COMPARISON OF RESISTANCE TO TREATMENT PROFILE OF DIFFUSE LARGE B CELL LYMPHOMA CELLS DERIVED FROM DIFFERENT SUBTYPES

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Introduction and objective

Among non-Hodgkin's lymphoma (NHL), diffuse large B-cell lymphomas (LDGCB) constitute the most heterogeneous subgroup in terms of morphology, clinical presentation and response to treatment. Currently, almost half of the patients still die due to relapse and/or refractory related to treatment resistance. Therefore, this study aimed on identifying mechanisms of resistance to chemotherapy.

Material and Method

The human cell lines derived from DLBCL SUDHL4 and Toledo were compared in the study. Both cell lines were treated with different concentrations of doxorubicin, vincristine, cisplatin or etoposide (first and second line chemotherapeutic agents used in the clinical treatment) for different periods of time. To evaluate cytotoxicity, the MTT assay was employed. The evaluation of cell cycle profile (propidium iodide or PI incorporation) and cell death (Annexin V/PI assay) upon treatment with the chemotherapeutic agents was performed by flow cytometry. The detection of P-glycoprotein (Pgp) and MRP1 expression was performed by flow cytometry. Cells were incubated with the antibody clone UIC2-PE for detection of Pgp and QCRL-3-FITC for MRP1. The cell line Lucena was used as a positive control and K562 as a negative control for Pgp expression. The analysis of Pgp efflux activity was performed by flow cytometry. The cells were incubated or not with Rhodamine-123 (Rho), a fluorescent substrate of Pgp, and verapamil (VRP), a non-fluorescent substrate, used as a modulator to evaluate the activity of this efflux pump. The cell line Lucena was used as a positive control. The determination of the expression and activity of ABC proteins was achieved using the ratio between the average fluorescence intensity (RMIF) of cells incubated with the antibody and the control cells (no antibody). The expression of XIAP, Survivin, Bcl-2 and HSC70 proteins was analyzed by Western blot. The HSC70 protein was used as a protein loading control.

Results and conclusion

To verify the effect of first and second line chemotherapeutic agents on LDGCB cells, cell viability assays were performed on cells from two cell lines derived from LDGCB (SUDHL4 and Toledo). SUDHL4 cells are sensitive to first-line (vincristine and doxorubicin) chemotherapeutic agents and are responsive at the calculated patients' plasma concentrations. Treatment with vincristine induced accumulation of cells in the G2/M phase in both line cells. In the cell death assay, treatment with chemotherapeutic agents for 24 hours only cisplatin treatment induced an increase in the percentage of cells that exposed phosphatidylserine and yield DNA fragmentation. These data suggest that SUDHL4 cells are more sensitive to the evaluated first-line chemotherapeutic agents than Toledo cells, which is in agreement with the clinical sensitivity of each cell line subtype. To characterize the possible mechanisms of treatment resistance in DLBCL cells, the expression profile and the activity of efflux pumps were analyzed. SUDHL4 cells showed Pgp expression, with no efflux activity. The lack of efflux activity of these cells corroborates with the cells' sensitivity to the drugs that are substrates for Pgp. In the analysis of the expression profile of resistance related proteins, preliminary data suggests a possible relationship between the expression of Survivin, XIAP, and Bcl-2 to treatment response. In both lines cells there was a decrease in survivin content and no change in the XIAP content.

Chemotherapeutic	IC50 SUDHL4	IC50 Toledo	Plasma Concentration
Doxorrubicin	1.27 µM	9 µM	360 nM
Vincristin	3.27 nM	2.7 µM	3.7 nM
Cisplatin	16 µM	11.2 µM	130 µM
Etoposide	6.7 µM	10.8 µM	20 µM

Table 1: IC50 of each chemotherapeutic for each cell line calculated by four-parameter logistic regression and calculated patients' plasma concentration.

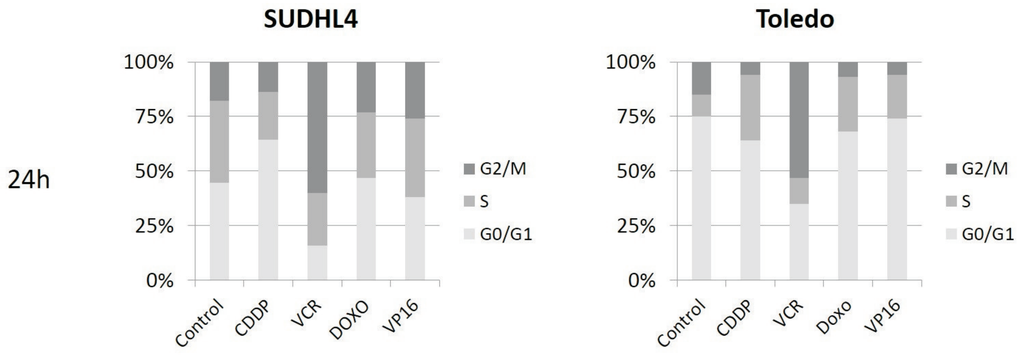

Figure 1: Distribution of cells at each phase of the cell cycle upon treatment with chemotherapeutics after 24 hours.

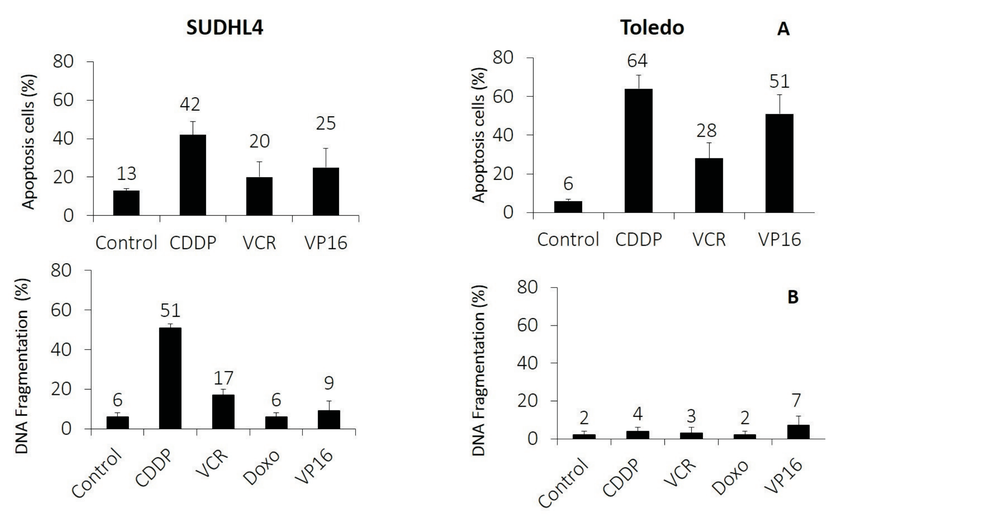

Figure 2: Comparison of the index of treatment-induced cell death with chemotherapeutics in LDGCB-derived cells. (A) Percentage of cells that exposed phosphatidylserine upon treatment with chemotherapeutics for 24 hours. (B) Percentage of cells with fragmented DNA (subG0) upontreatment with chemotherapeutic agents for 24 hours.

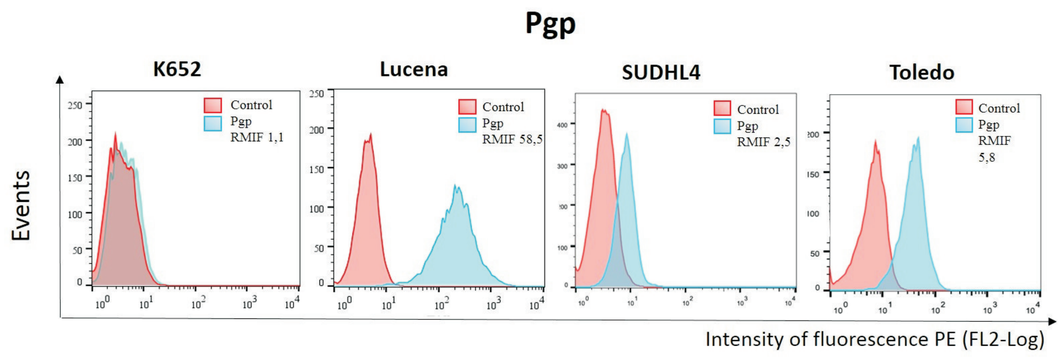

Figure 3: Expression of efflux pumps in LDGCB cells. Representative histogram of one experiment from three independent experiments.

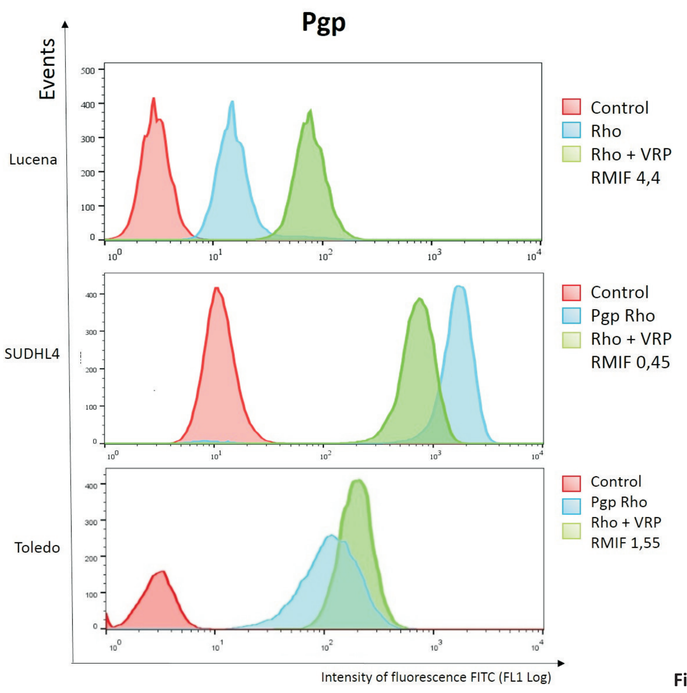

Figure 4: Activity of efflux pumps in LDGCB cells. Representative histogram of one experiment from three independent experiments.
RMIF: ratio of mean fluorescence intensity. VRP: verapamil; Rho-123: rhodamine-123.

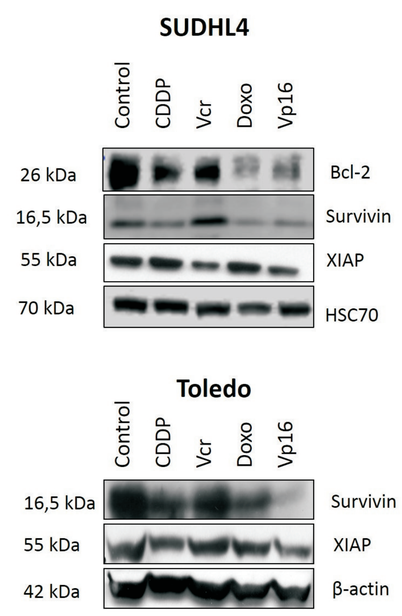

Figure 5: : Protein content of IAPs and Bcl-2 in Toledo cells (left) and SUDHL4 (right) upon treatment with chemotherapeutics after 24h. Representative image of one experiment, from three (Toledo) or two independent experiments (SUDHL4). Concentration of doxorubicin treatment of Toledo cells was 8 µM, vincristine 2 µM, cisplatin 11.2 µM and etoposide 10.8 µM. Concentration of doxorubicin treatment of SUDHL4 cells was 1.3 µM, vincristine 3.27 nM, cisplatin 16 µM and 10 µM etoposide.

Conclusion and perspectives

These data suggest that the LDGCB cells have distinct profiles of response to chemotherapy, and mechanisms of resistance, and that XIAP is a promising therapeutic target for LDGCB. To confirm these data, assays will be carried out to test the effect of chemotherapeutics on death and cell cycle of LDGCB derived cell lines for shorter treatment times. The inhibition of XIAP expression to determine if XIAP drives resistance and the activation of proteins associated to the apoptosis pathways will also be investigated.

Keywords: diffuse large B-cell lymphoma; resistance to treatment; cytotoxicity; cell death; P-glycoprotein; IAPs; Bcl-2.

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