

## Electrochemotherapy for digital chondrosarcoma

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**Abstract** Electrochemotherapy (ECT) delivers nonpermeable anticancer drugs to cell interiors by temporally increasing the permeability of the cytoplasmic membrane under locally applied pulsating electrical stimuli. This treatment results in consistent and enhanced pharmacological effects of drugs on the targeted tissue. ECT has been used for surface skin cancer but never for musculoskeletal tumors. This report describes a clinical trial of ECT for digital chondrosarcoma. A 74-year-old woman with a digital chondrosarcoma was administered electric stimulation with two surface electrodes 10 min after intratumoral multiple injection of bleomycin sulfate and 15 s after intraarterial perfusion of bleomycin sulfate. Biopsy performed after ECT showed 90% tumor necrosis. Marginal resection of the tumor was followed by autologous bone grafting to fill the bone defect. Although the follow-up period was short (3 years), the patient remained disease-free after ECT and was satisfied that amputation of the affected finger could be avoided. This preliminary result suggests that ECT is a viable modality for limb-preserving treatment of patients with sarcoma of the extremities.

**Key words** Electrochemotherapy · Chondrosarcoma · Finger

### Introduction

Electroporation (EP) is a technique that facilitates passage of chemical reagents or DNA into cells by applying electrical pulses, which create transient pores in the cell membrane.<sup>3,9</sup> The combination of EP and administration of an antineoplastic agent has been introduced as a new anticancer drug delivery method for treating the tumor burden; the whole procedure is known as electrochemotherapy (ECT).<sup>10,13</sup> ECT is effective because the electric pulses permeabilize tumor cell membranes, thereby allowing nonpermeant drugs,

such as bleomycin, to enter the cells, giving them free access to their intracellular target. ECT has been successful *in vivo* in animal models,<sup>7,14,17,18</sup> and for the treatment of patients with cutaneous malignancies in clinical trials.<sup>1,6,11</sup> The aim of the study presented here was to assess the results of a clinical trial of ECT for chondrosarcoma in the middle finger of a 74-year-old woman.

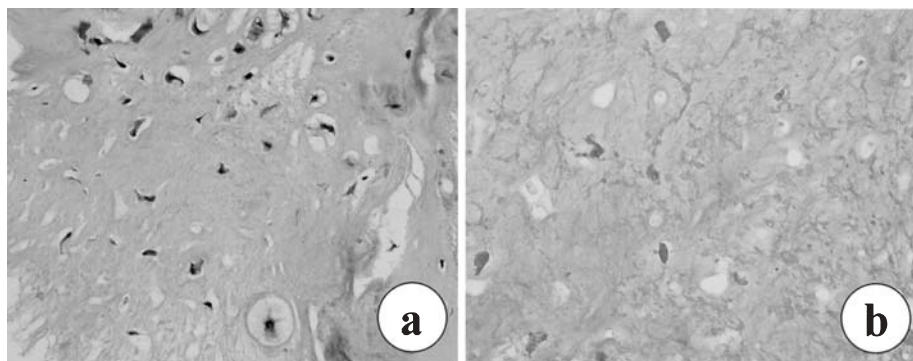
### Case presentation

The patient visited our hospital in March 1999 complaining of a 3-year history of swelling and soreness of her right middle finger, which had gradually increased in size (Fig. 1a). There was no disturbance of sensation, although flexion was limited owing to the swelling of the base of the finger. Radiological examination showed expansion and radiolucency of the proximal phalanx accompanied by cortical erosion (Fig. 1b). Core needle biopsy results led to a diagnosis of low-grade chondrosarcoma (Fig. 2a). Further radiological study did not show metastasis or any bony lesion of other sites. Because our usual strategy for digital chondrosarcoma is amputation, we suggested ray amputation. The patient strongly refused this, however, because she was right-hand dominant and for cosmetic reasons. This made us consider the possibility of electrochemotherapy, which has previously been proven effective for skin cancer. The treatment was approved by the Ethics Committee of our institution, and was undertaken with the informed consent of the patient and her family.

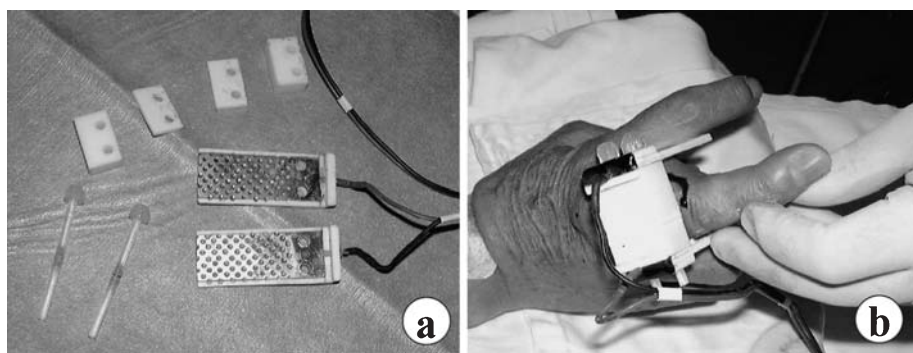
Electric pulse treatment using a Square Electroporator CUY-21 (TR Tech, Tokyo, Japan) was conducted percutaneously via two parallel stainless-steel surface electrodes with an uneven surface (stainless square plate 3 × 5 cm) that were placed on opposite sides of the tumor (Fig. 3). The electric pulses were applied at 150 V



**Fig. 1.** **a** Proximal phalanx of the middle finger is swollen. **b** Expanding radiolucent mass in the proximal phalangeal bone. **c** No recurrence was seen even 3 years after treatment



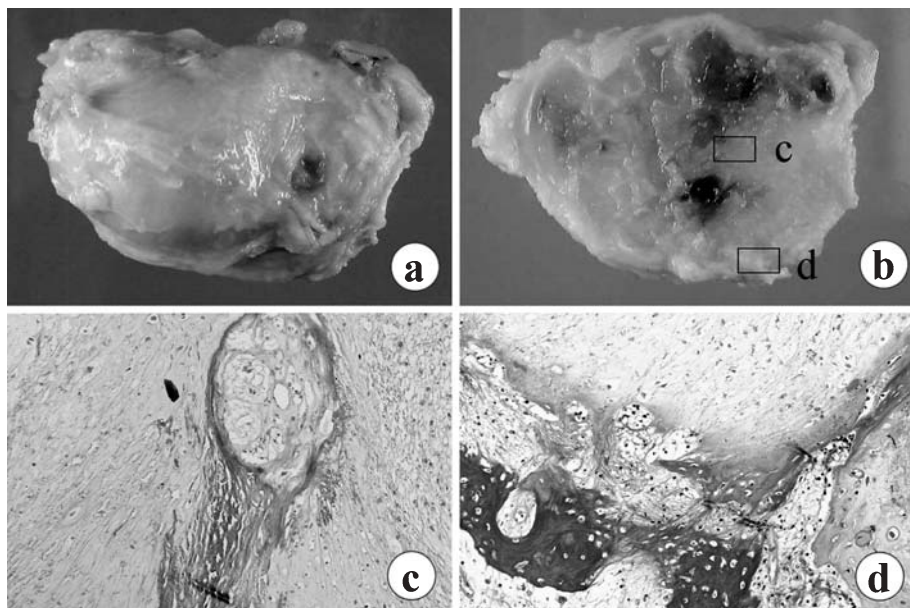
**Fig. 2.** Biopsy specimen. **a** Cells varying in size and shape with nuclear atypia and multivesicular vacuolation of cytoplasm. **b** Tumor necrosis after electrochemotherapy. H&E  $\times 200$



**Fig. 3.** Electrode and electroporation. **a** Stainless steel  $3 \times 5$  cm electrodes. **b** Electroporation with two parallel electrodes placed on opposite sides of the tumor

(50 V/cm, distance 3 cm; four pulses of 250 ms or six pulses of 167 ms). The pulses were applied 10 min after local injections of bleomycin sulfate 0.5 mg/0.1 ml (Nippon Kayaku, Tokyo, Japan) for a total dose of 5 mg. The injections were made into 10 parts of the tumor under axillary brachial plexus block. To monitor the characteristics of the electric pulses delivered for each treatment, voltage was measured at the generator output, and current traces were measured with a current probe. The condition of the electric pulses was examined at 150 V,  $1.1 \pm 0.5$  A, and  $245 \pm 101 \Omega$ . The patient tolerated the procedure well,

and no significant side effects were noted. Muscle contraction was evident during each electric pulse; the electrocardiogram showed a drop in the base of the wave, but it promptly recovered after the pulse. Two weeks after ECT, histological analysis of core needle biopsies revealed 90% tumor cell necrosis (Fig. 2b). ECT was performed again 1 month after the first application. An arteriogram of the finger, obtained prior to ECT, showed scanty vascularization in the central part of the tumor burden but a well-vascularized periphery. This time also the pulses were applied 10 times to the tumor 10 min after local injections of



**Fig. 4.** Gross and microscopic findings of the resected tumor. **a** Gross appearance of marginally resected tumor. **b** Cut surface. **c** Microphotograph of the central portion of the tumor (area *c* in panel **b**). **d** Peripheral region (area *d* in panel **b**). H&E  $\times 200$

bleomycin sulfate 0.5 mg/0.1 ml (total dose 5 mg) into 10 parts of the tumor 15s after intraarterial injection of 5 mg bleomycin under axillary brachial plexus block. The timing of electroporation was set at 15s based on the results of the arteriogram, which showed tumor staining 10–15s after contrast medium infusion. After 1 month of two courses of ECT, the tumor was resected marginally together with the subcutaneous connective tissue followed by bone grafting from the iliac bone to fill the bone defect. Hence the finger was rescued from amputation. A histological study of the resected tumor showed necrosis at the tumor margin (Fig. 4). Two months later bone union had been achieved at the proximal grafted part. No evidence of recurrence or distant metastasis was seen for 3 years after ECT (Fig. 1c).

## Discussion

A clinical trial was undertaken using ECT for chondrosarcoma in the middle finger of a 74-year-old woman. This is the first reported case of clinical application of ECT for a musculoskeletal tumor. ECT has so far proven to be effective for anticancer treatment, regardless of histological type, in a variety of animal models.<sup>14,17</sup> Based on studies concerning anticancer drugs used in combination with EP, bleomycin (BLM) was first selected for use with ECT. These studies found that administration of BLM in combination with EP has a stronger cytotoxic effect than other cancer drugs.<sup>4,16,18</sup> BLM is hydrophilic and highly cytotoxic, but it cannot permeate cancer cells, as it is

unable to cross the plasma membrane efficiently. When combined with EP, however, free access of BLM to its intracellular targets is facilitated by transient permeation of the cell membrane.<sup>10,15,16</sup>

Other anticancer drugs for use with ECT were also studied. Osteosarcoma is a common musculoskeletal tumor, and methotrexate (MTX) is a key for drug used treatment. Because MTX also has low ability to permeate sarcoma cells, a high dosage is needed, which results in unavoidable significant side effects such as myelosuppression or nephrotoxicity (or both) despite rescue treatment with citrovorum factor. We examined the efficacy of MTX for osteosarcoma in an experiment involving four groups of mice: controls, MTX only, EP only, and ECT (MTX+EP). The anticancer effect of MTX was enhanced by ECT, whereas EP or MTX alone could not devitalize the sarcoma, although either treatment slowed the tumor growth but with no evidence of massive tumor necrosis (unpublished data). If the capability of MTX can be enhanced in combination with EP, the dosage can be reduced, with a resultant lower incidence of side effects and no impairment of the antitumor effect of MTX. Clinically, soft tumors located in the surface (e.g., skin cancer) can be eliminated by ECT. Whereas it is easy to inject anticancer drugs and to insert the electrodes into surface tumors, deep-seated tumors, such as most musculoskeletal sarcomas, do not feature such advantages. The tumor in our patient was located in the finger, making drug injection and electrode application easy. The positioning of the electrodes and the drug delivery system remain to be determined for deeply situated tumors. The electroporating effect is limited to cells

exposed to the electric field generated between electrodes. Therefore, multidirectional application of the electrical stimulation is essential to cover the whole tumor with the effective electrical field for maximal effect. This means that more sophisticated positioning of the electrodes is needed as well as better designed electrodes to generate an electrical field covering the whole tumor.

Most tumors have abundant blood supply at the periphery of the tumor and scant blood supply in the central region, so EP after intraarterial perfusion of the anticancer drug must be followed by surgical removal. If tumor cell death in the peripheral region is achieved (i.e., the margins of the tumor contain no viable cells so recurrence is prevented), surgical removal on a reduced scale, such as marginal excision, may be selected.

Until now, all studies have used high-voltage EP (1000–2000 V/cm).<sup>2,6,8,14,18</sup> For safer, more convenient treatment, we decided to try low-voltage EP. The quantity of heat is given by the following equation:  $Q = V^2 \times T/4.2R$ , where  $Q$  is the heat (in joules),  $V$  is voltage,  $R$  is resistance (in ohms), and  $T$  is the loading period (in seconds).<sup>5</sup> Consequently, in terms of heat generated, electric pulses of 1000 V/cm for 100  $\mu$ s are the equivalent of 50 V/cm for 150 ms (i.e., one pulse of 150 ms).<sup>12</sup> In our preliminary experiments involving ECT with BLM for leiomyosarcoma-bearing mice<sup>7</sup> and using various voltages (50, 100, 150, 200, or 250 V/cm for each pulse), an effect was observed at 100 V/cm or more. Therefore, for our patient the voltage applied was 50 V/cm for two courses.

## Conclusions

In the case reported here, EP was performed after local and transarterial administration of BLM for chondrosarcoma. When it is necessary to remove the tumor after ECT, as in our case, the margins of the tumor should contain no viable cells so recurrence can be prevented. For an effective drug delivery system, therefore, intraarterial administration in addition to local tumor injection of BLM is considered necessary. Although this is a preliminary trial report, we concluded that ECT appears to be a feasible limb-preserving treatment for patients with sarcoma of an extremity.

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