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Consensus Statement

Electrochemotherapy as treatment option for hepatocellular carcinoma, a prospective pilot study

Mihajlo Djokic [a], Maja Cemazar [b, c], Peter Popovic [d], Bor Kos [e], Rok Dezman [d], Masa Bosnjak [b], Martina Niksic Zakelj [b], Damijan Miklavcic [e], Stojan Potrc [f], Borut Stabuc [g], Ales Tomazic [a], Gregor Sersa [b, h, *], Blaz Trotovsek [a, **]

[a] University Medical Centre Ljubljana, Department of Abdominal Surgery, Zaloska 7, SI-1000 Ljubljana, Slovenia
[b] Institute of Oncology Ljubljana, Department of Experimental Oncology, Zaloska 2, SI-1000 Ljubljana, Slovenia
[c] University of Primorska, Faculty of Health Sciences, Polje 42, SI-6310 Izola, Slovenia
[d] University Medical Centre Ljubljana, Institute of Radiology, Zaloska 7, SI-1000 Ljubljana, Slovenia
[e] University of Ljubljana, Faculty of Electrical Engineering, Trzaska 25, Ljubljana SI-1000, Slovenia
[f] University Clinical Centre Maribor, Department of Abdominal Surgery, Ljubljanska ulica 5, SI-2000 Maribor, Slovenia
[g] University Medical Centre Ljubljana, Department of Gastroenterology, Zaloska 7, SI-1000 Ljubljana, Slovenia
[h] University of Ljubljana, Faculty of Health Sciences, Zdravstvena pot 5, SI-1000 Ljubljana, Slovenia

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ABSTRACT

Background and objectives: Electrochemotherapy provides non-thermal ablation of cutaneous as well as deep seated tumors. Based on positive results of the treatment of colorectal liver metastases, we conducted a prospective pilot study on hepatocellular carcinomas with the aim of testing the feasibility, safety and effectiveness of electrochemotherapy.
Patients and methods: Electrochemotherapy with bleomycin was performed on 17 hepatocellular carcinomas in 10 patients using a previously established protocol. The procedure was performed during open surgery and the patients were followed for median 20.5 months.
Results: Electrochemotherapy was feasible for all 17 lesions, and no treatment-related adverse events or major post-operative complications were observed. The median size of the treated lesions was 24 mm (range 8–41 mm), located either centrally, *i.e.,* near the major hepatic vessels, or peripherally. The complete response rate at 3–6 months was 80% per patient and 88% per treated lesion.
Conclusions: Electrochemotherapy of hepatocellular carcinoma proved to be a feasible and safe treatment in all 10 patients included in this study. To evaluate the effectiveness of this method, longer observation period is needed; however the results at medium observation time of 20.5 months after treatment are encouraging, in 15 out of 17 lesions complete response was obtained. Electrochemotherapy is predominantly applicable in patients with impaired liver function due to liver cirrhosis and/or with lesions where a high-risk operation is needed to achieve curative intent, given the intra/perioperative risk for high morbidity and mortality.

Introduction

Hepatocellular carcinoma (HCC), together with intrahepatic cholangiocarcinoma, represents more than 98.5% of all primary liver tumors, and its incidence is rising worldwide. HCC is the third most common cause of cancer-related deaths in the world and is responsible for between 650,000 and one million deaths globally per year [1–3].

The optimal treatment options for patients with HCC are curative surgical resection, liver transplantation and, in rare cases, radio-frequency ablation (RFA). Other methods, such as local ablative techniques (percutaneous ethanol (PEI) or acetic acid injection (PAI) in tumor, RFA, microwave ablation (MWA)), transarterial chemoembolization (TACE), targeted therapy, chemo- and radiotherapy and others are used only as palliative treatment and in

* Corresponding author. Department of Experimental Oncology, Institute of Oncology Ljubljana, Zaloska 2, SI-1000 Ljubljana, Slovenia.
** Corresponding author.
E-mail addresses: gsersa@onko-i.si (G. Sersa), blaz.trotovsek@kclj.si (B. Trotovsek).

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some cases as bridging therapy (TACE, RFA) for possible curative liver transplantation. Most patients with HCC are complex, with only 20% having straight forward treatment scenarios. Therefore, the majority of patients (more than 60%) receive some combination of therapies, depending on the stage of the disease [4–10]. Despite intensive surveillance programs, considerable recent therapeutic advances, and the use of potentially radical treatments, prognosis and life expectancy remain low in patients with HCC [11].

Electroporation-based treatments, including irreversible electroporation (IRE) and electrochemotherapy, are new local treatment approaches that are gaining importance. Electrochemotherapy already has an established place among other local treatments for the treatment of cutaneous tumors [12]. While IRE is already quite well established among other ablative techniques for the treatment of liver tumors, among those also for the HCC, the translation of electrochemotherapy into deep-seeded tumors is lagging behind [13]. However, the first encouraging results for the treatment of colorectal liver metastases have already been published [14,15]. The technological approach has been described, based on the standard operating procedures for the cutaneous tumors [16], with the aid of treatment planning and synchronization of delivery of electric pulses with ECG during open surgery [17]. At this stage of development, intraoperative placement of electrodes enables higher accuracy than in percutaneous approach which is used in irreversible electroporation [18–20].

Based on the encouraging clinical results, we conducted a prospective pilot clinical study to establish the feasibility, safety, and effectiveness of electrochemotherapy in the treatment of HCC. In the study, patients not amenable to other therapeutic ablative techniques were included. Furthermore, electrochemotherapy was also employed to treat tumors located in the vicinity of the major blood vessels where other ablative techniques, such as RFA or MWA, would not have been efficient due to the heat sink effect.

Patients and methods

Study design

The study was designed as a prospective, pilot study, was registered at ClinicalTrials.gov (NCT02291133) and had regulatory approvals from the National Medical Ethics Committee 21k/02/14. Patients were presented at the multidisciplinary team meetings consisting of a surgeon, radiologist and gastro-oncologist. Before inclusion into the trial, all patients signed written informed consent.

The primary endpoint of the study was to assess the feasibility and safety of electrochemotherapy in the treatment of HCC according to the Common Terminology Criteria for Adverse Events (CTC-AE) ver. 4.0. The secondary aim was to determine the efficacy of ECT, based on a radiological evaluation of treated lesions, as measured by modified Choi criteria [21].

Electrochemotherapy was performed according to the Standard Operating Procedures for treatment of cutaneous tumors and the associated modifications for the treatment of liver tumors and reporting of data according the published recommendations [15,22,23].

Patients

In this trial, 10 patients with 17 lesions were enrolled from February 2014 to November 2016, based on the inclusion and exclusion criteria (Table 1). The diagnosis was confirmed either histologically (in 4 patients) [24], or by typical radiological appearance according to the EASL–EORTC Clinical Practice Guidelines for Management of hepatocellular carcinoma [5].

Table 1
Inclusion and exclusion criteria for electrochemotherapy in HCC.

Inclusion criteria
1. HCC confirmed by radiological imaging and/or histology.
2. Age more than 18 years.
3. Life expectancy more than 3 months.
4. Performance status Karnofsky $\geq$ 70 or WHO (World Health Organization) < 2.
5. Signed informed consent.
6. Unanimous decision of the multidisciplinary liver tumor team before entering the trial (surgeon, gastro-oncologist and radiologist).
Exclusion criteria
1. Multiple primary tumors.
2. Extrahepatic disease.
3. Poor performance status.
4. Clinically significant ascites.
5. Exposure to cumulative bleomycin doses in excess of 400 mg.
6. Allergic reaction to bleomycin.
7. Impaired kidney function (Creatinine > 150 μmol/L).

Patients' characteristics are presented in Table 2 and are divided into three groups according to the indications and previous treatments.

The first group represented patients in whom the local ablative technique was unsuccessful (TACE/RFA) and electrochemotherapy was offered as an additional treatment. In this group, 3 patients with 6 lesions were treated. Two of these patients underwent TACE, which was unsuccessful (progress of the treated lesion at the follow up), and further treatment with TACE was not indicated. According to the Barcelona Clinic Liver Cancer (BCLC) algorithm [25], treatment for advanced or terminal stage disease should be offered. The third patient in this group had previously been treated with RFA, which was unsuccessful, and other ablative techniques were not indicated.

The second group included patients for whom transplantation, radical surgery or other local ablative techniques were not indicated due to patients' performance status, the location of the lesions, or contraindications to the ablative techniques. However, electrochemotherapy was performed with curative intent. In this group, 6 patients with 9 lesions were treated.

The third group included a patient for whom electrochemotherapy was offered as “bridging” to transplantation option. In this patient, 2 lesions were treated. During the first procedure in 2009, right hemihepatectomy with resection of middle and right hepatic veins in un-cirrhotic liver was performed due to HCC. In 2015, the patient developed 2 new HCC lesions located in proximity to the left hepatic vein, so electrochemotherapy was offered to the patient as a “bridging” to liver transplantation.

Lesions were defined as “central” or “peripheral” based on their relation to the major blood vessels. The term “central” was used for the lesions in the close vicinity of the major blood vessels including the main hepatic or portal veins and the main hepatic arterial branches. The term “peripheral” was used for lesions not adjacent to the major blood vessels where RFA or other ablative techniques were not indicated by interventional radiologist blinded to the study [26,27].

Treatment procedure

All 10 patients enrolled in the study were treated during open surgery. Median laparotomy, extended to the right subcostal incision, was performed in 8/10 patients. In 2 patients, only upper median laparotomy was used.

The electrodes used for electric pulse delivery were either single long (20 cm) needle electrodes (variable geometry) with 3 or 4 cm active part or 7-needle electrodes fixed on the holder in hexagonal geometry and 3 cm active part [28,29]. The choice of electrode use was dependent on the location of the lesion. Electrodes with

Table 2
Patients, clinical features, tumor characteristics, response to treatment and postoperative course.

Patient					Previous treatments		Tumor characteristics			Electrodes used		Tumor response to ECT treatment				Clinical outcome
No.	Age	Sex	Child-Pugh score	Esophageal varices	Type	Days before ECT	Tumor size (mm)[a]	Position related to the major vessels	Liver segment	Geometry Electrodes used	No. of electrodes/No. of applications[b]	1st radiological evaluation	2nd radiological evaluation	Last radiological evaluation		
												1 month after ECT	3-6 months after ECT	Months after ECT	Response	
***Group I: Previous local ablative techniques unsuccessful*:**																
3	73	M	A	Yes	TACE	167	14	Peripheral	8	Fixed	7/3	CR	CR	12	CR	Progress with new liver lesions; treated with TACE
5	73	F	B	No	TACE	105	35	Central	8	Variable	5/1	CR	CR	19	CR	Progress with new liver lesions; treated with TACE
9	68	M	B	Yes	RFA + TACE	445	30	Central	8	Fixed	7/4	CR	CR	/	/	Patient died after the second radiological evaluation due to variceal bleeding
							20	Peripheral	5	Variable	5/1	CR	CR			
							16	Peripheral	5	Fixed	7/1	CR	CR			
							12	Peripheral	8	Fixed	7/1	CR	CR			
***Group II: Other treatment options not indicated*[c]:**																
1	78	F	A	No	None		40	Central	6	Variable	5/4	PR	SD	/	/	Patient died after the second radiological evaluation due to pneumonia
2	57	M	A	Yes	None		32	Peripheral	4a	Fixed	7/6	CR	CR	12	CR	No progression of the disease
							26	Peripheral	4b	Fixed	7/2	CR	CR		CR	
4	67	M	B	Yes	None		25	Peripheral	2	Fixed	7/3	CR	CR	22	CR	No progression of the disease
7	75	M	A	Yes	None		22	Peripheral	8	Fixed	7/4	CR	CR	21	CR	Progress with new liver lesions; treated with TACE
8	71	M	B	Yes	None		40	Central	8	Variable	5/2	CR	CR	31	CR	No progression of the disease
10	68	M	B	Yes	None		41	Central	4a	Variable	5/1	PR	SD	20	SD	No progression of the disease
							16	Central	6	Variable	5/1	CR	CR		CR	
							8	Peripheral	3	Fixed	7/1	CR	CR		CR	
***Group III: Bridging to transplantation*:**																
6	63	F	A	No	OP	6 years	24	Central	2	Variable	5/1	CR	CR	27	CR	No progression of the disease
							8	Central	2	Variable	4/1	CR	CR		CR	

No. – number; ECT – electrochemotherapy; TACE – transarterial chemoembolization; RFA – radiofrequency ablation; OP – operative treatment; CR – complete response; PR – partial response; SD – stable disease; PD – progress of the disease.

[a] Median tumor size 24 mm (range 8–41 mm).

[b] The electrodes were repositioned in the treatment of the specific tumor in order to cover the whole tumor area if necessary.

[c] Due to the patients' performance status, the location of the lesions (for example close vicinity of large vessels), or contraindications to the ablative techniques.

variable geometry *i.e.,* long needle electrodes were used for deep-seated tumors located more than 3 cm below the surface of the liver. The hexagonal electrodes were used for more superficial tumors that had their deepest margins less than 3 cm from the liver surface [28,29]. Specifically, the treatment of lesions in segment 8 included the use of both types of the electrodes, where for the use of hexagonal electrodes mobilization of the liver was required.

The intraoperative ultrasound was used to identify lesions and aid the positioning of the electrodes into and around the tumor. The long needle electrodes were positioned according to the pre-treatment plan prepared for each patient and specific tumor individually using previously developed procedures [30,31]. Plans were developed based on computed tomography and/or magnetic resonance scans taken less than 30 days prior to treatment. Target lesions (up to 41 mm in the largest diameter) were segmented. 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 the volume of healthy liver parenchyma above the irreversible electroporation threshold (700 V/cm)—see Supplementary Data I: An example of the treatment plan.

The intravenous bolus of bleomycin was given to the patient after the intra-operative ultrasound confirmed the correct electrode placement. Trains of eight electric pulses (electrodes with variable geometry) or 24 (fixed geometry) electric pulses (each pulse 100 μs long) were delivered to each pair of electrodes consecutively (Supplementary Data I). 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 (Bleomycin medac, Medac, Hamburg, Germany), as being determined to be the optimal pharmacological peak for the bleomycin in the tumors [16]. Maximal patient safety was achieved by synchronization of the delivery of electric pulses with the absolute refractory period of the heart to prevent the electrical pulses from being delivered during the vulnerable ventricle period [32].

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 h after the surgery using an ambulatory ECG device (SpiderView, ELA Medical, France).

Efficacy assessment based on radiology

Lesions treated in the study were assessed before electrochemotherapy by contrast-enhanced computed tomography (CECT) or with magnetic resonance imaging (CEMRI) using a distinct hepatocyte contrast (gadoliniumethoxybenzyl-diethylenetriaminepentaacetic acid—Gd-EOB-DTPA, Primovist, Bayer, Berlin, Germany). The follow-up was performed by CECT in all the patients but one in whom the CEMRI was performed at the 3-months follow-up. Images were evaluated by two radiologists, one of whom was blinded to the trial. Modified Choi criteria were used to assess the treatment response [21]. Evaluations of both radiologists were in complete consensus.

Statistical analysis

All analyses are descriptive. According to type and level of distribution, the data are presented as medians, means, standard deviations (SD), percentages and frequencies, as appropriate.

Results

Tumor and treatment characteristics, clinical features and lesion response rates are presented in Table 2. Feasibility, safety, and response to treatment evaluation were evaluated for all 10 patients and 17 lesions.

Feasibility

Electrochemotherapy was feasible in all 10 patients enrolled in the study, according to the inclusion and exclusion criteria (Table 1). Three females and 7 males were included, with a median age 69.5 years (range: 57–78 years). Three patients had undergone previous treatment with TACE and/or RFA, and one had undergone liver resection. Six patients had received electrochemotherapy as a primary treatment. The previously operated patient had electrochemotherapy as a bridging procedure for liver transplantation. Patients were treated according to the Standard Operating Procedures for cutaneous tumors, modified for the liver tumors during open surgery [16,28]. Electric pulses were delivered either by electrodes with fixed geometry for superficially located tumors (9/17 lesions) or with long needle electrodes for deeper seated tumors (8/17 lesions). Treatment of tumors with long needle electrodes was performed according to the prepared treatment plan for each lesion to predict electrode positioning and the electrical parameters (Fig. 1). Delivery of electric pulses with either fixed or long needle electrodes was synchronized with ECG [32]. The median size of the treated lesions was 24 mm with a range of 8–41 mm. Electrochemotherapy was also feasible in patients with centrally located lesions in the vicinity of major hepatic vessels (8/17 lesions).

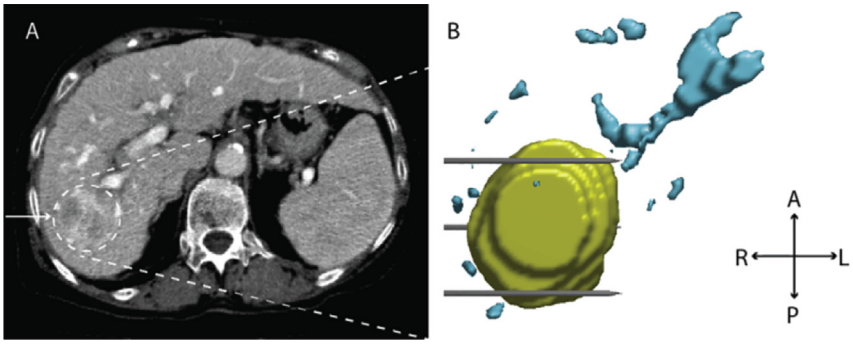

Fig. 1. Tumor located near the portal vein and right hepatic vein (A), 41 mm in diameter treated by long needle electrodes positioned according to the treatment plan (B).

Safety

Adverse reactions related to electrochemotherapy did not occur, despite the American Society of Anesthesiologists (ASA) score of 3 (8/10 patients) and ASA score of 2 (2/10 patients). No intraoperative or postoperative complications during the first 24 h occurred. The exceptions were the two patients with the ascites production after the procedure due to transient liver failure. Ascites was resolved by conservative measures. Nevertheless, patients were classified as Clavien-Dindo 3a and 3b because in both additional diagnostic/intervention was necessary: in one patient the ERCP was performed due to choledocholithiasis; the second patient had elevation of cholestatic enzymes and the endoscopic ultrasound was performed to clarify the origin of elevated enzymes. The impaired liver function was a result of liver cirrhosis before the operation and was not related to the electrochemotherapy. All 10 patients were discharged from the hospital after a median hospitalization of 5.5 days (range: 2–20 days) and were followed on an outpatient basis.

ECG signals were recorded during and 24 h after the electrochemotherapy. No onset of new or worsening of existing pathological morphological changes was recorded. There were no signs of myocardial ischemia, new-onset of atrial and/or ventricular extrasystoles, or increased frequency of abnormal heartbeats in relation to the electrochemotherapy procedure.

Centrally located tumor lesions (8/17) near major hepatic vessels were successfully treated without adverse events.

Effectiveness

The first radiologic follow-up was 1 month after treatment (median 31 days; range from 23 to 45 days). All 17 lesions were evaluated (Table 2), and a complete response was found in 15/17 lesions (88%). Two lesions had a partial response according to the modified Choi criteria, due to the small field of enhancement, without changes in lesion size.

The second radiological follow-up was 3–6 months after the treatment (median 194 days; range from 100 to 218 days). All 17 lesions were evaluated, and according to the modified Choi criteria, all of the lesions that had been initially evaluated after 1 month, i.e., the 15/17 lesions, remained in complete response. Two lesions identified in the previous follow-up as partial responses (2/17, 12%) remained in the stage of partial response and the patients were considered being in stable disease. An example of the complete response to electrochemotherapy is presented in Fig. 2.

The last radiological follow-up was 12–31 months after the treatment (median 626.5 days (20.5 months); range from 352 to 930 days). All treated lesions remained in the same radiological response as at the second follow-up.

The tumors treated with the long needle electrodes (variable geometry) were larger (median diameter 35 mm) than tumors treated with fixed electrodes (median diameter 20 mm). Eleven lesions were smaller than 3 cm in diameter and responded completely and were treated either with electrodes with fixed or variable geometry. However, 6 lesions that were larger than 3 cm in diameter were predominantly treated with electrodes using variable geometry, and 2/6 treated lesions responded partially and were also located centrally. These two patients were followed according to the Guidelines for treatment of hepatocellular carcinoma [33].

The complete response rate of the treated lesions after electrochemotherapy per patient was 80% (8/10) at the 3–6 months as well as at the last follow up.

Discussion

This study confirmed the feasibility, safety and effectiveness of electrochemotherapy in the treatment of HCC. No treatment or postoperative adverse events were recorded, including in patients with lesions located near the major hepatic vessels. The overall response was high, 88% (15/17) of the treated lesions and 80% (8/10) of patients had complete responses.

The feasibility and safety of electrochemotherapy was already demonstrated in a previous study on the treatment of colorectal liver metastases. The response rate of that study was 85% complete responses on 29 metastases in 16 patients which is comparable to the 88% complete response rate in this prospective pilot study on HCC [15]. The technology that has proven to be feasible and safe in the previous study was also confirmed in this study and another recently published study [14], in cases where other ablative techniques are not indicated. The technology is adopted for the treatment of deep seated tumors with the use of long needle electrodes that are inserted individually around and centrally into the tumor. Placement is aided with US guidance and electrodes are placed according to the treatment plan [15]. The pre-treatment plan was used in treatment of deep seated tumors, as well as in tumors larger than 3 cm in diameter and those in close vicinity of the major hepatic vessels [34,35]. Both the previous and the current study provided evidence that such a technological approach is feasible and safe with a high tumor response rate.

In this study, not only patients with previously unsuccessfully treated tumors, but also patients for whom standard treatment with curative intent could not be offered were included. In one patient, this method was used as a bridging to liver transplantation. Two patients had post-operative complications in the form of transient liver function failure with consequent ascites production. Based on poor performance status of the recruited patients in

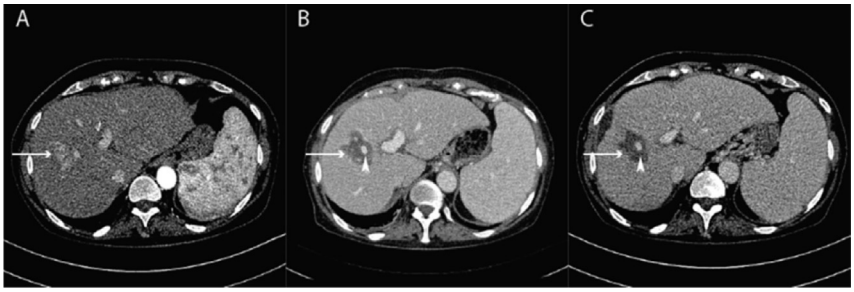

Fig. 2. HCC before and after successful treatment with electrochemotherapy. A: before the treatment with tumor indicated by long arrow. B: 1 month after electrochemotherapy. The edematous rim (long arrow) and unobstructed hepatic vein are visible. C: Four months after electrochemotherapy, shrinkage of the treated volume is visible (long arrow) whereas the vein remains unobstructed (short arrow).

whom other treatments were not feasible, electrochemotherapy provides effective treatment of lesions in such patients. Therefore, it could be considered as a technique with curative intent, which, however, needs to be confirmed in a phase II study in a larger cohort of patients.

One of the limitations of this approach is that larger tumors tend to have lower response rates. As indicated by several other studies, tumors larger than 3 cm in diameter seldom have complete responses [36,37]. The method in our case was upgraded by the treatment plan that intended to predict the optimal electrode placement for effective electroporation of the tumors. This certainly aided better execution of the electroporation of lesions larger than 3 cm in diameter, but the step for effective verification of the tumor coverage after electroporation is still missing. This can be executed either by US verification, as noted in IRE and also by electrochemotherapy, or by development of measuring tools for the current distribution in the tumors by MRI, which is still in progress [38–40].

HCC were already effectively treated by IRE, where a similar response rate and limitations on the size of the tumors were observed [41]. IRE has the advantage of relying only on tumor cell destruction by electroporation without the drug, as electrochemotherapy does [42,43]. The advantages and disadvantages of either approach can be discussed. However, the rationale between their modes of action is obvious; IRE has an immediate and predominantly ablative mode of action [42]. Electrochemotherapy executes its antitumor action by the drug itself (e.g., bleomycin) that drives the cells into mitotic cell death. Therefore, the tumors resolve slowly without immediate tumor necrosis [44,45]. This may prove to be an advantage for electrochemotherapy over IRE. A combined approach has however been suggested, which could enable larger treatment volumes [46,47].

Technologically, IRE can also be performed percutaneously, which is an advantage, but the first report of percutaneous electrochemotherapy for the resolution of tumor thrombi in large vessels in the liver has also been published [48]. Percutaneous insertion of electrodes needs, however, further technological advancements and navigation guided placement of electrodes would be among these solutions [18–20]. When these technological advancements will be feasible, the effectiveness of electrochemotherapy and IRE on comparable groups of patients and tumors will be possible.

The advantage of electroporation-based technology, such as IRE and electrochemotherapy, is that these are non-thermal techniques that are effective on tumors that are near large vessels where RFA is not indicated. The safety and effectiveness of electrochemotherapy and IRE has been demonstrated in such cases [49]. Additionally, this study demonstrated that tumors located near the major vessels responded with complete responses, even though they were larger than 3 cm in diameter. This further supports the previous observations in the treatment of liver colorectal metastases [14,15,50].

This study, however, has some limitations. Based on the fact that it is a pilot study and on small cohort of patients, only ten, the conclusion about the effectiveness of electrochemotherapy is premature. Nevertheless, the preliminary data foster the continuation of this study in phase II one.

In conclusion, this study is the first prospective pilot study to demonstrate feasibility, safety and provides preliminary data on the high effectiveness of electrochemotherapy in HCC. Electrochemotherapy could be predominantly applicable in patients with impaired liver function due to liver cirrhosis and/or with lesions where a high-risk operation is needed to achieve curative intent.

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Conflict of interest statement

All authors declare no conflict of interest, except Damijan Miklavcic, who holds patents on electrochemotherapy that have been licensed to IGEA S.p.a. and is also a consultant to IGEA.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejso.2018.01.090.

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