Buffered lidocaine 1%, epinephrine 1:100'000 with sodium bicarbonate (hydrogen carbonate) in a 3:1 ratio is less painful than a 9:1 ratio: A double-blind, randomized, placebo-controlled, crossover trial

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PII: S0190-9622(20)30066-9

DOI: https://doi.org/10.1016/j.jaad.2019.09.088

Reference: YMJD 14145

To appear in: *Journal of the American Academy of Dermatology*

Received Date: 12 December 2018

Revised Date: 1 September 2019

Accepted Date: 12 September 2019

Please cite this article as: Vent A, Surber C, Graf Johansen NT, Figueiredo V, Schönbächler G, Imhof L, Buset C, Hafner J, Buffered lidocaine 1%, epinephrine 1:100'000 with sodium bicarbonate (hydrogen carbonate) in a 3:1 ratio is less painful than a 9:1 ratio: A double-blind, randomized, placebo-controlled, crossover trial, *Journal of the American Academy of Dermatology* (2020), doi: https://doi.org/10.1016/j.jaad.2019.09.088.

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Funding sources:

JH Rahn Foundation, Zurich (www.jhrahn-foundation.ch)

Bruno Bloch Foundation, Zurich (https://www.brunoblochstiftung.ch)

Conflict of interest:

The authors have no conflict of interest to declare.

Prior presentation:

This study has not yet been presented.

Reprint request:

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Word count (main text):	2473
Word count (abstract):	195
Word count (capsule summary):	45
References:	20
Figures:	5
Table:	0

Consort statement:	Included
Trial protocol statistical analysis:	Included
Ethical approval of local ethical committee (KEK):	Included
Ethical approval of Swissmedic:	Included
Trial Registration:	ClinicalTrials.gov NCT03238625

Abstract

Background

Neutralizing (buffering) lidocaine 1%, epinephrine 1:100,000 solutions (Lido/Epi) with sodium hydrogen carbonate ($NaHCO_3$) (bicarbonate) is widely used to reduce burning sensations during infiltration of Lido/Epi. Optimal mixing ratios have not been systematically investigated.

Objectives

To determine whether the Lido/Epi-$NaHCO_3$ mixing ratio 3:1 (IMP1) causes less pain during infiltration than the mixing ratio 9:1 (IMP2) or unbuffered Lido/Epi (IMP3).

Methods

Double-blind, randomized, placebo-controlled, crossover trial (n=2x24) with 4 investigational medicinal products (IMP1-4).

Results

The 3:1 mixing ratio was significantly less painful than the 9:1 ratio (p = 0.044). Unbuffered Lido/Epi was more painful than the buffered Lido/Epi (p=0.001 *vs* IMP1; p=0.033 *vs* IMP2). IMP4 (NaCl 0.9%=placebo) was more painful than any of the anesthetic solutions (p=0.001 *vs* IMP1; p=0.001 *vs* IMP2; p=0.016 *vs* IMP3;). In all cases the anesthesia was effective for at least 3 hours.

Limitations

Results of this trial cannot be transferred to other local anesthetics such as prilocaine, bupivacaine, or ropivacaine which precipitate with $NaHCO_3$ admixtures.

Conclusions

Lido/Epi-$NaHCO_3$ mixtures effectively reduce burning pain during infiltration. The 3:1 mixing ratio is significantly less painful than the 9:1 ratio. Reported findings are of high practical relevance given the extensive use of local anesthesia today.

Introduction

Lidocaine, an anesthetic of the amide class, is one of the most commonly used local anesthetics. It is available in a variety of concentrations (0.5% to 2.5%). A concentration of 1%, with or without epinephrine, is the most commonly used. Epinephrine is added at a concentration of 1:100,000. It causes vasoconstriction resulting in less bleeding, longer action, and less systemic toxicity.[1]

To aid manufacture and stability, commercial lidocaine products with or without epinephrine have a pH of 2.5-4.0.[2-4] Acidity is assumed to be responsible for the burning sensation during infiltration.[5] Seventeen peer-reviewed studies have confirmed significant pain reduction during infiltration of lidocaine when sodium hydrogen carbonate ($NaHCO_3$) 8.4% (synonymous: bicarbonate 8.4%) was added in various mixing ratios (10:1-5:1) to buffer the solution at a neutral, more physiologic pH.[1,6] All studies consistently reported that buffering did not reduce or shorten the anesthetic effect.

Based on this information it has become common to mix Lidocaine 1%, Epinephrine 1:100'000 (Lido/Epi) with $NaHCO_3$ at a 9:1 ratio (9ml Lido/Epi plus 1ml $NaHCO_3$ 8.4%). In daily practice, however, many patients still report distressing pain during infiltration. We therefore empirically extended mixing ratios and found that a 3:1 ratio led to virtually painless infiltrations.

In order to scientifically substantiate our observation, we conducted a phase II, monocentric, double-blind, randomized, placebo-controlled, crossover trial to assess pain during infiltration of two solutions with different ratios of Lido/Epi-$NaHCO_3$ (3:1 and 9:1), unbuffered Lido/Epi, and sodium chloride (NaCl) 0.9% (placebo).

Materials and Methods

Approvals

The study was approved by the local Ethical Committee (KEK-ZH, Nr.2015-0531), and by *Swissmedic* (national authorization and supervisory authority of Switzerland for drugs and medical products). Written informed consent was obtained from all volunteers. The trial was registered at www.clinicaltrial.gov (NCT03110393).

Volunteers

48 healthy volunteers were included and distributed to two groups. In each group every volunteer was randomly allocated a different order of injections (figure 1).

Inclusion criteria were age (18-75 years), proficiency in German, and sufficient intellectual and linguistic abilities to fully understand and follow all trial procedures and instructions.

Exclusion criteria were hypersensitivity or allergies to local anesthetics of the amide type or to auxiliary substances such as sulfites, pregnancy (secured with testing), damaged skin on the arms, or inability to give informed consent.

Investigational Medicinal Products (IMP)

Four IMP (IMP1,2,3,4) were prepared and labelled according to a packaging- and randomization-plan by the hospital pharmacy of the University Hospital Zurich according to current Good Manufacturing Guidelines. To ensure a blind test and to guarantee product conformity/stability on the day of injection, IMP1,2,3,4 were prepared as sets of two identical vials (5ml) – one containing $NaHCO_3$ and one containing Lido/Epi in appropriate concentrations. For further details see figure 2. The mixing took place within 1 minute prior to infiltration. This procedure was explicitly chosen to guarantee product conformity/stability.

Injection Sites and Injection Procedure

Group 1 received two infiltrations. The injection sites were on the right (A) and left (B) palmar forearm.

Group 2 received four infiltrations into the palmar forearms. The injection sites were alphabetically assigned: (A) right radial, (B) right ulnar, and (C) left ulnar and (D) left radial palmar forearm, respectively.

All sites were 5 cm distal from the cubital fossa. Five seconds after skin puncture 2ml of the IMP was slowly infiltrated into the superficial subcutis over a period of 15s with a 30G needle. The residual 8ml in the syringe was discarded. The injections were performed by the same study physician throughout the investigation. The mixed study medication was at room temperature.

pH and Osmolality of Investigational Medicinal Products (IMPs)

The pH of the IMPs was determined with the potentiometer Titrando 906 (Metrohm AG, Herisau, Switzerland), according to European Pharmacopoeia (PhEur) 2.2.3. Osmolality of the IMPs was determined with the Advanced-3320 micro-osmometer (Advanced Instruments, Norwood MA, USA) according to PhEur2.2.35.

Measuring Parameters and Hypotheses

The following three measurements were recorded 1) pain during infiltration, 2) patient comfort during infiltration and 3) duration of local anesthesia (numbness).

Quantitative rating of pain during infiltration was recorded on a 10-point numerical rating scale (NRS) immediately after infiltration of the study solution (a few seconds after removing the needle). For each volunteer data was recorded on a sheet of paper with a printed numerical rating scale (from 0 to 10; 0=no pain; 10=unacceptable pain).[7]

Qualitative rating of patient comfort during infiltration was recorded using a sheet of paper with a choice of four categorical terms – *desirable* (wünschenswert), *acceptable* (akzeptabel), *less acceptable* (weniger akzeptabel), and *almost* or *totally unacceptable* (kaum oder gar nicht akzeptabel) which could be ticked by the volunteers. This was done directly after recording the *pain during infiltration (NSR)*-measurement.

Duration of local anesthesia (numbness) was recorded after infiltration (after recording the pain during infiltration and patient comfort during infiltration) within 3-5 minutes of removing of the needle, and at 30-minute intervals up to 3 hours, each time using a standardized laser stimulus which left the skin barrier intact (Erbium: Glass non-ablative fractional laser [NAFL], 1540nm, 10mm tip, fluence 30mJ, pulse width 15ms, Cynosure Inc.).[8] Numbness was recorded as present (YES) or *absent* (NO).

The following hypotheses were tested for *pain during infiltration*:

Group 1 (primary end point):

- IMP1 (Lido/Epi-$NaHCO_3$=3:1) causes less *pain during infiltration* than IMP2 (Lido/Epi-$NaHCO_3$=9:1)

Group 2 (secondary end points):

- IMP1 (Lido/Epi-$NaHCO_3$=3:1) causes less *pain during infiltration* than IMP3 (unbuffered Lido/Epi) and IMP4 (placebo)
- IMP2 (Lido/Epi-$NaHCO_3$=9:1) causes less *pain during infiltration* than IMP3 (unbuffered Lido/Epi) and IMP 4 (placebo).

Statistics

Group 1, primary end point (n=24): We planned for a two-sided Mann-Whitney test of the CROS-estimator, measured as the score for *pain on infiltration* of the first injection minus the score for *pain on infiltration* of the second injection. We estimated that 24 cross-overs would be needed to have at least 80% power with significance testing at the α=0.05 level to detect a difference between the two randomization groups (two different order of injection sequence) of 3 NRS values, assuming a standard deviation of 2.5. Variability was estimated based on a 2010 Cochrane review.[6]

Group 2, secondary end points (n=24): We decided to test all four IMPs on a second group of volunteers to avoid possible interferences with the investigation of the primary end point (IMP1 *vs* IMP2). Every volunteer of group 2 was randomly allocated a different order of injections.

The randomization list was computer generated by the hospital pharmacy, using block sizes of 6 for Group 1 (4 blocks of 6 participants, 3 start with IMP1, and 3 with IMP 2) and of one block of 24 participants for Group 2 (this block contains 24 possible combinations). The tests for differences in *pain during infiltration* are based on a two-sided exact Mann-Whitney test of the CROS-estimator.

Patient comfort during infiltration and *local anesthesia* (numbness) information were analyzed descriptively. All analyses were performed in the R-programming language (version 3.3.3).[9]

Results

48 volunteers were included (21 males, 27 females), with mean age 31.4 (21–62) years. All completed the study.

Pain measurements

Quantitative rating of pain during infiltration

Group 1: IMP1 (Lido/Epi-$NaHCO_3$=3:1) was significantly less painful than IMP2 (Lido/Epi-$NaHCO_3$=9:1) (p = 0.044). When IMP1 was followed by IMP2, IMP1 had a median pain score 1.5 points lower (less painful) than IMP2 (1^{st} to 3^{rd} quartile range -3.0 – -1.0). When IMP 2 was followed by IMP 1, the IMP 1 median pain score was 0.5 points lower than for IMP2 (1^{st} to 3^{rd} quartile range -2.0 – 1.25) (figure 3).

Group 2: IMP3 (unbuffered Lido/Epi) was more painful than both IMP1 (Lido/Epi-$NaHCO_3$=3:1) (p=0.001) and IMP2 (Lido/Epi-$NaHCO_3$=9:1) (p=0.033). IMP4 (placebo) was more painful than IMP1 (p=0.001), IMP2 (p=0.001), and IMP 3 (p=0.016) (figure 4).

[IMP1: median NRS 2.0 (1^{st} to 3^{rd} quartile range 1.0 – 4.0); IMP2: median NRS 3.0 (1^{st} to 3^{rd} quartile range 2.0 – 4.25); IMP3: median NRS 4.5 (1^{st} to 3^{rd} quartile range 3.0 – 7.0); IMP4: median NRS 6.0 (1^{st} to 3^{rd} quartile range 3.75 – 8.0)]

Qualitative rating of patient comfort during infiltration

Qualitative rating score data is presented in figure 5.

Duration of local anesthesia (numbness)

In all volunteers, laser-induced pain was absent in the injection areas of IMP1,2,3 and present in the injection areas of IMP4 between 5 minutes and 3 hours after infiltration.

pH and Osmolality of IMPs

pH information is presented in figure 2. Osmolality (mosm/kg) of IMP1,2,3,4 was 674.6, 467.1, 315.0, 285.3, respectively.

Adverse events

No serious adverse events occurred during the study. Five test persons experienced local tenderness for 1-2 days. One test person had a deep subcutaneous nodule of approximately

5mm diameter which completely disappeared after 2 months. We assume it must have been a small hematoma in the deeper subcutis.

Discussion

In a phase II, monocentric, double-blind, randomized, placebo-controlled, crossover trial we scientifically substantiated that the mixing of Lido/Epi-$NaHCO_3$ at a 3:1 ratio causes less *pain during infiltration* than at a 9:1 ratio (fig 3). Furthermore, we showed that unbuffered Lido/Epi is more painful than buffered Lido/Epi at ratios 3:1 and 9:1, and that the placebo was more painful than any of the anesthetic solutions (fig 4). *Patient comfort during infiltration* assessed with qualitative pain scores showed a clear preference for the buffered Lido/Epi at a ratio of 3:1. Placebo received the most negative scores (fig 5). All anesthetic solutions led to numbness in the injection areas between 5 minutes and 3 hours after infiltration. The lower lidocaine concentration in the 3:1 Lido/Epi-$NaHCO_3$ did not affect local anesthesia, compared to the 9:1 ratio, within the observation time.

Acidity causes the burning sensation during infiltration

Acidity has been assumed to be responsible for the burning sensation during infiltration. Meanwhile the detection of acid-sensing ion channel receptors or nociceptors fully supports this explanation.[5] The causal link between pH and burning pain during infiltration is also supported by our trial with the unbuffered Lido/Epi solution (IMP3) at pH 3.4 and with the pharmacologically inactive placebo (IMP4) at pH 6.2. The pain during infiltration with the neutralized solutions (IMP1: pH 7.5, IMP2: pH 7.3) was significantly reduced.

At neutral pH, lidocaine is predominantly present in its active form

Injection solutions contain lidocaine in an uncharged, non-ionized and in a charged, ionized form, respectively. The uncharged form - also known as the active form - is lipophilic and in contrast to the charged (hydrophilic) form readily permeates the nerve membrane to bind from the cytosol to the acid-sensing ion channel receptors.[10-11] According to the Henderson-Hasselbalch equation, in any sample of a lidocaine solution the ratio of the non-ionized (active form) to ionized species of the anesthetic depends on the pH. At a more acidic pH, the ionized, cationic form predominates. For instance, at a pH of 3.8, a typical cartridge of Lido/Epi contains only 1 molecule of non-ionized (active form) anesthetic for every 10,000 molecules of ionized anesthetic. On the other hand, closer to physiologic pH, more non-ionized (active form) anesthetic is present. For instance, at the physiologic pH 7.4 there is 1 molecule of non-ionized (active form) lidocaine in solution for every 4 molecules of ionized lidocaine. At the physiologic pH, there are 2'500 times more of the active form available than at a pH of 3.8.

NaCl 0.9% is more painful than unbuffered Lido/Epi

During injection of unbuffered Lido/Epi (pH 3.8), the buffering system of the body will bring – with a very short time lag - the unphysiologic pH of the solution to a more neutral level.[12] During neutralization the continuously formed non-ionized lidocaine (active form) can penetrate nerve cells and block the acid-sensing ion channel receptors from inside the synapse.[13,14] With NaCl 0.9% (pH 6.2) the buffering system of the body will also neutralize the solution. However, due to the absence of lidocaine, acidity will cause a noticeably longer duration of burning sensation until neutralization of the solution.

CO_2 has analgesic effects

When $NaHCO_3$ is mixed with acidic lidocaine hydrochloride solution, water (H_2O) and carbon dioxide (CO_2) are formed. Condouris et al. demonstrated that CO_2 develops an independent, direct local anesthetic effect.[15] Based on these observations, Catchlove et al. were able to demonstrate that CO_2 enhances the action of lidocaine. CO_2 directly deactivates the nerve axon, and indirectly increases the anesthetic effect of lidocaine by changing its electrical

charge.[16] This may explain why an excess of CO_2, as formed in 3:1 mixture, more effectively reduces the burning sensation of lidocaine relative to the 9:1 mixture. Both mixtures effectively neutralize the Lido/Epi solution, but the 3:1 mixture provides more local CO_2 anesthesia.

Osmolality and pain during infiltration

Osmolality may impair tolerability (including pain on infiltration) of injection solutions.[17] The general osmolality recommendations for injection and infusion solutions is to not greatly exceed the value of 600 and 1,000 mOsm/kg respectively.[17] As yet, no mOsm/kg-to-pain relationship could be found between 300 and 1100 mOsm/kg in a study after intra-muscular injection of vaccine suspensions in healthy adults.[18] Osmolality of Lido/Epi-$NaHCO_3$ at a ratio of 3:1 (IMP1) was 674.6 mosm/kg and higher than in the other solutions (467.1, 315.0, 285.3 mosm/kg). In a similar investigation, Parham et al. found that pain during infiltration of physiological NaCl 0.9% (285.3 mosm/kg) was significantly more painful than their more than twice as osmolar Lido/Epi-$NaHCO_3$ mixtures.[19] Our data confirms this observation.

Stability of bicarbonate neutralized Lido/Epi solution

The stability of neutralized Lido/Epi solutions with $NaHCO_3$ is limited[20] which is the major reason why there are no commercial products on the market. Neutralized Lido/Epi solutions are compounded in advance – either preoperatively by nurses or physicians, or by a specialized pharmacy.[20] The manner in which the solutions are mixed often depends on the available bulk containers (drug/buffer), syringes, and local situations and customs. This makes the storage and subsequent stability of the compounded product an important issue. Despite numerous examinations on the stability of neutralized anesthetics/epinephrine solutions, there is still a considerable degree of uncertainty on the topic. Due to the lack of proper method validation and study design, and the large number of influences when compounding (such as temperature, time, packaging material, light, and oxygen level), no general rules have emerged to simplify compounding and ensure product quality. In the context of our trial we therefore limited the time between mixing and infiltrating the $NaHCO_3$ - Lido/Epi solution to one minute so that degradation would not be a factor. In our everyday

practice we limit the shelf-life of the ad hoc prepared Lido/Epi-NaHCO3 solutions to the duration of the surgical intervention (less than 2 hours).

Conclusions

The Lido/Epi-$NaHCO_3$ mixtures effectively reduce burning pain during infiltration. The 3:1 mixing ratio was significantly less painful than the 9:1 ratio. The reported findings are of high practical relevance given the extensive use of local anesthesia today.

Limitations

The results of this clinical trial cannot be transferred to other local anesthetics, such as prilocaine, bupivacaine, or ropivacaine, which precipitate with bicarbonate admixture. $NaHCO_3$ admixtures with other concentrations of lidocaine, e.g., 0.5% or 2.0%, and with lidocaine without epinephrine are expected to function the same way, but this has not been formally tested.

Acknowledgements

This study was supported by the JH Rahn Foundation, Zurich (www.jhrahn-foundation.ch) and the Bruno Bloch Foundation, Zurich (www.brunoblochstiftung.ch). We thank all participants.

Conflict of interest

None.

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Figure legends

Figure 1: CONSORT study flow diagram.

IMP 1 (Lido/Epi-$NaHCO_3$ = 3:1)

IMP 2 (Lido/Epi-$NaHCO_3$ = 9:1)

IMP 3 (Lido/Epi without $NaHCO_3$)

IMP 4 (NaCl 0.9%)

Figure 2: Preparation, labelling and packaging of IMP 1-4

Investigational medicinal products 1-4 were prepared, labelled and packed according to a randomization list (double-blinded to study participant and physician)

Figure 3: Quantitative rating of pain during infiltration, IMP 1 vs IMP 2

Group 1 (n=24), primary end point. The volunteers of group 1 compared IMP 1 vs IMP 2 in a randomized sequence (block randomization, 4 blocks of 6 volunteers): IMP 1 was significantly less painful during infiltration than IMP 2 (p=0.044). When IMP1 was followed by IMP2, IMP1 had a median pain score 1.5 points lower (less painful) than IMP2. When IMP 2 was followed by IMP 1, the IMP 1 median pain score was 0.5 points lower than for IMP2.

IMP 1: Lido/Epi : $NaHCO_3$ = 3:1. IMP 2: Lido/Epi : $NaHCO_3$ = 9:1.
Box plots: The line in the box corresponds to the median, the lower and upper hinges correspond to the first and third quartiles, the upper/lower whisker extends from the hinge to the largest/smallest value no further than 1.5 * IQR from the hinge. Dots show each of the 24 NRS score points.

Figure 4: Quantitative rating of pain during infiltration, IMP 1 *vs* IMP3 and IMP4, IMP2 *vs* IMP3 and IMP 4

Group 2 (n=24), secondary end point. The volunteers of group 2 compared IMP 1 vs IMP3 and IMP4, and IMP 2 vs IMP3 and IMP4 in a randomized sequence (block randomization, 1 block of 24 different combinations):

Statistical significance:

IMP 1 *vs* IMP 3 (p = 0.001)
IMP 2 *vs* IMP 3 (p = 0.033)
IMP 1 *vs* IMP 4 (p = 0.001)
IMP 2 *vs* IMP 4 (p = 0.001)
IMP 3 *vs* IMP 4 (p = 0.016)

Group 2: IMP 1: Lido/Epi : $NaHCO_3$ = 3:1; IMP 2: Lido/Epi : $NaHCO_3$ = 9:1; IMP 3: Lido/Epi without $NaHCO_3$; IMP 4: NaCl 0.9% (placebo)
Box plots: The line in the box corresponds to the median, the lower and upper hinges correspond to the first and third quartiles, the upper/lower whisker extends from the hinge to the largest/smallest value no further than 1.5 * interquartile ranges from the hinge. Dots show each of the 24 NRS data.

Figure 5: Qualitative rating of patient comfort during infiltration of local anesthetics, captured with categorial terms. The first two bars represent results from group 1, while the next four bars represent results from group 2.

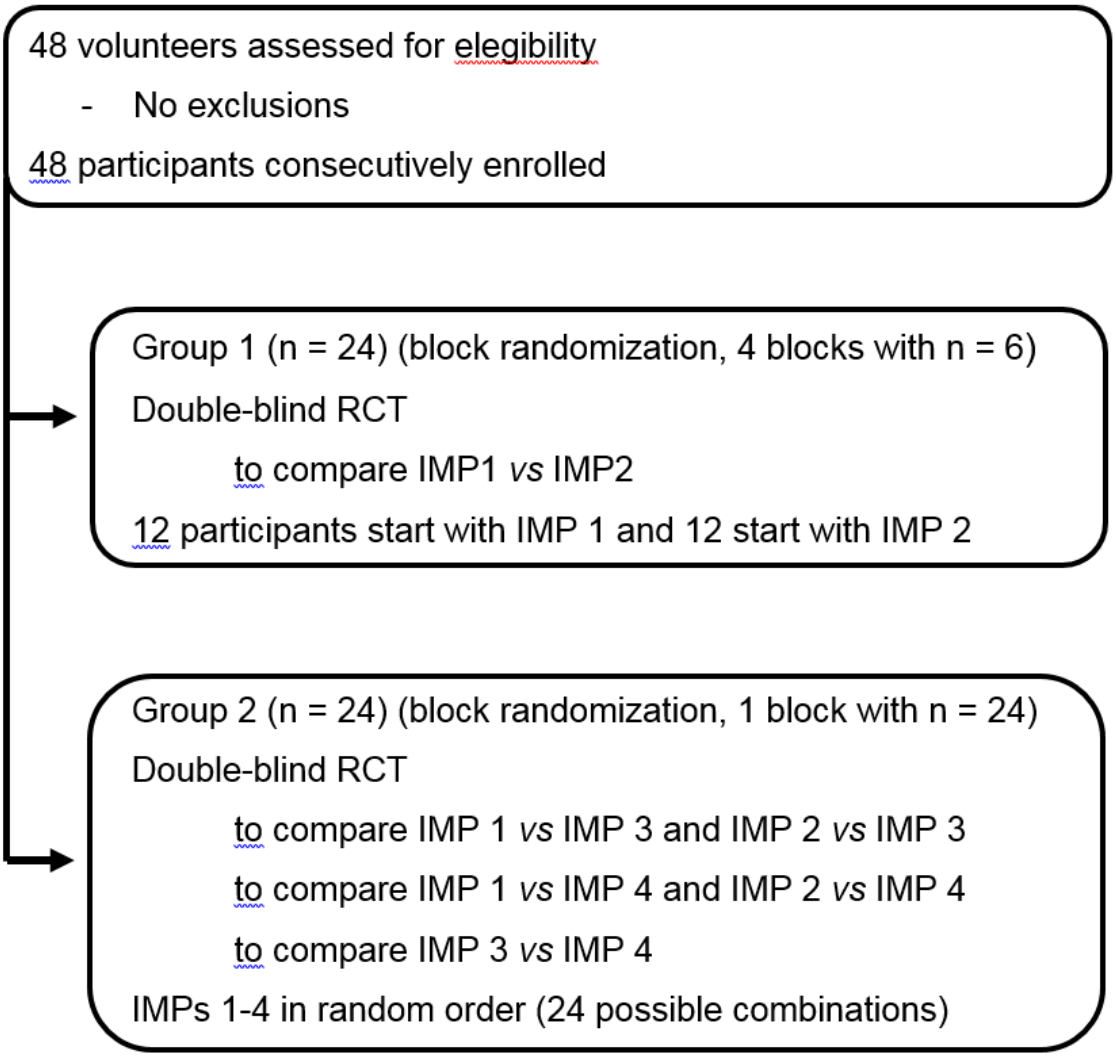
IMP 1 (Lido/Epi : $NaHCO_3$ = 3:1)
IMP 2 (Lido/Epi : $NaHCO_3$ = 9:1)
IMP 3 (Lido/Epi without $NaHCO_3$)
IMP 4 (NaCl 0.9%)

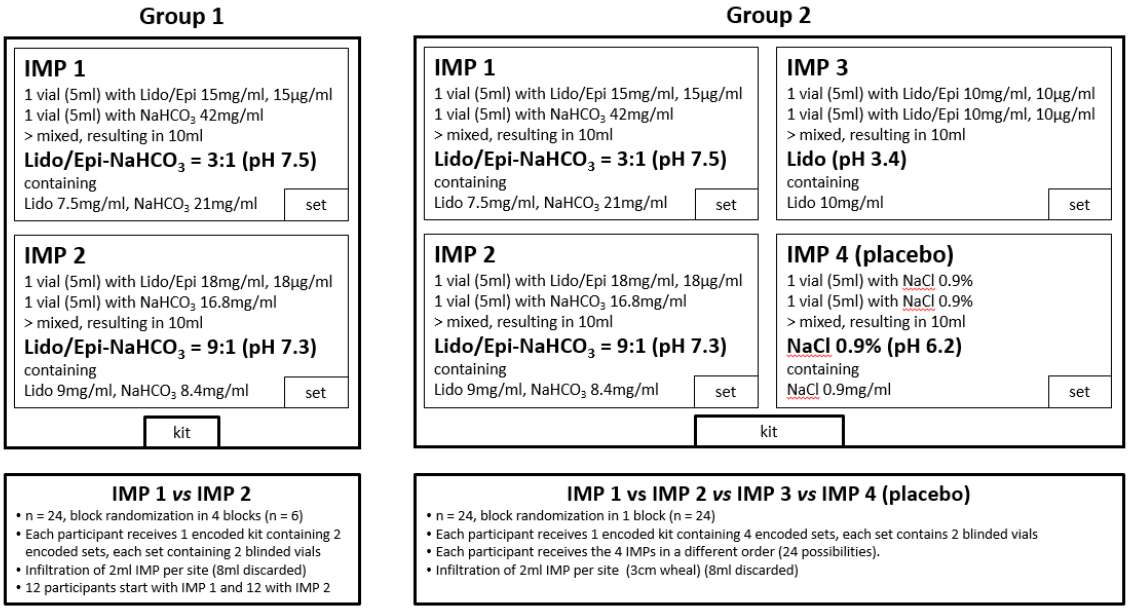
Abbreviation and acronym list

IMP:	Investigational Medicinal Product
GMP:	Good Manufactoring Production
NRS:	Numerical Rating Scale
PhEur:	European Pharmacopoeia
NaCl:	Sodium chloride
$NaHCO_3$	Sodium hydrogen carbonate
	(synonymous: bicarbonate)
Lido/Epi	Lidocaine 1%, Epinephrine 1:100’000

Key Words

Local anesthesia, lidocaine, epinephrine, sodium hydrogen carbonate, bicarbonate, admixture, ratio, 3:1, unbuffered, placebo, burning sensation, double-blind, randomized, controlled, trial


48 volunteers assessed for elegibility
- No exclusions
48 participants consecutively enrolled
Group 1 (n = 24) (block randomization, 4 blocks with n = 6)
Double-blind RCT
to compare IMP1 vs IMP2
12 participants start with IMP 1 and 12 start with IMP 2
Group 2 (n = 24) (block randomization, 1 block with n = 24)
Double-blind RCT
to compare IMP 1 vs IMP 3 and IMP 2 vs IMP 3
to compare IMP 1 vs IMP 4 and IMP 2 vs IMP 4
to compare IMP 3 vs IMP 4
IMPs 1-4 in random order (24 possible combinations)

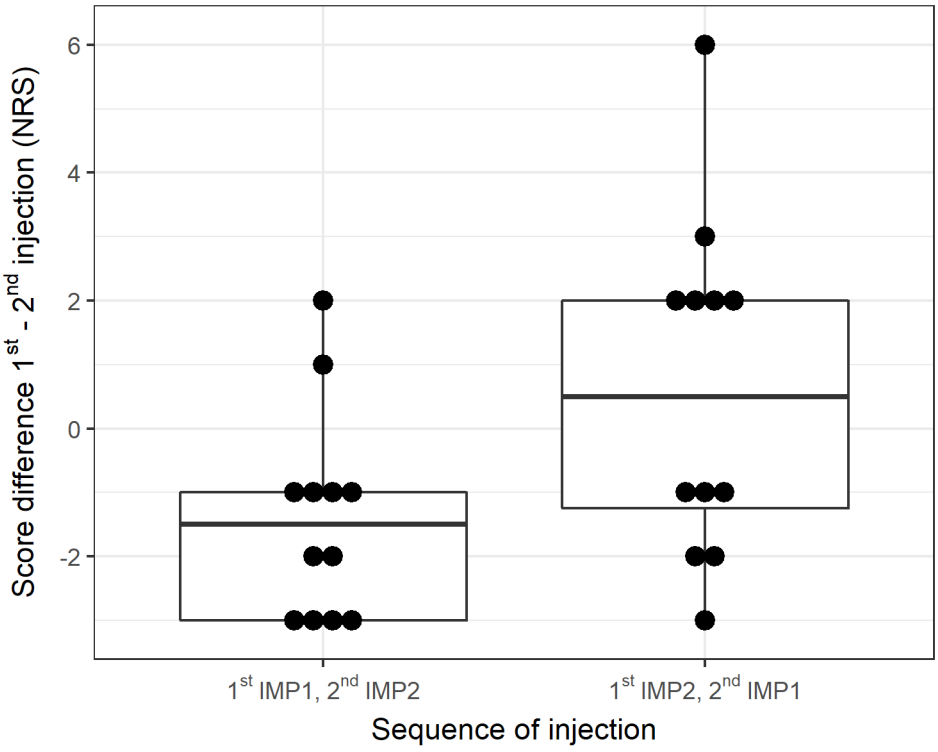


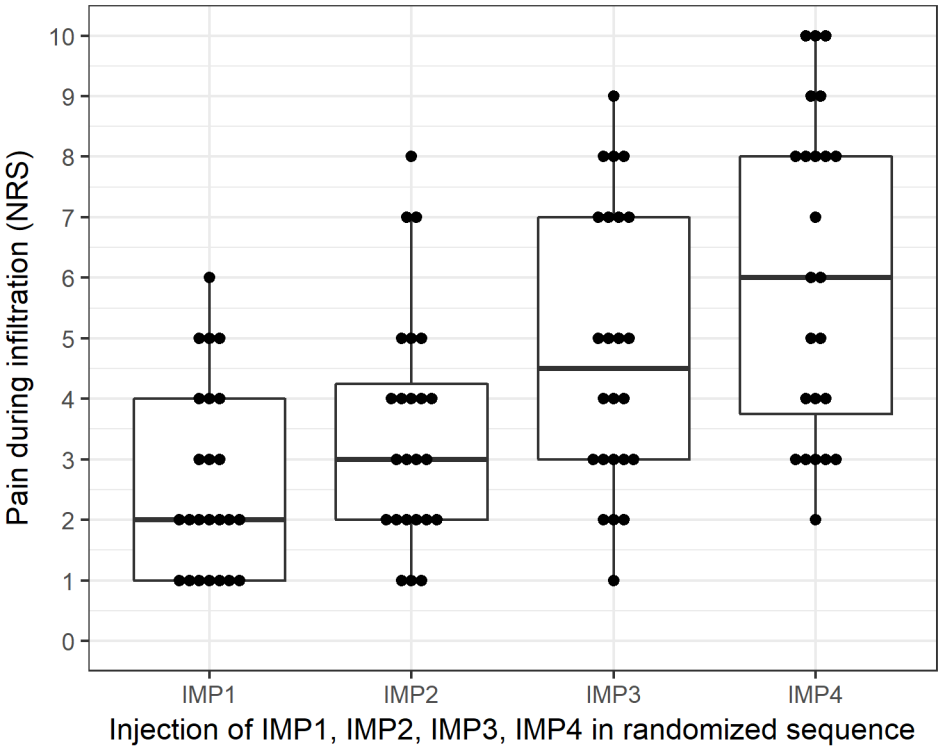
IMP 1 *vs* IMP 2

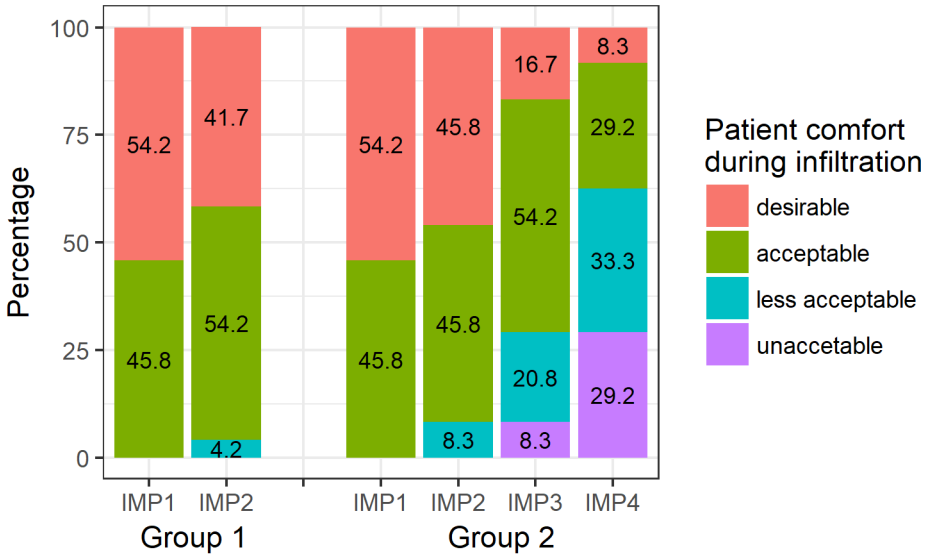
- n = 24, block randomization in 4 blocks (n = 6)
- Each participant receives 1 encoded kit containing 2 encoded sets, each set containing 2 blinded vials
- Infiltration of 2ml IMP per site (8ml discarded)
- 12 participants start with IMP 1 and 12 with IMP 2

IMP 1 vs IMP 2 *vs* IMP 3 *vs* IMP 4 (placebo)

- n = 24, block randomization in 1 block (n = 24)
- Each participant receives 1 encoded kit containing 4 encoded sets, each set contains 2 blinded vials
- Each participant receives the 4 IMPs in a different order (24 possibilities).
- Infiltration of 2ml IMP per site (3cm wheal) (8ml discarded)







Capsule Summary

- Admixture of sodium hydrogen carbonate significantly reduces the strong burning sensation during infiltration of lidocaine 1% with epinephrine (1:100000). The recommended ratios vary from 5:1 to 10:1.
- This double-blind, randomized, placebo-controlled, crossover trial demonstrates superiority of a 3:1 mixing ratio over 9:1 or unbuffered lidocaine.