Calcium Electroporation for Management of Cutaneous Metastases in HER2-Positive Breast Cancer: A Case Report

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Abstract
We report a case of successful treatment of cutaneous metastases in HER2-positive breast cancer with calcium electroporation (CaEP), in addition to trastuzumab, over a period of 5 years. CaEP is performed in local or general anesthesia, by injecting calcium chloride intratumorally and then electroporating cells in the area. Using a handheld needle electrode, a series of short, high-voltage electric pulses are delivered, which transiently permeabilizes cell membranes, causing toxic intracellular calcium levels. The treatment causes cancer cell death, while normal cells are less affected, making the treatment useful for local management of cutaneous lesions. This case presents a 66-year-old female, who had mastectomy surgery followed by adjuvant chemo- and radiotherapy for an ER-negative, HER2-positive breast cancer on her right side in 2003, and a mastectomy followed by endocrine therapy for an ER-positive, HER2-normal breast cancer on her left side in 2006. In 2015, the patient presented local cutaneous recurrence of the ER-negative, HER2-positive breast cancer. The patient was treated with trastuzumab alone, trastuzumab emtansine (TDM1), and a combination of trastuzumab and CaEP. TDM1 was found to have a slightly better effect on the cutaneous metastases than trastuzumab, but the side effects of TDM1 were not acceptable to the patient. The combination of continuous HER2-inhibition and intermittent CaEP, when needed, has been effective in keeping the cutaneous metastases under control for 5 years, and presumably more tolerable.
for the patient than chemotherapy. An interesting finding was local sparing of calcium electroporated skin from new recurrences, otherwise seen in the general area, which could be a sign of local immunity. This warrants further studies investigating local immunomodulation following CaEP. The patient reported appreciation of a treatment option without chemotherapy, and satisfaction with the outcome of the combination of HER2 inhibition and CaEP treatment. CaEP treatment is currently phase II treatment, and mechanisms and possible applications still need investigation. This novel anticancer treatment could potentially benefit many patients, due to its efficacy, low cost, and accessibility. This case provides observations, which may inspire future trials with CaEP for skin metastases of HER2-positive breast cancer.

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Introduction

Cutaneous Metastases from Breast Cancer – The Clinical Challenge

Breast cancer mortality is declining, partly due to earlier detection and partly due to improved treatment options [1]. Medical therapies, in the form of chemotherapy, endocrine therapy, and targeted agents, are often used either separately or combined, in the effort to keep systemic disease under control [2]. However, development of cutaneous metastases or locoregional recurrence in patients with breast cancer, even when antineoplastic treatment can control internal disease, is a relatively common phenomenon. A possible explanation could be lower drug concentration in the skin, especially in an area previously treated with radiotherapy, as radiation is known to cause hypoperfusion [3]. Breast cancer is more prone to metastasize to the skin than other cancers, and local treatments are often more efficient for management of cutaneous metastases than systemic treatments [4, 5]. The incidence of cutaneous metastases from breast cancer ranges from about 5% to as much as 30% in late-stage disease [4, 6]. As such, cutaneous metastases often occur when distant metastases are widely spread in internal organs, and can be associated with poor survival rates [6].

Calcium Electroporation for Cutaneous Metastases

Treatment with calcium electroporation (CaEP) is a novel anticancer treatment for malignant cutaneous and subcutaneous tumors – both primary tumors and metastases [7, 8]. In local anesthesia (or general anesthesia if indicated), calcium chloride is injected intratumorally (with a margin of normal tissue), which causes a high extracellular concentration of calcium. Immediately after injection, the area is electroporated with a handheld needle electrode inserted through the tumor tissue. Once inserted, a series of high-voltage electric pulses are sequentially applied (shown in Fig. 1) [9, 10].

The cell membranes in the electroporated area transiently permeabilize, which allows diffusion of calcium ions into the cell. The cell membrane will reseal shortly after, which traps the calcium inside the cytosol and creates a toxic intracellular calcium concentration [9]. In normal cells, homeostasis is restored as calcium is chelated, compartmentalized into mitochondria and endoplasmic reticulum, or extruded through ATPases and exchangers [11]. In cancer cells, mutations may alter these mechanisms, and calcium regulation becomes less efficient, whereby the toxic intracellular calcium levels may induce cell death [12]. As normal cells are less sensitive to CaEP than cancer cells, the treatment can spare healthy tissues around treated tumors [12]. Previous clinical studies that have investigated CaEP treatment of cutaneous metastases from breast cancer (Table 1) have shown that CaEP is a feasible, effective, and safe treatment option with only mild adverse events.
In one study, 6 breast cancer patients and a single patient with melanoma were treated. The patients had an objective response rate of 72% and the study proved CaEP non-inferior to electrochemotherapy (ECT) after 6 months of follow-up [8]. Another study, that included 1 breast cancer patient and 6 melanoma patients showed an objective response rate of 33%, with CaEP non-inferior to ECT after 1-year follow-up [13].
Case Presentation

This paper describes a case of recurring breast cancer metastases managed with intermittent CaEP treatments. A 66-year-old woman presented with an ER-negative and HER2-positive breast cancer in her right breast and lymph node metastases in 2003. The patient had a mastectomy on the right side with radical axillary lymph node dissection, followed by radiation therapy and chemotherapy (Fig. 2 provides a treatment overview). A tumor was discovered in the patient’s left breast in 2006, with an ER-positive and HER2 normal phenotype, which was managed with mastectomy and endocrine therapy. Disseminated disease was uncovered during routine follow-up in 2010. Biopsies verified a metastatic tumor in the periclavicular connective tissues, and an axillary lymph node metastasis with ER-negative and HER2-positive phenotypes, compatible with the initial tumor of the right breast. Standard treatment with chemotherapy and HER2-targeted antibodies (vinorelbine and trastuzumab) was initiated.

After 11 months of chemotherapy, the metastatic tumors had fully remitted, and the treating oncologist discontinued vinorelbine, while treatment with trastuzumab was continued. Heart failure is a well-known side effect of trastuzumab [14], and multigated acquisition scans were performed regularly. In 2015, her left ventricular ejection fraction (LVEF) had dropped to 45%, and trastuzumab treatment was paused.

A couple of months later, the patient presented with a pale red rash around the mastectomy scar on the right side. Although clinically described as nonsuspicious, a biopsy proved cutaneous metastases, concordant with the primary ER-negative and HER2-positive cancer of the right breast. The patient started treatment with trastuzumab emtansine (TDM1).

After 10 weeks of TDM1 treatment, the metastatic area on the chest had fully remitted, and it was recommended to continue treatment with a lower dose due to side effects, including fatigue and nausea. However, the patient decided to end the TDM1 treatment due to side effects.

A recurrence reappeared in the area after 5 months without treatment. The patient did not wish to resume chemotherapy, but agreed to resume trastuzumab treatment. Trastuzumab induced remission of the cutaneous recurrence, and over the course of the next 6 months, the appearance waxed and waned following trastuzumab treatments.

In 2016, the patient was referred to receive ECT due to the presence of cutaneous lesions. However, there was an ongoing study using CaEP for cutaneous metastases with promising results (Falk et al. [8]; see Table 1), and the patient requested CaEP instead of ECT and was treated outside protocol (CaEP-1). The target area measured 5.5 × 5.5 cm and was situated...
on the upper medial quadrant of the right chest (Fig. 3). After 11 weeks, the initial crusted wound had healed leaving a scar with no sign of residual tumor. The patient received trastuzumab treatment concurrently without side effects.

In 2018, new metastases appeared adjacent to the area treated with CaEP. The patient requested retreatment with CaEP for symptom relief from pruritus. The patient initially requested CaEP in order to avoid chemotherapy. As the patient had received CaEP once, standard treatment ECT was agreed upon with the patient, to see if this would give a longer response. The patient received ECT on four skin metastases. The metastases partly remitted, but the itching sensation persisted, and eventually, there was progression. Trastuzumab was paused concurrently due to a low LVEF. Since the cutaneous metastases kept growing, the patient resumed trastuzumab after a month, despite a declining LVEF. The patient never had clinical symptoms of heart failure, but began prophylactic medication with an angiotensin-II receptor-blocker and beta-blocker.

Four months after ECT, the patient was retreated with CaEP on part of the affected area (CaEP-2), upon her request. A larger area on the chest was treated two months later in general anesthesia (CaEP-3), covering an area of 6 × 8 cm (Fig. 4). In the meantime, the patient...
switched from trastuzumab treatment to TDM1, due to progression in the skin. Plastic surgeons offered the patient surgical removal and reconstruction of the affected skin, but she declined.

After 2 months of TDM1 therapy, oncocardiologists recommended a dose reduction, due to declining LVEF. Instead, the patient requested substitution of TDM1 with trastuzumab due to side effects.

In 2019, cutaneous metastases reappeared, now involving the first area treated with CaEP, otherwise without recurrence for 3 years (Fig. 4). After a few months of observation, the area was retreated with CaEP with complete response (CaEP-4). Recurrence emerged after 6 months, and the area was retreated (CaEP-5). After about 6 months, recurrence appeared just outside the previously treated area. Shortly after, a routine follow-up CT scan showed a suspicious lymph node in the left lung, and the patient resumed TDM1 therapy (subsequently reduced to a 60% dose, due to side effects with fatigue and local infections). After observation for 6 months, the nonresponsive skin recurrence was treated with CaEP twice in late 2020 (CaEP-6 and CaEP-7), with complete response and pruritus relief. The patient had to reduce her TDM1 treatment dose further to 50%, due to severe fatigue and bleeding from the mucosa.

In August 2021, a more nodular skin recurrence was treated (CaEP-8). The patient discontinued TDM1 after 18 series, because of side effects and continuous development of cutaneous metastases. As the metastases were now her only sign of residual disease, she went back to receiving trastuzumab. One of the smaller cutaneous elements kept presenting with itching, which the patient recognized as metastatic activity, and the single tumor was therefore treated again shortly after (CaEP-9). As of September 2021, after 5 years with a combination treatment of continuous HER2 inhibition and CaEP when needed, the patient had no other manifestation of breast cancer disease, than recurrent skin metastases (Fig. 3).

**Calcium Electroporation – The Procedure**

The CaEP treatments were performed in local anesthesia using a Cliniporator pulse generator (IGEA, Italy), which delivers eight 0.1 ms pulses with an amplitude of 1 kV/cm and a frequency of 1 Hz (CaEP-3 was performed under general anesthesia, due to the size of the target area, and discomfort during the previous treatment). Calcium chloride 220 mmol/L was injected intratumorally, and linear electrodes were used for pulse application. A schematic overview of the procedure and a response to treatment are shown in Figures 1 and 4, respectively.

The ECT treatment was performed in local anesthesia using the Cliniporator pulse generator. A total of 1.8 mL bleomycin 1,000 IU was injected intratumorally and a linear electrode was used for pulse application.

**Patient Experience**

The patient claimed to appreciate a treatment option without chemotherapy. The patient described the CaEP treatment as mild and tolerable. She experienced slight discomfort during pulse delivery, and some soreness lasting a day after treatment, that resembled muscle pain experienced after exercising. The symptoms that followed CaEP and ECT treatment were similar. The patient expressed preference of CaEP treatment for her cutaneous disease over ECT, as the ECT did not relieve pruritus, and she experienced that remission from ECT had shorter duration. She felt comfortable monitoring progression of the disease, and requesting treatment when needed. The patient would recommend CaEP to others in a similar situation.
Discussion

Cutaneous metastases can be distressing for patients, causing symptoms such as pruritus, pain, and often ulceration of the skin [10, 15]. Sometimes, progression in skin is treated with systemic agents, which target known immune markers such as HER2.

Researchers have previously hypothesized that TDM1 treatment is more effective than trastuzumab alone because the combination of trastuzumab and chemotherapy agents works through different mechanisms, thereby enhancing responses [5]. When treating metastases from HER2-positive breast cancers, CaEP could induce a local response, potentially enhanced when combined with trastuzumab.

Despite TDM1 being slightly more efficient than trastuzumab alone, TDM1 side effects were unacceptable to this patient. The cutaneous metastases have been successfully managed for 5 years with a combination of trastuzumab and CaEP, indicating that this could be an efficient treatment for metastases of HER2-positive breast cancer.

The long-term effects of CaEP are results of the toxic levels of cytoplasmic calcium inducing necrosis in the cancer cells, mainly in correlation with ATP depletion. A preclinical study has shown that CaEP has antivascular effects, which also play a part in the overall anti-tumor response [7, 16]. In the two clinical studies, with 6- and 12-month follow-up, respectively, high response rates were seen, warranting studies with a longer follow-up period, both to investigate recurrence rate and duration of response, as well as to investigate the cumulative effect of successive CaEP treatments.

Studies have suggested that CaEP can lead to an immune response [8, 17]. In this case, we observed that a previously treated area stayed clear of recurrence, even when new metastases arose outside the treated area. In a preclinical trial from 2017, mice were treated with CaEP and then rechallenged with either the same tumor cell type or a different tumor cell type, showing protective immunity toward the same tumor cell type, as well as an increase in immune-specific proteins and proinflammatory cytokines [17].

A clinical study from 2018 showed an indication of a possible systemic immune response, as a patient with melanoma had complete remission of both treated and untreated tumors after CaEP [8]. Possible immune responses related to CaEP are described in a few cases, yet the specific mechanisms are unknown. Future studies should further investigate possible effects of CaEP on both local and systemic immunity.

As ECT is currently a standard therapy for cutaneous metastases, CaEP builds on experience with ECT. CaEP is easily accessible, as most facilities using ECT already have the necessary equipment. Calcium is readily available at low costs, does not require the same authorization for handling as chemotherapeutic agents as it is not cytotoxic by itself, and has been shown to be a safe and efficient treatment, with few side effects [7, 8, 10]. This patient expressed appreciation for a cancer treatment without chemotherapy and was satisfied with the outcome of CaEP combined with HER2-antibody treatment.

This case supports CaEP as an effective and tolerable treatment for patients with cutaneous metastases, specifically metastases of HER2-positive breast cancer. CaEP may have a potential synergistic effect when combined with other treatments.

Statement of Ethics

The patient gave written informed consent to publication of medical history and anonymized data, including clinical images. Ethical approval was not required for this case report, in accordance with local and national guidelines.
Conflict of Interest Statement

Julie Gehl is a coinventor of a patent regarding calcium electroporation: therapeutic applications of calcium electroporation to effectively induce tumor necrosis (Grant: PCT/DK2012/050496).

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Author Contributions

Kathrine Borres Jensen was the primary author of the manuscript. Camilla Kjaer Lonkvist, Julie Gehl, and Mille Vissing contributed to the manuscript. Kathrine Borres Jensen, Camilla Kjaer Lonkvist, Julie Gehl, and Mille Vissing read and approved the final manuscript.

Data Availability Statement

Data extracted from the patient journal and the interview material are not publicly available on legal or ethical grounds. Access to the data requires patient consent. Further enquiries can be directed to the corresponding author.

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