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Pain Perception after Subcutaneous Injections of Media Containing Different Buffers

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Abstract: Several hormones are administered by daily subcutaneous injections. Pain caused by subcutaneous injection is an unpleasant condition, which can limit patient compliance. The objective of the present study was to evaluate the perception of pain by subcutaneous injection of two different and commercially available solutions for dispensing recombinant human growth hormone. The solutions are characterised by pH, conservation, and buffer. Isotonic saline was used as reference solution. Fifty-four healthy volunteers (mean age (±S.E.M.): 35.5±1.1 years) were recruited to the double-blind, randomised study. All injections were performed pairwise (right and left thigh) in one day by the same experienced nurse. Perception of pain was evaluated by the volunteers immediately after injection and 2 min. after injection into the thigh of three formulations, which differed with respect to pH and buffers (histidine, citrate and saline, respectively). Significantly more participants (38/54) found than the citrate buffer caused more pain than the histidine buffer immediately after injection (P=0.002). Histidine buffer did not cause more pain than saline (P=0.996). After 2 min., there was no difference between the histidine and the citrate buffer (P=1.00), nor between the histidine buffer and saline (P=1.00). In summary, the solution-containing citrate as buffer caused more pain after subcutaneous injection than the solution with histidine as buffer. Considering patient compliance, it seems advisable to employ histidine-buffered solution rather than citrate-buffered solution for dispensing recombinant human growth hormone by daily subcutaneous injections.

Several drugs such as erythropoietin and the hormones insulin, growth hormone and octreotide, are administered by frequent subcutaneous injections. The unpleasant effect of subcutaneous injection might limit patient compliance, in particular when the consequence of not taking the drug is not immediately life-threatening.

The effect on drug pharmacokinetics of substitution of buffers, alteration of pH and concentration of solvents have all been evaluated in previous studies, in which changes in injection pain, however, were not examined (Laursen *et al*. 1993; Vahl *et al*. 1996). Several factors influence the perception of pain associated with subcutaneous injections (Jørgensen 1994). Factors related to the solution: pH, temperature, conservation, injection volume, tonicity, and buffer, but also technique of injection, speed of injection, needle size, anatomical region, individual patient characteristics, and frequency of administration may contribute. Indeed, in a blinded study in volunteers the rate of sc administration of lidocaine played a greater role than did buffering (Scarfone *et al*. 1998). Still, the pain associated with subcutaneous injection of bupivacaine is reduced by pH buffering with sodium bicarbonate (Cheney *et al*. 1991), and the choice of buffer may also be important. In drug formulations for subcutaneous injections e.g. phosphate, carbonate, citrate or histidine, which buffer within various pH ranges, are commonly used.

The 22kDa peptide growth hormone is administered by subcutaneous injections. The majority of the patients are children, for whom experience of pain is of particular importance with respect to compliance to the daily injections. Growth hormone is a convenient model drug for the study of injection pain, as short-term administration of even large doses produces no side effects as opposed to e.g. insulin. The objective of the present study was to evaluate the perception of pain by subcutaneous injection of two commercially available solutions for dispensing recombinant human growth hormone (Norditropin® SimpleXx® and Nutropin AQ®), in which histidine or citrate, respectively, were used as buffer.

Materials and Methods

Participants. Healthy volunteers were recruited at the Medical Department, Aarhus University Hospital. Fifty-four (table 1) gave their written informed consent. All participants concluded the trial during one single visit according to the protocol, which was approved by the Regional Ethics Committee.

Experimental design. The study was carried out in a double-blind, randomised design. The perception of pain was evaluated after subcutaneous injection in the thigh of three different test media (table 2). The injection volume was 0.3 ml on all occasions, 30 G, 8 mm needles were employed, and all injections were performed in a 45° angle in a lifted skin-fold. Injections were given pair-wise, first one in the right thigh, and immediately after one in the left thigh, in all

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Table 1.

Demographic features of the trial population. Mean±S.E.M.

	Females (n=45)	Males (n=9)	All (n=54)
Age, years	34.9±1.2	38.6±2.6	35.5±1.1
Height, cm	169.3±0.8	182.4±1.2	171.5±0.99
Weight, kg	67.1±1.2	84.6±4.6	70.0±1.5
BMI, kg/m^2	23.5±0.45	25.5±1.6	23.8±0.47

two pairs of injections (4 injections). There was a 5 min. interval between the two pairs of injections. Injection pairs were given in random order. Randomising and blinding was performed by the Dispensary of the hospital. All participants received one injection pair including A and B, and one injection pair including A and C. All injections in each subject were performed in one day, and by the same experienced nurse, assuring that e.g. the rate of injection was kept constant.

After each pair of injection the participants evaluated, using a 5 point verbal rating scale (VRS) (see legends for figures), whether they experienced much more or more pain after one of the injections within the pair, or if there were no difference between the two injections within the pair. The VRS has been used in many studies of pain perception (Frenken *et al*. 1993; Jørgensen 1994; Grond *et al*. 1995). Evaluation of pain was made immediately after injection of each pair and 2 min. after injections.

Statistics. A one-sided binomial test with a 5% significance level was applied to assess if one buffer caused more pain than the other. With no difference between buffers, the distribution can be assumed to be symmetric around "score 3". The statistical results are reported according to the null hypotheses: i) solution B does not cause more pain than A, and ii) solution A does not cause more pain than C. As the most conservative test, the one-sided test is performed against the alternative that the probability of more or much more pain is >50%.

Results

Perception of pain immediately after injection (fig. 1).

No adverse events were recorded.

Thirty-one of 54 volunteers expected injections to be very painful, and 23 of 54 expected minor pain, before injections were performed. Significantly more volunteers (38/54) found that the citrate buffer caused more or much more pain than the histidine buffer immediately after injection (P=0.002). Compared with saline, the histidine buffer (18/54) did not cause significantly more pain immediately after injection (36/54) (P=0.996).

Perception of pain 2 min. after injection (fig. 2).

After 2 min., the majority (histidine versus citrate: 44/54; histidine versus saline: 36/54) scored 3 (no difference). Accordingly, no difference was found either between the histidine and the citrate buffer (P=1.00), or between the histidine buffer and saline (P=1.00) 2 min. after injections.

Table 2.

Test products.

Test solution	pH	Buffer	Preservative
A 2 ml	6.15	L-Histidine 1.36 mg	Phenol 6.0 mg
B 2 ml	6.00	Na-Citrate 10 mM	Phenol 5.0 mg
C 2 ml	8.30	0.9 % saline	Benzylalcohol 9 mg

Discussion

The benefit of minimizing the pain associated with subcutaneous injection of drugs is obvious, but is of particular importance when children are treated, or when drugs, for which at least short-term non-compliance does not severely affect health, are used. The present data show that subcutaneous injection of a solvent containing citrate causes more pain than one containing histidine. The lack of significant differences 2 min. after injection should not be overestimated, as even short-term exposure to pain will make the patients, in particular children, associate the treatment with inconvenience, which again may impair compliance. As 5/6 of the participants in the study were women, a theoretical gender difference cannot be excluded, but the results surely apply to women.

The patients acted as their own controls which more strongly allowed a judgement of which of the two different solutions that caused most pain in each individual following injection. As a control the histidine solution was compared with isotonic saline, which is supposed to cause the minimal degree of pain in connection with subcutaneous injections. The histidine solution did not cause significantly more pain than did isotonic saline immediately after injection or after 2 min. Previous studies have demonstrated that citrate solutions cause more pain than saline (Frenken *et al*. 1993). No absolute pain score associated with the injections was evaluated, since quantification of pain was not an objective of the study.

The few publications available in the literature on this topic provide limited information. However, an association between pain after subcutaneous injection and use of citrate as buffer has previously been described. In a double-blind, randomized, placebo-controlled study the pain after subcutaneous injection of erythropoietin was mainly caused by the citrate component of the buffered solution (Frenken *et al* 1993). In another well-controlled recent study in 60 haemodialysis patients, the pain of subcutaneous injection of erythropoietin was significantly reduced when phosphate was employed instead of citrate as buffer (Veys *et al*. 1998). In the present study the citrate buffer was slightly more acidic than the histidine buffer, but it seems unlikely that the difference is large enough to elicit the observed difference in perception of pain. Although it is unclear why citrate is more painful than histidine, the finding is in accordance with previous reports of pain associated with subcutaneous injections of citrate-buffered solutions (Frenken *et al*. 1993; Veys *et al*. 1998; Yu *et al*. 1998). The impact of buffer concentration and pH has also been examined for a formulation of insulin-like growth factor I, in which a phosphate buffer was used. In that study high concentrations of buffer turned out to be the main responsible factor as concerns injection pain (Fransson & Espander-Jansson 1996).

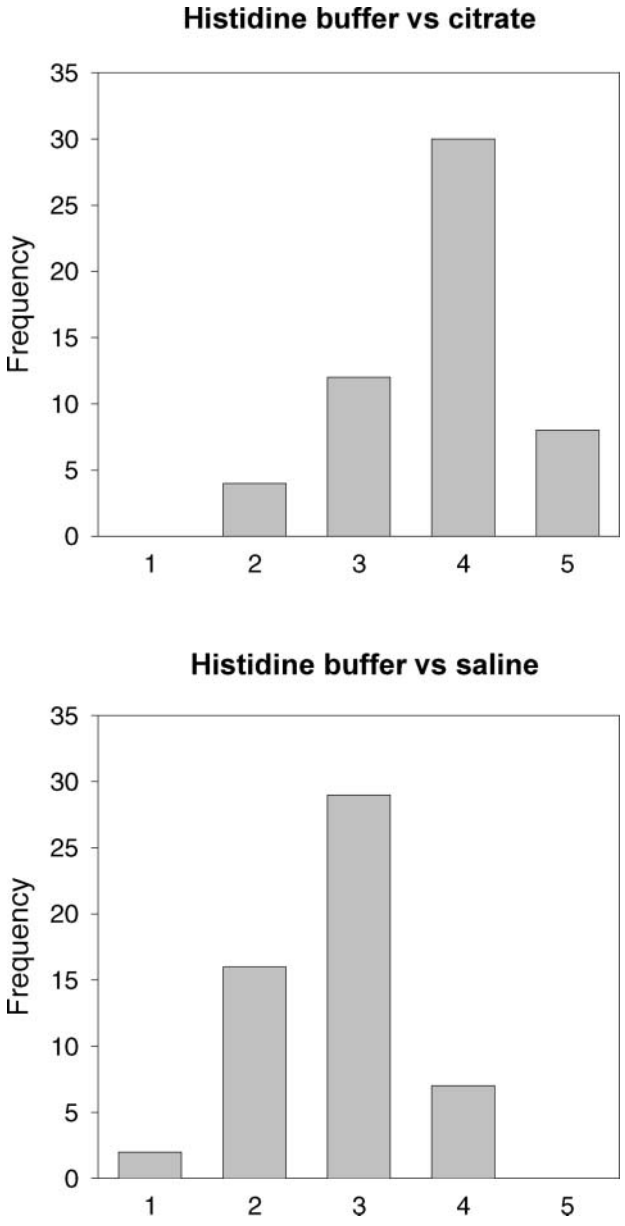

Fig. 1. Perception of pain at time=0 min. after injection of histidine (A) versus citrate (B) solutions (upper panel), and histidine (A) versus saline (C) (lower panel). The citrate solution caused more pain than histidine (P=0.002). Upper panel (fig. 1 and 2): score 1=histidine solution caused *much more* pain than citrate solution, score 2=histidine solution caused *more* pain than citrate solution, score 3=*no difference* in pain after the two injections, score 4=citrate solution caused *more* pain than histidine solution, score 5=citrate solution caused *much more* pain than histidine solution. Lower panel (fig. 1 and 2): score 1=histidine solution caused *much more* pain than saline solution, score 2=histidine solution caused *more* pain than saline solution, score 3=*no difference* in pain after the two injections, score 4=saline solution caused *more* pain than histidine solution, score 5=saline solution caused *much more* pain than histidine solution.

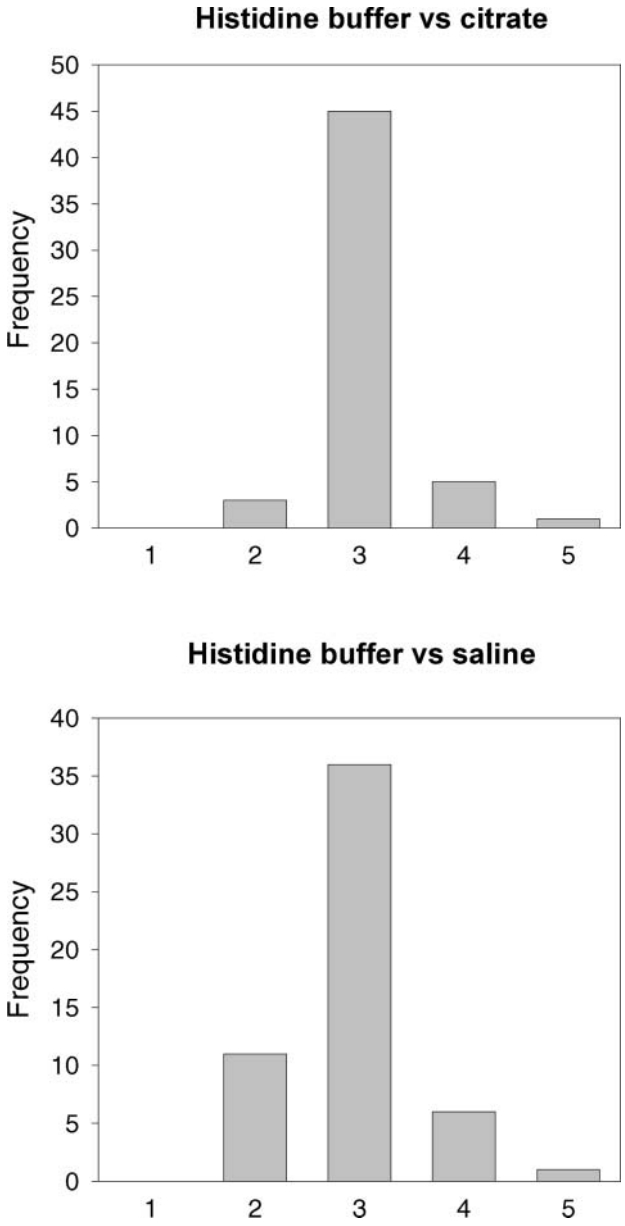

Fig. 2. Perception of pain at time=2 min. after injection of histidine (A) versus citrate (B) solutions (upper panel), and histidine (A) versus saline (C) (lower panel). Citrate versus histidine solution: N.S.

Solutions of growth hormone or insulin are multi-dose preparations, and must therefore contain a preservative, e.g. benzyl alcohol, *m*-cresol or as in the present study phenol, to protect the formulations from microbial degradation or contamination. The concentration of phenol was higher in the histidine than in the citrate solution (table 2), the influence of which, however, cannot be estimated since the overall evaluation was that the citrate buffer caused more pain. The concentration of phenol in the two solutions was identical to those of other growth hormone preparations (Müller *et al.* 1999). In a dose-toxicity study comparing the acute toxicity of phenol, *m*-cresol and benzyl alcohol high doses of *m*-cresol or benzyl alcohol caused tissue damage, whereas this was not the case for phenol (Svendsen & Carstensen 1997). On the other hand benzyl alcohol has been reported to cause less local discomfort as compared with *m*-cresol

(Rasmussen *et al*. 1989). The toxicology of these commonly used preservatives has been reviewed recently (Kappelgaard *et al*. 2004).

In summary, the solution containing citrate as buffer caused more pain immediately after subcutaneous injection than did the solution with histidine as buffer, which did not cause more pain than the control saline solution. In conclusion, considering the presumed inverse relation between injection pain and patient compliance, the histidine-buffered solution rather than the citrate-buffered solution should be employed for dispensing human growth hormone. Minimizing pain is presumably of particular importance when treating children with daily subcutaneous injections.

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