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CATS

In vitro chemosensitivity testing of the feline large granular lymphocyte cell line (S87)

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Abstract

Background: Feline large granular lymphocyte (LGL) lymphoma is an aggressive neoplasia characterised by short survival and poor response to chemotherapy.

Objectives: In this study, the effect of different chemotherapeutic agents on the growth kinetics of the feline cell line S87, a non-MHC-restricted feline LGL cell line, was investigated. Where possible, IC_{50} (inhibitory concentration 50) values were determined. The IC_{50} values of the cell line as lymphoma models can provide clues to the situation in vivo and serve as a basis for studying resistance mechanisms.

Methods: Cells were incubated with various concentrations of vincristine, doxorubicin, 4-hydroperoxycyclophosphamide, prednisolone, methotrexate and L-asparaginase for 24 and 48 h, respectively.

Results: The IC_{50} values could be determined as 14.57 (7.49–28.32) μg/mL at 24 h incubation and 5.72 (4.05–8.07) μg/mL at 48 h incubation for doxorubicin and 9.12 (7.72–10.76) μg/mL at 24 h incubation and 4.53 (3.74–5.47) μg/mL at 48 h incubation for 4-hydroperpoxycyclophosphamide. Treatment with vincristine and methotrexate resulted in relatively high cell resistance whereas L-asparaginase and prednisolone treatment led to a reduction in cell number compared to control while cell viability was not affected (cytostatic effect).

Conclusion: Overall, the feline LGL cell line S87 proves to be relatively sensitive to doxorubicin and 4-hydroperoxycyclophosphamide and relatively resistant to treatment with vincristine, prednisolone, methotrexate and L-asparaginase. The results of this study can be used for further investigations on resistance mechanisms in feline LGL lymphoma. Doxorubicin and cyclophosphamide can be interpreted as promising candidates for the therapy of feline LGL lymphomas.

KEYWORDS
chemosensitivity, feline, in vitro, large granular lymphocyte, lymphoma

1 | INTRODUCTION

While lymphoma is described as a common neoplastic disease in cats (Vail et al., 2019), large granular lymphocyte lymphoma (LGL lymphoma) is rare, accounting for approximately 7% of all feline lymphomas (Krick et al., 2008). Large granular lymphocytes (LGLs) are a heterogeneous population of circulating blood lymphocytes and include natural killer cells, cytotoxic T cells and natural killer T cells (Rola-Pleszczynski et al., 1985; Timonen et al., 1981). About 2%–13% of the peripheral blood cells are LGLs (Timonen et al., 1981; Wellman

TABLE 1 Review of literature: Case reports and case collections of feline large granular lymphoma with overview of therapy strategy and clinical outcome.

Author/year	Treatment	Patients	Outcome	Survival time
Collette et al. (2016)	**Modified Madison-Wisconsin protocol**	7	27 days progression-free interval (vs. 105 days in other lymphomas)	27 days mean survival time (vs. 57 days in other lymphomas)
Darbès et al. (1998)	-	6	5× euthanised due to bad prognosis, 1 died while examination	0–10 days
Finotello et al. (2018)	**CHOP-based protocol**	20	35.4% therapy response	60 days (53–67)
	CHOP-based protocol + enterectomy	9		42 days (4.6–79.3)
	Corticosteroids + enterotomy	1		42 days (4.6–79.3)
	Monotherapy with lomustine	5		90 days (47–132.9)
	Corticosteroids	42		15 days (9.4–20.6)
	No therapy	30		5 days (0–13.8)
Franks et al. (1986)	**CHOP-based protocol** (prednisone, L-asparaginase, cyclophosphamide, methotrexate, vincristine)	1	Euthanasia after 6 weeks due to cachexia	-
Goitsuka et al. (1988)	**Cyclophosphamide + prednisolone**	1	Death after 10 days	-
Kariya et al. (1997)	-	4	2× euthanasia, 2× dead after laparotomy	5–7 days
Krick et al. (2008)	**COP protocol**: Week 1: L-asparaginase, 400 IU/kg s.c. Week 2: vincristine, 0.5 mg/m^2 i.v. Week 3: cyclophosphamide, 100 mg/m^2 for 2 days Week 4: vincristine Week 5: methotrexate, 2.5 mg p.o.	20	1 complete remission (cat received COP protocol), 6× partial remission, 5× progression despite chemotherapy, 2× stable disease,	Mean survival time: 57 days (0–267)
	CHOP-based protocol: (2× modified Madison-Wisconsin protocol, 1× COP + mitoxantrone, 1× COP + doxorubicin)	4		
Meichner et al. (2014)	**Prednisone p.o.**	1	2 weeks without progression	Euthanasia after 1 month
Roccabianca et al. (2006)	**Not specified**	21 (18 leukemic)	15× euthanasia due to progression of disease, 3× not specified	Mean survival time: 18.8 days (0–84)
Rydzewski et al. (2016)	-	1	No therapy due to bad prognosis	Euthanasia
Sapierzynski et al. (2015)	**COP protocol**	2		Mean survival time: 9 months
	Masitinib	1		
	Glucocorticoids	2		Mean survival time: 1.5 months

et al., 1992). These cells are characterised by their typical azurophilic cytoplasmic granules, which enable them to lyse other cells, such as virus-infected cells or tumour cells (Timonen et al., 1981).

Feline LGL lymphoma is an aggressive neoplastic disease and is characterised by short survival times, even when treated with chemotherapy. Table 1 provides an overview of case reports and case collections on feline LGL lymphoma considering the therapeutic modality chosen and clinical outcomes (Collette et al., 2016; Darbes et al., 1998; Finotello et al., 2018; Franks et al., 1986; Goitsuka et al., 1988; Kariya et al., 1997; Krick et al., 2008; Meichner et al., 2014; Roccabianca et al., 2006; Rydzewski et al., 2016; Sapierzynski et al., 2015). Most common sites of LGL lymphoma are the gastrointestinal tract and the mesenteric lymph nodes (Darbes et al., 1998; Krick et al., 2008; Roccabianca et al., 2006; Wellman et al., 1992). Other tumour sites include liver, spleen and lung. Leukaemia has also been described (Darbes et al., 1998; Finotello et al., 2018). Anorexia, lethargy, fever, diarrhoea, vomitus and weight loss are common symptoms (Finotello et al., 2018; Roccabianca et al., 2006; Wellman et al., 1992).

The CHOP protocol is commonly used to treat feline LGL lymphoma. "*CHOP*" refers to combination therapy with alternating treatment with *c*yclophosphamide, *h*ydroxydaunorubicin (= doxorubicin), *o*ncovin (= vincristine) and *p*rednisolone. A 2017 retrospective study found a median survival time (MST) of 60 days in cats with LGL lymphoma treated with CHOP-based chemotherapy (Finotello et al., 2018). Cats treated with corticosteroids only had an MST of 15 days, whereas the nontreated animals had an MST of 5 days (Finotello et al., 2018; Meichner et al., 2014). Another study described a median progression-free survival time of 65.5 days in cats treated with COP-based protocols (cyclophosphamide, vincristine, prednisolone) and demonstrated that response to chemotherapy can be used as a prognostic factor in lymphoma treatment (Waite et al., 2013). In addition to the CHOP protocol, L-asparaginase and methotrexate are often used in the therapy of LGL lymphoma (Waite et al., 2013).

Due to the poor prognosis of feline LGL lymphoma even under chemotherapy, the aim of this study was to determine the in vitro chemosensitivity of the feline large granular lymphocyte cell line S87. This cell line was established from the abdominal effusion of a 12-year-old spayed female European Shorthair cat diagnosed with LGL lymphoma based on cytology. The cells were characterised as non-MHC-restricted cytotoxic T cells (Rydzewski et al., 2016).

There are few studies on in vitro drug sensitivity testing on feline tumour cell lines. Muleya et al. (1999) performed in vitro chemosensitivity assays on two feline mammary tumour cell lines and determined the antiproliferative effect of individual drugs as dose-response curves for IC_{50} determination. The IC_{50} is described as the concentration of 50% growth inhibition. Banerji et al. (2002) determined the in vitro chemosensitivity (IC_{50} and IC_{90} values) of four vaccine-associated feline sarcoma cell lines to vincristine and paclitaxel and, in a second study, to doxorubicin and mitoxantrone because for these tumours adjuvant chemotherapy is required in cases with incomplete tumour resection or small resection margins (Banerji et al., 2002; Williams et al., 2001). Okai et al. (2000) performed in vitro chemosensitivity testing on a feline T cell lymphoma cell line. The use of cell lines as models of the tumour disease in question to determine IC_{50} may help reduce the number of live animal clinical trials required to obtain information on the efficacy of chemotherapeutic agents (Banerji et al., 2002).

For in vitro chemosensitivity testing, vincristine sulphate, doxorubicin hydrochloride, 4-hydroperoxycyclophosphamide, prednisolone, L-asparaginase and methotrexate were selected for this study as commonly used chemotherapeutic agents in feline lymphoma therapy.

2 | METHODS

2.1 | Tumour cell line

S87 cells were incubated as a suspension culture in RPMI 1640 medium (Gibco®Life Technologies GmbH, Darmstadt, Germany) supplemented with 10% heat-inactivated foetal calf serum (Gibco®Life Technologies GmbH, Darmstadt, Germany) and 1% penicillin/streptomycin (Gibco®Life Technologies GmbH, Darmstadt, Germany) at 37°C, 95% humidity and 5% CO_2 in 75-cm^2 flasks (Sarstedt, Nümbrecht, Germany). Cells were passaged weekly, and the medium was also changed weekly. For each chemosensitivity assay, frozen cells from the same passage (passage 96) were recultured and passaged to the desired cell number.

2.2 | Cell line validation statement

Authentication of the cell line S87 was achieved by amplification of feline STR markers (F42, F141, FCA559, FCA749) (Schury et al., 2014).

2.3 | Chemotherapeutic agents

The following agents were used: vincristine sulphate (MP Biomedicals, Germany), doxorubicin hydrochloride (Merck KGaA, Darmstadt, Germany), 4-hydroperoxycyclophosphamide (Santa Cruz Biotechnology, Inc., Heidelberg, Germany), prednisolone (Merck KGaA, Darmstadt, Germany), L-asparaginase (Merck KGaA, Darmstadt, Germany) and methotrexate (Merck KGaA, Darmstadt, Germany). All substances were obtained as solids for laboratory use. Vincristine sulphate, 4-hydroperoxycyclophosphamide, L-asparaginase and prednisolone were dissolved in cell culture medium with the supplements described above. Doxorubicin hydrochloride was dissolved in 0.9% NaCl solution for infusions (B. Braun Melsungen AG, Melsungen, Germany) and methotrexate was dissolved in $Na_2CO_3/NaHCO_3$ buffer according to the manufacturer's recommendations. For the chemosensitivity assays, the dissolved chemotherapeutic agents were diluted with the cell culture medium described above. The initial tested concentrations were chosen based on concentrations tested in other publications (Banerji et al., 2002; Muleya et al., 1999; Okai et al., 2000; Vaidyanathan et al., 2016). The concentrations for subsequent experiments were then adjusted based on the initial results. Accordingly, six concentrations were finally tested for each drug in the following concentration ranges: for vincristine sulphate from 500 μg/mL to 0.01 μg/mL; for doxorubicin hydrochloride and 4-hydroperoxycyclophosphamide from 100 μg/mL to 0.001 μg/mL; for prednisolone from 500 μg/mL to 1 μg/mL; for methotrexate from 5000 μg/mL to 0.1 μg/mL and for L-asparaginase from 10 U/mL to 0.0001 U/mL.

As controls, cells were treated with control medium containing the same volume of the respective solvent (vincristine sulphate, 4-hydroperoxycyclophosphamide, prednisolone, and L-asparaginase: cell culture medium; doxorubicin: 0.9% NaCl solution and methotrexate: $Na_2CO_3/NaHCO_3$ buffer) without the chemotherapeutic agent.

Elacridar (Merck KGaA, Darmstadt, Germany) as an inhibitor of MDR-1 protein (P-Glycoprotein/ABCB1), among others, was used to evaluate the effect of this resistance protein on the effect of doxorubicin and vincristine as MDR-1 substrates with a concentration of 10 μM given to each well (Vaidyanathan et al., 2016).

2.4 | Chemosensitivity testing

For chemosensitivity testing, cells were plated out in 24-well plates (Sarstedt, Nümbrecht, Germany). Each well contained 1 mL of cell suspension per well and at a concentration of 1 million cells/mL. Four cell culture plates were used for each assay (two treated plates, two control plates, 24 and 48 h treatment time, respectively; 4 wells per tested concentration). Daily monitoring of cellular growth and morphology was performed using an inverted microscope (Olympus Optical Co. GmbH, Hamburg, Germany). Twenty-four hours after plating (day 2), 0.6 mL cell culture medium was changed. On the third day, cell culture plates were centrifuged at 200 × *g* for 7 min (Centrifuge 5810r, Eppendorf GmbH, Hamburg, Germany). The cell culture medium was replaced by culture medium containing the respective concentrations of the chemotherapeutic agents or by control medium. After 24 or 48 h of incubation, the cell number in the wells of the treated plates and control plates was determined. For this, the cells were washed 3 times in 1% phosphate buffered saline to remove the chemotherapeutic agents and resuspended in medium. Trypan blue staining was performed to determine viability. Cells were counted using the Countess II® automated cell counter (Thermo Fisher Scientific GmbH, Dreieich, Germany) in cell counting chambers with the devices software. Bright field mode and auto focus were used. Cells smaller than 7 μm and bigger than 20 μm were excluded. For each well, four replicate counts were performed for the treated cell group and two for the controls. Each assay was performed three times. This means that for each concentration 48 values were used in the calculation of the IC50 (4 wells per concentration, 4 replicate measurements per well (respectively 2 in the controls), 3 times repetition of the assay).

2.5 | Safety

To reduce the risk of contamination with the carcinogenic, mutagenic and reproductive toxic antineoplastic agents, a protocol with safety measures for the use of chemotherapeutics in chemosensitivity testing was developed and used (Henrich et al., 2021).

2.6 | Statistics

Arithmetic means were calculated from the replicates of each well in Microsoft Office 365 Excel® and used for further calculations. These were performed using the program BMDP/Dynamic, Release 8.1, (DIXON, 1993). To estimate the IC_{50}, the arithmetic means of each replicate of the assays were plotted as point clouds for each compound and incubation time. A function was then estimated by nonlinear regression using the BMDPAR program to fit the distribution of values. Because of the right-skewed distribution, the concentrations of the chemotherapeutic agents were logarithmised. This function was used to calculate the IC_{50} and the asymptotic standard deviation. Furthermore, a two-way-analysis of variance (two-way ANOVA) of the live cell count was performed using SAS statistical software (SAS® visual statistics 8.2, SAS institutes, Heidelberg, Germany) to show the influence of group (treated vs. control) and the drug concentration on significance. For this purpose, the unlogarithmised values were used, as they agree better with the adjusted least square means (LSmeans). These adjusted means were used for plotting the data (see Figure 1). Welsh's *t*-test was used to test the differences in the IC50 values of vincristine and vincristine with elacridar and of doxorubicin and doxorubicin with elacridar for statistical significance.

3 | RESULTS

The effects of the chemotherapeutic agents on the cell line were tested for six concentrations per agent and two different incubation times. Viability (percentage of live cells counted out of all cells counted) and total number of viable cells as a function of concentration are shown in Figure 1.

In all tests, the reduction in total number of viable cells was higher after 48 h of incubation than after 24 h. Treatments with doxorubicin (Figure 1a–d), vincristine (Figure 1e–h) (including coincubation with elacridar) and 4-hydroperoxycyclophosphamide (Figure 1i and j) showed a significant reduction in the total number of viable cells compared with controls (Table 2) at intermediate and higher concentrations. Treatment with methotrexate (Figure 1o and p) and L-asparaginase (Figure 1m and n) showed a reduction in the absolute number of viable cells compared with the cell number in the controls (Figure 1m and o), but the viability of the cells was hardly affected by the treatment (except for the 48-h incubation with methotrexate). Treatment with prednisolone (Figure 1k and l) showed little effect on viability and the number of viable cells only at the higher concentrations tested (100 μg/mL and above).

The results of the two-way ANOVA are shown in Table 2. Overall, there was a significant effect of concentration used and group (treated vs. control) for all agents tested, except for prednisolone after 24 h of treatment and combination therapy with vincristine and elacridar after 48 h of incubation. The higher the tested concentration was, the higher was the cytostatic or cytotoxic effect of the drug.

Furthermore, IC_{50} values were estimated (Table 3). Only for doxorubicin and 4-hydroperoxycyclophosphamide was it possible to estimate the values using the estimator function. For prednisolone, methotrexate and L-asparaginase treatment, a sufficiently high reduction in the number of living cells for a reliable estimation function could not be achieved in the experiments described, so that no reliable IC_{50} values could be determined. For vincristine and vincristine with elacridar, a mathematical estimation of the IC_{50} was only possible for the 24 h incubation and not possible for 48 h incubation due to technical reasons.

4 | DISCUSSION

Feline LGL lymphomas are commonly treated with combination therapy of various chemotherapeutic agents, but typically show high resistance to these therapies. In vitro chemosensitivity assays and

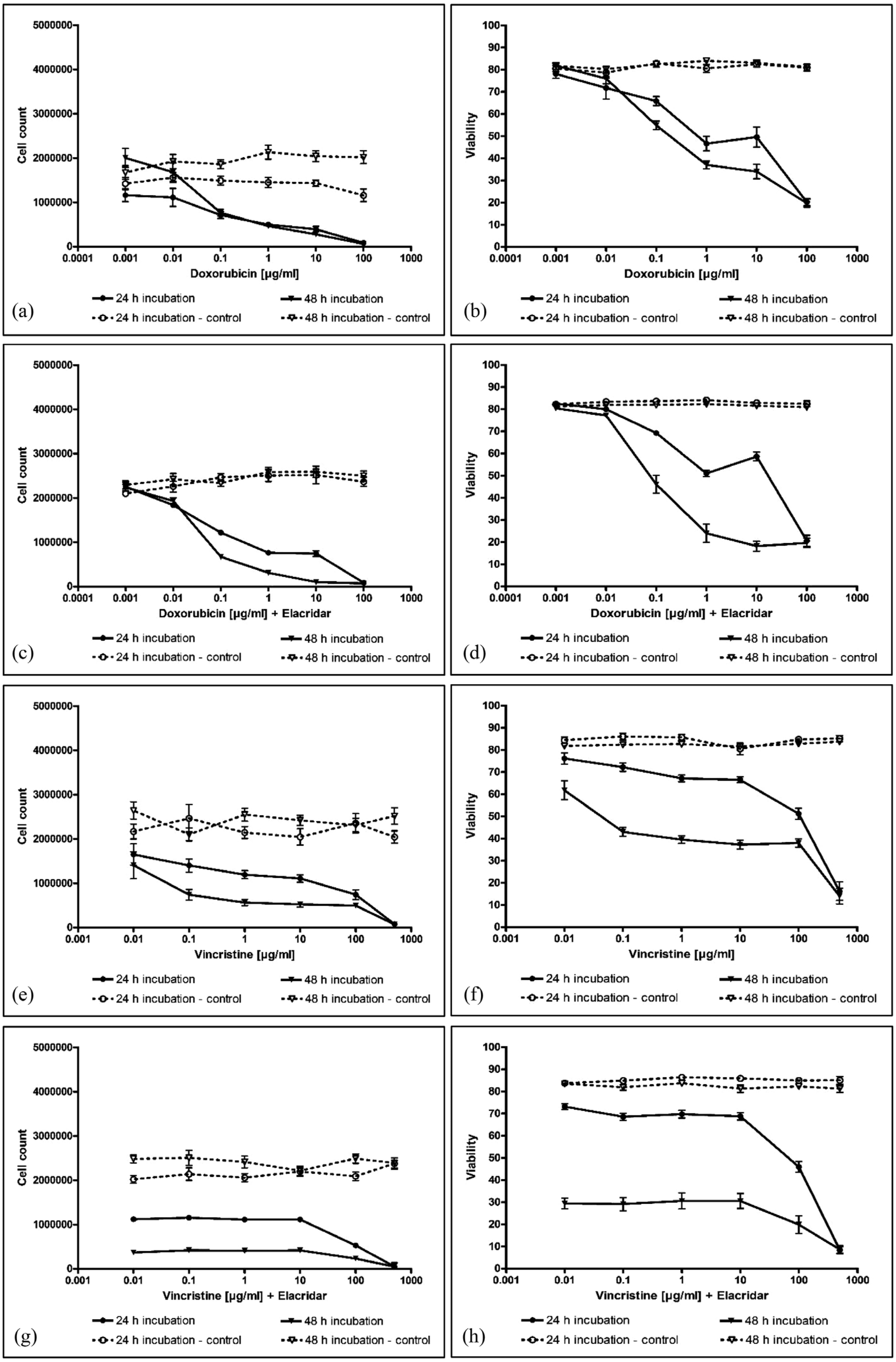

FIGURE 1 Cell count (left side) and viability (right side) for treatment of the cell line S87 with doxorubicin (a, b), doxorubicin and elacridar (c, d), vincristine (e, f), vincristine and elacridar (g, h), 4-hydroperoxycyclophosphamide (i, j), prednisolone (k, l), L-asparaginase (m, n) and methotrexate (o, p). The *x*-axis gives the concentrations of chemotherapeutic substances in μg/mL (U/mL respectively). The control was entered at the appropriate concentration of chemotherapeutic agent to reflect the corresponding amount of solvent in the cell culture medium of the control. Viability [%] was calculated as the proportion of live cells to total cell number.

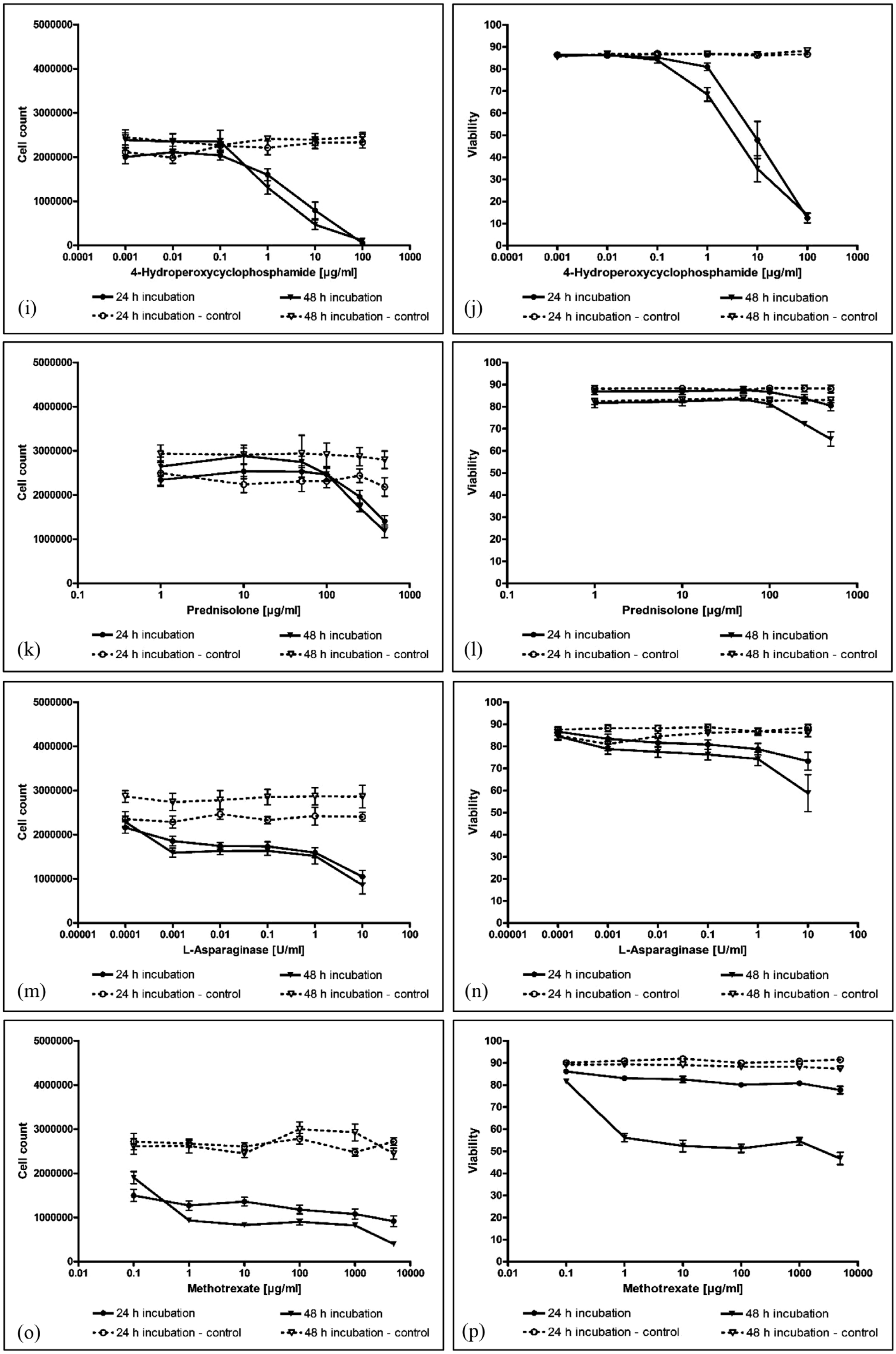

FIGURE 1 Continued

TABLE 2 Results of the two-way ANOVA performed on the cell count of living cells with SAS statistical software.

Chemotherapeutic agent	Incubation time	Main effects (*p* value) Group	Main effects (*p* value) Concentration	Interaction group × concentration (*p* value)
Doxorubicin	24 h	<0.0001*	<0.0001*	0.0011*
	48 h	<0.0001*	<0.0001*	<0.0001*
Vincristine	24 h	<0.0001*	<0.0001*	0.0006*
	48 h	<0.0001*	<0.0001*	0.0012*
4-Hydroperoxycyclophosphamide	24 h	<0.0001*	<0.0001*	<0.0001*
	48 h	<0.0001*	<0.0001*	<0.0001*
Prednisolone	24 h	0.2403	0.0034*	0.0182*
	48 h	<0.0001*	<0.0001*	0.0013*
L-asparaginase	24 h	<0.0001*	0.0032*	0.0004*
	48 h	<0.0001*	0.0049*	0.0047*
Methotrexate	24 h	<0.0001*	0.0438*	0.0867
	48 h	<0.0001*	<0.0001*	<0.0001*
Doxorubicin + elacridar	24 h	<0.0001*	<0.0001*	<0.0001*
	48 h	<0.0001*	<0.0001*	<0.0001*
Vincristine + elacridar	24 h	<0.0001*	<0.0001*	<0.0001*
	48 h	<0.0001*	0.1108	0.0600

Note: Main effects were calculated for the group (treated vs. control) and the concentration (6 concentrations per agent). The statistically significant *p* values are marked with an asterisk ($\alpha = 0.05$).

TABLE 3 Estimated IC_{50} values for 24 and 48 h incubation.

Substance	24 h incubation IC_{50} [μg/mL]	24 h incubation Interval (± asd)	48 h incubation IC_{50} [μg/mL]	48 h incubation Interval (± asd)
Doxorubicin	[14.57]*	7.49–28.32	[5.72]*	4.05–8.07
Doxorubicin and elacridar	[20.82]*	14.63–29.62	[2.30]*	2.09–2.54
4-Hydroperoxycyclophosphamide	9.12	7.72–10.76	4.53	3.74–5.47
Vincristine	[57.38]*	48.36–77.52	-	-
Vincristine and elacridar	[93.44]*	82.95–105.26	-	-

Note: All numbers are given in μg/mL. The interval shows IC_{50} ± asymptotic standard deviation. For vincristine (e.g., vincristine and elacridar) an estimation of IC_{50} for 48 h incubation was not possible.
*Differences statistically significant (Welsh's *t*-test) with $p < 0.0001$.

calculation of IC_{50} values can be used as a tool to analyse and describe resistance to chemotherapeutic agents in cancer. Cell lines are a suitable tool for studying cancer biology, used in human and veterinary medicine and, in some respects, can help predict the clinical behaviour of neoplastic disease (MacLeod et al., 2008; Seiser et al., 2013). Although the transferability of the conclusions of the in vitro chemotherapy studies are generally limited by pharmacokinetics and toxicity of drugs in living organisms, the results can be used for further studies on resistance mechanisms and cancer biology despite these limitations.

In this study, the individual drugs of the combination therapy were tested to evaluate the effects of each drug on the cell line at different concentrations. In this context, we aimed also to determine whether the cell line is suitable as a potential model for studying the resistance mechanisms of feline lymphomas, especially LGL lymphomas.

Chemosensitivity evaluation demonstrated the dose-dependent effects of different cytotoxic and cytostatic drugs on the S87 cell line. Typical cytotoxic chemotherapeutic drugs are doxorubicin and 4-hydroperoxycyclophosphamide, which interfere with DNA synthesis.

Doxorubicin is an anthracycline that intercalates with DNA strands resulting in cross-links, double-strand breaks, DNA degradation by formation of reactive nitrogen species and induction of apoptosis (Baxter-Holland & Dass, 2018; Gewirtz, 1999; Meredith & Dass, 2016; Minotti et al., 2004).

4-Hydroperoxycyclophosphamide is a nitrogen LOST derivative that can be used in in vitro studies to demonstrate the effects of cyclophosphamide. Cyclophosphamide is an inactive prodrug that requires activation in the liver and therefore cannot be used in in vitro studies. 4-Hydroperoxycyclophosphamide decomposes in aqueous solution to 4-hydroxycyclophosphamide, the first hepatic metabolite of cyclophosphamide. 4-Hydroxycyclophosphamide or its tautomer aldophosphamide in turn decomposes in cells to acrolein and chloroethylphosphate acid amide, the active metabolites that initiate DNA cross-linking (Boddy & Yule, 2000; Colvin et al., 1976; Komatsu et al., 1987; Ozer et al., 1982). In the in vitro chemosensitivity tests with the S87 cells, both substances, doxorubicin and 4-hydroperoxycyclophosphamide, resulted in a decrease in viability and the number of living cells (Figure 1a, b, I and j). This correlates with their cytotoxic activity.

When treated with the cytostatic substances, only decrease lower absolute number of living cells was observed compared to the control, while viability was mostly unaffected. This observation is consistent with the cytostatic mechanism of action, which inhibits cell proliferation while not affecting the viability of preexisting cells. A corresponding cytostatic effect was observed during treatment with L-asparaginase, vincristine and methotrexate. L-asparaginase is an enzyme produced in *E. coli* that inhibits cell proliferation by cleaving the amino acid L-asparagine, which neoplastic cells often cannot synthesise on their own (Kidd, 1953; Lang & Uber, 1904). In feline lymphoma, monotherapy with L-asparaginase results in a response rate of only 30%. Based on these results, its use in combination therapy is recommended (LeBlanc et al., 2007). In this study, treatment with L-asparaginase resulted in a relatively constant cell number (cytostatic effect), which was, however, about twofold lower than of the control (Figure 1m). At the highest concentration tested (10 U/mL), a decrease in cell number was observed (Figure 1m). So, the highest tested concentrations maybe also lead to a cytotoxic effect with a further decrease in cell number.

Comparable findings were obtained with methotrexate treatment (Figure 1o and p). Methotrexate also has a cytostatic effect by inhibiting dihydrofolic acid reductase and consecutively purine synthesis by folic acid deficiency (Chabner et al., 1985), which leads to a decrease in cell cycle activity and cell proliferation.

Prednisolone as a glucocorticoid has lymphocytoclastic properties and leads to nuclear fragility and cellular lysis (Nicholson & Young, 1978). In this study, only minor effects on viability and cellular proliferation were observed (Figure 1k and l). There was no significant difference between the cell number in the treated group and the control after 24 h of incubation with prednisolone (Table 2). This could be due to general resistance of the cell line to prednisolone or that the concentrations tested were too low for a toxic effect. However, experiments with higher concentrations were not possible due to the limited solubility of prednisolone in water. Several case reports and series indicate that short survival times and no clinical improvement occurred in cats with LGL lymphoma treated with corticosteroids alone. In these cases, median survival reached from 14 days to 1.5 months (Finotello et al., 2018; Krick et al., 2008; Meichner et al., 2014; Sapierzynski et al., 2015).

Vincristine is a vinca alkaloid that inhibits the microtubule polymerisation and leads to cell cycle arrest in metaphase (Jordan et al., 1985). In this study, in contrast to treatment with doxorubicin and 4-hydroperoxycyclophosphamide, a reduction in the number of living cells and viability was observed only at high concentrations (Figure 1e and f). Resistance of the cells to vincristine could explain this observation. A complete decrease in cell number was achieved with a dose of 500 μg/mL (Figure 1e), which seems to be a relatively high dose compared to clinically used doses in cats, which are around 500–700 μg/m^2 i.v. (this corresponds to about 125–175 μg in a 4 kg cat) (Collette et al., 2016; Plump, 1995). This assessment is limited by the fact that drug concentrations used in in vitro assays have limited comparability with clinically used drug doses due to differences in pharmacokinetics, such as drug redistribution and metabolism. Nevertheless, results such as these could help to understand clinical phenomena when they correlate with poor response to therapies, as in this case. In addition, the response of S87 cells to common chemotherapeutic agents can be used for further studies on resistance mechanisms in feline LGL lymphoma.

Meaningful calculation of the IC_{50} was possible in this study only for the substances that caused a large reduction in cell number compared with controls (Table 3). For vincristine, an IC_{50} of 48–78 μg/mL was calculated. In one study, Banerji et al. (2002) published IC_{50} values between 5 and 60 μg/mL in feline sarcoma cell lines. In a feline T cell lymphoma cell line an IC_{50} of 0.225 ng/mL was published (Okai et al., 2000). This value is about 10^4 lower than the values estimated in this study. For doxorubicin, the estimated IC_{50} values were 7–28 μg/mL for 24 h incubation time and 4–8 μg/mL for 48 h respectively. Muleya et al published data from two mammary tumour cell lines with an IC_{50} of 103.35 and 432.83 ng/mL after 72 h incubation time (Muleya et al., 1999). These values are also lower than the values tested in this study, but a different incubation time was used. For L-asparaginase, prednisolone, and methotrexate, a determination of the IC_{50} by pure calculation would be possible, but not meaningful, since the underlying estimator function is to be classified as biologically inconclusive due to the small change in cell number.

That the duration of exposure to the chemotherapeutic agent had an effect was demonstrated by the lower estimated IC_{50} values after 48 h of incubation compared to 24 h. This result correlates with the observations on cell growth and viability for all substances for which IC_{50} values were calculated.

To evaluate the effect of MDR-1 protein (P-glycoprotein/ABCB1) inhibition on assay results, the experiments were performed with doxorubicin and vincristine with and without elacridar as a potent inhibitor of the resistance protein. The MDR-1 protein is a well-known mediator of drug resistance in neoplastic disease. In feline neoplasia, MDR-1 protein expression has been detected in T- and B-cell lymphomas, intestinal carcinoma, haemangiosarcoma, mammary gland tumours, meningiomas, bronchial carcinomas, and others (Van der Heyden et al., 2011). Although there were statistically significant differences in the IC_{50} values, the effect was not as expected. Instead of the inhibition of the MDR1 protein causing a decrease in the IC_{50} value, the addition of elacridar caused an increase in the value, which could indicate an increase in resistance. The biological mechanism behind this remains unclear for the time being. In conclusion, this study describes the response of a feline LGL cell line to various chemotherapeutic agents and concentrations in vitro. The results can be used for further experiments on resistance mechanisms in LGL lymphoma. In addition, the only mild inhibitory effects of some substances on cell growth and viability confirm a preexisting drug resistance of the S87 cell line, which correlates with the clinical studies on chemotherapy of feline LGL lymphoma.

AUTHOR CONTRIBUTIONS

Svenja Hartung: conceptualisation; data curation; formal analysis; investigation; methodology; resources; validation; visualisation; writing—original draft. **Christiane Herden:** conceptualisation; methodology; project administration; supervision; writing—review & editing. **Marion Sparenberg:** formal analysis; methodology; software; validation. **Manfred Henrich:** conceptualisation; formal analysis; investigation; methodology; project administration; software; supervision; validation; writing—review & editing.

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CONFLICT OF INTEREST STATEMENT

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ETHICS STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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PEER REVIEW

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