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Stearidonic acid, a plant-based dietary fatty acid, enhances the chemosensitivity of canine lymphoid tumor cells

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ABSTRACT

Lymphoma is the most common hematopoietic tumor in dogs and humans, with similar pathogenesis and therapeutic responses. Anticancer drugs like vincristine (VCR) and doxorubicin (DOX) are often used in treating lymphoma. However, the cure rate is generally poor due to chemoresistance. Here, we sought to determine whether stearidonic acid (SDA), a plant-based dietary fatty acid, sensitizes chemoresistant canine lymphoid-tumor cells. GL-1 B-cell lymphoid-tumor cells were found to be highly sensitive to the antitumor-activity of VCR and DOX, while OSW T-cell and 17-71 B-cell lymphoid-tumor cells were moderately and fully resistant, respectively. SDA, at its non-toxic concentrations, significantly promoted the antitumor action of VCR and DOX in both OSW and 17-71 cells. SDA-mediated chemosensitization was associated with SDA inhibition of P-glycoprotein (P-gp) function. This was confirmed in HEK293 cells stably expressing P-gp as well as by increased binding-affinity of SDA to P-gp in P-gp docking analysis. SDA at its chemosensitizing concentrations did not affect the viability of healthy dog peripheral blood mononuclear cells, suggesting that SDA is non-toxic to normal dog peripheral blood leucocytes at its chemosensitizing concentrations. Our study identifies a novel dietary fatty acid that may be used as a dietary supplement in combination with chemotherapy to promote the antitumor efficacy of the chemotherapy drugs in dogs and possibly in humans with chemoresistant lymphoma.

1. Introduction

Both human and canine lymphomas have similar clinical presentation, biological behavior, and response to chemotherapy [1–4]. In dogs, lymphoma is one of the most common cancers accounting for up to 25% of all canine cancers [5]. Both B-cell and T-cell lymphomas can affect any dog of any breed at any age [6]. Most untreated dogs diagnosed with malignant lymphoma generally survive less than 6 weeks [7]. Lymphoma is usually treated with aggressive chemotherapy protocols involving a combination of chemotherapeutics, including vincristine (VCR) and doxorubicin (DOX) [8–10]. While these multi-agent chemotherapy regimens improve the survival time, relapses are frequently seen [7]. Most importantly, relapsed lymphoma often displays chemoresistance [11,12], resulting in a poor prognosis.

Chemoresistance in a variety of human cancers is associated with upregulation of expression/function of multidrug transporters, particularly drug efflux pumps such as P-glycoprotein (P-gp) [13]. Likewise, chemoresistance in canine lymphoma was shown to be associated with P-glycoprotein (P-gp) [14]. Hence, there is a need to identify novel therapeutic approaches to combat chemoresistance by inhibiting the expression/activity of P-gp.

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Dietary fatty acids could significantly improve the activity of chemotherapy drugs in cancer cells by downregulating multidrug transporters. For instance, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) greatly enhance the antitumor activity of DOX or paclitaxel in chemoresistant human colon cancer cells by reducing the expression and activity of P-gp and other drug efflux pumps [15,16]. Similarly, DHA improves the antitumor activity of DOX in DOX-resistant human breast cancer cells by suppressing the expression of P-gp [17].

Recently, a plant-based stearidonic acid (SDA), which serves as an alternative source to fish-based fatty acids, has been shown to exert antitumorigenic effects in human cancers [18,19]. However, the chemosensitizing nature of SDA is unknown. In the current study, we investigated whether SDA can potentiate the antitumor activity of VCR and/or DOX in the chemoresistant canine lymphoid tumor cells, and whether SDA can inhibit P-gp activity at its chemosensitizing concentrations.

2. Materials and methods

2.1. Cell culture

Canine OSW T-cell and GL-1 and 17-71 B-cell lymphoid tumor cell lines were grown in RPMI-1640 medium (Lonza) supplemented with 10% FBS (HyClone). HEK293/pcDNA3 and HEK293/ABCB1 (HEK293 cells stably expressing P-gp) [20] were grown in DMEM (Lonza). The cells were cultured in an incubator with a humidified atmosphere maintained at 5% CO_2 and 95% air at 37°C. The lymphoma cell lines are well characterized to reflect *in vivo* properties and are well-established models for lymphoma studies [2,21,22].

2.2. Isolation of dog peripheral blood mononuclear cells

Peripheral blood mononuclear cells were isolated from mixed breed dogs, maintained in breeding colonies at Auburn University. All procedures performed with the dogs were approved by the Auburn University IACUC. Briefly, up to 50 ml of blood was collected by venipuncture, typically of the jugular vein, and was placed into tubes containing EDTA to prevent clotting. The blood was layered onto an equal volume of Ficoll (1.077) and centrifuged at 400 × G for 30 min. The opaque interface was collected and washed with isotonic phosphate buffered saline and then centrifuged at 250 × G for 10 min. The supernatant was carefully aspirated and the pellet was washed a second time using the same method. The pellet was then resuspended in RPMI media and cultured as described above.

2.3. Materials

Stearidonic acid (SDA) (Cayman Chemical) was reconstituted in ethanol (ETOH). Puromycin (PUR) (Cellgro) was reconstituted in PBS. Doxorubicin (DOX), Vincristine (VCR), Valspodar (PSC-833), and Rhodamine 123 (R123) were purchased from Sigma and reconstituted in dimethyl sulfoxide (DMSO).

2.4. Cell viability assays

The lymphoid tumor cells or healthy dog peripheral blood mononuclear cells were plated into 96-well culture plates (PerkinElmer) at a density of 10,000 cells per well. The cells were then either untreated or treated with PBS, DMSO, ETOH, VCR, DOXO, SDA ± VCR, or SDA ± DOX for 24 h. Next, the CellTiter-Glo luminescent cell viability assays (Promega) were performed to determine the number of viable cells by quantifying the ATP present, which indicates the presence of metabolically active cells [23,24]. Luminescence was measured with a FLUOstar Optima microplate reader (BMG Labtech).

2.5. Intracellular rhodamine 123 accumulation assays

The efflux activity of P-glycoprotein (P-gp) was determined by measuring the intracellular accumulation of the fluorescent P-gp probe rhodamine 123 (R123) [23]. Briefly, the cells were washed with HBBS (without Ca^{2+}, Mg^{2+} and phenol red) and incubated at 37 °C for 15 min with or without DMSO, ETOH, SDA or PSC-833 (P-gp specific inhibitor) in HBBS. R123 (5 μM) was added after 15 min to the cells in the presence or absence of DMSO, ETOH, SDA or PSC-833 and incubated for another 45 min. The cells were washed with ice-cold-HBBS and solubilized in Triton-HBBS. To determine the intracellular concentration of R123, the fluorescence was measured using Infinite microplate reader (TECAN) at an excitation wavelength of 485 nm and an emission wavelength of 538 nm.

2.6. Molecular modeling studies

The structure of SDA was built using the builder module of Maestro v 9.3.5 and subsequently energy minimized by Macromodel program v9.9 (Schrödinger, Inc., New York, NY, 2012) using the OPLSAA force field with the steepest descent followed by truncated Newton conjugate gradient protocol. The X-ray crystal structure of P-gp in apoprotein state (PDB ID: 3G5U) and in complex with inhibitors QZ59-RRR (PDB ID: 3G60) or QZ59-SSS (PDB ID: 3G61; obtained from the RCSB Protein Data Bank) were used to build the homology model of human P-gp as previously described [20,25,26]. The diverse conformational library of SDA was docked at each of the generated grids (sites-1–4 of P-gp) using the "Extra Precision" (XP) mode of Glide program v5.8 (Schrödinger, Inc., New York, NY, 2012) with the default functions. The top scoring conformations of SDA with P-gp were used for graphical analysis.

2.7. Data presentation and statistical analysis

Cell viability data are expressed as percentage of untreated cells (control), where control was set as 100% viability. The fluorescence intensity of the samples without R123 was considered as the background. Fluorescence intensity of all the samples with R123 was subtracted with the background fluorescence before data normalization. The efflux activity of P-gp is presented as relative R123 accumulation by normalizing the fluorescence intensity of the samples with ETOH, SDA or PSC-833 to the samples without ETOH, SDA or PSC-833. The unpaired Student's t-test was used to determine statistical significance ($^*p < 0.05$).

3. Results and discussion

3.1. Canine lymphoid tumor cells are resistant to chemotherapy drugs

Several multi-agent chemotherapy protocols, consisting of VCR and DOX, are clinically employed to treat dogs with lymphoma [8–10]. However, relapses associated with chemoresistance are often seen in the lymphoma patients [14]. We examined whether canine lymphoid tumor cells are resistant to VCR and/or DOX using cell viability assays. VCR and DOX significantly decreased the viability of GL-1 B-cell lymphoid tumor cells in a concentration-dependent manner (Fig. 1A and B). On the other hand, both VCR and DOX showed little and moderate effect on the viability of and 17-71 B-cell and OSW T-cell lymphoid tumor cells, respectively (Fig. 1A and B). Together, these results suggest that GL-1 cells are