Journal of Surgical Oncology 2014;110:320–327

Intraoperative Electrochemotherapy of Colorectal Liver Metastases

IBRAHIM EDHEMOVIC, MD, MSc,[1] ERIK BRECELJ, MD, PhD,[1] GORANA GASLJEVIC, MD,[1] MAJA MAROLT MUSIC, MD, PhD,[1] VOJKA GORJUP, MD, PhD,[2] BARBARA MALI, PhD,[3] TOMAZ JARM, PhD,[3] BOR KOS, PhD,[3] DENIS PAVLIHA, PhD,[3] BILJANA GRCAR KUZMANOV, MD, PhD,[1] MAJA CEMAZAR, PhD,[1] MARKO SNOJ, MD, PhD,[1] DAMIJAN MIKLAVCIC, PhD,[3] ELDAR M. GADZIJEV, MD, PhD,[1]** AND GREGOR SERSA, PhD[1]*

[1]*Institute of Oncology Ljubljana, Ljubljana, Slovenia*
[2]*University Medical Center Ljubljana, Ljubljana, Slovenia*
[3]*Department of Biomedical Engineering, Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia*

Background and Objectives: Electrochemotherapy is effective in treatment of various cutaneous tumors and could be translated into treatment of deep-seated tumors. With this aim a prospective pilot study was conducted to evaluate feasibility, safety, and efficacy of intraoperative electrochemotherapy in the treatment of colorectal liver metastases.

Methods: Electrochemotherapy with bleomycin was performed during open surgery, by insertion of long needle electrodes into and around the tumor according to the individualized pretreatment plan.

Results: A 29 metastases in 16 patients were treated in 16 electrochemotherapy sessions. No immediate (intraoperative) and/or postoperative serious adverse events related to electrochemotherapy were observed. Radiological evaluation of all the treated metastases showed 85% complete responses and 15% partial responses. In a group of seven patients that underwent a second operation at 6–12 weeks after the first one, during which electrochemotherapy was performed, the histology of resected metastases treated by electrochemotherapy showed less viable tissue ($P = 0.001$) compared to non-treated ones.

Conclusions: Electrochemotherapy of colorectal liver metastases proved to be feasible, safe, and efficient treatment modality, providing its specific place in difficult to treat metastases, located in the vicinity of major hepatic vessels, not amenable to surgery or radiofrequency ablation.
J. Surg. Oncol. 2014;110:320–327. © 2014 Wiley Periodicals, Inc.

KEY WORDS: **electroporation; bleomycin; liver neoplasms; safety; treatment effectiveness; ablation**

INTRODUCTION

The best management of patients with resectable colorectal liver metastases is surgical; however, many patients are presented with unresectable metastases, due to their size, location, and/or inadequate remnant liver volume. In such unresectable cases, several alternative local approaches are used, among which the most frequent is radiofrequency ablation [1]; however, its efficacy is reduced in the vicinity of major vessels due to heat sink effect [2]. In such special cases and also in other unresectable cases, new electroporation-based treatment modalities are available—electrochemotherapy and irreversible electroporation—that have a potential role, because they are non-thermal local tumor treatment modalities, and are expected not to have deleterious effects on major blood vessels [3,4].

Electrochemotherapy is a treatment that combines the use of poorly or non-permeant, but highly effective cytotoxic drugs such as bleomycin or cisplatin with reversible electroporation, which facilitates drugs diffusion into the cells, thus increasing their cytotoxicity [5,6]. The use of bleomycin is based on the clinical evidence showing that among other drugs tested bleomycin has the highest potentiation of cytotoxicity by electroporation (up to several 1,000 times) [7,8]. Furthermore, the electroporation is effective only for hydrophilic drugs, like bleomycin and cisplatin, not for the lipophylic drugs that are regularly used in chemotherapy for liver colorectal metastases, like 5-Fu and irinotecan which penetrate cell membrane much easier, and electroporation does not potentiate their cytotoxicity.

Electrochemotherapy with bleomycin is effective in different cutaneous and subcutaneous tumors [9], as well as on preclinical models of colorectal tumors [10,11]. To date electrochemotherapy has been shown to be very effective in treatment of superficial metastatic disease, such as melanoma and chest wall breast cancer recurrence [7,12–21]. Its value of treating metastatic or unresectable disease within the abdomen and chest has enormous potential [22,23]. Translation of electrochemotherapy into treatment of deep-seated tumors is being currently explored [22], with the description of the technological approach on a case with liver metastasis [24]. The

Grant sponsor: Slovenian Research Agency (ARRS); Grant numbers: P3-0003, P2-0249.

Conflict of interest: Damijan Miklavcic holds patents (US 7625729 B2; EP 1395333 B1; US 7306940 B2) of which some have been licensed to IGEA SpA, Carpi, Italy, the producers of a clinical device used for electrochemotherapy in Europe since 2006. He also consults for IGEA SpA. Other authors declare no conflicts of interests.

Ibrahim Edhemovic and Erik Brecelj contributed equally to this work.

The institution at which the work was performed: Institute of Oncology Ljubljana, Ljubljana, Slovenia

*Correspondence to: Gregor Sersa, PhD, Department of Experimental Oncology, Institute of Oncology Ljubljana, Zaloska 2, SI-1000 Ljubljana, Slovenia. Fax: +386-1-5879-434. E-mail: gsersa@onko-i.si
**Correspondence to: Eldar M. Gadzijev, MD, PhD, Department of Surgical Oncology, Institute of Oncology Ljubljana, Zaloska 2, SI-1000 Ljubljana, Slovenia. Fax: +386-1-5879-434. E-mail: eldar.gadzijev@gmail.com

Received 13 January 2014; Accepted 31 March 2014

DOI 10.1002/jso.23625

Published online 30 April 2014 in Wiley Online Library (wileyonlinelibrary.com).

mechanism of action allows one to potentially sterilize tumors that are adjacent to structures that cannot be resected, such as major vessels, which frequently limit a curative resection, especially in liver metastatic disease.

The purpose of this article is to report the feasibility, safety, and efficacy of electrochemotherapy in treatment of colorectal liver metastases based on the treatment parameters of the previous ESOPE study [7]. This has not been done before.

PATIENTS AND METHODS

Study

The study was prospective, pilot study, conducted at the Institute of Oncology Ljubljana, Ljubljana, Slovenia. Regulatory approvals from the Institutional Board, as well as from the National Medical Ethics Committee (#45/09/08) were obtained. The study is registered at ClinicalTrials.gov: NCT01264952. Informed consent has been obtained from all patients included in the trial. The trial was designed based on ESOPE trial for treatment of cutaneous tumors [7], where the dosage of bleomycin and electrical parameters were set in standard operating procedures for treatment of cutaneous tumors [8].

The primary objective of the study was evaluation of the feasibility and safety of intraoperative electrochemotherapy of colorectal liver metastases. The secondary objective was to determine the efficacy of electrochemotherapy treatment, based on histological and radiological evaluation of treated metastases. The endpoints are: toxicity according to the Common Terminology Criteria for Adverse Events (CTC-AE) ver. 4.0 and response rate measured by percentage of vital tumor cells and mRECIST criteria.

Patients

Patients were enrolled from November 2009 to June 2012. All patients included in this study were in AJCC stage IV, with the disease limited to the liver only. Up to three metastases not exceeding 3 cm in the diameter were treated with electrochemoterapy. All patients except one were treated with systemic therapy prior to the electrochemotherapy; however, no systemic treatment was given until the second operation or radiological evaluation (Supplementary Table SI).

Inclusion and exclusion criteria are shown in Table I. Three groups of patients with colorectal liver metastases were included in the study (Table II). The first two groups of patients included patients with intent to cure within standard of care using two-stage surgical approach. This two-stage surgical approach allowed adding electrochemotherapy during the first operation and tissue collection for histological analysis during the second operation.

The first group (group I) included patients with bilateral, multiple, metachronous metastases in whom standard treatment included two-stage liver resection, due to the extent of the disease and/or their general condition. During the first operation, right portal vein was ligated and metastases on the left side were excised or ablated with radiofrequency ablation. At the same time, up to three metastases on the right side were treated with electrochemotherapy. During the second operation, both treated and non-treated metastases on the right side were removed with right hemihepatectomy.

The second group (group II) included patients with synchronous metastases, but their general condition and extent of the disease did not allow simultaneous removal of the primary tumor and metastases. During the first operation, the primary tumor was removed (colorectal resection) and some of the liver metastases were treated by electrochemotherapy. About 6 weeks later, during the second operation for liver metastases, both treated and non-treated metastases were removed with liver resection.

TABLE I. Inclusion and Exclusion Criteria

Inclusion criteria	Exclusion criteria
Age $\geq$ 18	Pregnancy and lactation
Performance status $\leq$2 ECOG	Implanted pacemaker or defibrillator
Chemotherapy free interval 2–5 weeks, depending on the drugs used	Significant cardiac arrhythmias
Life expectancy more than 3 months	Coagulation disturbances
Written informed consent	Cumulative dose of $\geq$250,000 IU bleomycin received
	Previous allergic reaction to bleomycin
	Chronically impaired kidney function
	Significantly impaired lung function
	Epilepsy
	Ascites
	Life threatening infection or other serious systemic condition or disease
	Secondary primary tumor, except surgically treated non-invasive cancer of the cervix or surgically treated or irradiated basal cell carcinoma, and confirmed visceral, bone or diffuse metastases

The third group (group III) included patients with up to three metachronous, unresectable liver metastases, demanding too excessive resection, or untreatable by standard thermal ablative methods, due to the close proximity of major blood vessels. Electrochemotherapy was offered to these patients as the only treatment option.

Based on the relation of the metastases to the major blood vessels, they were segregated into "central" or "peripheral". The term "central" was used to describe the metastases located in the near vicinity or on the major vessels. The term "peripheral" was used to describe the metastasis away from the major vessels to such an extent, so these vessels would not be affected by the electric field.

Treatment Procedure

The treatment of colorectal liver metastases was performed during open surgery using electrodes with variable [24] or fixed geometry, depending of the location of the metastasis. The electrodes with fixed geometry consist of seven electrodes fixed in a plastic holder and all of them are placed simultaneously as one electrode. The smaller tumors up to 2 cm in diameter, located no deeper than the length of the electrodes, that is, 3 cm, were treated with the electrodes with fixed geometry which are easier to insert and the treatment is performed faster. The variable geometry was utilized when bigger and deeper-seated tumors were treated. Patient-specific pretreatment plans were prepared based on computed tomography or magnetic resonance scans: target lesions (up to 3 cm in the largest diameter) were segmented, and a gradient-based optimization algorithm was used to optimize voltage between each electrode pair to maximize tumor coverage above the reversible electroporation threshold (400 V/cm) and minimize volume of healthy liver parenchyma above the irreversible electroporation threshold (700 V/cm)—see Supplementary Data I: An example of the treatment plan [25–27]. Trains of eight electric pulses (each pulse 100 μs long) were delivered to each pair of electrodes consecutively (Supplementary Table SII) [24]. Electric pulses were delivered by electric pulse generator (IGEA SpA, Carpi, Italy) during an interval of 8–28 min after the intravenous injection of bleomycin 15,000 IU/m^2 in bolus (Heinrich Mack Nachf. GmbH & CO. KG, Illertissen, Germany), as being determined to be the optimal pharmacological peak for the

TABLE II. Patients, Clinical Features, Tumor Characteristics, Response to Treatment, Adverse Events, and Postoperative Course

Patient			Previous treatments		Tumor characteristics			Electrodes used	Tumor response to ECT treatment				Postoperative complications		
No.	Age	Sex	Type	Days before ECT	Radiological size (mm)	Position related to the major vessels	Liver segment	Geometry	No.	Pathological size (mm)	Vital cells (%)	1st radiological evaluation (days after ECT)	2nd radiological evaluation (days after ECT)	ECT related (CTC-AE grade)	Non-ECT related within first 24 hr (CTC-AE grade)	Non-ECT related after first 24 hr (CTC-AE grade)
Group I: Two stage operations for metachronous metastases:																
01	67	M	CHT	35	22	Peripheral	8	Variable	5	30	17.5	CR (29)		None	None	Infection NOS (1)
02	55	F	CHT + BT	36	29	Central	1	Variable	6	17	0	CR (54)		None	Pulmonary hypertension (3)	None
03	69	M	CHT + BT	10	19	Peripheral	4-5	Variable	5	10	0	CR (54)		None	Infection NOS (1)	Atrial fibrillation (2)
					15	Central	4-8	Variable	5	9	0	PR				Colon perforation (3)
					20	Peripheral	5	Variable	5	14	0	CR				
04	56	M	CHT + BT	22	19	Peripheral	5	Variable	6	28	17.5	CR (30)		None	None	None
					10	Peripheral	8	Variable	5	23	7.5	CR				
05	54	M	CHT + BT	41	26	Central	4-8	Variable	6	50	0.5	CR (26)		Fever (1)	None	None
					9	Peripheral	5	Variable	4	20	0.5	CR				
					18	Peripheral	8	Variable	5	35	0.5	CR				
06	69	M	CHT + RT (as RT sens. only)	492	21	Peripheral	4	Fixed	7	20	30	PR (44)		Fever (1)	None	Infection NOS (1)
					16	Peripheral	8	Fixed	7	15	27.5	CR				
Group II: Two stage operations for synchronous metastases:																
07	59	M	CHT + BT	53	15	Peripheral	5	Variable	5	21	27	CR (76)		None	Infection NOS (1)	Abdominal abscess (3)
																Pneumonia (1)
																Wound infection (2)
08	32	M	CHT	19	12	Peripheral	4	Variable	5		NA	NA		None	None	Transient liver failure (2)
					14	Peripheral	5	Variable	5		NA	NA				
Group III: One stage operations for metachronous metastases, untreatable with other methods:																
09	38	F	CHT + BT + LR	68	6	Central	8	Variable	5			CR (19)	CR (274)	None	None	Ascites (2)
					8	Central	5–8	Variable	5			CR	CR			Infection NOS (1)
10	69	M	CHT + BT	108	17	Central	5	Variable	5			CR (33)	PD (119)	None	None	Supraventricular tachycardia (2)
					14	Central	4–8	Variable	5			PR	PD			
11	44	M	CHT + BT	66	17	Peripheral	8	Variable	5			CR (33)	CR (189)	None	None	Small bowel obstruction (3)
					21	Peripheral	8	Variable	5			CR	CR			Infection NOS (1)
12	57	F	CHT + BT	37	25	Central	4	Fixed	7			PR (50)	PD (163)	None	Infection NOS (1)	Ascites (2)
					18	Peripheral	4	Fixed	7			CR	CR			Colon perforation (3)
																Pleural effusion (2)
13	63	M	CHT + BT + LR	176	25	Central	4	Variable	5			CR (14)	PD (128)	None	Infection NOS (1)	Abdominal abscess (3)
14	61	M	CHT + BT	53	13	Peripheral	8	Fixed	7			CR (59)	CR (242)	None	None	None
					10	Central	2	Fixed	7			CR	CR			
15	62	F	CHT + BT	237	26	Central	3–4	Variable	6			CR (14)	CR (41)	None	None	Cholestatic icterus (2)
					18	Central	3	Variable	6			CR	CR			Infection NOS (1)
																Biliary fistula (3)
																Transient liver failure (2)
																Transient renal failure (3)
																Pleural effusion (2)
16	64	M	CHT	261	15	Central	4	Variable	5			CR (30)	CR (131)	None	None	Infection NOS (1)
																Biliary fistula (3)

No., number; ECT, electrochemotherapy; CHT, chemotherapy; BT, biologic therapy; RT, radiotherapy; LR, liver resection; CR, complete response; PR, partial response; PD, progressive disease; NA, not available; NOS, not otherwise specified.

bleomycin in the tumors [8]. To maximize the safety of patients, the delivery of electric pulses was synchronized with the absolute refractory period of the heart (see [24] for details) to prevent the electric pulses from being delivered during the vulnerable period of the ventricles [28–31].

Safety Assessment

Adverse events were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTC-AE) version 4.0. The ECG was monitored continuously during the surgical procedure as well as for 24 hr before and after the surgery using an ambulatory ECG Holter device (SpiderView, ELA Medical, France). Processing of ECG signals included statistical comparison of average RR and QT intervals over different time intervals and heart rate variability (HRV) analysis.

Efficacy Assessment Based on Pathology

Tissue for histological analysis was available in seven patients that were operated twice (Table II). The samples were assessed semi-quantitatively by two pathologists independently. One of the pathologists was blinded with respect to clinical information, treatment regimen and outcome. The mean between the two scores was calculated. The proportion of residual vital tumor tissue and proportion of regressive changes in relation to total tumor area were estimated as described by Ribero et al. [32]. Regressive changes included infarct-like tumor necrosis, fibrosis, foamy macrophages and other reparative changes. Infarct-like tumor necrosis was considered to be a form of treatment effect as proposed by Chang et al. [33].

Efficacy Assessment Based on Radiology

Before and after electrochemotherapy, liver metastases were evaluated by magnetic resonance (MRI) using a specific hepatocyte contrast agent (gadoliniumethoxybenzyl-diethylenetriaminepentaacetic acid—Gd-EOB-DTPA, Primovist, Bayer, Berlin, Germany) or contrast enhanced computed tomography (CE-CT) examination. The treatment response was evaluated by CE-CT or MRI, using the mRECIST criteria [34,35]. In the eight patients (group III) who did not undergo a second operation, an additional radiographic follow-up was performed subsequently.

Statistical Analysis

All data were entered into a Microsoft Access 2010 database, which was used for all calculations except for statistical analysis. For statistical analysis, SigmaPlot Ver. 12 software was used (Systat Software, Inc., San Jose, CA). The pathohistological differences between the electrochemotherapy treated and non-treated metastases were statistically evaluated by the *t*-test after confirming data normality using the Shapiro–Wilk test. A chi-square test was used for statistical comparison of response of metastases located near major blood vessels (referred as "central") and response of metastases located away from the major blood vessels (referred as "peripheral"). A two-tailed P value for the *t*-test and P value less than 0.05 was considered to be statistically significant.

RESULTS

The clinical features, treatment characteristics and response, adverse events and postoperative course of the 16 patients with 29 metastases are presented in Table II. Safety assessment was possible in all 16 patients; however, response to the treatment was evaluable in 15 patients (27 evaluable metastases)—one patient developed numerous new liver metastases, so evaluation of the response of the treated metastases was not possible.

Adverse Events

No electrochemotherapy related serious adverse events occurred. All observed adverse events are reported in Table II. Only grade 1 fever could be attributed to electrochemotherapy. Postoperative complications within and after 24 hr post electrochemotherapy could not be attributed specifically to electrochemotherapy and were in the range grades 1–3.

Three patients required reoperation: two patients due to colon perforation and one due to small bowel obstruction. None of these complications were related to the electrochemotherapy itself (Supplementary Data II: Data on patients' complications). All three patients were successfully reoperated and all 16 patients were discharged from hospital—there was no perioperative mortality.

The median duration of the patient's hospitalization after electrochemotherapy was 14 days (range 7–42); including three patients (one patient from group I and two patients from group III) that needed prolonged hospitalization, due to their reoperations.

After discharge from hospital, patients were followed up on outpatients' basis. Seven out of eight patients from groups I and II underwent major hepatic resection as planned at median of 59 days (range 43–84 days) after the electrochemotherapy (one patient was not reoperated due the disease progression). After 90 days, no patient from group III had signs of liver, renal or lung dysfunction, including those with serious complications and reoperations. Biliary fistulas in two patients ceased without intervention.

The treatment of 13 metastases (48%), that were located near or in-between the major blood vessels of the liver (referred as "central" in Table II), was safe. Neither intraoperatively nor postoperatively bleeding was observed. In some cases, the withdrawal of the electrodes resulted in mild bleeding, which however was easily stopped by electrocoagulation.

Safety Aspect of Electrochemotherapy in the Context of Changes in the ECG

The safety aspect of electrochemotherapy of colorectal liver metastases was evaluated based on detected changes in ECG signals recorded during and after the surgical procedure. No significant arrhythmias or pathological morphological changes that would indicate development of myocardial ischemia after electrochemotherapy were detected. The procedure did not result in new-onset of abnormal heartbeats (atrial or ventricular extrasystoles) or in increased frequency of abnormal heartbeats in patients who rarely had minor arrhythmias present in ECG signal before the treatment. ECG and HRV analysis; however, revealed some statistically significant but clinically irrelevant changes in the properties of the ECG during and after the surgical procedure. The most obvious one was a mild increase in heart rate immediately after electrochemotherapy (two patients) and also during the first 24 hr after the procedure (three patients). In addition, there was a mild depression in the low frequency component of the HRV spectrum (three patients).

Pathologic Response Evaluation

Pathologic analysis was performed on metastases treated with electrochemotherapy during the first operation and resected at the second operation (groups I and II). Altogether, 13 liver metastases treated with electrochemotherapy were microscopically analyzed and compared with 22 non-treated metastases from the same patients. Pathologic analysis revealed that metastases which were not treated by electrochemotherapy had a significantly higher percentage of residual vital tumor tissue, than electrochemotherapy treated metastases. On average, electrochemotherapy treated metastases had $9.9 \pm 12.2\%$ ($AM \pm SD$) viable tissue, and control metastases had $34.1 \pm 22.5\%$ ($P = 0.001$, two-tailed *t*-test) (Fig. 1). Typical changes that were observed in the metastases with complete response were infarct-like necrosis of the

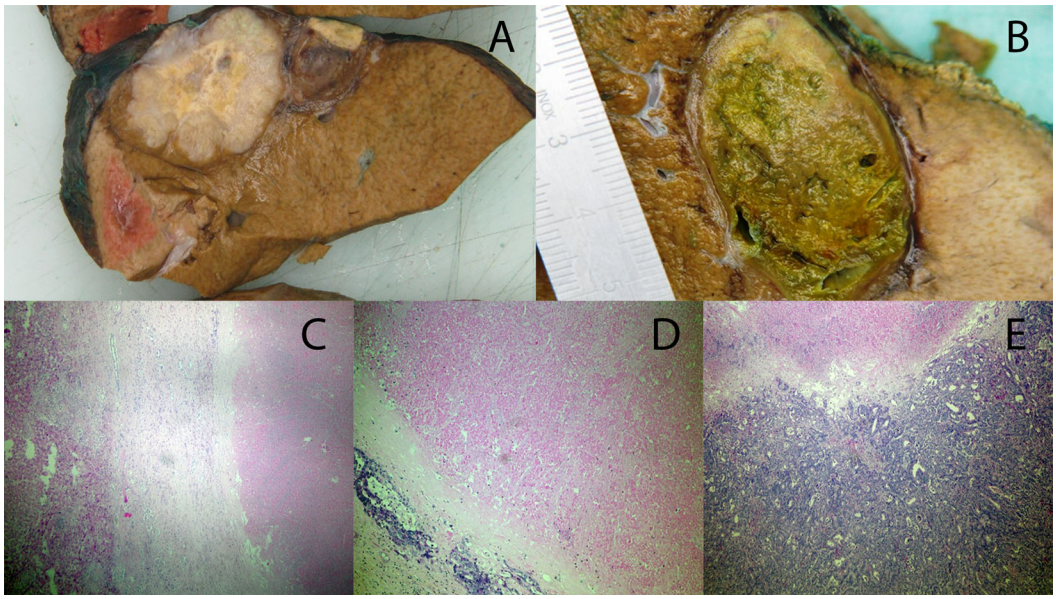


Fig. 1. Pathohistological features of tumors treated by electrochemotherapy in relation to those that were not. (**A**) Gross picture of two metastases: the large one corresponds to a metastasis treated by chemotherapy only, the small one corresponds to a metastasis treated by electrochemotherapy. The patient was in the group I where the two-stage operation was done. (**B**) Gross picture of metastasis treated by electrochemotherapy: complete necrosis of tumor and surrounding liver parenchyma. (**C**) Histological picture of completely necrotic tumor treated with electrochemotherapy: an infarct-like necrosis is in the right part of the picture, vital liver parenchyma in the left. In-between there is a fibrous pseudocapsule (H&E, 5×). (**D**) The only focus of residual vital tumor tissue in otherwise completely necrotic electrochemotherapy treated metastasis. An infarct-like necrosis is in the upper part of the picture (H&E, 10×). (**E**) Partial response in metastasis treated with chemotherapy only: an infarct-like necrosis is present in the upper field of the picture with lager amount of residual vital tumor tissue.

tumor tissue and the surrounding tumor parenchyma, with encapsulation of the treated tissue (fibrous pseudocapsule on the border between the normal liver tissue and the electrochemotherapy treated area).

Radiologic Response Evaluation

The median interval between the treatment and first radiological evaluation was 33 days (range 14–76). Twenty-seven metastases were evaluated (Table II), a complete response was observed in 23 (85%). In four metastases (15%) some enhancements of the treated lesion were seen, in both phases of liver enhancement, and they were evaluated as a partial response or local tumor progression.

In the group of eight patients (group III) with a single-stage operation, 14 metastases were treated by electrochemotherapy. These patients were evaluated radiologically twice. On the first follow-up examination at a median of 31.5 days (range 14–59) after electrochemotherapy, a complete response was seen in 12 metastases (86%). There was peripheral enhancement of the lesions in two metastases, which suggested a partial response. At the second follow-up, at median of 147 days (range 41–274) after electrochemotherapy, 10 (71%) metastases were still in complete response, while the other 4 progressed (Fig. 2). Response evaluated on a per patient basis was complete response for 5 (62.5%) patients and progressive disease for 3 (37.5%) patients.

Thirteen metastases were adjacent to major hepatic vessels. A 77% (10 metastases) were in complete response 33 days after electrochemotherapy (Table II). There was no difference detected in response of metastases located near major blood vessels and metastases located away from the major blood vessels ($P = 0.244$).

DISCUSSION

This translational study shows that electrochemotherapy is feasible, safe, and efficient treatment modality for the colorectal liver metastases. The simple physicochemical concept of electrochemotherapy procedure, using electric pulses to transiently increase the permeability of the cell membrane and facilitate the uptake of otherwise poorly permeant but highly effective cytotoxic drugs, provides a solid basis for its effectiveness in various tumor types, including colorectal tumors [10–12,18,36,37]. Translation of this treatment approach to the treatment in internal organs has recently begun. It is based on technological advance, with newly developed electric pulse generators and different sets of electrodes for specific organs [22,38].

Feasibility

In this study, we treated 16 patients with colorectal liver metastases, in different anatomical locations in the liver, including 13 metastases in the close vicinity of major hepatic vessels. Metastases positioned >3 cm deep in liver parenchyma were treated by long individual electrodes placed by ultrasound guidance according to the pretreatment plan [24]. For more superficially positioned metastases, electrodes with fixed geometry that were placed all at once were used without pretreatment plan. Eventually, further possible development of this method should provide percutaneous treatment, as in the case of radiofrequency ablation [39].

Safety

So far, no treatment related adverse events have been reported, either in the treatment of superficial tumors, or tumors in internal organs [12,16,24,40]. Local pain and transient erythema affecting the electroporated areas are among the most commonly reported side effects.

It is known that application of electric fields can affect implanted electrical devices (pacemakers) and interfere with cardiac function [24,30,31,41–43], therefore such patients were excluded for safety reasons. Electrochemotherapy of the cutaneous tumors has been demonstrated to have minimal risk of interfering with cardiac function,

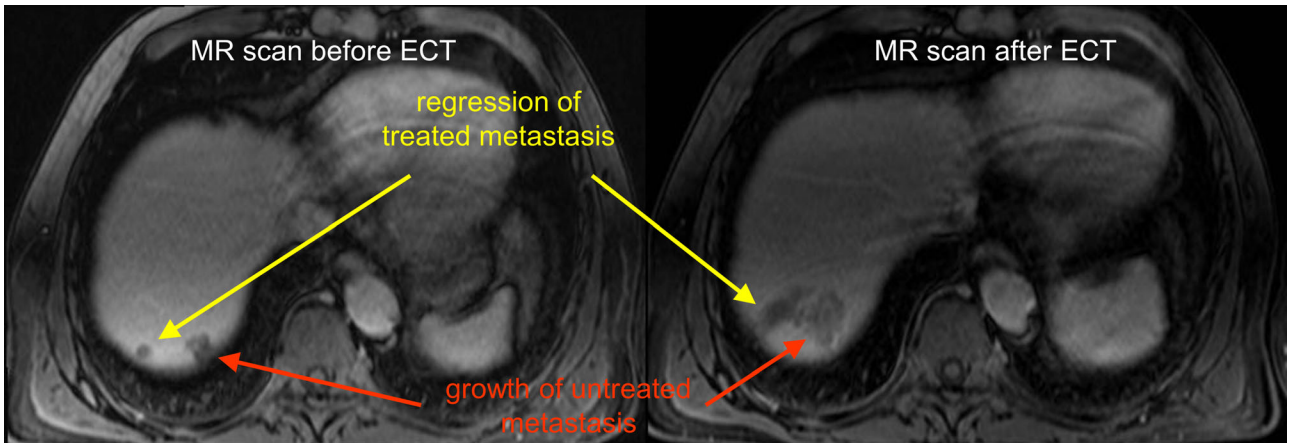


Fig. 2. Radiological changes in electrochemotherapy (ECT) treated and non-treated metastases, evaluated 30 days after the treatment.

even for tumors located on the chest wall near the heart [29]. Due to the proximity of the heart to the liver in our study, we synchronized the delivery of electric pulses with the ECG that resulted in uneventful and safe delivery of electric pulses to liver metastases. The mild changes detected in ECG and HRV parameters during and up to 24 hr after electrochemotherapy have no known clinical relevance.

The patients from groups I and II were in good condition and were treated with intent to cure within standard of care (two-stage R0 liver resection combined with systemic chemotherapy). In these two groups of patients there were no hepatic complications or any other serious complications related specifically to electrochemotherapy. A colon perforation was not caused by electrochemotherapy—it occurred at the same day when patient had an episode of the atrial fibrillation (1 week after the surgery) with subsequent partial thromboembolization of the colonic arteries that resulted in partial colon bowel necrosis and perforation.

All major complications; however, occurred in the group III (Table II). These patients were intensively treated previously and had unresectable or untreatable disease by conventional ablative techniques. These patients were offered electrochemotherapy as the only treatment option; however, majority of these patients had numerous previous major abdominal procedures and consequently required demanding liver mobilization due to very firm adhesions as well as additional liver resection along with electrochemotherapy. In this group of patients, two patients required reoperation. In the first case, mobilization of dense adhesions resulted in delayed perforation due to vascular compromise of the colonic wall. The second reoperated patient from this group had a typical obstruction of the small bowel caused by postoperative adhesions.

Efficacy

Significant reduction of viable tumor tissue in electrochemotherapy treated metastases versus control metastases was demonstrated. The typical changes that were observed in metastases with complete response were infarct-like necrosis of the tumor tissue and surrounding liver parenchyma, which supports evidence that electrochemotherapy has besides direct cytotoxic effect on the tumor cells also a vascular disrupting effect on small tumor blood vessels [44,45]. In contrast to the effect of electrochemotherapy on small tumor vessels, the effect on major blood vessels was not deleterious, similarly as it was demonstrated in non-thermal irreversible electroporation [3,4,46]. Namely, many of the metastases were located in-between, or in the vicinity of major blood vessels and no side effects on these vessels during or after electrochemotherapy procedure were observed. Furthermore, the electrochemotherapy was equally effective on these metastases and metastases located in a peripheral of liver tissue. Contrary, radiofrequency ablation does not work well close to the major vessels due to the heat sink effect. Previously, in the paper describing the technological approach of electrochemotherapy treatment, we reported histologically confirmed complete tumor response on a patient subjected to electrochemotherapy with liver metastasis located in-between inferior vena cava and the main hepatic veins [24]. The recent follow-up showed that this patient is still disease free 4 years after the procedure.

The high response rate is comparable to the effectiveness of non-thermal irreversible electroporation and radiofrequency ablation [2,47]. Furthermore, the radiological features of the treated metastases resemble those after radiofrequency ablation; the treated zone appeared as a well-defined area of low attenuation, usually larger than the former metastases. In some metastases, enhancement was seen in either the arterial or portal phase of liver enhancement.

Electrochemotherapy proved to be safe and effective in treatment of the metastases adjacent to structures that cannot be resected, such as major vessels that frequently limit a curative resection. Compared to thermal ablation techniques, electrochemotherapy, a non-thermal one, is safe and effective treatment also in the vicinity of the major blood vessels, because of lack of the heat sink effect [2,48,49]. Currently, electrochemotherapy is not a replacement for the radiofrequency ablation, but can be considered as a complementary method that may be used in situations where radiofrequency ablation would not be efficient.

CONCLUSION

This study provides the first evidence of the feasibility, safety and efficacy of electrochemotherapy in the treatment of colorectal liver metastases, which may also prove to be useful in the treatment of other tumors in the liver.

ACKNOWLEDGMENTS

The authors thank T. Pecnik Vavpotic, RN, BSc, from Institute of Oncology Ljubljana for technical assistance. This work was supported by the Slovenian Research Agency (ARRS), grant No. P3-0003 and No. P2-0249. The research was conducted within the scope of Electroporation in Biology and Medicine (EBAM), European Associated Laboratory (LEA), and COST Action TD1104. The electric pulses generator and electrodes for the study were generously provided by IGEA SpA (Carpi, Italy).

REFERENCES

1. Alberts SR: Update on the optimal management of patients with colorectal liver metastases. Crit Rev Oncol Hematol 2012;84:59–70.
2. Wong SL, Mangu PB, Choti MA, et al.: American Society of Clinical Oncology 2009 clinical evidence review on radiofrequency ablation of hepatic metastases from colorectal cancer. J Clin Oncol 2010;28:493–508.
3. Maor E, Ivorra A, Leor J, et al.: The effect of irreversible electroporation on blood vessels. Technol Cancer Res Treat 2007; 6:307–312.
4. Cheung W, Kavnoudias H, Roberts S, et al.: Irreversible electroporation for unresectable hepatocellular carcinoma: Initial experience and review of safety and outcomes. Technol Cancer Res Treat 2013;12:233–241.
5. Orlowski S, Belehradek J Jr, Paoletti C, et al.: Transient electropermeabilization of cells in culture. Increase of the cytotoxicity of anticancer drugs. Biochem Pharmacol 1988;37: 4727–4733.
6. Sersa G, Cemazar M, Miklavcic D: Antitumor effectiveness of electrochemotherapy with cis-diamminedichloroplatinum(II) in mice. Cancer Res 1995;55:3450–3455.
7. Marty M, Sersa G, Garbay JR, et al.: Electrochemotherapy—An easy, highly effective and safe treatment of cutaneous and subcutaneous metastases: Results of ESOPE (European Standard Operating Procedures of Electrochemotherapy) study. Eur J Cancer Suppl 2006;4:3–13.
8. Mir LM, Gehl J, Sersa G, et al.: Standard operating procedures of the electrochemotherapy: Instructions for the use of bleomycin or cisplatin administered either systemically or locally and electric pulses delivered by the Cliniporator^{TM} by means of invasive or non-invasive electrodes. Eur J Cancer Suppl 2006;4:14–25.
9. Eggermont AM: Treatment of melanoma in-transit metastases confined to the limb. Cancer Surv 1996;26:335–349.
10. Jaroszeski MJ, Dang V, Pottinger C, et al.: Toxicity of anticancer agents mediated by electroporation in vitro. Anticancer Drugs 2000;11:201–208.
11. Todorovic V, Sersa G, Flisar K, et al.: Enhanced cytotoxicity of bleomycin and cisplatin after electroporation in murine colorectal carcinoma cells. Radiol Oncol 2009;43:264–273.
12. Matthiessen LW, Chalmers RL, Sainsbury DCG, et al.: Management of cutaneous metastases using electrochemotherapy. Acta Oncol 2011;50:621–629.
13. Matthiessen LW, Johannesen HH, Hendel HW, et al.: Electrochemotherapy for large cutaneous recurrence of breast cancer: A phase II clinical trial. Acta Oncol 2012;51:713–721.
14. Campana LG, Valpione S, Mocellin S, et al.: Electrochemotherapy for disseminated superficial metastases from malignant melanoma. Br J Surg 2012;99:821–830.
15. Campana LG, Valpione S, Falci C, et al.: The activity and safety of electrochemotherapy in persistent chest wall recurrence from breast cancer after mastectomy: A phase-II study. Breast Cancer Res Treat 2012;134:1169–1178.
16. Sersa G, Cufer T, Paulin SM, et al.: Electrochemotherapy of chest wall breast cancer recurrence. Cancer Treat Rev 2012;38:379–386.
17. Latini A, Bonadies A, Trento E, et al.: Effective treatment of Kaposi's sarcoma by electrochemotherapy and intravenous bleomycin administration. Dermatol Ther 2012;25:214–218.
18. Gargiulo M, Papa A, Capasso P, et al.: Electrochemotherapy for non-melanoma head and neck cancers: Clinical outcomes in 25 patients. Ann Surg 2012;255:1158–1164.
19. Curatolo P, Quaglino P, Marenco F, et al.: Electrochemotherapy in the treatment of Kaposi sarcoma cutaneous lesions: A two-center prospective phase II trial. Ann Surg Oncol 2012;19:192–198.
20. Testori A, Faries MB, Thompson JF, et al.: Local and intralesional therapy of in-transit melanoma metastases. J Surg Oncol 2011; 104:391–396.
21. Mali B, Miklavcic D, Campana LG, et al.: Tumor size and effectiveness of electrochemotherapy. Radiol Oncol 2013;47: 32–41.
22. Miklavcic D, Sersa G, Brecelj E, et al.: Electrochemotherapy: Technological advancements for efficient electroporation-based treatment of internal tumors. Med Biol Eng Comput 2012;50:1213–1225.
23. Jahangeer S, Forde P, Soden D, et al.: Review of current thermal ablation treatment for lung cancer and the potential of electrochemotherapy as a means for treatment of lung tumours. Cancer Treat Rev 2013;39:862–871.
24. Edhemovic I, Gadzijev EM, Brecelj E, et al.: Electrochemotherapy: A new technological approach in treatment of metastases in the liver. Technol Cancer Res Treat 2011;10:475–485.
25. Miklavcic D, Snoj M, Zupanic A, et al.: Towards treatment planning and treatment of deep-seated solid tumors by electrochemotherapy. Biomed Eng OnLine 2010;9:10.
26. Pavliha D, Kos B, Zupanic A, et al.: Patient-specific treatment planning of electrochemotherapy: Procedure design and possible pitfalls. Bioelectrochemistry 2012;87:265–273.
27. Zupanic A, Kos B, Miklavcic D: Treatment planning of electroporation-based medical interventions: Electrochemotherapy, gene electrotransfer and irreversible electroporation. Phys Med Biol 2012;57:5425–5440.
28. Reilly JP, editor: Applied bioelectricity: From electrical stimulations to electropathology. New York: Springer; 1998.
29. Mali B, Jarm T, Corovic S, et al.: The effect of electroporation pulses on functioning of the heart. Med Biol Eng Comput 2008;46:745–757.
30. Ball C, Thomson KR, Kavnoudias H: Irreversible electroporation: A new challenge in 'out of operating theater' anesthesia. Anesth Analg 2010;110:1305–1309.
31. Deodhar A, Dickfeld T, Single GW, et al.: Irreversible electroporation near the heart: Ventricular arrhythmias can be prevented with ECG synchronization. Am J Roentgenol 2011;196:W330–W335.
32. Ribero D, Wang H, Donadon M, et al.: Bevacizumab improves pathologic response and protects against hepatic injury in patients treated with oxaliplatin-based chemotherapy for colorectal liver metastases. Cancer 2007;110:2761–2767.
33. Chang HHL, Leeper WR, Chan G, et al.: Infarct-like necrosis: A distinct form of necrosis seen in colorectal carcinoma liver metastases treated with perioperative chemotherapy. Am J Surg Pathol 2012;36:570–576.
34. Lencioni R, Llovet JM: Modified RECIST (mRECIST) assessment for hepatocellular carcinoma. Semin Liver Dis 2010;30:52–60.
35. Park M, Rhim H, Kim Y, et al.: Spectrum of CT findings after radiofrequency ablation of hepatic tumors. Radiographics 2008;28: 379–390.
36. Mali B, Jarm T, Snoj M, et al.: Antitumor effectiveness of electrochemotherapy: A systematic review and meta-analysis. EJSO 2013;39:4–16.
37. Soden DM, Larkin JO, Collins CG, et al.: Successful application of targeted electrochemotherapy using novel flexible electrodes and low dose bleomycin to solid tumours. Cancer Lett 2006;232:300–310.
38. Linnert M, Iversen HK, Gehl J: Multiple brain metastases—Current management and perspectives for treatment with electrochemotherapy. Radiol Oncol 2012;46:271–278.
39. Crocetti L, Lencioni R, Debeni S, et al.: Targeting liver lesions for radiofrequency ablation: An experimental feasibility study using a CT-US fusion imaging system. Invest Radiol 2008;43: 33–39.
40. Testori A, Tosti G, Martinoli C, et al.: Electrochemotherapy for cutaneous and subcutaneous tumor lesions: A novel therapeutic approach. Dermatol Ther 2010;23:651–661.
41. Thomson K: Human experience with irreversible electroporation. In: Rubinsky B, editor. Irreversible electroporation. Berlin Heidelberg: Springer-Verlag; 2010.pp. 249–254.
42. Pech M, Janitzky A, Wendler JJ, et al.: Irreversible electroporation of renal cell carcinoma: A first-in-man phase I clinical study. Cardiovasc Intervent Radiol 2011;34:132–138.
43. Bagla S, Papadouris D: Percutaneous irreversible electroporation of surgically unresectable pancreatic cancer: A case report. J Vasc Interv Radiol 2012;23:142–145.

44. Sersa G, Jarm T, Kotnik T, et al.: Vascular disrupting action of electroporation and electrochemotherapy with bleomycin in murine sarcoma. Br J Cancer 2008;98:388–398.
45. Jarm T, Cemazar M, Miklavcic D, et al.: Antivascular effects of electrochemotherapy: Implications in treatment of bleeding metastases. Expert Rev Anticancer Ther 2010;10:729–746.
46. Neal RE II, Rossmeisl JH Jr, Garcia PA, et al.: Successful treatment of a large soft tissue sarcoma with irreversible electroporation. J Clin Oncol 2011;29:e372–e377.
47. Charpentier KP: Irreversible electroporation for the ablation of liver tumors: Are we there yet? Arch Surg 2012;147:1053–1061.
48. Czymek R, Nassrallah J, Gebhard M, et al.: Intrahepatic radiofrequency ablation versus electrochemical treatment in vivo. Surg Oncol 2012;21:79–86.
49. Neal RE II, Kavnoudias H, Cheung W, et al.: Hepatic epithelioid hemangioendothelioma treated with irreversible electroporation and antibiotics. J Clin Oncol 2013;31:e422–e426.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

SUPPLEMENTARY MATERIALS

Supplementary Table I. The list of previous chemotherapy treatments for individual patient.

Patient ID	Age	Sex	Previous chemotherapy
Group one: Two stage operations for metachroneus metastases:			
01	67	M	XELOX x 7
02	55	F	Capecitabine x 8; XELOX + Bevacizumab x 3
03	69	M	XELIRI + Cetuximab x 6; Cetuximab (cont. maint.)
04	56	M	XELOX + Cetuximab x 8 Cetuximab (cont. maint.)
05	54	M	XELOX x 6; Capecitabine x 2; XELIRI + Bevacizumab x 4 XELIRI x 1
06	69	M	5FU + Leukov. (as sensitizer during RX) x 4
Group two: Two stage operations for synchroneus metastases:			
07	59	M	FOLFOX x 3; Cetuximab x 7; Capecitabine x 2 (conc. RX) XELOX + Cetuximab x 6
08	32	M	FOLFOX x 6
Group three: One stage operations for metachroneus metastases, untreatable with other methods:			
09	38	F	XELIRI + Cetuximab x6; XELIRI + Cetuximab x4; Cetuximab (cont. maint.); XELOX + Cetuximab; Cetuximab (cont. maint.); XELIRI + Bevacizumab; With ECT also HIPEC (Oxali + 5FU)

10	69	M	XELIRI + Bevacizumab; Bevacizumab (cont. maint.)
11	44	M	XELOX x 5; Capecitabine x 3; XELIRI + Bevacizumab x 3
12	57	F	FOLFOX + Cetuximab x 13; Cetuximab x 4; XELIRI + Bevacizumab x 8; FOLFOX + Bevacizumab x 3
13	63	M	XELIRI + Cetuximab x 7; Cetuximab x 6; XELOX x 2; XELOX + Cetuximab x 2; Cetuximab x 3; XELIRI + Cetuximab x 4; FOLFIRI + Cetuximab x 2; Cetuxumab x 12
14	61	M	XELIRI + Bevacizumab x 4 XELOX + Bevacizumab x 2 FOLFOX + Bevacizumab x 4
15	62	F	Capecitabine x 2; XELOX + Bevacizumab x 8
16	64	M	XELOX x 7

SUPPLEMENTARY MATERIALS

Supplementary Table II. Main characteristics of electrodes and electroporation (EP) pulse delivery in treatment of individual metastasis (M).

Patient number	Metastasis	Electrode geometry, length of active part	No. of electrodes used	No. of EP pulses delivered	Average voltage applied* [V]	Average current* [A]
01	M1	variable, 3 cm	5	32	3003	35.91
02	M1	variable, 4 cm	6	79	1702	48.86
03	M1	variable, 3 cm	5	64	2422	27.74
	M2		5	64	1938	18.12
	M3		5	64	1481	20.00
04	M1	variable, 3 cm	5	65	1695	16.69
	M2		6	72	1875	19.67
05	M1	variable, 3 cm	6	110	2810	29.72
	M2		5	64	2131	21.89
	M3		4	53	2024	31.21
06	M1	fixed, 3 cm	7	672	713	6.67
	M2		7	96	713	6.30
07	M1	variable, 3 cm	5	64	1052	10.58
	M2		5	64	1100	11.74
08	M1	variable, 4 cm	5	64	1778	24.84
	M2		5	64	2512	30.06
09	M1	variable, 3 cm	5	64	1778	26.82
	M2		5	64	1778	24.16
10	M1	variable, 3 cm	5	64	1924	24.40

	M2		5	64	1744	17.25
11	M1	variable, 4 cm	5	64	1633	20.83
	M2		5	64	2124	20.20
12	M1	fixed, 3 cm	7	288	713	9.02
	M2		7	384	713	8.24
13	M1	variable, 3 cm	5	75	2421	46.13
14	M1	fixed, 3 cm	7	96	718	4.59
	M2		7	288	718	3.77
15	M1	variable, 3 cm	6	104	2533	31.50
	M2		6	111	–	–
16	M1	variable, 3 cm	5	68	2383	31.67

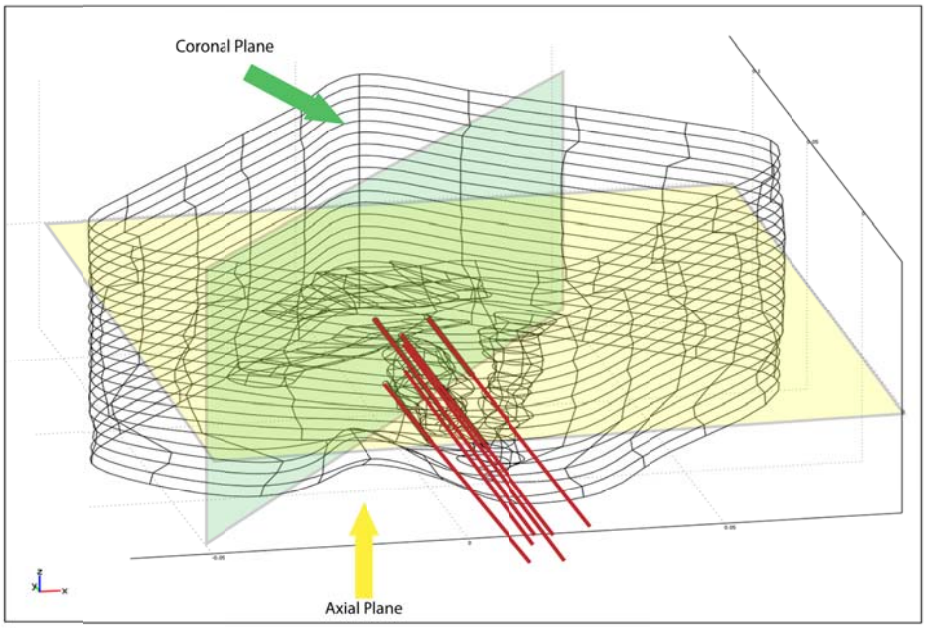
***** Average values of voltage and current were evaluated from recorded time course of voltage and current during electric pulse delivery. The median value of voltage and current delivered within each electric pulse was first calculated. Then, the median of these median values for all electric pulses delivered on individual tumor was calculated and used as measure for average value of voltage and current delivered on this tumor.
– data not available

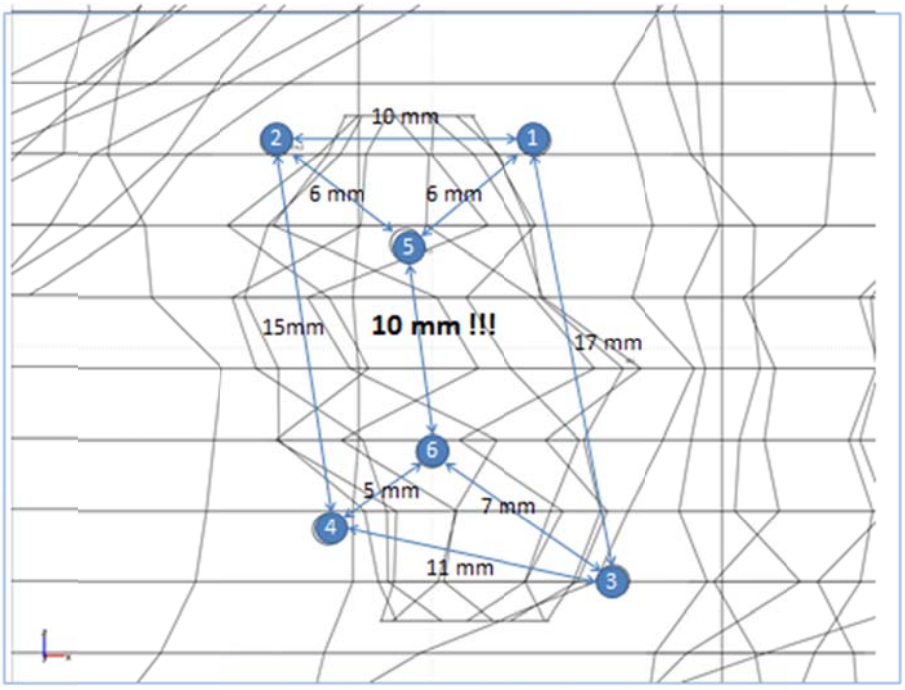
Treatment plan

Example treatment plan of Patient 2

Treatment report: Example case of metastasis in the liver

ECTplan





Electrode placement in the liver

Chapter 1. Electroporation pulses applied

Table 1.1 Electroporation pulses applied

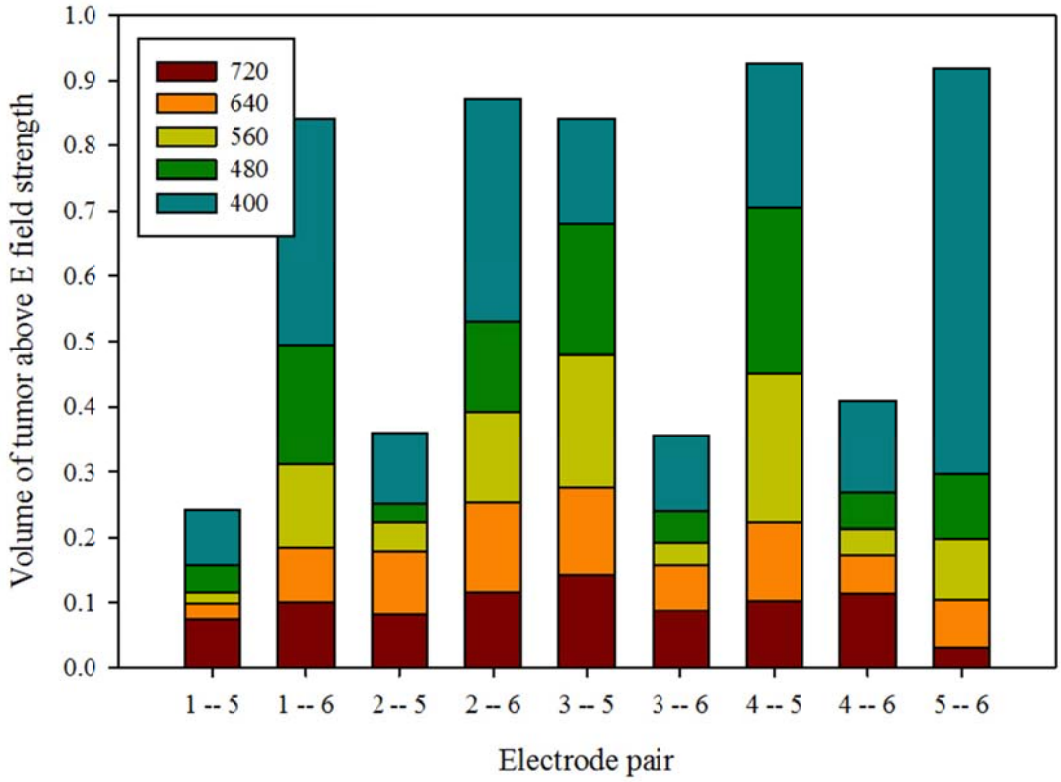
Electrode pair	Voltage	Predicted current
1-5	2100 V	31 A
2-5	2100	26 A
2-6	2100 V	25 A
1-6	2100 V	26 A
5-6	1700 V	40 A
3-5	2100 V	25 A
3-6	2100 V	29 A
4-5	2100 V	28 A
4-6	2100 V	33 A

The total volume of tumor treated above the reversible electroporation threshold (400 V/cm) was 100 %, the volume of tumor above 600 V/cm was 99 %.

The volume of liver tissue treated above the irreversible electroporation threshold was 27 cm^3.

Chapter 2. Electrode pair contributions

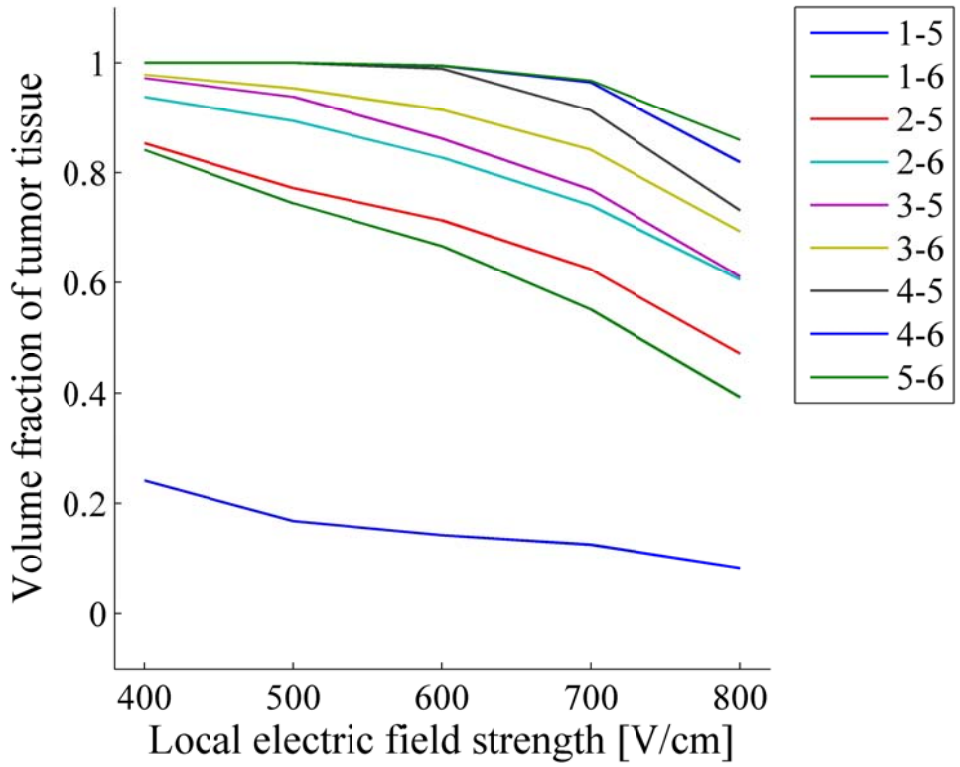
Figure 2.1. Electrode pair contributions



The figure shows the contribution of each electrode pair in the treatment to the final coverage of the tumor.

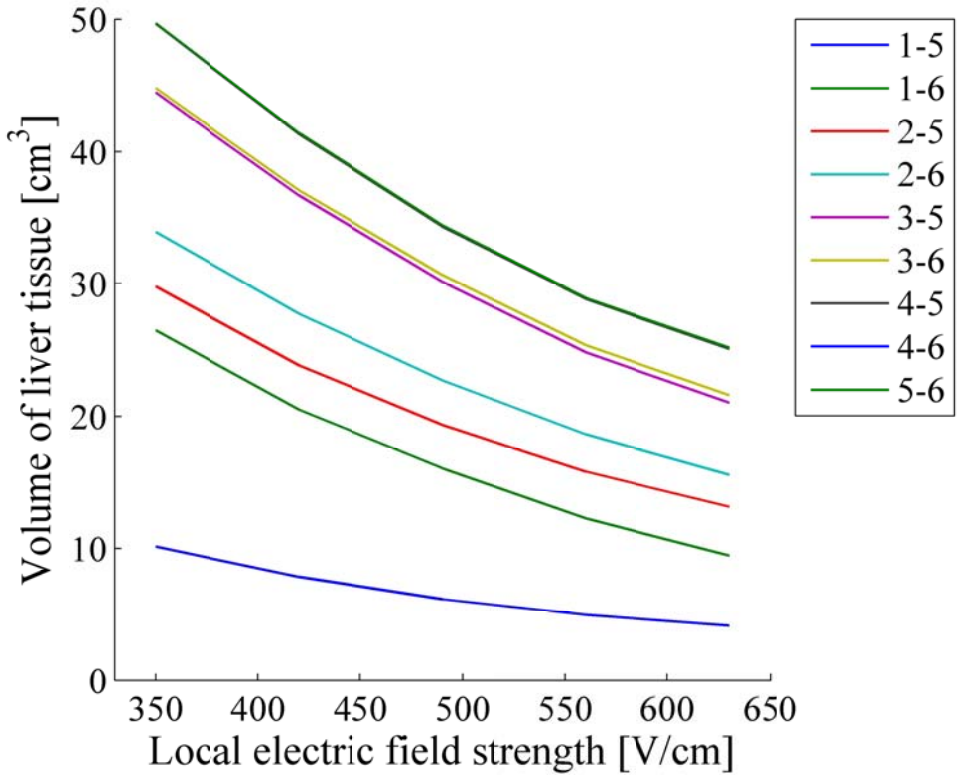
Chapter 3. Cumulative coverage curves

Figure 3.1. Cumulative coverage curves – tumor



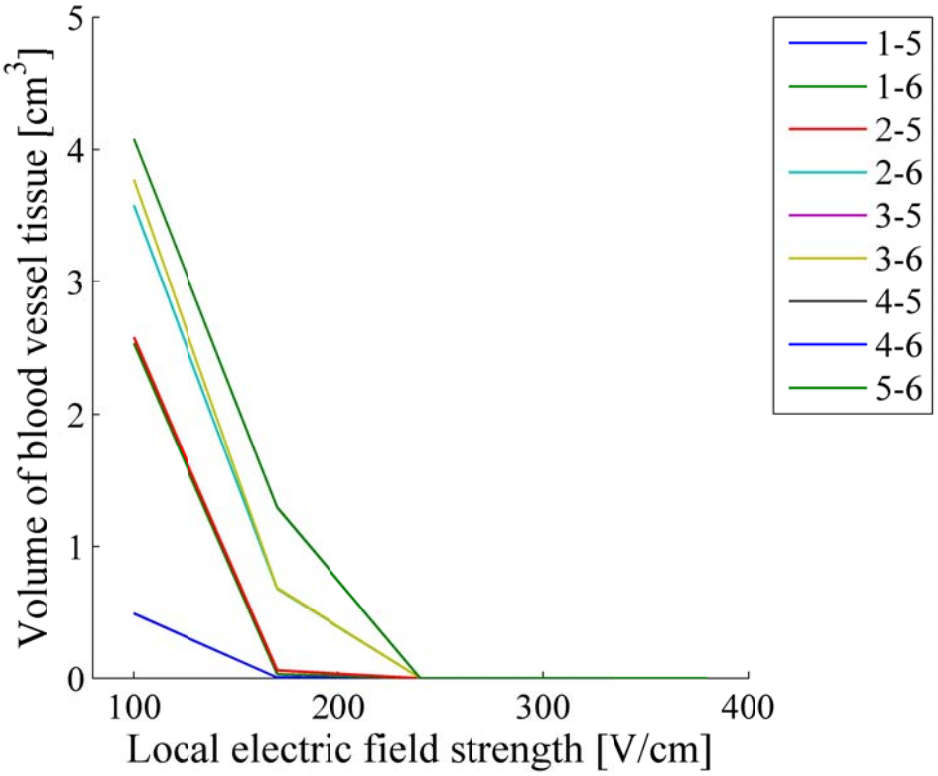
The figure shows the volume of tissue treated above the electric field strength indicated on the x axis.

Figure 3.2. Cumulative coverage curves – liver



The figure shows the volume of tissue treated above the electric field strength indicated on the x axis.

Figure 3.1. Cumulative coverage curves – veins



The figure shows the volume of tissue treated above the electric field strength indicated on the x axis.

Chapter 4. Electroporation cross-section images

Figure 4.1 Electroporation cross-section slice 6 of 21

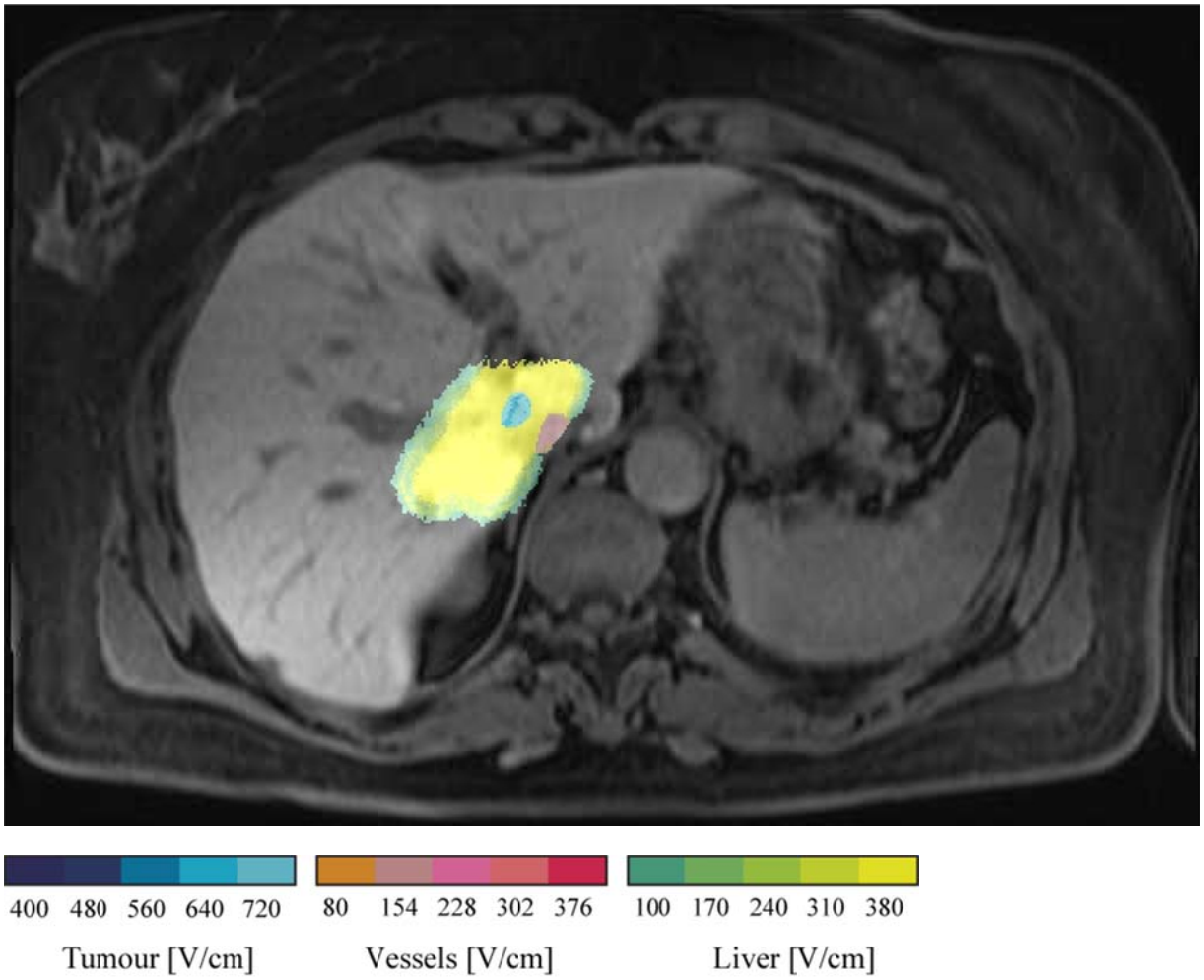


Figure 4.1 Electroporation cross-section slice 7 of 21

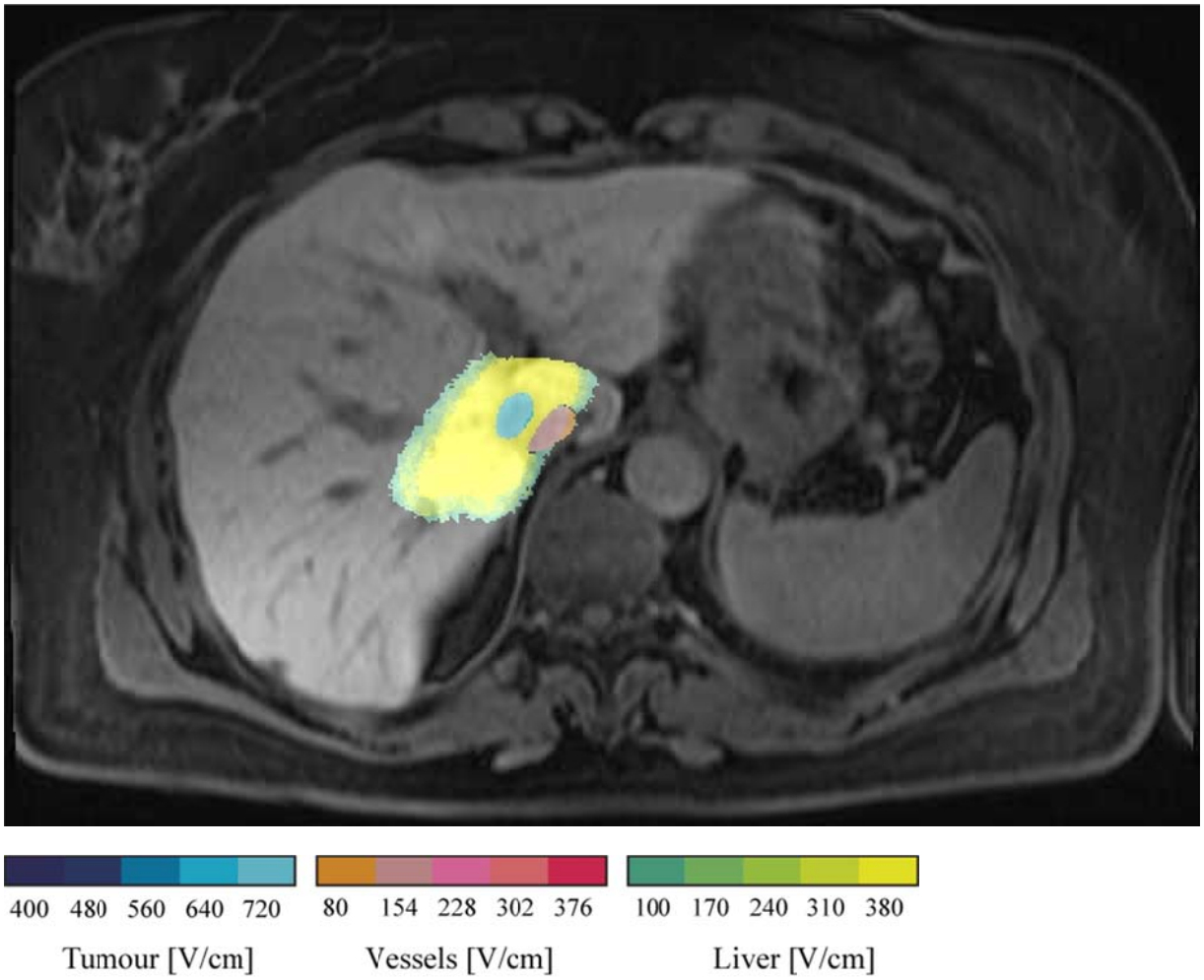


Figure 4.1 Electroporation cross-section slice 8 of 21

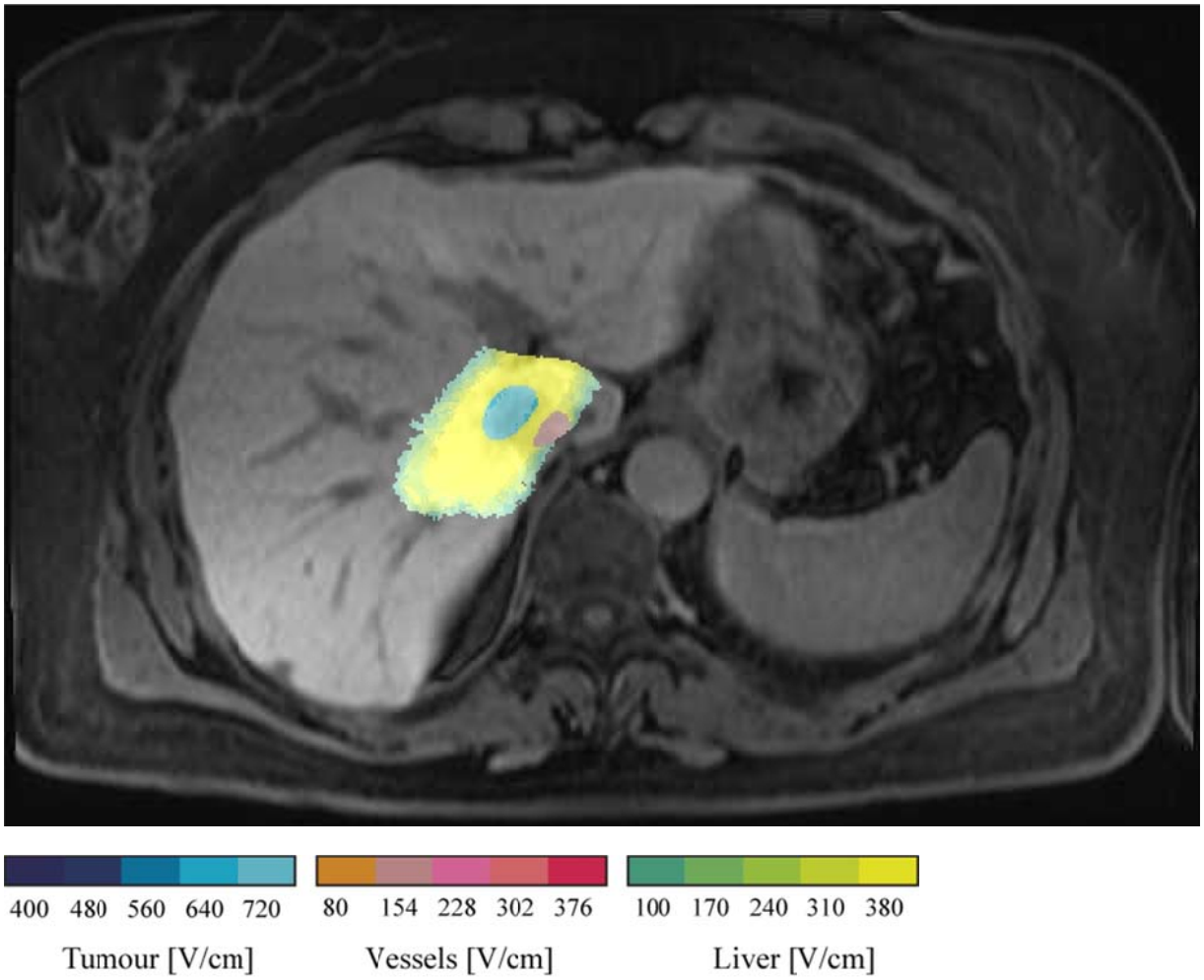


Figure 4.1 Electroporation cross-section slice 9 of 21

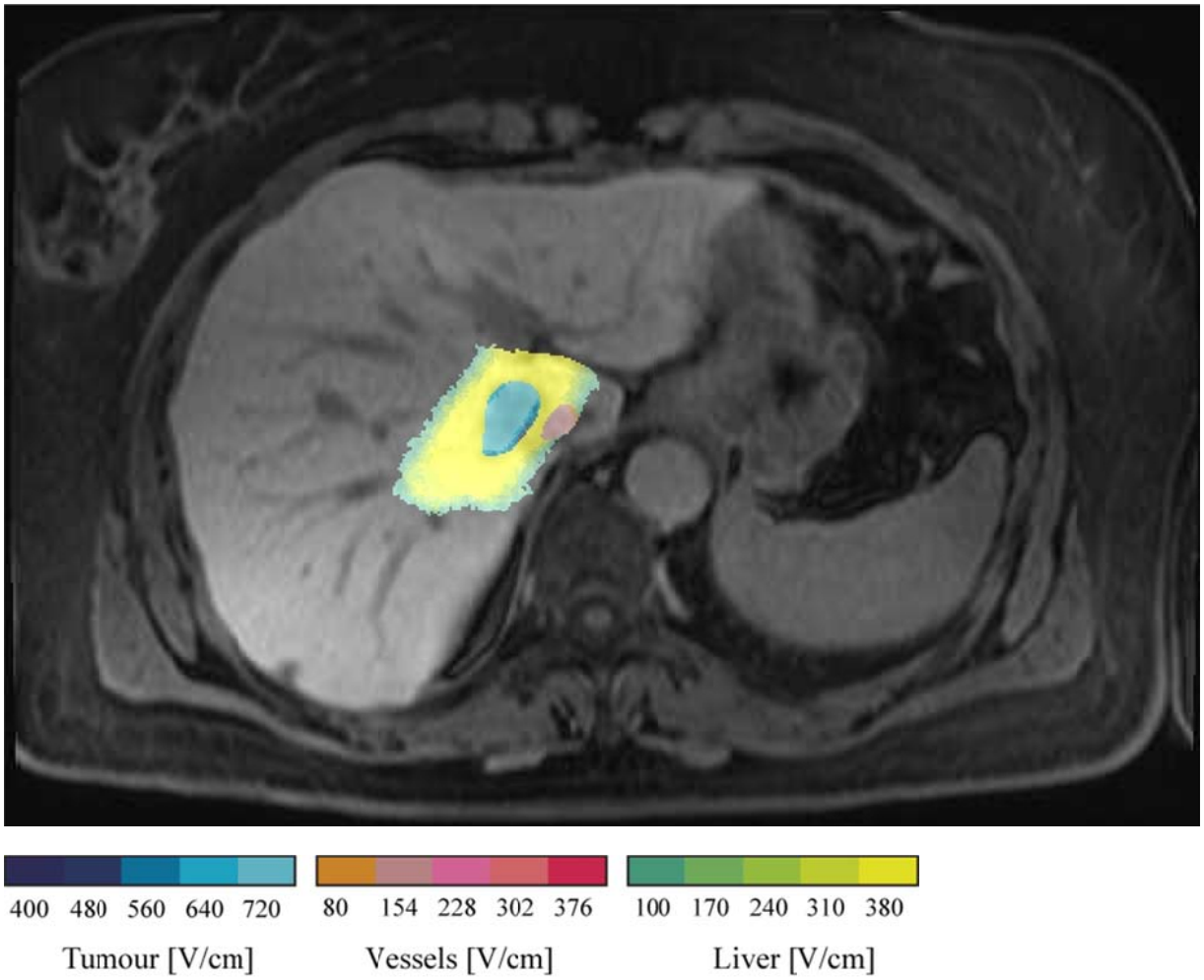


Figure 4.1 Electroporation cross-section slice 10 of 21

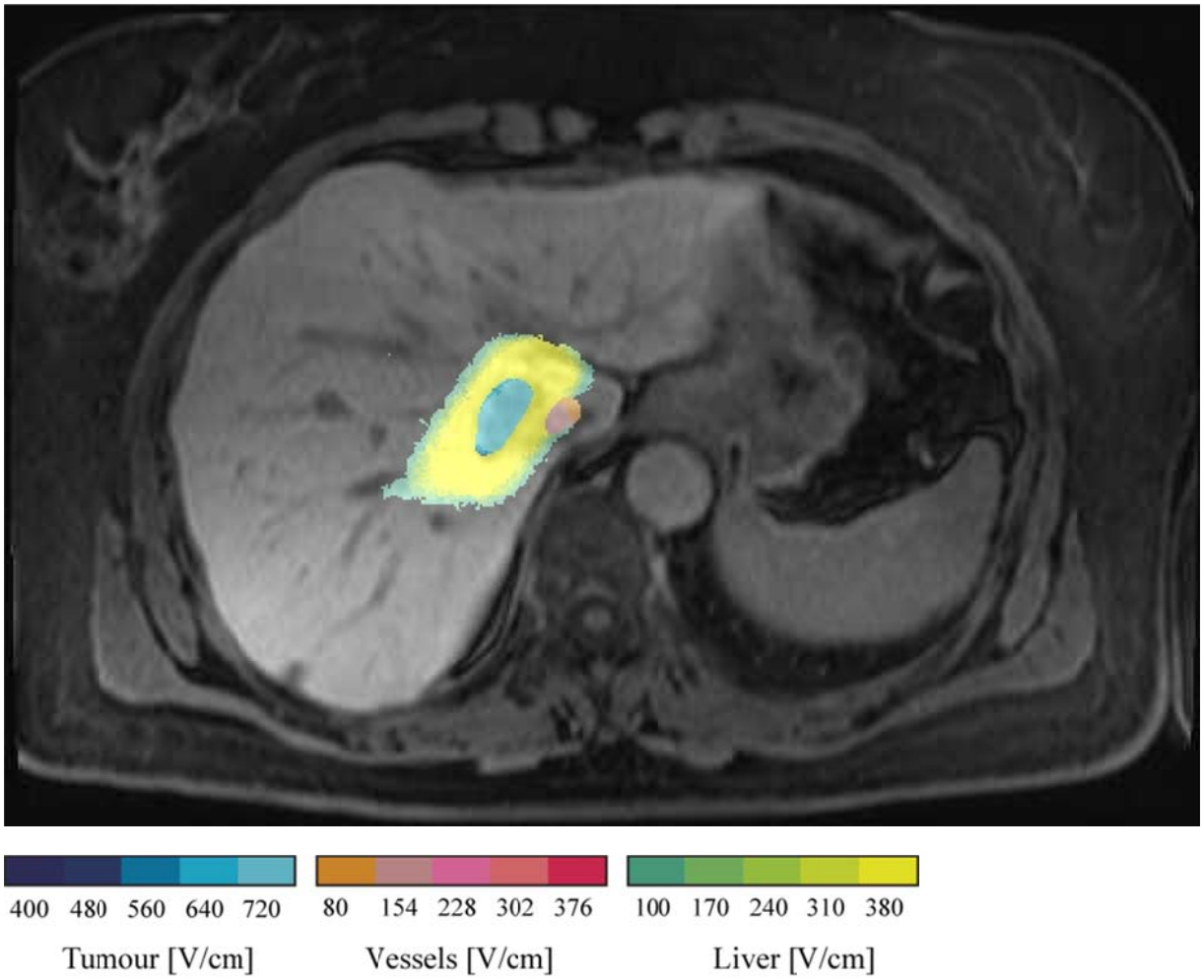


Figure 4.1 Electroporation cross-section slice 11 of 21

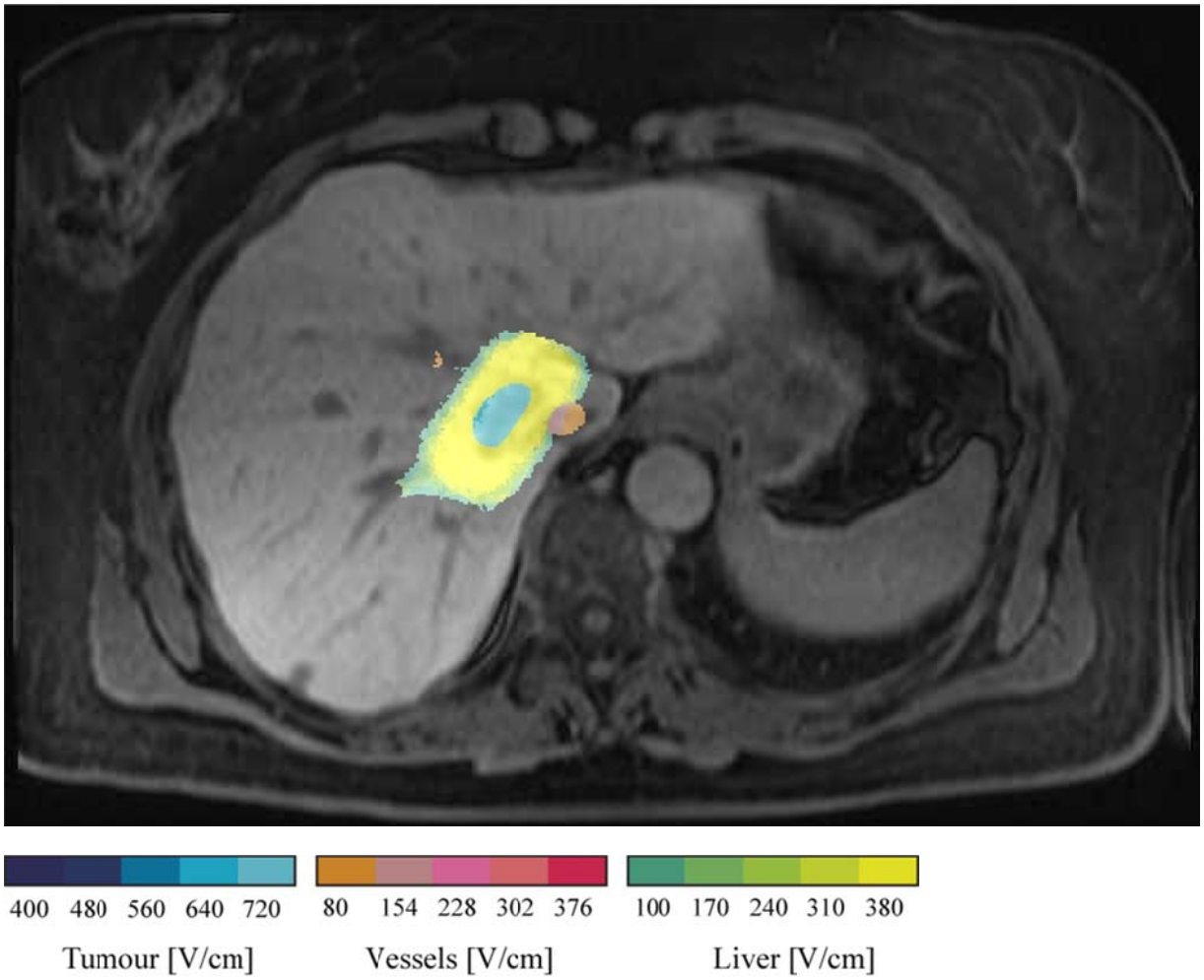


Figure 4.1 Electroporation cross-section slice 12 of 21

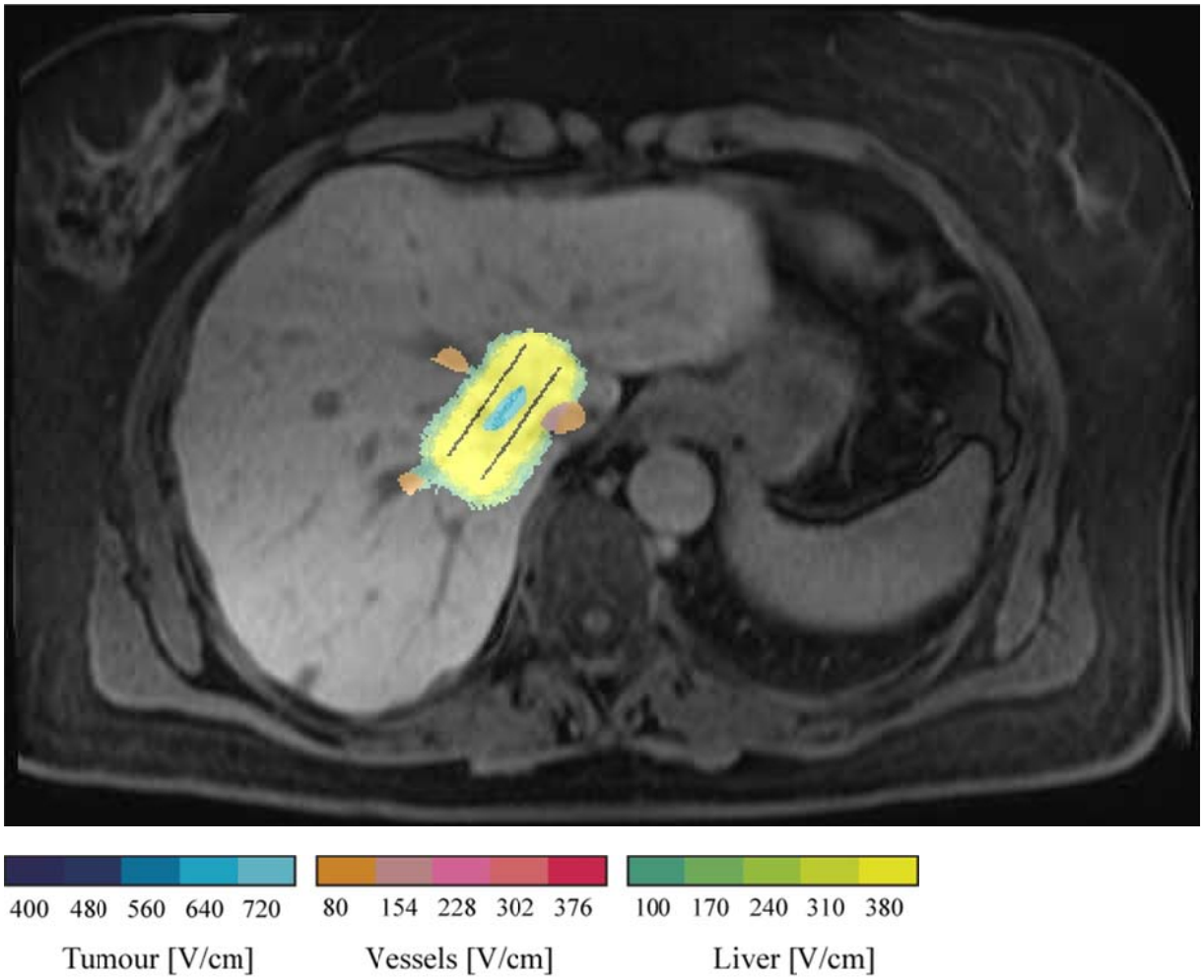
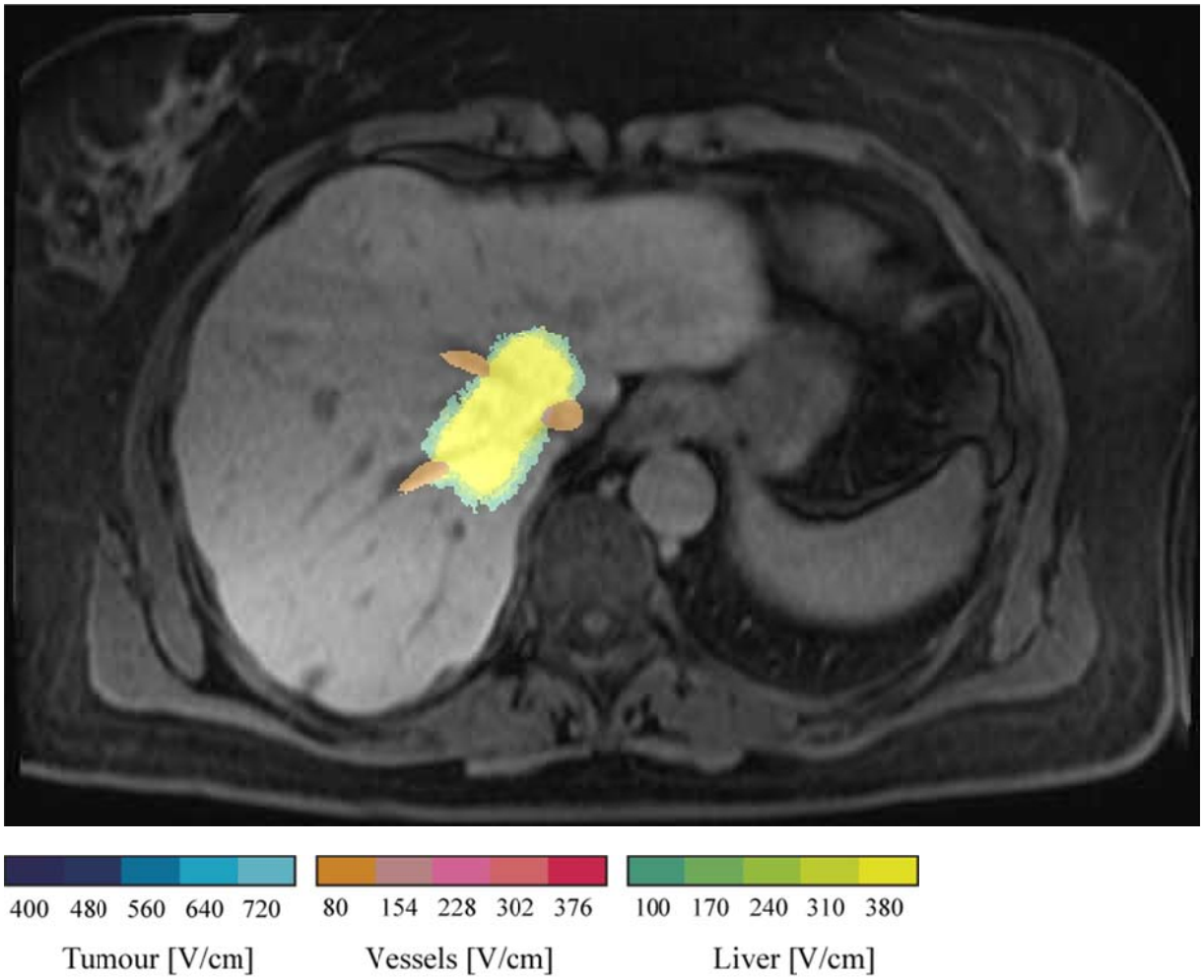


Figure 4.1 Electroporation cross-section slice 13 of 21



SUPPLEMENTARY MATERIALS

Supplementary Data II. Additional explanation of the patients with serious complications.

The patients from groups I and II were in good condition with curable disease. They were treated with intent to cure within standard of care and electrochemotherapy did not influence their standard treatment plan in any way. During the first operation, a relatively small procedure on the liver was performed (one or two metastasectomies on the left side and right portal vein ligation) along with electrochemotherapy. In these two groups of the patients there were no hepatic complications (ascites, biliary fistula, icterus, pleural effusion …) or any other serious complications. There was one reoperation indeed in patient # 03 due to the colonic perforation; however, this was the consequence of an episode of the atrial fibrillation (one week after the surgery). We assume that this patient suffered partial thromboembolisation of the colonic arteries resulting in partial colon wall necrosis and perforation which was confirmed during the reoperation. The intra-abdominal abscess in patient # 07 was drained percutaneously.

All major complications, however, occurred in the group III. These patients were extensively previously treated and had unresectable disease or untreatable disease with convenient ablative techniques. These patients were offered electrochemotherpy as the only treatment option; however, majority of these patients required demanding liver mobilization due to very firm adhesions and/or some kind of liver resection along with electrochemotherapy.

Illustration of cases with serious complications:

Patient # 11 which required reoperation had a typical obstruction of the small bowel caused by postoperative adhesions.

Patient # 12 had previously right hemihepatectomy and later metastasectomy due to the recurrence. Electrochemotherapy was the third operation during which we found colon very firmly adhered to the resection surface of the previously resected liver. These patients had also very firm adhesions and scars between liver and diaphragm. During the mobilization of the

hepatic flexure, we probably accidentally superficially injured/devascularized the colonic wall, which resulted in colon perforation one week later. We also had to mobilize the remnant of the liver from the diaphragm which resulted in both liver and diaphragm injury.

Abdominal abscess in patient # 13 was drained percutaneously.

Patient # 15 had previously surgically untouched liver; however, he had incurable bilateral disease. One of the metastases on the right side (Sg. 8) was 5 cm in diameter and ingrowing into the inferior caval vein. One of the two metastases on the left side (Sg. 3 and Sg. 3-4) was in contact with left hepatic vein and therefore untreatable with radiofrequency ablation. In this patient we performed the right hepatectomy with partial resection of the inferior caval vein wall and the electrochemotherapy of the both metastases on the left side.

Patient # 16 had three metastases (Sg. 7, Sg. 8 and in Sg. 4a which was in contact with median hepatic vein). Patients' performance status and the size of the left liver did not allow the extended right hemihepatectomy (which would have been the only potentially curable surgical option), so the metastasectomies from Sg. 7 and Sg. 8 were performed, while the metastasis from the Sg 4a was treated with electrochemotherapy.

Having in mind all these procedures, as well as the fact that in groups I and II there were no hepatic complications, we anticipate that additional procedures which had to be performed in group III caused these complications and not the electrochemotherapy itself.

It is true that without electrochemotherapy none of these patients would have been operated and none of them would have had these complications; however, it is also true that without electrochemotherapy the best supportive care would have been their only option.