Protocol of the clinical study

Clinico-pharmacological approach to optimize the therapeutic bleomycin concentration in patients undergoing electrochemotherapy

ISRCTN number: ISRCTN74054653

Acronym: ECT-BLM

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SYNOPSIS

TITLE	**Clinico-pharmacological approach to optimize the therapeutic bleomycin concentration in patients undergoing electrochemotherapy**
COORDINATING INVESTIGATORS	Asist. Ales Groselj, M.D. Prof. Gregor Sersa Dr. Francesca de Terlizzi
DISEASE	Cutaneous tumors suitable for electrochemotherapy
RATIONALE	Collection of pharmacological data on bleomycin (BLM) in blood and tumors, for optimization of electrochemotherapy (ECT) clinical protocol.
STUDY TYPE	Prospective single arm observational study
PRINCIPAL AIM	Pharmacokinetics and pharmacodynamics of BLM in patients treated with electrochemotherapy, for optimization of the treatment protocol. The objective will be by: • Collection of patient's sera undergoing electrochemotherapy according to the SOP and in compliance with the NICE recommendations for determination of in vivo BLM pharmacokinetics. • Collection of tumor tissue of the same patients at the time of application of electric pulses (8 min after intravenous 15 000 IU/m^2 BLM injection) for determination of BLM concentration in treated tumors.
SECONDARY AIMS	Data analysis for determination of: • Pharmacokinetics of BLM in patients within different age groups. • Correlation between the tumor response and BLM concentration in the tumors. • Possible variability in drug concentration according to the tumor type. • Impact of previous treatment(s) (radiotherapy, systemic therapies, surgery) on drug accumulation in the tumors. • Optimization of the therapeutic window.
INCLUSION CRITERIA	Patients eligible for electrochemotherapy of cutaneous tumors (basal cell carcinoma, squamous cell carcinoma,

	melanoma) and cutaneus metastases of melanoma and adenocarcinoma of the breast. • The selection of patients will be according to the NICE guidelines; patient's data will be included into the InspECT database. • The patients will be treated according to the current SOP for electrochemotherapy with intravenously injected BLM in the dose 15 000 IU/m^2. • Age ≥18 years. • Patients agreed to participate in this study and signed informed consent.
EXCLUSION CRITERIA	Patients not suitable for electrochemotherapy according to the NICE guidelines. Patients that do not comply with requirements for the blood collection, or samples were not collected according to the SOP for blood collection. Patients that do not comply with the requirements for tumor biopsy, or samples were not collected according to the SOP for tissue sampling.
PRE OPERATIVE EVALUATION	Compliance with indications for ECT.
PROCEDURE	Venous or arterial blood collection at the different time points after the infusion of BLM.
POST OPERATIVE EVALUATION	According to the case record form (CRF) in InspECT database.
ASSESMENT CRITERIA	Determination of BLM in blood and tumor tissue.
FOLLOW UP PERIOD	Minimum 6 months, as prescribed by InspECT database.
NUMBER OF PATIENTS	**80-100**
NUMBER OF REFERRAL CENTERS	3 • University Clinical Center Ljubljana • Institute of Oncology Ljubljana • Department of Dermatology and Allergology, University of Szeged
EXPECTED ACRURAL TIME	36 months (1.7.2018-30.6.2021)

Content

1. SUMMARY

Electrochemotherapy is a local ablative technique, which effectiveness favorably compares to other local ablative techniques. Approximately 80% objective responses of the treated tumors are recorded. Based on the physical principles of the delivery system the effectiveness is comparable in different tumor types. Electrochemotherapy is now used in more than 145 cancer centers throughout Europe, and several hundred patients are being treated yearly.

The technique is based on the increased drug delivery to cells which were exposed to electroporation. The drug used is bleomycin, with already established clinical validation, but with certain side effects, predominantly lung fibrosis and possible ulceration in the treated areas. Although the drug dosage is low (15 000 IU/m^2), further lowering of the dose would decrease the risk of side effects. Recently, we have developed a new analytical method for the bleomycin determination in tissue, serum and plasma samples. Based on this method and observed pharmacokinetics of the drug, we already suggested to lower the bleomycin dose in elderly patients, but for younger patients, pharmacokinetics has not been determined yet.

In this study we will set the clinical trial on pharmacokinetics and pharmacodynamics of bleomycin in the treated tumors. The goal is to optimize, based on the bleomycin serum elimination times in electrochemotherapy treated patients, the bleomycin dose. The aim is to determine the lowest dose of bleomycin that is still effective in patients treated with electrochemotherapy on the basis of length of tumor exposure to sufficiently high bleomycin concentrations. Furthermore, we will also collect tumor biopsies in order to determine tumor tissue concentration of bleomycin. Based on the results, we expect to determine minimal tumor concentration of the drug necessary for effective electrochemotherapy.

The results of the proposed international prospective multicentric trial will shed more light also on pharmacodynamics of the drug in the tumors and possibly set the stage for the lowering of the drug dose. Also, the differences in drug accumulation in different tumor types

might be recorded, which would explain the observed subtle differences in responsiveness of different tumors to electrochemotherapy.

2. INTRODUCTION AND RATIONALE OF THE STUDY

Scientific background

Electrochemotherapy is being performed according to the published Standard Operating Procedures (SOP) (*Mir at al., EJC Suppl 2006*). In the SOP, the drug dosage of bleomycin is 15 000 IU/m^2 when injected intravenously. Also, the lag time of 8 minutes and therapeutic window of 20 minutes are determined. Based on these guidelines several 1000 patients throughout the Europe in 145 centers were treated so far, and the number of the treated patients is steadily increasing. Electrochemotherapy is now enclosed in the National Institute for Health and Care Excellence (NICE) guidelines and is also reimbursed as one of the valid treatment options in certain clinical scenarios in many EU countries.

In recent meta-analysis, it was shown that non-melanoma tumor types have increased probability for achieving complete and objective responses compared to melanomas (*Mali EJSO 2013*), indicating that effectiveness of electrochemotherapy is dependent on tumor type.

In addition, so far only the tumors with the largest diameter of up to 3 cm were treated by electrochemotherapy, but lately also bigger tumors are treated with high success rate (*Campana et al 2016*). In the light of the recent technological developments, and increasing experience in electrochemotherapy, new SOP are in preparation.

At the time of preparation of existing SOP, no analysis of pharmacokinetics of bleomycin was done for electrochemotherapy in general and in different age groups of the patients. However, to refine the method, thorough knowledge is needed about the pharmacokinetics and pharmacodynamics of bleomycin, which would base on sensitive analytical method. This method was not available until recently. At the moment, the definition of therapeutic window of electrochemotherapy based on the observed responses of the tumors that were treated at different time points after the drug injection

We have recently developed an analytical method for determination of bleomycin concentrations in the sera and tumors of the patients. The method is based on liquid chromatography coupled with high resolution mass spectrometry (*Kosjek et al. Talanta 2016*). Furthermore, we also determined the pharmacokinetic parameters of bleomycin in 24

patients undergoing electrochemotherapy. For elderly patients (>65 years) subgroup, the results were recently published (*Groselj et al., Cancer Chemoth Pharm 2016*) and demonstrated slower elimination rate of bleomycin in this age group, with the half time of 30 min (Figure 1). Based on these data, we confirmed the 8 minutes interval between intravenous bleomycin administration and application of electric pulses to be adequate, but the data demonstrated that the therapeutic window can be extended from the previous 20 min to 40 min. Furthermore, we proposed that the dose of bleomycin can be reduced, specifically in patients older than 65 years. The decrease in bleomycin dosage can lead also to the less intense post treatment reaction of tumors, and lesser incidence of adverse effects while retaining the same effectiveness.

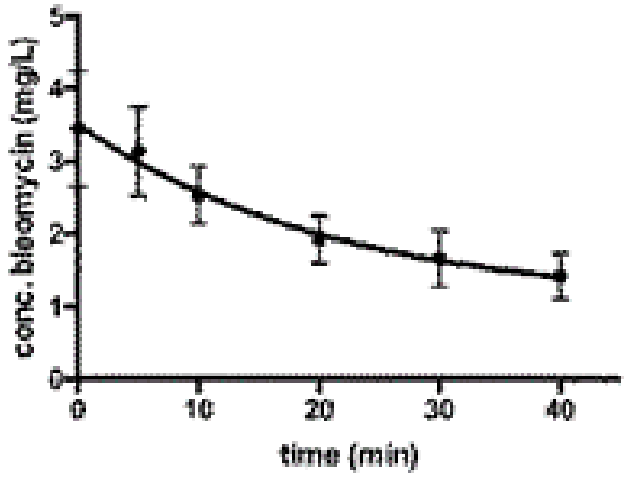

Figure 1. Elimination of Bleomycin from serum after bolus intravenous injection in elderly patients (Cancer Chemoth Pharm 2016)

Rationale of the proposed research

The preliminary data raised great interest and discussion in the community, as well as initiative of an international clinical study that would extend the knowledge about the pharmacokinetics of bleomycin.

The questions that arise are:

- What is the optimal drug concentration in the tumors that is needed for good antitumor effectiveness (objective response)?
- To what extent the intravenously administered drug dose can be safely decreased in elderly and younger patients' populations to attain the same effectiveness?

- Can lowering the dose of bleomycin lead to less intense post-treatment reaction of tumors, and less adverse side effects?
- Do different tumor histotypes respond differently because of the different drug accumulation in the tumors?
- Do pre-treated tumors respond differently because of the different drug accumulation in the tumors?
- Is it possible to further optimize currently proposed therapeutic window?

All these questions are clinically relevant for further development of electrochemotherapy, which should be evidence based.

Therefore, we propose implementation of the new analytical method to determine and resolve the above raised questions in prospective multicentric clinical study.

References:

Mir LM, Gehl J, SERŠA G, Collins CG, Garbay JR, Billard V, Geertsen P, Rudolf Z, O'Sullivan GC, Marty M. Standard operating procedures of the electrochemotherapy: Instructions for the use of bleomycin or cisplatin administered either systemically or locally and electric pulses delivered by Cliniporator ™ by means of invasive or non-invasive electrodes. EJC Suppl 2006; 4: 14-25.

Kosjek T, Krajnc A, Gornik T, Zigon D, Groselj A, Sersa G, Cemazar M. Identification and quantification of bleomycin in serum and tumor tissue by UHPLC-QTOF MS. Talanta 2016.

Groselj A, Krzan M, Kosjek T, Bosnjak M, Sersa G, Cemazar M. Bleomycin pharmacokinetics of bolus bleomycin dose in elderly cancer patients treated with electrochemotherapy. Cancer Chemoth Pharm 2016; 77(5): 939-947. doi: 10.1007/s00280-016-3004-z

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Campana LG, Testori A, Curatolo P, Quaglino P, Mocellin S, Framarini M, Borgognoni L, Ascierto PA, Mozzillo N, Guida M, Bucher S, Rotunno R, Marenco F, De Salvo GL, De Paoli A, Rossi CR, Bonadies A. Treatment efficacy with electrochemotherapy: A multi-institutional prospective observational study on 376 patients with superficial tumors. Eur J Surg 2016; pii: S0748-7983(16)30606-0. doi: 10.1016/j.ejso.2016.06.399. [Epub ahead of print]

3. STUDY OBJECTIVES

A. Primary objective

- Pharmacokinetics and pharmacodynamics of BLM in patients treated with electrochemotherapy, for optimization of the treatment protocol. The objective will be by:
    - Collection of patient's sera undergoing electrochemotherapy according to the SOP and in compliance with NICE recommendations for determination of BLM pharmacokinetics in patients.
    - Collection of tumor tissue at the time of application of electric pulses (8 min after intravenous 15 000 IU/m^2 bleomycin injection) for determination of BLM concentration in tumors at the time of electrochemotherapy.

B. Secondary objective

- Data analysis for determination of:
    - Pharmacokinetics of BLM in patients within different age groups
    - Minimal concentration of BLM in tumors for Objective Response.
    - Correlation between the tumor response and BLM concentration in the tumors.
    - Possible variability in drug concentration according to the tumor type?
    - Possible variability in drug concentration according to the eventual previous therapeutic intervention (radiotherapy, systemic therapies, surgery).
    - Optimization of the therapeutic window

4. SELECTION OF PATIENTS

4.1. Inclusion criteria

- In the study, patients eligible for electrochemotherapy of cutaneous tumors (basal cell carcinoma, squamous cell carcinoma, melanoma) and cutaneus metastases of melanoma and adenocarcinoma of the breast will be included. The selection of patients will be according to the NICE guidelines, with normal renal function, and must be included into the InspECT database.

- The patients will be treated according to the current SOP for electrochemotherapy with intravenously injected BLM in the dose 15 000 IU/m^2.
- Patients age ≥18 years. Patients will be divided into two groups: 18 - 65 years and older than 65 years (WHO definition of elderly person). Each group will comprise of 40-50 patients, in total 80-100 patients.
- Patients who agreed to participate in this study and signed informed consent.

4.2. Exclusion

- Patients not suitable for electrochemotherapy
- Patients who do not comply with requirements for the blood collection, or samples were not collected according to the SOP for blood collection
- Patients who do not comply with the requirements for tumor biopsy, or samples were not collected according to the SOP for tissue sampling.

5. METHODOLOGY

Electrochemotherapy and treatment response evaluation

All patients will be treated by electrochemotherapy according to the current SOP. The treatment reporting data will be included into the InspECT database. The prescribed route of BLM administration is intravenous, at the dose of 15 000 IU/m^2.

Treatment response will be evaluated according to the protocol prescribed in the InspECT database.

Renal function

Estimated Glomerular filtration rate will be calculated using the 2009 CKD-EPI formula. Web based calculator is available on: https://www.qxmd.com/calculate/calculator_251/egfr-using-ckd-epi

Blood collection

Venous or arterial blood will be collected from each patient that will sign the informed consent. All blood samples from specific patient must be drawn in the same way, either intravenous or arterial blood. The validation of the method provided evidence that the BLM

concentrations can be measured with the same sensitivity irrespective of the way of blood samples collection.

The blood will be collected in the following time points: 5, 10, 20, 30, 60, 120 min after drug administration, as defined in prepared SOP.

The time points of 60 and 120 min are not obligatory, when the patient cannot wait that long after the procedure.

The collected blood must be dully labelled, prepared for storage and stored according to the attached SOP for blood collection and storage.

Tumor biopsies

Punch needle biopsy will be obtained from tumors at the time point of 8 min after the end of intravenous bolus injection. Maximum three tumors per patient will be sampled.

The site of the biopsy must be in viable, well perfused part of the tumor, preferable on its margin.

The collected tissue sample must be dully labeled, prepared for storage and stored according to the attached SOP for tumor tissue sampling.

Four histological groups of tumors will be evaluated:

- Basal cell carcinoma
- Squamous cell carcinoma
- Melanoma
- Metastases of adenocarcinoma of the breast

Shipment of tissue samples

Serum or plasma samples will be shipped to the Jozef Stefan Institute, Ljubljana, at regular time intervals, or when samples from more than 10 patients will be gathered.

Address:
Jozef Stefan Institute
Department of Environmental Sciences
Jamova 39
SI-1000 Ljubljana
Slovenia
Phone: +386 31 535 086
Email: tina.kosjek@ijs.si.

The shipment will be organized by Francesca de Terlizzi from the InspECT group, and supervised as well.

CRF for the study

Case Record Form (CRF) will be created in the InspECT electronic database. In the CRF, the exact times of blood samples collection and the tumor biopsy collection will be recorded. All other clinical data and the treatment response will be in the InspECT database anyway, except glomerular filtration rate, which will be included in new CRF. Each participating center will have the overview of the data on patients that included into this study.

Bleomycin determination

All the determinations of the BLM content in blood samples and tumors will be done in Ljubljana, Jozef Stefan Institute. The centralization of the analytical part is for its precision and reproducibility. BLM determination is based on a new method recently published and validated also in serum and tumor samples.

Short description of the method is as follows: Frozen tumor biopsies will be grinded, sonicated and centrifuged, whereas sera or plasma samples will be diluted. Tumor and sera filtrates will be extracted by solid phase extraction, eluted and analyzed for BLM concentration by liquid chromatography coupled to tandem mass spectrometry. The separation will be carried out at ultra-high performance HILIC conditions of a liquid chromatograph, and the detection will employ a sensitive enhanced product ion mode of the mass spectrometer after electrospray ionization. The performance of the analytical method will be controlled and validated for every series of samples analyzed.

Kosjek T, Krajnc A, Gornik T, Zigon D, Groselj A, Sersa G, Cemazar M. Identification and quantification of bleomycin in serum and tumor tissue by UHPLC-QTOF MS. Talanta 2016.

Sample size calculation

The sample size was calculated based on two groups of patients segregated according to their age 18-65 and >65 years. A number of 40-50 patients in each group was calculated to be statistically safe sample size.

6. EVALUATION CRITERIA

A. Primary criteria

- BLM pharmacokinetic parameters will be calculated for each included patient. Further on, the different age groups will be formed (18-65 y; >65 y) and pooled pharmacokinetic parameters will be compared and statistically analyzed.

- BLM concentrations in tumor samples will be analyzed for minimal required BLM concentration in the tumors for achieving Objective Response of the tumors to electrochemotherapy.

B. Secondary criteria

- The data analysis will be to answer the following questions:
    - Is it possible to lower the BLM infusion dose also in patients who are younger than 65 years?
    - What is the therapeutic window for electrochemotherapy?
    - Does the treatment response correlate with the tumor concentration in the tumors at the time of application of electric pulses?
    - Does the pre-treatment (radiotherapy, systemic therapies, surgery) lower the drug accumulation in the tumors?
    - Is there a variability in the tumor response to electrochemotherapy according to the tumor type?
    - Is it possible to further optimize currently proposed therapeutic window?

7. DATA COLLECTION AND STATISTICAL ANALYSIS

All the data will be collected in the InspECT database. Statistical analysis of the data collected will be performed by a statistician using standard statistical tests.

8. STUDY DISCONTINUATION

The study could be interrupted or terminated by the participating centers in agreement with the coordinator and with competent authority for the following reasons:

- frequency and/or unexpected severity of the toxicity,
- poor recruitment of patients,
- poor quality of the data collected.

Supplementary files:

- Patients explanation of the study
- Patient's informed consent
- Instructions (SOP) for blood collection and storage
- Instructions (SOP) for tumor samples collection and storage
- Instructions (SOP) for blood and tumor samples shipment
- CRF of the InspECT database