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Treatment of equine sarcoids using recombinant poxviruses expressing feline interleukin-2

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Background – Interleukin (IL)-2 stimulates antitumour immunity and is successfully used for the treatment of different neoplasias.

Hypothesis/Objectives – Canarypox virus locally expressing feline IL-2 is safe and can be used to treat equine sarcoids.

Animals – Twenty horses of different breeds with a median age of eight years (interquartile range 6.0–13.3 years) and a total number of 59 sarcoids were included in the study.

Methods – In this prospective clinical trial, sarcoids were injected twice seven days apart, with a recombinant canarypox virus expressing feline IL-2. Complete blood counts (CBC) and fibrinogen levels were measured before treatment and on days 1, 2, 7 and 8.

Results – Complete regression was achieved in eight horses (40%) and partial regression in two horses (10%). No change in sarcoid size was observed in two horses (10%) and the disease progressed in five horses (25%). Sarcoids of three horses (15%) showed initial response followed by tumour growth. There were no significant changes in CBC and fibrinogen levels after either injection. One horse developed a mild fever the day after each injection, which subsided without treatment the following day.

Conclusions – Treatment of equine sarcoids with recombinant canarypox virus expressing feline IL-2 seems to be a safe therapy option. Although the expression of IL-2 after vector injection and its biological activity in horses were not proven in this study, the treatment resulted in regression and partial regression in 50% of the cases. Further studies are necessary to verify these findings and to establish a treatment protocol.

Introduction

Equine sarcoids are a locally invasive fibroblastic neoplasia, which represent 90% of skin tumours in horses. They occur in six different forms and at specific predilection sites throughout the body. Sarcoids show a high recurrence rate after treatment, and failure of treatment often is followed by more aggressive tumour growth. Numerous treatment options have been published since the first description of equine sarcoids in 1936,[1] yet none have been universally successful.[2–7] No licensed medication for the treatment of equine sarcoids is currently available and, therefore, human medicine or medication authorized for other species have to be used off-label. There is a considerable risk for the treating veterinarian while using some medicines, such as chemotherapeutics, as a result of their carcinogenic qualities. These circumstances have fuelled the search for safe and successful treatment options against equine sarcoids.

A novel focus of cancer research in human medicine is cancer immunology and the question of how the host's immune system can be stimulated to recognize and target tumour cells. Interleukin (IL)-2 is one of the key cytokines with stimulating effects on the immune system, and has been approved for systemic treatment of metastatic renal cell carcinomas and metastatic melanoma in humans.[8] As a growth factor, it stimulates the proliferation of cytotoxic T cells, T-helper (TH) cells (TH-1, TH-2 and TH-17), natural killer cells and lymphokine-activated killer cells, all of which contribute to antitumour responses.[8] Activation of lymphokine-activated killer cells by equine IL-2 has been demonstrated in horses[9] and is known to mediate tumour regression in humans and horses.[9–14]

IL-2 treatment has been used in human medicine since 1985. A wide variety of different application regimes have been tested. One of the major concerns of high-dose, recombinant, systemically injected IL-2 therapy in human cancer patients is severe adverse effects, including chills, fever and hypotension. These symptoms are not induced

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by the IL-2 itself and instead by the IL-2-promoted elevation of pyrogenic cytokines, such as tumour necrosis factor (TNF)α.[15] Local application systems have been tested, such as direct subcutaneous or intratumoural injection of low-dose recombinant IL-2, the use of viral (e.g. canarypox virus) or bacterial vectors or plasmid-based delivery systems, to avoid systemic adverse reactions.[16] The canarypox vector is a promising delivery system as its safety is well-established and antivector immunity does not interfere with the efficacy of subsequent injections. This delivery system is, therefore, successfully used in human patients with cutaneous metastasis of melanoma.[17] Locally applied IL-2 has been used in veterinary medicine to prevent the recurrence of feline fibrosarcomas after surgical resection.[18,19] Feline fibrosarcomas show similarities to equine sarcoids and have a high recurrence rate. Injections into the tumour bed of feline fibrosarcomas with canary poxvirus expressing feline IL-2 (Oncept IL-2, drug approval no. EU/2/13/150/001, Merial; Lyon, France) reduced the recurrence rate after surgical resection.[18] The published genetic sequences of feline and equine IL-2 show an homogeneity of 76%.

We investigated the safety of the application of canarypox virus expressing feline IL-2 in healthy horses in this prospective clinical trial. Thereafter, we evaluated the efficacy of the product to treat equine sarcoids.

Methods and materials

Ethics approval and consent to participate

The study was approved by the State Office of Health and Social Affairs Berlin (LaGeSo), sampling of control horses was approved (ref. no. G0220/18). The owners gave permission to include their horses in the study.

Safety study

Four clinic-owned horses (three mares, one gelding) with unremarkable clinical and dermatological examinations were included in the study. They were aged between five and 25 years (mean 16 ± 8.9 years) with a mean body weight of 489 ± 87 kg. Two of the horses were Arabians and two were Standardbreds. Each horse was injected twice, seven days apart, with Oncept IL-2 (lyophilizate + manufacturer's solvent) and with the manufacturer's solvent only as a control at two different sites. Injections were performed subcutaneously on the same side of the neck, ≥8 cm apart. The lyophilizate contained feline IL-2 recombinant canarypox virus (vCP1338) ≥ $10^{6.0}$ $EAID_{50}$ (ELISA infectious dose 50%) as the active substance, with sucrose, collagen hydrolysate, casein hydrolysate, sodium chloride, disodium phosphate dihydrate and potassium dihydrogen phosphate as excipients. Water was the solvent for injections. Clinical and dermatological examinations were performed before treatment [Day (D)0] and repeated daily (D1–D14). Skin biopsies were taken with a 4 mm biopsy punch (Dermal Biopsy Punch, WDT; Garbsen, Germany). They were formalin-fixed, bisected and paraffin-embedded. Paraffin sections were cut and stained with haematoxylin & eosin. A board-certified pathologist analysed the tissue sections for any aberrations. Skin biopsy 1 was taken on D0 from healthy, untreated skin. The first subcutaneous injections of Oncept IL-2 (Location A1) and solvent only (control, Location B1) were performed on D0 after the first biopsy was taken. The second subcutaneous injections with Oncept IL-2 (Location A2) and solvent only (Location B2) were performed on D7. Biopsies 2 and 3 were collected from each injection site five days after injection. All biopsy wounds were closed with staples.

In addition, CBC and fibrinogen levels were determined before the first treatment (D0) and on D1, D2, D5, D7, D8 and D12. The CBC were performed from ethylenediaminetetraacetic acid blood samples (VetScan HM 5, Abaxis Europe GmbH; Griesheim, Germany); fibrinogen levels were measured in heparinized blood samples (Coagulometer nach Schnitger und Gross, ABW Medizin und Technik GmbH; Lemgo, Germany).

Clinical trial

Horses. Twenty client-owned horses with clinically suspected sarcoids were recruited from the patient pool for this study. Horses were excluded from the study if they had systemic disease, sarcoids exceeding 10 x 10 cm, or if the clients were unable to document the development of the sarcoids after discharge from the clinic. Age, sex and breed of the horses enrolled can be seen in Table 1. Patient history and clinical examination results were obtained on admission to the clinic.

Sarcoids. The number, location, type and size of the sarcoids in all horses were recorded before the initiation of treatment. Sarcoids were confirmed in all cases by histological examination of a 2 mm punch biopsy taken from one sarcoid per case on the day of first treatment. Biopsy wounds were left untreated. Sarcoid type and locations are listed in Table 2; the location regions are depicted in Table 2.

Treatment

A maximum dose of three vials per horse (equalling a maximum of three sarcoids treated) were used. If horses had more than three sarcoids, the owners could choose which three sarcoids should be treated. Horses were sedated with detomidine (20–40 μg/kg BW intravenous, Cepesedan, CP Pharma; Burgdorf, Germany) and butorphanol (0.1 mg/kg BW i.v., Dolorex, MSD Animal Health; Unterschleißheim, Germany) if necessary. The surrounding area of the sarcoid was clipped as necessary. The skin was disinfected with alcohol swabs before injection. Horses received intratumoural treatment on D1 and D7 with a dose of ≥$10^{6.0}$ $EAID_{50}$ Oncept IL-2/mL (= one commercially available vial). Horses were observed for approximately 15 min post-treatment for any immediate local or generalized adverse reactions.

Additional examinations

Vital signs were obtained daily for eight days. The CBC (VetScan HM5, Abaxis Europe GmbH) and fibrinogen levels (Coagulometer nach Schnitger und Gross, ABW Medizin und Technik GmbH) were determined in-house before treatment and on D1, D2, D7 and D8. All sarcoids were photographed and measured (width and length) by two independent observers with a Vernier caliper on arrival and again before discharge from the clinic. The mean value of the measures was calculated each time. The horses were discharged from the clinic one day after the second treatment. Horse owners photographed and measured sarcoids at home once a week until eight weeks after the last treatment and then once a month until the end of the observation period (ranging from 22 to 40 months).

Statistics

Statistical analyses were carried out using SPSS Statistics v24 (IBM; Endicott, NY, USA). Descriptions included frequencies for categorical variables and means, medians, standard deviations and interquartile ranges for continuous data. Outcomes were categorized as binary variables. The first group comprised sarcoids that fully regressed (complete regression, CR) or partially responded by ≥50% (partial response, PR). The second group included those that were not reduced at all or by <50% (stable disease, SD), showed tumour growth (progression of disease, PD) or recurrence of tumour (RT) (Figure 1).[20]

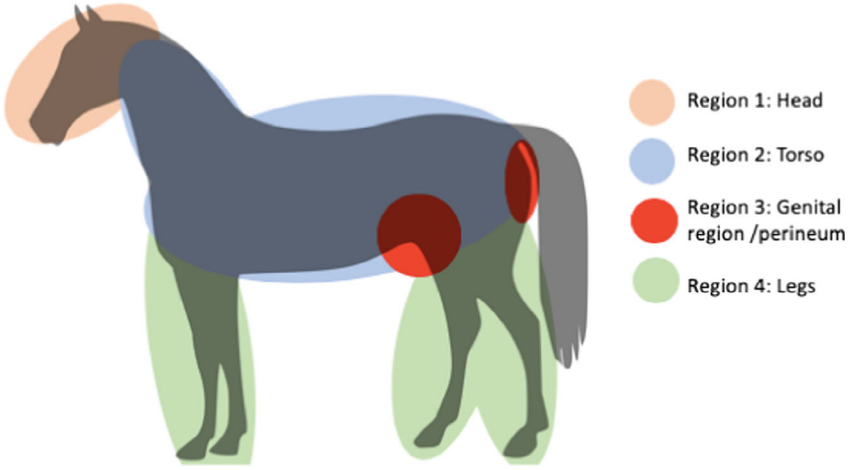
The binary variables were used as dependent variable in two multivariable mixed logistic regression models. The SD group was used as a reference group. The one sarcoid of fibroblastic type was excluded from the model. Some horses had more than one sarcoid and, therefore, animal IDs were included as random factors in the model, while location (reference: head), size (reference: 40–80 cm^2), type

Table 1. Animals in this study, types and number of sarcoids, and results.

	Breed	Age (years)	Sex	Number of sarcoids	Types of sarcoids	Result
1	Warmblood	8	Gelding	2	O, V	CR
2	Warmblood	7	Mare	3	O, V	RT
3	Warmblood	5	Gelding	6	O, V, N	CR
4	Pony	11	Mare	1	N	RT
5	Pony	6	Gelding	1	O	PR
6	Noriker	10	Gelding	11	V	CR
7	Warmblood	17	Mare	1	N	CR
8	Warmblood	8	Gelding	1	O	PD
9	Warmblood	18	Mare	3	V, N	PR
10	Warmblood	8	Gelding	1	V	SD
11	Quarter horse	2	Gelding	1	O	PD
12	Haflinger	22	Gelding	4	N	PD
13	Warmblood	6	Gelding	2	O, N	CR
14	Quarter horse	10	Mare	4	V	ST
15	Pony	7	Mare	2	O, N	CR
16	Pony	6	Gelding	3	N	PD
17	Warmblood	20	Mare	2	O, N	PD
18	Tinker	11	Gelding	13	V, N, F	CR
19	Warmblood	4	Gelding	1	N	RT
20	Warmblood	14	Mare	3	O, V	CR

F fibroblastic; O occult sarcoid; V verrucous; CR complete regression PD progression of disease; PR partial regression; RT recurrence of tumour; SD stable disease

Table 2. Locations and types of sarcoids found on the horses in this study



	Location	Number	Type of sarcoid	Number
Region 1	Head	18	Occult	14
Region 2	Torso	23	Verrucous	24
Region 3	Genital region /perineum	13	Fibroblastic	1
Region 4	Legs	5	Mixed	0
			Nodular	20
			Malevolent	0
	Total	59	Total	59

(reference: occult) and treatment (reference: no treatment) served as fixed factors. One model was fitted for all sarcoids and a second model included only those sarcoids that were treated in order to additionally investigate the influence of follow-up time. All factors were included in the model in a single step, and no selection process was carried out. Interactions between variables could not be included in the models owing to the small number of observations. Odds ratios (OR) and 95% confidence intervals (95% CI) are presented in the text. The significance level was set at $P < 0.05$ (Table S1).

Results

Safety study

All horses were clinically and dermatologically healthy before treatment (D0). Horse 3 showed a mild leukopenia on D0 (4.8 x 10^3/µL; reference range 5–10 x 10^3/µL). None of the horses showed any pathological changes in the clinical examination after the first or second

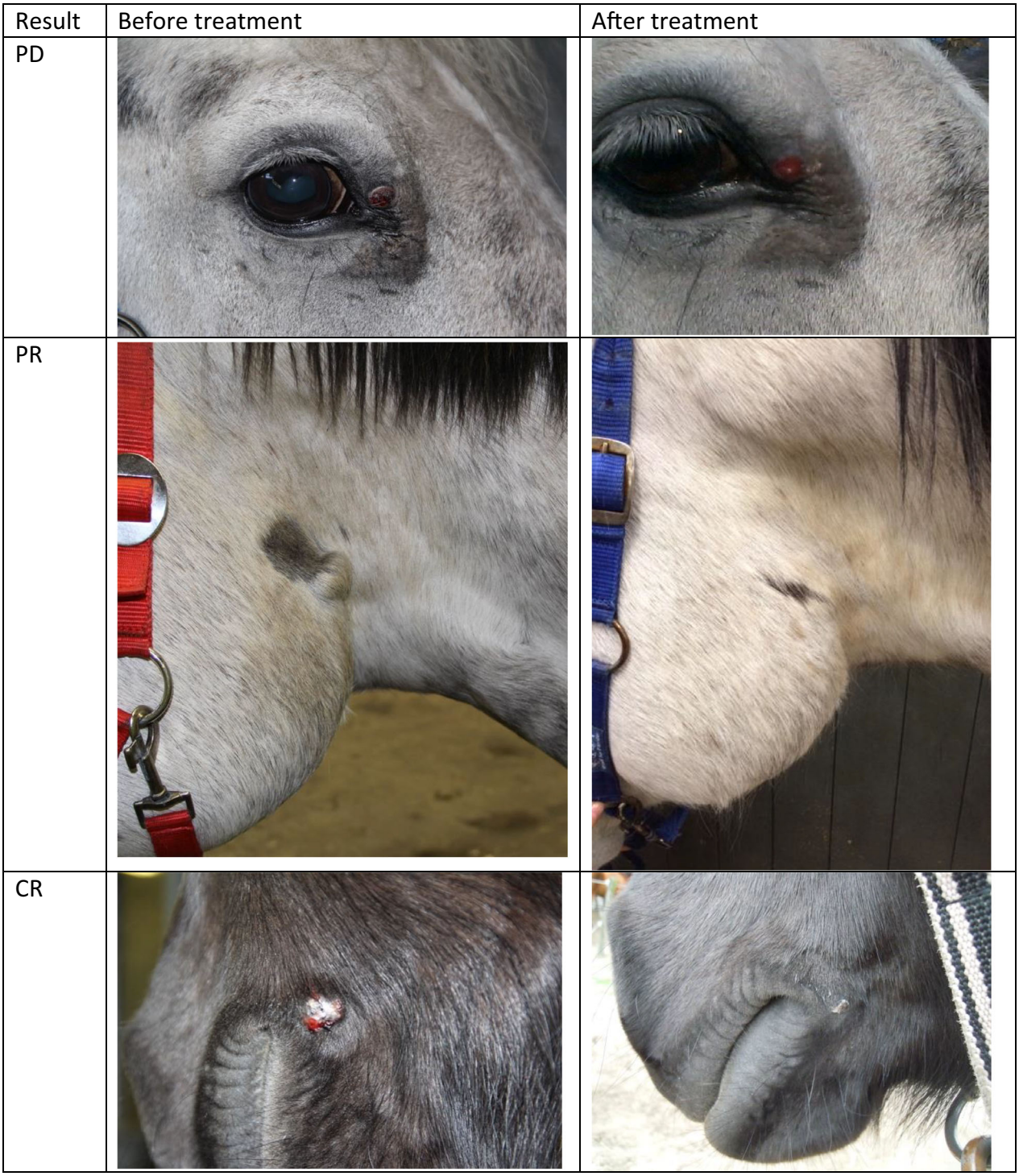


Figure 1. Example pictures of equine sarcoid and responses to recombinant canarypox virus expressing feline interleukin (IL)-2 treatment. PD, progressive disease; PR, partial response; CR, complete regression.

treatment. Horse 4 had a mild leukocytosis after the first treatment (D2; 10.9 x 10^3/μL; reference range 5–10 x 10^3/μL), which resolved by D5. The CBC and fibrinogen levels remained within the reference ranges in all horses after the second treatment. Horse 2 showed mild oedema at all four injection sites on D2 which resolved the next day. None of the other horses showed reactions at the injection sites. Horse 3 showed mild signs of inflammation (mild swelling and exudation) at all biopsy sites. In conclusion, none of the horses showed significant adverse effects after two treatments with Oncept IL-2.

The main findings of the histopathological examination were moderate to severe, diffuse perivascular lymphoplasmacellular dermatitis and panniculitis with mild oedema at all sites treated (A1 and A2). Biopsy A2 from Horse 4 additionally showed a moderate infiltration of eosinophilic granulocytes. Histopathological examinations of biopsy 1 (D0, before treatment) and control locations (B1 and B2) yielded no abnormal findings.

Clinical trial

Twenty horses with a total number of 59 sarcoids completed treatment and finished the trial. The number of sarcoids per horse ranged from one to 13. Nine of 59 sarcoids had received other treatments previously without success. One horse developed an upper airway infection after the first injection and was subsequently withdrawn before completing the trial. The mean observation period was 30 months (range 22–40 months).

A total of 38 sarcoids were treated in 20 horses, and one sarcoid of each horse was biopsied without signs of inflammation or complications of wound healing at the biopsy site. Treatment with Oncept IL-2 was well-tolerated by all horses. None of the horses showed haematological parameters outside the physiological range at any time point. Furthermore, all measured blood parameters were within the reference ranges before and one day after treatment. One horse developed mild fever the day after the first treatment which subsided the following day without requiring therapy.

Complete regression of all sarcoids, including those that had not been treated, was achieved in eight horses (40%) and PR in two horses (10%). Sarcoids did not change in size in two horses (10%), and the disease progressed in five horses (25%). Sarcoids showed response in three horses (15%) initially and then progressed again.

A total of 25% (n = 5) of the sarcoids injected with IL-2 progressed, whereas 10% (n = 2) did not change in size. In 10% (n = 2) of treated sarcoids PR was achieved in 10% (n = 2) of sarcoids treated and CR in 40%. A total of 15% (n = 3) of the treated sarcoids responded to treatment initially and then progressed again.

When the sarcoids that were not injected are included, then 16.95% of sarcoids (n = 10) progressed, 3.95% (n = 2) remained stable in size and PR was achieved in 3.95% (n = 2). A majority of 67.8% (n = 40) of sarcoids showed CR and 8.47 % (n = 5) of sarcoids recurred. Of the 67.8% of sarcoids that showed CR, regression started after a median time of 30.4 ± 5.4 weeks (Table 3).

Treatment proved to have a major impact on the probability of a reduction of ≥50% ($P < 0.001$) in the multivariable mixed logistic regression model including all sarcoids. Neither location ($P = 0.334$), size ($P = 0.828$) nor type ($P = 0.342$) played a major role. The classification of the model reached 100% and, thus, all observations were classified correctly by the model. This is a sign of possible overfitting of the model. The covariance analysis revealed that 88% of the variance was due to variance between horses. This means that individual effects played a major role in the probability of a reduction of 50% of the sarcoids treated.

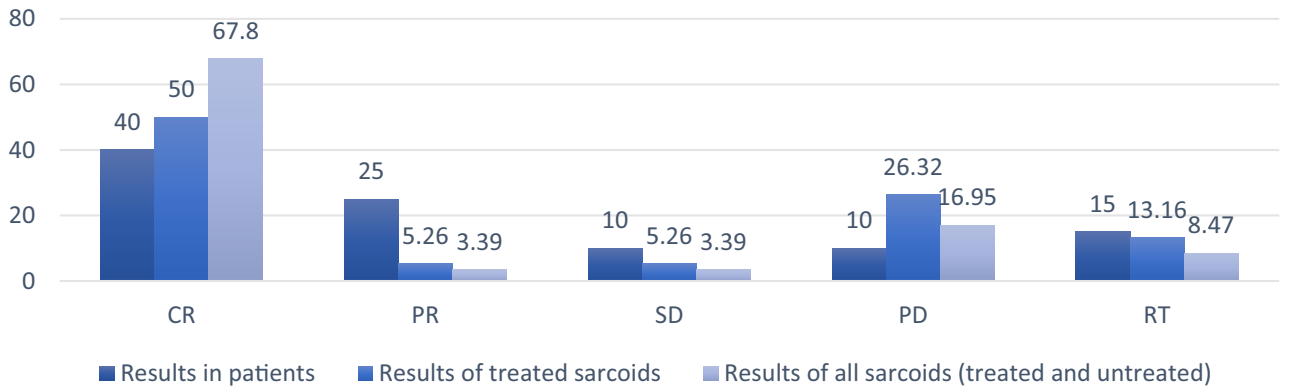
The multivariable mixed logistic regression model including only sarcoids treated showed that the follow-up time point of six months had the highest impact on the probability of a reduction of ≥50% ($P = 0.066$). The OR of 66.3 was extremely high and also had a large 95% CI (0.660–6648.1). Reduction was not strongly related to sarcoid location, with legs having the highest chance of reduction [OR 3.3 (95% CI: 0–27.690) compared to head] and core having the lowest chance [OR 0.085 (95% CI: 0–16.1) compared to head]. Sarcoids of the nodular type had the lowest chance of reduction, while verrucous sarcoids were more often reduced by >50%; the longer the observation period, the smaller the chance for reduction by >50%. The classification of the model reached 100%, so that all observations were classified correctly by the model. More than 90% of the variance was the result of variance between horses.

Discussion

Only mild local adverse effects and mild leukocytosis occurred in one horse after subcutaneous injection of Oncept IL-2 in the initial safety study with four healthy horses. In the prospective clinical study with 20 horses none of the horses with equine sarcoids showed significant adverse effects after intratumoural treatment. The canarypox vector generally has been found to be a safe delivery system. Systemic adverse effects after Oncept-IL 2 application have occurred rarely in cats and included transient apathy and fever.[19]

The canarypox vector has been used in horses previously and shows high biosafety because it is nonreplicative in mammalians, and genetically and physically stable.[21] It is, however, not certain whether the vector

Table 3. Results for 59 sarcoids from 20 horses (38 sarcoids from 20 horses were treated with recombinant poxviruses expressing feline interleukin-2; 21 sarcoids remained untreated) (n = 59) in %.



In Results in patients, the least responsive tumor of a patient was considered the overall result for evaluation.
PD, Progressive disease; SD, stable disease; PR, Partial regression; CR, Complete regression; RT, recurrence of tumor.

designed for cats actually induces expression of IL-2 in horses. Further in-depth studies on the effects of feline IL-2 on the equine immune system are needed.

Short- and long-term adverse effects of local treatment with human IL-2 also have been described in horses. They varied from tenderness and erythema at the injection site to slight oedema and localized swelling.[9] The combination of cisplatin and human IL-2, however, showed less severe adverse effects than generally seen after treatment with Bacillus Calmette–Guérin (BCG) or repeated cisplatin injections.[9] Additional advantages of Oncept IL-2 treatments are that the drug is safe to handle for humans due to the low biological activity of feline IL-2 in humans,[22] and that it is a licensed veterinary product in Europe.

A reduction in tumour size or complete resolution of the tumour after treatment with Oncept IL-2 occurred in 50% of the injected and 68% of all sarcoids in the present clinical study. Note that complete resolution was defined as no visible signs of tumour and/or skin alterations, and that biopsies were not repeated to confirm the resolution of the sarcoid. Better treatment results in horses with more than one sarcoid have been reported in the literature[9] with concomitant tumour rejection and/or systemic immunity after IL-2 therapy as possible explanations. It is possible that a systemic effect is induced by feline IL-2, as described for other immunostimulating treatments and cryosurgery.[23] One study showed that the odds of treatment failure were significantly lower for a sarcoid on a patient that received concurrent immunostimulating treatment (cryosurgery, BCG vaccine injection or imiquimod application). This would suggest that the immune system plays a considerable role in sarcoid treatment.[23] The influence of the horses' immune system in the aetiology of equine sarcoids is not yet completely understood and there might be differences in systemic immune reactions in horses with multiple sarcoids that lead to a changed responsiveness to IL-2 in these horses. Further studies are necessary to examine whether local intratumoural injections of Oncept IL-2 or a systemic parenteral application of IL-2 are more beneficial.

It also is possible that horses showed spontaneous remission during the study, as has been reported in equine sarcoids.[24] One major limitation of the present study is, therefore, the missing untreated control group. The inclusion of a negative control group was initially planned. All horses in this study were client-owned and placebo treatment, especially after performing a biopsy, resulted in low owner compliance. Therefore, no negative control group was included in the study. Two previous studies showed tumour size reductions or resolution in untreated control groups of 14%[25] and 16%.[26]. In another study, spontaneous remission was observed in ≤62% of horses during an observation period of five to seven years.[27]

A previous study on local treatment of equine sarcoids with human IL-2 reported a >50% size reduction or complete tumour regression after six months in four of 11 (36%) cases (IL-2 treatment alone intratumourally for five days, high dose), five of 10 (50%) cases (IL-2 treatment alone intratumourally for ten days, low dose) and in nine of 15 (60%) cases (IL-2 treatment combined with cisplatin).[9] Sarcoid types were comparable between the study mentioned above and our study. A reason for the slight difference in results could be the use of feline IL-2 and the different treatment regimes. The human IL-2 used in the other study[9] shows a homology with equine IL-2 of 72%, whereas feline IL-2 used in our study shows a slightly greater homology of 76%.

One disadvantage of the treatment with Oncept IL-2 was the long delay observed between the first injection and the beginning of tumour remission in the present study. Of the 68% of sarcoids that did show remission after 23 months, remission started after a median time of 30.4 ± 5.4 weeks. It is, therefore, not possible to say with certainty that remission was a consequence of the treatment as different ways and lag times of tumour regression after IL-2 treatment have been described in human patients and ruminants. An immediate regression has been described in which the tumour resolves within a few days. This is probably due to reduced blood flow to the tumour and subsequen necrosis.[28,29] A slower regression (up to one year) was observed in bovine ocular squamous cell carcinoma[30,31] and equine sarcoids.[9] In the latter study, histological changes in tumours 10–15 days after the last IL-2 treatment revealed a lymphocytic-plasma cellular infiltration that is seen as a late-change phenomenon.[9]

Oncept IL-2 is used in cats to prevent the recurrence of fibrosarcomas after surgical removal, and six treatments in a seven day interval are indicated.[19] The dosage and the seven day treatment interval for this pilot study were in accordance with the regime used in cats. However, more than two treatments may increase the efficacy in equine patients, and further examinations are necessary to establish the best treatment regime in horses.

In summary, in this clinical pilot study, the treatment of equine sarcoids with canarypox virus locally expressing IL-2 was easy and safe for both user and horse. Equine sarcoids injected with canarypox virus expressing feline IL-2 showed a reduction in tumour size or a complete remission of the tumour in 50% of cases. Further studies are necessary to research vector and feline IL-2 effects in horses, establish an optimal treatment regime for equines and verify the results in a larger population of patients.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Multivariable mixed logistic regression models.

Résumé

Contexte – L'interleukine (IL)-2 stimule l'immunité anti-tumorale et est efficacement utilisée pour le traitement de différentes néoplasies.

Hypothèses/Objectifs – Canarypoxvirus exprimant localement IL-2 félin est sûr et peut être utilisé pour traiter les sarcoïdes équins.

Sujets – Vingt chevaux de différentes races avec un âge médian de huit ans (écart interquartille de 6.0-13.3 ans) et un nombre total de 59 sarcoïdes ont été inclus dans l'étude.

Méthodes – Dans cette étude clinique prospective, les sarcoïdes ont été injectés deux fois à sept jours d'intervalle avec un canarypoxvirus recombinant exprimant IL-2 félin. Une formule sanguine (CBC) et les taux de fibrinogènes ont été mesurés avant traitement et à jours 1, 3, 7 et 8.

Résultats – Une régression complète a été obtenue pour huit chevaux (40%) et une régression partielle pour deux chevaux (10%). Aucun changement de taille des sarcoïdes n'a été observé pour deux chevaux (10%) et la maladie s'est aggravée pour cinq chevaux (25%). Les sarcoïdes de trois chevaux (15%) ont montré une réponse initiale puis une croissance de la tumeur. Il n'y avait pas de changement significatif dans le CBC et les taux de fibrinogènes après chacune des injections. Un cheval a développé une fièvre modérée le jour après chaque injection, qui disparaissait sans traitement le jour suivant.

Conclusions – Le traitement des sarcoïdes équins avec canarypoxvirus exprimant IL-2 félin semble être une option thérapeutique sure. Bien que l'expression d'IL-2 après injection du vecteur et son activité biologique chez les chevaux n'aient pas été prouvées dans cette étude, le traitement résultaient en une

régression et une régression partielle dans 50% des cas. D'autres études sont nécessaires pour vérifier ces données et pour établir un protocole thérapeutique.

Resumen

Introducción – la interleuquina 2 (IL-2) estimula la inmunidad antitumoral y se utiliza con éxito para el tratamiento de diferentes neoplasias.

Hipótesis/Objetivos – El poxvirus de canarios que expresa localmente IL-2 felina es seguro y puede usarse para tratar sarcoides equinos.

Animales – se incluyeron en el estudio veinte caballos de diferentes razas con una mediana de edad de ocho años (rango intercuartílico 6,0-13,3 años) y un total de 59 sarcoides.

Métodos – en este ensayo clínico prospectivo, se inyectaron sarcoides dos veces con siete días de diferencia, con un poxvirus de canarios recombinante que expresaba IL-2 felina. Se midieron los recuentos sanguíneos completos (CBC) y los niveles de fibrinógeno antes del tratamiento y en los días 1, 2, 7 y 8.

Resultados – se logró una regresión completa en ocho caballos (40%) y una regresión parcial en dos caballos (10%). No se observó ningún cambio en el tamaño del sarcoide en dos caballos (10%) y la enfermedad progresó en cinco caballos (25%). Los sarcoides de tres caballos (15%) mostraron una respuesta inicial seguida de crecimiento tumoral. No hubo cambios significativos en los niveles de CBC y fibrinógeno después de cualquiera de las inyecciones. Un caballo desarrolló una fiebre leve al día siguiente de cada inyección, que remitió sin tratamiento al día siguiente.

Conclusiones – El tratamiento de los sarcoides equinos con el poxvirus de canarios recombinante que expresa IL-2 felina parece ser una opción terapéutica segura. Aunque la expresión de IL-2 después de la inyección del vector y su actividad biológica en caballos no se probaron en este estudio, el tratamiento resultó en regresión y regresión parcial en el 50% de los casos. Se necesitan más estudios para verificar estos hallazgos y establecer un protocolo de tratamiento.

Zusammenfassung

Hintergrund – Interleukin (IL)-2 stimuliert die Antitumor Immunität und wird erfolgreich zur Behandlung verschiedener Neoplasien eingesetzt.

Hypothese/Ziele – Das Kanarienpoxvirus, welches lokal felines IL-2 exprimiert ist sicher und kann verwendet werden, um equine Sarkoide zu behandeln.

Tiere – Es wurden zwanzig Pferde verschiedener Rassen mit einem medianen Alter von acht Jahren (Interquartilsabstand 6,0-13,3 Jahre) und insgesamt 59 Sarkoide in die Studie aufgenommen.

Methoden – Eine völlige Remission wurde bei acht Pferden (40%) erzielt und eine partiale Remission bei zwei Pferden (10%). Es konnte bei zwei Pferden keine Veränderung der Sarkoidgröße beobachtet werden (10%) und bei fünf Pferden verlief die Erkrankung progressiv (25%). Die Sarkoide von drei Pferden (15%) zeigten anfangs eine Verbesserung, gefolgt von vermehrtem Tumorwachstum. Es gab nach der jeweiligen Injektion keine signifikanten Veränderungen bei Blutbild und Fibrinogenwerten. Ein Pferd hatte am Tag nach jeder Injektion ein mildes Fieber, welches ohne Behandlung am darauffolgenden Tag wieder verschwand.

Schlussfolgerungen – Eine Behandlung equiner Sarkoide mit rekombinantem Poxvirus, welches felines IL-2 exprimiert, scheint eine sichere Therapieoption darzustellen. Obwohl die Exprimierung von IL-2 nach einer Vektorinjektion und seine biologische Aktivität bei Pferden in dieser Studie nicht bestätigt werden konnten, resultierte die Behandlung ini 50% der Fälle in einer völligen bzw partialen Regression. Es sind weitere Studien nötig, um diese Befunde zu verifizieren und um ein Behandlungsprotokoll zu erstellen.

要約

背景 – インターロイキン (IL)-2 は抗腫瘍免疫を刺激し、さまざまな腫瘍の治療に使用されている。

仮説/目的 – ネコIL-2を局所的に発現するカナリアポックスウイルスは安全で、馬のサルコイドの治療に使用することができる。

被験動物 – 年齢中央値が8歳 (中間値範囲6.0~13.3歳) の異なる品種の馬20頭、合計59のサルコイドを研究に含めた。

方法 – 本前向き臨床試験では、サルコイドにネコIL-2を発現する組換えカナリアポックスウイルスを7日間隔で2回注射した。全血球計算(CBC)およびフィブリノーゲン値を治療前、治療後1、2、7、8日目に測定した。

結果 – 8頭 (40%) の馬で完全退行が、2頭 (10%) で部分退行を達成した。サルコイドのサイズの変化は2頭 (10%) に認められず、5頭 (25%) で病状が進行した。3頭の馬 (15%) のサルコイドは初期反応を示し、その後腫瘍の増殖が認められた。いずれの注射を行ってもCBCおよびフィブリノゲン値に有意な変化はなかった。1頭の馬は各注射の翌日に軽度の発熱を示したが、翌日には治療せずに治まった。

結論 – ネコIL-2を発現する組換えカナリアポックスウイルスを用いた馬のサルコイド治療は、安全な治療オプションの一つであると思われる。本研究では、ベクター注入後のIL-2の発現および馬における生物学的活性は証明されなかったが、治療により50%の症例で退行および部分的な退行が得られた。これらの知見を検証し、治療プロトコールを確立するためには、さらなる研究が必要である。

摘要
背景 — 白细胞介素(IL)-2刺激抗肿瘤免疫，并成功用于治疗不同的肿瘤。
假设/目的 — 局部表达猫IL-2的金丝雀痘病毒是安全的，可用于治疗马结节病。
动物 — 研究纳入了20匹不同品种的马，中位年龄为8岁（四分位距6.0-13.3岁），共59种结节病。
方法 — 在这项前瞻性临床试验中，结节病每隔七天注射两次，用表达猫IL-2的重组金丝雀痘病毒。在治疗前和第1、2、7和8天检测血常规（CBC）和纤维蛋白原水平。
结果 — 8匹马(40%)达到完全缓解，2匹马(10%)达到部分缓解。2匹马(10%)未观察到结节病大小变化，5匹马(25%)疾病有所发展。3匹马(15%)的结节病显示初始缓解，随后肿瘤生长。两次注射后，CBC和纤维蛋白原水平均无显著变化。1匹马在每次注射后第二天出现轻度发热，第二天未经治疗退烧。
结论 — 用表达猫IL-2的重组金丝雀痘病毒治疗马结节病，似乎是一种安全的治疗选择。尽管本研究未证实载体注射后IL-2的表达及其在马体内的生物活性，但该治疗使得50%的病例缓解和部分缓解。有必要进行进一步研究，以验证这些结果并确立治疗方案。

Resumo
Contexto – A interleucina (IL)-2 estimula a imunidade antitumoral e é utilizada com sucesso no tratamento de diferentes neoplasias.
Hipótese/Objetivos – O vírus canarypox que expressa IL-2 felina localmente é seguro e pode ser usado para tratar sarcóides equinos.
Animais – Vinte cavalos de raças diferentes com idade mediana de oito anos (intervalo interquartil 6,0-13,3 anos) e um número total de 59 sarcoides foram incluídos no estudo.
Métodos – Neste ensaio clínico prospectivo, o vírus canarypox recombinante expressando IL-2 felina foi injetado nos sarcoides duas vezes em um intervalo de sete dias. Hemogramas completos (HM) e níveis de fibrinogênio foram mensurados antes do tratamento e nos dias 1, 2, 7 e 8.
Resultados – Regressão completa foi obtida em oito cavalos (40%) e regressão parcial em dois cavalos (10%). Nenhuma mudança no tamanho do sarcóide foi observada em dois cavalos (10%) e a doença progrediu em cinco cavalos (25%). Os sarcoides de três cavalos (15%) apresentaram boa resposta inicial seguida de crescimento do tumor. Não houve alterações significativas no hemograma e nos níveis de fibrinogênio após qualquer injeção. Um cavalo apresentou febre moderada no dia seguinte de cada injeção, que cedeu sem tratamento em um dia.
Conclusões – O tratamento de sarcóides equinos com vírus canarypox recombinante que expressa IL-2 felina parece ser uma opção de terapia segura. Embora a expressão de IL-2 após a injeção do vetor e sua atividade biológica em equinos não tenham sido comprovadas neste estudo, o tratamento resultou em regressão e regressão parcial em 50% dos casos. Mais estudos são necessários para verificar esses achados e se estabelecer um protocolo de tratamento.