

# European Dermatology

V o l u m e 3

The Treatment of Cutaneous  
and Subcutaneous  
Lesions with  
Electrochemotherapy with  
Bleomycin

*a report by*

**A Testori, J Soteldo, A Di Pietro,  
F Verrecchia, M Rastrelli,  
M Zonta and G Spadola**

*Melanoma and Muscle Cutaneous Sarcoma Division,  
European Institute of Oncology*

# The Treatment of Cutaneous and Subcutaneous Lesions with Electrochemotherapy with Bleomycin

a report by

A Testori, J Soteldo, A Di Pietro, F Verrecchia, M Rastrelli, M Zonta and G Spadola

Melanoma and Muscle Cutaneous Sarcoma Division, European Institute of Oncology

Electrochemotherapy (ECT) is a local approach combining the electroporation phenomenon and drug administration (systemically and/or locally). Electroporation was introduced in the early 1980s to transfer genes into bacterial and mammalian cells.<sup>1</sup> Later, the technique was extended to facilitate cell uptake of DNA, drugs and chemotherapeutic agents. This technique is based on the use of pulsed, high-intensity, electric fields that temporarily increase cell membrane permeability by creating temporary pores, thus facilitating the transport of normally non-permanent molecules into cells.<sup>2</sup> One of the most commonly used drugs in this setting is bleomycin, a hydrophilic charged cytotoxic drug. As a local treatment modality, ECT has been proved to be effective in diverse tumour histotypes, although its first application was in skin tumours.<sup>3</sup> ECT consists of the administration of a 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.<sup>4</sup> Once the drug has reached a high concentration within the cytosol, cell-cycle arrest, mitotic arrest and apoptosis are induced by the formation of single- and double-stranded DNA breaks that interfere with the cell-cycle machinery. Only a local effect at the site of treatment is produced by electro-permeabilisation; the surrounding tissue, although exposed to electric pulses, is not affected. There are only a limited number of chemotherapeutic compounds suitable for ECT, as electroporation can facilitate only cell membrane transport of hydrophilic drugs. *In vitro* studies have shown that only two drugs are potential candidates for use in ECT: bleomycin and cisplatin.<sup>5,6</sup> In the normal cell membrane setting, bleomycin transport inside the cytosol is facilitated by carrier proteins internalising 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. In contrast, 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 its cytotoxicity. Electroporation induces a 300- to 700-fold increase of bleomycin cytotoxic activity.<sup>4,9,11</sup> Cisplatin transport inside the cell is also hampered, as only 50% is realised 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 an 80-fold increase in cisplatin cytotoxicity.<sup>7,8</sup> However, the cisplatin-induced DNA adduct formation is still lower than the one obtained with bleomycin. Methotrexate has also been proposed as suitable for use in combination with electroporation.<sup>9-11</sup>

Many *in vivo* studies on animals showed the efficacy of ECT techniques. The first study in 1987 proved that bleomycin associated with electrical pulses is very effective. Cemazar et al. demonstrated that cisplatin is also effective in the treatment of subcutaneous tumours transplanted in mice.<sup>12</sup> Many pre-clinical studies helped to set the parameters of the electrical pulses and the drug dosage.<sup>13-17</sup> Mir et al. showed that the response rate of human

tumour lines xenografted subcutaneously onto mice treated using electroporation and drugs ranges from 75 to 100%, and mice treated with drugs alone had complete response rates below 22%.<sup>18</sup> Some recent studies on canine sarcomas and tumours in animals also show the efficacy of ECT, thus suggesting possible new fields of application for this technique.<sup>19-21</sup>

## Fields of Application

Pre-clinical research began when the first clinical study with bleomycin was performed in 1991. This study demonstrated the effectiveness of the treatment for cutaneous metastases of head and neck carcinomas.<sup>22</sup> Several clinical studies using bleomycin or cisplatin (administered locally or systemically) were undertaken. ECT proved to be effective in the treatment of skin metastases of different tumour types such as head and neck squamous cell carcinoma, melanoma, Kaposi sarcoma and others. Objective responses were observed in 48-100% of the treated nodules.<sup>4</sup> The best results were recorded for smaller and superficial nodules.<sup>23,24</sup>

## Main Studies

A number of trials have been performed on ECT applied to different types of tumours. In 1991, Mir et al.<sup>22</sup> published the first clinical trial results in head and neck cancer patients, obtaining good results. The history of ECT can be divided into two periods: before and after the European Standard Operating Procedures on Electrochemotherapy (ESOPE) study. An extensive review of ECT before the ESOPE trial has been reported by Sersa et al.<sup>21</sup> (see *Table 1*). A total of 247 patients underwent clinical trials before the ESOPE study: 655 nodules from 202 patients were treated with ECT with bleomycin 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. Objective responses were obtained in almost 80% of cases. In melanomas, ECT induced 45 and 77% complete response when bleomycin was administered intratumourally or systemically, respectively. Cisplatin induced 67 and 48% complete response when administered intratumourally or systemically, respectively.<sup>21</sup> The ESOPE trial was a prospective, non-randomised, multi-institutional study conducted by a consortium of four cancer centres. Treatment response after ECT, used drugs, administration route and type of electrodes were investigated.<sup>25</sup> The trial enrolled 61 patients between 31 March 2003 and 20 April 2005. All of 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 (171 nodules evaluated). After ECT, objective response was obtained in 145 of the treated nodules (84.8%), with 11.1% being partial responses and 73.7% complete responses. In only a few cases was a negative response observed, with either no response in 10.5% of cases and progressive disease in 4.7%. The study showed no statistical difference in local tumour control between bleomycin given intratumourally (73.1%) or intravenously (88.2%) or cisplatin given intratumourally (74.5%). Evaluated nodules were divided into two groups: melanoma (57%) and non-melanoma. Although ECT

antitumoural activity was not statistically significant between the two groups, non-melanoma nodules showed a higher trend for response (overall response [OR] 90.4 versus 80.6%) supported by a higher complete response rate (83.6 versus 66.3%) compared with melanoma nodules.<sup>24,25</sup> The results of the ESOPE study were reported at an American Society of Clinical Oncology (ASCO) meeting and confirmed the effectiveness of ECT. As a result of the study, operating procedures were defined so that the oncologist can choose the most appropriate electrodes, drug and route of administration, with success rates in excess of 80%.

### Procedure Description

Electrochemotherapy is performed by means of an electric pulse generator (the CLINIPORATOR™, Igea, Italy) that produces squared wave electrical pulses with a variable amplitude with two options for electrical pulses frequency (1 or 5,000Hz). The device is computer-controlled.<sup>23</sup> There are several levels of the control over the machine manipulation and electrical parameter levels. In addition, thanks to specific software it is possible to store patients' characteristics as well as the electrical parameters used for the treatment, including traces of the voltage that was applied as well as the current delivered during the procedure. Either bleomycin or cisplatin can be used in ECT; good antitumour effectiveness has been obtained using both of the drugs.<sup>21,24</sup> Clinical data obtained so far have proved the antitumour effectiveness of bleomycin and cisplatin when given intratumourally; however, intravenous injection is recommended for bleomycin only (for large tumours). As the drug treatment can be performed either intratumourally or intravenously, it gives numerous possibilities for the varying treatment modality. Both solitary or multiple nodules can be treated, using local or systemic anaesthesia, respectively.<sup>21,24</sup> Bleomycin administration can be performed systemically or intratumourally; in both cases, the height and weight of patients have to be measured in order to calculate the surface area. Bleomycin is injected intravenously at a dose of 15,000IU/m<sup>2</sup> eight minutes before ECT in order to obtain the highest drug concentration inside the tumour tissue. Whether bleomycin or cisplatin are administered intratumourally, 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 *Tables 1* and *2*.

Electrical pulses can be delivered by three different types of electrodes that were developed along with the new electrical pulses generator. 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 the treatment of thicker and deeper-seated tumour nodules. There are two types of needle electrodes; two parallel arrays of needles (type II electrodes) with a 4mm gap between them, used for the treatment of small nodules, or a hexagonal array of electrodes (type III electrodes) for bigger (>1cm in diameter) nodules. This variety of different electrodes was

**Table 1: Bleomycin Lesion Volume and Drug Dose Calculation**

Tumour volume (V=ab <sup>2</sup> δ/6)	<0.5cm <sup>3</sup>	0.5cm <sup>3</sup> < < 1cm <sup>3</sup>	>1cm <sup>3</sup>
Bleomycin dose, concentration	1ml (1,000IU)/cm <sup>3</sup> of tumour	0.5ml (500IU)/cm <sup>3</sup> of tumour	0.25ml (250IU)/cm <sup>3</sup> of tumour
	1,000IU/ml		

**Table 2: Cisplatin Lesion Volume and Drug Dose Calculation**

Tumour volume (V=ab <sup>2</sup> δ/6)	<0.5cm <sup>3</sup>	0.5cm <sup>3</sup> < < 1cm <sup>3</sup>	>1cm <sup>3</sup>
Cisplatin dose, concentration	1ml (2mg)/cm <sup>3</sup> of tumour	0.5ml (1mg)/cm <sup>3</sup> of tumour	0.25ml (0.5mg)/cm <sup>3</sup> of tumour
	2mg/ml		

developed in order to encompass the varying cutaneous tumour nodules that may be suitable for a local treatment with electrochemotherapy.<sup>26</sup> The choice of the appropriate electrode depends on the dimension of the lesion. For lesions <1cm, plate or parallel array electrodes should be considered. For lesions larger than 1cm, hexagonal array electrodes should be used. The choice of electrode depends on the position (superficial or deep). In general and local anaesthesia a 5kHz frequency treatment reduces the number of contractions, although a frequency of 1HZ can also be used.

### Systemic and Local Treatment

In systemic treatment, eight minutes after drug injection electrical pulses must be applied and the surgeon should check the quality of the delivered pulse on the electroporator monitor. Many pulses can be used in order to obtain a complete treatment of the lesions. The treatment must finish within 38 minutes after the end of drug infusion because drug concentration after that time is too low to provide adequate treatment. In local treatment, for the intratumoural injection of bleomycin or cisplatin (see *Tables 1* and *2*), electrical pulses must be immediately applied to the lesions. The patient can be re-treated several times but the cumulative bleomycin dose should not exceed 450,000IU in order to avoid lung fibrosis.<sup>27</sup> In this case the patient should undergo pulmonary function tests.

### Conclusion

ECT is a feasible and safe method for the palliative treatment of metastatic melanoma. It can provide an immediate clinical benefit, especially in patients with multiple localised cutaneous and subcutaneous metastases that are unsuitable for surgery. Thanks to a low incidence of complications, ECT can be repeated several times in order to maintain local control of the disease. An emerging theme in ECT is the need for a new protocol combining ECT with another local approach in order to improve local control and obtain long-lasting objective responses. Finally, in a future perspective, ECT is a feasible tool for carrying out local gene and vaccine electrotransfer.<sup>28</sup> ■

- Neumann E, et al., *EMBO J*, 1982;1:841–5.
- Gehl J, *Acta Physiol Scand*, 2003;177:437–47.
- Sadadcharam M, Soden DM, O'Sullivan GC, *Int J Hyperthermia*, 2008;24(3):263–73.
- Gothelf A, et al., *Cancer Treat Revs*, 2003;29:371–87.
- Sersa G, et al., *Cancer Ther*, 2003;1:133–42.
- Domenge C, et al., *Cancer*, 1996;77:956–63.
- Marty M, Sersa G, Garbay JR, et al., *Expert Rev Anticancer Ther*, 2006;6:1–8.
- Larkin JO, et al., *Ann Surg*, 2007;245(3):469–79.
- Gehl J, Skovsgaard T, Mir LM, *Anticancer Drugs*, 1998;4: 319–25.
- Kuriyama S, et al., *Int J Oncol*, 1999;1:89–94.
- Orlowski S, et al., Transient electroporation of cells in culture. Increase of the cytotoxicity of anticancer drugs, *Biochem Pharmacol*, 1988;24:4727–33.
- Cemazar M, et al., *Eur J Cancer*, 2001;9:1166–72.
- Engstrom PE, et al., *Anticancer Res*, 2001;38:1817–22.
- Mitsui K, et al., *Drug Deliv*, 2002;4:249–52.
- Kitamura A, *Cancer Chemother Pharmacol*, 2003;4:359–62.
- Sersa G, *Anticancer Res*, 1999;58:4017–22.
- Entin I, *Clin Cancer Res*, 2003;8:3190–97.
- Mir LM, *Eur J Cancer*, 1991;1:68–72.
- Spugnini EP, et al., *In Vivo*, 2007;21(5):819–22.
- Spugnini EP, et al., *In Vivo*, 2007;21(5):897–9.
- Sersa G, et al., *Br J Cancer*, 2008;98(2):388–98.
- Mir LM, et al., *C R Acad Sci Paris*, 1991;313:613–18.
- Mir L, et al., *EJC*, 2006;(Suppl. 4):14–25.
- Giardino R, et al., *Biomed Pharmacother*, 2006;60(8):458–62.
- Mir LM, Electrochemotherapy for local tumour control. Results of the ESOPE European trials. In: Boiron M, Marty M (eds), *Eurocancer 2005*, Paris: Editions John Libbey Eurotext, 2005;319–30.
- Spugnini EP, et al., *J Exp Clin Cancer Res*, 2005;24(2):245–54.
- Comis RL, *Semin Oncol*, 1992;19(Suppl. 5):64–70.
- Mir LM, *Methods Mol Biol*, 2008;423:3–17.