# The treatment of cutaneous and subcutaneous lesions with electrochemotherapy with bleomycin

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## INTRODUCTION

Electrochemotherapy (ECT) is a local approach combining electroporation phenomenon and drug administration (systemically and/or locally). Electroporation was introduced in the early ‘80s to transfer genes into bacterial and mammalian cells (1). Later on the technique was extend to facilitate cell uptake of DNA, drugs and chemotherapeutic agents. This technique is based on the use of pulsed, high-intensity, electric fields which temporarily increase cell membrane permeability by creation of temporary pores, thus facilitating the transport of normally non permanent molecules into cells (2). One of the most used drugs in this setting is Bleomycin, a hydrophilic, charged cytotoxic drug.

As local treatment modality, ECT has already been proven to be effective in diverse tumour histotypes, although its first application was in skin tumours (3). Up to date, ECT remains a mainstay in the palliative local treatment of cutaneous and subcutaneous melanoma metastases.

## BACKGROUND

ECT consists of in the administration of poorly permanent chemotherapeutic agent, such as Bleomycin or cisplatin, soon after the application of electric pulses to the tumour nodules, in order to enhance drug uptake within the cell (4). Once the drug has reached a high concentration within the cytosol, cell-cycle arrest, mitotic arrest and apoptosis are induced, due to the formation of single- and double-stranded DNA breaks, interfering with the cell cycle machinery. Only a local effect at the site of treatment is produced by electro-permeabilization: the surrounding tissue (or the normal cells within the area of the treatment), although exposed to electric pulses, is not affected.

***-Preclinical studies:***

There are only a limited number of chemotherapeutic compounds suitable for ECT, as electroporation can facilitate only cell membrane transport of hydrophilic drugs, lacking transport

system. Although several drugs have been tested, *in vitro* studies identified that only two of them can be considered as potential candidates for ECT, i.e. Bleomycin and Cisplatin (5, 6).

In the normal cell membrane setting, bleomycin transport inside the cytosol is carried out by carrier proteins, internalizing the compounds via the endocytotic pathway. Such a process is limited by the low number of carrier proteins across the membrane and by their turn-over during the endocytotic pathway, thus constituting the limiting factor of bleomycin cytotxicity. On the contrary, cell exposure to electric pulses and the consequent electroporation lead to an increased membrane permeability and to the direct access of bleomycin to the cell. Once inside the cytoplasm, bleomycin can be transported to the nucleus where it exerts it cytotoxicity. Electroporation induces a 300- to 700-fold increase of bloemycin cytotoxic activity.

Cisplatin transport inside the cell is also hampered, as only 50% is realized by passive membrane diffusion and the rest is due to membrane carriers. Thanks to the increase in drug flux and accumulation inside the cell, electroporation produces a 80-fold increase in cisplatin cytotoxicity (7,8). However the cisplatin-induced DNA adducts formation is still lower than the one obtained with bleomycin. Methotrexate has also been proposed as suitable for use in combination with electroporation (9) (10, 11).

Many *in vivo* studies on animals showed the efficacy of ECT technique. First works on animals in 1987 proved that bleomycin associated with electrical pulses is a very effective drug. Cemazar et al. demonstrated that cisplatin is also effective in treatment of subcutaneous tumours transplanted in mice (12).

Many pre-clinical studies helped to set the parameters of the electrical pulses and the drug dosage to be subsequently used in clinical trials(13, 14, 15, 16, 17).

L.M. Mir et al. showed that the response rate of human tumour lines xenografted subcutaneously onto nude mice treated with electroporation and drugs ranges from 75% to 100% and mice treated with drugs alone had complete response rate in less than 22% (18).

Some recent studies on canine sarcomas and tumours in animals also show the efficacy of ECT, thus possibly suggesting new fields of application for this technique (19, 20, 21).

***-Fields of application:***

Pre-clinical research started with a first clinical study on ECT with bleomycin (BCM), performed in 1991. This study demonstrated the effectiveness of the treatment for cutaneous metastases of head and neck carcinomas (22). In the following years, several clinical studies using BCM or cisplatin, administered either locally or systemically, were carried out. ECT demonstrated to be effective in the treatment of skin metastases of different tumour types such as head and neck squamous cell carcinoma, basal cell carcinoma, melanoma, Kaposi sarcoma, adenocarcinoma of the breast and others. Objective responses were observed in 48-100% of the treated nodules (4). The best results were recorded for smaller and superficial nodules, than in larger and deep lesions where an optimal electroporation may be less effective (23, 24).

**MAIN STUDIES**

A number of trials have been performed on ECT applied to different kind of tumours. In 1991, Mir et al (22) published the first clinical trial on ECT in Head and Neck cancer patients, obtaining good results in terms of tumour response rate.The history of ECT can be mainly divided into two periods, before and after the ESOPE study.

An extensive review of the state of art before ESOPE trial has been reported by Sersa (21) Tab 1. A total number of 247 patients was included into several trials before ESOPE study; 655 nodules from 202 patients were treated with ECT with bloemycin and 354 nodules from 45 patients with ECT with cisplatin. The majority of treated nodules were melanoma metastases, followed by skin, head and neck, breast and ovarian cancer metastases, respectively. Objective responses were obtained in almost 80% of cases. As to melanoma, ECT induced 45% and 77% complete response, when bloemycin was administered intratumourally or systemically, respectively.

Cisplatin induced 67% and 48% complete response, when administered intratumourally or systemically, respectively (21).

ESOPE trial is a prospective non-randomised multi-institutional study, conducted by a consortium of 4 cancer centres gathered by ESOPE project. In this study treatment response after ECT, used drugs, administration route and type of electrodes were investigated (25). Esope trial enrolled 61 patients from 31 march 2003 to 20 April 2005. All the patients were assessable for toxicity related to the treatment, but only 41 of them completed clinical response evaluation for a period of at least 60 days, being 171 nodules evaluated. After ECT, objective response was obtained in 145 of the treated nodules (84.8%), being partial responds 11.1% and complete response 73.7%. Only in a few number of cases a negative response was observed, with either no-response in 10.5% and progressive disease in 4.7%. ESOPE study showed no statistical difference in local tumour control among bleomycin given intratumuorally (73.1%) or intravenously (88.2%) or cisplatin given intratumourally (74.5%). The results of the ESOPE study were reported at American Society of Clinical Oncology (ASCO) and confirmed the effectiveness of ECT. As a result of the study the operating procedures were defined and they now provide a great deal of flexibility so that the oncologist can choose the most appropriate electrodes, drug and route of administration with success rate above 80%. In ESOPE study, evaluated nodules were divided into two groups, melanoma and non-melanoma, gathering the latter all type of cancer other than melanoma. Melanoma nodules represented the 57% of all the nodules. Although ECT antitumoural activity was not statistically significant between the two groups, non-melanoma nodules showed a higher trend of response (OR 90.4% vs 80.6%) supported by a higher CR rate (83.6% vs 66.3%) in comparison with melanoma nodules (24, 25).

**PROCEDURE DESCRIPTION**

Electrochemotherapy is performed by means of an electric pulses generator (the CLINIPORATOR TM), generating squared wave electric pulses with a variable amplitude with

two options for delivered electric pulses frequency (1 or 5000 Hz). The device is computer controlled (23).

There are several levels of the control, both at the machine manipulation as well as at the electrical parameters levels. In addition, thanks to a specific software, it is possible to store patient's characteristics as well as of the electrical parameters used for the treatment including traces of the voltage which was actually applied as well as the current delivered during the procedure (Fig. 5).

Either bleomycin or cisplatin can be used in the treatment. Good antitumor effectiveness has been obtained by either of the drugs (21, 24). Clinical data obtained so far have proved antitumor effectiveness of bleomycin and cisplatin when given intratumorally, however intravenous injection is recommended for bleomycin only (for large tumours). Since the drug treatment can be performed either intratumorally or intravenously, it gives numerous possibilities for the varying treatment modality. Both solitary or multiple nodules can be treated, using local or systemic anesthesia respectively, as described in the various operating modalities of this SOP (21, 24).

Bleomycin administration can be done systemically or intratumorally, in both cases the height and weight of the patients have to be measured in order to calculate the surface area (in the electrochemotherapy modality treatments, only bleomycin can be administered intravenously).

Bleomycin is injected intravenous at a dose of 15 000 IU/m² 8 minutes before ECT in order to obtain the highest drug concentration inside the tumour tissue .

Whether BLM or Cisplatin are administered intratumorally, it is necessary to measure the two main diameters of every lesion, in order to calculate the correct dose of drug which has to be locally injected, according to the values reported in Table 1 and Table 2 .

When administered intratumorally, a BLM concentration of 1000 IU/ml is used and doses are calculated as follows:

| | | | |
|---|---|---|---|
| **Tumor volume (V=ab²ð/6)** | <0.5cm3 | 0.5 cm3 < < 1cm3 | > 1 cm3 |
| **BLM dose, concentration 1000 IU/ml** | 1ml (1000 IU/ cm3 of tumor | 0.5 ml (500 IU)/ cm3 of tumor | 0.25 ml (250 IU) / cm3 of tumor |

Table 1: BLM: lesion volume-drug dose calculation

Cisplatin is applied intratumorally only, at a concentration of 2 mg/ml, calculating the correct dose as follows:

| | | | |
|---|---|---|---|
| **Tumor volume(V=ab²ð/6)** | < 0.5 cm3 | 0.5 cm3 < < 1 cm3 | > 1 cm3 |
| **CDDP dose, Concentration 2mg/ml** | 1 ml (2mg) / cm3 of tumor | 0.5 ml (1mg) / cm3 of tumor | 0.25 ml (0.5mg) / cm3 of tumor |

Table 2: Cisplatin: lesion volume-drug dose calculation

Electric pulses can be delivered by three different types of electrodes that were developed along with the new electric pulses generator (Fig. 6). Type I electrodes are plate electrodes with different gaps between the plates. They are aimed to treat small superficial tumour nodules. Needle electrodes are suitable for treatment of thicker and deeper-seated tumour nodules. There are two types of needle electrodes with either two parallel arrays of needles (Type II electrodes) with a 4 mm gap between them, used for the treatment of small nodules, or an hexagonal array of electrodes (Type III electrodes) for bigger (>1cm in diameter) nodules. This variety of different electrodes was developed in order to encompass the varying cutaneous tumour nodules, which may be suitable for a local treatment with electrochemotherapy (26).

The choise of the appropriate electrode depends on the dimension of the lesion. For lesions smaller than 1 cm, plate or parallel array electrodes should be considered. For lesions larger than 1

cm, hexagonal array electrodes should be used. The choose of electrodes is mainly depending on the position (superficial or deep).

Either in general or local anaesthesia, a 5 kHz frequency treatment reduces the number of contraction, although a frequency of 1 HZ is used too.

Eight minute after drug injection, electric pulses must be applied and the surgeon should wacht the quality of the delivered pulse (Fig 7). Many pulses can be used in order to obtain a complete treatment of the lesions. The treatment must finish within 30 minutes after the end of drug infusion, because drug concentration after that time is too low for an adequate treatment.

The patient can be retreated several times, but the surgeon has to be aware that cumulative BLM dose should not exceed 450000 IU, in order to avoid lung fibrosis (27). In this case the patients should undergo pulmonary function tests.

**CONCLUSION**

ECT is a feasible and safe method for palliative treatment of metastatic melanoma. It can provide an immediate clinical benefit, especially in patients with multiple localized cutaneous and subcutaneous metastasis, which are too extend to be suitable for surgery. Thanks to the few number and incidence of complications, ECT can be repeated several times in order to maintain local control of the disease, thus improving the quality of life of metastatic melanoma patients.

An emerging theme in ECT is represented by the need for new protocol combining ECT with at least another local approach, in order to improve local control and to obtain long-lasting objective tumour responses. Finally, in a future perspective, ECT can be a feasible tool to carry out local gene as well as vaccine electro-transfer (28, 29).

**Examples of ECT treatment results: patient with melanoma in-transit metastases**

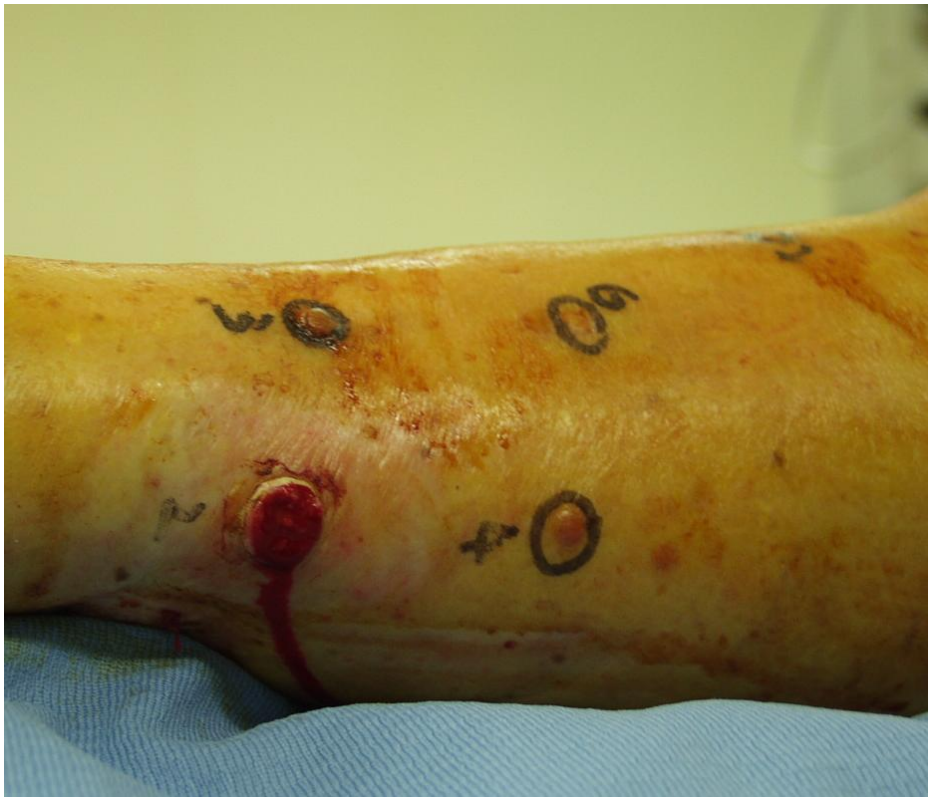

Fig.1 Preoperative field.

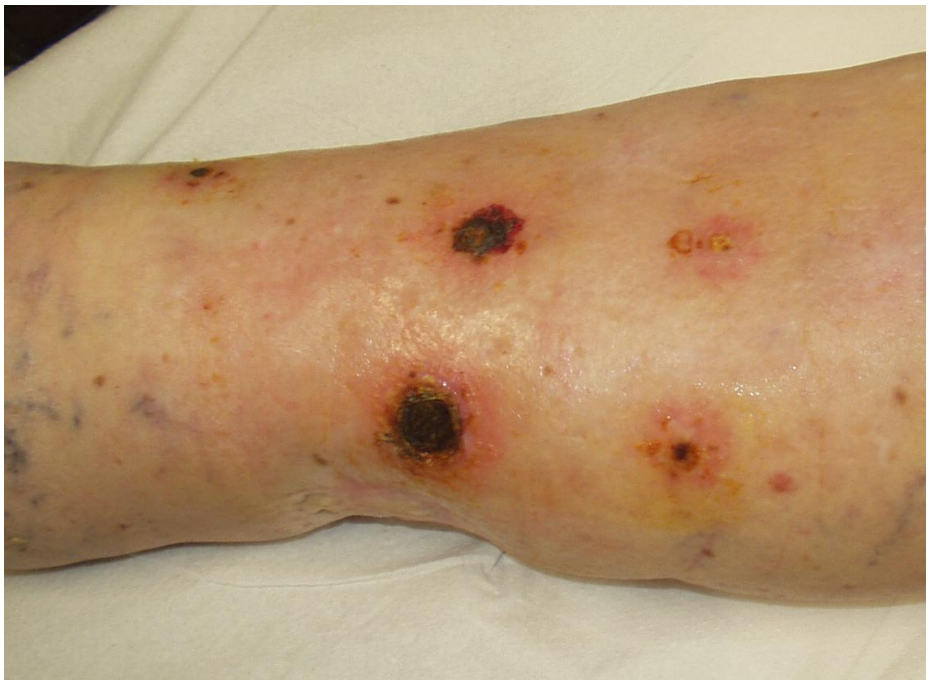

Fig.2 Same field at +19 Days after ECT with BLM

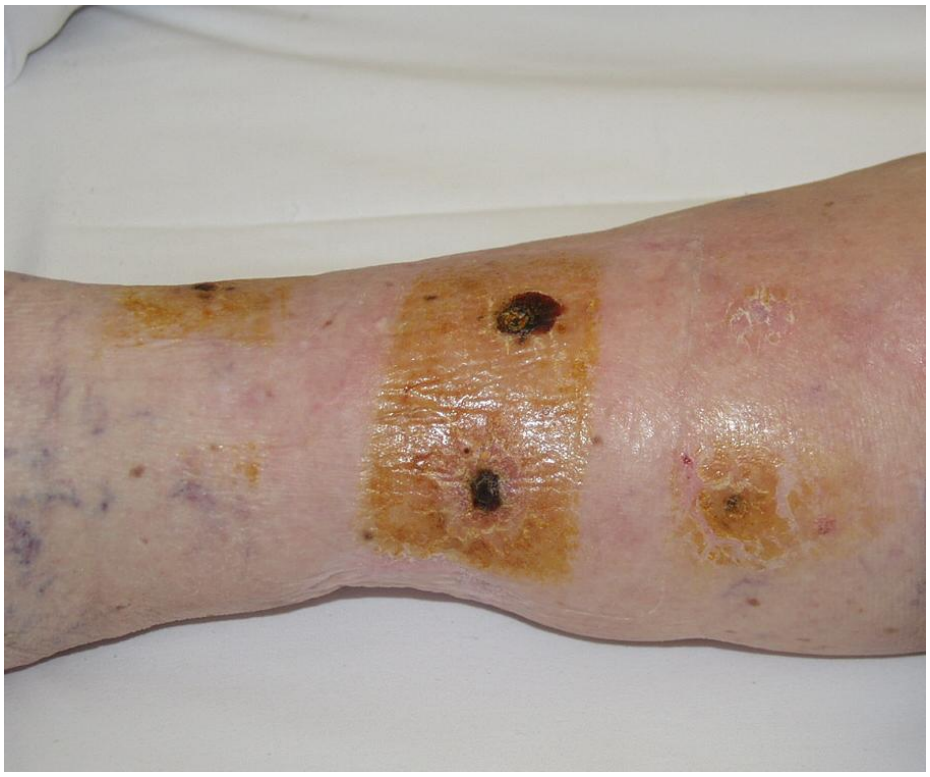

Fig. 3 Same field at +35 Days after ECT with BLM

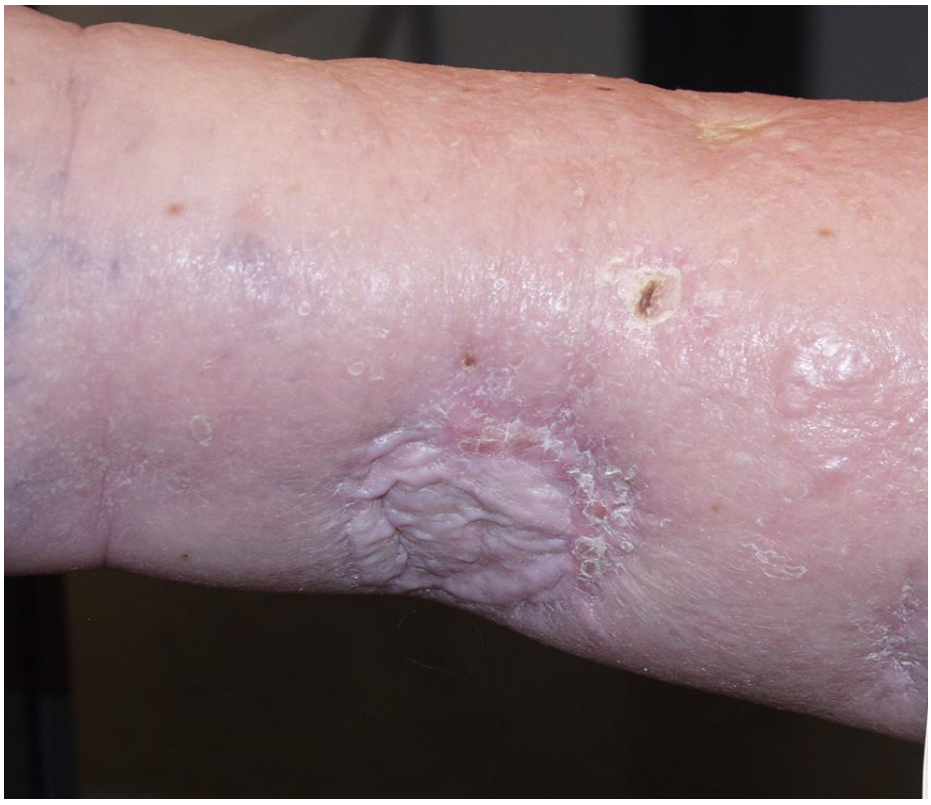

Fig.5  Same field at +35 Days after ECT with BLM

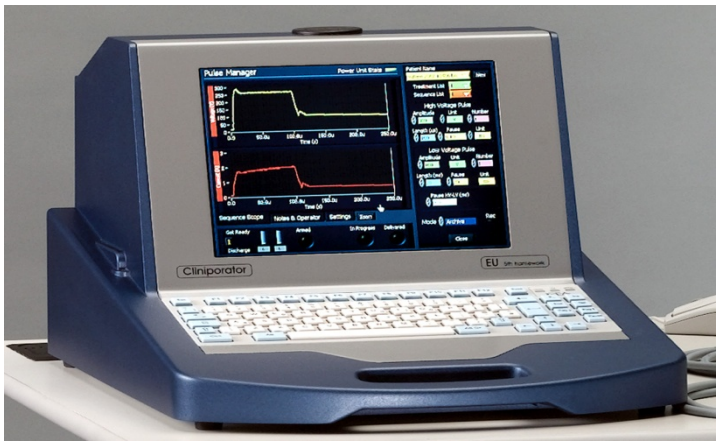


Fig 5. ECT gear

Fig.6 ECT electrode

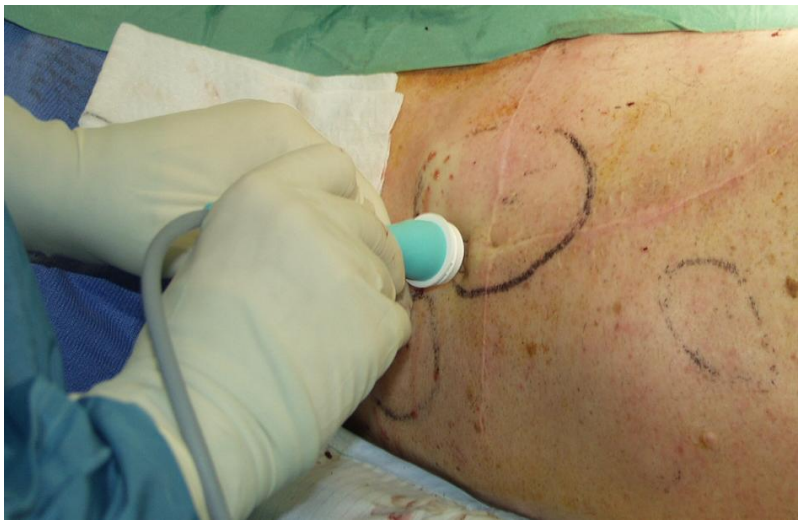

Fig. 7 intraoperative_ ECT procedure

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