First Horse Sarcoid Treatment by Electrochemotherapy: Preliminary Experimental Results (21-Nov-2003)

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Abstract
Electrochemotherapy (ECT) enhances the effectiveness of chemotherapeutic agents. First, intratumoral drugs are injected; then, short intense electric pulses are applied, which permeabilizes tumor cell membranes and allows the drug to reach significantly higher cell concentrations.

1. Introduction
Cutaneous tumors are frequent in equids. Sarcoids represent more than 50% of those tumors. Chemotherapy using cisplatin is the most widely used method among the conservative treatments; however, it is limited to small tumors (< 5 cm in diameter). This is because of its easy use, low cost, and high efficiency (up to 90% for sarcoids and up to 70 - 90% for carcinomas) [1]. However, the main disadvantage is the poor diffusion of the hydrophilic drug into the tumors. Therefore, cisplatin [a] is mixed with sesame oil to increase its permanence at the injection point [2].

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 of 20 [3]. This method, called electrochemotherapy (ECT), was introduced in the 90s, and it has already been successfully applied to mice and rats for a large variety of tumors [4]. Clinical trials have been performed in humans including the small nodes of head and neck squamous cell carcinoma, melanoma, basal cell carcinoma, and adenocarcinoma [5]. To date, very few data are available on domestic animals [6-8].

The hypothesis of this study was that increasing cisplatin concentration in equine sarcoids using ECT would enhance the cytotoxic effect, thus increasing treatment effectiveness. The treatment of equine sarcoids represents an interesting clinical model because of its high occurrence and specific localization to the skin of the horse.

2. Materials and Methods
Horse and Tumor Characteristics
From October 1999 to January 2003, 25 horses were included in this study. Selection criteria for inclusion in the study were horses with:

- Small tumors < 5 cm diameter, having either verrucous, fibroblastic, or nodular forms (Fig. 3A, Fig. 4A and Fig. 5A).
- Larger tumors (>5 cm in diameter) that were subjected to prior surgical excision (debulking) and left to heal for 2 weeks before ECT was implemented. (These cases were not included in the study.)

Cutaneous tumors were confirmed as sarcoids by histology. Forty-six different individual tumors were treated. The older cases have now completed a 3.5-yr, post-treatment surveillance period. Horses were of both sexes and averaged 8 yr of age (range = 3 - 19 yr). Tumor diameter ranged from 10 to 50 mm (with an average size of 26 mm and a sample standard deviation equal to 20 mm). Most of the horses that were previously treated by surgery had relapses.
Preparation of the Patients
The animals were treated under general anesthesia for short duration. Depending on the number of tumors to be treated, anesthesia ranged from 15 min for one tumor to 40 min for several tumors.

Treatment
First, the antimitotic drug was injected intratumorally and at 1 cm in the skin margins of the tumor (one more cm is added at the second treatment) (Fig. 2A). Second, 5 min after the injection of cisplatin, the electrical treatment was applied by bringing electrodes in contact with the skin (Fig. 2B).

Antimitotic Drug Injection - NaCl (0.15 m) at 1 mg/ml concentration of cisplatin [a] was prepared. It was then intratumorally injected in a standardized manner (0.2 - 0.3 ml every 0.6 cm) by using "luer-lock" or automatic syringes [2].

ECT Treatment - A specially designed set of wire contact electrodes was built. The distance between the electrodes was 0.9 cm, and their length was 0.9 cm. A PS15 Jouan Electropulsator [b] was used to deliver eight pulses of 0.1 ms at a 1-Hz frequency with a 1.3-kV voltage (Fig. 1). The pulse duration and current intensity were selected according to the recommendations of the Commission de l'Electricité Industrielle concerning the fibrillation risks to the patient (for a pulse duration of 0.1 ms, the intensity must be < 5 A).

Treatment Response Monitoring
During and immediately after the ECT treatment, horses were carefully monitored to determine the immediate effects. They were also examined 2 wk after ECT to determine treatment responses. Pictures were taken before ECT treatment and every 2 wk at each ECT session. Lesions were measured using a caliper. 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 [5]. A post-treatment surveillance period of 2 yr was required to close each case.

3. Results
Twenty-five horses with 46 individual tumors were treated (Fig. 3A, Fig. 4A, Fig. 5A). Eradication was obtained after less than four successive treatments in all horses (Fig. 3B, Fig. 4B, Fig. 5B).

No adverse reaction was elicited by the delivery of repeated pulses, except for the expected muscle contractions. Skin integrity was preserved. The day after ECT treatment, a slightly edematous reaction was noticed on some horses with lesions located on thin skin regions.
4. Discussion

Complete tumor regression was seen in this group of horses with no relapse up to 1 yr after the last ECT treatment. General anesthesia was used rather than local or regional anesthesia to prevent any uncontrolled horse reaction. A good tolerance to the delivery of a high number of pulses (an average of 160/animal over a 15-min period) was obtained. Horses were treated up to four times at 2-wk intervals without any adverse reaction. No negative effect was obtained. 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%. Small lesions with diameter < 10 mm responded faster to ECT than did larger ones with diameter > 10 mm (from 15 to 50 mm in this study). They ineluctably regressed after only one ECT treatment. This effect was probably linked to the depth of penetration of the electric field. The combination of intratumoral drug injection antielectrons in ECT treatments enhance the effectiveness of chemotherapy. It leads to significantly higher intracellular antimitotic drug concentration. Anti-tumor effect seems to be long-lived because of the stabilization of the treated lesions as observed 1 yr after ECT. Because the combination of cisplatin and ECT seemed to be a safe and effective treatment method, results of this preliminary trial on equine sarcoids are encouraging.

Footnotes

[a] Cisplatin (II)-diammine dichloride, P-4394, Sigma, St. Louis, MO, 63103.
[b] PS15 Jouan Electropulsator, St Herblain, France 44805.

References