How to cite this article: Ferreira BS, Silva WF, Souza J, Morini AC. Effects of carboxiterapy in the healing of skin wounds. J. Physiother. Res. 2019;9(1):28-36. doi: 10.17267/2238-2704rpf.v9i1.2203

Original article

Journals BAHIANA
SCHOOL OF MEDICINE AND PUBLIC HEALTH

Effects of carboxiterapy in the healing of skin wounds

Efeitos da carboxiterapia na cicatrização de feridas cutâneas

Brenno de Sousa Ferreira[1], Wanderson Fernandes Silva[2], Juarez de Souza[3], Adriana Caroprezo Morini[4]

[1]Federal University of West Pará. Santarém, Pará, Brazil. ORCID: 0000-0002-8117-8008. brenno_sousa@hotmail.com
[2]Autor para correspondência. Federal University of West Pará. Santarém, Pará, Brazil. ORCID: 0000-0001-9389-5795. wandersonfsm@outlook.com
[3]State University of Pará. Belém, Pará, Brazil. ORCID: 0000-0003-0852-2623. souza644@hotmail.com
[4]Federal University of West Pará. Santarém, Pará, Brazil. ORCID: 0000-0001-8731-9935. drimorini@gmail.com

RESUMO | INTRODUÇÃO: Novas terapêuticas para as lesões de difícil cicatrização têm-se tornado necessárias e estão sendo pesquisadas. Nesse contexto, a carboxiterapia tem se destacado pelos seus efeitos sobre a microcirculação tecidual. **OBJETIVO:** avaliar os efeitos da carboxiterapia, infusão no tecido subcutâneo de CO_2 medicinal, na cicatrização de lesões cutâneas em ratos Wistars. **MATERIAIS E MÉTODOS:** Trata-se de um estudo experimental e quantitativo, com uma amostra de 10 animais, divididos em grupo controle e grupo carboxiterapia. Todos os animais sofreram uma lesão com punch metálico de 5mm de diâmetro na região dorsal e, o grupo com terapia, tratado por 10 dias consecutivos. A região foi fotografada em vários momentos da pesquisa e, em seguida, as imagens foram analisadas pelo software ImageJ. **RESULTADOS:** Na análise descritiva dos dados o GC apresentou redução progressiva da área de 0,205±0,025 para 0,155±0,017 no 3° dia, 0,109±0,034 no 7° dia e 0,028±0,092 após o 10° dia. O GCa apresentou aumento da área de 0,198±0,040 para 0,207±0,035 no 3° dia, com redução para 0,109±0,012 no 7° dia e 0,044±0,030 após o 10 dia, demonstrando ser menos eficiente na cicatrização de feridas cutâneas do que a ausência de tratamento. **CONCLUSÃO:** A carboxiterapia não mostrou resultado significativo para acelerar o processo de cicatrização de lesões cutâneas na fase aguda, sugerindo esse período como não ideal para sua utilização.

PALAVRAS-CHAVE: Cicatrização. Ferimentos e lesões. Pele.

ABSTRACT | INTRODUCTION: New therapies for difficult-to-heal injuries have become necessary and are being researched. In this context, carboxytherapy has been highlighted by its effects on tissue microcirculation. **OBJECTIVE:** evaluate the effects of carboxytherapy, infusion in the subcutaneous tissue of medicinal CO_2, in the healing of skin lesions in Wistars rats. **MATERIALS AND METHODS:** This is an experimental and quantitative study, with a sample of 10 animals divided into a control group and a carboxytherapy group. All the animals suffered a metallic punch injury of 5mm diameter in the dorsal region and the group with therapy, treated for 10 consecutive days. The region was photographed at various times of the research and then the images were analyzed by ImageJ software. **RESULTS:** In the descriptive analysis of the data, the CG presented a progressive reduction of the area of 0.205 ± 0.025 to 0.155 ± 0.017 on the 3rd day, 0.109 ± 0.034 on the 7th day and 0.028 ± 0.092 after the 10th day, GCa presented an area increase of 0.198 ± 0.040 for 0.207 ± 0.035 on the 3rd day, with reduction to 0.109 ± 0.012 on the 7th day and 0.044 ± 0.030 after 10 days, demonstrating to be less efficient in the healing of cutaneous wounds than the absence of treatment. **CONCLUSION:** Carboxitherapy did not show significant results to accelerate the healing process of cutaneous lesions in the acute phase, suggesting this period as not ideal for its use.

KEYWORDS: Wound healing. Wounds and injuries. Skin.

Submitted 12/12/2018, Accepted 02/13/2019, Published 02/20/2019
J. Physiother. Res., Salvador, 2019 February;9(1):28-36
Doi: 10.17267/2238-2704rpf.v9i1.2203 | ISSN: 2238-2704
Responsible editor: Carina Oliveira dos Santos (Physiotherapy Dermatofunctional)

Introduction

New therapies for difficult-to-heal injuries have become necessary and are being researched. In this context, carboxytherapy has been highlighted by its effects on tissue microcirculation[1,2]. This technique consists of therapeutically administering anhydrous carbon dioxide (called carbon dioxide or CO_2) by injection into the subcutaneous tissue directly into the affected areas with the aim of improving tissue oxygenation[3,4].

Results of other experiments with the use of carboxytherapy[5,6] for the treatment of chronic lesions contextualize a positive outlook regarding the application of this technique to this condition. It is noteworthy that, in these experiments were reported reduced numbers of adverse reactions, increasing the expectation of good results.

The literature review revealed a small number of studies aimed at the application of carboxytherapy in the treatment of tissue repair[5-7] and an increasing number of studies aimed at its use in aesthetic treatments[4,8-11]. Therefore, it is necessary to carry out more studies to analyze the results of the application of carboxytherapy in tissue repair, under the light of the scientific method, offering more support to this application. Based on this, the present study aimed to evaluate the effects of carboxytherapy on the wound healing process in healthy Wistars rats.

Material and methods

Kind of study

This work was carried out through an experimental study, by means of a quantitative study, observing the behavior of a specific sample, during a time interval of 11 months.

Location and Period

The development of the research took place in the vivarium of the Instituto Esperança de Ensino Superior (IESPES) in Santarém. The period of the survey was between August 2017 and June 2018.

Ethical Principles of Research

All animals were managed according to the rules of the Brazilian College of Animal Experimentation (COBEA) and national legislation for animal vivisection in force (Federal Law 6,638 of May 8, 1979), which defines norms for conducting research with laboratory animals, and of law n. 9605/98 dealing with environmental crimes. This study was submitted to the Committee on Ethics in the Use of Animals of UFOPA protocol n ° 10014-2017, and duly approved.

Sample

For this research, 10 Wistar rats, all males, healthy adults, older than 90 (ninety) days and weighing between 250 (two hundred and fifty) and 300 (three hundred) grams were used. These animals were chosen because of their various advantages: they are easy to handle, occupy small spaces, have high resistance to infection and present clinical, laboratory and histopathological similarities with humans. Moreover, due to these advantages, they allow to work simultaneously with several experimental groups and subgroups.

During the research period, the rats were kept in appropriate polypropylene cages measuring 41cm x 34cm x 16cm, lined with wood, accommodating 5 (five) animals in each of these microenvironments. These animals were observed for a minimum period of 15 (fifteen) days before the beginning of the study itself. The objective of this period was to promote the best adaptation to the laboratory environment, following the guidelines of COBEA (2006), which defines this as the minimum period of pre-observation of the animals before any scientific study.

It is worth noting that the experimental laboratory had restricted access to the authors of this research and other members of the laboratory, presenting controlled environmental conditions (12 hours of light / dark cycle, sanitized environment, temperature of 22 ± 2°C and adequate ventilation). food and water ad libitum to the animals throughout the experiment.

Search Groups

For this research the animals were separated into 2 (two) groups regarding the type of treatment, composed of 5 (five) animals each. These groups were thus composed:

1 - Group GC (Control Group) received the lesion, however, without any treatment.
2 - Group GCa (Carboxytherapy Group) received the lesion and was treated with carboxytherapy.

The groups in this study suffered euthanasia on the 11th day after the injury. The reason for this choice is that medical prescriptions are generally around 10 sessions. From this routine, it was intended to bring this research closer to the treatments performed in the daily routine of professional practice.

Experiment

The technique of intraperitoneal induction of ketamine 10% (0.10ml / 100g), general anesthetic drug, and Xylazine 2% (0.25ml / 100mg), substance with sedative, analgesic and muscle relaxant. Euthanasia consisted of the application of overdose of thiopental.

After anesthesia, all the animals were submitted to standardized traumatic lesions. The procedure was started with trichotomy and antisepsis with povidine-iodine (PVPI). Next, the animal was positioned in the ventral decubitus with the four limbs extended and the head aligned to the trunk. Then, 1 (one) circular excisional wound was performed. For this, a 5mm metal punch with a cutting blade was used on its lower edge. Afterwards a cutaneous fragment was removed, with removal of the skin, until the exposure of the dorsal muscular fascia.

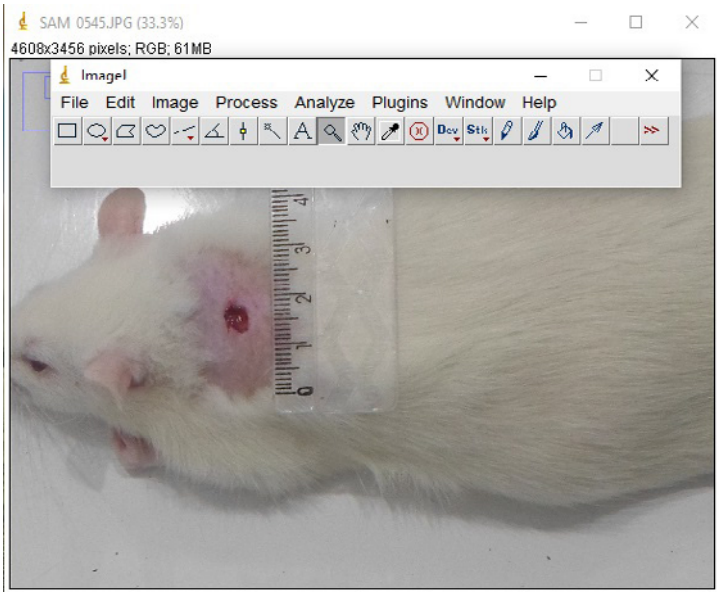
After the lesion occurred, the animals started daily therapy sessions lasting approximately 5 minutes / each for ten consecutive days. For the application of the carboxitherapy, the lesion was divided into quadrants and the application points had angular distance of approximately 90° from each other, a total of 4 application points. The gas was injected approximately 0.5cm from the edge of the lesion. Carbon dioxide was administered through 30G needles introduced at approximately 30° angulation to the skin with the tip of the needle toward the center of the lesion. This needle is connected to the carboxytherapy apparatus. In this research, the model of carboxytherapy apparatus was Ares produced by the company Ibramed®. The infusion flow used was 80 ml / min, with 10 ml being injected at each point.

Photogrammetric Analysis

The morphometric evaluation of the lesions consisted in the relative measurement of the physical dimensions of the lesions. For this, the technique of computerized photogrammetry was used from digital images of the lesions obtained in each session.

To obtain these images was used a digital camera of the brand Samsung®, model Samsung es95® of 16.2 megapixels. The images were obtained without zoom, always in a frontal plane and at a standard distance of 30cm from the lesions, with the aid of a tripod. These images fitted the lesion and a millimeter tape, which served as reference for calibration of the measurements obtained by the photogrammetric analysis software (Figure 1).

Figure 1. Using the ImageJ® Software. Framing of the lesion and the reference rule



Source: The authors (2019).

The software used to perform the analysis of morphometric parameters of this study was ImageJ®, which has been widely used in studies on the follow-up of the evolution of ulcers[12-14].

The morphometric evaluation of the lesions used in this study was the area. For this measurement, after visualization of the image of the lesion by the ImageJ® software, the programming was initially adjusted in the software so that the presentation of the final result demonstrates only the desired parameter. This setting was necessary only for each program use and for this you have clicked on the following commands: Analyze >>> Set Measurements ... In the window that has been opened (Set Measurements) the following command is selected: "Area".

Then, the calibration of each image was performed for analysis. This calibration consisted of drawing a line on the tape of 1cm distance (known distance) and then clicking on the following commands: Analyze >>> Set Scale. Within the window opened by the command (Set Scale,) the space "Konwn Distance" with the value "1" (corresponding to the distance of 1cm of the tape) was filled in and finally the unit of measure was reported in "cm" in the space "Unit of Length".

From these initial adjustments of the program, the evaluation parameter measurement procedure was started. With the tool "freehand selections" (" "), the exact contour defined by the edges of the lesions was drawn with the mouse.

Then it was clicked on the Analyze >>> Measure commands, which then presented a last window with the morphometry result of the lesion.

In order to avoid variation in the definition of the edges of the lesions, a single, previously trained evaluator performed these measurements, maintaining the same criteria for their execution.

Statistical treatment

The data related to these analyzes were tabulated in spreadsheets of the Excel software (Microsoft ® - USA) and presented in time intervals in hours 0h, beginning of the inflammatory process[15], 48h, beginning of wound contraction[16], 144h, increased proliferation of fibroblasts[17] and 240h, period corresponding to 10 daily treatment sessions. Subsequently the data received the statistical treatment by the software BioEstat 5.0. For this experimental study, the significance level of 0.05 (α = 0.05 or 5%) was accepted for a sampling error of 5% in all analyzes.

For the descriptive analysis we used measures of central tendency and dispersion. In the inferential analysis they were submitted to the Shapiro wilk normality test. The data with normal distribution were submitted to the parametric t-test of student.

Results

For a better demonstration of the data obtained in this research, table 01 shows all absolute sizes of lesion areas, both in the control group and in the carboxytherapy group, according to the time elapsed from injury. the CG presented progressive reduction of the area from 0.205 ± 0.025 to 0.155 ± 0.017 on the 3rd day, 0.109 ± 0.034 on the 7th day and 0.028 ± 0.092 after the 10th day, whereas the GCa presented an increase of the area from 0.198 ± 0.040 to 0.207 ± 0.035 at the 3rd day, with reduction to 0.109 ± 0.012 on the 7th day and 0.044 ± 0.030 after the 10th day.

Table 1. Areas in centimeters of wounds in Wistar rats, at 0h, 48h, 144h and 240h after injury, according to the groups in this study

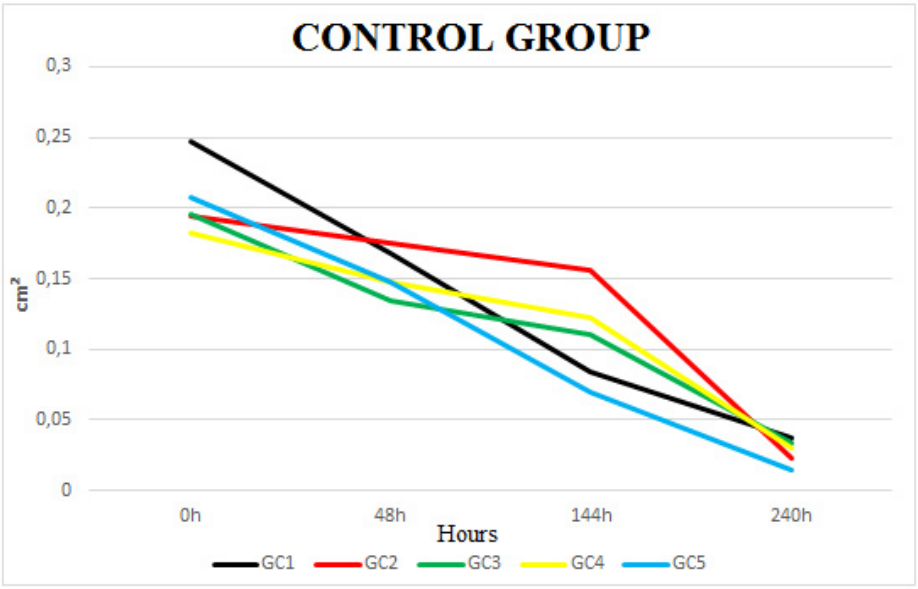
GC	0h	48h	144h	240h
GC1	0,247	0,168	0,084	0,037
GC2	0,194	0,175	0,156	0,023
GC3	0,196	0,134	0,110	0,034
GC4	0,182	0,148	0,123	0,030
GC5	0,208	0,148	0,070	0,014
GCa	**0h**	**48h**	**144h**	**240h**
GCa1	0,186	0,171	0,111	0,072
GCa2	0,250	0,200	0,089	0,081
GCa3	0,140	0,181	0,111	0,023
GCa4	0,204	0,256	0,109	0,025
GCa5	0,208	0,226	0,123	0,018

CG: control group; GCa: carboxytherapy group

Source: The authors (2019).

Through the observation of Figure 2, we can see the decreasing evolution of the lesion size in all evaluated moments and in all the animals of this study.

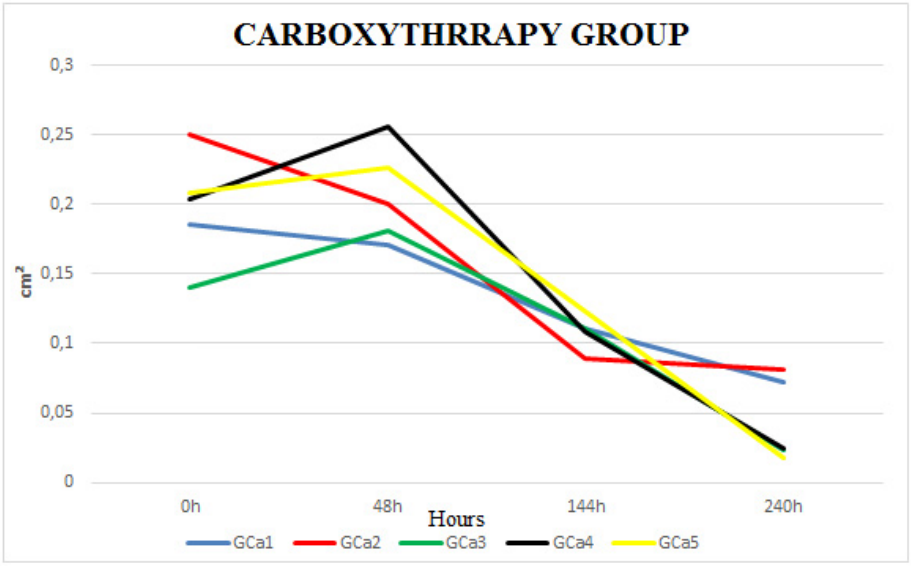
Figure 2. Evolution of the size of the area in cm² in the Control Group, according to the hours after the production of the lesion



Source: The authors (2019).

When evaluating Figure 3, it was observed that 3 animals from the group treated with carboxytherapy increased their areas in the first 48 hours and then showed a decreasing line for the remainder of the evaluated time. The other 2 animals decreased their lesions at all times in this study.

Figure 3. Evolution of the size of the area in cm² in the Carboxytherapy Group, according to the hours after the injury



Source: The authors (2019).

To further facilitate the visualization of the variations of the area, Table 2 and Figure 4 below present the results of the data through the statistical test Student's t test, in order to identify the significant correlations between the groups.

Table 2. Area in centimeters of wounds in Wistar rats at 0h, 48h, 144h and 240h of experiment

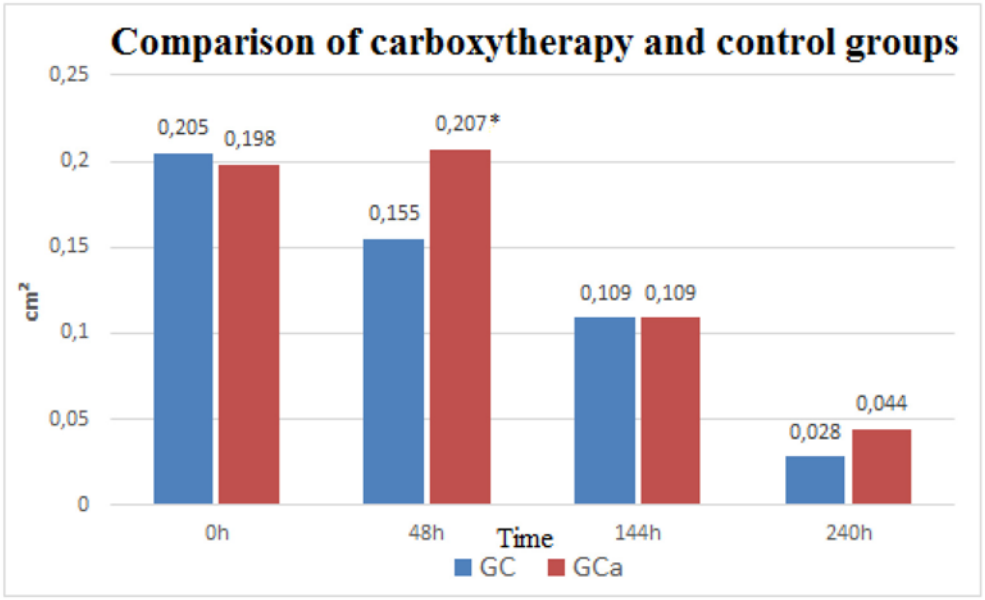
	0h		48h		144h		240h	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
GC	0,205	0,025	0,155	0,017	0,109	0,034	0,028	0,092
GCa	0,198	0,040	0,207	0,035	0,109	0,012	0,044	0,030
T	0,371		-3,042		0,000		-1,150	
P	0,720		0,016		1,000		0,314	

SD: Standard Deviation; CG: Control Group; GCa: Carboxytherapy Group; Student t test for correlation between groups, with t and p values.

Source: The authors (2019).

Student's t-test showed a significant change ($p < 0.05$) between the control group and the carboxytherapy group, however, it is an inverted alteration with negative t-value.

Figure 4. Correlation between the sizes of the areas of the groups of this research, by Student's t-Test



*p<0,05

Source: The authors (2019).

Discussion

The Wistar mouse is widely used in research for the evaluation of healing. The skin of this animal is quite similar to that of humans, but it is not the same. Wistar does not form hypertrophic or keloid scars, it does not form subcutaneous adipose tissue, there is no definition between papillary dermis and reticular dermis, its dermis is thicker, its blood vessels are subdermal[18]. These peculiarities can generate different results between animal and human research.

Carboxyterapy may have a great influence on its administration in wistar rats, the gas, being a readily dispersible substance, diffuses rapidly due to the lack of subcutaneous adipose tissue in the rat. This would hinder the action of the gas which is to stimulate the release of oxygen to the tissue and improve the microcirculation of the site, the so-called Bohr effect[19-21].

The healing process is hampered by internal factors of the organism and factors related to the injury. Patients with diabetes, vasculitis, immunosuppressed, use of steroids, leprosy are more likely to chronicle cutaneous lesions[22]. Infected wounds, burns, necroses

have a greater difficulty in healing[23]. This research performed injuries on healthy rats and clean wounds. After 240h (10 days) after injury, all wounds had not yet closed completely.

A relevant data is observed in the results of the present study. The mean area of the lesion increased with the application of carboxytherapy in the first 48 hours after 2 sessions. The possible explanation for this occurrence may be the possible proinflammatory effects of carbon dioxide therapy, including the vasodilatory effect of carbon dioxide and the inflammatory process caused by the penetration of the needle into the tissue[21], thus accentuating the period of inflammation of the tissue repair process that occurs soon after an injury, which has an intense leakage of liquid and immune cells[23,24]. In this context, carboxytherapy would enhance inflammation, increasing the area of the lesion, proving to be less efficient in the healing of cutaneous wounds than the absence of treatment.

The study supports other studies that sought to evaluate the effects of carboxytherapy on healing in cutaneous tissue. In one study, volumetric analysis and histology of the lesion in rats with septic wounds were performed, the authors did not observe statistically significant differences in the inflammatory infiltrate, necrotic area, edema and wound volume for 10 days of treatment when compared to control[25]. In another study the treatment was performed in a group of rats subjected to a 2nd degree burn lesion where the proliferation of fibroblasts, neoangiogenesis and inflammatory infiltrates were evaluated, the study did not present significant statistical differences between the treatment group and the control group[26].

This study did not show positive effects on the healing of cutaneous wounds with the use of carboxytherapy, however it is worth noting that the lesions were in their acute phase, which does not rule out the possibility of treatment with carboxytherapy in the use of chronic wounds. As limitations to the current research it can be pointed out the absence of histological or immunological analysis and the limited size of the sample.

Conclusion

At the end of the present study it was possible to observe that the carboxytherapy does not present improvement effects in the healing process in culturing wounds of healthy Wistars rats, presenting an increase of the area in the inflammatory period of the tissue repair process, suggesting this period as not ideal for its use. However, new researches with other methodologies are necessary to better evaluate the effects of carbon dioxide in the wound healing process.

Contributions of authors

Silva WF participated in the conception of the study design, search and statistical analysis of the research data, interpretation of the results and writing of the scientific article. Sousa B participated in the collection and interpretation of the research data. Souza J participated in the conception, research design and interpretation of the results. Morini AC participated in the research design and interpretation of the results.

Competing interests

No financial, legal or political competing interests with third parties (government, commercial, private foundation, etc.) were disclosed for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.).

References

1. Seidel R, Moy R. Effect of Carbon Dioxide Facial Therapy on Skin Oxygenation. J Drugs Dermatol. 2015;14(9):976-80.

2. Pianez LR, Custódio FS, Guidi RM, Freitas JN, Sant'Ana E. Effectiveness of carboxytherapy in the treatment of cellulite in healthy women: a pilot study. Clin Cosmet Investig Dermatol. 2016;9:183-90. doi: 10.2147/CCID.S102503

3. Brochado TMM, Schweich LC, Simões NP, Oliveira RJ, Antoniolli-Silva ACMB. Carboxytherapy: Controls the inflammation and enhances the production of fibronectin on wound healing under venous insufficiency. Int Wound J. 2018;1-9. doi: 10.1111/iwj.13031

4. Kołodziejczak A, Podgórna K, Rotsztejn H. Is carboxytherapy a good alternative method in the removal of various skin defects? Dermatol Ther. 2018;31(5):e12699. doi: 10.1111/dth.12699

5. Brandi C, Grimaldi L, Nisi G, Brafa A, Campa A, Calabrò M et al. The role of carbon dioxide therapy in the treatment of chronic wounds. In Vivo. 2010;24(2):223-6.

6. Piazzolla LP, Louzada LL, Scoralick FM, Martins ME, Sousa JB. Preliminary experience with carbon dioxide therapy in the treatment of pressure ulcers in a bedridden elderly patient. J Am Geriatr Soc. 2012;60(2):378-9. doi: 10.1111/j.1532-5415.2011.03806.x

7. Morales BT. La carboxiterapia em el tratamiento de las úlceras. Revista panamericana de Flebología y Linfología. 2008;13(1).

8. Brandi C, D'Aniello C, Grimaldi L, Caiazzo E, Stanghellini E. Carbon dioxide therapy: effects on skin irregularity and its use as a complement to liposuction. Aesthetic Plast Surg. 2004;28(4):222-5. doi: 10.1007/s00266-004-2068-z

9. Park JH, Wee SY, Chang J, Hong S, Lee JH, Cho KW et al. Carboxytherapy-Induced Fat loss is Associated with VEGF-Mediated Vascularization. Aesthetic Plast Surg. 2018;42(6):1681-1688. doi: 10.1007/s00266-018-1222-y

10. Ferreira JC, Haddad A, Tavares SA. Increase in collagen turnover induced by intradermal injection of carbon dioxide in rats. J Drugs Dermatol. 2008;7(3):201-6.

11. Kołodziejczak A, Podgórna K, Rotsztejn H. Is carboxytherapy a good alternative method in the removal of various skin defects?. Dermatol Ther. 2018;31(5):e12699. doi: 10.1111/dth.12699

12. Minatel DG, Enwemeka CS, França SC, Frade MAC. Fototerapia (LEDs 660/890nm) no tratamento de úlceras de perna em pacientes diabéticos: estudo de caso. An Bras Dermatol. 2009;84(3):279-83. doi: 10.1590/S0365-05962009000300011

13. Korelo RIG, Valderramas S, Ternoski B, Medeiros DS, Andres LS, Adolph SMM. Aplicação da microcorrente como recurso para tratamento de úlceras venosas: um estudo piloto. Rev Latino-Am Enfermagem. 2012;20(4).

14. Salmona KBC, Santana LA, Neves RS, Guadagnin RV. Estudo comparativo entre as técnicas manual e automática de demarcação de borda para avaliação de área de ulceras por pressão. Enfermagem Foco. 2016;7(2):42-46. doi: 10.21675/2357-707X.2016.v7.n2.793

15. Portou MJ, Baker D, Abraham D, Tsui J. The innate immune system, toll-like receptors and dermal wound healing: A review. Vascul Pharmacol. 2015;71:31-36. doi: 10.1016/j.vph.2015.02.007

16. Kattan WM, Alarfaj SF, Alnooh BM, Alsaif HF, Alabdul Karim HS, Al-Qattan NM et al. Myofibroblast-Mediated Contraction. J Coll Physicians Surg Pak. 2017;27(1):38-43. doi: 2519

17. Park J.E., Barbul A. Understanding the role of immune regulation in wound healing. Am J Surg. 2004;187(5A):11S-16S. doi: 10.1016/S0002-9610(03)00296-4

18. Santos MFS, Czeczko NG, Nassif PAN, Ribas-Filho JM, Alencar BLF, Malafaia O et al. Avaliação do uso do extrato bruto de Jatropha gossypiifolia L. na cicatrização de feridas cutâneas em ratos. Acta Cir Bras. 2008;21(supl 3):2-7. doi: 10.1590/S0102-86502006000900002

19. Scorza FA, Borges FS. Carboxiterapia: uma revisão. Revista Fisioterapia Ser. 2008;3(4).

20. Lee GSK. Carbon Dioxide Therapy in the Treatment of Cellulite: An Audit of Clinical Practice. Aesth Plast Surg. 2010;34(2):239-243. doi: 10.1007/s00266-009-9459-0

21. Lyra MC, Leão Júnior H, Previde Neto S, Orgaes FAFS, Gonella HA. Tratamento de queimaduras de carboxiterapia em modelo experimental. Rev Bras Queimaduras. 2012;11(1):2-5.

22. Okonkwo UA, DiPietro LA. Diabetes and Wound Angiogenesis. Int J Mol Sci. 2017; 18(7):1419. doi: 10.3390/ijms18071419

23. Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res. 2010;89(3):219-29. doi: 10.1177/0022034509359125

24. Alves NA, Fernandes KPS, Deana AM, Bussadori SK, Mesquita-Ferrari RA. Effects of Low-Level Laser Therapy on Skeletal Muscle Repair. Am J Phys Med Rehabil. 2014;93(12):1073-1085. doi: 10.1097/PHM.0000000000000158

25. Paulo DNS, Pavan CC, Almeida LDLF. Efeito da carboxiterapia na cicatrização de ferida cutânea contaminada de ratos. Salus J Health Sci. 2016;2(2):19-26. doi: 10.5935/2447-7826.20160013

26. Lyra MC, Leão Júnior H, Previde Neto S, Orgaes FAFS, Gonella HA.Tratamento de queimaduras de carboxiterapia em modelo experimental. Rev Bras Queimaduras. 2012;11(1):2-5.