Cutaneous tumor treatment by electrochemotherapy: preliminary clinical results in horse sarcoïds

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SUMMARY

Electrochemotherapy is a new anticancer therapy where the transient permeabilization of cells by electric field pulses induces a significant increase of antitumoral drug concentration and toxicity in tumor cells. It has been successfully applied to the treatment of tumors in animals and humans by using antimitic drugs. This report describes its first use in the treatment of horse sarcoïds. Several horses have been enrolled. They had several tumors located at different positions. Treatment was performed under short duration general anesthesia. Intra tumoral injections of cisplatin were followed by short and intense electric pulses directly applied on the skin at the tumor sites. Two to four successive treatments were applied at two-week intervals. Antitumor effect was obtained. To date, three horses have a two-years period of surveillance. Objective responses were obtained in 100% of the treated lesions. All horses tolerated the treatment well. No adverse effect from the electric pulses was observed even in the case of a high number of pulses, or when several consecutive treatments were applied.

KEY-WORDS: horses - sarcoïds - cisplatin - electrochemotherapy.

Introduction

Electric field pulses can induce the transient permeabilization of cell plasma membrane. This method is widely used for introduction of molecules such as DNA, antibodies, enzymes, and drugs into cells [9]. For the last ten years, it has been developed to facilitate delivery of drugs into cancer cells [8]. The critical intracellular target for cytotoxicity of drugs such as cisplatin and bleomycin is DNA. Bleomycin causes breaks in DNA, whereas cisplatin forms DNA adducts. The cytotoxicity of drugs is dependent on their intracellular concentration which is controlled by membrane permeability. Permeabilization of cells by electric pulses allows the hydrophilic drug to penetrate into the cells. Antitumoral drugs have therefore a direct access to the cytosol where they can fully exert their cytotoxic potential and can be used at lower doses than the ones required in classical protocols of chemotherapy [14]. It has been shown that in vitro electropermeabilization of cells potentiates cytotoxicity of bleomycin by several hundred times and cytotoxicity of cisplatin up to 70 times. In vivo, electropermeabilization of cells potentiates anti tumor effectiveness of cisplatin by a factor 20 [2]. This method, called electrochemotherapy (ECT), introduced in the 90's [8, 7], has already been successfully applied to mice and rats for a large variety of tumors [1]. Clinical trials have been performed in humans including small nodes of...
head and neck squamous cell carcinoma, melanoma, basal cell carcinoma and adenocarcinoma [6-11]. To date, very few data are available on domestic animals [5, 12].

Sarcoids are nonmetastatic skin tumors which constitute one of the most frequent skin tumors in horses. These tumors adversely affect the material value of horses and often compromise the use of the animal because of their location. They indeed occur on any part of the body either singly or in clusters. The head, ventral abdomen and limbs are most commonly affected. The gross appearance of sarcoids can vary. They are classified in several groups: occult, verrucous, nodular, fibroblastic and mixed (verrucous an fibroblastic) [3]. Therapies using classical surgical excision or laser photovaporization, as well as cryotherapy are used to treat them. However, such non-conservatire methods do not lead to a total cure of the disease and relapses are frequent. Moreover, these methods usually require specific training or special facilities and equipment, and their implementation in routine limited [13]. More conservative methods such as brachytherapy while effective face the same shortcomings. Chemotherapy using cisplatin is the most widely used method among the conservative treatments limited however to small tumors (less than 5 cm in diameter). This is due to its easy use, its rather low cost and its high efficiency (up to 90 % for sarcoids and up to 70 to 90 % for carcinomas). However, the main disadvantage is the poor diffusion of the hydrophilic drug into the tumors. Cisplatin is therefore mixed with sesame oil in order to increase its remanence at the injection point [13].

Increasing cisplatin concentration in sarcoid by using ECT would therefore enhance the cytotoxic effect thereby increasing treatment effectiveness. That was the aim of the study, horse sarcoids representing an interesting clinical model due to its high occurrence and specific localization to skin.

**Materials and methods**

**HORSE AND TUMOR CHARACTERISTICS**

3 horses were treated. They were from the two sexes and aging from 5 to 8 years. All of them were previously treated by surgery but had relapses. Cutaneous tumors were confirmed as sarcoids by histology. All horses are still under observation and the results presented up below are those observed two years after completion of the last ECT treatment.

**PREPARATION OF THE PATIENTS**

The animals are treated under general anaesthesia of short duration. Depending on the number of tumors to be treated, anaesthesia ranged from 15 minutes for 1 tumor to 40 minutes for several tumors.

**TREATMENT**

Firstly, the antimitotic drug was injected intra tumorally and secondly, 5 min after, the electrical treatment was applied by bringing electrodes in contact with the skin.

1) **Antimitotic drug injection**

Cisplatin* was prepared in sterile 0.15 M NaCl at 1 mg/ml concentration. It was then intra tumorally injected in a standardized manner (0.2 to 0.3 ml every 0.6 cm) by using «luer-lock» or automatic syringes [13] (figure 1a).

2) **ECT treatment**

A specially designed set of wire contact electrodes was built (figure 1b). The distance between the electrodes was 0.9 cm and their length was 0.9 cm. A PS15 Jouan Electrosurator was used to deliver 8 pulses of 0.1 ms at a 1 Hz frequency with a 1.3 kV voltage. The pulse duration and current intensity were selected to take into account the recommendations of «the Commission de l'Electricité Industrielle» concerning the fibrillation risks (for a pulse duration of 0.1 ms, the intensity must be less than 5 A).

The contact of the electrodes with the skin was obtained by a conductive paste. Multiple electrotreatments were applied by moving the electrodes on the tumor surface on adjacent positions. This allowed the treatment of all the tumor surface.

Several successive treatments were performed with a two-week interval.

**TREATMENT RESPONSES MONITORING**

During and immediately following the ECT treatment, horses were carefully monitored to determine immediate effects. They were examined 2 weeks after ECT to determine treatment responses. Pictures were taken prior to ECT treatment and every 2 weeks at each ECT session. Lesions were measured using a calliper. Responses were scored as follows: no response (NR); partial response (PR: > 50 % reduction in tumor volume); complete response (CR: absence of any trace of tumor), and relapse [6]. A post treatment surveillance period of 2 years is required to close each case.

**Results**

To date, numerous horses are under treatment for different kinds of tumors. Only 3 cases which were treated for sarcoids have completed a two years post treatment surveillance period. Lesions to be treated were selected on each horse according to severity, size or localization. The results of these cases are presented here.

**TREATMENT RESPONSES**

— For horse 1, multiple sarcoids were treated. They were present under fibroblastic and verrucous forms located on the head, at the cheek region. The size of the tumors varied from 1.5 to 5 cm in diameter. 4 ECT sessions were performed, the last one being a safety one (figures 2a and 2b).

— For horse 2, a single tumor (1.8 cm width, 3.1 cm length) was located at the ear level as a relapse to a surgical treatment performed 4 months before ECT treatment. 3 ECT

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* cis-platinium (II)-diammine dichloride, P-4394, SIGMA, St-

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FIGURE 1. — ECT procedure. - a: intratumoral cisplatin injection; b: electrodes in contact with skin lesions.

FIGURE 2. — Case 1: multiple fibroblastic and mixed sarcoïds. - a: before ECT; b: one year after ECT.

FIGURE 3. — Case 2: mixed sarcoïd as a relapse to a prior surgical excision. - a: before second surgery and ECT; b: one year after ECT.

FIGURE 4. — Case 3: verrucous sarcoïd. - a: before ECT; b: one year after ECT.
sessions were performed starting two weeks after a new surgical treatment (figures 3a and 3b).

— For horse 3, 2 sarcomas were treated: one at the limb (fibroblastic, 3 cm width, 7 cm length) and the other at the nose level (verrucous, 1 cm width, 2 cm length). 2 ECT sessions were performed (figures 4a and 4b).

For all horses, complete regression of all lesions was obtained even for the largest ones. After each ECT session, the size of the lesions decreased. Their complete regression was obtained with a number of ECT sessions which varied from 1 (for the smallest one) to 3 (for the largest ones). No relapse was observed two years after the last treatment.

**TREATMENT SAFETY**

All horses tolerated ECT well. Muscular contractions were observed during each pulse application. The amplitude of these movements depended on the tumor localisation, being more pronounced when the application was close to a limb root and at the ear level. These effects are expected when electric stimulation is used.

The day after ECT treatment and the followings, a slightly oedematous reaction was noticed on some horses for lesions located on thin skin regions. No other adverse reaction was observed.

**Discussion**

3 horses were fully treated totaling 10 tumors ranging from 1.5 to 5 cm in diameter. Complete regression was observed whatever the size of the tumor with no relapse up to two years following the last ECT treatment. General anaesthesia was used rather than local or loco-regional anaesthesia in order to prevent any uncontrolled horse reaction. A good tolerance to the delivery of a high number of pulses (an average of 160 per animal over a 15 min period) was obtained. Horses were treated up to 4 times, at two-weeks intervals, without any adverse reaction. An approximate total surface of 150 cm² of skin was treated. No negative effect was observed. Skin integrity was preserved even in regions previously submitted to surgical treatment. Objective responses were seen in 100% of the treated lesions with a complete response percentage of 100%. It must be noticed that small lesions with diameter < 10 mm responded faster to ECT than larger ones with diameter > 10 mm (from 15 to 50 mm in that study). They ineluctably regressed after only one ECT treatment. This effect was probably linked to the depth of penetration of the electric field.

As a conclusion of this electro-mediated cutaneous tumor treatment of horses, antitumor effect seems to be long-lived due to the stabilization of the treated lesions as observed two years after ECT. Because ECT is observed to be a safe method, results of this preliminary trial on horse sarcomas are encouraging.

**Reference cited**