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The Effect of Common Antineoplastic Agents on Induction of Apoptosis in Canine Lymphoma and Leukemia Cell Lines

ALEKSANDRA PAWLAK[1], ANDRZEJ RAPAK[2], IWONA ZBYRYT[3] and BOŻENA OBMIŃSKA-MRUKOWICZ[1]

[1]*Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Wroclaw, Poland;*
[2]*Laboratory of Tumor Molecular Immunobiology, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Wroclaw, Poland;*
[3]*Laboratory of Flow Cytometry, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland*

Abstract. *Background/Aim: Lymphoma, the most common hematopoietic cancer in dogs is sensitive to chemotherapy which is the dominant treatment method. The aim of the present study was to evaluate the concentration-dependent cytotoxicity and ability to induce apoptosis of the anti-neoplastic agents cyclophosphamide (CYC), chlorambucil (CBL), cytosine arabinoside (ARA), dexamethasone (DEX), doxorubicin (DOX), etoposide (ETO), lomustine (LOM), prednisone (PRED) and vincristine (VINK) against GL-1, CL-1, CLBL-1 and Jurkat cell lines. Materials and Methods: To determine cell viability and level of apoptosis, three different tests were performed: Thiazolyl Blue Tetrazolium Bromide (MTT), annexin V/propidium iodide (An/PI) staining and flow cytometric DNA fragmentation. Results: All tested substances exhibited concentration-dependent inhibitory effects on the proliferation of the examined cell lines with a different level of apoptosis induction. VINK and DOX strongly decreased the viability of canine cell lines, whereas CYC induced the highest level of apoptosis. Conclusion: Canine lymphoma (CL-1, CLBL-1) and leukemia (GL-1) cell lines are a useful tool for developing new and more effective treatment regimes for canine neoplasia.*

Lymphoma is the most common hematopoietic cancer in dogs, while leukemia and multiple myeloma are less frequent. Hematopoietic neoplasms are the third most common type of tumor diagnosed in dogs, accounting for approximately 8-9% of all canine malignant tumors. Canine lymphomas show strong similarities to high-grade non-Hodgkin's lymphomas (NHLs) in humans with respect to morphology, tumor genetics, disease progression and response to therapies (1). Dogs represent a suitable large animal model for the study of lymphoma in people; including the investigation into new therapeutic agents, due to the presence of a spontaneous disease. This is in contrast to an experimentally-induced disease in xenograft and genetically modified mouse models (2, 3). Additionally, dogs have shared with people a common environment for thousands of years, thus canine cancers have greater similarities to human diseases than any other animal model (4). The enormous heterogeneity between entities in lymphoma requires an intense need for the development of specific treatment schemes for particular types of the disease and therefore, individual patients (5). Chemotherapy is the routine method for the treatment of leukemia and lymphoma. Various chemotherapy protocols, containing cytostatic drugs with a different antineoplastic mechanism of action and specificity to cell cycle or phase, are used. As a rule for the induction of remission in dogs with lymphoma, the following protocols are applied: COP (CYC, VINK, PRED), CHOP (CYC, DOX,VINK, PRED), COAP (CYC, VINK, ARA, PRED) or CLOP (CYC, ASP: L-asparaginase, VINK, PRED). For maintenance treatment, the biggest role has the LMP (CBL, MTX: methotrexate, PRED) protocol while the most important rescue protocol is D-MAC (DEX, ACT: actinomycin D, ARA, MEL: melphalan).

For investigating the therapeutic methods and cancer biology, many different cancer cell lines are used. Chemosensitivity studies on cell lines do not always reflect the actual performance in a patient, more so, in many cases cell line-derived data has been shown to be of a predictive value for many tumors (6). Until recently, investigations of

Correspondence to: Aleksandra Pawlak, Department of Biochemistry, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, C.K. Norwida 31, 50-375 Wroclaw, Poland. Tel: +48 713205241, Fax: +48 713205455, e-mail: aleksandra.pawlak@up.wroc.pl

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spontaneous canine lymphoma/leukemia have been limited by a lack of validated, well-characterized and widely available cell lines (2). So far, a few canine cell lines have been described in the literature. Some of the best-characterized cell lines are: GL-1, derived from the peripheral blood of a dog with acute B-cell leukemia (7), CL-1, a T-cell lymphoma cell line derived from the pleural fluid from a dog with thymic lymphoma (8), OSW, established from the malignant pleural effusion of a dog with peripheral T-cell lymphoma (2) or CLBL-1, a cell line established from the lymph node of a dog with diffuse large B-cell lymphoma (9). Of the remaining canine lymphoma cell lines the T-cell line DCL-01 established from the lymph node of a dog with cutaneous lymphoma (10) and DLC-02 established from the peripheral lymphocytes of a dog with large granular lymphocyte (LGL) leukemia (11) can be distinguished. More recently four T-cell (Ema, CLK, Nody-1 and UL-1) and one B-cell (CLC) lymphoma cell lines were described (12).

The established canine cell lines should be a useful tool for cancer cell biology and new anticancer drug development. However, there is a lack of data describing the effect of various antiproliferative agents on induction of apoptosis in the established canine lymphoma/leukemia cell lines. One study describes the sensitivity of the derived cell lines on dexamethasone (13) showing that inhibition of the transcription factor NF-κB in CL-1 and GL-1 cells restored glucocorticoid receptor expression and sensitized these cells to DEX.

In the present study, the concentration-dependent cytotoxicity of CYC, CBL, ARA, DEX, DOX, ETO, LOM, PRED and VINK was assessed against selected canine hematopoietic cell lines, GL-1, CL-1 and CLBL-1, representing different types of canine hematopoietic cancers. In addition, effects on canine cancer cell lines have been compared to the effects on the human acute leukemia cell line Jurkat which is well-characterized and frequently investigated. Evaluation of the viability of the cells was performed using the Thiazolyl Blue Tetrazolium Bromide (MTT) test. Induction of apoptosis was determined by An/PI staining and DNA fragmentation using flow cytometry.

Materials and Methods

Cell lines and cell culture. The canine cell lines CL-1 (T-lymphoblastoid cell line) and GL-1 (B-cell leukemia) were obtained from Yasuhito Fujino and Hajime Tsujimoto from the University of Tokyo, Department of Veterinary Internal Medicine, Tokyo, Japan (7, 8) and the CLBL-1 (B-cell lymphoma cell line) from Barbara C. Ruetgen, Institute of Immunology, Department of Pathobiology of the University of Veterinary Medicine, Vienna, Austria (9). The human Jurkat cell line (T-cell leukemia) was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and kindly provided from the collection of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland.

All cell lines were maintained in RPMI 1640 culture medium (Institute of Immunology and Experimental Therapy, Wroclaw, Poland) supplemented with 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin and 10% fetal bovine serum (FBS) (Sigma-Aldrich, Steinheim, Germany) for GL-1 and Jurkat cells and 20% FBS for CL-1 and CLBL-1 cells. The culture was maintained in a CO_2 incubator at 37˚C in a humidified atmosphere. Cells were cultured in 75-cm^2 cell culture flask (Corning, NY, USA) and sub-cultivated every other day to keep at optimal density (50-70% of confluence).

Reagents. DOX, DEX, PRED, VINK, ETO, CBL, LOM, ARA, FBS, L-glutamine, penicillin and streptomycin solution, MTT, sodium dodecyl sulfate (SDS), and *N,N*-dimethylformamide (DMF), were purchased from Sigma-Aldrich (Steinheim, Germany). 4-hydroperoxycyclophosphamide (4-HC) was obtained from NIOMECH - IIT GmbH (Bielefeld, Germany). Dimethyl sulphoxide (DMSO) and ethanol were from POCH (Gliwice, Poland). Phosphate buffered saline (PBS) and RPMI 1640 culture medium were obtained from the Institute of Immunology and Experimental Therapy, Wroclaw, Poland.

Preparation of drugs. The initial solutions of the tested compounds were freshly-prepared for each experiment by dissolving each compound in 1 ml DMSO, ethanol or distillated water. The culture media was used as a solvent for obtaining further solutions. Each compound was tested within a concentration range of 0.05-10 μg/ml for DOX, 0.1-100 μg/ml for CYC, 0.005-24 μg/ml for DEX, 0.01-10 μg/ml for PRED, 0.000001-1 μg/ml for VINK, 0.005-20 μg/ml for ETO, 0.01-20 μg/ml for CBL, 0.05-50 μg/ml for LOM and 0.01-1 μg/ml for ARA.

Determination of cell viability. To determine cell viability, 1×10^4 cells *per* well were seeded in 96-well-plates (NUNC, Roskilde, Denmark). In parallel, the examined substances were prepared at several dilutions in culture medium (DMSO or ethanol concentration was less than 1% in each dilution) and added to the plates. The cells were incubated in medium-alone or medium containing either the vehicle control or increasing concentrations of the tested substances for 72 h. After the respective incubation time, 20 μl of MTT solution (5 mg/ml) was added to every well for a further 4 hours, then 80 μl of lysis buffer (225 ml DMF, 67.5 g SDS, 275 ml distillated water) was added. The optical density of culture wells was measured after 24 h using a spectrophotometric microplate reader (Elx800, BioTek, Winooski, USA) at a reference wavelength of 570 nm. The optical density of formed formazan in control (untreated) cells was taken as 100%. Viability of test samples was determined as: % Viability=(average OD for test group/average O.D. for control group) ×100. The results are calculated as an IC_{50} value (anticancer drug concentration inhibiting cell viability by 50%). The IC_{50} values were obtained from more than 3 independent experiments (four wells each).

Detection of apoptosis. Early apoptotic events were detected using an Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich, Steinheim, Germany) according to the manufacturer's instructions. Briefly, cells were treated with ETO, LOM, PRED and CYC in a final concentration of 10 μg/ml, CBL (5 μg/ml), DEX (24 μg/ml), VINK (1 μg/ml), ARA (1 μg/ml) or DOX (0.5 μg/ml) for 24 h. The concentrations of the test compounds used in this study were chosen based on the results of the MTT viability test carried out for a wide

Table I. Anticancer drug concentration inhibiting cell viability by 50% (IC_{50}) compared to untreated control cells. The results are presented as the mean±standard deviation (SD) of at least three separate experiments, with 4 wells each; n.i. – not investigated.

Chemical name of drug	Cell Line							
	GL-1		CL-1		CLBL-1		Jurkat	
	IC_{50} µg/ml	±SD	IC_{50} µg/ml	±SD	IC_{50} µg/ml	±SD	IC_{50} µg/ml	±SD
Cytosine arabinoside (ARA)	0.53	0.07	>1		0.06	0.01	0.315	0.11
Dexamethasone (DEX)	>24		>24		5.11	1.39	>24	
Chlorambucil (CBL)	9.55	2.27	>20		0.33	0.08	10.65	1.01
4-HO-cyclophosphamide (CYC)	1.85	0.82	3,97	0.10	<0.1		2.53	1.27
Doxorubicin (DOX)	0.08	0.10	0.08	0.8	<0.05		0.96	1.50
Etoposide (ETO)	4,04	1.14	>20		0.02	1.00	3.54	0.98
Prednisolone (PRED)	>10		>10		4.14	2,43	>10	
Vincrystine (VINK)	0.01	0.00	>1		0.0005	0.04	0.08	0.20
Lomustine (LOM)	9.36	5,83	n.i.		0.69	0.03	15.16	0.79

range of concentrations. After harvesting the cells and washing twice with cold PBS the cells were suspended in binding buffer and stained with An/PI for 10 min at room temperature in the dark. After the respective incubation time, flow cytometric analysis was immediately performed using a FACS Calibur (BD, Biosciences, San Jose, USA). The CellQuest 3.lf. software (BD, Biosciences, San Jose, USA) was used for data analysis on the basis of dot plots of FL1-H and FL2-H showing population of dead cells and those undergoing phosphatydylserine externalization.

Flow cytometry analysis of DNA fragmentation. DNA fragmentation (apart from phosphatydylserine externalization) is a common feature of apoptosis. For the detection of DNA fragmentation, flow cytometric assay with ethanol-fixed propidium iodide (PI) stained cells was performed. PI binds according to the stoichiometric ratio with DNA allowing the observation of cells in which DNA fragmentation can be observed as a specific peak designed as sub-G1 (14). Briefly, cells were treated with antineoplastics substances in the same way as for the apoptosis assay. After the respective incubation time, cells were washed twice with PBS and fixed with 70% ice-cold ethanol for 30 min at 4˚C. Then cells were then washed with PBS and stained with PI (50 µg/ml in PBS) overnight at 4˚C. For flow cytometry analysis, FACS Calibur and CellQuest 3.lf. software were used. On the basis of histograms, cells with oligonucleosomal DNA fragmentation were quantified as the percentage of cells with hypodiploidal DNA content (15).

Results

Determination of cell viability. After treatment of canine cell lines with various concentrations of the selected drugs, the metabolically active cells were measured by MTT. The effect of tested substances on cell viability is shown in Table I as an IC_{50} (concentration of tested compound which inhibits 50% of the cell viability). All tested substances exhibited dose-dependent inhibitory effects on the proliferation of the examined cell lines. Table I shows the IC_{50} for all four cell lines. The MTT viability showed the various effects of the tested drugs on canine leukemia/lymphoma cell lines. In general, the CL-1 cell line exhibited the lowest sensitivity to the largest number of the tested anticancer substances. This cell line represents a T-cell phenotype of lymphoma that is clinically characterized by drug resistance greater than that of the B-cell types. However, under the microscope, it was possible to observe morphological changes of the shrunken cells and many cell debris. The CLBL-1 cell line which represents the most common form of canine lymphoma (DLBCL) was the most sensitive cell line to all the tested cytostatic drugs. Moreover, this cell line, in contrast to CL-1 and GL-1 cell lines, was sensitive to glucocorticosteroids represented by DEX and PRED. GL-1 cell line, derived from a dog with B-cell leukemia, and Jurkat (human T-cell acute leukemia) showed intermediate sensitivity to most of the tested substances. Among the alkylating agents (CBL, CYC, LOM), CYC shows the strongest activity while CBL and LOM acted somewhat less. The most sensitive to this group of tested substances was the CLBL-1 cell line (IC_{50} value for CYC amounts <0.1 µg/ml, for CBL 0.33 µg/ml and for LOM 0.69 µg/ml), GL-1 and Jurkat cell lines exhibited average sensitivity while the CL-1 cell line was the least sensitive with IC_{50} values of 3,97 µg/ml for CYC and >20 µg/ml for CBL. A representative of antimetabolites, whose mechanism of action is to interfere with DNA synthesis, through a competitive antagonism with normal nucleotides, was ARA. This drug demonstrated a high efficiency even at low concentrations, in particular with regard to the CLBL-1 cell line (IC_{50} 0.06 µg/ml), but also was effective against Jurkat (IC_{50} 0.31 µg/ml) and GL-1 (0.53 µg/ml) cell lines. CL-1 remained resistant to the action of ARA over the tested concentration range (IC_{50} >1 µg/ml). Vinca alkaloids (VINK) acts on cells by distorting the mitotic spindle, leading to a

failed distribution of chromosomes and cell death. In the present study this substance exhibited a strong cytotoxic activity. The weakest effect was in relation to CL-1 (IC_{50} >1 μg/ml) and Jurkat (IC_{50} 0.08 μg/ml) cell lines while the strongest effect VINK has on CLBL-1 (0.0005 μg/ml). Among the anticancer antibiotics tested substance was DOX (an anthracycline), which works by inhibiting the synthesis of RNA. Our study showed high efficacy of the drug for all tested cell lines: the most sensitive was CLBL-1 (IC_{50} <0.05 μg/ml) while the other lines were characterized by similar sensitivity. The most resistant, however, turned out to be the Jurkat cell line (IC_{50} 0.96 μg/ml). ETO (exerting its action mainly through the influence of the topoisomerase II) affected strongly the CLBL-1 line (IC_{50} 0.02 μg/ml), had a lesser action on GL-1 (4.04 μg/ml) and Jurkat (3.54 μg/ml) and very little on the CL-1 (IC_{50} >20 μg/ml) cell line.

Apoptosis assay. The staining of cells with An/PI shows that the mode of action of the tested chemotherapeutic anti-cancer drugs on the examined canine lymphoma and leukemia cell line is via the induction of apoptosis as shown in Figure 1. The amount of double negative (An–/PI–) cells shows the percentage of live cells in the tested samples. Cells positive for annexin V (An+) and/or piopidium iodide (PI+) are considered as apoptotic or dead cells. After 24 hours of incubation, phosphatydylserine externalization to the outer side of the cell membrane (which is one of the signs of early stages of apoptosis) had occurred in all examined samples. As determined by the MTT test, CLBL-1 is a cell line with the highest sensitivity to all of the tested drugs. The results of An/PI tests on CL-1 cell line showed that all of the analyzed substances induce apoptosis to approximately 50%, while 10% of the cells are stained with PI or An and PI simultaneously. In this study, CL-1 appears to be more sensitive to the effects of the test substances than in MTT test. ETO, CYC, VINK and DOX had the highest capacity to induce apoptosis in all of the tested cell lines. Detailed results are presented on Figure 1. The apoptosis assay confirmed the sensitivity of CLBL-1 cell line to the action of DEX (Figure 2A). In contrast, the GL-1 cell line is resistant to the action of glucocorticoids represented by DEX (Figure 2B).

Flow cytometry analysis of DNA fragmentation. The results of DNA fragmentation coincide with those obtained in the An/PI test and confirmed apoptosis induction in the examined cell lines. Figure 3 shows the percentage of apoptotic CLBL-1, GL-1 and Jurkat cells as it results from their incubation with the tested samples. The present study showed that the cell line the most sensitive for a pro-apoptotic effect of the tested substance was CLBL-1. Performing a test on the CL-1 cell line is a problematic issue because the cells form aggregates, making the analysis difficult. Therefore, in this test the cell line with the lowest sensitivity was GL-1, whereas the Jurkat cell line revealed the average sensitivity to the applied substances. CYC, ETO and VINK had the highest apoptotic potency in this assay. DOX, ARA, CBL and LOM demonstrated a weeker apoptosis induction pattern in the tested cells. The use of PI also allowed observing the absence of cytotoxic effect of the glucocorticoids PRED and DEX on GL-1 and Jurkat cell lines. At the same time, both DEX and PRED strongly induced apoptosis in the CLBL-1 cell line.

Discussion

Hematopoietic neoplasms in dogs represents approximately 10% of all cancers and, among them, lymphoma, is the one most commonly diagnosed. For their treatment, traditional chemotherapy protocols as applied for humans, using classical cytostatics (DOX, VINK, PRED, CYC) administered alone or in combination, are used. Simultaneously, the dog is considered as a good model for the study of spontaneously occurring non-Hodgkin lymphoma (NHL) in humans because canine lymphomas show strong similarities to high-grade NHLs in humans with respect to morphology, tumor genetics, disease progression and response to therapies (1).

Anticancer therapy strategies are initially elaborated on primary and established cell lines. A few well-characterized canine hematopoietic cell lines have been established so far and there is a lack of data describing the effects of different antiproliferative agents on the induction of apoptosis on these cell lines. Only one paper describes the mechanism of the sensitivity of established cell lines to the action of DEX (13).

The presented study describes the sensitivity of three different canine lymphoma and leukemia cell lines under the influence of various commonly used cytotoxic drugs applied in human and veterinary medicine. Most frequently in dogs, lymphomas derived from highly malignant B-cells called DLBCL (diffuse large B-cell lymphoma) occur (16). For this reason, CLBL-1 derived from a dog with DLBCL was chosen for our research. About 26-38% of canine lymphoma is a hyperplasia derived from T cells (17) and therefore the study also included a canine B-cell leukemia (GL-1) and T-cell lymphoma (CL-1) cell lines.

The results of the MTT test, using a panel of anticancer drugs, allowed defining the sensitivity of the tested CLBL-1, GL-1, CL-1 and Jurkat cell lines to the cytostatic substances we chose for our research.

The heterogeneity of canine lymphoma creates problems for prognosis and therapy as we deal with different sub-populations of lymphocytes, malignancy grade or patient status. Therefore, there is a need for novel and detailed diagnostic procedures based on proper treatment regimes. The so far clinical investigations on T-cell canine lymphoma have a low prognostic value and a weak pharmacotherapeutic approach (18).

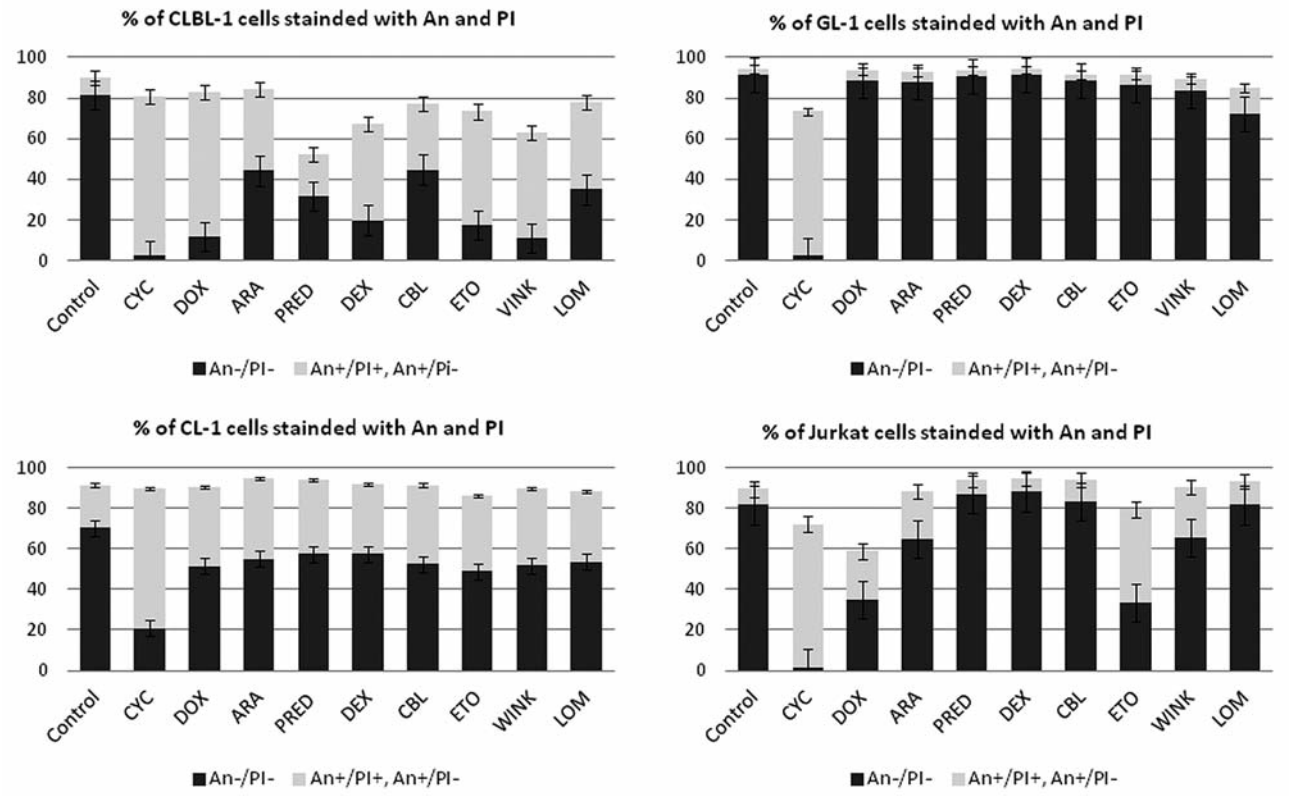

Figure 1. *Impact of 4-HO-cyclophosphamide (CYC, 10 μg/ml), doxorubicin (DOX, 0,5 μg/ml), cytosine arabinoside (ARA, 1 μg/ml), dexamethasone (DEX, 24 μg/ml), prednisolone (PRED,10 μg/ml), chlorambucil (CBL, 5 μg/ml), etoposide (ETO, 10 μg/ml), vincristine (VINK, 1 μg/ml) and lomustine (LOM, 10 μg/ml) on phosphatydylserine exposure and plasma membrane integrity determined by flow cytometry using Annexin V-FITC/PI staining after 24h of incubation. The results are presented as means±SD of three independent experiments. Amount of double negative (An–/PI–) cells demonstrates the percentage of live cells in tested samples. Cells positive for annexin V (An+) and/or propidium iodide (PI+) are treated as apoptotic or dead cells.*

The present study showed that the CL-1 cell line (T-cell lymphoma) is the one most resistant, from the investigated cell lines, to all tested substances, which agrees with clinical data on T-cell lymphomas. Among all anticancer substances used in this study only DOX and CYC at specific concentrations exhibited a satisfactory cytotoxic activity (IC_{50} 0,08 and 3,97 μg/ml, respectively). The observed degree of sensitivity of CL-1 cells, however, differs between the various tests employed in the present work. Specifically, highly resistant cells, as assessed by MTT, seem morphologically dying and/or disintegrating under light microscopy. Also, in tests with An/PI, cells are sensitive to the substances employed. It is well-known that MTT shows only metabolically active cells and the results obtained do not agree with those of other classical apoptosis assays.

The most sensitive cell population was the CLBL-1 cell line which, in contrast to the CL-1 and GL-1, is also sensitive to the action of steroidal anti-inflammatory drugs represented by DEX and PRED.

Steroidal anti-inflammatory drugs are widely used in the treatment of lymphomas and leukemias in dogs, particularly due to their availability, safety and relatively low price. As part of complex anticancer protocols, glucocorticosteroids exhibit the expected therapeutic efficacy. We have also found that, primary cultured cells taken from dogs with lymphoma and leukemia are sensitive to the *in vitro* steroid anti-inflammatory drugs (unpublished own research), which is opposite in relation to the GL-1 and CL-1 cell line. Attention is recently focused on the response of patients who were treated with steroids prior to chemotherapy (19) since the results that will be issued from cell line experimentation will be a valuable tool for therapeutic intervention. For example, the activated B-cell diffuse large B-cell lymphoma (ABC-DLBCL) which is an aggressive, poorly-chemoresponsive lymphoid malignancy is characterized by constitutive canonical NF-κB activity that promotes lymphomagenesis and chemotherapy resistance *via* an overexpression of antiapoptotic NF-κB target genes. Inhibition of the canonical NF-κB pathway may therefore have therapeutic relevance in ABC-DLBCL (20). Matsuda *et al*. (13) have shown that inhibition of the NF-κB transcription factor restores the glucocorticoid receptor expression and sensitivity of the CL-1 and GL-1 cells to the effects of DEX.

Among the treatment regimes used in the therapy of dogs, we need to distinguish between those that have induced remission of cancer, maintaining protocols and life-saving schemes. The most common chemotherapeutic drugs used in the treatment of canine lymphoma are CYC, VINK, DOX and PRED included in treatment programs with acronyms COP and CHOP. Therapy with these drugs may be further supplemented by the use of ASP or ARA in case of bone marrow involvement (21). Drugs included in the listed therapeutic protocols have the strongest influence on the viability of CLBL-1 cells, which is in agreement with clinical observations (19). Cell death is also effectively induced by ETO, LOM or ARA, commonly used in rescue protocols for obtaining further remission. The previously mentioned sensitivity of this cell line to glucocorticosteroids makes it a good model for developing new therapeutic strategies for DLBCL in dogs.

GL-1 also responds well to VINK, DOX, ETO and CYC following, however, a weaker pattern than that of the CLBL-1 cell line. It is known that T-type cells are more resistant to chemotherapy than B-type. In the present study, the sensitivity profile of GL-1 (B-type) is similar to T-type cells (CL-1 and Jurkat). Originally, the GL-1 cell line was described as B-cell leukemia, but further research on the characteristics of GL-1 showed positive receptor rearrangement for TCR characterising them as of T-cell origin (9).

Apoptosis is a programmed, physiological mode of cell death that plays an important role in tissue homeostasis and cancer development through pathological cell accumulations. Phosphatydylserine externalization and DNA fragmentation is a common feature of cell death which can be easily detected using An and PI testing. We herein detected that all employed anticancer substances induce cell death via apoptosis, an effect seen after 24 h of treatment. Staining with fluorescein-conjugated annexin V and PI demonstrates the percentage of apoptotic cells in all of the examined cell lines. Our results on DNA fragmentation confirm the induction of apoptosis in all cell lines. We consider that this method is suitable for all cell lines except CL-1 where clumping of cells hampered the experiments. VINK, DOX, ETO and CYC are the strongest apoptosis inducers in the tested lines.

Conclusion

Canine lymphoma (CL-1, CLBL-1) and leukemia (GL-1) cell lines are a useful tool for the better understanding of most common hematopoietic diseases in dogs and may help in developing new and more effective treatment regimes for canine neoplasia. Establishment and characterization of various cell lines and investigation of their sensitivity to anti-proliferative effects of different factors, will allow for creation of a database similar to the existing human for leukemia and lymphoma classification, since studies on canine cell lines will be the base for safer and specific targeted therapies for the treatment of various types of canine leukemias and lymphomas.

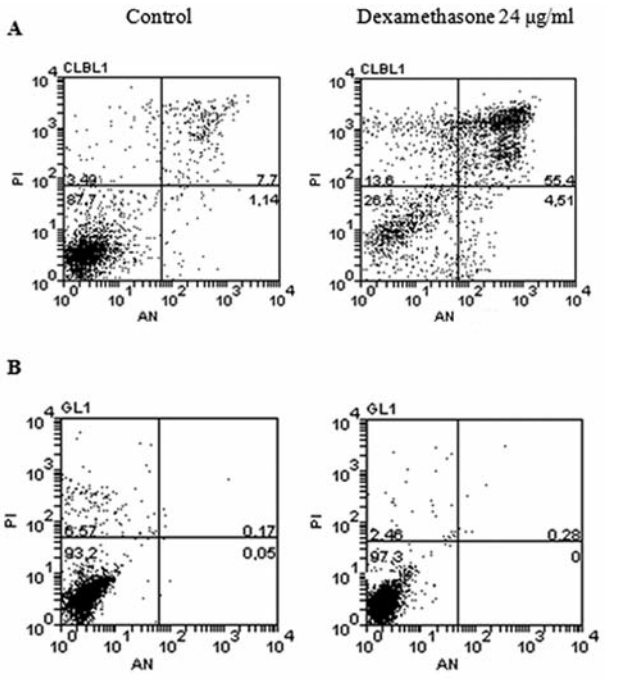

Figure 2. *Representative FACS dot plots show the effect of dexamethasone (DEX) on phosphatydylserine exposure and plasma membrane integrity after 24 h of incubation in CLBL-1(A) and GL-1 (B) cells determined by Annexin V-FITC/PI staining.*

Conflicts of Interest

The Authors disclose any financial and personal relationships with other people or organizations that could influence (bias) their work.

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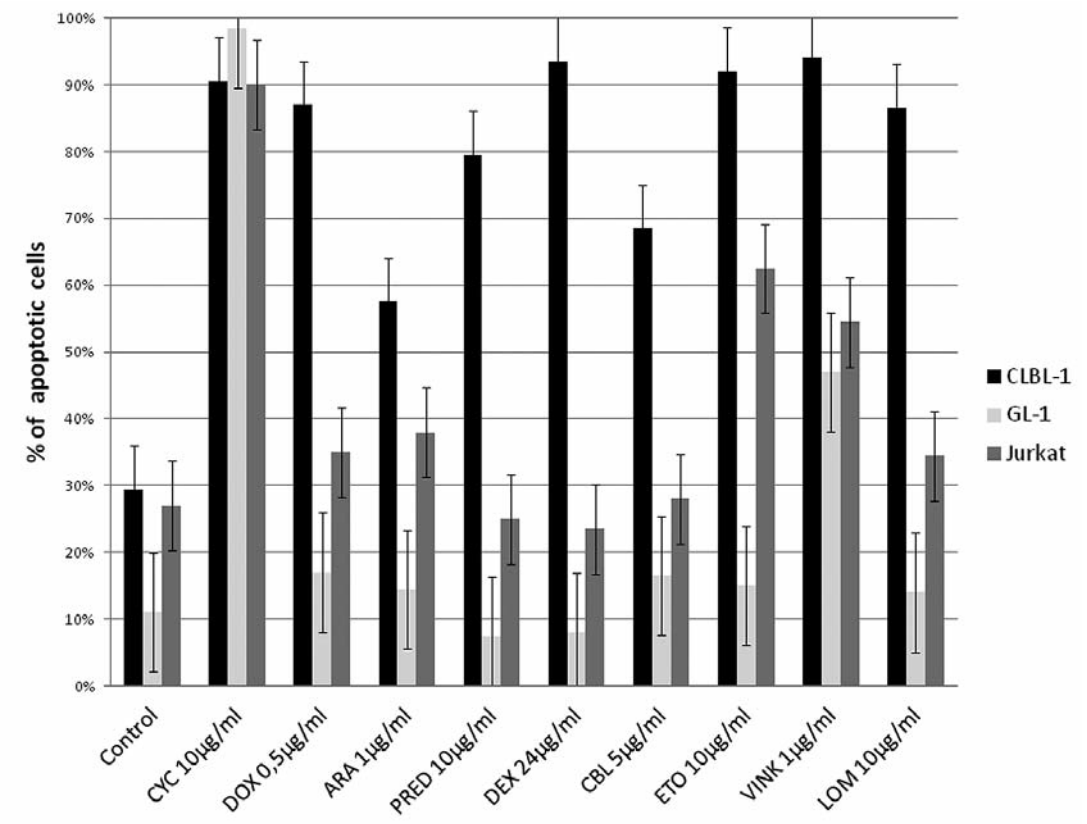

Figure 3. *Influence of 4-HO-cyclophosphamide (CYC), doxorubicin (DOX), cytosine arabinoside (ARA), dexamethasone (DEX), prednisolone (PRED), chlorambucil (CBL), etoposide (ETO), vincristine (VINK) and lomustine (LOM) on increase in population of cells with hypodiploidal DNA content corresponding to sub-G_1 peak on cytometry histogram. The results are presented as means±standard deviation (SD) of three independents experiments and shows the percentage of apoptotic cells.*

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