Calcium Electroporation for Keloids: A First-in-Man Phase I Study

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Abstract

Background: Keloid scarring is a pathologic proliferation of scar tissue that often causes pruritus, pain, and disfigurement. Keloids can be difficult to treat and have a high risk of recurrence. Recent studies have shown promising results in the treatment of cutaneous metastases with intralesional calcium combined with electroporation (calcium electroporation). As calcium electroporation has shown limited side effects it has advantages when treating benign keloid lesions, and on this indication we performed a phase I study.

Methods: Patients with keloids were treated with at least 1 session of calcium electroporation and followed up for 2 years. Calcium was administered intralesionally (220 mM) followed by the application of eight 100-µs pulses (400 V) using linear-array electrodes and Cliniporator (IGEA, Italy). Treatment efficacy was evaluated clinically (size, shape, erythema), by patient self-assessment (pruritus, pain, other) and assessed histologically.

Results: Six patients were included in this small proof of concept study. Treatment was well tolerated, with all patients requesting further treatment. Two out of 6 patients experienced a decrease in keloid thickness over 30%. A mean reduction of 11% was observed in volume size, and a mean flattening of 22% was observed (not statistically significant). Five out of 6 patients reported decreased pain and pruritus. No serious adverse effects or recurrences were observed over a mean follow-up period of 338 days.

Conclusion: In this first phase I clinical study on calcium electroporation for keloids, treatment was found to be safe with minor side effects. Overall, patients experienced symptom relief, and in some patients keloid thickness was reduced.

Introduction

A keloid is a result of abnormal wound healing with pathologic proliferation of scar tissue and occurs in about 15% of the population [1]. A keloid is characterized by aberrant fibroblast activity with excessive formation of extracellular matrix components such as collagen. Growths of keloids neither undergo spontaneous regression nor respect original scar boundaries, differentiating them from other hypertrophic scars. Following trauma or surgical procedures, keloids can lead to both loss of func-
tion and stigmatizing disfigurement, and present a major clinical issue [2]. Other symptoms may include pain, itching, and irritation. Surgical excisions of keloids can lead to unsatisfactory outcomes with recurrence [3]. Recommended management of keloids includes mechanical treatment (e.g., silicone gel bandaging or compression) [4], cryotherapy [5], radiation therapy [6], or pharmacological treatment with intrallesional injection of corticosteroids [7], and more recently systemic- or locally administered bleomycin [7–10]. This study aims to explore the effect of treatment of keloids with calcium electroporation, which is a novel treatment that has been investigated for malignant cutaneous tumours as well as recurrence of head and neck cancer [11–13] (Fig. 1).

Calcium is an important non-permeable ion involved in numerous cellular processes as a second messenger, including apoptosis. The concentration gradient of calcium across the cell membrane is tightly regulated to maintain cell homeostasis. Introducing high concentrations of calcium into cells will in some dysregulated cells, such as cancer cells, induce cell death [14].

Electroporation is a method used to introduce high concentrations of therapeutic agents into cells. Using an electrode, short, high-voltage pulses are applied to induce transient permeabilization of cell plasma membranes in targeted tissue. The increased permeability permits the passage of otherwise non-permeable ions and molecules such as calcium or bleomycin [15, 16] (Fig. 2).

The combination of chemotherapy (e.g., bleomycin) and electroporation is called electrochemotherapy and is already a widespread standard treatment for cutaneous metastasis [17–19]. Recent publications have shown that electroporation with the cytotoxic antibiotic drug bleomycin is an effective treatment for keloids as well as hypertrophic scars [20, 21].

In vitro and in vivo studies have shown that calcium can be used instead of bleomycin to induce cancer cell death [16, 22, 23]. These results have led to a recent clinical study showing calcium used instead of chemotherapy also has high efficacy in treating cutaneous metastasis, whilst also achieving more cosmetically satisfying results [12]. Electrochemotherapy is proven to be safe and effective in the treatment of solid tumours but can cause hyperpigmentation of the skin [24, 25], also seen in the treatment of keloids [21].

We hypothesized that calcium electroporation could be an effective, safe, and cost-efficient treatment option for keloids, potentially with limited adverse effects. It was also of interest to assess whether the treatment could be used in cases where applying cytotoxic agents is relatively contraindicated, which is a debated issue when treating benign diseases such as keloid scarring, especially known to affect young individuals.

**Patients and Methods**

We conducted a first-in-man phase I study to assess the efficacy and safety of calcium electroporation for keloids unresponsive to other known treatments, or where known treatments had not been deemed appropriate. The primary endpoint was to assess
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Keloid response to treatment with calcium electroporation. The secondary endpoint was to register any adverse or serious adverse events in patients treated with calcium electroporation for keloids.

The inclusion criteria were: a minimum of 1 keloid accessible for calcium electroporation; patients should have been offered current standard treatment and undergone at least 8 weeks since previous treatment (surgery, radiation, steroid injection, or pressure); patients had to be at least 18 years old, ECOG performance status previous treatment (surgery, radiation, steroid injection, or pressure); patients had to be at least 18 years old, ECOG performance status <2, platelet count ≥ 50 × 10^9/L, and prothrombin time ≥ 40 s; sexually active men and women with childbearing potential had to use adequate contraception during the trial; inclusion required the ability to understand given information and sign an informed consent form. The exclusion criteria were: uncorrectable coagulation disorders, pregnancy or ongoing nursing, participation in other clinical trials involving experimental drugs or involvement in a trial within 4 weeks prior to study drug administration.

Initially, only 1 keloid per patient was treated. As this was a first-in-man study we chose only to treat half of the lesion at the first treatment, the keloid was divided into 2 equal parts marked with a pen (as seen in Fig. 4a). Calcium was injected intralesionally using standard syringes, in lines parallel to the marking and only in 1 half of the keloid. Pulses were delivered immediately after using electrodes with 2 parallel linear arrays of needles [18]: 8 pulses of 0.1 ms duration and 400 V at a frequency of 5 kHz were delivered using a square wave pulse generator (Cliniporator, IGEA, Italy).

### Evaluation of Treatment Response
Assessment of response was performed by clinical examination at baseline with follow-up after 15, 30, 60, 90, and 180 days and after 10, 14, 18, and 24 months to detect any relapse. Before treatment and at follow-up, lesions were measured with the longest diameter (a), the diameter perpendicular to a, and thickness in millimetres. The treated target lesion response was clinically determined individually and documented by digital photography (including a ruler for scale). Only the initially treated lesion was included in the final response evaluation. Change in size of the keloid was evaluated according to RECIST-like criteria [26] and defined as: complete response – disappearance of the lesion; partial response – at least a 30% decrease in the thickness of the keloid; progressive disease – at least 20% increase in the thickness of the keloid, and stable disease – neither 30% decrease nor 20% increase in the thickness of the keloid (Table 2).

In addition, the keloids were evaluated using the Patient and Observer Scar Assessment Scale (POSAS) [27] at each follow-up, including the evaluation of vascularization, pigmentation, thickness, symptom relief, pliability, and surface area. Adverse events were recorded on the days of clinical examination using the Com-

| Patient No. | Sex | Age, years | Skin type | Lesion cause | Localisation | Lesion duration, months | Previous treatments | Sessions, n | Total lesions, n | Lesions treated, n | Smoking status |
|------------|-----|------------|-----------|-------------|--------------|------------------------|-------------------|-------------|----------------|------------------|---------------|
| 1          | F   | 44         | 2         | Acne        | Upper back   | 240                    | Surgery, steroid injection | 2            | 9              | 4                | Former smoker  |
| 2          | F   | 27         | 3         | Acne        | Sternum      | 108                    | Surgery, steroid injection | 2            | 1              | 1                | Smoker         |
| 3          | F   | 40         | 2         | Ear piercing | Back of ear  | 216                    | Surgery, steroid injection, laser therapy | 2            | 1              | 1                | Non-smoker     |
| 4          | F   | 46         | 3         | Dermato-fibroma | Sternum      | 48                     | Surgery, steroid injection | 2            | 1              | 1                | Non-smoker     |
| 5          | F   | 20         | 2         | Ear piercing | Back of ear  | 142                    | Surgery, steroid injection, pressure | 3            | 1              | 1                | Non-smoker     |
| 6          | F   | 25         | 2         | Acne        | Sternum, shoulders and back | 156                    | Steroid injection, PDT, laser therapy, cryotherapy | 3 >20        | 3              | 3                | Non-smoker     |

PDT, photo-dynamic therapy.
Table 2. Lesion details

| Patient No. | Evaluation point | Day | Lesion thickness, mm | Lesion length, mm | Lesion diameter, mm | Lesion volume, cm³ | % change (volume) | % change (thickness) | Response |
|-------------|------------------|-----|----------------------|-------------------|--------------------|-------------------|------------------|---------------------|----------|
| 1           | Before treatment | 0   | 6                    | 4                 | 20                 | 15                | 6.29             | –                   | –        |
|             | End of follow-up| 473 | 6                    | 4                 | 20                 | 15                | 6.29             | 0.00                | SD       |
| 2           | Before treatment | 0   | 3                    | 3                 | 40                 | 18                | 9.05             | –                   | –        |
|             | End of follow-up| 476 | 3                    | 3                 | 45                 | 28                | 7.92             | –75.00              | PD       |
| 3           | Before treatment | 0   | 8                    | 8                 | 22                 | 12                | 4.42             | –                   | –        |
|             | End of follow-up| 308 | 9                    | 4                 | 23                 | 13                | 2.51             | 8.14                | 43.37    |
| 4           | Before treatment | 0   | 20                   | 10                | 33                 | 17                | 11.75            | –                   | –        |
|             | End of follow-up| 287 | –                    | 4                 | 35                 | 20                | 5.87             | 50.09               | 60.00    |
| 5           | Before treatment | 0   | 5                    | 20                 | 10                 | 2.10             | –                | –                   | –        |
|             | End of follow-up| 210 | –                    | 4                 | 20                 | 12                | 2.01             | 4.00                | 20.00    |
| 6           | Before treatment | 0   | 3                    | 3                 | 15                 | 8                 | 0.75             | –                   | –        |
|             | End of follow-up| 274 | 3                    | 3                 | 15                 | 8                 | 0.75             | 0.00                | SD       |

Change in size of the keloid was evaluated according to RECIST-like criteria [26] and defined as: CR, complete response – disappearance of the lesion; PR, partial response – at least 30% decrease in thickness; PD, progressive disease – at least 20% increase in thickness; SD, stable disease – neither 30% decrease nor 20% increase in thickness.
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Before treatment | After treatment

Fig. 4. Patient cases. a Patient No. 1, lesion before treatment (left) and 388 days after treatment (right). Female (aged 44 years) with keloids located on the upper back reported reduced colour, no adverse effects, and stagnant growth. The patient requested retreatment because of stationary growth and self-assessed reduction of thickness. Number 1 indicates the treated lesion. There was no measured reduction of thickness or volume during follow-up. b Patient No. 2, lesion before treatment (left) and 273 days after treatment (right). Female (aged 27 years) with a large keloid located on the chest developed from an acne scar. During follow-up, the patient reported the softening of scar tissue, reduced pain and pruritus, and a slower growth rate. Following treatment, the keloid developed a crusted wound that healed spontaneously (shown in Fig. 5). The patient requested retreatment because of symptom relief but withdrew from the trial after retreatment because of radiation therapy. After a follow-up period of 273 days an increase in scar volume was observed, although the scar thickness remained unchanged. c Patient No. 5, lesion before treatment (left) and 512 days after treatment (right). Female (aged 20 years) with a keloid scar on the antihelix of the ear after an ear-piercing. The patient reported a calming effect following treatment as well as flattening and less redness. The patient requested retreatment.

Monday Toxicity Criteria for Adverse Events (CTCAE) version 4.0 before and after treatment.

Punch biopsies were collected from the keloids before and after treatment (day 7). The biopsies were cut, frozen, and HE stained. The degree of necrosis, inflammation, and fibrosis was described using a point scale: 0, none; 1, slight; 2, moderate; 3, severe.

Statistical Analysis

This was set up as a phase I study and only descriptive statistics were used. Where appropriate, a 2-sided Student t test was performed. A p value < 0.05 was considered significant. Parametric data are presented as the mean ± SD. Non-parametric data are presented as the median with interquartile ranges (IQR).

Results

Patients

Seven patients were included (6 female, 1 male). One patient was excluded after treatment since the histology from biopsies showed the lesion was a non-keloid scar.

The median duration of keloid lesions prior to treatment was 142 months (IQR 78–186) and follow-up time was 338 ± 101 days (mean + SD). Included patients had skin types 2 or 3. For further information see Table 1.

Keloid Thickness

Regarding the primary endpoint of the study, 2 of the 6 patients (33%) experienced a more than 30% reduction of the thickness of the treated keloid. No increase in lesion thickness was observed in any of the 6 treated patients during a mean follow-up period of 338 days (Fig. 3, 4).

Keloid Volume

As an additional measure, the calculated volume was also used to assess response. Taking into account the shape of most keloids, the volume was calculated as a half oblate ellipsoid: 
\[ V = \frac{4}{3} \times \pi \times 0.5a \times 0.5b \times c \] 
(a, longest diameter; b, longest diameter perpendicular to a; c, thickness). Treatment led to a decrease in lesion volume in 3 out of 6 patients, with an observed mean reduction of 10.7 ± 43.6 (p = 0.57). One patient (No. 2) exhibited an increase in scar volume after a follow-up period of 565 days, although the scar thickness remained unchanged (Fig. 3).

Histology

Biopsies taken before treatment and on day 7 were compared and graded on a scale of 0–4 for signs of keloid, necrosis, inflammation, and fibrosis. The biopsy evaluation was limited by the number of samples that were eval-
uable (3 of 6 pre-treatment samples and 4 of 6 post-treatment samples). The results suggested a small decrease in signs of keloid after treatment but limited changes in other parameters.

**Response to Treatment on Subjective Symptoms**

Five out of 6 patients reported decreased pain and pruritus (Table 3). Treatment was well tolerated, with all patients requesting more than 1 treatment session.

**Adverse Effects**

We did not observe any unexpected adverse reactions compared to the untreated part and all 6 patients were offered retreatment of the entire keloid. No serious adverse effects or recurrences were observed in the study period. Some patients experienced pain during treatment in relation to the injection of calcium and lidocaine and application of electric pulses (Table 3). Transient ulceration was observed in 3 of the 6 cases (Table 3; Fig. 5).

**Table 3. Observations**

| Patient No. | Observations                                      | Reason for continuation/withdrawal                                                                 | Histology | Follow-up time, days | Relapse |
|-------------|--------------------------------------------------|-------------------------------------------------------------------------------------------------|-----------|----------------------|---------|
| 1           | Paleness, no adverse effects, stagnant growth     | Requested continued treatment because of stationary growth and self-assessed reduction of height | Keloid    | 473                  | No      |
| 2           | Softening, crusted wound, lessening of pain and pruritus, slower growth rate | Requested retreatment because of symptom relief; withdrawal because of radiation therapy       | Keloid    | 476                  | Yes     |
| 3           | Pain in treated area, less pruritus, flattening  | Requested retreatment because of self-assessed decline in lesion size; withdrawal because of pain during treatment | Keloid    | 308                  | No      |
| 4           | Crusted wound, contraction, pain, less pruritus, flattening | Requested retreatment because of decline in pruritus and lesion size; withdrawal because of desired surgical intervention | Keloid    | 287                  | No      |
| 5           | Transient oedema, crusted wound, flattening, less pruritus, less pain | Requested retreatment because of flattening and symptom relief                               | Keloid    | 210                  | No      |
| 6           | Calming effect, paleness, flattening             | Requested retreatment because of colour improvement and symptom relief                        | Keloid    | 274                  | No      |

![Patient 2 before treatment](image1)

![Immediately after re-treatment (day 163)](image2)

![1 month after re-treatment (day 196)](image3)

![6½ months after re-treatment (day 273)](image4)

**Fig. 5.** Lesion response to treatment: patient No. 2, a 27-year-old female with a large keloid on the chest resulting from an acne scar. Prior interventions included surgery and steroid injections. The patient requested retreatment because of a decline in pruritus and lesion size. Ulceration with a crusted wound was observed 1 month after retreatment, followed by contraction of the scar.
Discussion

This study is an investigative phase I study of calcium electroporation treatment for keloid scars. Treatment was well tolerated, and 2 out of 6 patients experienced a more than 30% reduction in thickness (primary endpoint), whilst 5 of the 6 patients reported symptom relief with decreased pruritus.

Keloid scars are difficult to treat, with most known treatments having a high recurrence rate. Intralesional injection with bleomycin as well as electrochemotherapy have shown promising results in the treatment of keloids [20, 21]. As treatment without chemotherapy would have advantages when treating benign keloid lesions, we performed the investigation of calcium electroporation for keloids on this indication.

Manca et al. [21] showed a median reduction of lesion thickness of 83% (range 49–100%) in 20 patients with 38 keloids and hypertrophic scars treated with electrochemotherapy. They reported a significant reduction in pliability, erythema, hitching, and pain [21]. Here we introduce a mean reduction of thickness of 22% (range 0–60%) with an objective reduction in lesions of 33% and a patient reported decrease in pain and pruritus in 5 out of 6. However, the 2 studies are not comparable due to the difference in number of treated lesions, and we only present descriptive data. A larger phase II study with calcium electroporation will be needed. Hyperpigmentation was seen in patients treated with ECT [21], which is a well-known side effect to bleomycin-based ECT. We did not observe any hyperpigmentation after calcium electroporation, which confirms previous findings [12].

Calcium in place of chemotherapeutics has the benefit of being non-mutagenic. Patients treated with steroid injections for keloid scars also risk an induced lower bone density, resulting in osteoporosis and osteopenia. Such adverse effects could potentially be avoided using alternative treatments such as calcium electroporation. Thus, calcium electroporation could be further investigated as a treatment modality for benign proliferative disorders of the skin, especially in young patients.

The limited medical implications of low-dose cutaneous injections of calcium allow it to be administered outside hospitals. Calcium electroporation can therefore be performed at clinics provided with electroporators and appurtenant facilities. Electroporation equipment is already widely used to enhance the local effect of chemotherapy in superficial tumours, with trials on internal tumours in progress [28–30]. The availability, safety, and low cost of calcium electroporation can therefore easily be introduced to clinical use and further studies.

Prior to the study we expected very few adverse effects to treatment as previous studies have shown calcium electroporation to be a safe and well-tolerated treatment. The basic mechanism of drug delivery in electroporation requires a lipid bilayer. A greater treatment efficacy is therefore expected in tissue with a high cellular component, as is the case in cutaneous metastasis. The effect in tumours

| Patient No. | Day of treatment | Calcium (9 mg/mL), mL | Highest given current, A | % of treated keloid | Localisation |
|------------|-----------------|-----------------------|------------------------|--------------------|-------------|
| 1          | 0               | 0.4                   | 3–5                    | Not registered     | 50          | Upper back |
|            | 300             | 1.2                   |                        |                    |             | Upper back |
| 2          | 0               | 1.7                   | 7–10                   | 50                 | Sternum     |
|            | 90              | 3.4                   | 7–10                   | 100                | Sternum     |
| 3          | 0               | 0.38                  | 1.5–3                  | 50                 | Back of ear |
|            | 60              | 1.34                  | 3–5                    | 100                | Back of ear |
| 4          | 0               | 1.13                  | >10                    | Not registered     | 50          | Sternum     |
|            | 180             | 2                     |                        |                    |             | Sternum     |
| 5          | 0               | 1                     | 7–10                   | 100                | Back of ear |
|            | 270             | 0.9                   | 1.5–3                  | 100                | Back of ear |
|            | 360             | 1.2                   | 7–10                   | 100                | Back of ear |
| 6          | 0               | 0.25                  | 3–5                    | 100                | Shoulder    |
|            | 90              | 0.13                  | 3–5                    | 100                | Shoulder    |
|            | 240             | 0.15                  | 5–7                    | 100                | Shoulder    |
with a low cellular component and high degree of extracellular matrix is less predictable. Interestingly we observed no progression, and a non-significant reduction in lesion volume.

In this study, a high dose of calcium was used (220 mM), injected to 50% of the keloid volume (Table 4). As we observed limited side effects, we would propose that this strategy is useful, but it is clear that the optimal dose for the treatment of keloids requires further examination.

Calcium electroporation as well as electrochemotherapy has been proven to spare normal, healthy tissue in therapeutic doses [31] and, interestingly, a study on 3D cellular spheroid models observed a very low impact on normal fibroblasts compared to spheroids composed of malignant cells [32]. It was of interest to investigate the effect of calcium electroporation on keloid growth, correlating to the activity and proliferation of the cellular components of scar tissue. Calcium electroporation was not expected to greatly influence the abundance or presence of non-cellular material. As collagen is the main component of keloids, we did not expect an immediate reduction in keloid size in response to treatment. It could be interesting to further investigate whether calcium electroporation could assist in aiding keloid scars into an appropriate remodelling phase instead of a pathologic chronic scar proliferation phase.

All patients requested retreatment, in most cases because of symptom relief, self-assessed flattening, and colour improvement. The symptom relief alone observed in this study encourages further investigation of the possibilities for calcium electroporation in the treatment of keloid scars. Larger phase II studies are needed to clarify these possibilities as well as the actual response rate.

The limitations in this study are the small number of patients, and keloid thickness measurement is subject to variation due to manual measurement. Furthermore, patient outcome reporting may be influenced by a placebo effect.

Thus, with moderate improvement of keloid thickness and symptoms in a small study population, further randomized studies comparing calcium electroporation to other known treatments for keloids are warranted and should include endpoints such as keloid thickness and symptoms such as pruritus.

Conclusion

In this study, which is the first investigating calcium electroporation for keloids, one third of patients experienced a reduction of lesion thickness, and a majority experienced symptom relief from pruritus. The treatment was well tolerated. Further studies should consider comparisons between calcium electroporation and other available treatments for keloids.

Key Message

Calcium electroporation for keloids was found to be safe and resulted in symptom relief.

Statement of Ethics

The protocol was approved by the Danish Medicine Agency, The Regional Ethics Committee, and the Danish Data Protection Agency. The study was performed according to Good Clinical Practice (GCP). Clinicaltrials.gov identifier: NCT01941914.

Conflict of Interest Statement

Julie Gehl is co-inventor of a granted patent (therapeutic applications of calcium electroporation to effectively induce tumour necrosis): PCT/DK2012/050496.

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Author Contributions

H.F. wrote the protocol and treated the patients, including during follow-up. M.V. collected data and drafted the manuscript. G.W. performed the histological analyses. J.G. supervised writing of the protocol, treated and followed-up patients. All authors contributed to and approved the final manuscript.

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