



Electrochemotherapy: A New Technological Approach in Treatment of Metastases in the Liver

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Electrochemotherapy is now in development for treatment of deep-seated tumors, like in bones and internal organs, such as liver. The technology is available with a newly developed electric pulse generator and long needle electrodes; however the procedures for the treatment are not standardized yet. In order to describe the treatment procedure, including treatment planning, within the ongoing clinical study, a case of successful treatment of a solitary metastasis in the liver of colorectal cancer is presented. The procedure was performed intraoperatively by inserting long needle electrodes, two in the center of the tumor and four around the tumor into the normal tissue. The insertion of electrodes proved to be feasible and was done according to the treatment plan, prepared by numerical modeling. After intravenous bolus injection of bleomycin the tumor was exposed to electric pulses. The delivery of the electric pulses did not interfere with functioning of the heart, since the pulses were synchronized with electrocardiogram in order to be delivered outside the vulnerable period of the ventricles. Also the post treatment period was uneventful without side effects. Re-operation of the treated metastasis demonstrated feasibility of the reoperation, without secondary effects of electrochemotherapy on normal tissue. Good antitumor effectiveness with complete tumor destruction was confirmed with histological analysis. The patient is disease-free 16 months after the procedure. In conclusion, treatment procedure for electrochemotherapy proved to be a feasible technological approach for treatment of liver metastasis. Due to the absence of the side effects and the first complete destruction of the treated tumor, treatment procedure for electrochemotherapy seems to be a safe method for treatment of liver metastases with good treatment effectiveness even in difficult-to-reach locations.

Key words: Electrochemotherapy; Liver metastases; Colorectal cancer.

Introduction

Electrochemotherapy is a local treatment that uses electroporation of the tumors to increase uptake of cytotoxic drugs, such as bleomycin or cisplatin (1). In the case of bleomycin, up to a 1000-fold increase in cytotoxicity was observed (1-3). Currently electrochemotherapy is used in treatment of cutaneous and subcutaneous tumors of different histological types with response rate of 80% and long lasting complete responses of 70% (4, 5). The treatment has been predominantly used with palliative intent for melanoma metastases and other cutaneous tumors, whereas the colorectal liver metastases (CRLM) have not been treated yet. In a preclinical *in vitro* study on CMT-93 colorectal carcinoma cells it was demonstrated that exposure of cells to electric field potentiates cytotoxicity of bleomycin 500-fold (6).

Abbreviations: CRLM: Colorectal Liver Metastases; IVC: Inferior Vena Cava; Sg: Segment; MHV: Middle Hepatic Vein; LHV: Left Hepatic Vein; sRHV: Superior Hepatic Vein; US: Ultrasound; CEA: Carcinoembryonic Antigen; H&E: Haematoxylin and Eosin; ECG: Electrocardiogram.

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Further development of electrochemotherapy is oriented into treatment of bigger as well as deep-seated tumors; in internal organs or at least 1 cm below the skin. The first reported case was treatment of deep-seated melanoma metastasis in the thigh (7), in which the treatment feasibility was demonstrated, but also a sensitivity study was performed which demonstrated that positioning of electrodes with respect to the initial treatment plan is important for the success of the treatment (8). The treatment of deep-seated tumors is now possible with the new electroporation device and the newly developed long-needle electrodes. In contrast to cutaneous tumor lesions, the abdominal tumors (such as metastases in the liver) are located in electrically highly conductive medium and the treatment region can be in close proximity of the heart. Therefore, the delivery of electroporation pulses should be synchronized with the refractory period of the cardiac cycle to minimize the probability of interaction of electric pulses with the heart function (9-11).

CRLM located in between inferior vena cava (IVC) and the main hepatic veins represent a challenge for a liver surgeon. Although the paracaval region and caudate process in the angle of the main hepatic veins inflow into IVC can be approached with segmental resection of segment 1 (Sg 1) it is often impossible to achieve a sufficient safety margin due to veins proximity. Available alternatives depend on the hepatic veins involved: if both, the middle (MHV) and left hepatic veins (LHV) are involved or the common trunk is involved, extended left hemihepatectomy together with Sg 1 (caudate lobe) is possible. When the superior right hepatic vein (sRHV) and MHV are involved and the LHV is unaffected, the right trisectionectomy may be performed (12, 13). It has become accepted as surgical standard that as much liver tissue as possible is preserved in treatment of CRLM (14). Another possibility; if both sRHV and common trunk are involved, in rare cases, when there are strong inferior and/or middle right hepatic veins present (15), the liver resection which includes left hemihepatectomy and Sg 8 and Sg 7 with preservation of Sg 5 and Sg 4 is possible. Using ablative method like RFA in this region has limitations because of the heat sink from cooling effect of hepatic veins (16-18).

Here we describe for the first time the treatment procedure of electrochemotherapy of CRLM, in a case of a patient with liver metastasis in between IVC and the origin of the main hepatic veins. We demonstrate the feasibility of the procedure and describe the procedure steps needed for electrochemotherapy, safety of the treatment and the treatment effectiveness.

Material and Methods

Clinical Study

In the on-going Phase I/II study (EudraCT number 2008-008290-54; ClinicalTrials.gov (NCT01264952)) so far

8 patients were recruited: 5 patients with CRLM in both hemilivers planned for two-step surgical procedure and 3 patients with solitary metastasis on the MHV inflow to the IVC. The study was approved by Institutional Medical Board and Ethical Committee of the Republic of Slovenia.

Patient

The patient was a 55 years old lady who had been operated for sigmoid colon carcinoma (July 2007), stage pT3, N2 (6 positive out of 19 removed lymph nodes), M0. A R0 resection of the colon was performed, followed by 8 cycles of chemotherapy with Capecitabine. After 24 months a hypoechoic lesion close to IVC was found on ultrasound (US), measuring 24 × 21 mm. The lesion was confirmed to be a metastasis by the PET/CT and MRI scans. It was located in between the IVC and the main hepatic veins. The metastasis increased in a month to 35 × 20 mm. Due to its direct contact with IVC, MHV and sRHV, the metastasis was considered as non-resectable. Chemotherapy (capecitabine and oxaliplatin (XELOX) + bevacizumab) was introduced. After three cycles of chemotherapy, in the mid of November 2009, MRI using liver specific contrast Gd-EOB-DTPA showed partial downsizing of the lesion to 34 × 15 mm. It was still close to IVC and main hepatic veins; however there was a possibility that it could have become resectable. RFA was not considered as an option due to the proximity of the IVC and main hepatic veins and their cooling effect. The patient was offered an exploration and electrochemotherapy if the metastasis would still be found non-resectable during the surgery in the end of December 2009. The patient agreed and signed informed consent for electrochemotherapy.

Radiology of the Metastasis

Before electrochemotherapy, the liver metastasis was evaluated by contrast-enhanced Gd-EOB-DTPA MRI of liver. Pre contrast enhanced images included in- and opposed phase transversal images, T1W transversal images and T2W transversal and coronal images. Contrast enhanced images were obtained in late arterial (25 s), portal-venous (70 s) and late phase (5 and 20 min) in transversal and coronal planes. Post treatment the effect was evaluated by multislice contrast enhanced computed tomography (CT) in late portal phase (70 s), 5 mm slice reconstructions were made, in axial and coronary planes.

Treatment Planning

Prior to surgery and electrochemotherapy, numerical treatment planning was performed using a method established and reported earlier (7, 8, 19). Briefly, a 3-D model geometry was built based on segmented MRI images of the patient as described previously (20). The images were segmented into

three tissues: liver, tumor and blood vessels, the IVC and the main hepatic veins. Next, several different electrode configurations were designed in consultation with the surgeon based on the limited number of possible access routes. Using an optimization algorithm coupled with a finite-element model of electroporation, the minimum required voltage for each electrode pair in each electrode configuration was computed to guarantee adequate electric field distribution in the tumor, as this is the major indication for successful electroporation (21). In contrast to previous work (7), a gradient optimization algorithm was used to optimize the voltages between each electrode pair, while positions of electrodes were determined by using the so-called forward modeling approach. Finally, the optimal design using 6 electrodes was used, as it provided the most robust treatment, and the surgeon was able to execute the plan.

Electrochemotherapy

On the exposed liver the needle electrodes (1.2 mm in diameter with 4 cm non-isolated tip length) were inserted into the metastasis under US guidance. The positioning followed the provided treatment plan. The electrodes were connected to the electric pulse generator (Cliniporator *VITAE*, IGEA SpA, Carpi, Italy). Thereafter, the patient was given 15,000 U/m² of bleomycin (27.45 mg) intravenously in bolus. Eight electric pulses of 100 μs duration were delivered between pairs of electrodes 8 min after the bleomycin injection, when the maximal pharmacological peak of bleomycin in the metastases was expected (22). The amplitudes and protocol of electric pulses delivery according to the treatment plan for this particular metastasis is given in Table I.

ECG Synchronization

Delivery of electroporation pulses was synchronized with electrocardiogram (ECG); one pulse per heart-beat was delivered. Namely, Cliniporator *VITAE* provides an option for

synchronization of electroporation pulse delivery with ECG. The ECG triggering device AccuSync 42 (AccuSync Medical Research Corp., Milford, CT, USA) was used. The AccuSync is a 3-lead electrocardiograph which detects the R-wave from one of the preselected standard leads early on the ascending slope of the R-wave. ECG lead II was selected due to prominence of the R wave. This ECG signal was acquired independently of the regular ECG monitoring performed by the anesthesiologist. The Cliniporator *VITAE* is programmed to deliver a single electroporation pulse 50 ms after receiving a valid trigger from the AccuSync (provided that the latest R-R interval was within the 0.5-3.5 s range) thus avoiding the so-called vulnerable period of the ventricles, the T wave. Both the ECG signal used for synchronization and the trigger signal were recorded and stored for post processing and further analysis.

Surgery

1st Operation – Electrochemotherapy: One month after the third cycle of chemotherapy (XELOX + bevacizumab), the patient was operated. Despite the downsizing which was visible on MRI images, intraoperative US assessment still showed direct contact of the metastasis with the IVC, and main hepatic veins at their origin, so the lesion was considered non-resectable. Due to the reasons explained earlier, RFA was not an option, so electrochemotherapy remained the only possibility. After the mobilization of the left liver, the area between IVC and the origin of the main hepatic veins was exposed. The electrodes were placed and electrochemotherapy was performed, as described. No single adverse effect was noted and blood loss was minimal. Postoperative course was uneventful and the patient was discharged from hospital on the day 10. After this operation patient did not receive any systemic treatment.

2nd Operation – Excision of the Metastasis: Due to changes visible on CT scan (homogenously hypo dense lesion, without

Table I

Summary of planned voltages, number of pulses and predicted currents based on numerical model as well as the actually delivered voltages, number of pulses and measured currents.

Electrode pair	Voltage according to plan [V]	No. of pulses according to plan	Predicted current [A]	Delivered voltage [V]	Delivered No. of pulses	Measured current [A]
1-5	2100	8	31	1300	20	32.3
1-6	2100	8	26	2100	8	45.2
2-5	2100	8	26	1700	21	44.7
2-6	2100	8	25	2100	8	48.3
3-5	2100	8	25	2100	8	48.9
3-6	2100	8	29	1900	8	48.8
4-5	2100	8	28	2100	8	47.5
4-6	2100	8	33	1700	16	41.2
5-6	1700	8	40	1700	8	48.9
Total		72			105	

changes in size) the second operation was performed three months after the first one. Furthermore, intraoperative US scan showed that the metastasis was hypo-echogenic, which was interpreted as probable necrotic changes. The metastasis was found no longer firmly fixed to the surrounding structures, so it was decided to excise it. Postoperatively, a moderate subcutaneous wound infection occurred, which was treated with partial wound dehiscence. No other adverse effects were noted.

Histology

The excised tissue was fixed in 10% buffered formalin. After the macroscopic examination, the specimen was sectioned and entirely taken for microscopic examination. Tissue was embedded in paraffin; 3 μ m thick sections were cut and stained using haematoxylin and eosin (H&E). Immunohistochemical studies were performed by peroxidase avidin-biotin method using the formalin fixed and paraffin embedded material. The following primary antibodies were used: against carcinoembryonic antigen (CEA) (DAKO, Denmark; polyclonal; dilution 1:8000) and CK20 (DAKO, Denmark; monoclonal; dilution 1:20) for staining tumor tissue and Hepar (DAKO, Denmark; polyclonal; dilution 1:20) for staining liver tissue.

Results

Location of the Liver Metastasis

The metastasis treated with electrochemotherapy was confirmed by MRI using liver specific contrast Gd-EOB-DTPA before the operation (Figure 1). Twenty minutes after contrast application, a metastasis (34 mm \times 15 mm) located in the paracaval region and caudate process, in contact with IVC and main hepatic veins was identified. No other lesions were seen in the liver.

Treatment Plan

Several treatment plans were prepared based on the MRI of the patient. The treatment plans were evaluated based on the quality of the predicted electric field distribution (for details see ref. 7) and on how difficult it would be for the surgeon to execute the plan. Finally, the setup with 6 electrodes presented in Figure 2 was selected for the treatment, with other treatment plans, including those with 4- and 5-electrode configurations (not presented) also prepared for back-up purposes. The final voltages and predicted currents, as well as actually delivered voltages and currents are shown in Table I with an overlay of the computational model and MRIs of the patient anatomy shown in Figure 3A. Two electrodes were positioned centrally in the tumor in order to provide sufficiently high electric field in the center of the tumor. The other 4 electrodes were positioned around the tumor in the normal liver tissue in order to provide the treatment of safety margins. These peripheral electrodes were positioned approximately 1-2 mm away from the tumor tissue.

Treatment Procedure

Identification of Metastasis and Preparatory Procedures Needed Before Delivery of Electric Pulses: During the operation in general anesthesia (December 2009) the left liver was mobilized, so that electrochemotherapy could be performed. The exact location of the tumor as well as the location of needle insertion according to the treatment plan was intraoperatively verified by US. Insertion of the needle electrodes was attempted to be as close as possible according to the treatment plan. The exact location of the electrodes was determined and was found later on to be in close match to the predetermined locations (Figure 2). Electrodes were inserted into the tumor and around it without any problem and without injury of any major blood vessel (Figure 3B).

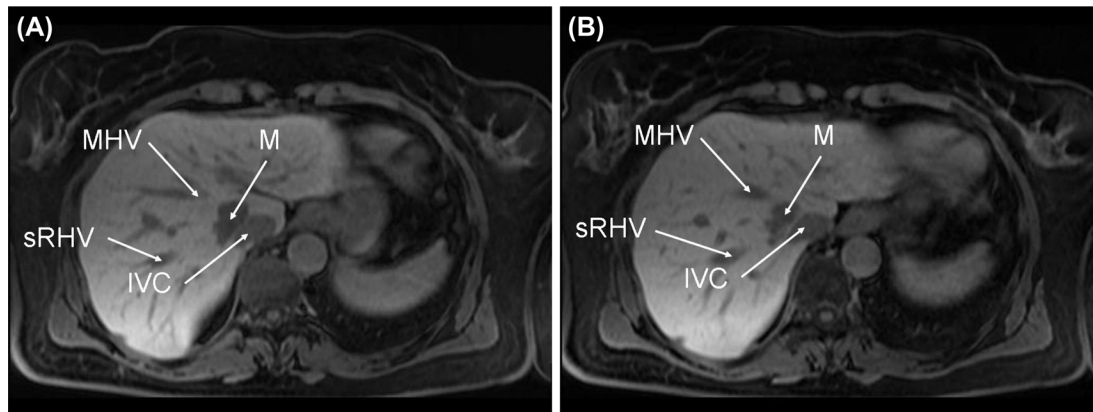


Figure 1: Axial T1W MRI image, showing a hypointense lesion (M) in between IVC and sRHV and MHV, in late liver phase, 20 min post Gd-EOB-DTPA. Images A and B are two consecutive images in 5 mm slice thickness.

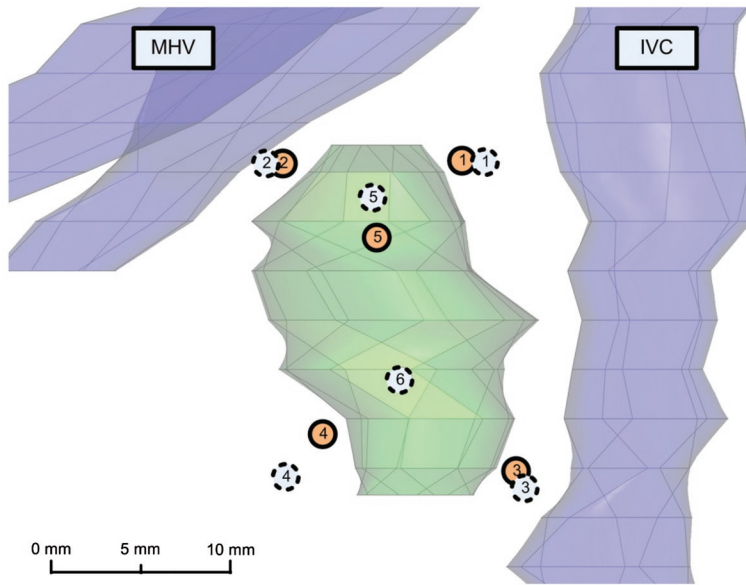


Figure 2: Design of treatment plan. The Figure shows the location of the tumor (green color) between the IVC and main hepatic veins (MHV, IVC, blue color). The solid circles represent electrode locations according to the original treatment plan and the dashed circles represent reconstructed electrode positions achieved *in situ*. The electrode 6 is in the same location in both cases.

After insertion into tissue the electrodes were connected to the Cliniporator *VITAE*, with special attention to correct wiring between the electrodes and the appropriate channel ports on the Cliniporator *VITAE*. Thereafter bleomycin (15000U/m² of the patient) was injected intravenously in bolus. After 8 minutes, the time needed by the circulating bleomycin to

reach the pharmacological peak in the tumor, the preparations for delivery of electric pulses were completed.

Delivery of Electric Pulses: The generic electroporation procedure is described first. The delivery of electroporation pulses is always preceded by a sequence of low-voltage pre-pulses.

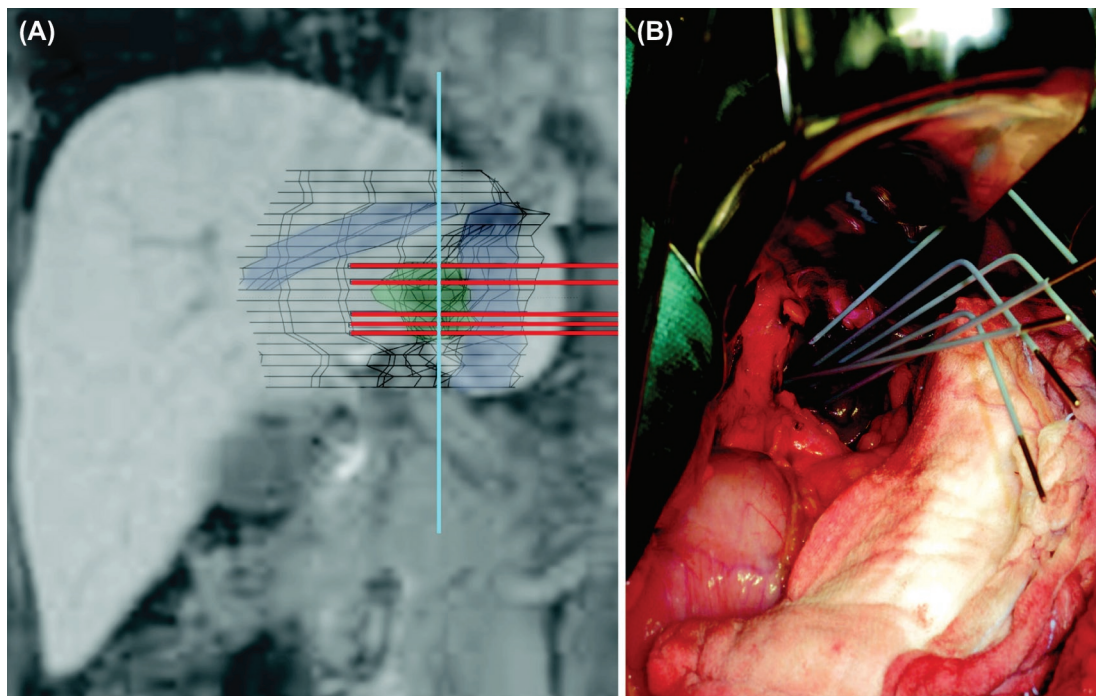


Figure 3: **A:** Overlay of the computational geometry and patient's anatomy. The red lines represent the direction of insertion of the electrodes, while the blue line represents the cross-section of Figure 2. **B:** Photograph of the surgical setup with electrodes penetrating into the tumor is clearly seen (cables not connected).

One pre-pulse is delivered to each pair of electrodes which is later to be used for actual electroporation according to the treatment plan. The purpose of pre-pulses is to verify the connections between the electrodes and Cliniporator *VITAE* outputs and also to predict (based on the current measured at low voltage) the current levels for the imminent electroporation pulses. For any pair of electrodes for which either a poor connection is found or the predicted current level exceeds 50 A (upper limit of the Cliniporator *VITAE*), the delivery of electroporation pulses is automatically suspended. Immediately after completion of the pre-pulse sequence, the high-voltage electric pulses, 8 per electrode pair, are delivered synchronized with the ECG (see the following subsection for details). The Cliniporator *VITAE* automatically suspends the delivery of electroporation pulses for: a) electrode pairs for which the pre-pulse sequence resulted in invalid values (current too low or too high); and b) for electrode pairs for which the actual current measured during electroporation itself exceeds the upper limit of 50 A, even though the predicted value based on the pre-pulse was below this limit. This completes the electroporation procedure. However, if needed, the procedure must then be repeated for all electrode pairs for which the delivery was suspended. Depending on the reason of suspension, the connections between the electrodes are checked and/or parameters of electroporation pulses are adjusted (voltage must be lowered if either the predicted or actual current exceeded 50 A). The whole electroporation procedure is then repeated just for the remaining electrode pairs. Sometimes more than one repetition may be required.

In our case, in the original treatment plan, 72 pulses were planned. However, a total of 105 electroporation pulses were delivered, because the electroporation procedure had to be repeated 5 times to complete delivery of all pulses. The larger number of actually delivered electroporation pulses is explained in the discussion. The entire electroporation procedure was finished 23.5 min after injection of bleomycin, *i.e.* within 15 minutes.

ECG Synchronization: The triggering of electric pulses was synchronized with ECG signals, through the ECG triggering device AccuSync, as described in previous section. The Cliniporator *VITAE* delivered one electroporation pulse per valid trigger pulse, which means that there was one pulse per heartbeat delivered. Whenever there was a transient loss of the ECG signal (due to ECG artifacts caused by muscle contractions after the previous pulse), the Cliniporator *VITAE* temporarily suspended pulse delivery until ECG and valid trigger pulse sequence were restored. The subsequent analysis of ECG signals showed that the synchronization procedure implemented in the Cliniporator *VITAE* resulted in safe and uneventful delivery of the pulses since all pulses were delivered outside the vulnerable period of the ventricles.

The preliminary evaluation of ECG signals recorded during the entire surgical procedure also revealed no heart arrhythmias or any other pathological morphological changes in any of the recorded signals either during or immediately after the application of pulses. However, some transient and statistically significant changes in the duration of some intervals in the PQRST complex were observed during the heartbeats coinciding with the delivery of electroporation pulses. Currently the practical significance of these changes (if there is any) is not clear and can only be elucidated when data from more patients treated in similar conditions become available.

Safety of Treatment

During and after the electrochemotherapy, there were no adverse effects which could be attributed to the procedure itself. Postoperative course was uneventful and the patient was released from the hospital on day 10.

Post Treatment Follow-up and Treatment

Two months after electrochemotherapy CT was performed. In the paracaval region toward the Sg 1 there was a homogenous hypodense ovoid lesion (30 mm × 15 mm) without any new lesions (Figure 4).

There was no change in size of the metastasis treated with electrochemotherapy, however, CT image showed that margins were blurred, which demonstrated that treatment had some effect (Figure 4). At that time it was unclear what kind of the effect it was. The patient was suggested another exploration, which was accepted and the operation was performed three months after the first one. Intraoperative US examination showed hypo-echogenic changes, probably caused by necrosis. We mobilized left and right hemiliver and resected the part of paracaval region with Sg 1, preserving the main

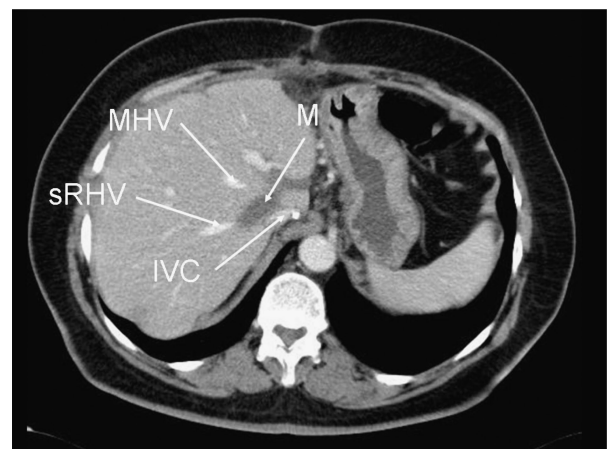


Figure 4: Axial CT image taken before the metastasis (M) was removed shows homogenous hypodense lesion in paracaval region toward Sg 1 (30 mm × 15 mm), with no signs of any new lesion.

hepatic veins and IVC (Figure 5). Using ultrasound dissector the Sg 1 was easily resected from sRHV and common trunk. The walls of both vessels were firm and not as tender as usually. There was very little blood loss and no transfusion was needed during and after the operation.

Gross pathological examination of the excised specimen showed oval, relatively sharply demarcated area of amorphous and yellowish tissue in the liver parenchyma. Histologically, complete necrosis was found and, in the vicinity of it, focal necrosis of the narrow zone of the liver parenchyma was present. In the excised lesion there was no viable tumor tissue. Taking into account that etiology of necrosis can be different, immunohistochemical staining for CK20, CEA and Hepar was performed. CK20 is an intermediate filament (part of cytoskeleton) mainly expressed in gastrointestinal type epithelia and carcinomas deriving from it. CEA is found in a large variety of carcinomas of gastrointestinal, respiratory and genitourinary tract while Hepar stains normal human hepatocytes. Although CK20 and CEA are not entirely specific for colorectal carcinoma, vast majority of them are strongly positive for both markers. In our case, necrotic tissue was immunohistochemically positive for CK20 and CEA, and negative for Hepar in contrast to vital liver parenchyma which was positive for Hepar and negative for CEA and CK20 (Figure 6). Findings like that support that necrosis arose from the metastatic carcinoma. Although it was not possible to discriminate between necrosis induced by ECT or chemotherapy only, there was indirect evidence of the effect of electrochemotherapy and that is the presence of necrosis of the narrow zone of the liver parenchyma surrounding the tumor. On the border between necrotic tumor tissue and vital liver parenchyma, focal proliferation of fibroblastic tissue with some chronic inflammatory reaction, foamy macrophages and pigmentophages were observed. In the remaining liver parenchyma slight portal fibrosis was present.

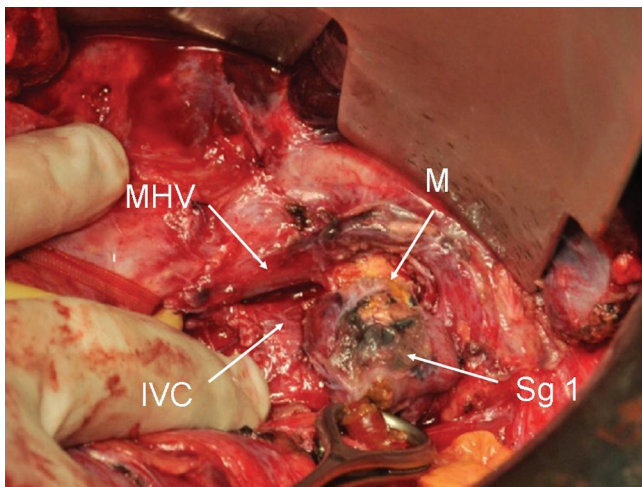


Figure 5: Resection of Sg 1 with common trunk and MHV exposed. Necrotic metastasis (M) is visible in Sg 1, close to the MHV and IVC.

Discussion

The results of the study show that electrochemotherapy on liver metastases can be performed safely and effectively. Treatment procedure for electrochemotherapy of liver metastases is presented and described on a patient with CRLM in paracaval region extending to Sg 1. This single metastasis in difficult location was successfully treated, as evident by histological examination of the removed metastasis after the second operation. The described procedure demonstrates its complexity, where several specific steps have to be taken in consideration; exact treatment planning for electrode positioning and the delivery of electric pulses, positioning of the electrodes before injection of bleomycin, as well as synchronization of electric pulses delivery with ECG for safety reasons. The patient is disease free 16 months after the electrochemotherapy procedure.

Radical removal of any malignant tumor from the paracaval region and Sg 1, close to the main hepatic veins inflow to IVC is challenging. In the presented case, the radical resection of the metastasis would have been potentially possible by doing right trisectionectomy leaving probably too little liver remnant (lateral part of the left liver). Considering the specific location, RFA would not be effective because of the cooling effect of the veins. All possibilities of treatment had been presented to the patient who decided to be treated with electrochemotherapy. The treatment was performed following very closely the treatment plan, without post-treatment side effects.

Treatment planning is needed for the exact positioning of the electrodes, in order to predict successful electroporation of the whole tumor mass. As it is known, two conditions have to be met for effective electrochemotherapy: presence of the drug during the electroporation in the tissue and electroporation of the whole tumor mass. Based on the presumption that intravenous drug administration would adequately deliver bleomycin to the liver metastasis, emphasis was put on electroporation treatment plan. Several treatment plans were prepared, in order to satisfy all possible situations that might have occurred (7). According to the numerical model, it was decided that one or two electrodes should preferentially be inserted in the tumor to improve the electric field distribution and guarantee complete tumor coverage. This was reported previously (7), and we have shown that positioning of electrodes outside the tumor is very sensitive to errors in electrode placement and differences in tissue conductivities between the target (tumor) and surrounding tissue (8).

Despite the effort to precisely follow the treatment plan for the positioning of the electrodes, the final positions of the electrodes did not match the original treatment plan exactly. These deviations from the original treatment plan (see Figure 2 and Table I) were considered in the post-surgery numerical

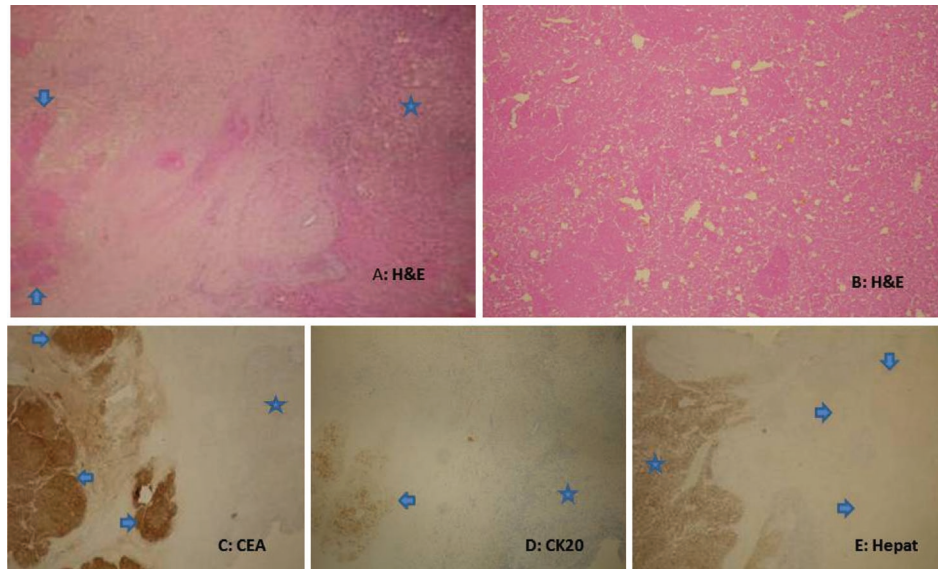


Figure 6: A: H&E, 4x; complete necrosis of the tumor tissue (arrows) and vital liver parenchyma (star). B: H&E, 10x; completely necrotic tumor tissue. C: IHC CEA 4x; CEA positive staining in the necrotic tumor tissue (arrows) and negative in the vital liver parenchyma (star). D: IHC CK20 4x; CK20 positive staining in necrotic tumor tissue (arrow) and negative in vital liver tissue (star). E: IHC Hepat 4x; positive staining for Hepat in the liver (star) and negative in the necrotic tumor tissue (arrows).

evaluation that used the same numerical model as in treatment planning. The calculations showed that the treatment plan was still followed closely enough and that the electric field distribution was adequate for the procedure, *i.e.* the entire tumor volume was exposed to the electric field above 450 V/cm, with 90% being higher than 900 V/cm.

In the delivery of pulses between electrodes 1-5, 2-5, 3-6 and 4-6 (see Table 1) the originally planned amplitude of voltage for pulses could not be used due to excessive current values experienced during the first attempt to deliver the pulses (more than 50 A – a limitation of the Cliniporator VITAE device). Therefore the amplitude was lowered and a greater number of pulses at decreased voltage were delivered. It is known that electroporation can be effectively carried out (and the desired effect achieved) if a weaker electric field is used but with a greater number of pulses (23, 24). For this reason the number of pulses delivered to some of the electrode pairs was increased.

In all reports on clinical use of electrochemotherapy for treatment of cutaneous or subcutaneous malignant tumors the method has been described as completely safe. No serious side effects for the patient have ever been reported. The minor side effects reported in the literature include localized transient lesions in normal tissue in immediate vicinity of the treated region and the acute pain associated with contraction of skeletal muscles in vicinity of the electrodes which was caused either by direct electrical stimulation of the

muscles or of the nerves innervating these muscles (25-27). The acute pain is the reason for use of either local or general anesthesia (depending on the location and number of tumors to be treated) during treatment of cutaneous or subcutaneous tumor lesions. Such electrochemotherapy treatment was shown to have no effect on the function of the heart apart from a transient and mild tachycardia attributed to anxiety of the patient in case of local anesthesia (10). This result was not surprising taking into account the high level of treatment localization (the electrodes are positioned close together and far away from the heart) and the very short duration of electric pulses. However, according to the results of the study on numerical calculations of electric field and current distribution for a tissue model it may be theoretically possible to affect functioning of the heart even in case of subcutaneous tumors located on the chest close to the heart and for deep insertion of needle electrodes (*e.g.* approximately 4 cm) (10). Under such extreme conditions the threshold value of current for induction of ventricular fibrillation (set at 100 mA for the given duration of electric pulses) could be exceeded (10). Furthermore, with recent development of new electrochemotherapy modalities for treatment of internal tumors using surgical procedures or endoscopic routes (28) to gain access to treatment area, could result in the treated region located in close proximity of the heart. Due to the absence of protective barrier of the skin and relatively large electrical conductivity of internal tissues and organs the electrical current delivered during electrochemotherapy using invasive access, can propagate through a larger volume of

tissue surrounding the treated region. Therefore, an increased probability for electroporation pulses interfering with the heart function is present. In recently published studies on non-thermal irreversible electroporation, different minor and major hemodynamic and cardiologic changes due to unsynchronized irreversible electroporation pulse delivery were reported, such as systolic hypertension, supraventricular tachycardia, ventricular tachycardia with pressure drop, ventricular fibrillation, ST segment elevation and changes in T wave (11, 29). Deodhar *et al.* (11) showed that unsynchronized irreversible electroporation pulses delivered at less than or equal to 1.7 cm from the heart provoked fatal events (such as ventricular fibrillation) whereas pulses delivered more than 3 cm from the heart did not provoke any changes from baseline ECG (11). On the other hand, they reported that synchronized irreversible electroporation did not provoke any (fatal or minor) events at more than 1.7 cm distance from the heart. In the case reported here, the electroporation pulses were delivered at a location more than 10 cm away from the heart. Regardless of an unlikely event of serious consequences (such as induction of various arrhythmias or, in worst case, ventricular fibrillation), the synchronization of electroporation pulse delivery with the cardiac rhythm should be a prerequisite step for treatment of tumors in all internal organs, and especially those in close vicinity of the heart, to maximize the safety of the patient. The synchronization algorithm currently implemented in Cliniporator VITAE device coupled with the external triggering device AccuSync proved to be effective in preventing external stimulation of the heart during the so-called vulnerable period of the ventricles. As a result all electroporation pulses in our study were delivered outside the vulnerable period and no heart arrhythmias or any other pathological morphological changes were observed.

The safety of the treatment was demonstrated also by absence of side effects during and after electrochemotherapy. The use of electrochemotherapy did not extend hospital stay. Electrochemotherapy was performed in the vicinity of the big blood vessels (IVC, MHC, RHV), which may pose a specific problem. The electrodes are 1.2 mm in diameter and puncturing the vessels, specifically after retracting the electrodes may induce bleeding. However, also in this case, where electrodes were positioned nearby or even in or through the vessels no adverse events were recorded. No bleeding of the tissue after retraction of the electrodes was noticed. In the case of bleeding, the electrodes may be used as electrocoagulation tip by bringing them in contact with electrical surgical knife. This also has a preventive effect against bleeding. The mechanisms of action of electric pulses on vessels are known, for normal and tumor vessels (30-32). Electroporation induces immediate vasoconstrictive effect on vessels that is gradually

released after a few hours. This immediate effect can be continued by vascular disrupting effect when the drug is present leading also to cytotoxicity of endothelial cells and abrogation of perfusion for a long period of time. This vascular disrupting effect of electrochemotherapy may add substantial part to overall effectiveness of electrochemotherapy in treatment of well vascularized tumors (31). However, there was no damage observed on normal liver tissue in our case. The results are in agreement with the results on liver tissue with irreversible electroporation where safe use of electroporation on bigger vessels in the liver was described (33).

Based on histological analysis, electrochemotherapy treated metastasis underwent complete necrotization within two months after the treatment. However, we are aware, that this patient was not treated with electrochemotherapy solely. After the metastasis was diagnosed, the patient was treated with systemic chemotherapy (XELOX + bevacizumab), and 5 weeks later received electrochemotherapy; so it is hard to put all credits for good treatment result to electrochemotherapy only, although chemotherapy was discontinued 5 weeks before electrochemotherapy. Although full responses to systemic chemotherapy are rare, they do occur, but histologically these metastases tend to develop central fibrosis (34). The usual responses to electrochemotherapy are non-necrotic, however also necrotic responses were recorded, as in the case of treatment of brain metastases (35, 36). To clarify and evaluate the treatment effectiveness on larger number of patients, there is an ongoing phase I/II clinical trial at the Institute of Oncology Ljubljana. The trial is designed to evaluate the effectiveness, safety and toxicity of the electrochemotherapy with bleomycin in treatment of the liver metastases originating from colorectal cancer. After recruiting a sufficient number of patients, we will have a much clearer picture about effectiveness of electrochemotherapy in treatment of liver metastases. Therefore, we must await further experience with this technique to get proof of clinical efficacy.

In case of encouraging results of the on-going clinical trial, further clinical trials performed on other liver tumors or liver metastases will be performed. With well elaborated and tested treatment planning system even percutaneous treatment of tumors using a guided system could be performed, that would be considerable improvement over invasive procedure described here (37).

In conclusion, electrochemotherapy proved to be feasible technological approach for treatment of liver metastases. Due to absence of side effects and the first reported complete destruction of the treated tumor, electrochemotherapy in treatment of liver metastases proved to be safe with good treatment effectiveness even in a difficult-to-reach location.

Conflict of Interest

We certify that regarding this paper, no actual or potential conflict of interest exists; the work is original, has not been accepted for publication nor is concurrently under consideration elsewhere, and will not be published elsewhere without the permission of the Editor and that all the authors have contributed directly to the planning, execution or analysis of the work reported or to the writing of the paper.

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