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Calcium Electroporation for Recurrent Head and Neck Cancer: A Clinical Phase I Study

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Background: Calcium electroporation is a novel cancer treatment, which combines temporary cell permeability from electroporation with a high influx of calcium intracellularly resulting in cancer cell necrosis.

Methods: A phase I trial performing calcium electroporation on 6 patients suffering from recurrent head and neck cancer. In general anesthesia, intratumoral calcium injections were followed by electroporation. Safety was monitored by adverse events registration, serum Ca^{2+}, ECG, and pain scores. Tumor response was measured on PET/MRI scans.

Results: Procedures were performed without complications. No serious adverse events, signs of hypercalcemia, or cardiac arrhythmias were observed. Two months post-treatment tumor responses on MRI: three partial responses, one stable disease, and two progression. Responses on PET: one partial metabolic disease, four with stable metabolic disease, and one not evaluable. One patient was without clinical evidence of disease after 12 months of observation.

Conclusion: Calcium electroporation is feasible and safe in head and neck tumors. Clinical responses were observed in three of six patients, warranting further studies.

Key Words: Electroporation, calcium, head and neck cancer, PET/MRI imaging, phase I trial.

Level of Evidence: Level 4

INTRODUCTION

Calcium electroporation is a new cancer treatment that causes cell necrosis by inducing a high influx of calcium intracellularly.[1] In electroporation, short electric pulses applied to the cell are creating a temporary permeability in the cell membrane, during which molecules in the extracellular compartment can diffuse into the cell cytosol.[2–5]

Currently, electroporation is clinically used in combination with chemotherapy, termed electrochemotherapy (ECT). ECT is mainly used on skin tumors and metastases[6–9] but several studies[10–13] including a recently published multi-institutional trial[14] have shown that the treatment also can be applied on mucosal head and neck cancers with good tumor response.

Ionized calcium (Ca^{2+}) functions as an intracellular signaling molecule involved in numerous processes such as apoptosis, muscle contraction, gene transcription, metabolism, etc.[15] Normal cells will have a low intracellular Ca^{2+} concentration of 10^{-7} mol/L, whereas the high extracellular concentration will be around 10^{-3} mol/L.[15,16] This low intracellular Ca^{2+} concentration is mandatory for the signaling process, consequently, a rise in intracellular concentration can be cell toxic.[17] When combining calcium with electroporation the result is a rapid rise in intracellular Ca^{2+} concentration, which is associated with acute and severe ATP depletion.[1,18] Primarily, an influx of Ca^{2+} activates the calcium-ATPase. Secondly, Ca^{2+} is being absorbed into the mitochondria, which can lead to a lowering of the electrochemical gradient of the mitochondrial membrane and to mitochondrial collapse.[1,18] The overall ATP depletion will contribute to necrosis of the cell. Calcium electroporation as well as nanosecond pulsed electric fields can induce intracellular calcium overload leading to necrosis[19] and it has been shown that the increased intracellular calcium concentration change the expression level of different calcium transporters as well as change the cytoskeleton structure.[20]

Trials using calcium electroporation both in vivo, in vitro, and in clinical trials have demonstrated cell necrosis on different tumor histologies.[1,18,21,22] Additionally, recent studies testing calcium electroporation on different tumor lines against normal cells showed an increased effect on tumor cells indicating that normal

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tissue surrounding the tumor would be less affected by the treatment.[20,23–26] A new clinical trial randomizing calcium electroporation against ECT on small skin metastases has shown calcium electroporation to have a similar objective response rate compared to ECT with only limited side effects.[21]

Based on our previous experience with ECT on mucosal head and neck cancers[14] and the current knowledge on calcium electroporation, we wanted to test the safety of using calcium electroporation on mucosal, head and neck cancers. The use of calcium instead of a chemotherapeutic agent would offer advantages, in particular for readiness of use and simplicity of treatment.

METHODS

This is a phase I, observational, non-comparative trial for the safety of calcium electroporation on mucosal head and neck tumors. ClinicalTrials.gov identifier: NCT03051269. EudraCT no. 2015-005050-35. The trial was approved by the Regional Ethics Committee, the Danish Medicines Agency and the Danish Data Protection Agency. It was externally monitored by the Good Clinical Practice unit at the University of Copenhagen. Treatment was intended as palliative and the study was pre-planned to include one treatment cohort of six patients. The study protocol included subjects with head and neck cancer with either: 1) recurrence after surgery and radiation, 2) subjects who would potentially badly tolerate major surgery, 3) if primary surgery would cause severe disfiguration or loss of function, or 4) if the subjects poorly tolerated or did not wish to receive (further) palliative chemotherapy.

All subjects should have been offered the standard treatment according to DAHANCA guidelines (Danish Head and Neck Cancer Group).[27] Subjects should have an understanding of the trial, written informed consent was mandatory and they had to meet all inclusion and exclusion criteria (Table I).

Primary outcome was evaluation of the safety measures of using calcium electroporation on mucosal head and neck cancer. This was done by registration of side effects by CTCAE (Common Terminology Criteria for Adverse Events) into Adverse Events (AE) and Serious Adverse Events (SAE), by measurement of se-Ca^{2+} in blood samples after treatment and continuously evaluating pain by NRS (numeric rating scale).[28]

Secondary outcomes were evaluation of response 2 months post-treatment by ^{18}F-FDG-PET/MRI imaging (^{18}F-flourodeoxyglucosis positron emission tomography/magnetic resonance imaging), clinical photography and biopsies from the tumor site. The subjects' evaluation of the treatment and post-treatment period was performed through Quality-of-life questionnaires, EORTC (European Organization of Research and Treatment of Cancer) QLQ-C30 and H&N35: two different questionnaires both validated for head and neck cancer patients.[29]

Calcium Dose

Tumor volume was calculated by the following formula: $V = ab^2\pi \,/\, 6$ (a = the longest tumor diameter in cm; b = the perpendicular diameter to a). Tumor volume was measured and calculated from a PET/MRI baseline scan by a radiologist and controlled by one of the investigators.

The calcium dose in this trial was chosen to be similar as in the trial testing calcium electroporation on small cutaneous tumors.[21] We used calcium chloride (Amgros, Denmark) with a concentration of 0.5 mmol/ml or 20 mg/ml as stock solution. The calcium chloride was dispensed in an isotonic NaCl solution resulting in a calcium dose of 0.225 mmol/ml or 9 mg/ml. The dose to tumor volume ratio was calculated according to the ESOPE guidelines (European Standard Operating Procedure of Electrochemotherapy)[30]:

- Tumor <0.5 cm^3 need 1 ml/cm^3 tumor tissue
- Tumor from 0.5 cm^3 to 1 cm^3 should be 0.5 ml/cm^3 of tumor tissue
- Tumor >1 cm^3 should be 0.25 ml/cm^3 of tumor tissue

It was important that the entire tumor volume with surrounding tissue would be treated. However, it was not possible to predict the injected volume with exact precision due to spill from the mucosa. Consequently, when treating with calcium chloride, the tumor would be

TABLE I.
Inclusion and Exclusion Criteria.

Inclusion criteria	Exclusion Criteria
1. Age > 18 years.	1. Symptomatic progression of the subject's cancer disease that requires another intervention.
2. Verified cancer in the head and neck region of any histology.	
3. At least one tumor lesion accessible for electroporation.	
4. Performance status WHO ≤2.	2. Allergy to constituents of the planned anesthesia.
5. Progressive and/or metastatic disease.	3. Coagulation disorder that cannot be corrected.
6. Expected survival of >3 months.	4. Chronic renal dysfunction with creatinine >200 mmol/L will trigger a Cr-51-EDTA clearance.
7. A treatment-free interval of >4 weeks.	5. Pregnancy or lactation.
8. The subject should have been offered all curative treatment options. If there are no further curative treatments to offer or if the subject declines the treatments offered, the subject may be included in the trial.	6. If participating in other clinical trials involving experimental drugs or involved in a trial within 4 weeks prior to study drug administration.
9. The subject should be able to understand the information for participants and comply with the follow up.	7. Other disorders investigator finds incompatible with participation in the trial.
10. Platelets ≥50 billion/L, INR > 1.5. Medical correction is allowed.	
11. Sexually active men and fertile women must use adequate contraception during this trial.	
12. Signed informed consent.	

injected with a minimum volume derived from the calculated tumor volume. To ensure a sufficient treatment of the surrounding tissue as well, the injected volume would be between the calculated calcium chloride volume and the maximum dosage.

As a safety measure, in this study, the maximum dosage was 20 ml (180 mg) of calcium chloride. This was calculated from a desired maximum rise in serum-Ca^{2+} of 0.3 mmol/l extracellular volume in a person weighing 70 kg. The maximum tumor volume became: 20 ml / (0.25 ml/cm^3) = 80 cm^3. A mucosal tumor this large in the head and neck region would most likely not be encountered or included, and we therefore found the dosage of calcium chloride reasonable and safe.

Procedure

Calcium electroporation was performed in general anesthesia with the use of muscular relaxation. The tumor area including a safety margin of 1 cm tissue surrounding the tumor was treated. To ensure a high concentration of calcium in the tissue, the tumor was treated in smaller sections alternating between direct intratumoral injection of calcium chloride (9 mg/ml) and immediately thereafter electroporation, performed in a systematic fashion to cover the treatment area. This technique is similar to electrochemotherapy using intratumoral injection and directly thereafter electroporation, whereas systemic infusion of bleomycin dictates a time-window of 8 minutes to allow distribution of drug from the systemic circulation to the tumor.[30,31] As we injected calcium locally, pulses were administered immediately thereafter.

For electroporation, we used a Cliniporator (model EPS02, IGEA, Carpi, Italy), which delivers a series of eight consecutive pulses of 0.1 msec each with 1 kV/cm and a frequency of 1 Hz or 5000 Hz.[30] The electrodes (IGEA, Carpi, Italy) could be chosen according to the anatomic locations as either a small, flexible finger-electrode with linear array needles (10 mm long) or as hexagonal- or linear-electrodes on a handle (20 or 30 mm long).[31,32]

All patients were treated perioperatively with systemic antibiotics (cefuroxime and metronidazole) to reduce the risk of infections and with hydrocortisone (16 mg dexamethasone administered once) to reduce swelling. Systemic antibiotic treatment was continued for 3 days post-treatment.

Safety Measures

To detect any signs of hypercalcemia, serum ionized calcium (Ca^{2+}) was measured pre-treatment, 30 minutes and 6 hours post-treatment. Also, electrocardiography (ECG) was performed pre-treatment, monitored continuously during treatment, and performed again within the first hours post-treatment to detect possible signs of cardiac arrhythmias.

Hypercalcemia was graded according to CTCAE version 4.03[33].

PET/MRI

^{18}F-FDG-PET/MRI imaging was performed at baseline, 1, and 2 months follow-up on a 3 Tesla Biograph mMR (Siemens Medical Solutions). PET imaging was performed 60 minutes after administration of ^{18}F-FDG (4 MBq/kg), using a single-bed acquisition over 20 minutes, reconstruction with three iterations, 24 subsets using 344 x 344 matrices and a 4-mm Gaussian post-filter and Siemens standard four-compartment-attenuation map. MRI was performed with administration of gadolinium contrast. MRI imaging included T1 (Turbo Spin Echo), T2 (Blade), and Diffusion Weighted Imaging with b-values 0 and 800 (with and without RESOLVE).

PET/MR images were reviewed by an experienced PET/MR radiologist and nuclear medicine physician. Tumor responses were evaluated on the MRI images according to RECIST criteria[34] and on the PET according to PERCIST.[35] Tumor response was evaluated as: 1) tumor size on MRI; 2) SUV_{peak}; and 3) total lesion glycolysis ($TLG_{60\%}$ = MTV*SUV_{mean}) with an isocontour of 60% of SUV_{max}. The biopsy location was correlated to the PET/MRI with measurements of ADC (apparent diffusion coefficient) and FDG-uptake. The latter was measured as maximum and peak standardized uptake value (SUV_{max} and SUV_{peak})[35] normalized to body weight.

Statistics

The usual number of patients for dose finding in a phase I study is six. This study was preplanned to only test a single dose level and therefore tested six subjects. All statistical analyses of the EORTC questionnaires were performed using SPSS.

RESULTS

The study was conducted from January 2016 to September 2017. All six patients were included, treated, and could be evaluated for safety of the procedure. All suffered from recurrent cancer in the oral cavity and all had squamous cell carcinoma except one with epithelial myo-epithelial carcinoma. After tumor response evaluation at 2 months post-treatment, four patients were referred to standard palliative treatment in the form of systemic chemotherapy and one patient (patient 6) stayed for continuous follow-up due to good response. One patient (patient 5) could not be evaluated for tumor response at 2 months post-treatment due to progression and referral to radiotherapy. Patient characteristics as well as details on the treatment are described in Table II.

Safety Results

The injected calcium chloride volume was median 8 ml (72 mg), range 4.8–10 ml. No signs of hypercalcemia were detected, neither in se-Ca^{2+} (Fig. 1), in ECG or in CTCAE registrations.

All ECG's taken preoperatively and post-treatment were without signs of arrhythmia. CTCAE registrations were recorded during hospitalization and in the

TABLE II.
Details on Patients and Treatment.

Patient no.	Age Sex PS	Histology and location	TNM at and time of debut	Previous treatment	Current treatment area	Tumor volume cm^3	Largest tumor diameter (cm)	Calculated min. ml of CaCl	Used ml of CaCl	No. of pulses	Type of electrode	Surgery time (min)
1	70 M 1	SCC Baseos oris	$T_4N_0M_0$ 1999	Surgery x 4 (1999, 2004, 2013, 2015) + radiotherapy x 1 (2004)	Baseos oris	25	4.5	6.25	8	144	Hex	55
2	69 F 1	SCC Tongue base	$T_{4a}N_{2c}M_0$ 2015	Radiotherapy (2015) + TORS (2015) + brachytherapy (2016)	Baseos oris and tongue base	0.3	1.7	0.3	8	40	Hex	19
3	71 M 1	SCC Tongue	$T_4N_0M_0$ 2009	Radiotherapy x 1 (2009), electrochemotherapy and chemotherapy (2016)	Tongue	30	4.2	7.5	10	44	Hex	28
4	86 M 1	SCC Oropharynx	$T_3N_0M_0$ 1991	Radiotherapy x 1 (2015) + surgery x 1 (2016)	Hard palate	8.14	3.8	2.03	4.8	20	Hex	10
5	89 F 2	EMC Gl. Subman-dibularis	$T_4N_0M_0$ 2010	Surgery x 3 (2010, 2014, 2016). Refuses radiotherapy and chemotherapy	Cheek and baseos oris	58.7	5.8	14.7	10	43	Fin + Hex	22
6	61 M 1	SCC Baseos oris	$T_{4b}N_{2c}M_0$ 2016	Radiotherapy x 1 (2016-2017)	Baseos oris	1.04	NE	0.5	5	52	Hex	18

EMC = epithelial-myoepithelial carcinoma; Fin = finger electrode; Hex = hexagonal electrode; PS = performance status; SCC = squamous cell carcinoma; TORS = trans oral robotic surgery.

outpatient clinic follow-up. There were no grade 4 or 5 events. Grade 3 events were recorded in "dysphagia," "oral mucositis," and "pharyngeal mucositis" in the follow-up period. All CTCAE events are shown in Table III.

Pain score was recorded at baseline, during hospitalization and during outpatient follow-up. The results are depicted in Fig. 2. Statistically, this patient group is too small to conclude the impact of the treatment in relation to pain. However, in the first days after treatment the patients generally reported low NRS. In the following 2 months, the NRS score was very much dependent on whether there was a tumor progression or response to treatment with lower NRS.

Tumor Response

The results are described in Table IV. Tumor response on MRI using RECIST criteria v.1.1[34] demonstrated three partial response (PR), one standard disease (SD), and two progression (PD). See Figure 3 for example of PET/MRI.

Patient 6 with PR had no clinical evidence of disease demonstrated in post-treatment biopsies without tumor cells; however, the ulcus at tumor site was still visible at 2 months post-treatment and therefore categorized as a PR (Fig. 4). At 12 months post-treatment the patient was still without clinical evidence of disease. Patient 5 suffered from progression and could not cooperate to further imaging post-treatment.

PET SUV_{peak} demonstrated PMR (partial metabolic response—a decrease of more than 30%) in patient 6. A stable metabolic response (SMR) was seen in the remaining four patients.

$TLG_{60\%}$ decreased in three patients and increased in two patients. The results tended to resemble the tumor size results.

ADC was also measured on MRI, but could only be determined in three patients post-treatment due to metal artefacts disturbing the image quality.

Biopsies were performed post-treatment in all except patient 5. At 1 month post-treatment only one biopsy demonstrated malignant cells. Two months post-treatment three patients had biopsies with malignant cells (Table V).

Mucosal Reaction

The mucosal reaction to calcium electroporation was a temporary local swelling (days) followed by tumor necrosis with shedding of yellow-grey colored dead tissue. After several weeks, the treated lesion healed with new mucosa (Fig. 4).

Quality of Life

Results from EORTC questionnaires are listed in Supplementary Materials 1 and 2. Due to the limited number of patients only descriptive statistics are used. The EORTC QLQ-C30 was affected in "fatigue," "pain,"

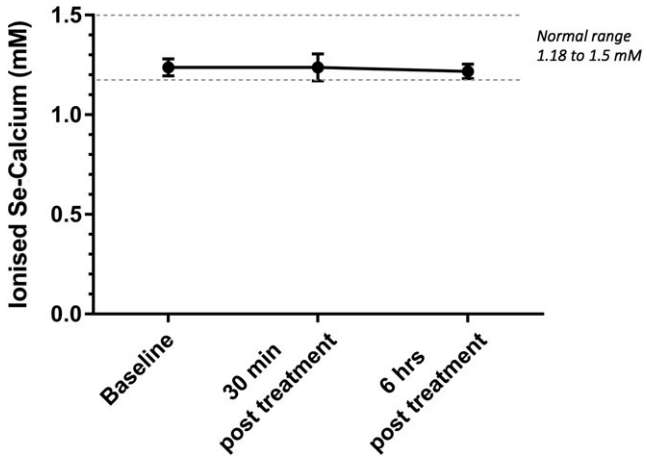

Fig. 1. Diagram showing serum levels of ionized calcium (median and range) taken at baseline, 30 minutes and 6 hours after treatment. The dotted lines depict normal ionized calcium serum range.

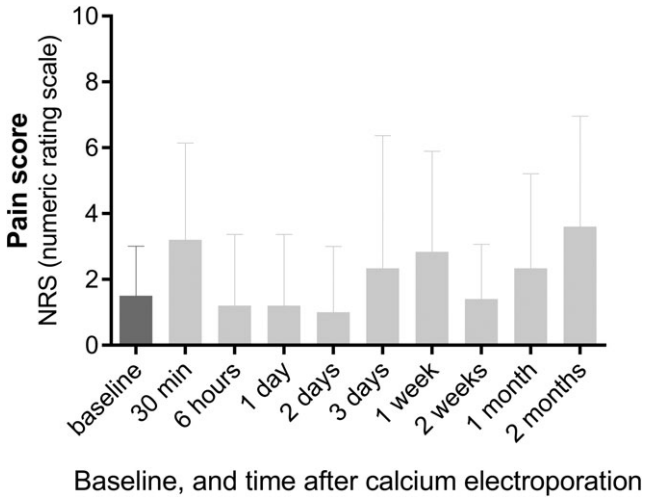

Fig. 2. Pain score diagram depicting NRS as mean with SD before and after treatment.NRS = numeric rating scale; SD = standard deviation.

and "appetite loss," whereas EORTC QLQ-H&N35 was affected in "social eating" and "opening mouth."

DISCUSSION

This is the first clinical study to use calcium electroporation on mucosal tumors within the head and neck region. The primary endpoint regarding safety of the treatment was successfully fulfilled, showing no signs of hypercalcemia, cardiac arrhythmias, or severe adverse events. The treatment was found to be reasonably easy to implement with electroporation already being a used treatment modality in our department and calcium chloride a very affordable agent and straightforward to use.

TABLE III.
Common Terminology Criteria for Adverse Events.

Adverse events Grade 3	Baseline	Follow-up period from day 1 to 2 months post-treatment
Skin ulceration	0/6	0/6
Dysphagia	**3/6**	**4/6**
Blurred vision	0/6	0/6
Tumor pain	0/6	0/6
Trismus	0/6	0/6
Ear pain	0/6	0/6
Oral mucositis	0/6	**1/6**
Laryngeal edema	0/6	0/6
Pharyngeal mucositis	0/6	**1/6**
Fatigue	0/6	0/6
Infection	0/6	0/6
Localized edema (tongue, lip)	0/6	0/6
Vomiting	0/6	0/6
Epistaxis	0/6	0/6

In the follow-up period, all adverse events were registered even though some of the events only lasted one day. The more severe grade 3 events are listed here as no. events/6 patients. There were no grade 4 or 5 events.

Calcium electroporation is a new treatment modality that utilizes a high influx of calcium intracellularly resulting in cell necrosis. The only study available so far is a randomized study on small cutaneous tumors recently published testing ECT against calcium electroporation[21]: metastases were treated with a calcium chloride volume of median 0.24 ml (2.16 mg calcium) (range 0.03–1.21 ml). No severe adverse events were observed and calcium electroporation response was similar to the one in the ECT treated lesions. In comparison, we used a median calcium chloride volume of 8 ml (72 mg calcium). Despite the volume increase, no safety issues were encountered, indeed se-calcium was unaffected (Fig. 1). In our opinion, calcium chloride can therefore safely be injected intratumorally up to a volume of 10 ml, 9 mg/ml, and possibly higher, pending further investigation.

The exact calcium chloride volume was difficult to predefine prior to treatment. Primarily, these heterogenic, pre-irradiated mucosal tumors will display some spilling through the mucosa when injecting a fluid. Secondly, the tumor formula applied ($V = ab^2\pi / 6$) did not include the surrounding 1-cm macroscopic normal tissue, which we in a surgical setting normally would include in the treatment. As a practical solution, we found it more important to cover the entire tumor than to restrict ourselves to a predefined dose.

In our current trial treating head and neck cancer patients with ECT (ClinicalTrials.gov Identifier: NCT02549742), we have found that the best imaging modalities for evaluating tumor response are MRI and PET. MRI depicts the soft tissue and the tumor borders in the head and neck region much clearer than CT, and PET is excellent to display progression or inflammation in the treated lesion. In this trial, we had the opportunity to combine these two imaging modalities and the results support our previous experience. Especially with a treatment like electroporation resulting in necrosis and debris of tumor, the combination of PET and MRI can visualize this process. In the cases, were MRI cannot demonstrate

TABLE IV.
Results from PET/MRI Scan at Baseline, 1, and 2 Months Post-Treatment.

	MRI				PET						
	Largest tumor diameter				SUV_{peak}				$TLG_{60\%}$		
Patient no.	B	1	2	Response RECIST	B	1	2	Response	B	1	2
1	4.5	4.3	5.1 (+13%)	SD	14.1	11.3	10.8 (-23%)	SMD	97	119	130 (+34%)
2	1.7	2.2	2.9 (+71%)	PD	7.6	7.4	7.9 (+4%)	SMD	15	30	41 (+173%)
3	4.2	2.5	2.9 (-31%)	PR	10	8.5	8.2 (-18%)	SMD	62	39	50 (-19%)
4	3.8	1.2	0.5 (-87%)	PR	5.3	4.9	4.9 (-8%)	SMD	28	21	14 (-50%)
5	5.8	ND	ND	PD*	ND	ND	ND	ND	ND	ND	ND
6	NE	NE	NE	PR†	4.2	3.9	2.9 (-31%)	PMR	5	8	4 (-20%)

*Patient no. 5 only had a baseline PET/MRI scan performed. PD by clinical evaluation.
†Patient no. 6: tumor in the floor of the mouth was not visible on MRI. Tumor volume was calculated from the tumor diameters seen by clinical inspection and PR by clinical evaluation.
SUV = standardized uptake values; TLG = total lesion glycolysis; B = Baseline; 1 = 1 month's follow-up; 2 = 2 months' follow-up; ND = not done; NE = not evaluable (not visible due to size or metal artefacts from previous surgery); SD = stable disease (<30% tumor reduction); PR = partial response (≥30% tumor reduction); PD = progression (>20% progression); SMD = stable metabolic disease (<30% metabolic reduction and < 30% metabolic increase); PMR = partial metabolic disease (≥30% metabolic reduction).

the tumor due to size or metal artefacts, FDG-PET can still present the tumor and measure the FDG uptake.

The overall appearance of the post-treatment mucosal reaction to calcium electroporation with swelling and necrosis was similar to the one shown with electrochemotherapy.[10,12] Regarding tumor response, a recent trial using electrochemotherapy on 43 patients showed an objective response rate (CR + PR) of 56% with complete response in 19%.[14] Currently, we cannot compare tumor responses between these two electroporation modalities since the calcium data is based on only six patients.

This first phase I study shows that calcium electroporation in head and neck cancer is safe, and interestingly shows objective responses in three of six patients. The sequel to this study must be larger phase II trials, to better estimate tumor response rates, and the side effects

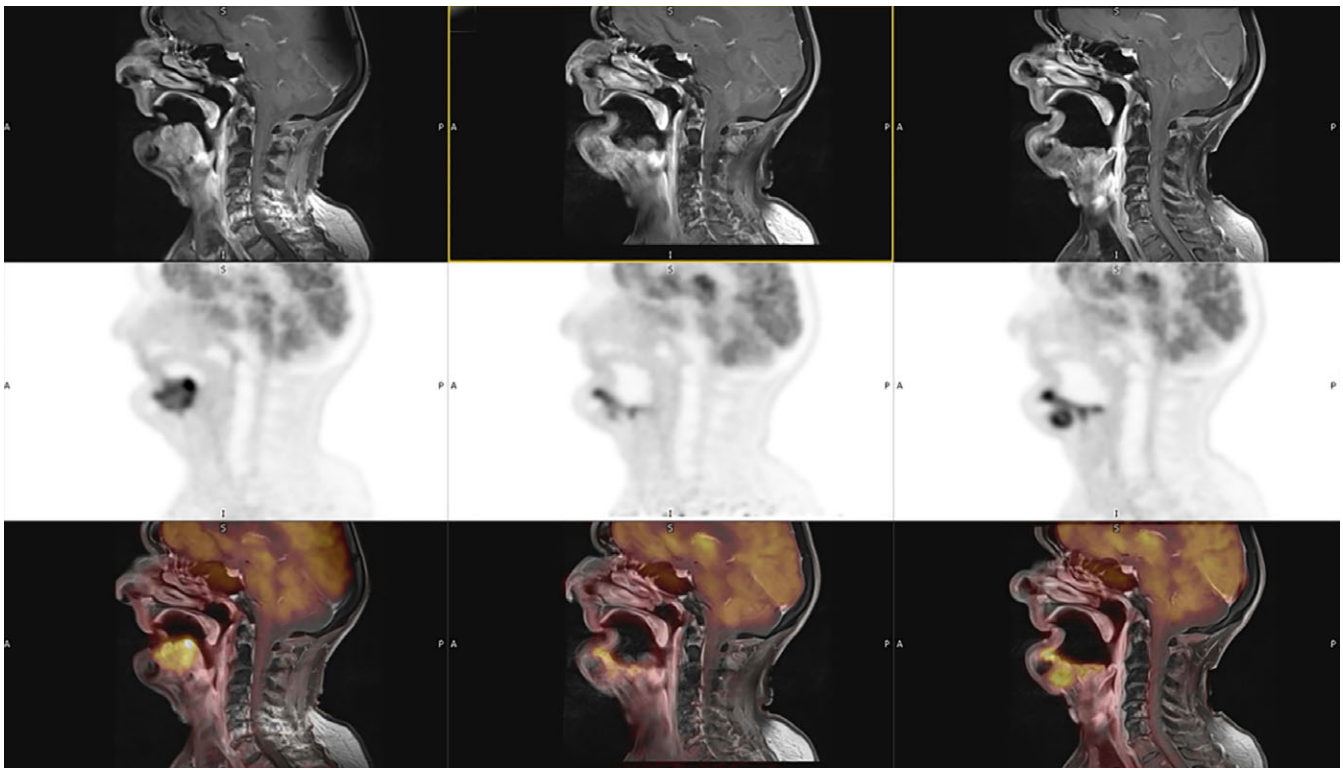

Fig. 3. PET/MRI imaging of patient 3 with tumor infiltration in the tongue and floor of mouth. The tongue was immobile and without function. From left to right PET/MRI imaging from baseline, 1, and 2 months post-treatment. Top images: MRI; middle images: FDG-PET; bottom images: PET/MRI alignment. On MRI a PR with 31% tumor reduction was seen whereas the FDG-PET demonstrated SMD with 18% reduction in SUVpeak. FDG-PET = flourodeoxyglucosis positron emission tomography; MRI = magnetic resonance imaging; PET = positron emission tomography; SMD = stable metabolic disease; SUV = standardized uptake values.

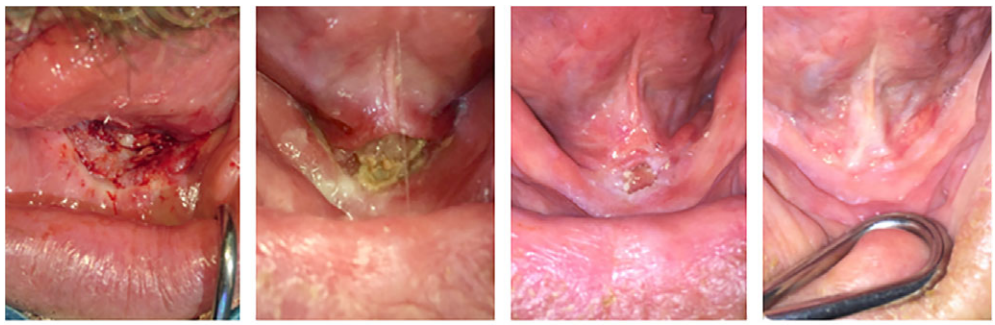

Fig. 4. Patient 6 with recurrence of SCC in the floor of the mouth. From left to right; before calcium electroporation; 1 month post-treatment; 2 months post-treatment; 5 months post-treatment with complete healed mucosa. The patient was seen again 12 months after treatment without clinical signs of tumor. A biopsy at 5 months was without signs of malignancy.SCC = squamous cell carcinoma.

TABLE V.
Biopsies Performed at Baseline, 1, and 2 Months Post-Treatment.

Time	Baseline				1 month				2 months			
Patient no.	Biopsy	SUV_{max}	SUV_{peak}	ADC	Biopsy	SUV_{max}	SUV_{peak}	ADC	Biopsy	SUV_{max}	SUV_{peak}	ADC
1	Pos.	12.3	10.2	675	Neg.	6.5	5.8	NE	ND	ND	ND	ND
2	Pos.	9.9	7.6	751	Pos.	9.4	7.4	982	Pos.	10.0	7.9	843
3	Pos.	12.2	10.0	1350	Neg.	1.3	1.9	NE	Pos.	5.2	5.0	1409
4	Pos.	6.2	5.3	NE	Neg.	3.8	3.7	NE	Pos.	2.8	2.9	NE
6	Pos.	4.8	3.7	NE	Neg.	5.2	3.9	NE	Neg.	4.0	2.9	NE

Biopsies were performed at the same location each time. For each biopsy, the correlation position has been measured on PET/MRI with SUV_{max}, SUV_{peak} and ADC.

Pos = malignant cells in the biopsy; Neg = no findings of malignant cells in the biopsy; ND = Not done; NE = Not evaluable.

to calcium electroporation. Additionally, calcium electroporation has so far been tested with a similar calcium chloride dose of 9 mg/ml.[21,36] A calcium chloride dose-optimization trial would bring further information on optimal dosing ranges.[37] Finally, it is of interest that a case report, and preclinical evidence supports an immune response to calcium electroporation.[36,38] This leads to another interesting perspective, namely combining calcium electroporation with eg, check point inhibitors for patients with recurrent head and neck cancer.

Future perspectives for calcium electroporation are looking promising. The treatment modality is fairly easy to implement in both surgical and oncological departments, and from a surgical point of view it is easier and preferable to avoid using chemotherapy in a department not used to handle cytostatics. In principle, this treatment can be repeated whereby the patients can be spared from otherwise mutilating surgical procedures. Calcium chloride is an inexpensive drug with long shelf life, which makes this treatment modality a plausible solution also for low income countries. Hopefully, this trial will be a stepping stone for further studies on larger patient populations to elucidate the potentials of calcium electroporation.

CONCLUSION

Calcium electroporation has shown to be a safe treatment on mucosal head and neck tumors with no signs of hypercalcemia, cardiac arrhythmias, or severe adverse events. Objective tumor responses were observed in three of the six treated patients with one patient in complete clinical remission one year after treatment, warranting further studies. The procedure was quickly adapted into the surgical setting and patients could be discharged in good condition shortly after.

CONFLICT OF INTEREST

A patent on calcium electroporation has been granted (PCT/DK2012/050496), with co-inventor Julie Gehl.

FINANCIAL DISCLOSURE

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