Zdravljenje jetrnih zasevkov z elektrokemoterapijo

Treatment of liver metastases with electrochemotherapy

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1 PURPOSE OF EXTENDING THE STUDY

The purpose of extending the study was to evaluate the efficacy of bleomycin-based electrochemotherapy in treating colorectal liver metastases in a phase II clinical study.

The purpose will be achieved by:

- applying treatment in a controlled manner based on pre-clinical experience and the clinical experience of the ESOPE project;
- evaluating procedures and results achieved;
- preparing standardized procedures;
- disseminating these new approaches to treatment using written and audio-visual materials as well as by attending and organizing expert meetings.

The study is designed as an institutional study (Institute of Oncology Ljubljana) conducted in cooperation with the Faculty of Electrical Engineering (University of Ljubljana), University Medical Centre Ljubljana and industrial partner, IGEA srl., Carpi, Italy, which will provide technical support for the study. An additional 15 patients who meet the inclusion criteria will be included in the extended part of the study.

The study will be carried out within the P3-0003 "Razvoj in ovrednotenje novih terapij za zdravljenje malignih tumorjev" (Development and evaluation of new therapies for treating malignant tumours) research programme headed by Prof. Gregor Serša, PhD.

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2 STARTING POINTS

Principles of electroporation and electrochemotherapy (ECT)

By exposing a cell to an external electric field, transmembrane voltage is established on the cell membrane. When the threshold value is exceeded, this voltage causes electroporation of the cell membrane, i.e. the formation of hydrophilic pores, which allow the increased passage of molecules into the cell.[1]

The principle of electrochemotherapy (ECT) is based on exposing the tumour to electroporation pulses delivered by specific electrodes. This allows the passage of previously applied hydrophilic cytostatics, e.g. bleomycin and cisplatin, into cells.[2] Because the intake of cytostatics is increased only in areas exposed to electroporation pulses, ECT is a local treatment method. In addition to the direct action of cytostatics on tumour cells, ECT has an indirect vascular-disrupting effect, because cytostatics act on the endothelial cells in tumour vasculature.[3] Doses of cytostatics used in ECT are extremely low, resulting in minimal or no systemic toxicity.[4] Furthermore, the application of electroporation pulses reduces blood flow in the tumour, which keeps the cytostatics in the tumour for a longer period of time, thus making the treatment more effective.[3]

Clinical applications of ECT

In the early 1990s, our team took an active part in pioneering clinical studies on treating cutaneous and subcutaneous tumours with ECT.[5] According to the findings of these first clinical trials, 80% of objective responses of treated tumour nodules are achieved using ECT.[6] Our team also helped draw up and publish standard surgical procedures for treating cutaneous and subcutaneous tumours using ECT. Today, ECT is a well-established method for treating malignant melanoma metastases, especially as part of palliative care, in 15 European countries. In 2011, 2,000 patients were treated with ECT and, according to several databases, ECT is now used in 100 oncology centres.

In the first recorded clinical case of treating a deep-seated tumour with ECT, melanoma metastases in thigh muscles were treated.[7] Experience in treating other types of soft tissue tumours with ECT is good, but limited to a few clinical cases and studies involving a small number of patients.[8-11] In addition to treating deep-seated soft tissue tumours, ECT is also used to treat deep-seated tumours in internal organs. Brain and liver metastases have already been shown to be effective targets in ECT.[12–14]

Treatment of liver tumours

Colorectal cancer is the most common cancer and the second most common cause of cancer death in Europe, taking into account both sexes[15]. At the time of diagnosis, more than 23% of patients have liver metastases,[16] while after the first treatment, metastases occur in a further 20% of patients.[17] In Slovenia, the incidence of colorectal cancer metastases that are eligible for surgical treatment is from 12 to 15 per 100,000 population, and is higher than the incidence of other liver metastases. In these cases, radical liver resection is the only promising treatment method, having an almost 50% 5-year survival, but due to the scale of the disease, it is only feasible in a minority of cases.[18] The distant form of the disease is usually treated with systemic chemotherapy or biological drugs, which are only partially successful while producing high systemic toxicity. Where individual small metastases are present, patients are offered a local form of treatment: a thermoablation method called radiofrequency ablation (RFA). Metastases that grow into the inferior vena cava or into the area of large hepatic and portal veins, and on which RFA has small or no effect due to cooling, are particularly problematic. Therefore, new, more effective approaches to liver metastases treatment are being sought for such patients.

A phase I/II clinical study is currently underway at Institute of Oncology Ljubljana to assess the safety and efficacy of treatment for colorectal liver metastases using ECT. We wish to include an additional fifteen (15) patients within phase II using this protocol. To date, 17 patients have been included in the clinical study, and preliminary results in 16 of these patients indicate that treating colorectal liver metastases and one HCC patient using ECT is a safe method for treating tumours even in hard-to-reach locations. Preliminary MRI results in all patients testify to radiological changes similar to those observed after RFA treatment. The response to ECT treatment was complete in 50% of patients and partial in the remaining 50% of patients. Preliminary histological analysis results for all ECT-treated metastases after surgical removal indicate progressive degenerative changes within metastases and a statistically significant reduction in viable tumour tissue in ECT-treated metastases compared to non-ECT-treated metastases ($p < 0.001$). We were the first to publish the technological part of the procedure in the clinical case.[14]

Planning ECT treatment

ECT treatment of deep-seated tumours in internal organs requires planning based on diagnostic radiology imaging. Electrode type is selected based on the precise location and measured size of the

tumour nodules; for metastases located up to 3 cm (lower edge of the metastasis) below the liver surface, which are more easily accessible to ECT, short needle electrodes with a fixed hexagonal geometric arrangement are appropriate, while for hard-to-reach and deep-seated metastases, long needle electrodes with an arbitrary geometric arrangement have recently been made available. In line with technological advances and the development of new electrode types, experts at the Italian company IGEA designed a new electric pulse generator called the Cliniporator VITAE for treating larger and deep-seated tumours, such as liver metastases. The generator meets the essential requirements for consumer safety, and health or environmental protection as defined by EU guidelines or regulations, as it has the CE mark.

The distribution of the electric field within the tissue is an important predictor for the success of ECT tumour treatment, as the change in the transmembrane potential of the cell membrane, which is a prerequisite for successful electroporation, is directly proportional to the electric field strength.[19] Numerical modelling is currently the only effective method for predicting electric field distribution in tissue, as its structure is, by definition, inhomogeneous, nonlinear, and in some cases anisotropic. When using long needle electrodes with an arbitrary geometric arrangement, it is necessary to determine the depth of electrode insertion into the tumour nodule, and the voltage, length and frequency of the supplied electroporation pulses based on numerical modelling in order to achieve optimal coverage of tumour nodules with an electric field.[7]

Electroporation pulse synchronization using electrocardiogram (ECG)

In ECT treatment, the electric current reaches the surroundings of the treated area due to the high electrical conductivity of the tissue. This increases the likelihood of possible interaction between electroporation pulses and heart function when treating deep-seated tumours in internal organs due to the anatomical proximity of the heart. In our clinical study, we used a synchronization procedure built into the electroporation pulse generator (Cliniporator VITAE) to avoid cardiac dysfunction caused by ECT. So far, this procedure has proven to be effective in preventing cardiac pacing during the ventricular sensitivity period.[14] Nevertheless, further research is urgently needed to verify the effectiveness of the existing synchronization procedure and to determine any short- or long-term effects of electroporation pulses on heart function.

Preliminary results suggest that ECT does not seriously affect cardiac function, but there are statistically significant changes in some parameters of heart rate variability during and after therapy relative to pre-therapy parameter values.

The analysis of ECG signals recorded during ECT applied to liver tumours shows short-term effects on cardiac function, even though the delivery of electroporation pulses was synchronized with the ECG. The effects were expressed as a temporary shortening of the corrected QT interval and an increase in short-term heart rate variability. The analysis of ECG signals recorded before and after ECT was applied to liver tumours indicates that there are longer-term effects of ECT on cardiac function, which are expressed as increased heart rate or shortened RR interval and as reduced long-term heart rate variability parameters (low-frequency component). We find that these changes are most likely at least partly attributable to the effects of analgesics and other medications received by patients in intensive care, and to postoperative pain, and not to the effects of electrochemotherapy itself, although these results have yet to be confirmed. These results could be confirmed by capturing ECG signals before, during, and after abdominal surgery, which would include procedures similar to those performed during ECT of liver tumours. The only difference should be that ECT is not performed during these surgical procedures. The ECG signals captured in this way would serve as a control and would allow these assumptions to be confirmed.

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3 PATIENT SELECTION

The suitability of patients for inclusion in the study will be assessed using inclusion and exclusion criteria.

3.1 Inclusion criteria

1. Patients with one to three (1–3) metachronous colorectal liver metastases, up to 5 cm in size, that are difficult to resect due to their position (likelihood of insufficient healthy liver after resection) or inaccessible for treatment with standard ablation techniques because they are located on large hepatic vascular structures (portal veins, hepatic veins or their inflow into the inferior vena cava, immediate vicinity of vena cava), but are otherwise operable.
2. Patients with up to three (3) recurring colorectal liver metastases, each with a maximum diameter not exceeding five (5) cm, and for which other treatment is either unacceptably risky due to possible insufficient residual liver volume after resection or expected to be less effective due to the proximity of veins in RFA.

   Patients in groups 1 and 2 are patients in whom standard treatment options (systemic and/or surgical) have been exhausted. ECT will be offered to these patients as the only therapeutic option. If a patient has a large number of metastases that are unresectable and at the same time unsuitable for RFA, the metastases will be treated with ECT, while any other metastases will be resected or treated with RFA.
3. Patients with synchronous colorectal liver metastases, in whom—due to their poor general health or the extent of the disease—first the removal of the primary tumour is planned, and after 2–3 months, surgery on the metastases, with systemic treatment taking place in the meantime. ECT would be used to treat metastases up to 5 cm in maximum diameter during the first surgical procedure, i.e. when the primary tumour is removed.
4. Patients with bilateral metachronous colorectal liver metastases who require two-stage surgery due to the extent of the disease, i.e. right portal vein ligation and excision or RFA of metastases in the left hepatic lobe at the first stage and right hepatic lobe resection at the second stage. During the right portal vein ligation surgery, the metastases should be accessible in order to apply electroporation pulses without major manipulation and mobilization of the liver, so as not to create additional problems for the second planned procedure, i.e. the removal of metastases by resecting the right hepatic lobe.

   Patients in groups 3 and 4 are potentially curable using standard treatment. The fact that these patients will be operated on twice as part of standard treatment will be used to apply ECT during the first procedure and to resect treated and untreated metastases during the second. The added ECT used on these patients will not affect the standard treatment recommended in the current guidelines.
5. The patient is offered ECT treatment even if the patient refuses standard treatment options.
6. Histologically and/or cytologically confirmed colorectal cancer.
7. Age above 18.
8. Life expectancy over 3 months.
9. Performance status (PS) ≥ 70 according to Karnofsky PS or ≥ 2 according to WHO recommendations.
10. Depending on the type of active substance, 2–5 weeks have passed since the last treatment, if any.

11. The patient must understand the treatment process and any side effects.
12. The patient must be able to give consent to participate in a clinical trial ('informed consent').
13. Prior to inclusion in the study, the patient should be treated by a multidisciplinary gastrointestinal tumour team.

3.2 Exclusion criteria

1. Any previously confirmed form of cancer other than surgically treated non-invasive uterine cancer or basal cell carcinoma treated with surgery or radiotherapy.
2. Proven visceral and/or bone metastases or diffuse metastases.
3. Life-threatening infection and/or heart failure and/or liver failure and/or other life-threatening systemic conditions.
4. Ascites.
5. Significantly reduced lung function.
6. Age under 18.
7. Coagulation system disorders.
8. Received cumulative dose of bleomycin ≥ 250 mg/m^2.
9. Allergic reactions to previous treatment with bleomycin.
10. Chronic decrease in renal function (creatinine > 150 µmol/L).
11. Epilepsy
12. Heart rhythm disorders.
13. Implanted pacemaker or defibrillator.
14. Pregnancy.
15. Patients who are unable to understand the purpose of the study.

3.3 Examinations and tests before treatment

Two weeks before inclusion in the study, the following will be performed:

- clinical examination, anamnesis
- radiological imaging techniques to discover liver metastases:
  - dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI)
  - dynamic contrast-enhanced ultrasound (DCE-US)
  - computed tomography (CT) perfusion
- Testing for systemic metastases
  - CT of the lungs
  - other examinations depending on the clinical picture (bone scintigraphy, PET/CT)

One week before inclusion in the study, the following will be performed:

- laboratory tests:
  - haematological tests with a complete blood count
  - coagulation tests
  - biochemical tests: electrolytes, creatinine, transaminases
  - immunohistochemical examinations: tumour markers
- ECG

In patients for whom two-stage surgery is planned, the following will be performed:

- histological analysis of liver metastases and comparison of pathohistological changes between treated and untreated metastases.

4 TREATMENT PLAN

When there are liver metastases (up to 3 cm in maximum diameter) with the lower edge located up to 3 cm below the liver capsule, ECT using short needle electrodes with a hexagonal geometric arrangement will be performed in line with standard surgical procedures for treating cutaneous and subcutaneous tumours/metastases with ECT.

When there are larger liver metastases (up to 5 cm) located near the vena cava or along large hepatic or portal veins, ECT using long needle electrodes with an arbitrary geometric arrangement will be performed. Using numerical modelling, we will prepare a treatment plan to determine the optimal position of the electrodes and the required electric current voltage for optimal electroporation of the target tissue. The preparation of a treatment plan using numerical modelling will take place in two basic steps (see below) based on radiological images captured up to 2 weeks before the procedure.

1. Decomposing medical images and constructing a model:
   - importing medical radiology images into an appropriate program,
   - determining important areas,
   - pre-treating and decomposing images,
   - constructing a 3D model.
2. Planning treatment:
   - The 3D model will be supplemented with electrodes supplying electroporation pulses, and with corresponding tissue properties and boundary conditions based on the calculation using the finite element method and the calculation processed by automatic algorithms for determining the criterion function,

- the possibility of statistical and probability functions to determine the probability of electroporation will be examined, the effect probability being a function of the electric field strength applied.

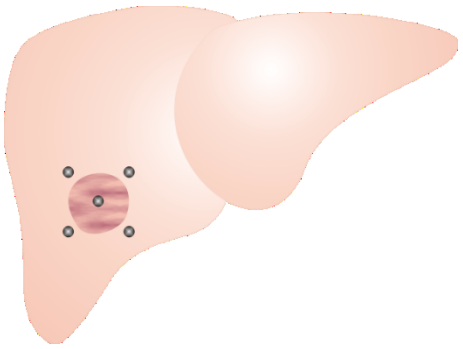

Figure 1: **Schematic representation of electrode placement in the middle and the periphery of a metastasis in healthy liver tissue.**

5 TREATMENT: ELECTROCHEMOTHERAPY (ECT)

Patients with multiple bilateral metastases scheduled for two-stage surgery depending on their general condition and extent of the disease will have their right portal vein ligated and up to 3 metastases treated on the right hepatic lobe with up to 3 cm in maximum diameter using ECT during the first surgical procedure. On the left, metastases will be treated using established methods, such as surgery and RFA. During the second procedure, the entire right half of the liver will be removed.

In patients with liver metastases in areas where, due to the proximity of the main hepatic veins, surgery is not possible and the success of other ablation techniques cannot be predicted, ECT will be performed.

5.1 Chemotherapeutic agents

- The cytostatic used will be bleomycin (BLM) manufactured by Heinrich Mack Nachf. GmbH & Co. KG, Illertissen, Germany.
- BLM will be dissolved in saline solution and administered intravenously with a bolus injection at a dose of 15 mg/m^2.
- 8–28 min after BLM application, electroporation pulses will be delivered to the metastases.

5.2 Electroporation

5.2.1 Electric pulse generator

- We will use the Cliniporator VITAE electric pulse generator manufactured by IGEA, Carpi, Italy, designed to treat larger and deep-seated tumours, such as liver metastases.
- It has the CE mark and is authorized for use in a clinical setting.

5.2.2 Electrodes

Two types of electrodes will be used:

a) short needle electrodes with a fixed hexagonal geometric arrangement:
   - for metastases with their lower edge located up to 3 cm below the liver capsule;
   - ECT will be performed in line with standard surgical procedures for cutaneous and subcutaneous tumours/metastases;

b) long needle electrodes with an arbitrary geometric arrangement:
   - for deeper-seated tumours and tumours located ≥ 3 cm below the liver capsule;
   - to capture the safety margin, the required number of electrodes (presumably from 4 to 6) will be inserted into the metastasis and its surroundings using ultrasound guidance in line with the treatment plan prepared based on numerical modelling before the procedure.

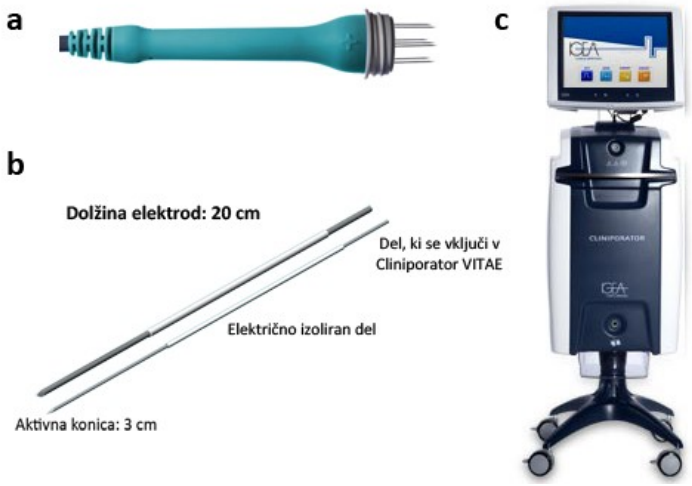

Figure 2 **Short needle electrodes with a fixed hexagonal geometric arrangement (a), long needle electrodes with an arbitrary geometric arrangement (b) and Cliniporator VITAE (c).**

5.2.3 Electroporation pulses

- Between each pair of electrodes, 8 rectangular electroporation pulses (length: 100 µs) will be supplied to the metastases in two trains, each containing 4 electroporation pulses of reversed polarity and a repeating frequency of 1000 Hz.
- The delivery of individual pulse trains will be synchronized with the occurrence of successive heartbeats.
- When using long needle electrodes with an arbitrary geometric arrangement, the voltage of electroporation pulses for each electrode pair will be determined on the basis of numerical modelling, and when using short needle electrodes with a fixed hexagonal geometric arrangement, a voltage of 730 V will be applied.
- If excessive electric current (> 50 A) makes it impossible to supply a sufficiently high voltage, a larger number of lower-voltage electroporation pulses will be supplied.

5.3 Synchronization of electroporation pulses with ECG and Holter ECG recording

- To ensure synchronization, the Cliniporator VITAE will be connected to an AccuSync 42 external unit, which produces trigger pulses based on the measured ECG signal to deliver electroporation pulses on the R wave of each heartbeat.
- For interoperative patient monitoring, patients will be connected to AccuSync via three ECG electrodes independently of ECG capture.
- On the selected ECG lead (standard leads I, II, III; the most suitable lead is chosen depending the intensity of the R wave), AccuSync detects the R wave of individual heartbeats in the early phase of increasing R wave slope, and produces a trigger pulse.
- The trigger pulse is brought to the Cliniporator VITAE, which uses its built-in synchronization algorithm for a further verification of trigger pulse suitability, and, meeting a series of conditions, produces electroporation pulses with a 50 ms delay behind the trigger pulse to avoid ventricular sensitivity (T wave range).
- ECG signal and trigger pulses will be captured and stored for later analysis of the synchronization algorithm function and of any immediate effects of treatment on heart function.

6 FOLLOW-UP AFTER TREATMENT

Follow-up will be carried out at three levels. First, defining the early and late effects of treating colorectal liver metastases using ECT; second, evaluating short- and/or long-term effects of electroporation pulses, BLM or ECT as a whole on heart function; and third, recording adverse effects of ECT.

6.1. Effects of treating colorectal liver metastases using ECT

- Early treatment effects will be assessed using DCE-US or CT based on changes in tumour blood flow after ECT compared to pre-ECT blood flow.
- Late effects will be assessed based on modified RECIST criteria, taking into account the change in the size and density of treated tumour nodules before and after ECT. These will be assessed based on DCE-MRI images; the volume of the metastases will be calculated using the formula $V = \frac{a \times b^2 \times 6}{\pi}$, where a is the shorter and b the longer metastasis diameter.

Based on the assessment of the late ECT effects, the response to treatment will be evaluated in line with the WHO classification:

- Complete response (CR): complete disappearance of the tumour nodule
- Partial response (PR): reduction of tumour nodule size by more than 50%
- No change (NC): decrease in tumour nodule size by less than 50% or increase in tumour nodule size by less than 25%
- Progressive disease (PD): growth of the tumour nodule by more than 25%

- The direct effect will be assessed by histological analysis of those metastases that will be surgically removed.
- EORTC Quality of Life questionnaire according to QLQ C30 standards

EXAMINATION/TEST	Screening period until D –14	D –1	D 7	D 30	D 60	D 90	D 120
clinical examination, anamnesis	X	X	X	X	X	X	X
ECG	X	X					

DCE-MRI	X			X		X	
DCE-US	X			X	X		X
CT perfusion	X			X			X
Blood count	X	X	X				
Coagulation tests	X	X	X				
Biochemical blood analysis	X	X	X	X	X		X
EORTC QLQ C30 questionnaire	X		X	X			

***D = day**

6.2 Effects of electroporation pulses, BLM or ECT as a whole on heart function

- To study the short-term effect, we will use the ECG signal captured during surgery, which will cover the period before, during and immediately after ECT, including BLM injection and electroporation pulses.
- To study possible late and/or long-term effects, ECG signal using a standard Holter system will be recorded in 24-hour periods immediately before and after surgery. 5–10 patients will be included. ECG recordings will also be performed on 10–15 patients who will undergo abdominal surgery without ECT at the Institute of Oncology Ljubljana.

6.3 ECT adverse effects will be recorded in line with the criteria of the National Cancer Institute (NCI)

An adverse effect is an undesirable and unexpected sign, symptom or disease, regardless of the cause, which develops or worsens during the study and which includes pathological clinical observations and deviations in the values of laboratory-measured parameters. The latter are considered a side effect only if they require additional treatment or result in premature withdrawal of the patient from the study.

We will record any side effects that occur during the study and up to 30 days after the end of treatment. If a serious adverse reaction occurs more than 30 days after the end of treatment, the principal investigator will decide whether the complication is study-related or not.

We will record the start and end time of any adverse effect, and the cause-and-effect relationship between the adverse effect and ECT, cancer or other diseases, as well as assessing the severity of the adverse effect and describing the actions that will need to be taken.

The causal relationship between the adverse effect and the ECT will be assessed as follows:

- Not related to the study.
- Probably not related to the study.
- Possibly related to the study.
- Probably related to the study.
- Certainly related to the study.

The severity of side effects will be assessed using the following grades:

- Grade 1 – mild
- Grade 2 – moderate
- Grade 3 – a difficult complication requiring medical care
- Grade 4 – a life-threatening condition requiring immediate medical assistance
- Grade 5 – death

If a serious adverse effect is recorded during the study, the principal investigator will notify the responsible person at the National Medical Ethics Committee of the Republic of Slovenia in writing within 24 hours. A serious adverse effect is a complication that results in the following:

- death,
- a life-threatening condition requiring immediate medical assistance,
- hospitalization or extended hospitalization,
- prolonged inability of the patient to perform any task that they were able to perform before the onset of the complication,
- any event or condition which endangers the life or health of the patient and which, according to a medical assessment, could lead to any of the serious complications referred to above.

7 DATA PROCESSING

The results of the proposed clinical trial will be analyzed descriptively by describing the antitumour effect, any adverse effects and the patients' quality of life after treatment of colorectal liver metastases using ECT.

8 STATISTICAL ANALSIS

All data will be entered into a Microsoft Access 2010 database, which will be used for all calculations except for statistical analysis, which will be performed with GraphPad Software (La Jolla, CA, USA). The log-rank (Mantel-Cox) test will be performed on the Kaplan-Meier estimates. A chi-squared test will

be used for the statistical comparison of response according to tumor location. A two-tailed P value less than 0.05 will be considered to be statistically significant.