

# Long-term outcome associated with intratumoral chemotherapy with cisplatin for cutaneous tumors in equidae: 573 cases (1995–2004)

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**Objective**—To determine outcome associated with cutaneous tumors treated via intratumoral chemotherapy with cisplatin and identify risk factors affecting local tumor control and complications in equidae.

**Design**—Retrospective case series.

**Animals**—573 equidae with 630 cutaneous tumors.

**Procedures**—Medical records of horses, mules, donkeys, and ponies with cutaneous tumors treated via intratumoral chemotherapy with cisplatin were analyzed.

**Results**—549 horses, 13 mules, 8 donkeys, and 3 ponies with 630 histologically confirmed cutaneous tumors were included. Tumors included sarcoids ( $n = 409$ ), squamous cell carcinomas (151), soft tissue sarcomas (28), cutaneous lymphomas (26), and melanomas (16). Overall cure rate, defined as local control at 4 years, was 93.3%. For all tumor stages combined, cure rates after 1 course of treatment were 96.3% for sarcoids, 96% for lymphomas, 88% for squamous cell carcinomas, 85% for soft tissue sarcomas, and 81% for melanomas. Treatment protocol, tumor stage, and prior treatment were significant prognostic factors for tumor control. Treatment efficacy was lower for large tumors, those with gross postoperative residual disease, and those that had been treated previously with other modalities. Treatment was well tolerated. Local reactions were more likely to occur and to be more severe after the third and fourth treatment sessions.

**Conclusions and Clinical Relevance**—Results confirmed the value of intratumoral chemotherapy with cisplatin for treatment of cutaneous tumors in equidae. The results cannot be extrapolated to other formulations of cisplatin or other protocols that might be used. (*J Am Vet Med Assoc* 2007;230:1506–1513)

Cisplatin (cis-diamminedichloroplatinum [II]) is one of the most effective anticancer agents used in the treatment of solid tumors in humans.<sup>1</sup> However, its use as systemic agent has been limited by its toxicity, as well as by the occurrence of resistance. Several approaches have been developed to decrease its toxicity without impairing its therapeutic activity. Intratumoral administration of cisplatin exploits dose-response effects by maximizing drug concentrations in localized tumors while minimizing exposure of normal tissue.<sup>2</sup> Cisplatin is an ideal drug for intratumoral chemotherapy because it does not cause tissue necrosis, its effects are dose and time dependent, and its cytotoxicity is independent of tumor growth rate.<sup>3–5</sup> The therapeutic index of intratumoral administration of cisplatin may be optimized by increasing drug exposure via slow-release drug carrier<sup>6</sup> (concentration  $\times$  duration of exposure) or by increasing cellular uptake via electroporation.<sup>7</sup>

Two distinct approaches have emerged for controlled-release formulations of cisplatin administered

## ABBREVIATIONS

SCC	Squamous cell carcinoma
STS	Soft tissue sarcoma
CI	Confidence interval

intratumorally; the first involves percutaneously injecting a viscous fluid, slow-release drug solution via a needle,<sup>6,8</sup> and the second involves surgically implanting a solid polymeric sustained-release matrix loaded with the drug within the tumor.<sup>9,10</sup> The use of solid polymer matrices loaded with cisplatin has been limited for cutaneous tumors because the matrices require surgical implantation and do not allow dose adjustment. Conversely, intratumoral chemotherapy with cisplatin formulated in a viscous fluid formulation is practical and effective for treatment of a wide variety of human,<sup>11–14</sup> canine,<sup>15–17</sup> and equine cutaneous tumors.<sup>8,18–21</sup>

Most reports of intratumoral chemotherapy with cisplatin in equidae have included only a limited number of cases. The purpose of the study reported here was to evaluate the therapeutic value of intratumoral chemotherapy with cisplatin in sesame oil emulsion in a large series of equidae with various common skin tumors and determine prognostic factors associated with response to treatment and toxicosis.

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## Criteria for Selection of Cases

Medical records of horses, mules, donkeys, and ponies (all termed horses unless otherwise specified) with a diagnosis of cutaneous tumors treated at the Veterinary Medical Teaching Hospital of the University of California from January 1995 to June 2004 were analyzed. A subset of these horses was included in 2 prospective studies<sup>18,22</sup> published previously. Horses were required to have histologically confirmed measurable tumors, good general health, no evidence of systemic dissemination of tumor, and complete medical records with a minimum of 2 years of follow-up.

## Procedures

Data obtained from the oncologic, medical, and surgical records included species, age, breed, weight, sex, duration of clinical signs, histopathologic diagnosis, tumor histologic findings, clinical tumor type, tumor size, tumor stage, treatment protocol, and previous and concurrent treatments. Horses were evaluated clinically every 2 weeks during the course of treatment; follow-up examinations were performed at 2 weeks; 1, 2, 3, and 6 months; and approximately every year after treatment. Tumor staging was done by use of a modified tumor/node/metastasis (TNM) classification system<sup>23</sup> in which the T category indicated maximum diameter of tumors (T0, microscopic disease after surgery = 0 cm; T1, > 0 to < 2 cm; T2, 2 to 5 cm; T3, > 5 to 10 cm; T4, > 10 cm). The letter s was used to designate tumor size after grossly incomplete surgical resection. Normal tissue reactions and tumor response were recorded and photographed.

Treatment protocol was defined as intratumoral chemotherapy administered alone or in combination with surgery. When treatment was combined with surgery, cisplatin was administered after surgery following wound healing or during the perioperative period, with or without primary wound closure, as described.<sup>18</sup> Surgical procedures were either a subtotal tumor resection, defined as piecemeal removal of the tumor with evidence of gross residual tumor, or a microscopically incomplete resection. The presence of residual tumor at the surgical margins was confirmed histologically.

For intratumoral administration, crystalline lyophilized cisplatin powder was reconstituted with sterile water at a concentration of 10 mg/mL and mixed with medical grade sesame seed oil (60%) and sorbitan monooleate (7%) by use of the pumping method<sup>24</sup> just before administration (3.3 mg of cisplatin/mL of mixture). For treatment of horses with melanomas and tumors of vascular origin, the formulation included epinephrine.<sup>25</sup> Treatment included a series of 4 intratumoral administrations of cisplatin given at 2-week intervals. Intratumoral administrations of cisplatin consisted of a series of intratumoral and peritumoral injections in 1 or 2 parallel planes, depending on the tumor size. The cisplatin emulsion was injected through narrow-bore needles (22 or 25 gauge). The spacing between injection rows was kept as uniform as possible with a separation of 6 to 8 mm. The spacing between planes of injection was approximately 1 cm. Planned dosages were 1 mg of cisplatin for each cubic centimeter of tissue in the

target field (tumor bed and a margin of normal tissue). Tumor volume was calculated from the formula:

$$V = \pi \times D1 \times D2 \times D3/6,$$

where D1 through D3 are tumor diameters measured with Vernier calipers. All treatments were performed on an outpatient basis as described elsewhere.<sup>25</sup> Most treatments were performed via sedation with xylazine (0.5 to 1 mg/kg [0.23 to 0.45 mg/lb], IV) or detomidine (0.01 to 0.03 mg/kg [0.005 to 0.014 mg/lb], IV) and butorphanol (0.01 to 0.02 mg/kg [0.005 to 0.009 mg/lb], IV), with the horses standing. Short-term general anesthesia with xylazine (1.1 mg/kg [0.5 mg/lb], IV) and ketamine (2.2 mg/kg [1.0 mg/lb], IV) was used to facilitate treatment of 19 horses that were either uncooperative or had tumors in unfavorable locations. Horses were administered either trimethoprim-sulfamethoxazole (5 mg of trimethoprim fraction/kg [2.3 mg/lb], PO, q 12 h), doxycycline (10 mg/kg [4.5 mg/lb], PO, q 12 h in 3 horses), or procaine-penicillin G (22,000 U/kg [10,000 U/lb], IM, q 12 h in 1 horse) for 5 days after each treatment. Procedures for proper handling and disposal of cisplatin were followed as previously described.<sup>25</sup>

**Statistical analysis**—The endpoints evaluated were local tumor control after treatment and acute and chronic local toxicoses. Duration of local control was defined as the time interval between completion of the treatment series and measurable local tumor regrowth within (infield recurrence) or at the periphery (marginal recurrence) of the treated area. Tumor response and toxicosis of normal (nonneoplastic) tissue were assessed during the course of treatment and at 2 weeks, 4 weeks, 1 month, 6 months, and yearly after completion of treatment. The date of tumor recurrence detected by the owner or clinician and confirmed by histologic findings was recorded. All horses alive without tumor recurrence or dead for reasons unrelated to the disease (n = 3 deaths) were censored at the time of last follow-up for the calculation of local control rate.

Exact local control rate at 2 years after treatment was calculated as the ratio of disease-free horses to the total number of horses. Beyond 2 years after treatment, rate and duration of local control were estimated by use of the product-limit method.<sup>26</sup> The Cox proportional hazards regression model was used to identify significant prognostic factors of local tumor control.<sup>27</sup> Variables examined as indicators of prognosis included breed, age, tumor stage, histologic type, clinical type, site of primary tumor, treatment delays, and prior treatments. Treatment delay was defined as an interval of > 2 weeks between surgery and initiation of chemotherapy or between 2 consecutive administrations of cisplatin. Analysis of patterns of tumor recurrence was done by use of the  $\chi^2$  test. Variables examined as indicators of infield versus marginal recurrence included tumor stage, histologic type, clinical type, site of primary tumor, and treatment protocol.

Local treatment-induced toxicosis was assessed on the basis of severity of reactions in normal (nonneoplastic) tissues included in the treatment volume. Acute local toxicoses observed during the treatment and chronic local toxicoses that developed after treatment completion were recorded. Scoring reactions of nonneoplastic tissue was performed as described previ-

ously.<sup>8</sup> Acute reactions were graded as follows: grade 0 = no change from condition before treatment; 1 = tenderness, bright erythema, and slight edema; 2 = moderate edema lasting  $\leq 3$  days and focal crusting; 3 = moderate edema lasting  $> 3$  days and patchy crusting; and 4 = pitting edema and crusting over  $> 50\%$  of the treatment field or necrosis, necessitating withdrawal from further treatment. Wound complications were categorized as impaired healing (infection, dehiscence, necrosis, and hematoma) and seroma formation (collection of  $> 100$  mL of fluid). Wound complications were classified as major, moderate, or minor, as previously described.<sup>28</sup> Chronic local complications were categorized as persistent ulcers, fibrosis, and skin atrophy with tissue deformity or organ dysfunction. Diagnosis of systemic toxicosis was based on owner's evaluation and objective clinical signs (weight loss, abortion, or colic). Variables examined for associations with toxicosis included breed, tumor stage, wound closure, tumor location, clinical type (exophytic vs invasive), cumulative dose of cisplatin, treatment delays, and previous treatments. Logistic regression analyses were used to assess the independent effect of each of the variables on risk and severity of skin reactions.<sup>29</sup> All computations were performed by use of statistical software.<sup>a</sup> Differences were considered significant at  $P < 0.05$ .

## Results

**Animals**—Medical records of 549 horses, 13 mules, 8 donkeys, and 3 ponies with 630 histologically confirmed cutaneous tumors were included in this study. Follow-up intervals ranged from 25 to 74 months (mean, 42 months; median, 39 months). Mean and median ages were 9.9 and 8.5 years, respectively (range, 2 to 26 years), and the male-to-female ratio was 1.8. There were 12 stallions and 6 pregnant mares. Four hundred ninety-one horses were purebred, and 58 horses were of mixed breeding. Twenty-nine breeds were represented, the most common being Quarter Horse ( $n = 157$ ), Thoroughbred (63), Arabian (60), American Paint Horse (56), and Appaloosa (51).

Tumor histologic types included sarcoid (409 tumors in 386 horses), squamous cell carcinoma (151 in 144 horses), STS (in 28 horses), cutaneous lymphoma (26 in 10 horses), and melanoma (16 in 13 horses). Sarcoid clinical types included verrucous ( $n = 43$  tumors), fibroblastic exophytic with ulcerated surface (86), fibroblastic invasive (194), and mixed types (86). Squamous cell carcinomas were of cutaneous or conjunctival origin. Clinical types included exophytic type with ulcerated surface ( $n = 49$  tumors) and invasive ulcerative (102). Soft tissue sarcomas included fibrosarcoma ( $n = 7$  tumors), spindle cell sarcoma (5), undifferentiated sarcoma (4), Schwannoma (4), myxosarcoma (3), angiosarcoma (3), and angiomatosis (2). Melanoma clinical types included dermal ( $n = 11$  tumors) and subcutaneous (5) types. Lymphomas were of skin or conjunctival origin ( $n = 26$  tumors).

Tumor locations included the periorbital region (192 sarcoids, 103 SCCs, 11 STSs, 3 melanomas, and 15 lymphomas), face and ear pinnae (76 sarcoids, 6 SCCs, 3 STSs, 2 melanomas, and 4 lymphomas), trunk and

neck (45 sarcoids, 6 STSs, 2 melanomas, and 7 lymphomas), extremities (91 sarcoids, 3 SCCs, and 9 STS), male genitalia (5 sarcoids and 18 SCCs), female genitalia (15 SCCs and 2 melanomas), and perianal region (6 SCCs and 8 melanomas).

Tumor volumes prior to treatment ranged from 4.2 to 167 cm<sup>3</sup> (median, 66 cm<sup>3</sup>). Tumor stage included T0 (52 sarcoids, 21 SCCs, and 7 STSs), T1 (24 sarcoids, 23 SCCs, and 13 melanomas), T1s (15 SCCs), T2 (32 sarcoids, 17 SCCs, 4 STSs, 3 melanomas, and 18 lymphomas), T2s (112 sarcoids, 45 SCCs, and 7 STSs), T3 (8 sarcoids, 1 STS, and 8 lymphomas), T3s (137 sarcoids, 21 SCCs, and 5 STSs), and T4s (44 sarcoids, 9 SCC, 4 STSs). In horses that received surgery in combination with intratumoral cisplatin chemotherapy, mean  $\pm$  SD scar length was 12.4  $\pm$  4.3 cm.

Various treatments had been attempted on 205 lesions 4 to 12 months before treatment with cisplatin. One hundred twenty-six lesions had previously been treated with at least 1 cytoreductive procedure including surgery ( $n = 80$ ), laser vaporization (7), or cryotherapy (42). Topical application of escharotics was performed for 57 lesions with zinc chloride-based caustic ointments<sup>b-d</sup> ( $n = 49$ ) or 5% fluorouracil cream<sup>e</sup> (8). Twenty-one lesions were treated with immunotherapy by use of BCG cell wall derivatives<sup>f</sup> ( $n = 19$ ) administered intralesionally or heat-killed *Propionibacterium acnes*<sup>g</sup> (2) administered systemically. Nonspecific treatment included oral administration of piroxicam ( $n = 2$ ) and intralesional administration of triamcinolone (3).

**Treatment efficacy**—All horses received the planned course of treatment. For horses that received postoperative chemotherapy, the time interval between surgery and treatment ranged from 10 to 14 days. Twelve horses had a treatment delay between surgery and initiation of chemotherapy (mean delay, 8 days) because of surgical complications. Acute skin reactions ( $n = 12$ ) and owner compliance (8) resulted in treatment delays during the course of treatment with cisplatin (mean delay, 7 days). The cumulative dose of cisplatin injected per lesion during the course of treatment ranged from 20 to 180 mg. The maximum dose per treatment session in horses with multiple lesions was 85 mg.

**Local tumor control**—Exact local control rates at 2 years and estimates of cure rates (local control rates at 4 years) were determined (Table 1). After 1 course of treatment, overall control rate at 2 years was 95.9% (SE, 2) and overall cure rate was 93.3% (SE, 3). Cure rates after 1 course of treatment were 96.3% (SE, 1%) for sarcoids, 88% (SE, 3%) for SCCs, 85% (SE, 7%) for STSs, 81% (SE, 10%) for melanomas, and 96% (SE, 4%) for lymphomas. Of the 39 lesions that grew back, 22 of 25 were retreated successfully with a second course of intratumoral administration of cisplatin, 5 of 9 with radical surgery, and 2 of 3 with surgery combined with external beam radiation. The owner declined further treatment for 2 horses. Multivariate analysis of tumor control revealed that the treatment protocol ( $P = 0.005$ ), tumor stage (relative risk, 2.22; 95% CI, 1.3 to 6.1;  $P < 0.001$ ), and prior treatment ( $P < 0.001$ ) were significant prognostic factors. Treatment efficacy was lower for high stage (large) tumors (Figure 1), tumors with gross postoperative residual disease, and tumors that had been

Table 1—Exact local control rates and cure rates (local control at 4 years) in 573 equidae with 630 cutaneous tumors treated by intratumoral administration of cisplatin alone or in combination with surgery.

Treatment protocol	2-year local control rate (% [No. of equidae]) by stage					2-year local control rate (% [SE]), all stages	IFR	MR	Cure rate (% [SE])
	T0	T1	T2	T3	T4				
<b>Cisplatin alone</b>									
Sarcoid	—	100 (24)	97 (32)	87 (8)	—	97 (1)	3	1	93.7 (3)
SCC	—	100 (23)	88 (17)	—	—	95 (5)	3	3	85.0 (6)
STS	—	—	100 (4)	100 (1)	—	100	0	1	80.0 (17)
Melanoma	—	100 (13)	100 (3)	—	—	100	2	0	87.5 (8)
Lymphoma	—	—	100 (18)	87 (8)	—	96.2 (4)	0	1	96.2 (4)
<b>Perioperative cisplatin, open wound, gross residual disease</b>									
Sarcoid	—	—	—	92 (24)	87 (23)	89 (6)	1	5	87.2 (5)
SCC	—	—	—	82 (11)	97 (3)	79 (10)	1	2	78.5 (10)
<b>Perioperative cisplatin, closed wound, gross residual disease</b>									
Sarcoid	—	—	100 (63)	97 (36)	—	99 (1)	3	0	96.9 (2)
SCC	—	100 (15)	97 (33)	75 (4)	—	96 (4)	4	1	90.4 (4)
STS	—	—	100 (7)	80 (5)	—	92 (8)	1	0	91.7 (8)
<b>Postoperative cisplatin, closed wound, gross residual disease</b>									
Sarcoid	—	—	100 (49)	99 (77)	95 (21)	99 (1)	0	2	98.6 (1)
SCC	—	—	100 (12)	100 (6)	67 (6)	87 (4)	1	2	87.5 (6)
STS	—	—	—	—	75 (4)	75 (21)*	0	1	75.0 (21)*
<b>Postoperative cisplatin, closed wound, microscopic residual disease</b>									
Sarcoid	100 (52)	—	—	—	—	100	0	0	100
SCC	100 (21)	—	—	—	—	100	0	0	100
STS	100 (7)	—	—	—	—	100	0	1	85.7 (13)†

\*Significantly ( $P = 0.001$ ) different from other tumor types within the same treatment protocol. †Significantly ( $P = 0.005$ ) different from other tumor types within the same treatment protocol.  
IFR = Infield recurrence. MR = Marginal recurrence. — = Not applicable.

treated previously with other modalities. Histologic type, clinical type, site of primary tumor, and treatment delays were not significantly associated with prognosis. The lack of prognostic value of treatment delays should be interpreted cautiously because few horses had delayed treatments. A stratified analysis of the association between tumor stage and local tumor control revealed significant differences for lesions of different clinical stages treated with or without surgery and for the type of unsuccessful treatments prior to cisplatin treatment at our institution, with or without surgery. Lesions treated with cisplatin alone (relative risk, 5.2; 95% CI, 1.6 to 16.9;  $P = 0.007$ ) and lesions treated with cisplatin administered in the perioperative period without primary wound closure (relative risk, 4.1; 95% CI, 1.29 to 13;  $P = 0.017$ ) were more likely to recur, compared with lesions treated after surgery that had microscopic residual disease. No significant differences in risk of tumor recurrence were found between lesions with microscopic residual disease and lesions with residual tumors treated in the perioperative or postoperative periods. These findings should be interpreted cautiously because of the low power of the test. Among all treatments performed prior to treatment with cisplatin, cryotherapy was most likely (relative risk 7.46; 95% CI, 4.8 to 11.5;  $P < 0.001$ ) to negatively affect the prognosis.

**Patterns of treatment failure**—Treatment failure was histologically confirmed for 38 lesions. There were 19 infield recurrences and 20 marginal recurrences. The pattern of local failure (infield vs marginal) was significantly affected by treatment protocol ( $P = 0.005$ ) and clinical type ( $P = 0.01$ ). The risk of marginal failure was higher for exophytic tumors, lesions treated after

surgery with a closed wound, and lesions with an open wound treated in the perioperative period, whereas the risk of infield recurrence was higher for deeply invasive tumors and for lesions treated with cisplatin alone. The risk of recurrence on the surgical scar was higher for lesions with a closed wound treated in the perioperative period. Furthermore, the time to failure was significantly ( $P = 0.001$ ) associated with the pattern of failure. Infield recurrences were more likely to recur early, compared with marginal recurrences. Although not significant, SCCs often recurred earlier in the posttreatment period than did sarcoids. Seven horses with an SCC developed regional metastasis 22 to 62 months after treatment. Forty-two horses with sarcoids, 6 horses with SCCs, and 14 horses with melanomas developed new tumors outside the treated area during the follow-up period.

**Toxicosis**—Reactions observed in nonneoplastic tissue during the course of chemotherapy ranged from grades 0 to 3. Grade 4 toxicosis was not reported in any horse. There were 1 ( $n = 11$  horses) or 2 delays (5) during the course of chemotherapy because of acute skin reactions. The severity of acute local reactions was significantly ( $P = 0.015$ ) associated with the number of treatment sessions (Figure 2). Local reactions were more likely to be worse after the third and fourth treatment sessions. Breed, skin pigmentation, hair color, tumor type, tumor stage, wound closure, tumor location, cumulative dose of cisplatin, treatment delay, and previous treatments were not associated with the risk and severity of treatment reactions. However, peritumoral edema was more likely to develop in horses with periorbital tumors. Cisplatin-related systemic toxicosis was not observed. The reproduc-

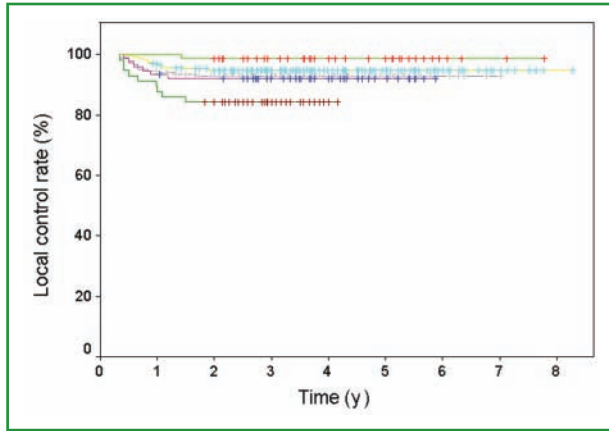


Figure 1—Local control rates for tumors in 573 equidae treated with intratumoral administration of cisplatin. Colored lines indicate tumor stage (light green = T0, magenta = T1, yellow = T2, gray = T3, and dark green = T4). Tick marks represent horses that were alive and disease free.

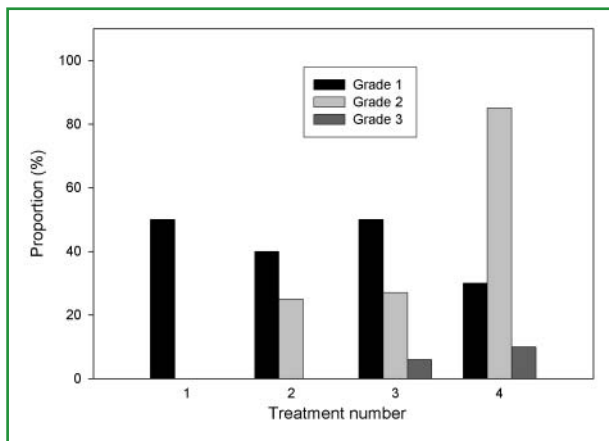


Figure 2—Proportions of equidae (n = 573) undergoing intratumoral chemotherapy with cisplatin that had skin reactions after 1 to 4 treatment sessions. Skin reactions were categorized as grade 1 (tenderness with slight edema), grade 2 (moderate edema lasting  $\leq$  3 days and focal crusting), and grade 3 (moderate edema lasting  $>$  3 days and patchy crusting).

tive performance of 12 stallions during treatment and in the follow-up period (24 to 51 months) and 6 mares that were pregnant during treatment was not associated with cisplatin treatment. Three of these mares were successfully rebred during the follow-up period (32 to 60 months). In horses treated after surgery, the time interval between surgery and initiation of chemotherapy ranged from 10 to 14 days. Time interval between surgery and chemotherapy was increased (mean, 19 days) in 7 horses because of postoperative complications, compared with horses without postoperative complications. Intratumoral injection of cisplatin was not associated with prolongation of healing of open or closed surgical wounds. Minor wound complications were observed in 6 horses with periocular lesions and in 2 horses with sarcoids involving the distal portions of the limbs. Late effects, such as nonhealing skin necrosis or fibrosis, were not observed in the follow-up period after treatment.

## Discussion

Results of this study confirmed the efficacy of intratumoral chemotherapy with cisplatin for treatment of the

most common skin tumors in horses, mules, donkeys, and ponies. These findings are consistent with data reported in dogs and humans.<sup>13,15,18-27</sup> Cisplatin is a broad-spectrum antineoplastic agent and is one of the most potent and widely used anticancer agents in the treatment of various solid tumors. Intratumoral administration of cisplatin in dogs is effective against cutaneous carcinomas, STSs, melanomas, and lymphomas.<sup>15,16,30</sup> Although drug resistance is a major obstacle when cisplatin is administered systemically in clinical treatment, drug resistance did not appear to be associated with outcomes in the present study. All tumors were initially susceptible to the drug, and retreatment was also effective for recurring tumors. Twenty-two of 25 lesions were retreated successfully with a second course of intratumoral administration of cisplatin.

For all tumors combined, the overall control rate at 2 years and the cure rate were 96% and 93%, respectively. Although the difference was not significant, results support the necessity for long-term follow-up to determine true efficacy and to assess the therapeutic benefit of antineoplastic treatments in horses. Local control rate at 4 years after treatment was an accurate estimate of the cure rate because no horses had tumor recurrence in the 3- to 4-year interval. For all tumor stages combined, cure rates after 1 course of treatment with or without surgery for tumors ranging in size from 4.2 to 167 cm<sup>3</sup> were 81% for melanomas, 96% for sarcoids and lymphomas, 88% for SCCs, and 85% for STSs. These data compared favorably with results of other means of treatment of cutaneous tumors in equidae.<sup>25,31-34</sup> The pattern of failure was consistent with a previous report.<sup>18</sup> Because drug resistance did not appear to be associated with tumor response, treatment failure was primarily technique related. Infield recurrence reflected an inadequate dose concentration in the target tissues because of bleeding at the injection site, leakage of the surgical suture, and heterogeneous dose distribution. Marginal failure represented a geographic miss because part of the tumor margin was not included in the treatment field. This type of failure reflected an underestimation of the extent of the tumor during treatment planning, rather than a true lack of efficacy.

Prognostic factors that were significantly associated with treatment efficacy were tumor size prior to cisplatin treatment and previous unsuccessful treatment. Large tumors and tumors that recurred after treatment with methods other than intratumoral cisplatin were less likely to respond to treatment with cisplatin. When combined with surgery, optimal results were obtained after gross resection of the tumor that left only microscopic or minimal residual tumor. Cure rates for small tumors or microscopic residual tumors approached 100% for most tumors. The timing of the 2 treatment modalities depended on several factors including tumor location, extent of surgical resection, type of surgical wound, and volume of residual tumor. Intratumoral chemotherapy was started at the time of surgery when primary closure was not possible or after incomplete removal of gross tumor when the risk of postoperative complications was low. Intratumoral chemotherapy was initiated after complete skin healing (10 to 14 days after surgery) in those horses with microscopically incomplete surgical resection and in those judged to be at high risk for surgical complications and

wound dehiscence after extensive tumor resection. The delay between incomplete excision and chemotherapy was kept as short as possible to minimize the negative impact of active tumor regrowth.<sup>18</sup> A delay of up to 1 week between 2 consecutive treatments during the course of treatment did not appear to affect treatment efficacy. However, these data should be interpreted with caution because the small number of horses at risk resulted in low statistical power and because the tumor cell repopulation that occurs during the intertreatment time interval may decrease treatment efficacy.<sup>18</sup> It is recommended that the time interval between surgery and intratumoral chemotherapy be kept as short as possible to allow skin healing, minimize active tumor repopulation, and complete treatment in the planned 6 weeks unless acute adverse effects are severe and cannot be managed medically.

Results of this study confirmed the importance of using effective treatment modalities for first-line treatment of cutaneous tumors and the negative association between tumor recurrence and prognosis. Management of a tumor recurrence after inadequate primary treatment requires more expensive, and often less cosmetically acceptable, treatments and leads to higher local recurrence rates. In the present study, tumor regrowth after unsuccessful treatment other than cisplatin chemotherapy was significantly less likely to be controlled, compared with tumors treated primarily with intratumoral cisplatin chemotherapy. Further analysis revealed that recurrences after cryotherapy were the least likely to be successfully treated with cisplatin. Recurrences after the use of caustic agents or cryotherapy were associated with fibrosis and, often, necrosis. These tissue changes likely compromised drug diffusion and prevented uniform drug distribution in the target tissue, resulting in an increased risk of infield recurrence. In addition, fibrosis in normal tissue surrounding the tumor obscured the transition between normal and abnormal tissue, thereby increasing the risk of marginal recurrence.

Although epidemiologic data suggest that the risk of developing specific skin tumors may be related to age, skin pigmentation, location, and breed,<sup>31,32,34</sup> results of the present study indicated that these factors did not influence the prognosis. Treatment efficacy was not associated with tumor histologic type, clinical appearance, clinical type, or location. This confirms the broad spectrum of activity of cisplatin against the most common skin tumor types in horses. This wide range of activity, independent of histologic type, clinical type, and appearance, makes this treatment unique and represents a substantial advantage over other nonsurgical treatment methods that are indicated only for specific types of tumor, tumor appearance, or locations.

Results of the present study confirmed that among the drugs tested for intratumoral administration of equine skin tumors, cisplatin is the anticancer drug of choice. It is inexpensive (a generic form is available), widely available, and more effective than any other reported drugs.<sup>16</sup> Cisplatin is a better choice than bleomycin for intratumoral chemotherapy of eyelid carcinomas.<sup>22</sup> Treatment outcomes of sarcoids treated with cisplatin in the present study compared favorably with

those in a previous report<sup>35</sup> on 4 horses with 8 sarcoids treated with intratumoral administration of bleomycin. In that study, tumor control at 3 months was observed in 5 of the sarcoids; however, no long-term follow-up was available. In rodent models, cisplatin is more effective against sarcomas and carcinomas than 5-fluorouracil when administered intratumorally.<sup>36,37</sup> In humans, intratumoral administration of 5-fluorouracil is used only for benign skin conditions including early basal cell carcinomas,<sup>38-40</sup> whereas cisplatin is used for malignant tumors.<sup>11,14,41</sup> In horses with sarcoids and carcinomas, cisplatin is also more effective than 5-fluorouracil when administered intratumorally.<sup>16</sup> Findings of a recent study<sup>42</sup> of horses with small sarcoids ranging in size from 0.5 to 33 cm<sup>3</sup> (median, 5.2 cm<sup>3</sup>) and treated with intratumoral injection of 5-fluorouracil, with or without surgery, confirm the superiority of cisplatin.<sup>42</sup> In that study, tumors in 6 of 14 horses did not respond or recurred after treatment with 2 to 7 intratumoral administrations of 5-fluorouracil. Although small tumors responded better than larger ones, 3 lesions of  $\leq 0.6$  cm<sup>3</sup> treated with 5-fluorouracil alone and 2 lesions of  $\leq 10.5$  cm<sup>3</sup> treated in combination with surgery failed to respond after 7 intratumoral administrations.

Direct delivery of free antineoplastic drug in aqueous solution into the tumor leads to a high local concentration, but the time of exposure is short and varies as a function of tissue affinity and vascularity.<sup>2</sup> The pharmacologic advantage of intratumoral drug delivery is optimized by techniques that prolong dwelling times within the tumor mass or increase cellular uptake of the drugs. Exposure of tumors to high drug concentration can be achieved by use of a crystalline suspension of drug in a slow-release carrier. The slow dissolution rate of the drug crystals in the formulation<sup>43</sup> and the slow-release rate<sup>3,6</sup> in tissues from the drug carrier increased the drug-exposure time in the tumor while avoiding systemic toxicosis. Electrochemotherapy is another approach to maximizing drug exposure of tumor cells.<sup>44</sup> Intratumoral administration of drug is combined with electroporation, which enhances drug passage into cells. This technique has been used with bleomycin and cisplatin. However, the usefulness of this approach is limited in equine oncology because of the equipment needed for electroporation and the need for general anesthesia.<sup>7</sup>

In the present study, a crystalline suspension of cisplatin in a sesame seed oil emulsion was used successfully. The use of aqueous solution of cisplatin (1 mg/mL) alone<sup>19</sup> or mixed with almond oil<sup>20</sup> reduces treatment efficacy. The use of cisplatin powder contributes substantially to the increased duration of exposure and allows delivery of a high dose in a small volume of mixture. Because the mean  $\pm$  SEM amount of drug that can be injected intratumorally is approximately  $0.29 \pm 0.05$  mL of mixture/cm<sup>3</sup> of tissue injected,<sup>8</sup> it is critical that the drug concentration in the formulation be as high as possible to achieve the prescribed dosage of 1 mg of cisplatin/cm<sup>3</sup>. As a result, the use of aqueous solution of cisplatin requires a volume of drug-containing material too large to be practical.<sup>20</sup>

The sesame oil-based formulation used in the present study is safe and releases cisplatin slowly into tissues.<sup>8,43,45</sup> Because cisplatin strongly and irreversibly

binds to proteins,<sup>46</sup> vegetable oil-based, slow-release formulations are better suited than proteinaceous carriers for high-dose intratumoral administration of cisplatin.<sup>8,43,45,47</sup> Collagen matrix<sup>h</sup> has been used successfully because the extensive cross-linking of collagen fibers during the manufacturing process minimizes binding to cisplatin. Other vegetable oil emulsions, including arachid<sup>48</sup> and almond<sup>20</sup> oils, have not been satisfactory for intratumoral administration of cisplatin.

The formulation in this study was improved, compared with earlier reports,<sup>8,28</sup> by adding an emulsifier, Sorbitan monooleate, to improve the stability and consistency of the mixture.<sup>45</sup> In addition, epinephrine was added to the formulation for treatment of melanomas and heavily vascularized tumors to prevent bleeding and increase dwelling times.<sup>25</sup> Previous studies in horses<sup>49</sup> and dogs<sup>15</sup> indicate that epinephrine in the formulation is necessary for optimal effect against melanomas.

Results of this study confirmed previous findings that cisplatin treatment is well tolerated and does not affect wound healing when combined with surgery.<sup>18,28</sup> The lack of local toxicosis in horses that received additional treatment for recurrence indicated that tolerance was not exceeded in normal tissue with a course of treatment. During the course of intratumoral injection of cisplatin in water oil emulsion, a cumulative effect for local reactions was observed. The effects of treatment were local and resolved quickly with no evidence of permanent cosmetic or functional deficits. Treatment did not cause tissue fibrosis or necrosis or affect hair regrowth and hair color. Drug-related systemic toxicosis was not observed, and treatment was used safely in pregnant mares and stallions.

- a. SPSS Inc, version 11.5, Chicago, Ill.
- b. Indian Mud, Original Cream Co, Magnolia, Ark.
- c. XTERRA, Larson Laboratories Inc, Fort Collins, Colo.
- d. Animex, NIES, Las Vegas, Nev.
- e. Efudex cream, Hoffmann La Roche Inc, Nutley, NJ.
- f. Nomagen, Fort Dodge Laboratories Inc, Fort Dodge, Iowa.
- g. Eqstim, Vetoquinol/ImmunoVet, Lavaltrie, QC, Canada.
- h. Matrix Pharmaceutical Inc, Enlo Park, Calif.

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## Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Plasma concentration and local anesthetic activity of procaine hydrochloride following subcutaneous administration to horses

Natasha L. Kuchembuck et al

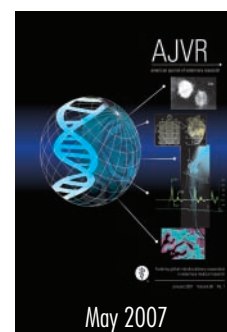
**Objective**—To determine the durations of the local anesthetic effect and plasma procaine concentrations associated with 5- and 10-mg doses of procaine hydrochloride (with or without 100 µg of epinephrine) administered SC over the lateral palmar digital nerves of horses.

**Animals**—6 healthy adult horses.

**Procedures**—The hoof withdrawal reflex latency (HWRL) period was determined by use of a focused heat lamp before and after administration of procaine with and without epinephrine. Blood samples were collected immediately before determination of each HWRL period to assess plasma concentrations of procaine via liquid chromatography–mass spectrometry–mass spectrometry (LC-MS-MS).

**Results**—10 but not 5 mg of procaine alone and 5 and 10 mg of procaine administered with epinephrine significantly prolonged the HWRL period (mean durations of effect, 5, 120, and 180 minutes, respectively), compared with baseline values. Plasma procaine concentrations did not correlate well with local anesthetic activity; for example, although the HWRL period was prolonged to the maximum permitted duration of 20 seconds at 60 to 180 minutes following administration of the 5-mg dose of procaine with epinephrine in certain horses, plasma procaine concentrations were less than the limit of quantitation of the LC-MS-MS assay.

**Conclusions and Clinical Relevance**—Small doses of procaine coadministered with epinephrine provided long-lasting local analgesia and resulted in plasma procaine concentrations that were not always detectable via LC-MS-MS. On the basis of these results, the use of regulatory limits or thresholds for procaine concentration in equine plasma samples obtained after racing should be seriously reconsidered. (*Am J Vet Res* 2007;68:495–500)



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