Paper

Electrochemotherapy as a single or adjuvant treatment to surgery of cutaneous sarcoid tumours in horses: a 31-case retrospective study

N. Tozon, P. Kramaric, V. Kos Kadunc, G. Sersa, M. Cemazar

The aim of our study was to evaluate the efficacy of electrochemotherapy (ECT) with cisplatin as a single or adjuvant treatment for sarcoids in equids. Different treatment options with different success rates were proposed. Thirty-one horses and one donkey with different clinical type, size and location of tumours were treated with ECT as a single treatment (18 animals with 52 tumour nodules) or as adjuvant treatment with marginal surgical excision (14 animals with 18 tumour nodules). In animals treated only with ECT with cisplatin, complete response was obtained in 48/52 (92.3 per cent) nodules and partial response in the other 4 nodules (7.7 per cent). In most cases, one to three sessions, only in two cases four and in one case five sessions, every 4 weeks were needed to obtain the measurable response. During the observation time, only in one case was the recurrence noted 60 months after treatment. Complete response in all 18 tumour nodules treated with surgery and adjuvant ECT was obtained and only one recurrence was noted after 14 months during the observation time. The results of this study show that ECT with cisplatin is an effective, safe, and simple local treatment of sarcoids in equids. According to the tumour size and location, single or combined treatment should be performed.

Introduction

Sarcoids are the most frequent cutaneous neoplastic diseases in horses, with a prevalence of between 1 and 2 per cent (Mattil-Fritz and others 2008). They are locally aggressive fibroblastic tumours occurring in six clinically recognisable forms: occult, verrucouse, nodular, fibroblastic, mixed and malignant (Knottenbelt and Walker 1994, Knottenbelt and others 1995, 2005). An individual horse may have more than one sarcoid type and for the purpose of clinical description, each lesion is classified individually. The rate of progression is variable, there is no predictable course of the progression of disease, except the dramatic changes that can follow interference or injury of a sarcoid. Complete, spontaneous regression of tumours is occasionally seen (Broström 1995). The various treatment methods are described, which are more or less applicable to specific types of sarcoid and include different surgical methods (Marti and others 1993), including cryosurgery (Lane 1977) and laser vaporisation (Vingerhoets and others 1988, Carstaniene and others 1997). Besides surgery, which has a high recurrence rate (Ragland and others 1970, Broström 1995), many other treatments are currently available, such as photodynamic therapy, brachytherapy, immunotherapy using BCG (Owen and Jagger 1987), intratumoural application of cisplatin oil emulsion (Theon 1998, Theon and others 1993, 2007) and numerous topical medications (Knottenbelt and others 1994, Knottenbelt and Kelly 2000, Nogueira and others 2006, Stadler and others 2011). A major influence on the selection of treatment options is the number of tumours, their type, distribution and extent. In the case of recurrence, a combination of treatment methods is usually the best option.

Different treatments have shown different success rates for the various forms of the disease. Frequent recurrences after surgical treatment alone are reported. Moreover, in many cases multiple lesions and unfavourable location render it impossible to provide wide surgical excision, which is needed to prevent possible recurrences (Bergvall 2013).

Intra-tumoural injection of cisplatin, dissolved in sesame seed oil (Theon and others 1993) and later cisplatin in biodegradable beads was used for the treatment of sarcoids in horses (Hewes and Sullins 2006) with a high success rate. These treatment options are easy and cheap, however the major problem remains the safety of the operator performing the injection of cisplatin, the people who prepare the solution, and the people who take care of the patient during the elimination time period of the drug.

Electrochemotherapy (ECT) using different treatment protocols has been shown to be an alternative treatment option for local control of tumours of different histological types in different animal species (Cemazar and others 2008). ECT is the combined use of chemotherapeutic drugs, for example cisplatin or bleomycin, in combination with high-voltage electric pulses that

Veterinary Record (2016) doi: 10.1136/vr.103867

N. Tozon, DVM, PhD,
P. Kramaric, DVM, PhD,
V. Kos Kadunc, DVM, PhD,
Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia
G. Sersa, PhD,
M. Cemazar, PhD,
Institute of Oncology Ljubljana, Ljubljana, Slovenia

E-mail for correspondence: natasa.tozon@vf.uni-lj.si

Some of the results were presented as a poster at the 6th Conference on Experimental and Translational Oncology, Kranjska Gora, Slovenia, March, 24–28, 2010.

Provenance: not commissioned; externally peer reviewed

Accepted September 12, 2016

cause reversible permeabilisation of the cell membrane, enabling the entry of chemotherapeutic drugs into the cells. Consequently the concentration of the injected drug is much higher which leads to much higher efficacy in the treated area (Cemazar and others 1998). ECT is also used for the treatment of cutaneous and subcutaneous tumours in humans in more than 150 oncological centres throughout Europe (Miklavčič and others 2012, Mali and others 2013a,b, Campana and others 2016).

There are only a few reports that use ECT with intralesional injection of cisplatin in horses. The first preliminary study in three horses showed 100 per cent objective response (OR) without recurrence within 18-month follow-up period (Rols and others 2002). In an enlarged clinical study, in which 34 horses, two ponies, 11 donkeys and one mule with 194 tumour nodules were treated, the efficacy of ECT as a single or adjuvant treatment to surgery was confirmed (Tamzali and others 2012).

The aim of our study was to further evaluate and independently confirm the efficacy of ECT with intralesional cisplatin injection as a single or adjuvant treatment to surgery for sarcoids in horses.

Material and methods

Animals

Thirty-one horses and one donkey with different clinical type (fibroblastic, nodular verrucous and mixed according to clinical classification; Knottenbelt 2005), size and location of cutaneous sarcoids were treated between 2003 and 2010.

The animals were divided into two groups according to the treatment option used. The treatment protocol was chosen according to the location and clinical type of tumours.

Eighteen horses with 53 tumour nodules that were mainly verrucous or nodular were treated with ECT as a single treatment and 14 horses with 17 tumour nodules that were mainly fibroblastic were treated with marginal surgical excision as a primary treatment followed by adjuvant ECT immediately after surgery.

Animals included in the study were the ones whose owners refused any other type of standard treatment at the time of inclusion. Prior to inclusion, written consent for participation in the clinical study for each animal was obtained from the owners.

Treatment protocol

Animals were sedated with detomidine (Domosedan sol. 10 mg/ml, dosage 10 μg/kg bodyweight; Pfizer) and anaesthesia was induced with midazolam (Dormicum sol. 5 mg/ml, dosage 0.1 mg/kg bodyweight; Roche) and ketamine (Bioketan sol. 100 mg/ml, dosage 2.2 mg/kg bodyweight; Vetoquinol) and maintained with isoflurane (Forane sol. 100 ml, maintenance with 3 per cent, Aesica Queenbotough).

Before the treatment, tumour nodules were measured and the volume of the tumour nodules was calculated by the formula $V=ab^2\pi/6$ (where 'a' is the larger diameter of the tumour nodule and 'b' the diameter of the tumour nodule perpendicular to 'a'). Cisplatin (cis-diamminedichloroplatinum II, Cisplatyl; Aventis) was dissolved in distillate water at a concentration of 2 mg/ml and the dose injected in the tumour nodules was ~2 mg/cm^3.

ECT consisted of intralesional injection of the cisplatin followed by exposure of tumours to electric pulses 1–2 minutes after drug administration. The same protocol was used in both groups. Two different types of electrodes and corresponding electric pulses parameters were used for treatment depending on the size of the tumours. For smaller tumours, electric pulses of 100 μseconds duration, 1300 V/cm amplitude to electrode distance ratio, and 5000 Hz frequency were delivered through two parallel stainless steel plate electrodes (thickness, 1 mm; width, 7 mm; length, 8 mm, with rounded tips and an inner distance between them of 7 mm; IGEA S. r. l.) delivered in two trains of four pulses with 1 seconds interval in two perpendicular directions. Good contact between the electrodes and the skin was assured by depilation and application of a conductive gel to the treatment area. In the second protocol for bigger tumours, the electric pulses delivered through needle electrodes (four needles in a row, two rows, 4 mm apart; IGEA S.R.L.) consisted of eight electric pulses of 100 μsecond duration, 1000 V/cm amplitude to electrode distance ratio and 5000 Hz frequency. Electric pulses were generated by the electric pulse generators Jouan GHT 1287 or Cliniporator (IGEA S.R.L.).

Treatment evaluation

The horses were examined at four-weeks intervals to evaluate the treatment effectiveness and possible local and systemic side effects. At each visit, tumours were measured with a Vernier calliper and photographed. Response to the treatment was scored after four weeks and at the end of the observation period, as complete response (CR) when the tumour was not palpable or as partial response (PR) when a decrease of more than 50 per cent of the nodule volume was determined. A reduction of less than 50 per cent and an increase of less than 25 per cent of the above measurements was defined as no change. Progressive disease was defined by an increase of more than 25 per cent. The number of ORs was determined by combining the number of CRs and PRs (WHO 1997). Observation time was calculated as the interval between the date of the first treatment and the date of the last examination of the patient.

In cases in which we did not achieve the CR, the same treatment protocol of ECT was repeated four weeks after the previous treatment.

Statistical analysis

Sigma Plot software (Systat Software, London, UK) was used for calculation of descriptive statistics and graphical presentation. The values were expressed as median.

Results

Both groups were composed of different breeds, different ages (3–14 years) and both sexes.

All clinical data are presented in Tables 1 and 2.

Eighteen horses with 52 tumour nodules, included as in the first group and treated with ECT as a single treatment, had a different number (mostly one, but up to 13 tumour nodules), located on different body regions and of different types: 27 mixed (52 per cent), 14 (27 per cent) fibroblastic, 9 (17 per cent) verrucous and 2 (4 per cent) nodular type. The nodule volumes ranged from very small (the smallest was 0.02 cm^3) to very large masses (the largest was 88.97 cm^3). Six nodules were treated only once, 25 nodules two times, 10 three times, two nodules were treated four times and only one nodule required five sessions to obtain an OR, as shown in Table 1. In all nodules the OR was obtained 4 weeks after the last ECT treatment. The size of the tumour nodules at the time of first treatment was related to the number of sessions needed to obtain OR. Smaller tumours (median 0.5 cm^3) required one or two sessions, while bigger tumours required three or more sessions (Fig 1).

At the end of the observation time (median 54 months), CR was obtained in 48 tumour nodules (92.3 per cent) and PR in the remaining four (7.7 per cent) nodules. Observation time in two animals was <2 years (12 and 18 months), in all others it was >2 years (24–108 months). Only in one horse with three verrucous type tumour nodules was the recurrence of one tumour nodule observed, 5 years after ECT treatment. Fig 2a, b shows the mixed type of sarcoid on the head (patient no. 7 in Table 1) before treatment and 4 weeks after the first treatment. The CR in all treated tumours in this patient was obtained after two ECT sessions and lasted during 72 months of observation time.

In the second group, 14 animals (13 horses and one donkey) were included with 18 fibroblastic tumours and one mixed type of tumour nodule. Volumes of tumour nodules ranged from 0.5 to 267.95 cm^3. CR was obtained in all 18 tumour nodules treated with surgery and adjuvant ECT. For the majority of nodules (15), mostly one (11 nodules) or two (four nodules) additional sessions of ECT were required to achieve CR. The

TABLE 1: Patients and tumour data and treatment response to ECT with cisplatin

Patient No.	Breed	Age/ sex	Nodules (n)/volume (cm^3)	Location/type	No. of sessions	Response at the end of observation time	Observation time (months)
1	Warmblood	5/M	1/0.58	Head/mixed	1	CR	108
2	Slo Warmblood	8/M	1/2.09	Abdominal/fibroblastic	1	CR	65
3	Arabian	6/M	9/0.25–1.7	Whole body/3 verrucouse, 6 fibroblastic	1	CR	24
4	Haflinger	4/M	3/0.5, 2.35, 16.72	Head, thorax/mixed	2	CR	108
5	Haflinger	8/M	3/0.52	Auricular 1, sternal 3/ verrucouse	2	CR	98
6	Warmblood	12 /F	3/0.54, 0.64, 3.57	Breast/fibroblastic	2	CR	96
7	Warmblood	5/M	3/3.77, 2.09×2	Head, thorax, hind leg/mixed	2	CR	73
8	Lipizzan	3/M	10/0.52; 2/2.09*; 1/ 12.55*	Whole body/mixed	2	10 CR; 3 PR*	12
9	Lipizzan	3/M	3/1.05×3	Auricular, sternal/ verrucouse	3	CR (recidiv 1×)	60
10	Arabian	4/M	1/88.97	Thorax/mixed	3	CR	72
11	Holstein	9/F	1/47.1	Gluteal/nodular	3	CR	87
12	Icelandic	5/M	1/3.77	Orbital/nodular	3	CR	38
13	Lipizzan	9/F	2/22.63, 0.52	Head/nodular; fore leg/ fibroblastic	3	CR	120
14	Holstein	6/F	1/0.02	Sternal/fibroblastic	3	CR	101
15	Coldblood	7/M	1/84.40	Sternal/fibroblastic	3	CR	36
16	Warmblood	7/F	4/0.70, 0.63, 0.23, 18.13†	Neck, abdomen, hind leg/ mixed	1/4†	CR	78
17	Holstein	7/F	1/12.55	Auricular/mixed	4	CR	18
18	Coldblood	5/F	1/41.87	Neck/mixed	5	PR	42
			52 nodules			48 CR, 4 PR	Median 72.5

*Nodules in which PR was achieved
†Tumour nodule treated with four ECT sessions
CR, complete response; ECT, electrochemotherapy; PR, partial response

TABLE 2: Patients and tumour data and treatment response to combined surgical and ECT with cisplatin treatment

Patient No.	Breed	Age/ sex	Nodules (n)/volume (cm^3)	Location/type	No. of sessions	Response at the end of observation time	Observation time (months)
1	Holstein	7/F	1/12.55	Inguinal/fibroblastic	1	CR	84
2	Warmblood	8/M	1/1.25	Sternal/fibroblastic	1	CR	44
3	Donkey	4/F	1/47.07	Abdominal/fibroblastic	1	CR	38
4	Haflinger	5/F	1/2.09	Sternal/fibroblastic	1	CR	28
5	Warmblood	5/M	1/2.09	Fibroblastic	1	CR	72
6	Arabian	3/F	1/12.55	Fibroblastic	1	CR	48
7	Coldblood	6/F	1/25.10	Inguinal/fibroblastic	1	CR	8
8	Coldblood	3/F	1/175.00	Fibroblastic	1	CR	17
9	Warmblood	8/F	1/6.28	Inguinal/fibroblastic	2	CR	36
10	Arabian	6/M	1/2.09	Sternal/fibroblastic	2	CR	34
11	Lipizzan	5/M	4/0.5, 1.0, 1.0, 4.71*	Auricular/fibroblastic	1/2*	CR (recidiv 1×)	14
12	Trotter	7/M	1/18.84	Preputii/fibroblastic	2	CR	96
13	Coldblood	8/F	2/14.13, 267.95	Thorax/mixed, fibroblastic	3	CR	62
14	Lipizzan	14/M	1/48.00	Inguinal/fibroblastic	4	CR	58
			18 nodules			18 CR	Median 41

*Tumour nodules treated with two ECT sessions
CR, complete response; ECT, electrochemotherapy

remaining three nodules were treated with three (two nodules) and four (one nodule) ECT sessions. Three to four sessions were needed mostly for larger and infiltrative tumour nodules. The observation time in this group was 28–62 months in nine animals and 8–17 months in three animals. In only one case was recurrence noted after 14 months, but the owner refused any additional treatment. The results are presented in Table 2.

Treatment was very well tolerated by all animals. Only mild to moderate local inflammatory reaction was noted, which was characterised by mild oedema and erythema. Ulceration, induced by treatment was noted mostly in more aggressive tumour nodules with infiltrative growth. No additional treatment was used in these cases, except routine wound care.

Discussion

The results of the presented clinical study confirm the safety and efficacy of ECT with cisplatin as a single or adjuvant treatment to surgery in equine sarcoids.

OR was observed in all treated animals with 92.3 per cent (48/52) CR and 7.7 per cent (4/52) PR at the end of observation period, which lasted from one to more than seven years (average four years). PR was observed in one bigger, mixed tumour and in three of the biggest mixed tumour nodules in horses with multiple lesions (horse numbers 8 and 18 in Table 1). All tumour nodules that responded with PR clinically presented with infiltrative growth, which could be a reason for less effective treatment and more treatment sessions necessary to obtain local control. In the only published clinical study using ECT in equids (Tamzali and others 2012), the authors report about 97.9 per cent of CR for animals (47/48) and 99.5 per cent of CR (193/194) for tumour nodules without any recurrence during the four-year observation period (Tamzali and others 2012). The results obtained in a preliminary study of ECT in equine sarcoids showed even 100 per cent of OR without recurrence within an 18-months follow-up period (Rols and others 2002). The disease-free interval at the end of the observation period (more than five

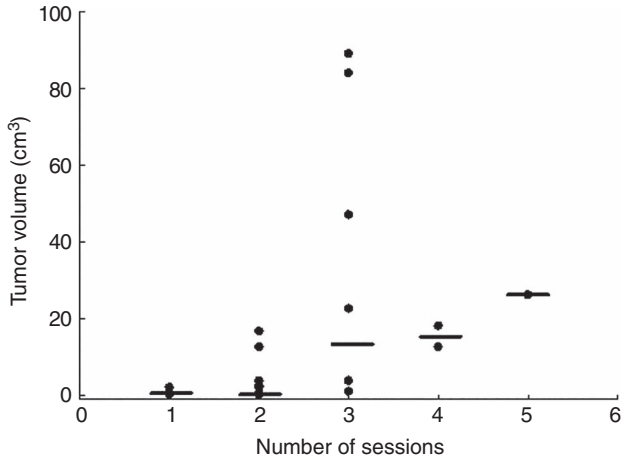

FIG 1: The dependence between the numbers of electrochemotherapy sessions required to obtained objective response and tumour volumes

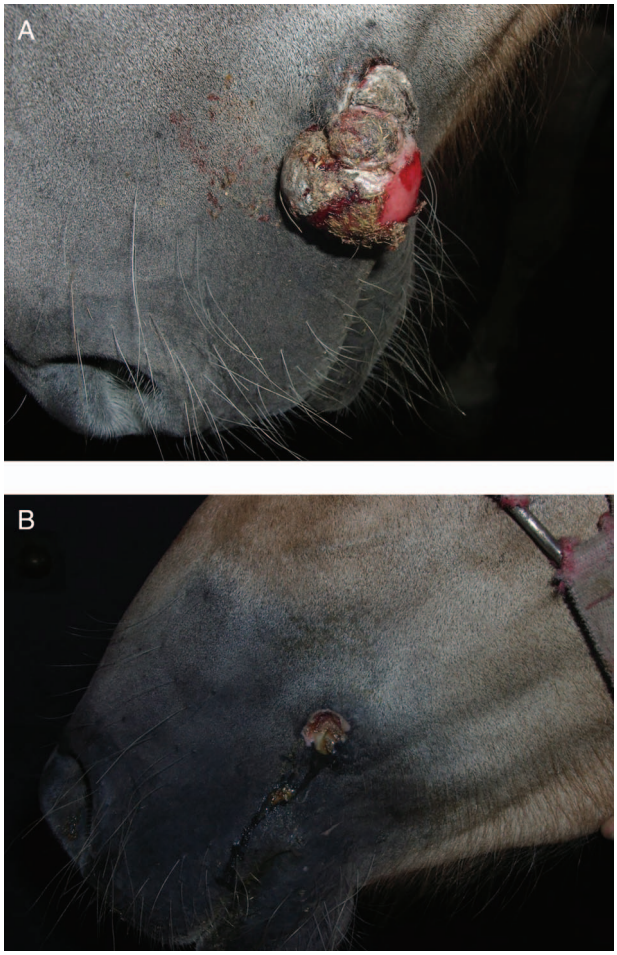

FIG 2: Fibroblastic sarcoid on the head (patient no. 7) before (a) and 4 weeks after (b) first treatment with electrochemotherapy with cisplatin. Complete response was obtained

years) in our study is almost the same as in previously published studies. The same results also apply for the response to treatment; for example, 92.3 per cent (48/52) of CR per tumour nodule and 88.8 per cent (16/18) of CR per animal for animals treated only with ECT. A slightly lower response rate is most probably because of a significantly lower number of treated animals and tumour nodules in our study. A recurrence of the tumour was observed in only one horse with multiple verrucous type of tumour nodules, 60 months after ECT treatment. In a study performed by Theon and others, relapse-free rates of 96 per cent using intralesional injection of cisplatin oil emulsion (409 sarcoids of 386 horses) were reported (Theon and others 2007). In another study, 83 per cent relapse-free rates were obtained using cisplatin beads in 22 sarcoids of 13 horses (Hewes and Sullins 2006). Therefore, our findings and previous results of ECT studies confer a higher efficacy of cisplatin compared with other methods, when electroporation is used for increased delivery of intralesionally injected cisplatin into the tumour cells. Such results could be expected based on the fact that electroporation was shown to increase and prolong increased cisplatin concentrations in the tumours. In preclinical studies more than two times higher concentrations of platinum were obtained in tumours as well as bound to the DNA of tumour cells at 72 and 96 hours after ECT compared with intralesional injection of cisplatin alone (Cemazar and others 1998). Furthermore, a higher amount of platinum bound to the DNA was also observed at shorter time points (1, 4 and 18 hours) after the ECT treatment with intravenously injected cisplatin (Cemazar and others 1999). The main mechanism of electrochemotherapy is thus increased cytotoxicity of cisplatin due to its higher intracellular concentration. Other mechanisms, such as prolonged accumulation of the drug in the tumours due to the vascular lock caused by application of electric pulses to the tumour and induction of immune response due to the tumour antigen shedding into the tumour microenvironment and blood stream were also documented (Cemazar and others 2015, Campana and others 2016).

Another reason for lower effectiveness of ECT in bigger sarcoid tumours, which are usually very dense, is adequate intralesional injections. Namely, it is very difficult to ensure the proper and uniform drug distribution, which may result in non-optimal effects of treatment. Consequently, to achieve good tumour response, at least two consecutive ECT sessions are needed. One to three sessions were required in most cases and only two horses were treated four and five times. In our study, 2.5 ECT sessions per animal were needed in order to achieve the final result, which is comparable to 2.6±1.1 sessions required in the only published paper on ECT in equids by Tamzali (Tamzali and others 2012).

Similar to the response in the group treated with ECT only, CR was achieved in all 14 animals with 17 tumour nodules in the group of horses treated with incomplete or marginal surgery followed by adjuvant ECT. Mostly one (11 nodules) or two (four nodules) sessions were needed to obtain the final result. Similar to the first group, larger and infiltrative tumour nodules required more sessions; three and four sessions, respectively, were performed in the remaining three nodules. During the observation time (28–62 months), only one recurrence was noted after 14 months in a Lipizzaner horse with a fibroblastic type of sarcoid, which represents a 5.6 per cent recurrence rate per tumour nodule and 7.1 per cent recurrence rate per animal. At the end of the observation time, 94.4 per cent (17/18) of CRs calculated according to tumour nodule numbers, and 92.8 per cent (13/14) of CRs calculated according to animals treated with ECT combined with surgery, support the fact that the presented treatment option is very successful.

Although the numbers of horses of different breeds are not high enough to perform adequate statistical analysis, the interesting observation from our study is that all recurrences were noted in Lipizzaner horses despite the fact that there is no evidence about specific breed predisposition or prevalence of specific types of sarcoidosis.

Tamzali and others (2012) showed that when ECT was used as a single treatment, tumour size significantly influenced the treatment sessions required ($\rho=0.55$). Similarly, in our study it appears that the tumour size plays an important role in treatment response, although it was not confirmed statistically because of the unequal number of tumours in different treatment groups regarding the required number of sessions. In addition, tumour type and infiltrative growth of the tumour nodule

seem to be important prognostic factors for the number of treatment sessions needed and the final outcome.

The most common adverse effect in the previous study on horses (Tamzali and others 2012) was a slight oedematous reaction for lesions located on thin skin regions. Also in our patients, minimal side effects, typically mild to moderate oedema with mild erythema and ulceration, were noted. Local inflammatory reaction was more pronounced in tumour nodules located on the head and limbs compared with the nodules located on the rest of the body and in tumour nodules with infiltrative growth.

In conclusion, our results independently confirmed the effectiveness of ECT in equine sarcoids. Tamzali with his co-workers demonstrated the effectiveness of very similar treatment protocols with minimal adverse reactions. Inclusion criteria for ECT as a single or adjuvant treatment combined with surgery were the same. The only differences between the two studies are in the treatment protocol. In our study the interval between sessions was four weeks, compared with four-week intervals in the study by Tamzali's group. There is a comparable response rate and we can conclude that both intervals could be used. In cases when animals remain hospitalised during the whole treatment period, the interval will reduce the cost of the treatment. However, we believe that a prolonged interval of four weeks, used in all our previous studies in other animal species, may reduce the number of sessions. In our studies we observed that the response rate could improve between the second and fourth week post treatment. Using different types of electrodes could be important in infiltrative tumour nodules in which plate electrodes cannot reach the deeper layer of the affected tissue. Needle electrodes in these cases seems to be more effective, without challenge to the result in more severe adverse effects. The last difference between both ECT protocols was the cisplatin concentration: 1 mg/ml solution in Tamzali's study and 2 mg/ml in our study. According to the results obtained in both studies, the higher concentration of the drug may not contribute substantially to the final outcome of the treatment; important is the total dose per tumour nodule, which was similar in both studies.

Conclusion

The results of this study show that ECT with cisplatin as a single or adjuvant treatment to surgery is an effective, safe and simple local treatment of sarcoids in horses. According to the tumour size and the location, single or combined treatment should be performed.

Funding This study was supported by the Slovenian Research Agency (project numbers P3-0003, J3-7044, P4-0053 and P4-0092).

Competing interests None declared.

References

BERGVALL, K. E. (2013) Sarcoids. *Veterinary Clinics of North America: Equine Practice* **29**, 657–671

BROSTRÖM, H. (1995) Equine sarcoids. A clinical and epidemiological study in relation to equine leucocyte antigens (ELA). *Acta Veterinaria Scandinavica* **36**, 223–236

CAMPANA, L., CLOVER, A. J. P., VALPIONE, S., QUAGLINO, P., GEHL, J., KUNTE, C., SNOJ, M., CEMAZAR, M., ROSSI, C. R., MIKLAVCIC, D. & SERSA, G. (2016) Recommendations for improving the quality of reporting clinical electrochemotherapy studies based on qualitative systematic review. *Radiology and Oncology* **50**, 1–12

CARSTANIENE, B., JORDAN, P. & LEPAGE, O. M. (1997) Carbon dioxide laser as a surgical instrument for sarcoid therapy—a retrospective study on 60 cases. *The Canadian Veterinary Journal* **38**, 773–776

CEMAZAR, M., MILACIC, R., MIKLAVCIC, D., DOLZAN, V. & SERSA, G. (1998) Intratumoral cisplatin administration in electrochemotherapy: antitumor effectiveness, sequence dependence and platinum content. *Anticancer Drugs* **9**, 525–530

CEMAZAR, M., MIKLAVCIC, D., SCANCAR, J., DOLZAN, V., GOLOUH, R. & SERSA, G. (1999) Increased platinum accumulation in SA-1 tumor cells after in vivo electrochemotherapy with cisplatin. *British Journal of Cancer* **79**, 1386–1391

CEMAZAR, M., TAMZALI, Y., SERSA, G., TOZON, N., MIR, L. M., MIKLAVCIC, D., LOWE, R. & TEISSIE, J. (2008) Electrochemotherapy in veterinary oncology. *Journal of Veterinary Internal Medicine* **22**, 826–831

CEMAZAR, M., TODOROVIC, V., SCANCAR, J., LAMPREHT, U., STIMAC, M., KAMENSEK, U., KRANJC, S., COER, A. & SERSA, G. (2015) Adjuvant TNF-α therapy to electrochemotherapy with intravenous cisplatin in murine sarcoma exerts synergistic antitumor effectiveness. *Radiology and Oncology* **49**, 32–40

HEWES, C. A. & SULLINS, K. E. (2006) Use of cisplatin-containing biodegradable beads for treatment of cutaneous neoplasia in equidae: 59 cases (2000–2004). *Journal of the American Veterinary Medical Association* **229**, 1617–1622

KNOTTENBELT, D. C. (2005) A suggested clinical classification for the equine sarcoid. *Clinical Techniques in Equine Practice* **4**, 278–295

KNOTTENBELT, D. C., EDWARDS, S. E. R. & DANIEL, E. A. (1995) The diagnosis and treatment of equine sarcoids. *In Practice* **17**, 123–129

KNOTTENBELT, D. C. & KELLY, D. F. (2000) The diagnosis and treatment of periorbital sarcoid in the Horse: 445 cases from 1974 to 1999. *Veterinary Ophtalmlogy* **3**, 169–191

KNOTTENBELT, D. C. & WALKER, J. A. (1994) Topical treatment of the equine sarcoid. *Equine Veterinary Education* **6**, 72–75

LANE, G. J. (1977) The treatment of equine sarcoids by cryosurgery. *Equine Veterinary Journal* **9**, 127–133

MALI, B., JARM, T., SNOJ, M., SERSA, G. & MIKLAVCIC, D. (2013a) Antitumor effectiveness of electrochemotherapy: a systematic review and meta-analysis. *European Journal of Surgical Oncology* **39**, 4–16

MALI, B., MIKLAVCIC, D., CAMPANA, L. G., CEMAZAR, M., SERSA, G., SNOJ, M. & TOMAZ, J. (2013b) Tumor size and effectiveness of electrochemotherapy. *Radiology and Oncology* **47**, 32–41

MARTI, E., LAZARY, S., ANTCZAK, D. F. & GERBER, H. (1993) Report of the first international workshop on equine sarcoid. *Equine Veterinary Journal* **25**, 397–407

MATTIL-FRITZ, S., SCHARNER, D., PIUKO, K., THÖNES, N., GISSMANN, L., MÜLLER, H. & MÜLLER, M. (2008) Immunotherapy of equine sarcoid: dose-escalation trial for the use of chimeric papillomavirus-like particles. *Journal of General Virology* **89**, 138–147

MIKLAVČIČ, D., SERŠA, G., BRECELJ, E., GEHL, J., SODEN, D., BIANCHI, G., RUGGIERI, P., ROSSI, C. R., CAMPANA, L. G. & JARM, T. (2012) Electrochemotherapy: technological advancements for efficient electroporation-based treatment of internal tumors. *Medical & Biological Engineering & Computing* **50**, 1213–1225

NOGUEIRA, S. A., TORRES, S. M., MALONE, E. D., DIAZ, S. F., JESSEN, C. & GILBERT, S. (2006) Efficacy of imiquimod 5% cream in the treatment of equine sarcoids: a pilot study. *Veterinary Dermatology* **17**, 259–265

OWEN, R. R. & JAGGER, D. W. (1987) Clinical observation on the use of BCG cell wall fraction in the treatment of periocular and other equine sarcoids. *Veterinary Record* **120**, 548–552

RAGLAND, K. W. L., KEOWN, G. F. H. & SPENCER, G. R. (1970) Equine sarcoid. *Equine Veterinary Journal* **2**, 2–11

ROLS, M. P., TAMZALI, Y. & TEISSIÉ, J. (2002) Electrochemotherapy of horses. A preliminary clinical report. *Bioelectrochemistry* **55**, 101–105

STADLER, S., KAINZBAUER, C., HARALAMBUS, R., BREHM, W., HAINISCH, E. & BRANDT, S. (2011) Successful treatment of equine sarcoids by topical aciclovir application. *Veterinary Record* **168**, 187

TAMZALI, Y., BORDE, L., ROLS, M. P., GOLZIO, M., LYAZRHI, F. & TEISSIE, J. (2012) Successful treatment of equine sarcoids with cisplatin electrochemotherapy: a retrospective study of 48 cases. *Equine Veterinary Journal* **44**, 214–220

THEON, A. P. (1998) Intralesional and topical chemotherapy and immunotherapy. *Veterinary Clinics of North America Equine Practice* **14**, 659–671

THEON, A. P., PASCOE, J. R., CARLSON, G. P. & KRAG, D. N. (1993) Intratumoral chemotherapy with cisplatin in oily emulsion in horses. *Journal of American Veterinary Medical Association* **202**, 261–267

THEON, A. P., WILSON, W. D., MAGDESIAN, K. G., PUSTERLA, N., SNYDER, J. R. & GALUPPO, L. D. (2007) Long-term outcome associated with intratumoral chemotherapy with cisplatin for cutaneous tumours in equidae: 573 cases (1995–2004). *Journal of American Veterinary Medical Association* **230**, 1506–1513

VINGERHOETS, M. DIEHL, M., GERBER, H. STORNETTA, D. & RAUSIS, C. (1988) Traitement de la sarcoide equine au laser a gaz carbonique. *Schweiz Arch Tierheilk* **130**, 113–126

WHO (1997) *WHO Handbook for Reporting Results of Cancer Treatment*. Vol 48. Geneva: WHO Offset Publications. pp 22–27

Veterinary Record

Electrochemotherapy as a single or adjuvant treatment to surgery of cutaneous sarcoid tumours in horses: a 31-case retrospective study

N. Tozon, P. Kramaric, V. Kos Kadunc, G. Sersa and M. Cemazar

Veterinary Record published online October 7, 2016