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Electrochemotherapy to Metastatic Spinal Melanoma

A Novel Treatment of Spinal Metastasis?

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Study Design. Preliminary report of new antitumor treatment.
Objective. To evaluate the effectiveness of electrochemotherapy as a novel treatment of spinal metastasis.
Summary of Background Data. Electrochemotherapy is a new antitumor treatment that combines systemic bleomycin with electric pulses delivered locally at the tumor site. These electric pulses permeabilize cell membranes in the tissue, allow bleomycin delivery diffusion inside the cells, and increase bleomycin cytotoxicity. Previous clinical studies have demonstrated the effectiveness of electrochemotherapy in the treatment of several primary and metastatic solid tumors.
Methods. Treatment planning for electrode positioning and electrical pulse parameters was prepared for 4 needle electrodes. Mini-open surgery with a left L5 laminectomy was performed to introduce the eletrodes. The patient was treated according to the established Electrochemotherapy Protocol with Bleomycin. Clinical efficacy of electrochemotherapy was evaluated according to a visual analog scale of pain, Oswestry Disability Index 2.0, the Karnofsky Performance Scale, and Response Evaluation Criteria in Solid Tumors.
Results. The assessed follow-up period was 48 months after the electrochemotherapy procedure. Neither serious electrochemotherapy-related adverse events, nor bleomycin toxicity were reported. Overall improvement in pain according to Oswestry Disability Index 2.0 and Karnofsky Performance Scale outcomes was better.
Conclusion. Our case represents, to our knowledge, the first one to test the potential role of electrochemotherapy as treatment of spinal metastasis. Electrochemotherapy allowed a successful treatment of metastatic spinal melanoma. However, we believe that there is a strong scientific rationale to support the potential utility of electrochemotherapy as a novel treatment of spinal metastasis, regardless of the histological types.
Key words: electroporation, electropermeabilization, electrochemotherapy, bleomycin, spinal tumor, metastasis, melanoma, cliniporator, tumor ablation, electric pulses.
Level of Evidence: 5
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Electroporation is a physical method in which high voltage direct current electric pulses cause non-selective plasma membrane permeabilization. This method is widely used for introduction of molecules such as DNA, antibodies, enzymes, dyes, and drugs into cells.[1] The use of electric pulses to facilitate delivery of chemotherapeutic drugs into tumour cells was termed electrochemotherapy (ECT) and was introduced by Okino *et al*[2] and Mir *et al*[3] ECT efficacy is independent of tumor histology and is an effective local therapy for many solid tumors, such as malignant melanoma, basal cell carcinoma, squamous cell carcinoma, adenocarcinoma and others. Pore formation on the cell membrane allows low permeant chemotheratic drugs like bleomycin or cisplatin to enter the cell and thus locally increase their toxicity: up to 10.000 times for bleomycin and 80 times for cisplatin. Studies have shown high incidence of complete responses between 70% and 90% of cases with cost effectiveness and has few local and systemic side effects.[4,5]

ECT has been used clinically since 2005 and is currently in use in 83 centers all over Europe (European Standard Operating Procedures of the Electrochemotherapy—ESOPE).[5,6] New indications using ECT have emerged in the medical literature, especially in bone tumors.[7] In our Institute, the Laboratory of Preclinical and Surgical Studies has previously published about ablation of bone cells by electroporation. The results of this study show that electroporation can induce ablation of bone cells without affecting the recovery of osteogenic activity.[8,9] Based on this study

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and previous successful treatments of other tumour types, we report a case of metastatic spinal melanoma treated with ECT. We present here the first reported clinical case using ECT as a novel option of treatment to spinal metastasis.

MATERIALS AND METHODS

Clinical Data of the Patient

A 51-year-old female Caucasian patient presented 4 successive occurrences of epistaxis. Nasal examination revealed a polypoid mass lesion of right nasal cavity originating from the inferior concha. Endoscopic excisional biopsy was performed for this lesion and histopathological diagnosis was a mucosal malignant melanoma. Next the patient underwent maxillectomy associated with turbinectomy and sphenoidectomy. 4 years later the patient had lesion recurrence and again it underwent surgical treatment. Radiotherapy was also planned. 1 year after completion of radiotherapy, the patient presented atypical low back pain (VAS 10). MR imaging showed a suspected lesion at the L5 level measuring $30 \times 20 \times 15$ mm (Figure 1).

Subsequently the patient was referred to our department of oncologic spine surgery and a percutaneous biopsy of the L5 vertebral body was performed. Histopathological examination and immunohistochemistry were consistent with metastatic melanoma (Figure 2A). Regarding the surgical strategy, oncologic scores of the patient (Karnofky = 80%, Tokuhashi = 11, Tomita = 5) indicated a locoregional treatment for the tumor at the L5 level. At the time neither spine instability nor neurological signals were found. Thereafter ECT was offered as a local/palliative treatment option to prevent rapid disease progression and was intended to spare patients from any intolerable morbidity coming from extended surgical resection. The patient agreed with this option and gave her informed consent.

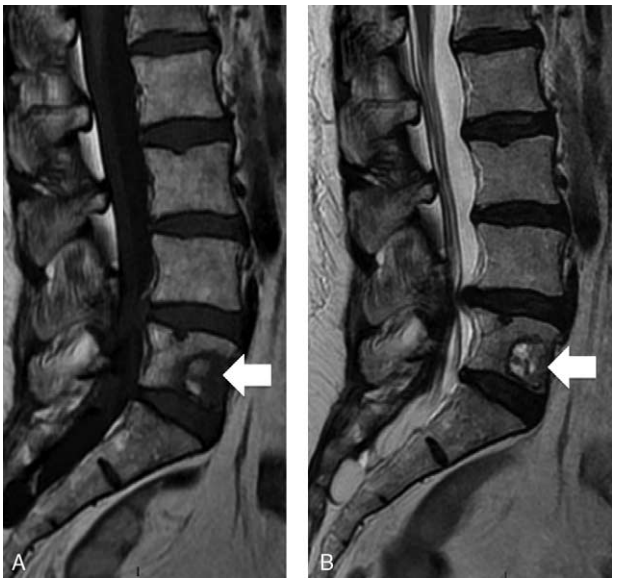

Figure 1. (A) Sagittal T1-weighted MR imaging showed a heterogenic 30-mm lesion at the L5 level with mixed hyperintense/hypointense signal (arrow). This hyperintense signal intensity was attributed to hemorrhage or melanin associated with melanoma metastasis. On (B) sagittal T2-weighted MRI, the hypointense side of the lesion became an isointense signal (arrow).

The patient was monitored intraoperative and for 48 hours after ECT. Clinical and radiological examination was scheduled at 1, and 4 weeks and at 3-month intervals thereafter. The patient's reported outcome was collected using the Visual Analogic Scale (VAS), Oswestry Disability Index 2.0 (ODI), and the Karnofsky Performance Scale (KPS). Tumor response and control were clinically assessed on target lesion according to response evaluation criteria in solid tumors (RECIST [version 1.1; http://www.eortc.be/recist/]). Percutaneous vertebral biopsy was performed on the 3rd postoperative month. Local and systemic toxicity of bleomycin were graded according to Common Terminology Criteria for Adverse Events (CTCAE 4.0 [http://ctep.cancer.gov]).

Operation

Under general anesthesia, the patient was placed in the prone position, and then a posterior operative approach with a left L5 laminectomy was performed, providing an adequate exposure of the pedicle area. Care was taken not to injury the root and dural sac. 4 needle eletrodes were placed around the vertebral tumor at the L5 level under fluoroscopic and neuronavigator control (Figure 3).

Bleomycin Administration

Systemic bleomycin (Bristol-Myers Squibb, Princeton, NJ) was delivered by intravenous (i.v.) push (i.e., in 30–45 s) at a dose of 26 mg after connections of eletrodes and 8 minutes before electrical pulse delivery. Bleomycin administration was executed according to ESOPE (10 or 15 mg/m^2).[10]

Electrodes, Pulse Generator and Pulse Parameters

4 custom-made electrodes made of stainless steel, 1.8 mm in diameter with sharpened tips and insulated were used. The electrodes were connected to independently controlled generator outputs of the Cliniporator Vitae (*IGEA, Carpi, Italy*). The Cliniporator Vitae device is a pulse generator with 6 independently controlled and electrically insulated outputs each providing up to 3000 V, max current 50 A, delivering 8 rectangular electrical pulses (rise time 1 μs) of 100 μs duration at a pulse repetition frequency of 4 Hz.[11] Pulse deliverance was monitored to assure the effectiveness of the applied electrical field 1.5 A. The current and voltage are measured and logged with a precision better than 3%, which allows for pulse delivery control and post-treatment evaluation. Pulses were delivered 8 minutes after i.v. Bolus injection of bleomycin. During electrical pulse delivery the patient was partially curarized.

Electric Field Monitoring

An oscilloscope and a standard current monitor were used to monitor pulses that were delivered to the patient. Applied voltages were monitored across the outputs of the RIX instrument. The oscilloscope had the capability to store up to 4 digitized waves. This storage function was used

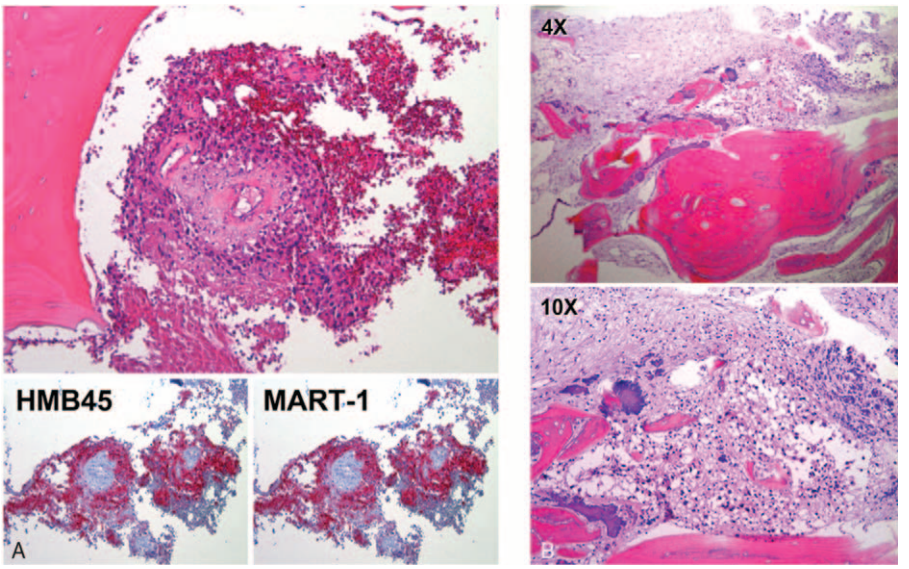

Figure 2. Histopathological exam with H&E staining. (**A**) Pre-ECT biopsy showed nests of tumoral poorly differentiated cells with positive HMB45 and MART-1 antibodies. (**B**) Post-ECT biopsy showing intertrabecular fibrosis and necrosis (4 ×) and focal residual nests of viable tumoral cells (10 ×). The overall percentage of necrosis obtained using ECT was >95%.

to save experimental signals for analysis following treatment. Signal data from the oscilloscope was stored and analyzed through interface of the computer coupled to the Cliniporator Vitae.

RESULTS

Tolerance of ECT

No significant modification of hemodynamic or cardiologic parameters was noticed during the ECT. Despite the neuromuscular block, some mild contractions affecting the muscles of low back was observed after each electrical pulse. Contractions were instantaneous, disappearing immediately at the end of each electrical pulse. There were no local or general side effects. Postoperatively, the patient did not have any disagreeable impression, either on waking or in the hours or days that followed the treatment. The patient was discharged on the third day reporting just mild pain related to surgical site and no neurological symptom.

Clinical Outcomes

The assessed follow-up period was 48 months after the ECT procedure. All outcome details of present case are provided in Table 1. Neither serious ECT-related adverse events, nor bleomycin toxicity were reported. At the 3-month follow-

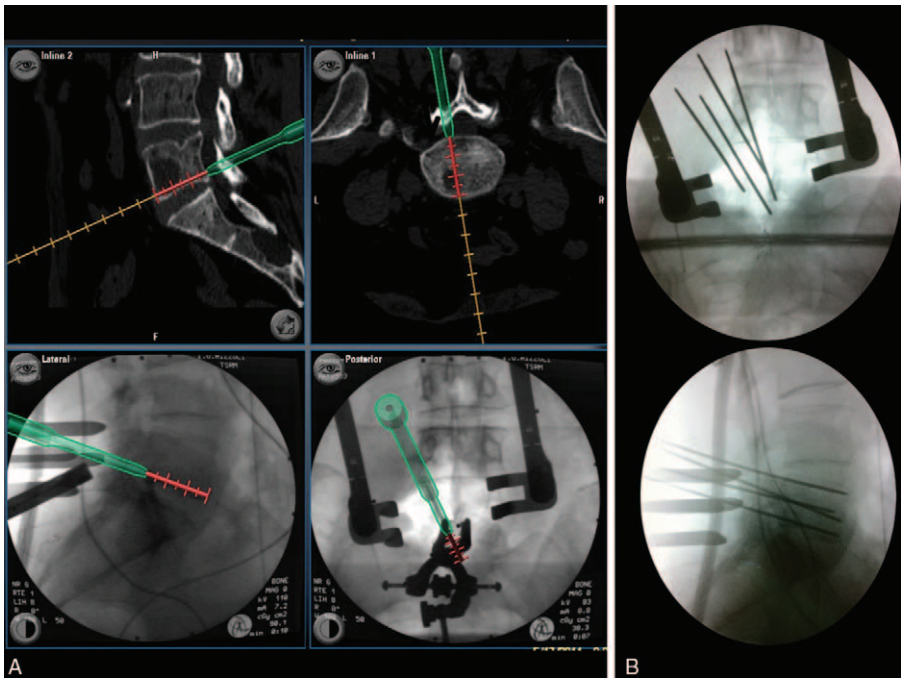

Figure 3. Intraoperative image control. The accurate placement of needle electrodes around the metastatic lesion was achieved under navigation (A) and fluoroscopic (B) control of placed electrodes.

TABLE 1. Summary of ECT Treatment for Metastatic Melanoma to the Spine: Clinical Outcome and the Time Point Response According to Response Evaluation Criteria in Solid Tumors (RECIST)

	Pre-ECT	1st mo	3rd mo	6th mo*	9th mo	12th mo†	24th mo	48th mo
VAS	10	2	2	5	2	0	0	0
ODI	65	18	9	25	12	5	3	3
Tokuhashi score	11							
Tomita score	5							
KPS	80	90	100	70	90	90	100	100
BLM toxicity	—	—	—	—	—	—	—	—
Histology (vertebral biopsy)	Melanoma		>95% of necrosis					
MRI findings (spine)								
Contrast-enhanced	+	—	—	—		—	—	—
Spinal Instability	—	—	—	—		—	—	—
Tumor size	30 mm	30 mm	30 mm	30 mm	30 mm	30 mm	30 mm	30 mm
Tumor response evaluation								
PET/CT (body)	—	—	—	—	—	Lung	Lung	Lung
PET/CT (spine)	+++	—	—	+	+	+	+	+
Target lesions	CR	CR	CR	CR	CR	CR	CR	CR
Non-target lesions		CR (spine)	CR (spine)	SD (spine)	SD (spine)	SD (spine)	SD (spine)	SD (spine)
							PR (lung)	PR (lung)
New lesions	*Yes*	No	No	No	No	*Yes*	No	No
Overall response	*PD*	CR	CR	SD	SD	*PD*	SD	SD

*Radiotherapy.
†Chemotherapy.
CR indicates complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, invaluable.

up, histopathological examination showed more than 95% of necrosis following a new percutaneous vertebral biopsy (Figure 2B). Likewise, no lesion was identified on basis of FDG-PET/CT scans (Figure 4). Nevertheless, a positive FDG-PET/CT at 6-month follow-up was found. In this time, the patient also presented back pain again (Table 1). Therefore, this image finding was interpreted as probably local recurrence. Immediately, radiotherapy treatment (10 session × 3 Gy = 30 Gy) in the L5 vertebra was performed. Subsequently, the tumor size at the L5 level was stable and there were no new lesions on MRI scans of the spine until the last follow-up.

Overall improvement in pain outcomes was excellent, with pain intensity (VAS) preoperatively of 10 and postoperatively of 2 ($P < 0.0001$) before the radiotherapy (6th mo), and of 0 after radiotherapy at last follow-up ($P < 0.0001$). The interference with global function (ODI) was 65 preoperatively and reduced to 9 ($P < 0.0001$) before the radiotherapy (6th mo), and 3 after radiotherapy at last follow-up ($P < 0.0001$). In addition, the patient improved her Karnofsky rating (KPS) from 80 preoperatively to 100 postoperatively until the 6-follow-up and at last follow-up ($P < 0.005$). Despite the questionable local recurrence of metastasis in the L5 vertebra at 6-month follow-up, no local tumor progress occurred after the radiotherapy treatment. Thereafter, according to RECIST criteria, this target lesion was considered stable disease.

DISCUSSION

Spinal metastases are the most frequent tumor of bone and the most frequent tumor of the spinal column regardless of the origin of the primary tumor. More than 90% of spinal tumors are metastatic. The treatment of spinal tumors represents a challenge to spine care professionals. To date, treatment options include medical therapy, surgery, chemotherapy and radiation. Historically, surgery was considered the most appropriate initial therapy in patients with spinal metastasis with the goals of eradication of gross disease. However, operative intervention is often palliative, with pain control and maintenance of function and stability

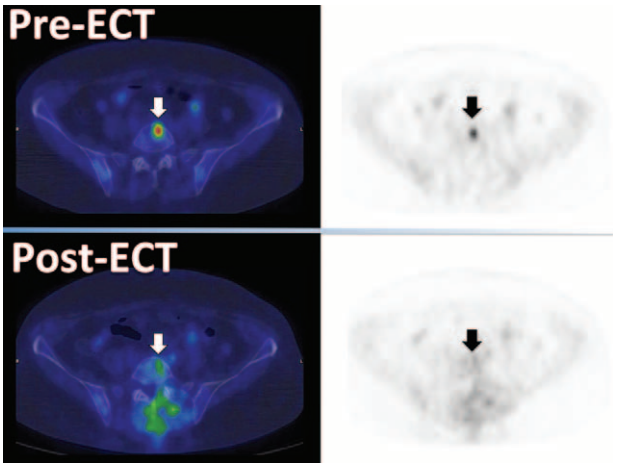

Figure 4. Axial view FDG-PET/CT and scintigraphy scans in present patient comparing the result of ECT treatment. Pre-ECT images showed intense focal area of 18-FDG activity in L5 vertebral body. Post-ECT images showed no metabolic activity.

the major goals. Furthermore, such an aggressive approach has not been practical for many patients.[12–15]

Spinal metastasis from melanoma is an uncommon event and its natural history still is unclear in the medical literature. Gokaslan *et al*[16] reported a review of 133 cases and concluded that melanoma metastatic to the spine represents a late event in the evolution of this illness. Although the median survival for these patients was 4 months, surprisingly in our patient the last follow up was 48 months so far. Therefore, our patient's survival fueled tremendous excitement in the efforts to elucidate the mechanism associated to ECT as a novel treatment of spinal metastasis, regardless of the histological types.

Developments in cancer therapy have advanced along with the introduction of new technologies coming from the basic research to clinical use. Regarding the theoretical knowledge of electropulsation technology, it has been crucial to obtain the most suitable protocols for chemotheratic drug delivery.[2] The molecular mechanisms remain rather obscure, however 2 key phenomena are induced in the cell membrane: the induced transmembrane voltage, which is crucial for electropermeabilization, and the diffusion of the drug molecules through the permeabilized cell membrane after electrical pulse delivery. ECT is a novel type of tumor treatment that combines the use of specific chemotherapeutic drugs (bleomycin or cisplatin) that have an intracellular target and low membrane permeability, with application of electric pulses to the tumors to increase drug uptake into cells.[17] Extension of the ECT to nodules of other origins preclinical studies supported the rationale that ECT may be applied to tumors of quite different origins, such as melanomas, carcinomas, sarcomas and gliomas.[4,5,17–21]

In fact, once inside the cell, the chemical reaction of chemotherapeutic drugs on DNA is very rapid and recognizably independent of the origin of the neoplasic cell. Because electropermeabilization is a universal phenomenon, in principle any kind of tumor can be treated by ECT, whatever its usual sensitivity to bleomycin or cisplatin.[22] In the present study, we chose the bleomycin as the chemotherapeutic drug based on ESOPE protocol and because its local toxicity increases up to 10.000 times.[4,5] This cytotoxic effect of the bleomycin enhanced by ECT was confirmed in present study by histological examination. We found more than 95% necrosis in the biopsy specimen at 3-month follow-up period (Figure 2B). According to histopathology report and its importance, our result was excellent. In general, oncologic response to chemotherapy is noted based on percentage necrosis of tumor cells in bone tumours. For patients who receive chemotherapy prior to surgery, the degree of tumor necrosis observed postoperatively is highly predictive of disease-free survival, local recurrence, and overall survival. Patients showing 90% and greater necrosis in the resected tumor specimen have a better outcome than patients with a poorer histologic response.[23]

Nevertheless, in present case FDG uptake on PET/CT scans was showed at 6-month follow-up. It is well known that FDG-PET is mostly used in oncology for staging and therapy control. On the other hand, it is important to keep in mind that although PET is a sensitive tool for detecting malignancy, FDG uptake is not tumor specific. It can also be seen in healthy tissue or in benign disease as inflammation or post-traumatic repair and could be mistaken for cancer.[24] However, as this FDG uptake on PET/CT scans could be local recurrence, radiotherapy was proposed as the safest treatment option for the patient. There were no new lesions on MRI scans of the spine until the last follow-up (Table 1).

Beside the cytotoxic effect of BLM input, several studies have evidenced that other mechanisms also contribute to the observed anti-tumour effectiveness of ECT, such as the immune system and modification of tumour blood flow. Because of the massive tumor antigen shedding in the animals after ECT, systemic immunity can be induced and up-regulated by additional treatment with biological response modifiers such as interleukins 2 and 12, granulocyte–macrophage colony-stimulating factor, and tumor necrosis factor alpha.[25–27]

Application of electroporation also induces a rapid, profound but transient two-phase decrease in local blood flow. The first phase is instantaneous, extreme (almost complete shut-down of blood flow), but short-lived (several minutes) and is a result of vasoconstriction of afferent arterioles. The second component is slower, less extreme, but longer-lived (24 h) and is a result of transient disruption of cytoskeletal structures and compromised barrier function of the microvascular endothelium.[28,29]

ECT has been an effective means of local control for a variety of cancers, mainly melanoma, with an approximately 80% objective response rate.[30–32] As a local treatment, ECT can be an excellent alternative therapy. The healing of a site after ECT is by secondary intention. A potential advantage of this type of healing is that the defect is usually less extensive and less mutilating than excisional surgery, resulting in less scarring. An additional advantage is

that ECT is a tissue-sparing procedure that maintains the functionality of the adjacent tissue associated to a progressive and highly selective destruction of the tumours cells.[33,34]

Miklavcic *et al*[17] reported the first clinical case deep-seated tumor electrochemotherapy based on numerical treatment planning. However, this study had an inadequate tumor electroporation, supposedly associated with failure in the positioning of the electrodes. A word of caution, however, should be added concerning this point. Another study has shown that the effectiveness of the treatment is not affected significantly by small single errors in electrode positioning. Obviously, when many errors occur simultaneously, the resulting drop in effectiveness is larger.[35] In the present study, care was taken to place the electrode using both fluoroscopic control and neuronavigator system (Figure 3).

ECT has also been used in combination with radiation therapy. As well known, hypoxic cells are radioresistant and special attention has to be taken into account when scheduling the combined treatment. Recent studies have shown that both drugs used in ECT, bleomycin and cisplatin, are also radio-sensitizing drugs, and are significantly more radiosensitizing when used in ECT.[36,37] Based on this rationale, conventional external beam radiotherapy was performed in present case. However, stereotactic radiosurgery have been offered a better therapeutic modality for the safe delivery of large dose fractions of radiation therapy in a single fraction for the management of spinal metastases, even in patients with previously irradiated lesions.[38,39]

Choosing the best approach for metastatic spinal tumours is often difficult, and depends on many factors, including life expectancy and the balance of the risk of surgery or another treatment against the likelihood of improving quality of life. In some particular cancer patients, studies with ECT have shown several advantages on other anti-tumor techniques: ease of administration, low cost, minimal toxicity (usually limited to mild focal inflammation at the treated site), and high response rate. Furthermore, ECT may be combined with other therapies that could produce a local control associated to optimizing of a potent systemic therapy. Moreover, ECT could also represent a palliative treatment for spinal metastases patients with local or regional disease who are not candidates for surgical resection and have failed radiation therapy and/or chemotherapy.

Our case represents, to our knowledge, the first to test the potential role of ECT as treatment of spinal metastasis. ECT allowed a successful treatment of metastatic spinal melanoma with local control reducing the risks, downtime, and adverse effects related to surgery, radiotherapy, and systemic chemotherapy. In conclusion, we believe that there is a strong scientific rationale to support the potential utility of ECT as a novel and adjuvant treatment of spinal metastasis, regardless of the histological types. However, large, well-designed randomized, controlled, long-term studies are needed to confirm the clinical efficacy of this new treatment.

➢ Key Points

- ❑ More than 90% of spinal tumors are metastatic and the treatment represents a challenge to oncologic multidisciplinary team.
- ❑ Electrochemotherapy (ECT) is a recently described therapy that relies on the permeation of cancer cell membranes by electrical pulses to enhance cytotoxic drug penetration.
- ❑ There are many authors supporting the potential utility of ECT as a novel and adjuvant treatment of local tumor, regardless of the histological types.
- ❑ Based on this biological rationale, we reported the first case of metastatic spinal tumor treated with electrochemotherapy.

Acknowledgments

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