Published online: 2020-06-30

Changes in pH as a result of galvanic currents used in percutaneous needle electrolysis

Ramón Margalef[1] Francisco Minaya-Muñoz[2,3] Fermín Valera-Garrido[2,3] Marc Bosque[1] Manel M. Santafé[1]

[1] Unit of Histology and Neurobiology, Department of Basic Medical Sciences, Faculty of Medicine and Health Sciences, Rovira i Virgili University, Reus, Spain
[2] MVClinic Institute, Madrid, Spain
[3] Department of Physiotherapy, CEU San Pablo University, Madrid, Spain

Address for correspondence Dr. Manel M. Santafé, MD, PhD, Unit of Histology and Neurobiology, Department of Basic Medical Sciences, Faculty of Medicine and Health Sciences, Rovira I Virgili University, carrer St Llorenç num 21, 43201-Reus, Spain (e-mail: manuel.santafe@urv.cat).

Rev Fisioter Invasiva 2020;3:2–6.

Abstract

Aim To determine whether sodium chloride electrolysis causes a change in the pH of tissues.

Methods The effects of a 3 mA galvanic current has been evaluated, applied for 3 seconds and 3 repetitions (3:3:3). In vitro pH changes were evaluated in three experiments: 1) Eppendorf® tubes filled with Ringer's solution; 2) a very small volume of Ringer's solution (100µl); 3) Eppendorf® tubes filled with saline solution (NaCl 0.9%). The pH changes in the gastrocnemius of mice were evaluated, using the left limb as a control and the right limb for the intervention. The gastrocnemius muscles were ground up and the pH of each group was determined.

Results In the in vitro experiments 1 and 2, no variation was observed in the pH of either the cathode in the Ringer's solution or the anode in the Ringer's solution (the variation did not exceed 16% in either of the cases, $p > 0.05$). In the third in vitro study, the pH after galvanic current application increased by 70% in the saline solution of the cathode and the anode pH decreased by 34% ($p < 0.05$ in both cases). In the in vivo experiments, no change in pH was obtained (% variation: 0.00 ± 0.00).

Conclusions The galvanic current used in percutaneous needle electrolysis applying the 3:3:3 parameters generates very small changes in the pH, in the area near the needle, which the body is able to rapidly compensate for.

Keywords
- galvanic current
- in vitro electrolysis
- in vivo electrolysis
- pH

Introduction

In recent years, the popularity of percutaneous needle electrolysis (PNE) has increased for the application of invasive physical therapy treatments. The theoretical model of the biological effects of PNE states that a galvanic current (GC) applied through a solid metal needle causes an inflammatory response in the treated tissue, favoring its repair.[1] This inflammatory response is described as being caused by a local, non-thermal, electrochemical reaction, which uses the cathode needle as the treatment electrode.[2] Biological tissues and body compartments basically contain water (H_2O) and salts such as sodium chloride (NaCl). Applying a GC through the cathode generates an electrolytic dissociation of NaCl and H_2O, producing gases and sodium hydroxide (NaOH), colloquially known as "caustic soda," with an extremely alkaline pH.[1] The generation of the inflammatory response in the tissues mentioned above is attributed to this compound.[2] Despite evidence of NaCl electrolysis, there are no studies evaluating the typical pH change of tissues

received
December 16, 2019
accepted
April 8, 2020

DOI https://doi.org/10.1055/s-0040-1712511.
ISSN 2386-4591.

(pH = 7.2) toward more alkaline values. Therefore, the aim of this study was to determine whether NaCl electrolysis causes a change in tissue pH.

Material and Methods

The study was conducted at the Histology and Neurobiology Unit (UHN) of the Faculty of Medicine and Health Sciences of the Rovira i Virgili University, in Reus. This experiment comprised both in vitro and in toto experiments. Adult male mice were used, aged 40 days old. The mice were treated according to the regulations of the European Community Council Directive of November 1986 (86/609/EEC) for the handling of laboratory animals. The protocol was approved by the Ethics Committee of the Rovira i Virgili University with reference number 0259GC. These mice were anaesthetized with 0.7 ml of intraperitoneal tribromoethanol (TBE 2%: 2 g of Tribromoethanol in 100 ml of bi-distilled water). To verify that the mouse was sedated, the inexistence of the ocular and plantar reflex was assessed. All the experiments were performed in the laboratory, maintaining a constant temperature of 26° and a humidity of 50%. Physio Invasiva® needles (PRIM Physio. C/ F n° 15, Polígono Industrial n°1 - 28938. Móstoles, Spain) measuring 0.30 mm × 40 mm were used in all procedures. The evaluated protocol was 3 mA for 3 seconds and 3 repetitions (3:3:3) as this is a typical dosage in clinical applications of percutaneous needle electrolysis.[3,4] The equipment used to generate the GC was Physio Invasiva® CE0120 (PRIM Physio. C/ F n° 15, Polígono Industrial n°1 - 28938. Móstoles, Spain).

pH Assessment

pH changes were evaluated in vitro, in a test tube, using the gastrocnemius muscles of mice. All pH measurements were made with the Crison GLP 21+ pH meter (Crison Instruments, SA. Riera Principal, 34, 36. E-08328 Alella, Spain).

The in vitro experiments were performed under three study conditions. In the first study condition, the effect of the 3:3:3 GC treatment protocol was evaluated, with 10 seconds between applications, by immersing the needles in Eppendorf® tubes filled with Ringer's solution (NaCl 137 mM, KCl 5 mM, $NaHCO_3$ 12 mM, Na_2HPO_4 1 mM, $CaCl_2$ 2 mM, $MgSO_4$ 1 mM). These Eppendorf® tubes contained Ringer's solution at a volume of 0.6 ml. The pH was determined for each vial before applying the current. The pH of the cathode (four tubes) and the anode (four tubes) was determined. An agar-filled glass bridge (3.5% agar at 137 mM NaCl) was used to electrically communicate the two Eppendorf® tubes (1 for the cathode, 1 for the anode) keeping the effect of each pole isolated (►**Fig. 1.A**). Once the protocol was applied, the contents of each group of tubes were collected and grouped into a single test tube for the anodes and another for the cathodes (total volume 2.4 ml). The pH was then determined. This procedure was repeated three times with three sets of needles and test tubes on each occasion.

In the second experiment, the pH changes of the Ringer's solution exposed to the 3:3:3 GC treatment protocol were evaluated in a very small volume of 100µl (►**Fig. 1.B**). As in the previous situations, a conventional test tube was used for the pH readings, one for the Ringer's solution exposed to the cathode and one for the anode. Subsequently 2 ml of Ringer's solution was poured into each test tube and the pH was determined. The contents of each test tube were poured into the 100µl test tubes which were distributed into two groups: cathode ($n = 20$) and anode ($n = 20$). An agar-filled glass bridge (3.5% agar at 137 mM NaCl) was used to communicate the electric current between the two test tubes, keeping the effect of each pole isolated. Once the protocol was applied, the contents of each group of test tubes were collected and deposited in the conventional test tube and the pH measurements were made. This procedure was repeated three times with test tubes, 100µl test tubes and new needles.

For the third experiment, the procedure described in the first experiment was repeated, however the Ringer's solution was replaced with saline solution (SF; $H_2O + NaCl$ 0.9%). This procedure was also repeated three times with test tubes, Eppendorf® test tubes and new needles on each occasion.

During the in toto animal experiments, (see ►**Fig. 1.C**) Physio Invasiva® needles of 0.30 mm × 40 mm were used. The same treatment protocol was applied: 3:3:3, leaving 10 seconds between applications. With the mouse previously anesthetized, in prone position and the hind legs stretched and waxed, the needle was inserted from the proximal end to the distal insertion of each gastrocnemius, the left side was used as the control group and the right side was used as the treatment group. The gastrocnemius muscles were then removed and freed of connective tissue (tendons and fascia). To avoid a possible diffusion of substances throughout the entire muscle volume, only the area of the muscle where the inserted needles were located was dried out. The sample was then weighed and Ringer's solution was added in a ratio of 1 g of muscle to 2 g of Ringer's solution. Then, the samples were ground up using a VWR /VDI 12 homogenizer (VWR International Eurolab, S.L. C/ De la Tecnología, 5–17 A7 08450 Llinars del Vallès). Finally, the pH of the tube corresponding to each experiment was determined.

Statistical Analysis

SPSS v17.0 © statistical software was used to analyze the results. The values were expressed as the mean ± SD. To evaluate differences between groups, the Student's *t*-test was used. Differences were considered significant if $p < 0.05$.

Results

pH Study

To mimic the normal biological environment, experiments were performed with normal, pre-oxygenated Ringer's solution. This solution is rich in ions and sugars and with a pH within the physiological range. The pH was determined before and after applying the 3:3:3 GC to the Ringer's solution exposed to the cathode and to the Ringer's solution exposed to the anode. In both cases, there was no variation in pH (≈ 1.15% variation, $n = 3$ readings, $p > 0.05$ from initial values in both cases; see ►**Table 1**).

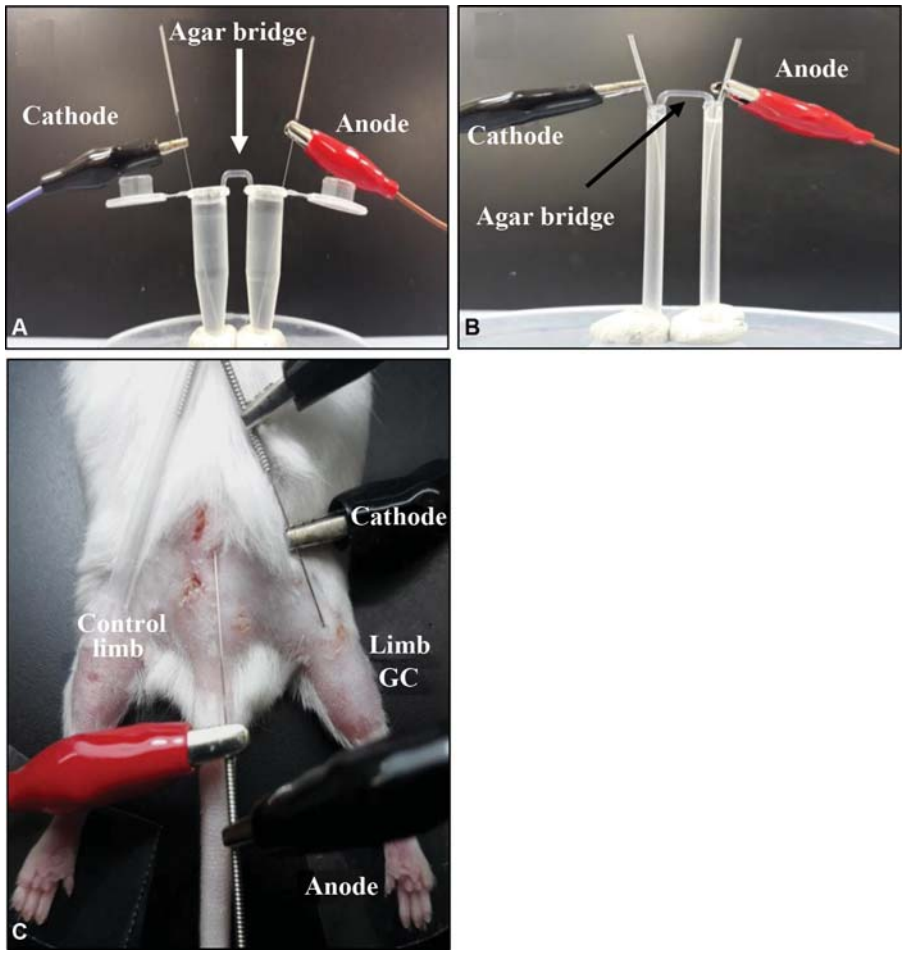

Fig. 1 In vitro pH study. (**A**). The anode and cathode were immersed in Eppendorf® tubes filled with Ringer's Solution or saline, according to protocol 1 or 3. The 2 tubes were connected by a glass bridge filled with conductive agar. (**B**). An experiment performed with Ringer but in very small volumes. As in the previous figure, the anode and cathode are immersed in Ringer's solution and the tubes are electrically connected by glass bridges filled with conductive agar. In vivo pH study. (C). With the mouse anesthetized, in prone position and the hind legs stretched and waxed, the needle is inserted. Insertion is performed in the distal direction on each gastrocnemius. For each animal, the right gastrocnemius was treated (the cathode was inserted) and the left gastrocnemius was used as a control. The needle used as the anode was inserted at the base of the tail. In all cases, 3 mA galvanic current (CG) was applied for 3 seconds repeated 3 times (3:3:3).

The lack of results led us to suppose that the NaOH generated was very low for the volume of liquid used and therefore it was excessively diluted. Therefore, a set of experiments was designed with a very reduced volume of liquid (►**Fig. 1.B**; see experiment number two in Material and Methods). On this occasion, the initial pH of Ringer's solution was also determined and after applying a GC following a 3:3:3 protocol to the cathode (12.01% variation) and Ringer's solution exposed to the anode (15.26% variation), no significant pH variations were obtained, ($n = 3$ readings, $p > 0.05$ compared with the initial values in both cases; see ►**Table 1**).

Since electrolysis is a phenomenon based on the dissociation of water and salt, we decided to carry out experiments in 0.9% NaCl saline solution. The pH after applying GC 3:3:3 increased by 70% in the SF of the cathode and in parallel, the pH of the anode decreased by 34% ($n = 3$ determinations, $p < 0.05$ compared with initial values in both cases; see ►**Table 1**).

Table 1 In vitro determination of pH

			After GC	
Solution	**Volume**	**Before**	**Cathode**	**Anode**
Ringer Normal Oxygenated	0.6 ml	6.13 ± 0.07	6.20 ± 0.20	6.20 ± 0.10
	100 µl	6.16 ± 0.03	6.90 ± 0.10	7.10 ± 0.05
NaCl (0.9%)	0.6 ml	$5{,}90 \pm 0.04$	$10.09 \pm 0.20^{*}$	$3.90 \pm 0.50^{*}$

Galvanic current (GC) of 3 mA during 3 seconds, repeated 3 times (3:3:3).
$^{*}p < 0.05$ compared with pH values before application of GC.

For the in vivo experiments, the 3 mA protocol based on 3 seconds and 3 applications was applied in the right gastrocnemius of three mice and compared with the results obtained in the left leg, control. No change was obtained (% variation: 0.00 ± 0.00).

Discussion

The literature on this subject explains that when two electrodes are immersed in a conductive medium and a direct current (galvanic current) passes between them, electrochemical reactions take place around the electrodes and in the medium containing the same.[5] The present study found that the pH change only takes place in a simple solution of NaCl. Using normal oxygenated Ringer's solution, no pH change was observed, neither did this occur with a live sample. However, an increase of protons in the area of the anode, i.e., acidic pH, and a decrease around the cathode, i.e., alkaline pH, is described. These extreme pHs can, in some cases cause denaturation of proteins, even cell death. For example, Eva Nilsson et al,[6] working with GCs applied using platinum electrodes, described that the most important reaction that occurs is the decomposition of water into H_2 and hydroxyl ions (OH-) (formulated as: $2H_2O + 2e^- \leftrightarrow H_2 + 2OH^-$). It is well known that during the application of GC, hydrogen bubbles are formed in the cathode needle. This accumulates in the tissue surrounding the needle and some escapes along the line where the needle is inserted. Under physiological conditions, this gas has a low electrochemical reactivity and its possible effect is limited to a mechanical effect related to pressure.[7] However, when this hydrogen combines with oxygen to form hydroxyl, this ion can cause tissue destruction, however, under biological conditions the tissue buffering systems, including bicarbonate, proteins and organic phosphate, are capable of neutralizing the destructive role of this ion.[6] Bicarbonate is a buffer system present in plasma and interstitial fluid. When a tissue is exposed to strong alkalinity, the bicarbonate buffer system acts as an open system allowing it to compensate for the changes.[8] In the experiments of the present work the pH did not change when working with the normal oxygenated Ringer's solution. This solution is isotonic and has an ionic set with a discrete oncotic and pH buffering capacity. It is possible that the ex vivo experiments made with Ringer's solution may have been buffered while with simple saline solution this did not occur and therefore this produced the change of pH.

In addition, proteins, via the prosthetic groups (non-amino acidic component of proteins that is necessary for this to be functional) also contribute toward cushioning the changes that hydrogen can generate.[6] Finally, the buffering role of the organic phosphate present in the adenosine triphosphate molecule (ATP; very present in muscle tissue) should be highlighted. Most likely, the experiments performed in the present study using muscle samples did not show any variation in pH possibly due to the buffering effect of proteins and ATP.[6]

A pioneering work by Li et al[9] applied a GC protocol of 8.5 Volts at 30 mA during 69 minutes, obtaining changes in the concentrations of Na^+ and K^+ ions in the cathode ($pH = 12.9$) and the Cl^- concentrations in the anode ($pH = 2.1$). Subsequently, several papers were published[5,7,10] that considered the pH change as being the main mechanism of necrosis in tissues treated with electrical currents. This change in pH within the human body requires a temporary application of ~30 minutes to achieve these effects and also requires both electrodes to be present in the treated tissue. In the present study, the GC used was of much lower amperage (3 mA) and the duration only lasted a few seconds. It is possible that the low values used in this study failed to generate a sufficient change in pH to be detected and that it is also easily buffered.

In summary, the pH changes detected in saline could have occurred because there were no ionic buffers such as in the Ringer's solution or no biological buffers as in the muscle experiments. Additionally, the amperage and duration used were too low to generate a large change in pH and this is easily buffered by the Ringer's solution or biological tissues.

According to our study findings, the clinical benefits obtained via treatment using the 3 mA protocol for 3 seconds and 3 repetitions do not come significantly from the pH, rather from other sources such as changes in the membrane potential of the cells in the treated tissues. For example, since the 1950s the activity of osteocytes and osteoblasts is known to depend on variations in membrane potential.[11] This is still a current topic today (see the book by Zhao [12]). Many other tissues benefit from changes in the membrane potential of their cells, such as the skin[13,14] or the cornea cells.[15] Furthermore, it is known that cells involved in the inflammatory or immune response such as lymphocytes[16] or macrophages[17] are attracted by the galvanic current. Immediate and transient local vasodilation in medium and small caliber vessels have been described after the application of GC[18] which would expedite the arrival of these cells. Given the involvement of cells in the inflammatory response, the participation of the NLRP3/ASC/CASP1 inflammasome could also be proposed, which generates an increased release of interleukin (IL)-1β, from the ionic decompensation caused by the GC in the resident macrophages in the tissue, generating a drop in intracellular potassium (K^+),[19] and thus inducing the first pro-inflammatory phase of tissue regeneration.

In this sense, the minimal changes found in local pH fail to support the electrochemical hypothesis and are not sufficient to justify the inflammatory response associated with percutaneous needle electrolysis.

However, given the small radius of action of the electrical current around the needle tip, it would be interesting to evaluate the local pH as initially described by Eva Nilsson et al,[6] mentioned above, using galvanic current applied to the tissue using platinum electrodes.

Conclusion

The GC used in percutaneous needle electrolysis applied according to the established 3 mA, 3 seconds and 3 applications parameters, generates very small changes in the pH in the area near the needle, which the body is able to

compensate for in a short period of time. The relevance of pH changes in a tissue treated with GC is linked to the intensity and time parameters, which in the present study reproduced the usual form of clinical application, so the therapeutic effects do not seem to be linked to pH changes, but rather to other factors.

Conflict of Interests
The authors have no conflict of interests to declare.

Acknowledgments
The authors would like to thank PRIM Physio, the company that selflessly donated the needles and the device for generating galvanic current used in this study.

References

1 Valera Garrido F, Minaya Muñoz F. Fisioterapia Invasiva. 1ª ed. Barcelona: Elsevier; 2013. Capítulo 9: Mecanismos moleculares de la electrolisis percutánea intratisular; 187–203
2 Finch JG, Fosh B, Anthony A, et al. Liver electrolysis: pH can reliably monitor the extent of hepatic ablation in pigs. Clin Sci (Lond) 2002;102(04):389–395
3 Valera Garrido F, Polidori F, Benavent Canet J, et al. Criterios clínicos de aplicación de electrolisis percutánea en las tendinopatías: documento de consenso de expertos y estudio transversal en fisioterapeutas. Rev Fisioter Invasiva 2019;2(02):55–61
4 Valera-Garrido F, Minaya-Muñoz F. Fisioterapia Invasiva. 2ª ed. Barcelona: Elsevier; 2016. Capítulo 17: Aplicaciones clínicas de la electrolisis percutánea; 417–451
5 Perkons NR, Stein EJ, Nwaezeapu C, et al. Electrolytic ablation enables cancer cell targeting through pH modulation. Commun Biol 2018;1:48
6 Nilsson E, Fontes E. Mathematical modelling of physicochemical reactions and transport processes occurring around a platinum cathode during the electrochemical treatment of tumours. Bioelectrochemistry 2001;53(02):213–224
7 Vijh AK. Phenomenology and Mechanisms of Electrochemical Treatment (ECT) of Tumors. In: Modern Aspects of Electrochemistry. Springer US; 2007:231–274
8 Arieff AI, DeFronzo RA. Fluid, Electrolyte and Acid–Base Disorders. 2nd ed. Churchill LivingstoneNew York1995
9 Li K, Xin Y, Gu Y, Xu B, Fan D, Ni B. Effects of direct current on dog liver: possible mechanisms for tumor electrochemical treatment. Bioelectromagnetics 1997;18(01):2–7
10 Ciria HMC, González MM, Zamora LO, et al. Antitumor effects of electrochemical treatment. Chin J Cancer Res 2013;25(02): 223–234
11 Wiesmann HP, Hartig M, Stratmann U, Meyer U, Joos U. Electrical stimulation influences mineral formation of osteoblast-like cells in vitro. Biochim Biophys Acta - Mol. Cell Res 2001;1538(01):28–37
12 Zhao M. Electrical fields in wound healing-An overriding signal that directs cell migration. Semin Cell Dev Biol 2009;20(06): 674–682
13 Ravikumar K, Boda SK, Basu B. Synergy of substrate conductivity and intermittent electrical stimulation towards osteogenic differentiation of human mesenchymal stem cells. Bioelectrochemistry 2017;116:52–64
14 Foulds IS, Barker AT. Human skin battery potentials and their possible role in wound healing. Br J Dermatol 1983;109(05): 515–522
15 Levin MH, Kim JK, Hu J, Verkman AS. Potential difference measurements of ocular surface Na+ absorption analyzed using an electrokinetic model. Invest Ophthalmol Vis Sci 2006;47(01):306–316
16 Lin F, Baldessari F, Gyenge CC, et al. Lymphocyte electrotaxis in vitro and in vivo. J Immunol 2008;181(04):2465–2471
17 Sun YS, Peng SW, Cheng JY. In vitro electrical-stimulated wound-healing chip for studying electric field-assisted wound-healing process. Biomicrofluidics 2012;6(03):34117
18 Margalef R, Minaya Muñoz F, Valera Garrido F, Santafe MM. Vasodilation secondary to exposure to galvanic currents. J Invasive Tech Phys Ther 2019;2(02):107
19 García Vidal JA, Pelegrín P, Escolar Reina P, Medina i Mirapeix F. Inflammatory response of two invasive techniques in the mouse with collagenase induced tendinopathy. J Invasive Tech Phys Ther 2019;2(02):80