusion

A: Mean VAS scores of the first (blue solid line) and second (blue dotted line) infusion of pH 5.0 acidic saline (N=8) and the first (green solid line) and second (green dotted line) infusion

of pH 7.4 neutral phosphate buffered saline (N=4) into the left brachioradialis muscle in healthy humans (N=12). B: Means of % changes (relative changes to the baseline of the respective days) of pressure pain threshold from the baseline at the T2 testing site after the first (blue) and second (black) infusion of pH 5.0 acidic saline into the left brachioradialis muscle in healthy humans. The relative changes of PPTs were significantly lower during infusion of pH 5.0 acidic saline, but no significant difference was detected between two repeated sessions.

Figure

Fig. 1

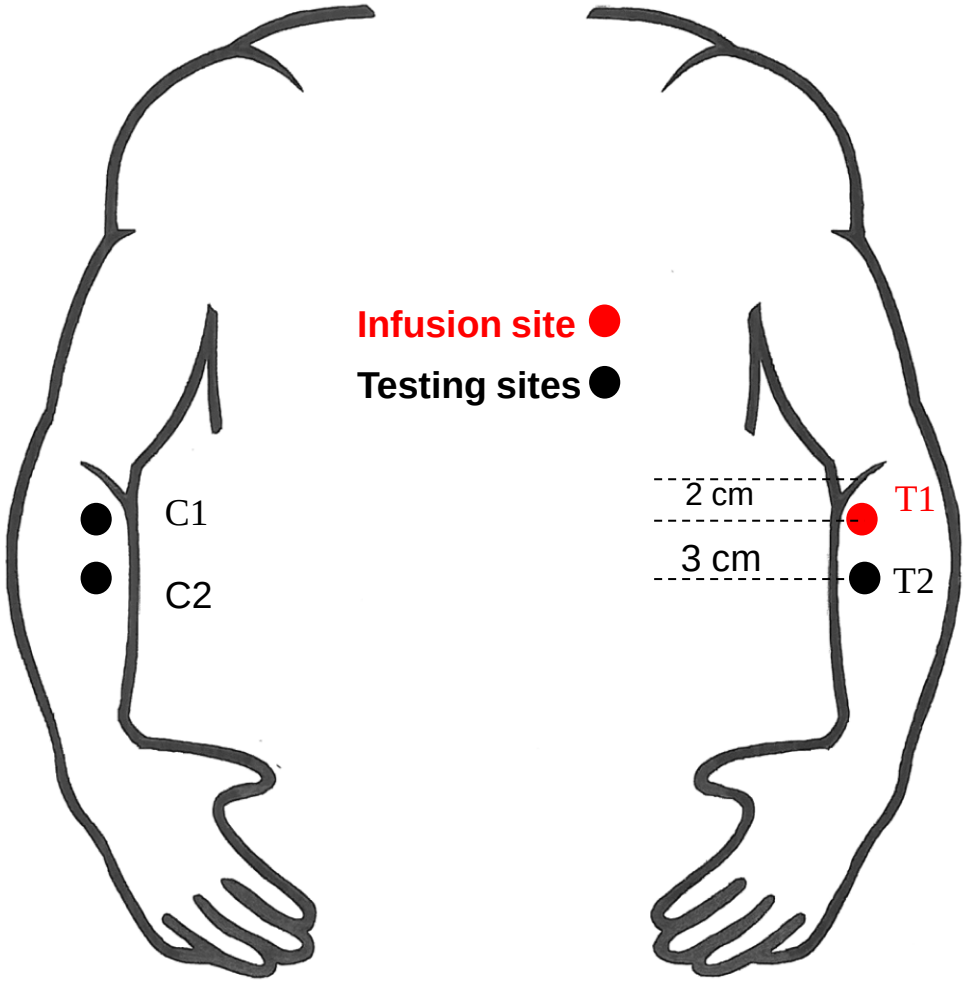


Fig. 2

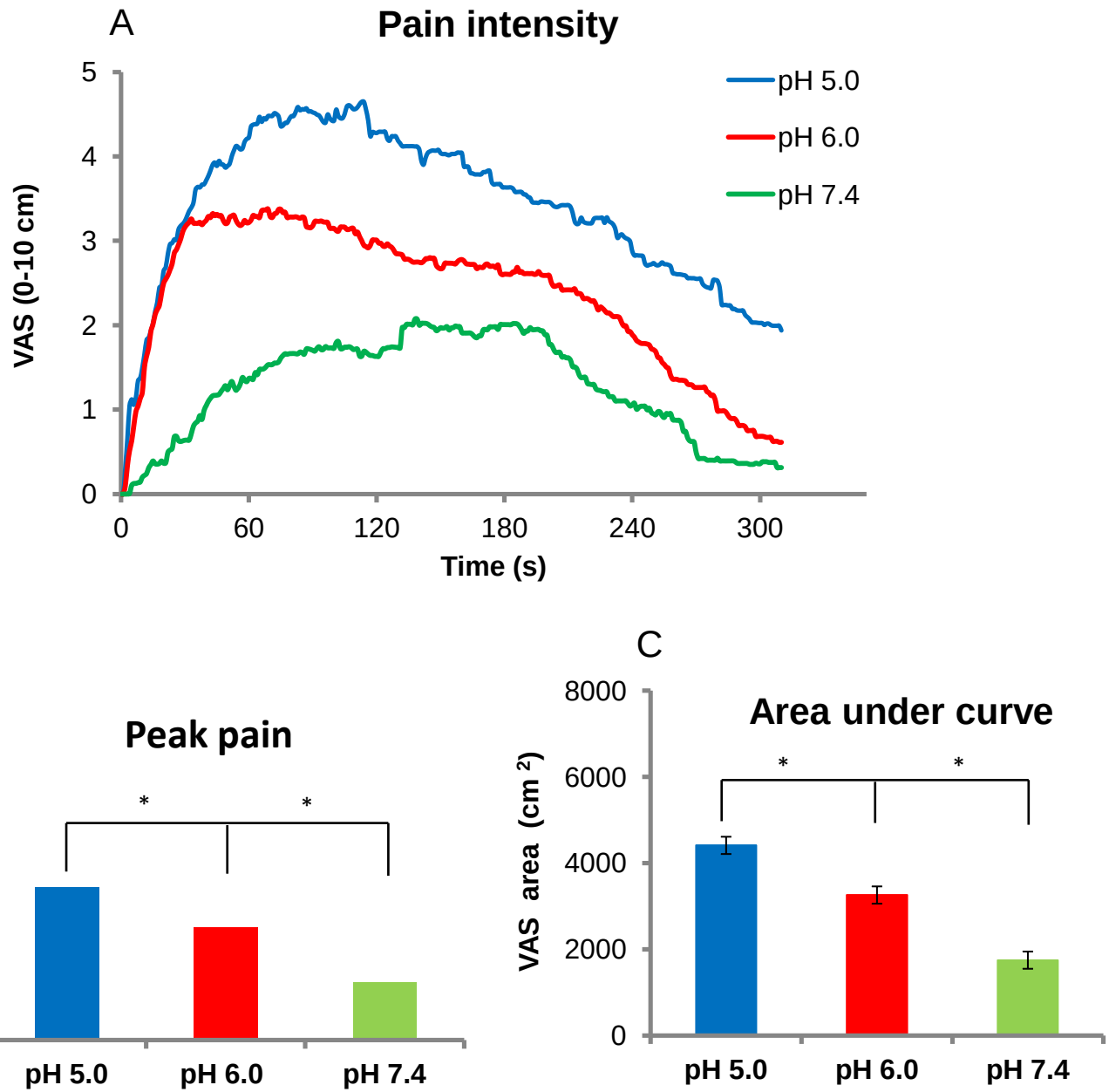

A
Pain intensity
VAS (0-10 cm)
Time (s)
pH 5.0
pH 6.0
pH 7.4
0
1
2
3
4
5
0
60
120
180
240
300
B
Peak pain
VAS (0-10 cm)
0
2
4
6
8
10
*
*
pH 5.0
pH 6.0
pH 7.4
C
Area under curve
VAS area (cm 2)
0
2000
4000
6000
8000
*
*
pH 5.0
pH 6.0
pH 7.4

Fig. 3

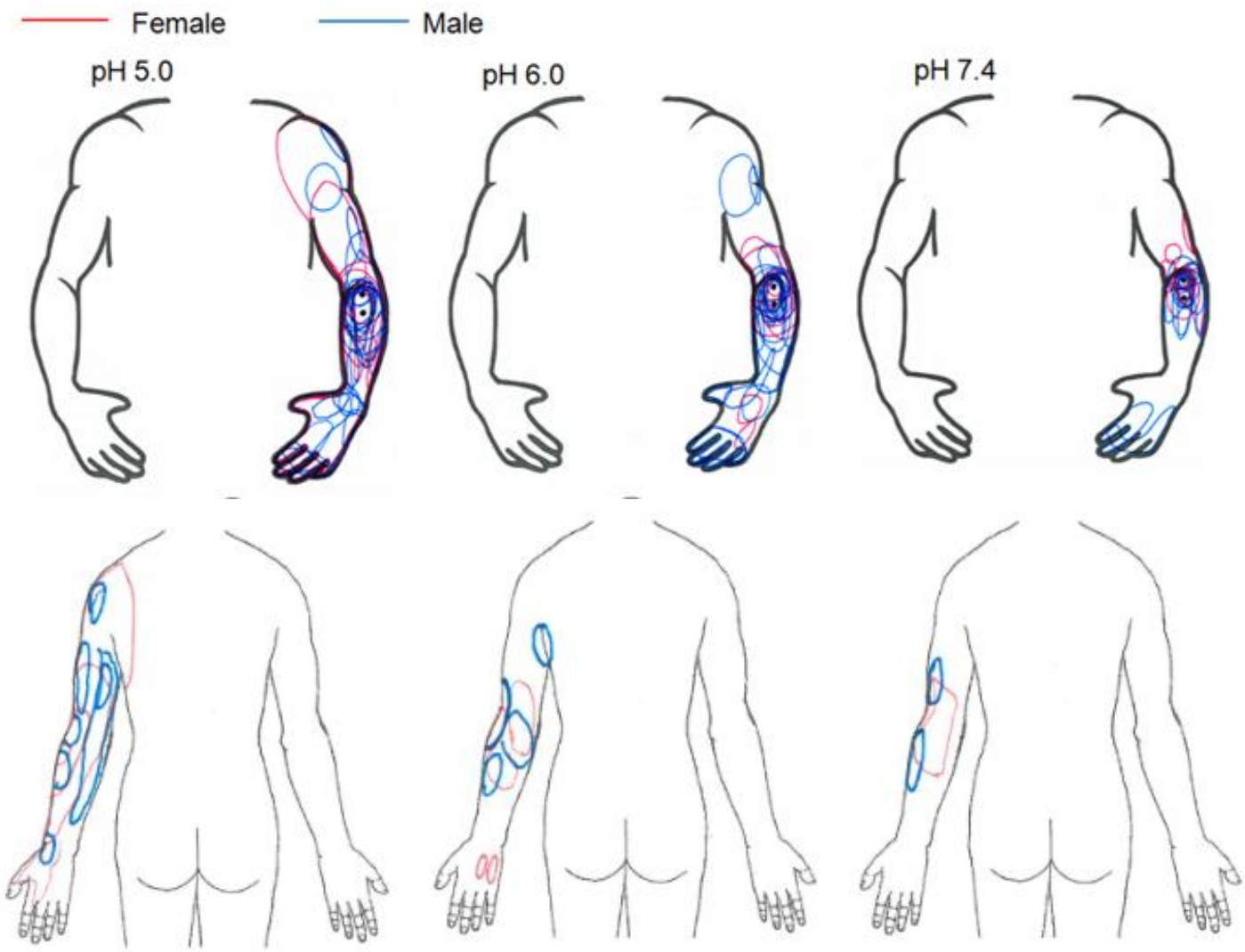


Fig. 4

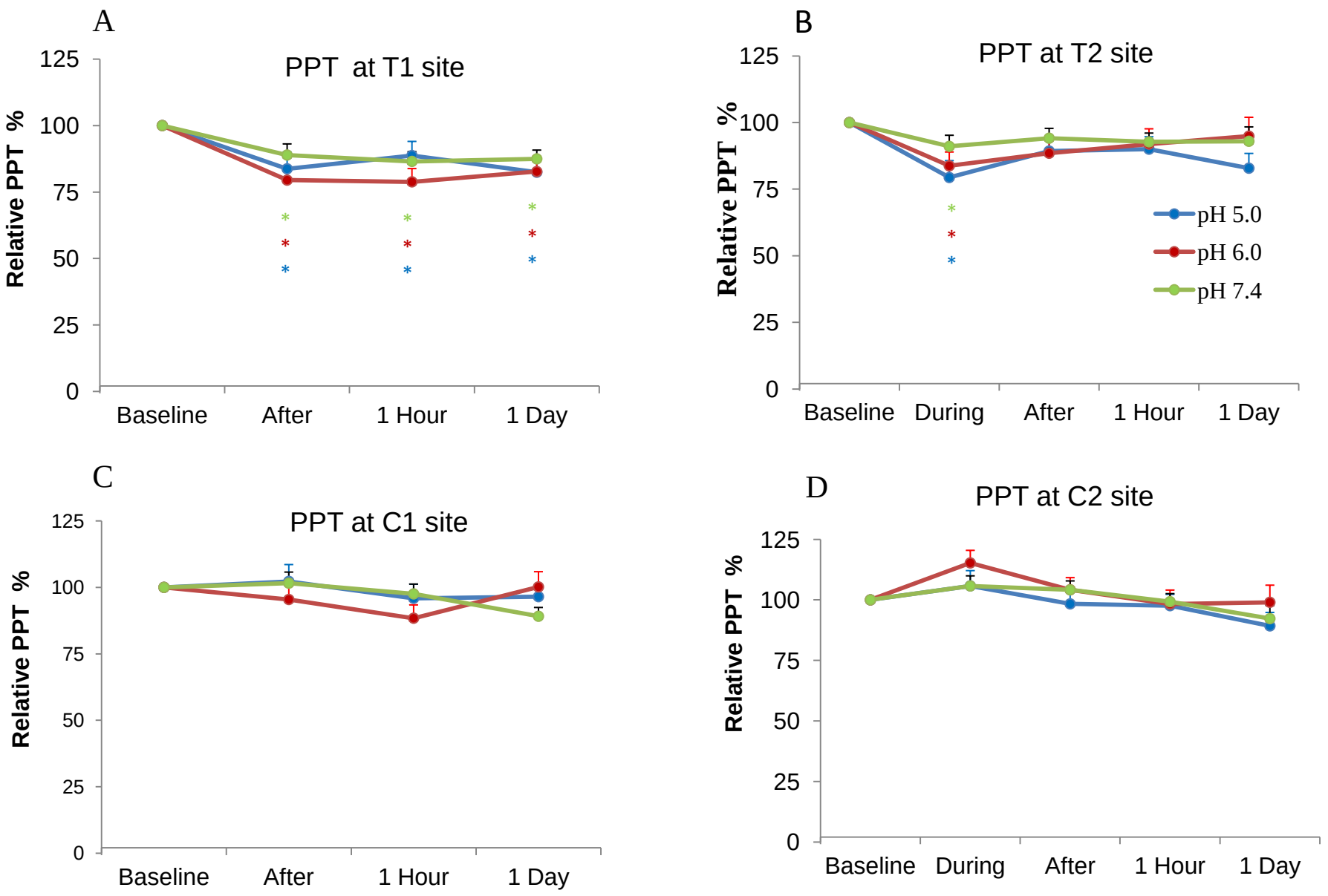
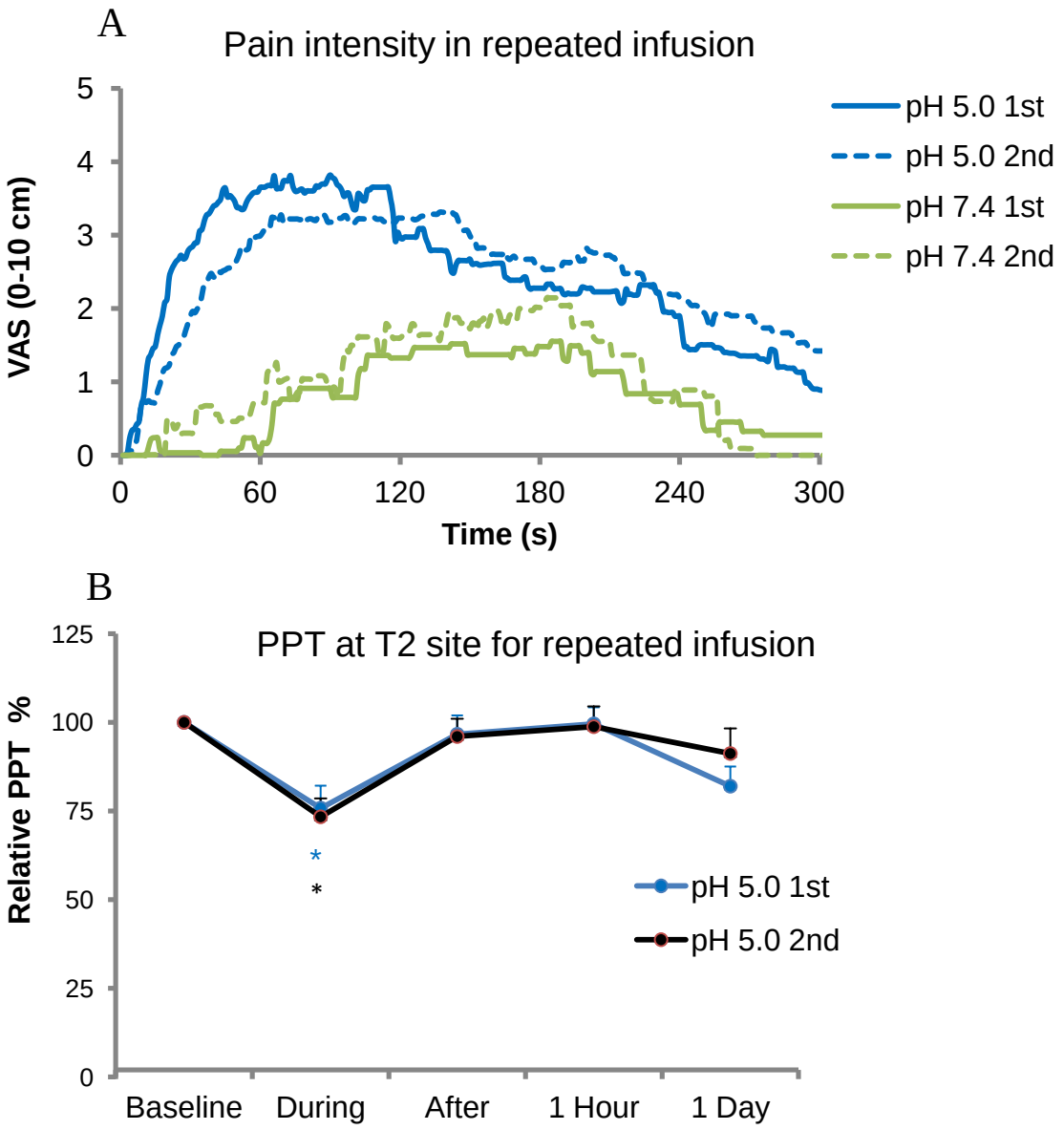

A
PPT at T1 site
Relative PPT %
125
100
75
50
25
0
Baseline
After
1 Hour
1 Day
B
PPT at T2 site
Relative PPT %
Baseline
During
After
1 Hour
1 Day
pH 5.0
pH 6.0
pH 7.4
C
PPT at C1 site
Relative PPT %
D
PPT at C2 site
Relative PPT %

Fig. 5


A
Pain intensity in repeated infusion
VAS (0-10 cm)
Time (s)
pH 5.0 1st
pH 5.0 2nd
pH 7.4 1st
pH 7.4 2nd
B
PPT at T2 site for repeated infusion
Relative PPT %
Baseline
During
After
1 Hour
1 Day
pH 5.0 1st
pH 5.0 2nd

ACID-INDUCED EXPERIMENTAL MUSCLE PAIN AND HYPERALGESIA WITH SINGLE AND REPEATED INFUSION IN HUMAN FOREARM

Kelun Wang[*1], Yi Luo[1], Toshiyuki Asaki[1], Thomas Graven-Nielsen[2], Brian E Cairns[1,2], Thomas Arendt-Nielsen[1], Lars Arendt-Nielsen[1,2]

[1]SMI, Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Denmark
[2]Center for Neuroplasticity and Pain (CNAP), SMI, Department of Health Science and Technology, Aalborg University

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Correspondence: Kelun Wang

SMI, Department of Health Science and Technology, The Faculty of Medicine, Aalborg University, Fredrik Bajers Vej 7D-3, 9220 Aalborg, Denmark.

Tel.: +45 9940 8745, Fax: +45 9815 4008

E-mail: kelun@hst.aau.dk

ABSTRACT

Background and purpose: Acid has long been thought to play an important role in the pain process. Animal study showed that repeated acid stimulation induced central sensitization. The purpose of the study is to investigate muscle pain and hyperalgesia evoked by intramuscular infusion of saline at different pH levels, and to compare the effect of a single versus repeated acid infusions.

Methods: Twenty healthy subjects received infusions of buffered saline (pH 5.0, 6.0, and 7.4) into the brachioradialis muscle in a randomized order. Twelve of the subjects received repeated infusions. The subjects rated the pain intensity on visual analogue scale (VAS). Thermal pain sensitivity, and pressure pain threshold (PPT) were assessed in both arm before, during, immediately after, one hour after, and one day after the infusion. A McGill Pain Questionnaire and pain mapping were completed after each infusion.

Results: The pH 5 solution caused significantly higher pain and larger areas than pH 6.0 or 7.4. The local PPTs were significantly decreased (hyperalgesia) during and immediately after infusion of all three solutions. No significant differences were detected between the first and second infusion.

Conclusions: The intensity of acid-induced muscle pain is pH-dependent. All three solutions induced pressure hyperalgesia at the infusion site. Repeated infusions did not induce increased pain or prolonged hyperalgesia as compared with a single injection. Human intramuscular acidic saline infusion could not produce chronic pain model.

Implications: The acid-induced pain model may reflect the early stage responses to tissue injury of clinical conditions. Repeated intramuscular acidic saline injection model of prolonged hyperalgesia in rodents could not be translated into a human for modelling chronic musculoskeletal pain.

Key words: Acid-induced pain; Hyperalgesia; Muscle pain; Experimental pain; Gender

difference

1. Introduction

Tissue acidosis has been observed as a regular phenomenon following inflammation, ischemia, arthritis, cancer, hematomas, and exercise [1, 2, 3]. Local tissue pH has been found to drop to 5.4 in purulent exudates, 4.7 in fracture-related hematomas, 5.0-4.0 in bone cancer, and 6.0 in patients with occlusive arterial diseases in the leg [3]. Considering the painful nature of all the conditions above, the high proton concentration might be a significant contributor to the associated pain [1, 4]. Previous studies also suggest that a strong reciprocal pain potentiating interaction exists between acidic pH and several inflammatory mediators and neurotransmitters, with low pH playing the dominant role [5, 6, 7]. The acid-sensing ion channels (ASICs) play an important role in the activation of nociceptors by low pH and thus may serve as potential targets for analgesic drug developing [3, 4, 6, 8, 9, 10].

Human and animal studies have shown that acid can induce both transient and sustained pain [3, 4, 11]. An acid-induced pain model in rats has been proven to be safe and without significant tissue damage by histological biopsy compared with other inflammation or tumour-induced pain models in rodents [11]. Using acid to produce pain in human skin and muscle has also proven safe [12, 13, 14]. Primary mechanical hyperalgesia was reported to be observed following the acid stimulation. In spite of the different methods adopted, the intensity of the acid-induced pain is pH-dependent [3, 7, 13, 14]. A previous study including intramuscular acidic stimulation reported that women experienced higher referred pain and exhibited a stronger correlation between local and referred pain than men [13].

Animal studies have reported that repeated intramuscular acidic stimulations induced spinal hyperexcitability with contralaterally spreading hyperalgesia [8, 11, 15, 16]. Local anaesthetics applied to the muscle previously injected with acidic saline could not inhibit the acid-induced contralateral spreading of hyperalgesia [11] indicating a central origin of the phenomenon. However, spreading of pain has not been found after repeated acid injection

into the masseter muscle in neither human [12] nor animal studies [17]. Since the results from human research within this field are limited, further studies should be conducted to elucidate the possible central mechanism of acid-induced muscle pain.

The aims of this human study were to investigate: 1) whether acid-induced muscle pain and 2) pressure hyperalgesia were pH dependent; 3) if spreading sensitization could be evoked by repeated versus single injection of acid stimulation; and 4) if there were gender differences in any of the parameters.

2. Methods

2.1 Subjects

Twenty healthy subjects (7 women, 24.3±3.1 years) participated in a three-session study with a single infusion in each session. Further, 12 of the 20 subjects (4 women, 24.1±2.8 years) participated in sessions with repeated infusions. None of the subjects had a history of pain or injuries or medical conditions that could interfere with normal somatosensory functioning. Women in the menstrual period were avoided. The study was approved by the local ethics committee (N 2011-0081) and conducted in accordance with the Declaration of Helsinki. All subjects gave written informed consent.

2.2 Experimental Protocol

The subjects participated in three sessions; each with a single infusion of buffered saline with one of three different pH levels (pH 5.0, 6.0 and 7.4). The infusions were conducted in random order and with a one-week interval between sessions. Further, 12 subjects received repeated infusions of either pH 5.0 or pH 7.4 solution with a one-day interval between infusions. In each session, neutral phosphate buffered saline (10 ml) was infused into the brachioradialis muscle over 20 min using a computer-controlled infusion pump.

Cold pain threshold (CPT), heat pain threshold (HPT), mechanical pain sensitivity (MPS), and pressure pain threshold (PPT) were assessed before, during, after, one hour after, and one day after the infusion. The pain intensity was rated by means of an electronic visual analogue scale (VAS). A McGill Pain Questionnaire and a pain map were completed after each infusion.

2.3 Acidic Infusions and Pain Assessment

The pH adjusted phosphate buffered saline (10 mL, Hospital Pharmacy of Aalborg University Hospital, Aalborg, Denmark) was randomly infused into the left brachioradialis muscle (2 cm from the superior border of cubital fossa) in a double-blinded manner with respect to the pH level. The infusion site was cleaned with alcohol and dried prior to the needle insertion. The needle (27 G, 19 mm, BD Microlance 3, Becton Dickinson, Ireland) was inserted into middle part of the brachioradialis muscle with a depth of 15 mm. The inserted needle was fixed to the skin using surgical tape and sterile cotton. A tube (200 cm, 1.5 ml, G30303M, Care Fusion, Switzerland) was connected to the needle from the syringe. The sterile buffers were infused at a constant rate of 30 ml/h for 20 minutes using a computer-controlled infusion pump (Asena CC MK-III, Alaris medical systems, USA). The needle and tube were removed immediately after completion of the infusion.

The subjects rated the induced pain intensity on an electronic VAS on which "0 cm" indicated "no pain" and "10 cm" represented "most pain imaginable". The VAS signal was sampled every 2 seconds from the beginning of the infusion until the pain intensity had returned to zero. The maximal pain (VAS peak) and the area under the curve (VAS area) were calculated. After the infusion, the subjects were asked to draw the pain areas on an arm drawing describe the quality of the pain on the McGill Pain Questionnaire (MPQ).

2.4 Assessment Sites

Two sites in the infusion side and two sites in the contralateral side were assessed (Fig. 1). CPT, HPT MPT, and PPT were assessed 1 cm from the infusion site (T2). All sensory assessments were also conducted at the infusion site (T1) at the same time points except during infusion. Further, the assessments were performed on the contralateral arm at the corresponding sites as controls (C2 and C1).

2.5 Cutaneous Thermal Pain Sensitivity

Cold pain threshold (CPT) and heat pain threshold (HPT) were measured (TSA 2001 II (CHEPS, Medoc, Israel) at T1 and C1 sites. The contact area of the thermode was 9 cm^2. The baseline temperature was 32 C° (centre of neutral range). The method of limits was used by applying ramp stimuli at a velocity of 1 C°/s. The cut-off temperatures were 0 C° and 55 C°. The volunteers were asked to press a button when the respective thermal sensations were perceived. The mean threshold temperature of three consecutive measurements was calculated.

2.6 Cutaneous Mechanical Pain Sensitivity

The cutaneous sensitivity was assessed using weight-calibrated pins (128 mN, custom made Aalborg University) at all assessment sites. The subjects rated the cutaneous mechanical pain sensitivity (MPS) on a 0-5-10 Numeric Rating Scale (NRS) on which "0" represented “no sensation", "5" represented "pain threshold", and "10" presented "worst pain imaginable". The mean of the three measurements was used in the statistical analysis.

2.7 Pressure Pain Sensitivity

A hand-held pressure algometer (Somedic AB, Sweden) was used to assess the PPTs. The

pressure was applied to all assessment sites at a constant rate of 30 kPa/s through a 1 cm^2 probe. The subjects were instructed to push a button immediately when they felt the pressure turning into pain. The PPTs were measured twice at each site. The interval between the two PPT trials was at least 40 sec and the mean of the two measurements was used in the statistical analysis.

2.9 Statistics

The normal distribution was checked for all data. The necessary logarithmic transformation was performed [18]. QST data were then analysed using a 3-way repeated measure analysis of variance (ANOVA) with gender as between-subject factor and testing site (T1, T2, C1, C2), pH levels (pH 5.0, 6.0, 7.4) and time (baseline, during, after, one hour after, and one day after) as within-subject factors. The VAS scores and pain areas of the different pH levels and of the single and repeated infusions were analysed by 2-way (pH level and gender) repeated measure of ANOVA. A Bonferroni test was employed for post-hoc comparisons in case of significant ANOVAs. All statistical calculations were performed using the Statistical Package for Social Sciences version 20 (SPSS, IBM). The significance level was set at $P<0.05$. The data are presented as mean and standard error of the mean (SEM).

3. Results

3.1 Acidic-evoked Pain

The VAS profiles of the three infusions with different pH levels are shown in Fig. 2A. Significant differences were detected in VAS peak (ANOVA: $P < 0.023$, Fig. 2B) and VAS area (ANOVA: $P < 0.012$, Fig. 2C). The infusion of the pH 5.0 solution caused higher VAS scores (Peak 4.65) than the pH 6.0 solution (Peak 3.37) ($P= 0.015$) and the 7.4 solution (1.68) ($P = 0.001$). In addition, the pH 7.4 solution caused the lowest VAS scores compared with the

pH 6.0 solution (P= 0.016).

The pain areas following the three different solutions are illustrated in Fig. 3. Infusion of the pH 5.0 solution evoked a larger area than infusion of pH 6.0 (P =0.036) and 7.4 (P =0.004), whereas the pH 7.4 solution evoked the smallest area compared with pH 6.0 and pH 5.0 solution (P< 0.021).

No gender differences were detected in VAS peak, VAS area, or drawing areas (P > 0.092).

3.2 Cutaneous Thermal and Mechanical Pain Sensitivity

The ANOVA of the CPT, HPT, or MPS among the different solutions at any of the tested sites were not significant (ANOVA: P > 0.073). Likewise, no significant change was detected before, during, immediately after, one hour after, and one day after the infusion (ANOVA: P > 0.103,) or between gender (ANOVA: P > 0.087, data not presented).

3.3 Pressure Pain Sensitivity

The ANOVA demonstrated that the PPTs were different over time (ANOVA: P < 0.017) but not between solutions pH levels (P > 0.092). Compared with baseline, relative PPTs decreased immediately after, 1 hour after, and 1 day after the infusion at T1 (p<0.017) and during infusion at the T2 site (p<0.030) (Fig. 4AB). No significant difference was detected in the contralateral sites (ANOVA: P > 0.105; Fig. 4CD) and no gender difference was found (ANOVA: P > 0.087).

3.4 Repeated Infusions

Eight out of 12 subjects received repeated infusion of pH 5.0 solution, and 4 of them received repeated infusion of pH 7.4 solution No significant difference in VAS scores was detected

between the two repeated sessions for neither pH 5.0 nor pH 7.4 ($P > 0.195$) (Fig. 5A). No difference was detected in CPT, HPT, MPS or PPT between the two repeated sessions for either pH 5.0 or pH 7.4 (ANOVA: $P > 0.106$, data not presented). Normalized PPT values after the 1st and 2nd infusion of pH 5.0 is shown in Fig. 5B.

4. Discussion

The intensity of the acid-induced muscle pain was pH dependent whereas the deep tissue pressure hyperalgesia was not pH dependent. Repeated acid stimulation did not induce more pain or prolonged pressure hyperalgesia as compared with a single injection. No gender differences were found.

4.1 Acid-induced Muscle Pain

In the present study, the single infusions of the pH solution into the human forearm muscle produced significantly higher pain intensities than the neutral buffer (pH 7.4) and the pH 6.0 buffer infusions, which is consistent with previous studies on acidic muscle pain models [13, 19,]. The study is the first to use infusion of buffered saline with different pH levels into the same group of human muscles. The results provide clear evidence that the acid-evoked pain was pH dependent. The thin myelinated Group III and unmyelinated Group IV nerve fibres in muscle are responsible for transmitting muscular nociceptive information and their endings are sensitive to inflammatory mediators including low pH stimulation [20, 21]. The decrease in tissue pH following muscle ischemia is believed to activate ASICs in muscle nociceptors, thus contributing to e.g. ischaemic muscle pain [8, 19, 22, 23]. Clinically it is known that local anaesthetics with pH levels as low as 5 occasionally produce transient pain upon injection [24, 25]. Thus acidic infusion could be used as a muscle pain model in both animal and human studies.

4.2 Factors affecting the pain evoked by acid infusions

Factors such as infusion volume and muscle size could affect acid-evoked pain intensity. In the present study, a total of 10 ml of buffered acid saline was infused into the brachioradialis muscle at a rate of 30ml/h. The average peak pain intensity of the evoked pain was 4.65 cm for the pH 5.0, 3.37 cm for the pH 6.0, and 1.68 pH 7.4 solution (Fig. 2). In previous animal and human studies, different volumes were used to induce pain. In studies examining male rats, acidic saline was injected in volumes of 20 μL into the rat masseter [26] and rat gastrocnemius muscle [8,] which successfully evoked pain and hyperalgesia. However, in human studies with injection of 0.5 ml unbuffered acid saline, approximately 3% of the total volume of the human masseter muscle which was comparable to the relative injection volume used in the rats, no significant pain was induced when compared with neutral saline injections [12]. Infusion of five times the volume (2.5 mL) of unbuffered acidic saline into the masseter muscle of human subjects induced pain levels similar to the results of single injection (0.5 ml) in previous study [27]. Thus, the results of the previous human studies did not provide evidence that the injection/infusion volume had a major impact on the acid-induced pain intensity. It seems other factors, such as infusion rate, and using buffered saline may play more important role in the acid-evoked pain intensity. However, the injection or infusion volume should be considered according to the muscle volume when using an acid infusion as pain model.

The infusion rate may play an important role in the acid-evoked pain. A previous study on acid-induced human skin pain indicated that raising the infusion rate leads to increasing pain by lowering the local pH more effectively and by increasing the tissue volume in which the proton concentration exceeds the threshold to excite nociceptors [28]. Infusion of isotonic pH 5.2 phosphate buffer into the flexor carpi radialis muscle produced pain correlated with

the flow rate of the infusion [19]. In a recent human study, acid saline (pH 3.3) was infused into the masseter muscle with a slow infusion rate of 15ml/h. The infusion evoked only mild pain and no mechanical allodynia or increased release of algesic substances assessed by microdialysis were detected [27]. In our previous study, acid saline (pH 5.2) was infused into the anterior tibialis muscle and the pain level was higher and PPTs were lower following an infusion rate of 40 mL/h compared to the infusion rate of 20 ml/h [13]. In the present study, the infusion rate of 30 mL/h was selected since the expected pain intensity was evoked by both pH 5.0 and pH 6.0 buffered saline during the pilot experiments when the different infusion rates of 10ml, 20 ml, 30ml, and 40 ml were tested.

In addition, using buffered saline instead of unbuffered saline might be the necessary to evoke pain. Previous human studies have suggested that acid-induced muscle pain may be more effectively produced by infusion of low pH (~5) phosphate buffers [13, 28] than by injections of unbuffered acidic saline [12, 27]. Recent human studies did not evoke the expected pain by means of unbuffered acid saline with pH 3.3 [12, 27]. This difference is possibly due to the ability of the muscle tissue to rapidly buffer pH changes after injections of acidic solutions. Compared with a buffered saline solution, an unbuffered saline solution could physiologically regain pH level more quickly because of the buffering capacity of the muscle tissue. Since ACIC3 channels generate sustained currents as long as the pH is acidic [29], the longer the pH in the muscle remains acidic and the longer the ASIC3 channels will be activated.

4.3 Mechanical Hyperalgesia

In the present healthy human study, PPT values at the infusion site (T1) and around the infused site (T2) were significantly decreased during the acidic infusion compared with baseline. However, no mechanical hyperalgesia was observed in the contralateral side

indicating that the acidic infusion caused local sensitisation without central mechanisms being involved. Further, no significant difference was detected in the three different pH solutions indicating that the local mechanical hyperalgesia was not pH-dependent but most likely a volume effect. Similar mechanical hyperalgesia was observed in the experimental muscle pain model conducted by injecting acidic buffer into the anterior tibialis muscle [13]. In contrast, in a recent human study acid-infusion into the masseter muscle did not evoke mechanical hyperalgesia in either the local or contralateral side [27]. It seems that only localised pain and short period local hyperalgesia were observed after infusion of acidic buffer in human studies.

4.4 Gender Differences

No sex-related differences in pain intensity, pain areas, or induced local pressure hyperalgesia were observed among the three different infusions in the present study. A previous study of intramuscular acidic stimulation reported that women experienced higher referred pain and exhibited a stronger correlation between local and referred pain than men [13]. In rats, expression of $ASIC_3$ receptors is greater in masseter muscle sensory afferent fibers in females compared with males [30]. It is unclear if a similar difference in ASIC channel expression occurs in humans or if the expression of ASIC channels by sensory afferent fibers varies depending on the muscle assessed. The present study only included a relatively small study sample. The non-significant findings may have resulted from inadequate statistical power. Future studies in humans may help to address whether there indeed sex-related differences in acid induced muscle pain.

4.5 Effect of Repeated Acid Infusions

In animal studies, repeated intramuscular injections of acidic saline produced a prolonged bilateral mechanical hyperalgesia lasting up to 30 days [11] providing the first insight into the molecular mechanisms underlying the development of chronic muscle hyperalgesia [8]. However, in the present human study, contralateral spreading of pain and hyperalgesia was not observed following repeated infusions of acidic saline. In line, repeated infusion of unbuffered acidic saline into human masseter muscle did not evoke any mechanical hyperalgesia in either the local or the contralateral side [12, 27]. Repeated infusions into the tibialis muscle induced short-lasting (20 minutes) local hyperalgesia without involving the contralateral side [13]. It is not clear why the repeated acidic infusion in humans did not reproduce any long-lasting and widespread hyperalgesia similar to those in animals. It should be noted that conflicting results were also found in a previous animal study where the mechanical allodynia could not be detected after two repeated injections of acidic saline into the masseter muscle [17]. The modality differences between acidic saline, buffered or unbuffered, flow rate, infusion volume, intervals between repeated infusions, different muscles, trigeminal region vs spinal region, and evoked pain intensity are likely to contribute to the controversial results. Another explanation might be the difference in the total amount of acid stimulation between the animal and human studies as a larger part of the muscle was actually stimulated in the animals; whereas only a small part of the muscle was affected in humans. Again, the relatively smaller sample size may also contribute to the negative result.

5. Conclusions

Infusions into the brachioradialis muscle induced pain that was pH-dependent and mechanical hyperalgesia that was pH independent. However, repeated intramuscular acidic saline injection model of prolonged hyperalgesia in rodents could not be translated into a

human for modelling chronic musculoskeletal pain.

6. Implications

The acid-induced pain model may reflect the early stage responses to tissue injury of clinical conditions. It was not possible to use this human intramuscular acidic saline infusion model to produce the type of prolonged local and widespread hyperalgesia that has previously been demonstrated to occur in animal models.

Ethical issues

None to declare

Conflict of Interest

None of the authors have potential conflicts of interest to be disclosed.

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Figure legends

Fig. 1. Infusion Sites and Testing Sites

T1: infusion site on the left brachioradialis muscle; T2: local testing site, 1 cm from infusion site. C1 and C2: testing sites on the contralateral brachioradialis muscle

Fig. 2. Visual Analogue Scale (VAS) Profile and Pain Area Under the Curve

Mean VAS scores after pH 5.0 (blue), pH 6.0 (red), or pH 7.4 (green) infusion of acidic buffered saline into the left brachioradialis muscle in healthy humans (Mean ± Standard Error, N=20). * indicates significant difference ($P < 0.05$).

Fig. 3. Pain Drawing Area

The pain distribution after pH 5.0, pH 6.0, or pH 7.4 infusion of acidic buffered saline into the left brachioradialis muscle in healthy humans (N=20). Blue and red lines represent men and women, respectively.

Fig. 4. Pressure Pain Threshold

Mean (± standard error of the mean, N=20) pressure pain thresholds relative (%) to baseline measures on the infused site T1 (A), local site T2 (B) and contralateral side C1 (C), C2 (D) by the infusion of pH 5.0 (blue), pH 6.0 (red), or pH 7.4 (green) buffered saline into the left brachioradialis muscle in healthy humans. * indicates significant difference ($P < 0.05$) compared with baseline.

Fig. 5. VAS scores and PPT Changes after Repeated Infusion

A: Mean VAS scores of the first (blue solid line) and second (blue dotted line) infusion of pH 5.0 acidic saline (N=8) and the first (green solid line) and second (green dotted line) infusion

of pH 7.4 neutral phosphate buffered saline (N=4) into the left brachioradialis muscle in healthy humans (N=12). B: Means of % changes (relative changes to the baseline of the respective days) of pressure pain threshold from the baseline at the T2 testing site after the first (blue) and second (black) infusion of pH 5.0 acidic saline into the left brachioradialis muscle in healthy humans. The relative changes of PPTs were significantly lower during infusion of pH 5.0 acidic saline, but no significant difference was detected between two repeated sessions.