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ORIGINAL REPORT

An Explorative Study on Calcium Electroporation for Low-risk Basal Cell Carcinoma

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In electrochemotherapy, permeabilization of the cell membrane by electric pulses increases the anti-tumour effect of chemotherapeutics. In calcium electroporation, chemotherapy is replaced by calcium chloride with obvious benefits. This study explores the effect and underlying mechanisms of calcium electroporation on basal cell carcinomas using either high- or low-frequency electroporation. Low-risk primary basal cell carcinomas were treated in local anaesthesia with intratumoral calcium chloride followed by electroporation with high (167 kHz) or low (5 kHz) frequencies. Non-complete responders were retreated after 3 months. The primary endpoint was tumour response 3 months after last calcium electroporation. Plasma membrane calcium ATPase was examined in various cell lines as plasma membrane calcium ATPase levels have been associated with calcium electroporation efficacy. Twenty-two out of 25 included patients complete the study and 7 of these (32%) achieved complete response at 3 months with no difference in efficacy between high- and low-frequency pulses. High-frequency calcium electroporation was significantly less painful (p=0.03). Plasma membrane calcium ATPase was increased 16–32-fold in basal cell carcinoma cell lines compared with 4 other cancer cell lines. Calcium electroporation for low-risk basal cell carcinomas does not fulfil the requirements of a new dermatological basal cell carcinoma treatment but may be useful as adjuvant treatment to surgery in more advanced basal cell carcinomas. The elevated PMCA levels in basal cell carcinomas may contribute to low efficacy.

Key words: calcium electroporation; electrochemotherapy; basal cell carcinoma; high-frequency electroporation.

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SIGNIFICANCE

In electrochemotherapy, permeabilization of the cell membrane by electric pulses increases the anti-tumour effect of chemotherapeutics. In calcium electroporation, chemotherapy is replaced by calcium chloride with obvious benefits in dermatology. This explorative prospective study was the first to evaluate the efficacy of calcium electroporation for low-risk basal cell carcinomas. Twenty-five patients received 1–2 calcium electroporation treatments. Complete BCC clearance was seen in 7 patients (32%) in the 22 patients completing the study. Calcium electroporation for low-risk basal cell carcinomas does not fulfil the requirements of a new dermatological basal cell carcinoma treatment but may be useful as adjuvant treatment to surgery in more advanced basal cell carcinomas.

Basal cell carcinoma (BCC) is the most common malignant tumour in fair-skinned populations and accounts for 75% of all skin cancers (1). Ultraviolet radiation is believed to be the predominant causative risk factor and the incidence of BCC is inversely related to a country's geographic latitude combined with the pigment status of its inhabitants (1, 2). The incidence rates of BCC are increasing worldwide, and this imposes an important health problem (1).

There are several treatments options for BCC. Superficial and low-risk tumours are often treated with conventional surgery, topical therapies (imiquimod, fluorouracil, or photodynamic therapy), or destructive therapies (curettage, cryotherapy, or laser ablation), while high-risk, recurrent, and critical anatomically located BCCs are treated with microscopically controlled surgery (Mohs surgery) or superficial radiotherapy (3). Factors such as comorbidities, anatomical location, patient preference, and cosmetic concerns create the need for additional therapeutic options for BCCs.

Electroporation is a method whereby short, high-voltage pulses induce a transient permeabilization of the cell membrane, allowing passage of ions and molecules into and out of the cell (4). Electroporation is used in combination with chemotherapeutic drugs (electrochemotherapy, ECT) such as bleomycin and cisplatin, and is an effective treatment for various cancers including skin cancer (5).

Calcium electroporation (CaEP) is a novel cancer treatment in which the combination of intratumoral calcium and electroporation increases calcium uptake in cancer cells, leading to acute and severe ATP depletion associated with cell death (6, 7). Levels of plasma membrane

ATPase (PMCA) expression has been associated with the efficacy of CaEP (8). High PMCA expression will allow the cell to restore the low intracellular calcium level more efficiently and thereby decrease the effect of CaEP. Therefore, low levels of PMCA expression have been associated with better response to CaEP (8).

The effect of CaEP has been shown to be comparable to ECT using bleomycin in the treatment of cutaneous breast cancer and malignant melanoma metastasis, in smaller phase II trials (9, 10). In addition, CaEP has been investigated in phase I–II studies on cutaneous metastases across cancer histologies, in patients with recurrent head and neck cancer, in patients with oesophageal cancer, and in colorectal cancer (11–14). No previous study has reported on the effect of CaEP for BCC.

As calcium would be simpler to administer than chemotherapy, especially in non-hospital settings, there would potentially be an advantage of being able to use calcium rather than chemotherapy for electroporation-based treatment in dermatology. Furthermore, it has been shown that the mechanism of action of CaEP does not involve DNA damage, which may be beneficial especially in treating BCC in younger patients (15).

The aim of this explorative study was to evaluate the efficacy and side effects of calcium electroporation for low-risk basal cell carcinomas using either high-frequency (167 kHz) or low-frequency (5 kHz) electroporation.

MATERIALS AND METHODS

Study design

This explorative study was a prospective, non-randomized, non-blinded single-centre study. Signed informed consent was obtained from all participants prior to entry into the study and the Good Clinical Practice Unit, Copenhagen University, performed external monitoring. The patients in this manuscript have given written informed consent to publication of their case details. The protocol was approved by the Danish Medicine Agency (EudraCT 2019-002730-36), the Regional Ethics Committee of Region Hovedstaden (H-19042561), and the Danish Data Protection Agency. The study is listed at clinicaltrials.gov (NCT05046262).

Patients

Patients with histologically proven low-risk BCCs were included. Low-risk BCCs were defined as primary tumours less than 3 cm in diameter, histologically not aggressive tumours (excluding infiltrative, micronodular, morpheaform, basosquamous), located outside the lip, scalp, nose, eyelids, and ears. The exclusion criteria were organ transplant recipients and immunosuppression, allergy to local anaesthetics, and pregnant or breastfeeding women. Patients were recruited from January 2020 to April 2021 among patients referred to the Department of Dermatology, Bispebjerg University Hospital, Copenhagen, Denmark for treatment of BCC.

Treatment

The tumour area and a safety margin of 5 mm around the tumour was marked and a template corresponding to the entire treatment area was created for later identification of the treatment area. The tumour-free margin was confirmed using optical coherence tomography (OCT) (Vivosight, Michelson Diagnostics, Maidstone, UK). The treatment was performed in local anaesthesia injected in a square around the treatment area. Carbocaine with adrenaline was used in 21 patients and lidocaine with adrenaline in 4 patients (due to short supply of carbocaine with adrenaline). The calcium chloride solution was prepared in the recommended concentration of 9 mg/mL (220 mmol/L) (9). The volume used depended on tumour size and was approximately 0.5 mL/cm^3 tumour volume (9). The calcium chloride solution was injected in superficial parallel rows, ensuring that both the tumour and safety margin were covered.

Electric pulses were delivered immediately after calcium injection. Needle electrodes with two parallel rows of 4 needles with a 4 mm gap were used (CUTIS, Mirai Medical, Galway, Ireland) to deliver either biphasic pulses of 2 µs duration, and 200 µs energized time at 1300 V/cm with a frequency of 167 kHz (high-frequency electroporation), or 8 unipolar pulses of 100 µs duration, 1,000 V/cm with a frequency of 5 kHz (low-frequency electroporation, ESOPE settings) using the pulse generator ePore (Mirai Medical, Galway, Ireland). The term V/cm describes voltage applied relative to the distance between opposing electrodes measured in cm. Needle electroporation was repeated until the whole treatment area was electroporated.

Tumours with partial or no response 3 months after the first CaEP treatment received a second CaEP treatment.

Primary endpoint

The primary objective was tumour response 90 days after the last treatment. Response was defined as complete response – disappearance of the lesion confirmed by histology; partial response – at least 30% decrease in the largest diameter of the lesion; or no response – less than 30% decrease in lesion diameter. Response was assessed by clinical examination and confirmed by dermoscopy and OCT-guided punch biopsy at 90 days and 365 days after the first (and second) CaEP treatment. Tumour responses were documented by digital photography including a ruler.

Secondary endpoints

Secondary endpoints were lesion recurrences at 12 months after last CaEP, pain score, local skin reactions after treatment, and cosmetic outcome.

Pain was recorded on a numeric rating scale (0: no pain; 10: worst imaginable pain) before, during, and after CaEP. A study nurse contacted the patients weekly until follow-up, recording the pain score, ulceration, oedema, and redness in the treated area. At follow-up, side effects such as ulceration, scarring, and hypo- and hyperpigmentation were recorded on a 4-point scale (0: none, 1: mild, 2: moderate, 3: severe). Patients and investigator rated the cosmetic outcome as poor, fair, good, or excellent.

In vitro plasma membrane calcium ATPase expression

To measure the expression of pan PMCA (all 4 isoforms) in different cell lines *in vitro* studies were performed. Eight cell lines were analysed and cultivated: HDF-n (human fibroblasts from surgical specimens), HaCat (immortalized keratinocytes), A431 (epidermoid carcinoma), UWBCC1 (BCC) (16, 17), HT29 (human colorectal adenocarcinoma) in SMEM+glutamx (Gibco) + 10% FBS (HyClone), BCC77015 (BCC) in human basal cell carcinoma cell line complete medium (Celprogen) (17) + 10% FBS (Hyclone), SCC25 (squamous cell carcinoma) in DMEM/F12 1:1 (Gibco) + 20% FBS (Hyclone), and MDA-MB-231 (human breast adenocarcinoma) in RPMI-1640 (Gibco) + 10% FBS (Hyclone). The culture media were supplemented with 1% penicillin/streptomycin.

Total cellular protein was extracted using lysis buffer (150 mM NaCL, 50 mM Tris-HCL, 1% NP-40, 0.5% Na-deoxycholate, 0.1% SDS) with protease inhibitor (Aprotinin 25mg/mL, 1:1000). Total protein (10 µg) was separated on a 4–12% Bis-Tris SDS gel (Invitrogen) and the proteins, which were blocked with TBST buffer (20 mM Tris, 150 mM NaCl, 0.1% Tween20 containing 5% skimmed milk powder), were transferred onto a PVDF membrane. Membranes were incubated with 1:1,000 PMCA monoclonal antibody (Invitrogen) in TBST at 4ºC overnight, then 1:5,000 goat, anti-mouse poly HRP (Invitrogen) for 2 hours (22ºC). Positive bands were detected with enhanced chemiluminescence (SuperSignal West Femto, ThermoFisher Scientific, Waltham, MA, USA) and quantified using GeneTools image analysis software (Syngene, https://www.syngene.com/). Western blots were repeated 3 times and data were normalized to HT29 and expressed as the mean± standard deviation.

Statistical analysis

All statistics were performed using GraphPad Prism version 9.4.1 (GraphPad Software Inc, San Diego, CA, USA). We used non-parametric statistics and Wilcoxon signed-rank test to compare data. p-values < 0.05 were considered significant.

RESULTS

Patients

Twelve female and 13 male patients were included in the study and had a median age of 74 years (range 50–93). In total, 25 biopsy-verified primary BCCs were treated with CaEP (one BCC per patient). The BCCs' median diameter was 24 mm (range: 15–50 mm) and OCT measured thickness 0.61 mm (range: 0.35–1.05 mm). Histology showed superficial BCC in 7 tumours and nodular BCCs in 18 tumours. Five BCCs were located in the face (cheek, temple, and forehead), 4 BCCs on the arm, 8 BCCs on the back, and 8 BCCs on the chest/abdomen (**Table I**).

Thirteen patients were treated with-high frequency CaEP and no patients were lost to follow-up. The remaining 12 patients received low-frequency CaEP. In this group, 2 patients refused re-treatment due to pain during electroporation, lack of efficacy, and fear of Covid infection. In addition, 1 patient left the study before 3-month follow-up due to recurrence of internal cancer and initiation of chemotherapy. An additional patient was lost to 12-month follow up due to terminal cancer.

Table I. Baseline characteristics

Factor	High-frequency CaEP $n = 13$	Low-frequency CaEP $n = 12$
Age, years, median (range)	75 (54–93)	73 (50–91)
Sex, *n*		
Female	6	6
Male	7	6
Histological type, *n*		
Superficial basal cell carcinoma	2	5
Nodular basal cell carcinoma	11	7
Localization, *n*		
Face	4	1
Trunk	8	8
Arms	1	3
Tumour diameter , *n*		
< 10 mm	2	3
10–20 mm	8	9
21–30 mm	3	0
Tumour thickness, mm, median (range)	0.60 (0.38–1.05)	0.62 (0.35–0.86)

CaEP: calcium electroporation.

Efficacy

Seven out of 22 patients completing primary endpoint (32%) had complete response at 3-month follow-up after last CaEP. No overall difference in efficacy was seen between the 13 patients treated with high-frequency CaEP and 9 patients treated with low-frequency CaEP for low-risk BCC. At follow-up, treatment areas were evaluated using dermoscopy and OCT-guided biopsies were obtained to prove efficacy (**Fig. 1**). Three months after last CaEP, 3 patients had no clinical sign of BCC but dermoscopy and OCT gave a suspicion of remaining tumour, which was confirmed by histology. Four patients with biopsy-proven partial response 3 months after treatment had no residual tumour after routine treatment (curettage or Mohs surgery).

High-frequency CaEP

Three patients out of 13 (23%) had histology-proven complete clearance of the BCC after one treatment of high-frequency CaEP with no recurrences at 12-month follow-up (**Table II**). The remaining 10 patients experienced partial response (4 patients) or no response (6 patients) at 3 months after the second CaEP (**Fig. 2**).

Low-frequency CaEP

No BCC had complete clearance after the first low-frequency CaEP. Of the 9 patients receiving a second CaEP treatment, 4 patients had complete clearance (44%), 4 patients had partial response, and 1 patient no response (Fig. 2). At the 12-month follow-up 2 patients had recurrences of their BCC (see Table II).

Safety and cosmetic outcome

CaEP using high-frequency electroporation was significantly less painful than using low-frequency electroporation with a median pain score of 2 (range 0–9) compared with 5 (0–9) ($p = 0.03$). No differences in pain score were found between first and second CaEP ($p = 0.67$). During the 4 treatments using lidocaine, patients had a pain score of 7, 6, 6, and 9, respectively. Excluding these lidocaine treatments, the median pain score during low-frequency electroporation was as low as 1 (range 1–8).

Two patients (1 treated with high-frequency CaEP and 1 with low-frequency CaEP) with BCC on the arm reported muscular pain after treatment for up to 1 week. Otherwise, no pain was reported after treatment except for 1 patient with severe ulceration.

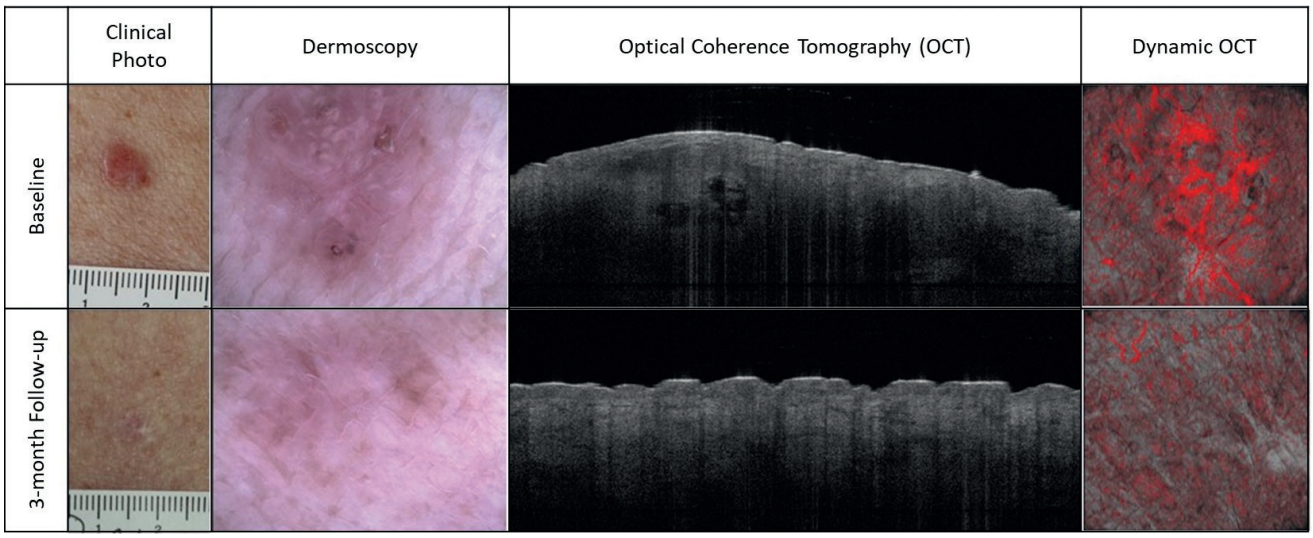

Fig. 1. Clinical photo, dermoscopy, optical coherence tomography (OCT), and dynamic OCT images of a nodular basal cell carcinoma on the back. At baseline a necrotic black area and high vascularization is seen by OCT and dynamic OCT respectively. At 3-month follow-up complete clearance of the BCC is observed, and skin structure and vascularization are normalized.

After high-frequency electroporation of a small BCC under the breast, one patient developed necrosis of the underlying tissue, resulting in severe ulceration spreading outside the treatment area. The patient was treated with systemic antibiotics and wound care. The ulceration healed within 11 weeks with large scar formation (**Fig. 3**).

Ulceration after treatment was seen in 19/23 (83%) and 11/19 (58%) patients after first and second CaEP, respectively. Ulcerations healed within 2–10 weeks (median 3.5 weeks) except for 2 patients who still had ulcerating tumours after both treatments. Most patients in complete response had mild scar formation and mild hyper- or hypopigmentation (see Fig. 2). However, 4 out of 7 patients rated the cosmetic outcome excellent or good, 2 patients fair, and 1 patient poor.

Plasma membrane ATPase expression

In vitro results suggest that the protein expression of pan PMCA is high in the SCC cell line (SCC25) and the 2 BCC cell lines (UWBCC1 and BCC77015), with a mean fold change of 9.0, 32.0, and 15.7, respectively (normalized to HT29). MDA-MB-231 and HDF-n had low pan PMCA expression, with a mean fold change of 1.81 and 1.92, respectively, normalized to HT29 (**Fig. 4**).

Table II. Efficacy evaluation at 3-month follow-up after last calcium electroporation (CaEP)

Efficacy	High-frequency CaEP $n=13$	Low-frequency CaEP $n=12$
Complete response	3[a]	4[b]
Partial response	4	4
No response	6	1
Dropouts	0	3

Complete response was seen after one CaEP treatment in the high-frequency group and after two CaEP treatments in the low-frequency group. Complete responses were located on the back (2), chest (4), and arm (1).
[a]No recurrence at 12-month follow-up. [b]2 recurrences and 1 dropout at 12-month follow-up.

DISCUSSION

Calcium electroporation of low-risk basal cell carcinoma resulted in complete clearance in 7 out of 22 patients and 2 of these patients had recurrences within 12 months.

These results are inferior to what is seen in studies using bleomycin ECT for basal cell carcinomas (18). In a randomized controlled trial by Clover and colleagues, the efficacy of ECT with intra-tumoral bleomycin was compared with surgery for primary BCC in 87 patients (19). All lesions treated with ECT were in complete clinical response 2 months after 1–2 treatments. At 5 years' follow-up, 5 recurrences were seen in the ECT-treated patients and 1 in patients receiving surgery, resulting in an overall complete response rate of 89% after ECT, which was non-inferior to the 97% in the surgical arm (p=0.33). These results are much more favourable than our overall complete response rate of 32%. Two notable differences between the 2 studies are the use of calcium instead of bleomycin as well as the use of different pulse generators (ePore, Mirai, Ireland versus Cliniporator, IGEA, Italy).

No studies have previously evaluated the efficacy of CaEP for basal cell carcinoma. The original clinical study by Falk et al. compared CaEP with bleomycin ECT in the treatment of 37 cutaneous breast cancer and malignant melanoma metastases in an intra-individual design (9). Complete clearance of 66% of the metastases was seen after CaEP, which was comparable to 68% after ECT ($p=0.5$).

During CaEP, a high influx of calcium triggers cell death. Studies have indicated that cancer cell lines are more sensitive to CaEP than normal dermal fibroblasts (8). It has been shown that the influx of calcium is similar in normal and cancer cells, yet calcium content decreases rapidly in normal cells and endures at high levels in cancer cells (8). This difference might be due to

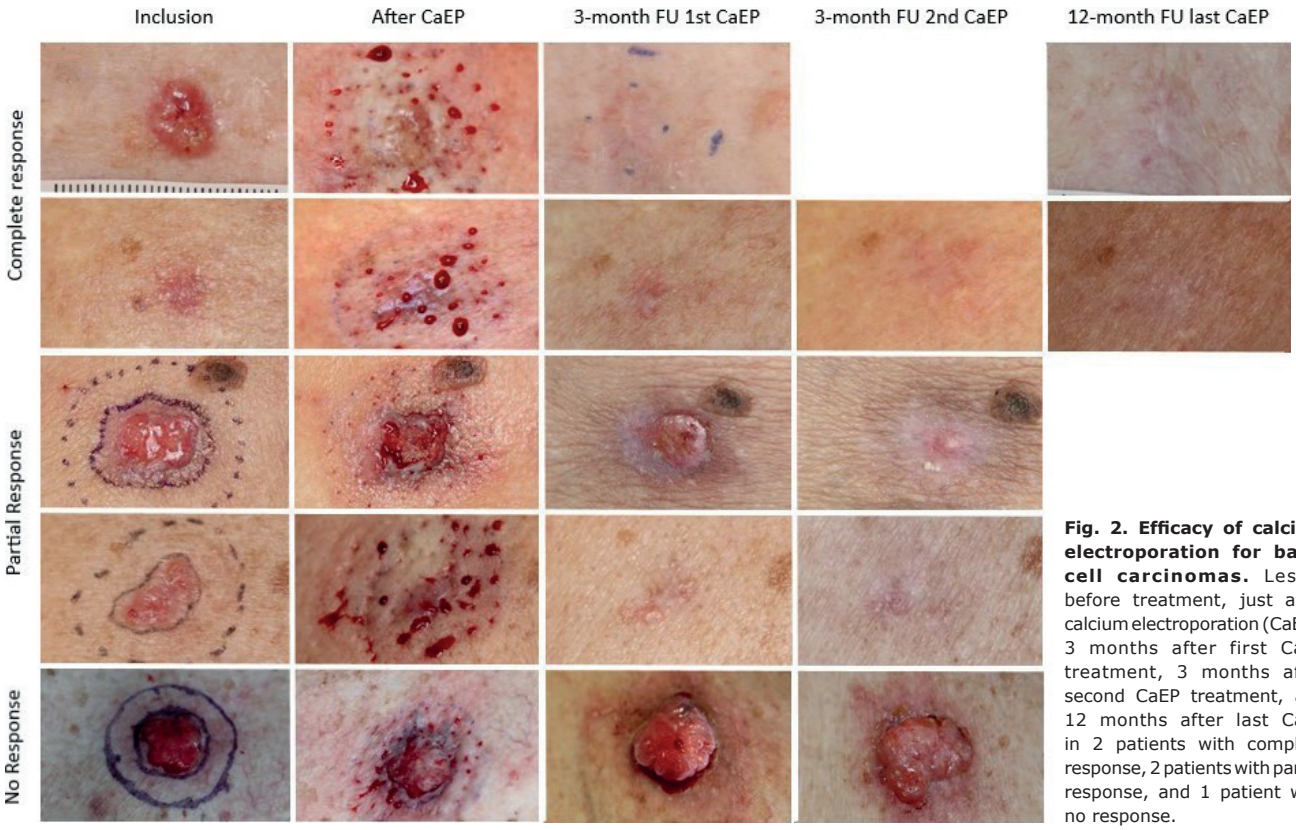

Fig. 2. Efficacy of calcium electroporation for basal cell carcinomas. Lesion before treatment, just after calcium electroporation (CaEP), 3 months after first CaEP treatment, 3 months after second CaEP treatment, and 12 months after last CaEP in 2 patients with complete response, 2 patients with partial response, and 1 patient with no response.

differences in protein expression of the plasma membrane Ca2+-ATPase (PMCA), which has been shown to be lower in lung, colon, breast, and bladder cancer cell lines compared with normal fibroblast (8). An explanation for low efficacy of CaEP for basal cell carcinoma could be that this slow-growing, minimally invasive tumour is similar to normal cells with a more normal expression of PMCA compared with other cancer cells. This theory is supported by our preliminary in vitro study showing that the expression of pan PMCA is increased 16- to 32-fold in BCC cell lines compared with other cancer cell lines, such as colon and breast cancer.

The expression of pan PMCA varied greatly between the 2 different BCC cell lines, which could contribute to explaining why some BCCs had partial or complete response after CaEP whilst others had no response at all. Further studies should be performed to validate the findings in primary BCC samples in addition to the eva-

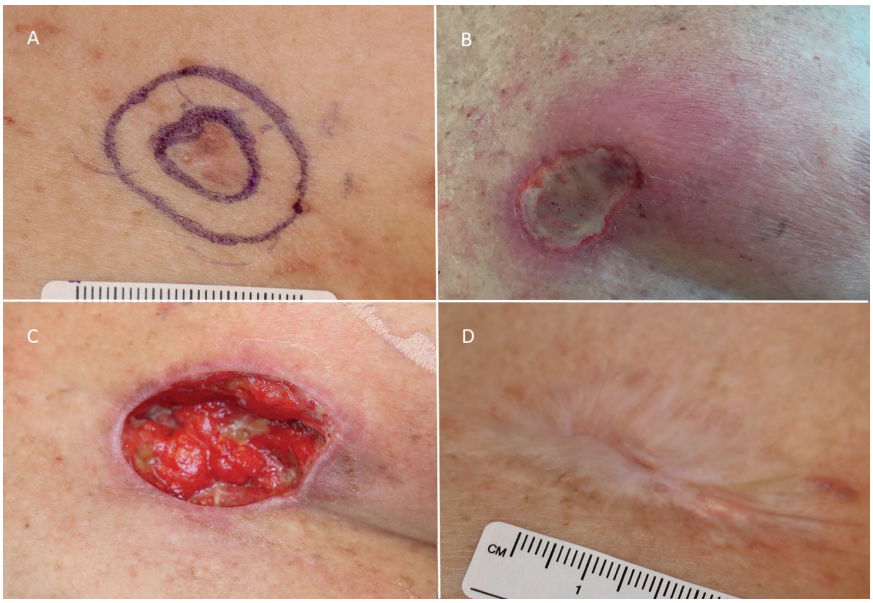

Fig. 3. Severe tissue necrosis after calcium electroporation (CaEP) of a small basal cell carcinoma under the left breast. (A) At inclusion before CaEP. (B) Severe erythema, oedema, induration, and ulceration 7 days after CaEP. (C) Severe ulceration and tissue loss 12 days after CaEP. (D) Ulceration healed within 11 weeks and severe scar formation was seen at 12 months' follow-up.

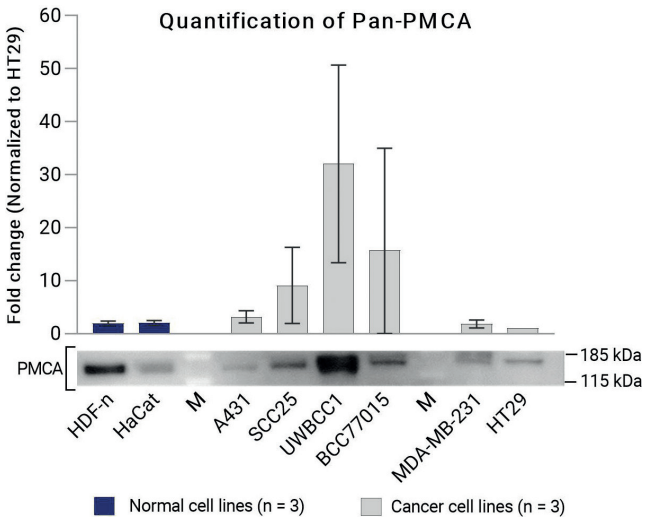

Fig. 4. Pan plasma membrane ATPase (PMCA) protein expression level in normal and cancer cell lines. Pan PMCA protein expression in HDF-n, HaCat, and 6 cancer cell lines (HT29, MDA-MB-231, A431, SCC25, UWBCC1, and BCC77015) measured by Western blotting. The PMCA markers are titled M (from 185 to 115 kDa). Mean ± standard deviation, $n = 3$ for all cell lines.

luation of levels of different PMCA isoforms in cultured BCC cell lines.

The ePore electroporator uses short bipolar squared wave pulses to minimize muscle contraction, making it possible to treat patients in local instead of general anaesthetics. After the poor responses in the first 13 patients, we changed the setting of the ePore electroporation to longer unipolar squared wave pulses (ESOPE settings), which resembles the pulses generated by the Cliniporator. However, this setting did not improve the efficacy of the treatment. Only 2 papers using the ePore electroporator have been published to date, reporting single cases of ECT for gynaecological and colorectal cancer, respectively (20, 21). As such, studies of ECT for skin cancer have all used the Cliniporator. Randomized clinical studies comparing high- and low-frequency electroporation using different certified electroporators are needed.

Contrary to previous studies of ECT for BCC, we used dermoscopy and OCT-guided biopsies to evaluate treatment response and recurrences. This enabled us to recognise very small remaining or recurrent tumours, which might have negatively affected our results (22). However, in the study by Clover et al. (19), treated tumours were followed for 5 years, which should make up for potentially missed remaining or recurrent tumours, as tumours are expected to grow in size and visibility within this time.

We found that high-frequency electroporation was less painful than low-frequency electroporation. Pain depended on localization of the treatment, with treatment on the arm resulting in muscle contraction and pain. In addition, the use of carbocaine as local anaesthetic seemed to be preferable to lidocaine, as the use of lidocaine increased pain during electroporation substantially.

One patient with a small BCC under the breast developed severe necrosis exceeding the treatment area by several centimetres. Several cases have been published of iatrogenic skin and tissue necrosis after calcium chloride has extravasated into soft tissue during intravenous administration (23). In a few cases, even topical exposure to calcium salts has resulted in skin necrosis (24). In our patient, the severe and large ulceration healed within 11 months after careful wound care leaving a large scar under the breast. No skin biopsies were taken, but skin necrosis and prolonged ulceration after calcium chloride infusion is usually caused by tissue calcification (calcinosis cutis) (25). This serious side effect has not previously been reported after CaEP and was not seen in other patients during the study.

In conclusion, calcium electroporation for low-risk basal cell carcinomas does not fulfil the requirements of a new dermatological BCC treatment, which needs to be effective, cheap, well tolerated, and resulting in excellent cosmesis. However, with a response in more than 50% of the patients, CaEP may be useful as an adjuvant treatment to surgery in more advanced basal cell carcinomas.

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IRB approval status: The protocol was approved by the Danish Medicine Agency (EudraCT 2019-002730-36), the Regional Ethics Committee of Region Hovedstaden (H-19042561), and the Danish Data Protection Agency. The study is listed at clinicaltrials.gov (NCT05046262).

Conflict of interest statement: JG is co-inventor of a patent entitled "Therapeutic applications of calcium electroporation to effectively induce tumour necrosis". MH has received equipment and a research grant from Mirai Medical. SRW, KH, CSKF, MV, SWB, JTT and GBEJ report no conflicts of interest.

REFERENCES

1. Dessinioti C, Antoniou C, Katsambas A, Stratigos AJ. Basal cell carcinoma: what's new under the sun. Photochem Photobiol 2010; 86: 481–491.
2. Verkouteren JAC, Ramdas KHR, Wakkee M, Nijsten T. Epidemiology of basal cell carcinoma: scholarly review. Br J Dermatol 2017; 177: 359–372.
3. Peris K, Fargnoli MC, Garbe C, Kaufmann R, Bastholt L, Seguin NB, et al. Diagnosis and treatment of basal cell carcinoma: European consensus-based interdisciplinary guidelines. Eur J Cancer 2019; 118: 10–34.
4. Gehl J. Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research. Acta Physiol Scand 2003; 177: 437–447.
5. Clover AJP, de Terlizzi F, Bertino G, Curatolo P, Odili J, Campana LG, et al. Electrochemotherapy in the treatment of cutaneous malignancy: outcomes and subgroup analysis from the cumulative results from the pan-European International Network for Sharing Practice in Electrochemotherapy database for 2482 lesions in 987 patients (2008–2019). Eur

J Cancer 2020; 138: 30–40.
6. Frandsen SK, Vissing M, Gehl J. A comprehensive review of calcium electroporation: a novel cancer treatment modality. Cancers 2020; 12: 290.
7. Frandsen SK, Gissel H, Hojman P, Tramm T, Eriksen J, Gehl J. Direct therapeutic applications of calcium electroporation to effectively induce tumor necrosis. Cancer Res 2012; 72: 1336–1341.
8. Frandsen SK, Krüger MB, Mangalanathan UM, Tramm T, Mahmood F, Novak I, et al. Normal and malignant cells exhibit differential responses to calcium electroporation. Cancer Res 2017; 77: 4389–4401.
9. Falk H, Matthiessen LW, Wooler G, Gehl J. Calcium electroporation for treatment of cutaneous metastasis: a randomized double-blinded phase II study, comparing the effect of calcium electroporation with electrochemotherapy. Acta Oncol 2018; 57: 311–319.
10. Ágoston D, Baltás E, Ócsai H, Rátkai S, Lázár PG, Korom I, et al. Evaluation of calcium electroporation for the treatment of cutaneous metastases: a double blinded randomised controlled phase II trial. Cancers 2020; 12: 179.
11. Vissing M, Pervan M, Ploen J, Schnefeldt M, Rafaelsen SR, Jensen LH, et al. Calcium electroporation in cutaneous metastases: a non-randomised phase II multicentre clinical trial. Eur J Surg Oncol 2023; 49: 106925.
12. Plaschke CC, Gehl J, Johannesen HH, Fischer BM, Kjaer A, Lomholt AF, et al. Calcium electroporation for recurrent head and neck cancer: a clinical phase I study. Laryngoscope Investig Otolaryngol 2019; 4: 49–56.
13. Egeland C, Baeksgaard L, Gehl J, Gögenur I, Achiam MP. Palliative treatment of esophageal cancer using calcium electroporation. Cancers 2022; 14: 5283.
14. Broholm M, Vogelsang R, Bulut M, Stigaard T, Falk H, Frandsen S, et al. Endoscopic calcium electroporation for colorectal cancer: a phase I study. Endosc Int Open 2023; 11: E451–459.
15. Gibot L, Montigny A, Baaziz H, Fourquaux I, Audebert M, Rols M-P. Calcium delivery by electroporation induces in vitro cell death through mitochondrial dysfunction without DNA damages. Cancers 2020; 12: 425.
16. Noubissi FK, Kim T, Kawahara TN, Aughenbaugh WD, Berg E, Longley BJ, et al. Role of CRD-BP in the growth of human basal cell carcinoma cells. J Invest Dermatol 2014; 134: 1718–1724.
17. Olesen UH, Bojesen S, Gehl J, Haedersdal M. Anticancer drugs and the regulation of Hedgehog genes GLI1 and PTCH1, a comparative study in nonmelanoma skin cancer cell lines. Anticancer Drugs 2017; 28: 1106–1117.
18. Hendel K, Jemec GBE, Haedersdal M, Wiegell SR. Electrochemotherapy with bleomycin for basal cell carcinomas: a systemic review. J Eur Acad Dermatol Venereol 2021; 35: 2208–2215.
19. Clover AJP, Salwa SP, Bourke MG, McKiernan J, Forde PF, O'Sullivan ST, et al. Electrochemotherapy for the treatment of primary basal cell carcinoma: a randomised control trial comparing electrochemotherapy and surgery with five year follow up. Eur J Surg Oncol 2020; 46: 847–854.
20. Ahmed-Salim Y, Saso S, Meehan HE, Galazis N, Phelps DL, Jones BP et al. A novel application of calcium electroporation to cutaneous manifestations of gynaecological cancer. Eur J Gynaecol Oncol 2021; 42: 662–672.
21. Hansen HF, Bourke M, Stigaard T, Clover J, Buckley M, O'Riordain M, et al. Electrochemotherapy for colorectal cancer using endoscopic electroporation: a phase 1 clinical study. Endosc Int Open 2020; 8: E124–E132.
22. Ulrich M, von Braunmuehl T, Kurzen H, Dirschka T, Kellner C, Sattler E, et al. The sensitivity and specificity of optical coherence tomography for the assisted diagnosis of nonpigmented basal cell carcinoma: an observational study. Br J Dermatol 2015; 173: 428–435.
23. Lin C-Y, Hsieh K-C, Yeh MC, Sheen-Chen S-M, Chou F-F. Skin necrosis after intravenous calcium chloride administration as a complication of parathyroidectomy for secondary hyperparathyroidism: report of four cases. Surg Today 2007; 37: 778–781.
24. Kim MP, Raho VJ, Mak J, Kaynar AM. Skin and soft tissue necrosis from calcium chloride in a deicer. J Emergency Med 2007; 32: 41–44.
25. Taogoshi T, Shibata Y, Uno H, Yokooji T, Tanaka M, Hide M, et al. Classification of skin injury risk caused by extravasation of electrolyte solutions or infusions in a rat model. Biol Pharm Bull 2022; 45: 1254–1258.