Evaluation of in vitro chemosensitivity of vaccine-associated feline sarcoma cell lines to vincristine and paclitaxel

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Objective—To determine the in vitro sensitivity of 4 vaccine-associated feline sarcoma (VAFS) cell lines to the chemotherapeutic agents vincristine and paclitaxel.

Sample Population—Cell lines derived from 4 VAFS specimens.

Procedures—Cell lines were cultured in vitro and individually exposed to various concentrations of vincristine and paclitaxel. Survival was estimated after 24 and 72 hours of drug exposure, and the drug concentrations that resulted in 50 and 90% reduction in number of viable cells (IC50 and IC90, respectively) were calculated.

Results—Both vincristine and paclitaxel had significant dose-dependent effects on the viability of the VAFS cell lines. After 72 hours of drug exposure, the IC50 and IC90 of vincristine for the 4 cell lines were between 0.005 to 0.039 µg/ml and 0.045 to 1.027 µg/ml, respectively. The IC50 and IC90 values for paclitaxel were between 0.037 to 0.092 µg/ml and 2.450 to 15.413 µg/ml, respectively.

Conclusions—Results of pharmacokinetic studies on vincristine and paclitaxel in other species suggest that concentrations greater than the IC50 values may be possible for both drugs in feline patients as well. The drug concentrations at which viable cell numbers were reduced by 90% may also be attained in vivo for some cases, but detailed information is needed regarding the distribution, concentration, duration of availability, and toxicity of various drugs in cats. Carefully chosen combinations of antineoplastic agents need to be screened to identify treatment protocols that may be further evaluated clinically for the treatment of VAFS. (Am J Vet Res 2002;63:728–732)

Clinical observations and epidemiologic analyses over the past decade have established a strong causal relationship between vaccination and the development of soft tissue sarcomas at injection sites in cats. It is estimated that between 3 and 13 cases of vaccine-associated feline sarcomas (VAFS) develop for every 10,000 cats that are vaccinated. Thus, a sizable number of cats are at risk of developing this disease, as approximately 40% of the household cat population in the United States (which is currently >66 million) receives at least 1 vaccination each year.

It is believed that VAFS develop because of neoplastic transformation of cells from injection site granulomas. These tumors may be distinguished from sarcomas arising at nonvaccination sites by the presence of granulation tissue and inflammatory infiltrates of lymphocytes and macrophages. Often, a grayish material containing aluminum is present in the macrophages. Aluminum (in the form of hydroxide or phosphate) is commonly used as a adjuvant in various vaccines and has been previously detected in vaccination-site granulomas in humans and animals. Aluminum hydroxide also has the potential to cause chromosomal damage or mutations in vitro and thus may be a risk factor for tumor development at vaccination sites. However, nonaluminum adjuvants and other inflammatory agents may also be associated with injection-site tumors in cats. Although viral involvement in tumorigenesis at vaccination sites is possible, experimental evidence as well as the age of onset of the disease in cats (generally between 8 and 9 years) indicate that infection with FeLV is probably not etiologically related to VAFS development.

Vaccine-associated feline sarcomas are highly invasive tumors with poorly defined margins. The tumors are predominantly fibrosarcomas and malignant fibrous histiocytomas, although other histologic subtypes have been reported. These aggressive tumors exhibit severe pleomorphism, high mitotic indices, and central areas of necrosis. The rate of local recurrence ranges from 27% for aggressive en bloc removal of the tumors at first chance to >60% for more limited excisions. Early detection and aggressive excision of tumors at referral hospitals also greatly extend median disease-free intervals (from 66 days to >300 days). But complete resection may be difficult, especially for tumors located in the interscapular area, where most vaccines were traditionally injected. Moreover, many of the tumors that do recur still appear <6 months after excision of the primary tumor. Thus, improvement of methods of treatment and management of VAFS are a priority within the veterinary medical community.

Determination of the true efficacy of various protocols used in the treatment of VAFS will require controlled long-term prospective studies. Thus far, adjuvant radiation therapy for local tumor management undertaken at various institutions has resulted in median disease-free intervals ranging from 112 to 398 days, and only limited information is available on the...
use of chemotherapeutics in the treatment of sarcomas in cats. A retrospective evaluation of 4 primary and 8 recurrent cases of VAFS indicated that combined treatment with doxorubicin and cyclophosphamide elicited a partial response (decrease in tumor burden by > 50%) in 6 cases. Partial and even complete responses have also been reported for treatment of a limited number of sarcomas using vincristine (alone or in combination with methotrexate and cyclophosphamide). Because vaccine site-associated tumors are highly invasive and distant metastasis (primarily to the lung) occurs in up to 28% of cases within approximately 2 years of diagnosis, the use of chemotherapeutic drugs in the treatment and control of VAFS needs to be carefully evaluated.

Given the rarity of VAFS patients that have undergone chemotherapy and the limited information on pharmacokinetics of anticancer drugs available in cats, we have undertaken studies to evaluate the effect of various drugs on VAFS cell lines in vitro. The purpose of the study reported here was to evaluate the effect of 2 tubulin binding antimitotic agents, vincristine and paclitaxel, on growth and viability of 4 VAFS cell lines (determination of the in vitro chemosensitivity of tumor cells provides a useful initial step in screening anticancer agents that may be suitable for further evaluation in vivo). Materials and Methods

Vaccine-associated feline sarcoma cell lines—Specimens (1 to 1.5 cm3) were collected at the time of en bloc surgical excision of vaccination-site tumors from 4 cats. Cats (2 male and 2 female) ranged in age from 5 to 10 years, and results of FeLV and feline immunodeficiency virus tests were negative. All 4 tumors had typical histopathologic features of vaccine associated sarcomas. Cell lines (FS1, FS2, FS3, and FS4) were established from these tumors as previously described and cultured in complete growth medium consisting of Dulbecco modified eagle medium supplemented with 5% heat-inactivated fetal bovine serum, penicillin (100 U/ml), streptomycin (100 mg/ml), and amphotericin B (0.25 mg/ml).

Chemotherapeutic agents—Vincristine and paclitaxel were stored at –20 C and 4 C, respectively, and the drugs were dissolved in minimum volumes of dimethyl sulfoxide (25 µl/mg of drug) prior to use. The solutions were diluted in complete growth medium to a working stock solution of 2 mg/ml, and serial dilutions prepared for addition to cell cultures.

In vitro drug sensitivity assays—Cells from the 4 cell lines were individually seeded at a density of 2 X 104 cells/well in 12-well cell culture plates containing 2 ml of complete growth medium in each well. Growth medium was changed every third day and sensitivity of the 4 cell lines to various concentrations of vincristine (1 X 10–3, 1 X 10–2, 5 X 10–2, 1 X 10–1, 1, 5, 10, 50 and 100 µg/ml) and paclitaxel (1 X 10–3, 5 X 10–2, 1 X 10–1, 1, 5, 10, 50, and 100 µg/ml) was measured during exponential growth (day 6). Each cell line was exposed (in triplicate wells) to the different concentrations of each drug for 24 hours or 72 hours (24 hour incubations were followed by 72 hours in drug-free medium). Cells that were not exposed to any drug served as drug-free controls. The numbers of viable cells (determined by trypan blue exclusion) were counted on a hemacytometer for the test and control wells. The mean cell densities determined for the various concentrations of the 2 drugs were expressed as percentages of the untreated controls (for each cell line). The 50% and 90% inhibitory concentrations (IC50 and IC90, respectively) of each drug were determined for the 4 cell lines from their dose response plots.

Figure 1—Viability of 4 vaccine-associated feline sarcoma cell lines (FS1 [A], FS2 [B], FS3 [C], and FS4 [D]) after 72 hours of exposure to vincristine (squares) and paclitaxel (circles). Cells were seeded in 12-well plates at 2 X 104 cells/well and various concentrations of each drug were added to triplicate wells on the 6th day of growth. Cell viability at each drug concentration was determined at the end of the exposure period and expressed as the percentage of cells remaining alive, compared with untreated controls.
Results

Both vincristine and paclitaxel had significant dose-dependent effects on the viability of the VAFS cell lines (Fig 1). Longer incubation with the drugs (72 hours vs 24 hours) was generally more effective in reducing cell viability (Table 1). The IC₅₀ of vincristine after incubation with the drug for 72 hours ranged from 0.005 to 0.039 µg/ml, and the IC₉₀ was between 0.045 to 1.027 µg/ml. Thus, under these experimental conditions, there was a 7.8-fold difference in IC₅₀ values and a 22.8-fold variation in IC₉₀ values among the 4 cell lines. The response to paclitaxel was more uniform between the cell lines tested, as the IC₅₀ for this drug (after incubation for 72 hours) ranged between 0.42 and 0.005 µg/ml (a 2.5-fold variation) and the IC₉₀ ranged from 0.54 to 2.45 µg/ml (a 4.8-fold variation). However, the relative effect of the drug on the different cell lines varied with concentration. For example, 1 of 2 cell lines with the highest IC₅₀ for paclitaxel required the lowest drug concentration for 90% reduction in viability (FS3). Similarly, the cell line with the lowest IC₅₀ (FS4) had the second highest IC₉₀. Despite the considerable effect of both drugs on the growth and viability of the VAFS cell lines, a small fraction of cells from each of the 4 lines tested remained viable after 72-hour exposure to drug concentrations above reported maximum in vivo concentrations.₄⁴-⁴⁶

Discussion

Tubulin-binding chemotherapeutic agents exert their antitumor activity through several mechanisms, including inhibition of mitosis and disruption of other essential cellular functions such as maintenance of shape, secretion, and transportation.⁴¹ Vincristine, an alkaloid from the periwinkle plant (Catharanthus roseus), is actively transported into cells where it interacts with tubulin, disrupting its polymerization into microtubules and causing dissolution of the mitotic apparatus.⁴²-⁴⁴ Paclitaxel, another antimitotic agent originally isolated from plant sources (the bark of the western yew, Taxus brevifolia) also binds to tubulin.⁴⁵ But unlike the vinca alkaloids, which inhibit microtubule assembly, paclitaxel prematurely stabilizes the polymerization process, thereby disrupting cytoskeletal functions and causing mitotic arrest.⁴⁶-⁴⁸

In humans, vincristine is widely used in the treatment of a host of pediatric and adult malignancies as well as immune-mediated diseases.⁴⁹ The drug is a component of combination therapy for the treatment of leukemias, nephroblastoma (Wilms’ tumor), osteosarcoma, rhabdomyosarcoma, and other malignant soft tissue sarcomas.⁴⁶-⁵⁰ The most common application of vincristine is in combination with bleomycin (or bleomycin and doxorubicin) for treatment of multiple idiopathic hemorrhagic sarcoma (Kaposi’s sarcoma), a commonly diagnosed malignancy in patients with acquired immunodeficiency syndrome (AIDS).⁵¹,⁵² In veterinary medicine, single agent vincristine sulfate chemotherapy has been used most effectively to attain remission of transmissible venereal tumors in dogs.⁵³ The drug is also used in combination with prednisone and cyclophosphamide or doxorubicin and cyclophosphamide to improve survival times in dogs with hematopoietic and lymphoid neoplasms.⁵⁴-⁵⁶ In cats, vincristine has been used for the treatment of lymphomas. A combination of the drug with i-asparaginase, doxorubicin, cyclophosphamide, and methotrexate elicits favorable responses in cats with alimentary malignant lymphoma.⁵⁷ Vincristine has also been used in the treatment of feline mammary neoplasms and partial response to single agent as well as combination therapy with vincristine has been reported in a few cases of feline sarcoma.⁵⁸-⁶⁰ Recommended dosages of vincristine for cats and dogs range from 0.025 to 0.05 mg/kg of body weight or 0.5 to 0.75 mg/m² of body surface, weekly, as a slow IV injection (extravascular injection of the drug results in localized irritation and necrosis).⁵⁹-⁶¹

Paclitaxel is a promising chemotherapeutic agent with demonstrated clinical activity against breast, head and neck, ovarian, and lung cancers.⁶²-⁶⁴ Results from recent clinical trials on breast and head and neck cancers indicate that paclitaxel remains effective in patients with recurrent or metastatic disease and even elicits a response in tumors that have become refractory to treatment with other agents.⁶⁵ Single agent paclitaxel is also one of the most effective drugs in the treatment of patients with advanced AIDS-associated multiple idiopathic hemorrhagic sarcoma.⁶⁶ The drug has been shown to elicit complete to partial responses in aggressive angiosarcomas that were refractory to other drugs and some cases of leiomyosarcoma, a disease that is generally resistant to chemotherapy.⁶⁷ The effect of taxanes on other soft tissue sarcomas needs to be carefully evaluated for individual histologic subtypes.

The use of paclitaxel as a chemotherapeutic agent is comparatively new in veterinary medicine, and administration of the drug in dogs (at 170 mg/m² every 3 weeks) and cats (5 mg/kg) is in the investigational stage.⁶⁸ In a recent study, biweekly administration of 40

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<th>Drug</th>
<th>IC₅₀ (mg/ml)</th>
<th>IC₉₀ (mg/ml)</th>
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<tr>
<td>VINCRISTINE</td>
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<tr>
<td>PACLITAXEL</td>
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Table 1—Inhibitory concentrations at 50% (IC₅₀) and 90% (IC₉₀) for 4 vaccine-associated feline sarcoma-derived cell lines on exposure to vincristine and paclitaxel.
mg of paclitaxel by the inhalation route to 15 dogs with primary and metastatic lung cancers resulted in complete remission of an osteosarcoma metastasis, regression in tumor volume in 2 metastatic mammary carcinomas (by > 50% and 47%, respectively), and long-term stabilization of 1 dog with wide-spread metastatic doxorubicin refractory liposarcoma. Inhaled paclitaxel caused little or no signs of local toxicosis at this dose, although at higher doses the drug is known to substantially deplete WBC counts. The concentrations of the drug in plasma ranged from 0.175 to 1.245 µg/ml, which may be less than a tenth of the maximum plasma concentration obtained after IV administration of 160 mg/m².62

Although in vitro chemosensitivity assays cannot model in vivo pharmacokinetics, results of in vitro chemosensitivity assays provide valuable insight on the inherent resistance or sensitivity of cancer cells, and the IC₅₀ value conventionally serves as a measurement for determining the efficacy of anticancer agents in such experiments. The IC₅₀ of vincristine (0.003 to 0.039 µg/ml) and paclitaxel (0.037 to 0.092 µg/ml) determined in our study for the 4 VAFS cell lines (Table 1) were comparable to values determined for a human osteosarcoma cell line (IC₅₀ of vincristine, approximately 0.017 µg/ml; paclitaxel, approximately 0.027 mg/ml). Results of pharmacokinetic studies performed in humans and other species indicate that concentrations greater than the IC₅₀ values determined in our in vitro study should be achievable for both antineoplastic drugs in cats.63-66 It is possible that some of the IC₅₀ values could be achieved in vivo, increasing the chances for a marked or prolonged response. However, detailed information regarding the distribution, concentration, and duration of availability of these drugs, as well as the undesirable adverse effects associated with their administration, need to be determined for cats. Additional drugs and cell-lines also need to be screened to gain a more comprehensive insight on the response of VAFS cells to chemotherapeutics.

Because chemotherapy of VAFS is still in the developmental stages, in vitro studies yield good candidates for clinical evaluation as therapeutic agents.33-35 Although administration of single agents are unlikely to produce complete or long-term remission in the treatment of VAFS, carefully chosen combinations of antineoplastic agents may be more effective, and in vitro screening of such drug combinations is in progress.33,35,38 In addition, because a considerable number of VAFS patients develop distant metastases, the antimetastatic properties of some of these drugs should also be evaluated.33-35,38

References