Characterization of the resistance profile and effect of 11a-N-Tosil-5-deoxiazapterocarpan (LQB-223) in diffuse large B-cell lymphoma cells

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INTRODUCTION AND OBJECTIVE

Diffuse large B-cell lymphoma (DLBCL) represents the most frequent type of non-Hodgkin's lymphoma (NHL) with aggressive clinical behavior (40% of cases) and that have the lowest response rate to conventional treatment (HENNESSY e col., 2004; NG, 2007). Due to the heterogeneity of responses to treatment and low cure rate observed in patients (BAI e col., 2005), it is important to identify new therapeutic possibilities. Data from our group and our partners suggest that the 11a-N-Tosyl-5-deoxiazapterocarpan compound (LQB-223) is a promising agent in the treatment of cancer (BUARQUE e col., 2014; LEMOS e col., 2016). Other results also indicate that the compound has low toxicity to healthy cells (BUARQUE e col., 2011; 2014). Furthermore, LQB-223 has no structural relation with the drugs used in the first or second lines of treatment of DLBCL, suggesting that its mechanisms of action are unrelated to these agents. Consequently, it has the potential to overcome the resistance to conventional treatment. Therefore, the objective of this study is to evaluate the potential effect of LQB-223 in two cell lines derived from DLBCL.

MATERIAL AND METHODS

The human cell lines derived from DLBCL SUDHL4 and Toledo were used in the study. The cell line SUDHL4 is characterized as GCB subtype and has a sensitivity profile of treatment response. The cell line Toledo is characterized as ABC subtype and has a profile of resistance to treatment. Both cell lines were treated with different concentrations of LQB-223 for different periods of time (24h, 48h, and 72h). Both cell lines were also treated with different concentrations of doxorubicin, vincristine, cisplatin or etoposide, chemotherapeutic agents used in the clinical treatment, for different periods of time (24h and 48h). To evaluate cytotoxicity, the MTT assay was employed. The evaluation of cell cycle profile and cell death upon treatment with the LQB-223 compound and the classical chemotherapeutic agents were performed by flow cytometry. Detection of 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 analysis of Pgp efflux activity was performed by flow cytometry. Cells were incubated or not with 123-Rhodamine (Rho), a fluorescent substrate of Pgp, and cyclosporin A (CsA) and verapamil (VRP), non-fluorescent substrates, used as modulators to evaluate the activity of this efflux pump. The evaluation of the expression and activity of ABC proteins were made using the ratio between the average fluorescence intensity (RIF) of cells incubated with the antibody and the control cells (no antibody) (VASCONCELOS e col., 2013). The expression of Pgp and HSC70 proteins was analyzed by Western blot. The HSC70 protein was used as a protein loading control. The cell line KB-3-1 was used as a negative control and MCF7-DoxR as a positive control for Pgp expression.

RESULTS

SUDHL4 cells were sensitive to some of the evaluated concentrations of conventional chemotherapy in 24h (Figure 1). IC50 concentrations at 24h were 1.4 uM for doxorubicin, 0.25 uM for vincristine, 16 uM for cisplatin, 6.7 uM for etoposide. Compared to the IC50 of Toledo cells previously determined by our group, SUDHL4 cells showed greater sensitivity to conventional first-line chemotherapeutic agents doxorubicin and vincristine (Table 1).

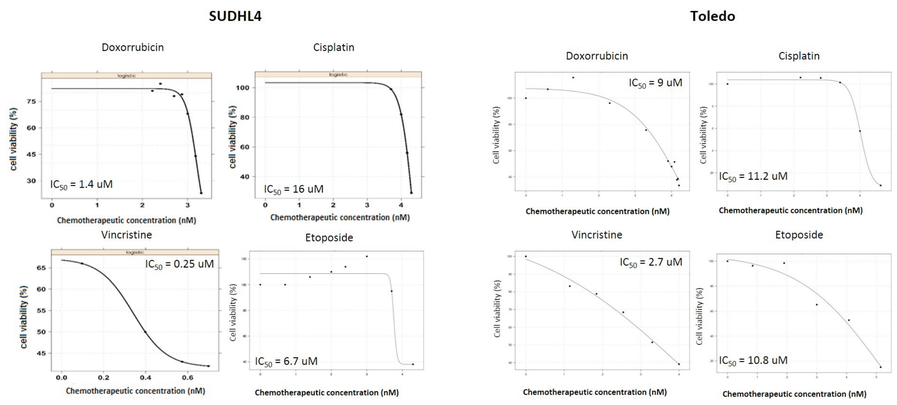

Figure 1: Sensitivity profile of cells derived from diffuse large B-cell lymphoma SUDHL4 and Toledo to chemotherapeutic agents used in the first (doxorubicin and vincristine) and second (carboplatin and etoposide) lines of clinical treatment (24h treatment).

chemotherapeutic agents	IC50 concentration in 24h	
	SUDHL4	Toledo
Doxorrubicin	1.4 uM	9 uM
Cisplatin	16 uM	11.2 uM
Vincristine	0.25 uM	2.7 uM
Etoposide	6.7 uM	10.8 uM

Table 1: IC50 concentrations in 24h of chemotherapeutic agents for the cells derived from diffuse large B-cell lymphoma SUDHL4 and Toledo. IC50 concentrations were calculated by four-parameters non-logistic regression in the R software.

The evaluation of chemotherapy-induced changes in the cell cycle profile of SUDHL4 cells suggested that 24h doxorubicin treatment induces an accumulation of cells at the G0/G1 phase. Treatment with vincristine induced an accumulation of cells at the G2/M phase. Treatment with cisplatin induced an accumulation of cells in G0/G1 phase. Treatment with etoposide induced an accumulation of cells in S phase (Figure 2). Our collaborators have further shown that LQB-223 induces accumulation of chronic myelogenous leukemia cells at the G2/M phase of the cell cycle. To check whether this mechanism of action also occurs in DLBCL cells is a perspective of the present work.

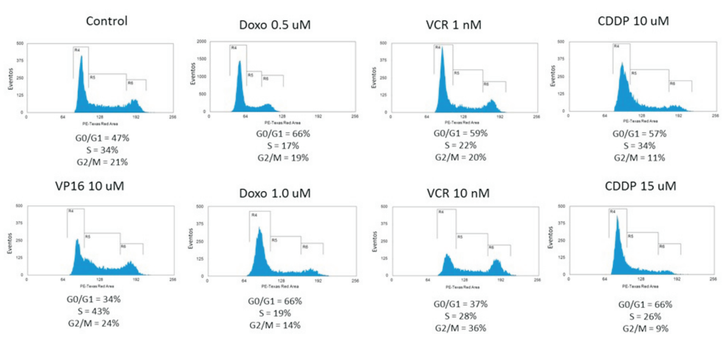

Figure 2: Cell cycle profile assessment by flow cytometry in SUDHL4 cells upon treatment with doxorubicin (Doxo), vincristine (VCR), cisplatin (CDDP) and etoposide (VP16) for 24h.

The evaluation of chemotherapy-induced cell death of SUDHL4 cells demonstrated that 24h treatment with doxorubicin and etoposide, mildly increased cell fragmentation. Treatment with vincristine and cisplatin induced a greater increase in the percentage of cells with fragmented DNA (Figure 3). Only treatment with etoposide induced an increase in the percentage of apoptotic cells (Figure 4). These data need to be confirmed in further experiments with longer times of treatment.

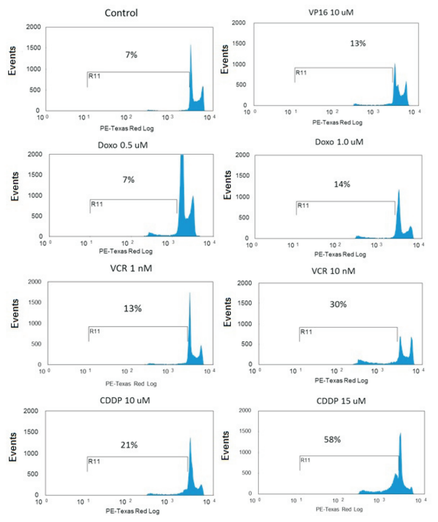

Figure 3: DNA fragmentation upon treatment with vincristine (VCR), cisplatin (CDDP), etoposide (VP16) and doxorrubicin (Doxo) for 24h.

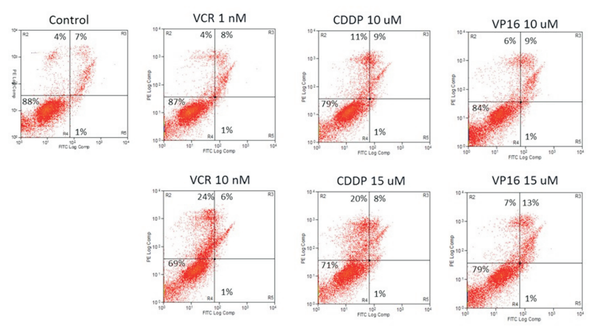

Figure 4: Cell death upon treatment with vincristine (VCR), cisplatin (CDDP) and etoposide (VP16) for 24h (Annexin V / PI cell death assay). Representative dot plots of one experiment.

Next, evaluating the resistance mechanisms in Toledo cells through flow cytometry, we found overexpression (Figure 5a) and overactivity (Figure 6) of efflux protein ABCB1 (P-glycoprotein or Pgp) compared to SUDHL4 cells. However, in the assessment of expression of Pgp by Western blot, it was not possible to detect protein expression in either cell lines derived from DLBCL SUDHL4 and Toledo (Figure 5b). To prove this hypothesis, we will carry out new tests to assess expression and activity of these proteins.

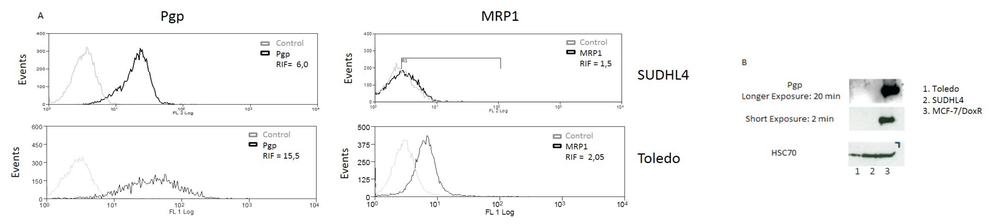

Figure 5: Profile of expression of P-glycoprotein (Pgp) and Multidrug Resistance-Associated Protein 1 (MRP1) of cells lines derived from diffuse large B-cell lymphoma SUDHL4 and Toledo. A) Flow cytometry B) Western blot. RIF: ratio of fluorescence intensity average.

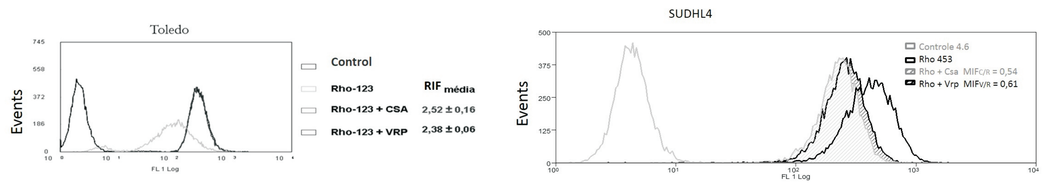

Figure 6: Eflux activity of P-glycoprotein (Pgp) of cells derived from diffuse large B-cell lymphoma SUDHL4 and Toledo. RIF: ratio of fluorescence intensity average. CSA: cyclosporin A; VRP: verapamil; Rho-123: rhodamine-123.

The LQB-223 compound had time and dose-dependent effects on both cell lines. For SUDHL4 cells it was not possible to achieve the IC50 after 24h of treatment. At 48h, the IC50 was 7.1 uM and at 72h, the IC50 was 6.9 uM. For Toledo cells at 24h it was also not possible to achieve the IC50. At 48h, the IC50 was 5.1 uM and at 72h, the IC50 was 4.3 uM (Figure 7; Table 2).

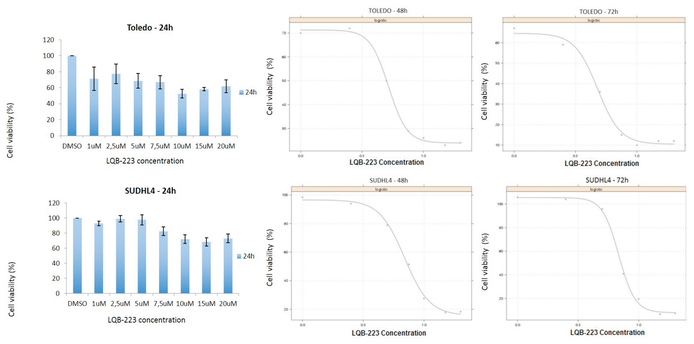

Figure 7: Sensitivity profile of cell lines derived from diffuse large B-cell lymphoma SUDHL4 and Toledo to LQB-223 (24h, 48h and 72h treatment). Bars display the mean of 3 independente experiments. Error bars represent the standard error.

Time-point	IC50	
	SUDHL4	Toledo
24h	>20 uM	>20 uM
48h	7.1 uM	5.1 uM
72h	6.9 uM	4.3 uM

Table 2: IC50 concentrations of the LQB-223 compound in cells lines derived from diffuse large B-cell lymphoma SUDHL4 and Toledo for each time-point studied.

CONCLUSIONS

In conclusion, although preliminary, these results suggest that LQB-223 may have a promising effect on sensitive or resistant to conventional treatment DLBCL cells, which display expression of Pgp and MRP1 and Pgp activity, and stress the need for further study of its effects on these cells.

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Keywords: New anticancer agents; LQB-223; Diffuse large B cell Lymphoma; P-glycoprotein.

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