The bacteriostatic effect of controlled-flux electrolyzed acidic solution on healthy hallucal skin

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ABSTRACT

Background: There are various treatment protocols to manage the increased bacterial load in plantar ulcers. Recently, Controlled-Flux Electrolyzed Acidic Solution (CFEAS), with a pH less than 3, has appeared to be an effective option since its antimicrobial effect could help in the healing of those ulcers. In order to evaluate its potential in this sense, the aim of the present study was to compare bacterial growth on healthy hallucal skin using two types of bandaging (control and Controlled-Flux Electrolysed Acidic Solution).

Material and methods: In a sample of 19 healthy subjects, two experiments were performed. In the first, for each subject, two identical hallux bandages were applied in the early morning. At random between left and right foot, either physiological saline (wetting every 2 h) was applied or nothing (control). In the second, two days later, new bandages were applied as before, but now either wetting with Controlled-Flux Electrolysed Acidic Solution (experimental, again wetting every 2 h) or nothing (control). In each experiment, the bacterial load in the nail fold was assessed at the first moment and after 10 h from standard counts of bacterial colony forming units (CFU).

Results: In the first experiment, the CFU counts had increased significantly ($p < 0.05$) in both toes after the 10-h period. In the second experiment, while the bacterial load increased significantly ($p = 0.001$) from $0.68 \pm 0.8 \times 10^4$ CFU/cm² (the “pre” sample) to $1.3 \pm 0.9 \times 10^4$ CFU/cm² (the “post” sample) in the control toe, in the experimental CFEAS toe, the pre sample bacterial load was $0.61 \pm 0.6 \times 10^4$ CFU/cm², and the post sample $0.9 \pm 0.8 \times 10^4$ CFU/cm², with no significant difference between them ($p = 0.221$). Negative cultures were obtained in 3 cases (15.78%) of the experimental toe post sample, and equal post and pre counts in 2 cases (10.5%).

Conclusion: Controlled-Flux Electrolyzed Acidic Solution has an effect on healthy hallucal skin that is bacteriostatic, and in some cases bactericidal. This effect could be very helpful in treating plantar ulcers when there is a greatly increased bacterial load in the wound, thus potentially favoring the normal formation of granulation tissue in the skin and normal healing and closure of the ulcer.

1. Introduction

The infection of wounds or ulcers in the foot is because the bacterial flora goes from being saprophytic (and thereby forming a protective barrier) to opportunistic and infectious. This is especially so in chronic diabetes patients due to their immune system's lack of response [1–3]. The infection provokes (together with other factors, such as macro- or micro-angiopathy) a delay in the healing of the lesion, and can even cause this tissue regeneration to be inviable [2,4]. Infections in the

diabetic foot are generally polymicrobial, with predominance of Gram-positive cocci (especially *S. aureus*), Gram-negative bacilli (*E. coli*), and obligate anaerobes [5,6]. Such infections are one of the main complications of diabetic foot syndrome, and, if they are maintained over time, may derive into osteomyelitis, amputation, and even death [2–4] – hence the importance of controlling the flora in high-risk patients, especially when a wound or ulcer is already present. This interest has led to experimentation with new substances of natural origin with which to control the skin's microbiological flora while minimizing drug

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interactions [7–10].

One such substance of recent appearance is Controlled-Flux Electrolyzed Acidic Solution (CFEAS), which can create an acidic environment in which microbial reproduction is prevented. This solution, of pH < 3, has been used to disinfect wounds (against aerobic or anaerobic bacteria), to treat diabetic ulcers, and to treat infected surgical wounds [11,12]. So, in cases where there is a major bacterial load in the ulcer fold, this solution might help reduce this load, and thus make the patient's earlier discharge possible. Before starting a randomized controlled trial in diabetic patients, we believed it might be helpful to investigate the real effect of CFEAS on the saprophytic flora of healthy skin in order to assess its bacteriostatic or bactericidal potential. In particular therefore, the aim of the present study was to examine its effect on bacterial growth of healthy hallux skin after 10 h of its being allowed to act.

2. Material and Methods

19 subjects (5 men, 15 women; mean age 23.9 ± 3 years, mean weight 67.6 ± 13.2 kg, and mean height 168.4 ± 9.7 m), all students of podiatry at our university, participated in the study. Two experiments were designed. In the first, at the beginning of the study day, first samples (PRE) were collected from the lateral fold of each hallux with sterile swabs (transport medium, Amies Viscosa). The samples were taken by making a 5-s distal-to-proximal smear along the nail fold involved. Identical bandages were then applied to each hallux. The left or right foot was selected at random for physiological saline to be applied every 2 h to the bandage on the hallux (in order to keep the bandage moist), and the other toe being the control with nothing applied to the bandage. A second samples were taken after 10 h (POST), following the same procedure as for the PRE sample. In the second experiment (after two days), the same procedure was followed, but now with CFEAS (solucion acida electrolizada de flujo controlado, Electrobioral®, Laboratorios Naturales EJ, Mexico) applied to keep the bandage of the experimental toe (again selected at random) moist instead of physiological saline.

The samples were taken to the laboratory for bacterial culture within 24 h. The swabs were re-suspended in 2 ml of 0.9% saline solution, and then 20 µl aliquots were plated in duplicate onto 5% blood agar, and cultured for 24–48 h at 35 °C. After this incubation time, the bacterial colony forming units (CFU) were counted, recording the results in units of CFU/cm [2]. A Student's t-test for paired samples was applied to identify differences in the means. Delta (Δ : post – pre) values were calculated to assess the minimum, maximum, mean, and standard deviation of the increase in CFU. Pearson tests were made to assess correlations between anthropometric characteristics and the increase (Δ) in CFU for the two toes. Statistical analyses were performed using SPSS software v21.0 (SPSS, Chicago, IL, UEX license Campus), setting a significance level of $p < 0.05$.

3. Results and discussion

In the first experiment, the CFU counts significantly increased in both toes after the 10-h period – from 0.6 ± 0.8 to $1.9 \pm 1.1 \times 10^4$ CFU/cm² ($p = 0.001$) in the physiological saline toes, and from 0.6 ± 0.4 to $1.2 \pm 0.6 \times 10^4$ CFU/cm² ($p = 0.001$) in the control toes (Table 1). Mean bacterial growth (delta, post-pre) were +0.6 in the control group, by $+1.3 \times 10^4$ CFU/cm² in the saline solution group (Table 1). Neither were any negative cultures observed in this first experiment nor any equal results or reduction of the bacterial load in the nail fold in either toe (Table 1).

The first experiment confirmed that moist conditions favour bacterial proliferation, since in occlusive bandaging under conditions of continuous humidity (wetting every 2 h with physiological saline over the course of the 10-h day) the bacterial colonies tripled, while in the dry control toe the bacterial colonies doubled (Table 1). In this first

Table 1

Bacterial load in the nail fold (Experiment 1: wet conditions). The results represent the mean, standard deviation, and p-value of the pre and post bacterial load expressed in Colony Forming Units (CFU) values (saline/control) with Paired t-test. And descriptive statistics of delta (post – pre) for the first experiment's CFU measurements.

		Mean	SD	p-value
Pair 1	PRE physiological saline	0.640	0.8173	0.001
	POST physiological saline	1.961	1.1791	
Pair 2	PRE control	0.602	0.3945	0.001
	POST control	1.227	0.5710	
				Min - max
Delta control		0.6249	0.49475	0.10 - 1.77
Delta physiological saline		1.3209	1.21102	0.16 - 5.00

experiment, there were no negative cultures nor any reduction in the number of bacterial colonies, thus confirming that the conditions of occlusion to which we subjected the foot can favour the infection of small lesions that otherwise, under normal immunodepression conditions, would be resolved without complications [2,4].

In the second experiment, the bacterial load increased significantly ($p = 0.001$) from $0.68 \pm 0.8 \times 10^4$ CFU/cm² (pre sample) to $1.71 \pm 1.3 \times 10^4$ CFU/cm² (post sample) in the control toes (Table 2). In the experimental toes, the pre sample bacterial load was $0.61 \pm 0.6 \times 10^4$ CFU/cm², and the post sample load was $0.9 \pm 0.8 \times 10^4$ CFU/cm², with the difference between them not being significant ($p = 0.221$, Table 2). Mean bacterial growth (delta, post-pre) in this second experiment were +0.6 in the control group, by $+0.3 \times 10^4$ CFU/cm² in the experimental group (Table 2).

Negative cultures were obtained after the 10-h period in 3 cases (15.78%) of the experimental toes, and equal post and pre counts in 2 cases (10.5%). In the control toes, no negative cultures or equal pre/post counts were observed. In Table 2, one observes that the minimum Δ (post – pre) was a –1.50 CFU reduction in the experimental toes, and, in the control toes, the minimum was a slight increase (0.07). There were no correlations between the increase in bacterial load in either toe and age or anthropometric characteristics.

Something similar might have been expected in the second experiment, since we kept one toe wet while the other remained bandaged, but dry. However, while moist conditions favour bacterial proliferation, one can see that the effect was not the same as before. The present work thus shows the overall bacteriostatic effect achieved with the CFEAS treatment protocol, with a doubling of CFU in the nail fold area of the control toe, but an increase of only 0.29 CFU ($p = 0.221$, Table 2) in the toe with CFEAS application. This greater than 0.05 p-value indicates that there was no significant growth of the bacterial colonies in the experimental toe, unlike the case in the control toe ($p = 0.001$, Table 2). Moreover, three of the cases gave negative cultures, showing a bactericidal effect, although this term is not extrapolatable to the overall sample. According to the results therefore, one can refer to CFEAS having a general bacteriostatic effect, because only in this

Table 2

Bacterial load in the nail fold (Experiment 2). The results represent the mean, standard deviation, and p-value of the pre and post CFU values (experimental/control) with Paired t-test. And descriptive statistics of delta (post – pre) for the CFU measurements.

		Mean	SD	p-value
Pair 1	PRE experimental	0.609	0.6062	0.221
	POST experimental	0.902	0.8271	
Pair 2	PRE control	0.688	0.8661	0.001
	POST control	1.294	0.8688	
				Min - max
Delta control		0.6060	0.55369	0.07 - 1.72
Delta experimental		0.2932	1.00706	− 1.50 - 3.37

experimental condition bacteria are not duplicated in number after the 10 h period.

Since ulcers without clinical infection do not require antibiotic therapy, CFEAS could be used as a prophylactic to control excess bacterial load and avoid possible subsequent infections. In ulcers already infected, it could be useful as an adjuvant to antibiotic treatment for Gram+ (mild) or Gram- and obligate anaerobe (moderate and severe) infections [10–14]. In this way, one would be contributing to the prevention of infections and hence of major amputations in the lower limbs, because 85% of amputations at this level are preceded by an uncontrolled infection [3,15].

The effect of CFEAS is determined by its low pH, less than 3, corresponding to an acidic medium in which bacteria encounter difficulties in their reproduction [12]. Thus, to ensure the effect of this treatment protocol, the bandage should be kept moist at all times, controlling the microbiological flora on the skin. Diabetics are usually polymedicated, so that CFEAS would be a good alternative with which to minimize drug interactions. In addition, CFEAS treatments would be compatible with dressings and wound drainage which have a proven bactericidal effect [16]. Other dressings or therapeutic approaches that can provide these benefits of CFEAS are less cost-effective and more complex methodologically [13].

Therefore, by applying CFEAS in a continuously moistened mode of cure, one can manage to control the bacterial load existing on the skin in conditions in which the immune system may be compromised. Since this could cause infections, and the literature found on the use of CFEAS provides no scientific evidence concerning the treatment of ulcers [11,12], a research path is opened to plan a controlled clinical trial with which to determine the effect on bacterial load in diabetic ulcers.

4. Conclusions

The implementation of a treatment protocol with bandaging and continual application of CFEAS has an effect that is bacteriostatic overall, and in some cases bactericidal. To ensure this effect, the bandage applied must be kept moist at all times. This control of the bacterial load could improve the normal formation of granulation tissue, and therefore foster the better closure of ulcers.

Declaration of competing interest

None.

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