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Electrochemotherapy of horses. A preliminary clinical report

M.P. Rols [a], Y. Tamzali [b], J. Teissié [a,*]

[a] *IPBS CNRS (UMR 5089), 205 route de Narbonne, 31077 Toulouse cedex, France*
[b] *Médecine Equine, Ecole Nationale Vétérinaire de Toulouse, 23 chemin des Capelles, 31076 Toulouse cedex, France*

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Abstract

Sarcoids are skin spontaneous tumours detected in horses. It can be cured by chemotherapy by using cisplatin. A multisequence treatment must be performed. Problems are present due to the poor diffusion of the hydrophilic product in the tumours. Electropulsation is known to drastically enhance the effect of antitumoral drugs in vivo. Taking into account the very successful results of the group in Ljubljana (Slovenia), we started a research clinical program where electropulsation was applied after local cisplatin injection. The size of sarcoids is large (several centimeters). 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. The contact with the skin was obtained by a conductive paste. A PS15 Jouan Electropulsator was used to deliver eight pulses of 0.1 ms at a 1-Hz frequency with a 1.3-kV voltage. The animal was anesthesized. Intratumoral cisplatin injections were operated every 0.6 cm (0.2 ml at a 1-mg/ml concentration). Five minutes after the first drug injection, multiple electrotreatments were applied by moving the electrodes between the pulse applications. This allows the treatment of all the tumour surface. Several successive treatments were performed with a delay of 2 weeks between each. All lesions completely responded. The sarcoids disappear after only 2 or 3 electrochemotherapies. 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. No regrowth was observed in the 18 months follow-up period.

Keywords: Horses; Sarcoids; Cisplatin; Electrochemotherapy

1. Introduction

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 and fibroblastic) [12]. Therapy using classical surgical excision or laser photovaporization, as well as cryotherapy, are used to treat them. However, such nonconservative 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 is 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, rather low cost and high efficiency (up to 90% for sarcoids and up to 70–90 % for carcinomas). However, the main disadvantage is the poor diffusion of the hydrophilic drug into the tumors. This reflects the organization of the tumorous tissue in the case of sarcoids. Cisplatin is, therefore, mixed with sesame oil in order to increase its remanence at the injection point [13].

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 [1]. For the last 10 years, it has been developed to facilitate delivery of drugs into cancer cells [2]. 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

* Corresponding author. Tel.: +33-5-61-17-58-12; fax: +33-5-61-17-59-94.
E-mail address: justin@ipbs.fr (J. Teissié).

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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 [3,4]. 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 antitumor effectiveness of cisplatin by a factor 20 [5]. This method, called electrochemotherapy (ECT), introduced in the 1990s [2,6], has already been successfully applied to mice and rats for a large variety of tumors [7]. Clinical trials have been performed in humans including small nodes of head and neck squamous cell carcinoma, melanoma, basal cell carcinoma and adenocarcinoma [8–10]. To date, very few data are available on domestic animals [11,12].

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. The problems were how to treat a large tumour volume where a good availability of cisplatin was present.

2. Experimental

2.1. Horse and tumor characteristics.

Three horses were treated from October 1999 to February 2000. They were of both sexes and were 5–8 years old. All

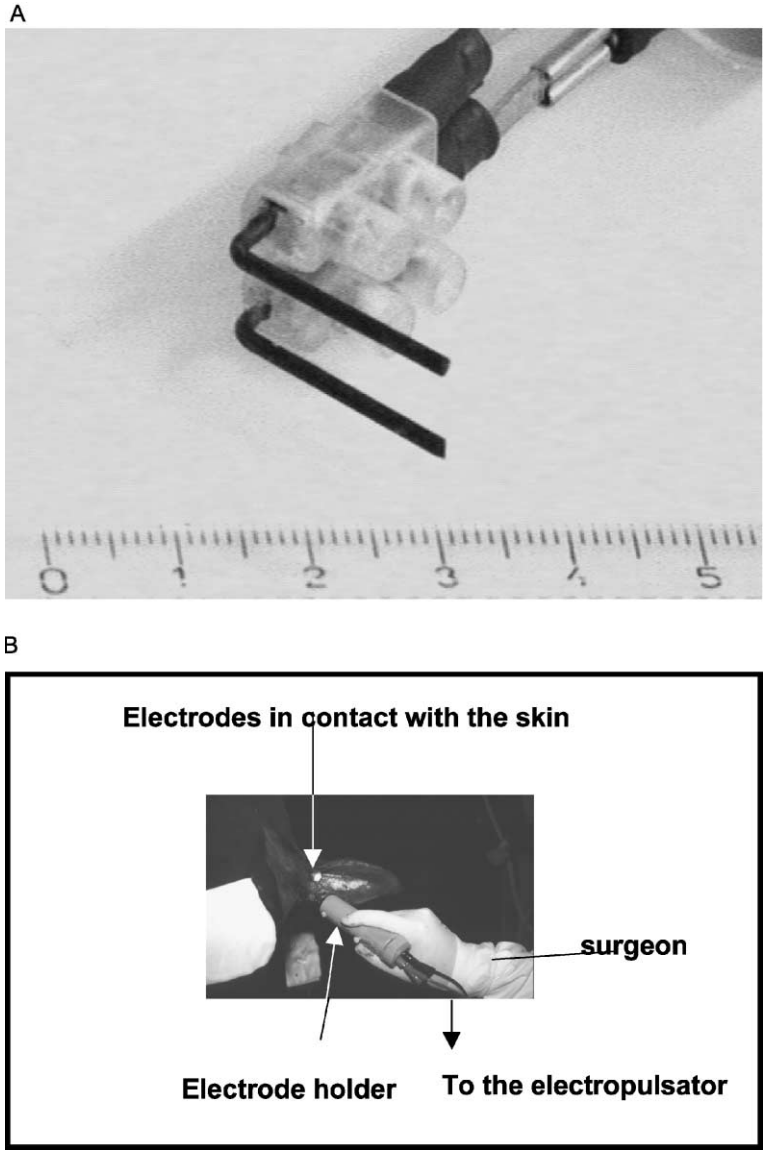

Fig. 1. Electrodes. (A) The contact wire electrodes had a fixed width given by the distance between the two stainless steel rods. Their length was fixed by an elastomer coating (not shown). They were inserted at the end of an insulating holder which was held by the surgeon. A centimetric scale is shown at the bottom of the picture. (B) The electrodes were brought into contact with the skin of the horse at the level of the sarcoid to be treated.

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 below are those observed 1 year and a half (June 2001) after completion of the last ECT treatment.

2.2. 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 min for one tumor to 40 min for several tumors.

2.3. Treatment

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

2.3.1. Antimitotic drug injection

Cisplatin (P-4394, Sigma, St Louis) was prepared in sterile 0.15 M NaCl at 1 mg/ml concentration. It was then intratumorally injected in a standardized manner (0.2–0.3 ml every 0.6 cm) by using ''luer-lock'' needles [13].

2.3.2. ECT treatment

A specially designed set of wire contact electrodes was built (Fig. 1). The optimized set-up was when the distance between the electrodes (1.2 mm diameter) was 0.9 cm (distance between the center of the wires) and their length was 0.9 cm (adjusted by a polymer coating). A PS15 Jouan Electropulsator was used to deliver eight 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 (Fig. 1). This allowed the treatment of all the tumor surface.

Several successive treatments were performed with a 2-week interval. The check list of the procedure is shown on Fig. 2.

2.4. 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 [3]. A posttreatment surveillance period of 2 years is required to close each case.

Experimental procedure

1- General anesthesia
2- IT Cisplatin injection
3- Electropulsation of the tumour through the skin by means of the contact electrodes
4- On line control of pulse delivery (muscle reaction and trace on the scope)
5- Delivery of 8 successive pulses at one given place
6- Pulse application at another site
7-When all the tumour surface has been treated, pulses are applied again with the electrodes in the perpendicular orientation
8- The treatment is applied during 3 successive sessions each other week.

Fig. 2. Steps in ECT on horse sarcoids.

3. Results

3.1. Injection procedures

Due to the tissue organization of sarcoids, the diffusion of injected soluble compounds is rather poor. In routine treatments, cisplatin is injected dissolved not in saline solution but in sesame oil. In our experimental trials, a NaCl solution was used. This brings us to select a multisite intratumoral injection procedure to obtain a rather homogeneous tumour irrigation by the drug. The intravenous procedure described in many other ECT procedure using bleomycin was not suitable due to the sarcoid organization and to the huge amount of drug which was needed due to the weight of horses.

3.2. Electrode design

Up to now, two electrode designs have been described in ECT procedures:

plate electrodes where the tumour is pinched in between the two electrodes;
needle electrodes which are inserted in the tumour.

This second design allows a deep penetration of the field of the tissue but with a very heterogeneous field distribution

while the first model gives a superficial but homogeneous field distribution.

In the present study, a new design was selected (Fig. 1): the wire contact electrodes where the two rod electrodes were in flat contact with the animal skin. This design appears well suited to treat large tumours by simply moving the electrodes between the successive pulses to cover the tumour surface. The electrodes were held in firm contact with the skin by the surgeon to correct from the muscular reaction between the successive pulses. The electrode distance and length were adjusted to limit the current during the pulse to less than 5 A.

Due to their geometry, as for plate electrodes, the field distribution is homogeneous between the electrodes but again intense field intensities are only present close to the skin surface. As described in other works, the distribution of the field is a key parameter to obtain an efficient eradication of the treated tissue cells [14]. With this electrode geometry, the field was very effective on the surface cell layer (data to be published). Destruction of these cells was obtained after only one treatment. To obtain the treatment of all the tumour volume, repetitive treatments were operated with a 2-week delay. This allows to affect cells which were initially in deeper layers of the tissue.

3.3. Pulse treatments

Electric pulses were rectangular as reported in most of the previous ECT procedure. The voltage to electrode distance was about 1400 V/cm. The direct on-line monitoring of the pulse with an oscilloscope allowed to observe the proper delivery of the field. It was observed that when blood was present on the tumour surface, an increase in the current was present bringing a cut off of the pulse by the safety current limit device.

Eight successive pulses of 0.1 ms were applied with a delay of less than 1 s. Two successive trains of pulses were applied with the electrodes in perpendicular directions to take advantage of the vectorial character of the field as previously reported in the case of mice [15].

3.4. Animal responses

Muscular contractions were observed during each pulse application. The amplitude of these movements was 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 colour of the tissue in the electrotreated region was observed to change. This appears to be related to the previous observation that electric pulses induced a temporary shut down of the blood flow [16].

In the days following an ECT treatment, a slightly oedemateous reaction was detected for lesions located on thin skin regions on some horses. No other adverse reaction was observed.

3.5. Tumour responses

Three cases which were treated for sarcoids have completed a 1-year posttreatment 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.

- For horse 1, multiple sarcoids were treated. They were present under fibroblastic and verrucous forms located on the head. The size of the tumors varied from 1.5 to 5 cm in diameter.
- 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.
- For horse 3, two sarcoids were treated : one at the limb (fibroblastic, 3 cm width, 5 cm length) and the other at the nose level (verrucous, 1 cm width, 2 cm length).

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

4. Discussion

Three horses were fully treated totalizing 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 1 year and a half following the last ECT treatment. General anaesthesia was used 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. About 150 cm^2 of skin was treated. No negative effect was obtained. Skin integrity was preserved even in regions previously submitted to surgical treatment. The electrical treatment was fast. This was clearly an advantage when taking into account the short time of residence of cisplatin in the tumour.

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 to a smaller number of ECT than larger ones with diameter >10 mm (up 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.

These positive observations give evidences that the new electrode design which was used in this study is very well suited for the treatment of sarcoids and as such of other skin tumours. The field which is generated brings electropermeabilization in layers of cells present in the volume limited at the skin surface by the electrodes (a 0.9×0.9-cm square). The depth of penetration of the field with permeabilization intensities was limited as shown by its limited effectiveness

with large, and as such thick, tumours. This limitation in the treatment was overcome by the use of successive treatments taking advantage of the necrosis of the treated cells during the 2-weeks delay. This is a direct illustration of the importance of the field distribution in anticancer electrotreatments.

This present study shows that ECT is effective on large tumors with a simple procedure. Positive results are obtained with verrucous as well as with fibroblastic sarcoids. Because ECT is observed to be a safe method, results of this preliminary trial on horse sarcoids are encouraging.

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