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The Therapeutical Potential of a Novel Pterocarpanquinone LQB-118 to Target Inhibitor of Apoptosis Proteins in Acute Myeloid Leukemia Cells

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Abstract: Acute myeloid leukemia (AML) is a challenging neoplasm that despite therapeutic advances requires efforts to overcome the multidrug resistance (MDR) phenotype, the major cause of relapse. The pterocarpanquinone LQB-118 is a new compound that induces apoptosis in leukemia cells. The objective of this work was to analyze the role of LQB-118 in inhibiting the inhibitor of apoptosis proteins (IAPs), XIAP and survivin, as well as in modulating the subcellular localization of NFκB, in comparison with idarubicin. LQB-118 was more effective in inducing apoptosis than idarubicin in both AML Kasumi-1 cell line and cells from patients despite their MDR phenotype. LQB-118-induced apoptosis was accompanied by a marked inhibition of IAPs, and cytoplasmatic NFκB subcellular localization. On the other hand, idarubicin increased the IAPs expression and translocated NFκB to the nucleus. The inhibition profile of survivin induced by LQB-118 was comparable to the survivin inhibition profile when we investigated the efficiency of survivin-small interfering RNA (siRNA) treatment. LQB-118 as well as survivin-siRNA contributed similarly to the increase in apoptosis rate of Kasumi-1 cells. The data indicated that there is a functional interaction between the survivin, XIAP and NFκB, which appears to be involved in idarubicin resistance of Kasumi-1 cells. The efficacy of LQB-118 to induce cell death through inhibiting survivin suggests that this IAP may be involved in the chemoresistance phenotype in AML cells. Our findings suggest that LQB-118 might be a promising therapeutic approach for AML patients through survivin downregulation.

Keywords: Acute myeloid leukemia, Chemoresistance, Idarubicin, Inhibitor of apoptosis proteins, NFκB, Pterocarpanquinone LQB-118, siRNA. Survivin, XIAP.

1. INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease that results in an accumulation of blast cells with variable degrees of granulocytic and monocytic differentiation. Although the majority of patients achieve an initial complete remission with standard remission induction therapy, most ultimately die due to refractory AML (mainly those aged 60 years and above) [1]. The idarubicin or daunorubicin anthracyclines associated with cytosine arabinoside (ara-c) have remained the gold standard treatment against AML [2]. However, AML response to therapy depends on many different characteristics such as patient age, number of peripheral white blood cells (WBC), performance status of the patient, *de novo* or secondary AML, cytogenetics and molecular abnormalities [3]. Indeed, mechanisms determining anthracycline chemoresistance in AML may be caused by the multidrug resistance (MDR) phenotype [1, 4]. This phenomenon comprises a group of different mechanisms such as the efflux pump *ABCB1/MDR1/*P-glycoprotein (Pgp) commonly found in AML patients [5], and related to response to induction therapy and overall survival [6]. In this situation, anthracyclines being a good substrate for Pgp, the AML cells' ability to accumulate and retain drugs is impaired due to the outward transport across the plasma membrane [7]. Although less understood, resistance to ara-c may also contribute to the unfavorable results in AML patients [8]. Recently, it was demonstrated that ara-c was able to increase *ABCB1* mRNA and Pgp expression in HL-60 drug-resistant and drug-sensitive AML cell line [9]. Therefore, the combination of ara-c with one anthracycline, commonly used in AML treatment protocols, has the potential to not only prevent the drugs from accumulating inside cells, but also to induce or increase the MDR phenotype. In addition, the inhibitor apoptosis proteins (IAPs), survivin and XIAP, have been correlated to a poor prognosis in adult AML patients [10, 11]. Such wide variability of drug resistance mechanisms, each one acting alone or in combination, may provide a big challenge for therapeutic approaches in AML patients. At present, intense scientific research is being carried out for the analysis of variations of genomic aberrations towards finding the gene expression profile to individualize therapy in AML patients [12]. Simultaneously, some randomized clinical studies are going on in an attempt to find an alternative strategy using known and emerging drugs such as hypomethylating and alkylating agents. However, no substantial advances related to complete remission or overall survival in AML patients has been observed in recent years [13]. Therefore, the development of novel compounds exhibiting potent pro-apoptotic role to target the diversity of drug resistance mechanisms is highly desirable.

Pterocarpanquinone LQB-118, a molecular hybrid between pterocarpans and quinones, structurally related to lapachol [2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone] [14], has emerged from among the many molecules being studied by our group. Recently, we have shown that LQB-118 was very effective in inducing apoptosis in cells from chronic myeloid leukemia (CML) patients exhibiting MDR phenotype [15]. In addition, LQB-118 was capable of inducing apoptosis by reducing the IAPs XIAP and survivin in the CML K562 cell line overexpressing Pgp. Beyond that, LQB-118 also reduced the Pgp expression levels. Based on these premises, the aim of the present study was to analyze the effect of this new synthetic compound LQB-118 in AML cells to observe if it would be equally effective in inhibiting IAPs as compared to idarubicin. For this purpose, we also analyzed

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NFκB subcellular localization since NFκB plays an important role in the regulation of a variety of biological processes, including cellular proliferation and apoptosis [16]. The nuclear localization of NFκB can promote activation of target genes such as *ABCB1, XIAP,* and *survivin* [17, 18]. Previous studies have characterized the activation level of NFκB in AML patients [17] and have demonstrated the loss of nuclear translocation of NFκB followed by the inhibition of proliferation after treatment with FLT3 inhibitor and methyltransferase and histone deacetylase inhibitors [19, 20]. However, the molecular mechanism of NFκB activation in AML remains unclear.

Here, we showed that LQB-118 was highly effective in inducing apoptosis in AML Kasumi-1 cell line as compared with idarubicin. The inhibition of survivin by LQB-118 was comparable to the inhibition observed after survivin-siRNA treatment. The difference in apoptosis rate observed between LQB-118 and idarubicin treatment was probably related to survivin and XIAP inhibition through regulation of NFκB. Moreover, LQB-118 triggered apoptosis in AML cells from patients exhibiting multifactorial drug resistance mechanism as well as some features related to unfavorable prognostic factors in AML.

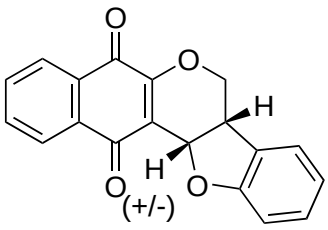
2. MATERIALS AND METHODS, AND PATIENTS

2.1. Drugs and Reagents

Pterocarpanquinone LQB-118 was synthesized in the Laboratory of Bio-organic Chemistry, NPPN, Federal University of Rio de Janeiro, Brazil by the oxyarylation reaction of a chromen derived from commercially available lawsone with *ortho*-iodophenol, in the presence of catalytic amounts of palladium acetate [14], and was kindly provided by Prof. Chaquip Daher Netto. Pterocarpanquinone LQB-118 was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St Louis, MO, USA). When this solvent produced a proapoptotic stimulus that might potentially influence protein expression levels, we analyzed all experiments with Kasumi-1 using these cells incubated with DMSO as a control. The chemical structure of LQB-118 is shown in Fig. (**1**). Idarubicin was provided by Pharmacia-Upjohn (Milan, Italy). The drug was diluted in distilled water and by serial dilutions in DMEM/F12 medium (GIBCO® Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12) (Invitrogen, Carlsbad, California, USA) prior to use. Tris (tris hidroximethyl aminomethane) was purchased from Merck (Darmstadt, Germany) and MTT (3-(4,5-dimethylthiazlol-2-yl)-2,5-diphenyltetrazolium bromide) from GE Healthcare, USA. Ficoll-Hypaque and RNAse (ribonuclease A) were purchased from Sigma-Aldrich, USA (St Louis, MO, USA).

2.2. Cell Line

Kasumi-1 cell line, derived from an AML with t(8;21) was obtained from American Tissue Culture Collection (ATCC, Rockville, MD) and kindly provided by Prof. Eduardo Rego from University of Ribeirão Preto, São Paulo, Brazil. This cell line was cultured in DMEM/F12 containing 20% fetal bovine serum (FBS) (Biomast, Brazil) and incubated at 37°C in a humidified atmosphere with 5% CO_2.



LQB 118

Fig. (1). LQB-118 chemical structure.

2.3. Patients and Primary AML Blasts

Bone marrow or peripheral blood samples were obtained from 17 consecutive AML patients newly diagnosed, at relapse or with secondary AML. The local Institutional Ethic Committee approved this study, which was conducted in accordance with the recommendations of the Helsinki Declaration. The diagnosis of AML was based on standard clinical data, cytogenetics and was according to the standard French-American-British (FAB) morphological criteria recommended by the World Health Organization [21]. Demographic, clinical and biological characteristics of patients are shown in Table **1**. Cells from patients were isolated within 4h after collection by Ficoll-Hipaque density gradient (Sigma, St Louis, MA, USA), washed three times with saline solution, re-suspended in RPMI-1640 (Sigma, St Louis, MA, USA) medium supplemented with 10% FBS (Biomast, Brazil), and adjusted to a concentration of 1 x 10^6/ml. Blast cells represented more than 70% of the WBC.

2.4. Measurement of Cell Viability in Kasumi-1 Cell Line

Growth inhibition of Kasumi cell line was determined by the colorimetric MTT cell viability/proliferation assay [22]. Briefly, cells were incubated for 24, 48 and 72h at 37°C in the presence or absence (control) of LQB-118 or idarubicin. MTT (20µl/well) was added to each well and plates were incubated for another 4h. The colored formazan product was then dissolved using 150µl of DMSO (Sigma-Aldrich, St Louis, MO, USA). Plates were read using the microtiter plate reader (*SpectraMAX® 190 Microplate Reader* – Molecular Devices) at 570 nm. The percentage of growth inhibition cells treated with different concentrations of LQB-118 or idarubicin was normalized to untreated controls. All of the assays were performed in triplicate.

2.5. Measurement of Apoptosis by Annexin V Assay in Kasumi-1 Cell Line and Cells from AML Patients

AML cells ($0.5x10^6$/well) from patients or from Kasumi cell line were cultured at 37°C in 96 well-culture plates in DMEN/F12 medium supplemented with 20% FBS in the presence of LQB-118 for 24h and 48h. The percentage of apoptotic cells was assessed using the Annexin V assay (Genzime Diagnostics, USA). Cells were stained with fluorescein isothiocyanate (FITC)-labeled Annexin V as described previously [18]. Briefly, cells were harvested and washed in phosphate buffer saline (PBS). The pellets were incubated with 5µg/ml Annexin V, 5µg/ml propidium iodide (PI) and binding buffer, for 15min in the dark on ice. The Annexin V and PI fluorescences were measured by flow cytometry using a CyAn ADP Analyzer (Dako, USA). PI was used to avoid necrotic cell detection (Annexin V^-/PI^+). Spontaneous apoptosis was analyzed in cells cultured in the absence of the drug. Drug-induced apoptotic index was calculated as percentage of early and late apoptosis in the presence of the drug, subtracting early and late apoptosis in the absence of the drug (spontaneous apoptosis). A cut-off point of 20% was empirically used to segregate the samples into those with higher or lower early and late apoptosis.

2.6. Cell Cycle Analysis

Evaluation of the DNA content in cell cycle analysis was performed as previously described [23]. Cells (5 x 10^5) were incubated with or without LQB-118, or idarubicin for 24h and 48h. Cells were washed once with PBS and incubated with 500µl of PI staining solution (PI 50µg/ml diluted in citrate buffer 4mM and Triton X-100 0.3%) and RNAse (100µg/ml diluted in citrate buffer 40mM) for 15min at room temperature. The DNA content was determined on a flow cytometer (CyAn ADP Analyzer, Dako, USA). A total of 10.000 events were acquired per sample, and cell populations within this sample in cell cycle phases were quantified.

Table 1. Demographic, Clinical and Biological Characteristics of the Acute Myeloid Leukemia Patients

Patient N.	Gender	Age (year)	AML status *	FAB	WBC (/μl)	Cytogenetics	Rho-123 Efflux (MFI)	Pgp Expression (MFI)	LQB-118 3.0μM			
									Apoptosis Index at 24h (%)	Apoptosis Index at 48h (%)	Survivin Expression (Fold Changes)	XIAP Expression (Fold Changes)
1	F	43	*de novo*	NC	33,700	normal	1.16	1.55	5.91	24.21	0.59	0.72
2	F	13	*de novo*	M3	142,000	t(15;17)	1.40	2.16	54.12	52.66	-	-
3	M	15	*de novo*	M4	72,200	t(8;21)	1.00	3.19	46.39	38.51	-	-
4	F	32	*de novo*	M2	50,400	normal	1.00	14.1	77.18	25.64	-	0.01
5	F	14	*de novo*	M3	29,400	t(15;17)	1.29	1.80	-	75.05	-	-
6	M	67	*de novo*	M2	200,000	Inv16	1.13	7.85	29.46	42.51	0.09	0.31
7	F	25	*de novo*	M2	340,000	normal	1.21	1.18	38.96	34.98	-	-
8	M	1	*de novo*	M5	89,100	t(1;8;11)	1.13	6.87	0.0	0.0	-	-
9	M	20	*de novo*	M2	35,000	t(8;21)	1.00	1.68	9.17	5.13	0.43	-
10	M	9	*de novo*	M3	180,000	t(15;17)	1.78	12.22	11.79	-	-	-
11	M	31	relapse	M2	146,000	normal	1.15	1.23	46.49	46.77	0.83	-
12	F	9	relapse	M2	45,500	normal	2.91	1.12	-	48.08	-	-
13	M	60	relapse	M1	3,000	normal	1.57	1.18	0.0	4,41	0.23	-
14	F	73	secondary	NC	36,900	t(8;13)	1.47	1.31	24.46	22.06	3.63	5.70
15	M	67	secondary	NC	25,500	normal	1.71	3.13	-	13.55	-	-
16	M	1.2	secondary	NC	318,000	t(2;11)	1.13	6.87	11.14	-	-	-
17	M	56	secondary	NC	59,000	normal	1.00	1.68	8.14	24.0	-	-

F= Female; M= Male; FAB= French-American-British classification; NC= Not classified; WBC= White blood cells; secondary = AML secondary to myelodysplastic syndrome or after chemotherapeutic drugs for solid tumor treatment. The rates of LQB-118-induced apoptosis were estimated by the difference between the percentage of early + late apoptosis in cells treated and not by LQB-118 3.0μM. The percentage of apoptosis in untreated cells (control) was considered spontaneous apoptosis. The fold changes were calculated by rate between densitometric units of the treated or not patient cells. Rho-123= Rhodamine-123 efflux. MFI= ratio of mean fluorescence intensity. *At laboratory study.

2.7. Survivin, XIAP, IκBα, Activated Caspase-3 and PARP Cleavage Detection by Western Blot Assay

Western blot was performed as described previously [24]. Briefly, cells were lysed by incubation at 4°C for 20min in lysis buffer. The protein content of lysates was determined using an adapted Lowry assay. Equal amounts of protein (50μg) were suspended in SDS-PAGE buffer and boiled in a bath for 10min. After that, the samples were submitted to 15% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and electro-transferred into a nitrocellulose membrane (Hybond - GE Healthcare, Piscataway, NJ, USA) in a buffer containing 25 mM Tris, 192 mM glycine, and 20% methanol at 100 V for 3h at 4°C. The membrane was blocked with tris buffered saline (TBS), pH 7.6, containing 0.1% Tween 20 (TBS-T) and 5% non-fat dry milk for 2h. Subsequently, the membrane was washed three times (10 min for each washing) with TBS-T and then incubated with each antibody rabbit anti-survivin (1 μg/ml; 1:1000) (R&D Systems, Minneapolis, MN, USA), goat anti-XIAP (1mg/mL; 1:2000) (R&D Systems, Minneapolis, MN, USA), or mouse anti-IκBα (100 μg/ml; 1:1000) (L35A5 clone, Cell Signaling, Danvers, MA, USA) overnight at 4°C. After washing (3x 10 min), the membrane was incubated with antibodies conjugated with horseradish peroxidase, and the antibody complexes were visualized by the ECL detection system through the manufacturer's instructions (GE Healthcare, Piscataway, NJ, USA). The membrane was reprobed with a monoclonal antibody against β-actin (1:2000) (Sigma-Aldrich, St. Louis, MO, USA) as a loading control. The relative expressions of survivin and XIAP proteins were determined with a chemiluminescence imaging system and quantified by image analysis (Labworks software).

As caspase and PARP-cleavage are events that indicates that the cascade of apoptosis was triggered, we evaluated the expressions of cleaved caspase-3 and PARP [25]. A total of 50 μg of lysates was loaded and primary antibodies against activated caspase-3 (1:500 dilution, BD Biosciences, San Jose, CA, USA), PARP (1:1000 dilution, Cell Signaling Technology, Danvers, MA, USA), and β-actin (1:1000 dilution, Sigma-Aldrich, St. Louis, MO, USA) were incubated overnight in TBS-milk. Membranes were probed with the secondary antibody horseradish peroxidase (HRP)-labeled anti-mouse antibody (1:5000 dilution; GE Healthcare, Buckinghamshire, UK).

2.8. NFκB Subcellular Localization by Immunofluorescence Assay

To observe the subcellular localization of NFκB, we performed an immunofluorescence assay using an anti p65 monoclonal antibody (100μg/ml; 1:50, C22B4 clone, Cell Signaling Technology, Danvers, MA, USA). Cytospins were prepared using 10^5 Kasumi-1 cells before and after treatment with idarubicin or LQB-118. Cytospins were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 for 10 min. Cells were blocked with PBS pH 8.0 containing 3% bovine serum albumin (BSA) for 1 h. Cells were then stained with a rabbit polyclonal antibody against NFκB/p65 overnight at 4ºC and washed three times with PBS pH 8.0 for 5min. The antibody–antigen complexes

were detected by incubation for 1 h with a secondary goat anti rabbit Alexa Fluor 488 immunoglobulin G antibody (1:500, Invitrogen Molecular probes, Carlsbad, CA, USA). Cells were washed three times in PBS pH 8.0 for 5 min and treated with DAPI (4',6-diamidino-2-phenylindole, 1:5000) for 10 min to stain the nucleus. Coverslips were mounted with n-propyl-gallate and the cells were subsequently analyzed with Nikon Eclipse E200 fluorescence microscope using software Nis Element F 2.30 (Nikon Instruments, Melville, NY, USA).

2.9. Downregulation of Survivin by siRNA

Kasumi-1 cells (10^5 cells/ml) were transfected with control (*scramble*) (*Silencer*® Select Negative Control, Applied biosystems) or survivin (*Silencer*® Select siRNA, s1457, Applied biosystems) siRNAs by using Lipofectamine™RNAiMax (Invitrogen) in serum-free OptiMem medium (Invitrogen) for 48h at 37°C in a humidified atmosphere with 5% CO_2 according to the manufacturer's recommendations. Then, the cells were incubated with DMEM/F12 medium, (with FBS 20%) with or without LQB-118 or idarubicin for 24h. The transfection effectiveness was evaluated by Western blot.

2.10. Measurement of Pgp Expression

To detect the extracellular Pgp epitopes, AML cells from patients (1×10^6) were incubated with phycoerythrin (PE)-conjugated monoclonal antibody against Pgp (clone UIC2, Coulter, USA) or PE-conjugated isotype IgG2a as control for 30 min at room temperature, washed and then analyzed by flow cytometry (summit v4.3 software, Dako, USA). 10,000 events were collected for each sample. Results were expressed as the ratio of mean fluorescence intensity (MFI) of cells incubated with UIC2 divided by MFI of untreated cells. Positivity was considered when MFI > 1.1 [5, 26].

2.11. Measurement of Pgp Activity

The Pgp activity was analyzed in AML cells from patients using Rhodamine-123 (Rho-123; Sigma-Aldrich, USA) as previously described by our group [5, 26]. Briefly, $5x10^5$ cells were suspended in RPMI 1640 or 200 ng/ml cyclosporin A (CSA; Novartis, Switzerland) and incubated with 200 ng/ml Rho-123 for 45 min at 37°C. Cells were washed in cold PBS after incubation. Medium or medium plus CSA was added and incubated for a further 45 min at 37°C in the absence of Rho-123. After the second incubation, cells were analyzed by flow cytometry (Summit v4.3 software, Dako, USA). Cells without Rho-123 or CSA were used to define fluorescence thresholds. Results were expressed as the ratio of MFI of cells with Rho-123 plus CSA divided by mean fluorescence intensity of cells with Rho-123 only. Positivity was considered when MFI $\geq$ 1.1.

2.12. Statistical Analysis

A standard one-way analysis of variance (ANOVA) was used to determine statistically significant differences of means and/or medians. A value of *P*-value < 0.05 was considered statistically significant. Statistical analysis was performed using the Graph Pad Prism 5.0 software.

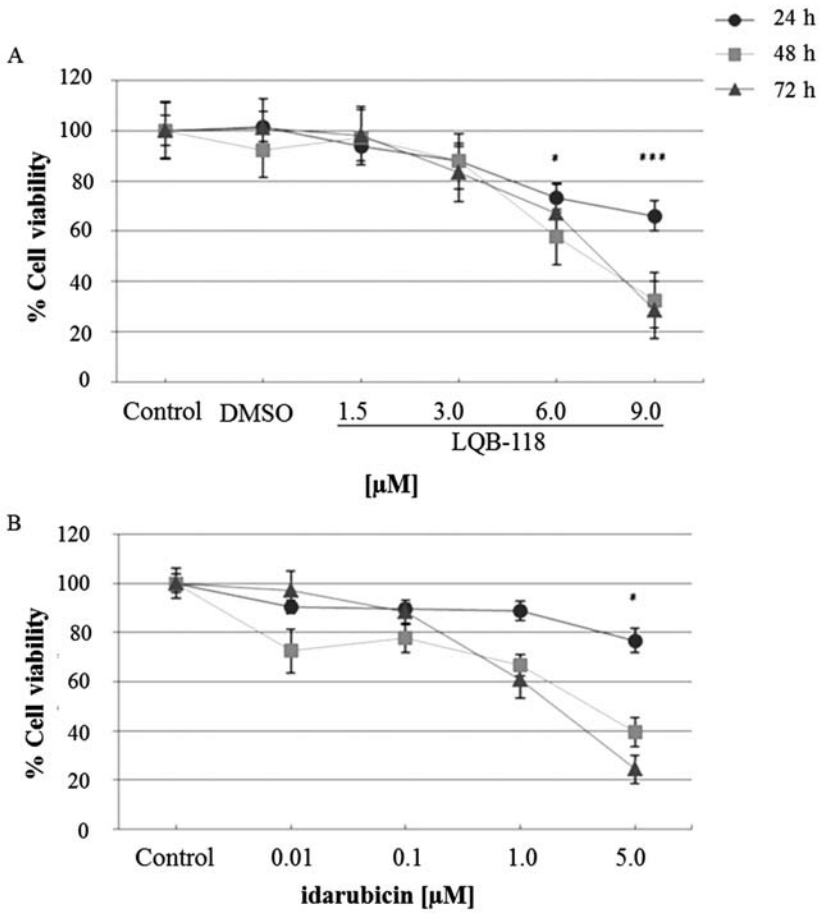


Fig. (2). Viability of AML Kasumi-1 cell line induced by (A) LQB-118 or (B) idarubicin by MTT assay. Cells were incubated with different (**A**) LQB-118 concentrations (3.0, 6.0 and 9.0µM) and (**B**) idarubicin (0.01, 0.1, 1.0 and 5.0µM) for 24, 48 and 72h. Cell viability was expressed as percentage. Error bars represent standard deviation of the mean of three independent experiments performed in triplicate. (**P*-value < 0.05; ****P*-value < 0.001).

3. RESULTS

3.1. LQB-118-induced Reduction of the Kasumi-1 Cells Viability

As a first step, we evaluated the effect of LQB-118 on the viability of the AML Kasumi-1 cell line compared with idarubicin using the MTT assay. This anthracycline was chosen as it is one of the most effective drugs employed in the treatment of AML patients [27]. As observed in Fig. (**2A**), LQB-118 3.0 μM reduced less than 20% of the cell viability as compared to controls. However, when the cells were incubated with LQB-118 6.0 μM, this compound reduced the cellular viability by about 40% (*P*-value < 0.05) after 48h or 72h incubation. The viability reduction with LQB-118 9.0μM was markedly higher (70%) after 48h and 72h incubation (*P*-value < 0.001). As observed in Fig. (**2B**), idarubicin 1.0 μM reduced cellular viability by 40% after 48h (*P*-value > 0.05) and by 40% after 72h incubation (*P*-value > 0.05). The concentration of 5.0 μM was able to decrease 60% of cell viability after 48h (*P*-value < 0.05) and 80% after 72h incubation (*P*-value < 0.05). However, this 5.0 μM concentration of idarubicin is higher than the peak concentration reached *in vivo* in patients' blood [28].

3.2. LQB-118-induced Apoptosis in Kasumi-1 Cells

To further explore the anti-apoptotic LQB-118 role in Kasumi-1 cells, we used the flow cytometric Annexin V assay. As compared to controls, LQB-118 at 3.0 μM concentration induced 30.7% (*P*-value > 0.05) and 54.2% (*P*-value < 0.01) of apoptosis after 24h and 48h incubations respectively. A marked increase in apoptosis index was observed when Kasumi-1 cells were incubated with LQB-118 9.0 μM in which this compound induced 85% (*P*-value < 0.01) and 92% (*P*-value < 0.001) of apoptosis after 24h and 48h respectively (Fig. **3**).

3.3. LQB-118 and Idarubicin-induced Alterations in Cell Cycle

To assess whether the antiproliferative effect of LQB-118 and idarubicin resulted from cell cycle arrest or apoptosis, cell cycle profile assay was carried out after 48h of treatment. Confirming our previous result [26], idarubicin at 0.1 and 1.0 μM induced G2/M arrest after 48h of treatment (*P*-value < 0.001; *P*-value < 0.01). At 5.0 μM idarubicin concentration, we observed a marked characteristic of DNA fragmentation (*P*-value < 0.01). Shorter treatment (24h) resulted in similar picture (data not shown). On the other hand, LQB-118 at 3.0 μM concentration did not induce arrest during any phase of the cell cycle (*P*-value > 0.05). DNA fragmentation was only observed at 9.0 μM concentration of LQB-118 (*P*-value < 0.01) (Fig. **4A** and **4B**).

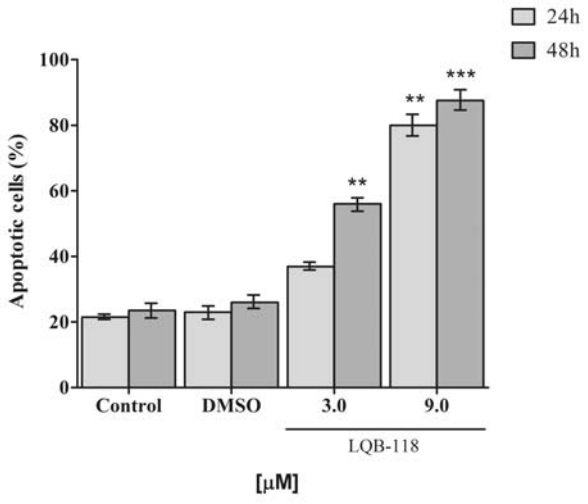


Fig. (3). Apoptosis of Kasumi-1 cell line induced by LQB-118 by the Annexin V assay using flow cytometry. After incubation with LQB-118 (3.0 and 9.0μM), cells were stained with Annexin V and propidium iodide (PI) for early and late apoptosis detection by flow cytometry. Kasumi-1 cells incubated without LQB-118 or with vehicle alone (DMSO) were used as control of spontaneous apoptosis. Values are ± mean of three independent experiments performed in triplicate. (***P*-value < 0.01; ****P*-value < 0.001).

3.4. LQB-118-induced Caspase-3 Activation, PARP Cleavage and Downregulation of Survivin and XIAP in Kasumi-1 Cell Line

To confirm that the cell death observed by Annexin V in Kasumi-1 cells was caused by LQB-118-induced apoptosis, we analyzed the activation of caspase-3 under LQB-118 treatment by Western blot. Corroborating the results presented in Fig. (**4**), there was a clear increase in caspase-3 activation and PARP cleavage at 3.0 and 9.0 μM concentrations of LQB-118 (Fig. **5**).

LQB-118 was also able to inhibit IAPs in AML cells, as observed previously in CML cells [15]. Using Western blot assay, survivin and XIAP were downregulated in AML Kasumi-1 cells after LQB-118 treatment, although XIAP was inhibited with 3.0 μM and 9.0 μM concentrations, and survivin was inhibited only after 9.0 μM LQB-118. In contrast, idarubicin induced the up regulation of survivin and XIAP, that of survivin being more prominent (Fig. **5**).

3.5. IκBα is Degraded after Incubation with Idarubicin

As a result of the observation that LQB-118 caused a marked downregulation of survivin and XIAP expression in Kasumi-1 cells, we investigated if the NFκB pathway was involved in the cellular response after treatment with either LQB-118 or idarubicin by evaluating the expression pattern of IκBα - the NFκB inhibitor. After treatment with LQB-118, we observed stable or elevated levels of IκBα in all the concentrations tested (3.0 and 9.0 μM). On the other hand, there was a drastic reduction in IκBα protein levels after treatment with idarubicin, suggesting that the complex IκBα-NFκB no longer exists, and thus, NFκB translocated from the cytoplasm to the nucleus to assume acting as a transcription factor (Fig. **6**).

3.6. LQB-118 and Idarubicin-induced Subcellular Alterations in NFκB

Immunofluorescence analysis was carried out to study the subcellular localization pattern of NFκB. We observed that after 24h incubation with a 3.0 μM concentration of LQB-118, NFκB was predominantly in the cytoplasm of the Kasumi-1 cells. On the other hand, incubation with idarubicin at 0.01 μM and 1.0 μM concentrations induced its translocation, since NFκB was observed predominantly in the nucleus of the Kasumi-1 cells (Fig. **7**). This result is in line with the observed increase in survivin and XIAP levels induced by idarubicin demonstrated in Fig. (**5**).

3.7. Survivin Inhibition by siRNA Sensitizes Kasumi Cell Line to LQB-118 and Idarubicin Treatment

To assess the relevance of survivin having a role in apoptosis resistance, Kasumi-1 cells were exposed either to idarubicin or to LQB-118 after transient silencing by siRNA. The obtained level of silencing was approximately 40%. LQB-118, in an isolated way, was able to reduce the expression of survivin, independent of silencing. Contrarily, idarubicin induced an increased expression of survivin that was partly reversed by siRNA (Fig. **8A**).

After silencing, LQB-118 was able to cause significantly higher levels of cell death (40%) after survivin inhibition, when compared with control (*P*-value = 0.01). Similarly, idarubicin induced significantly different cell death (approximately 35%) compared with control (*P*-value = 0.001) (Fig. **8B**).

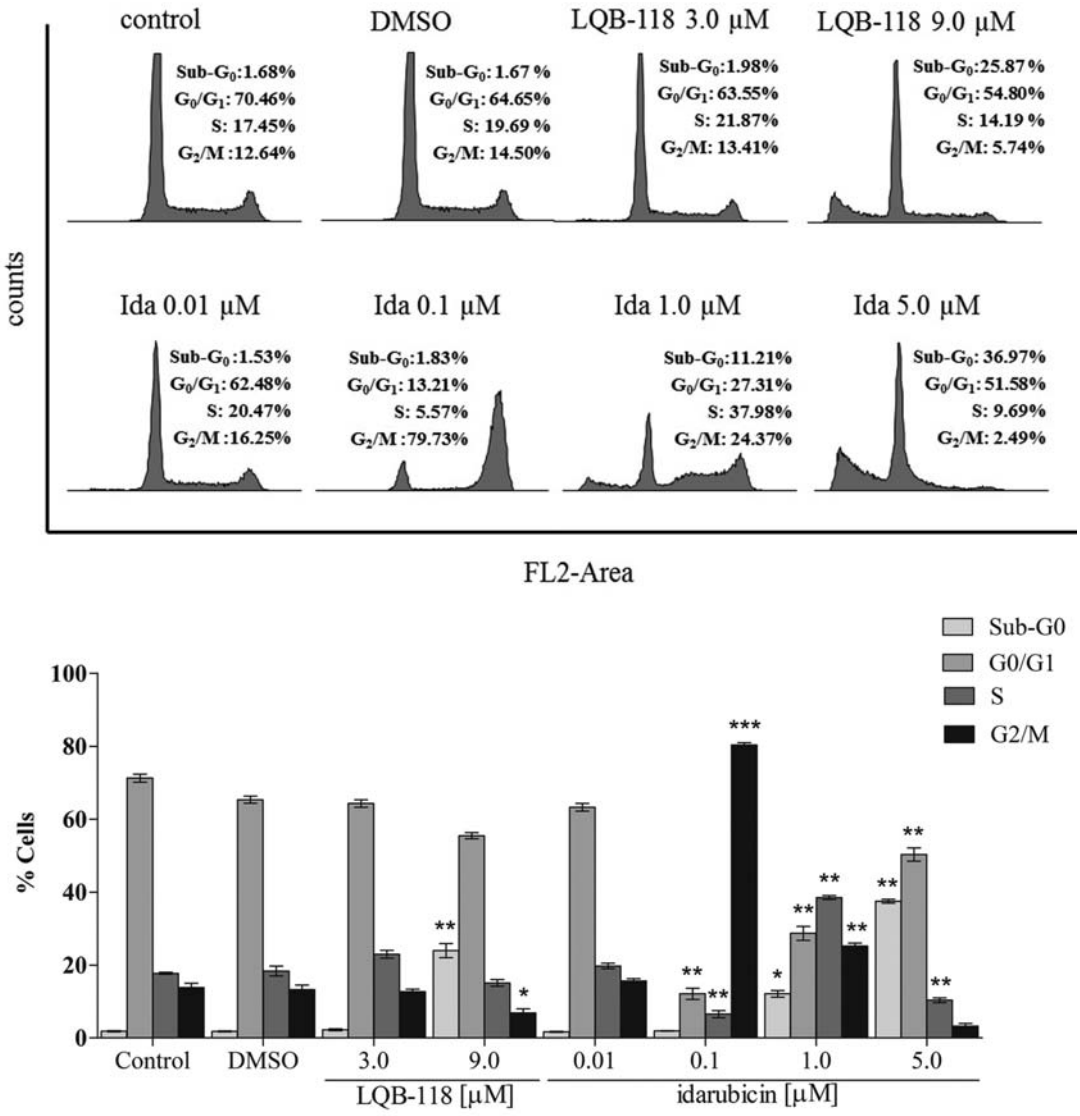


Fig. (4). Flow cytometry analysis of LQB-118- and idarubicin-induced alteration in Kasumi-1 cells DNA content. Cell cycle was analyzed using PI-stained Kasumi-1 cells after 48h of incubation with each drug. (**A**) The percentage of cells in each phase was calculated from the histograms. The data are representative of three independent experiments. (**B**) Values in the graph are ± mean of three independent experiments. (**P*-value < 0.05; ***P*-value < 0.01; *** *P*-value < 0.001).

3.8. LQB-118-induced Apoptosis in Leukemic Cells from AML Patients

After observing, through Annexin V, caspase-3 activation and PARP cleavage, that Kasumi-1 cells exhibited apoptosis features after LQB-118 treatment, and in order to gain more insight into the LQB-118 role in triggering the apoptotic process in AML cells, we investigated the rate of apoptosis induced by LQB-118 3.0 µM in fresh blast cells obtained from 17 AML patients. Using Annexin V assay by flow cytometry, leukemic cells from patients were incubated with LQB-118 for two different periods of time (24h and 48h). This concentration was chosen based on the LQB-118 effect on CML cells observed in our previous study [15]. At the lowest period of time incubation, LQB-118 had no effect in two out of 17 AML samples. In the other 12 samples, the apoptotic effect ranged from 5.91% to 77.18% (median= 29.46%). After 48h incubation, cells from 14 AML patients exhibited similar percentage of apoptotic cells as observed after 24h incubation (median= 32.39), ranging from 4.41 to 75.05% (Table **1**).

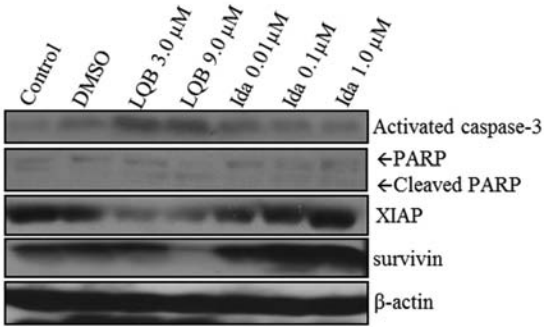


Fig. (5). Western blot analysis of caspase-3 activation, PARP cleavage, survivin and XIAP levels in response to increasing concentrations of LQB-118 and idarubicin for 24 h in Kasumi-1 cell line. Fig. (**5**) demonstrates that the treatment with LQB-118 increased activated caspase-3, cleaved PARP levels, and decreased survivin and XIAP levels, whereas idarubicin treatment increased survivin and XIAP levels. The data are representative of three independent experiments.

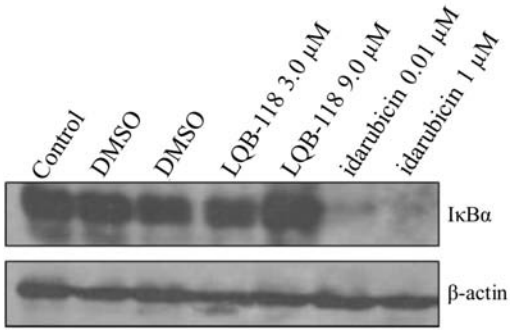


Fig. (6). Western blot analysis of IκBα levels in response to LQB-118 and idarubicin in Kasumi-1 cell line. Cells from Kasumi-1 cell line were treated with LQB-118 3.0 and 9.0 µM, and idarubicin 0.01 and 1.0 µM for 24h. Only cells treated with idarubicin presented reduction in IκBα levels as compared to cells with or without LQB-118 treatment. The protein expressions were normalized by β-actin. The data are representative of three independent experiments.

3.9. Pgp Expression and Pgp Activity in AML Patients

Positive Pgp activity, analyzed through Rho-123 efflux assay, was observed in 13 out of 17 (76.47%) AML patients. On the other hand, Pgp expression was observed in all patients, including four patients in which Rho-123 efflux assay was negative (Table **1**). Three out of four patients in which Pgp activity was negative were *de novo* AML and one secondary AML. Three out of three patients at relapse and three out of four with secondary AML displayed both Pgp overexpression and Pgp activity.

3.10. LQB-118-induced Apoptosis in Cells from AML Patients Presenting Adverse Prognostic Features

It has been demonstrated that high peripheral WBC is associated with bad prognosis in AML patients [3]. Ten (#2, 3, 4, 6, 7, 8, 10, 11, 16 and 17) out of 17 patients exhibited more than 50 x 10^9 l^{-1} leukocytes at diagnosis, and five (#2, 4, 6, 7 and 10) out of those ten patients had evolution to death prior to or during the induction therapy. Therefore, we confronted this variable with the apoptosis index induced by LQB-118. It was possible to observe

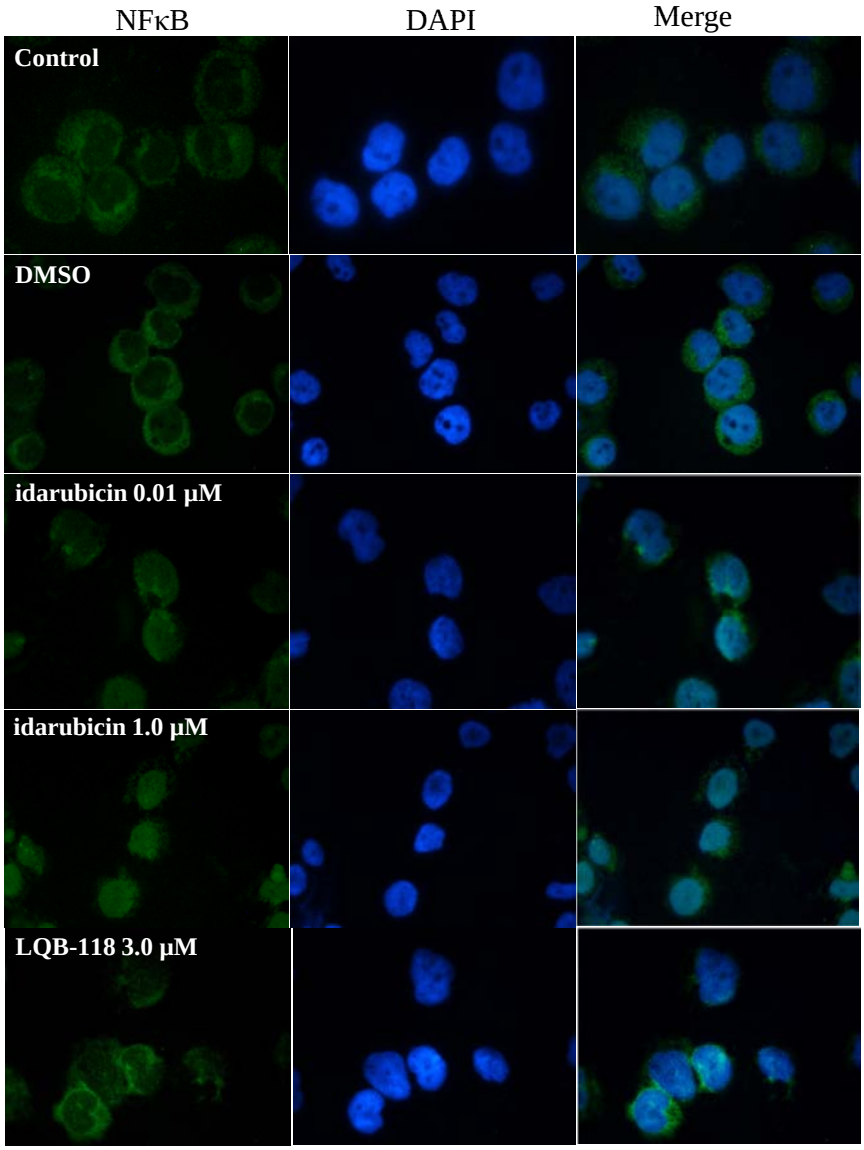


Fig. (7). Immunofluorescence analysis of NFκB subcellular location in response to LQB-118 and idarubicin after 24 h in Kasumi-1 cell line. AML cells, Kasumi-1, were treated with LQB-118 (3.0µM) and DMSO (vehicle) or idarubicin (0.01 and 1.0µM) for 24h, stained with DAPI (blue) and anti-NFκB antibody (green), and analyzed by immunofluorescence. Original magnification x1000. The data are representative of three independent experiments.

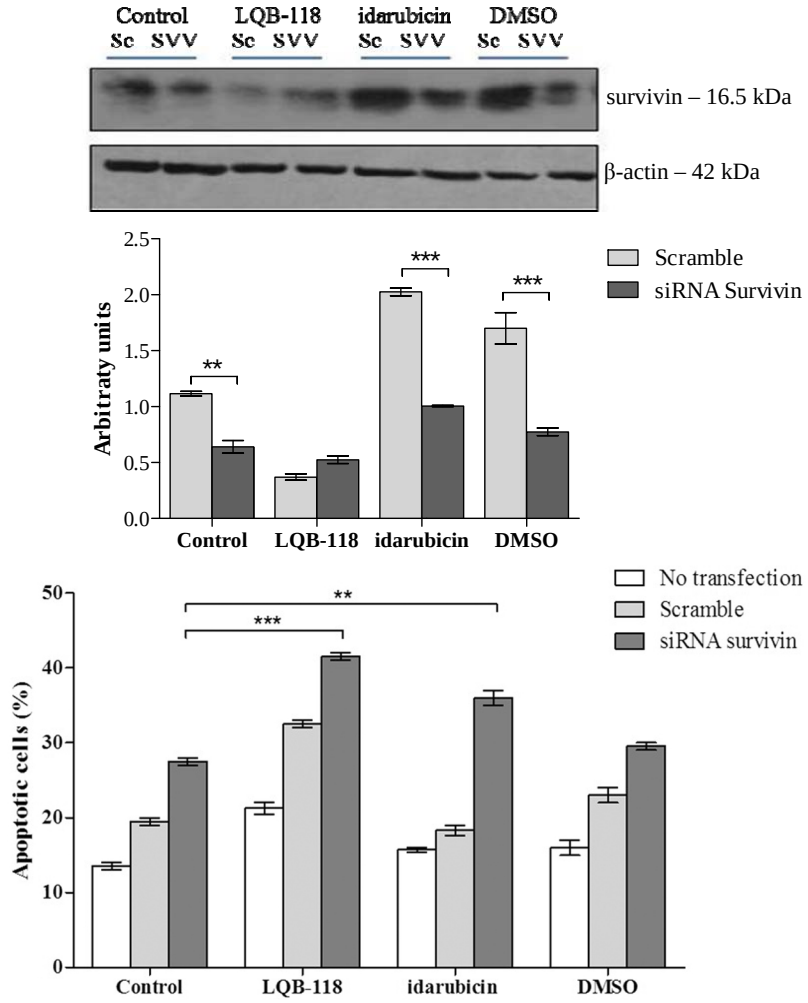


Fig. (8). Downregulation of survivin (SVV) by siRNA and apoptosis induction after exposition to LQB-118 and idarubicin. Kasumi-1 cells were transfected with siRNA to survivin or scramble (Sc) negative control for 48h and exposed to LQB-118 (3.0 μM) or idarubicin (0.01 μM) for additional 24h. (**A**) Survivin protein expression evaluated by Western blot as arbitrary unit of protein expression. (**B**) After incubation time cells were submitted to Annexin V assay by flow cytometry. Values are ± mean of three independent experiments performed in triplicate. (***P*-value < 0.01; ****P*-value < 0.001).

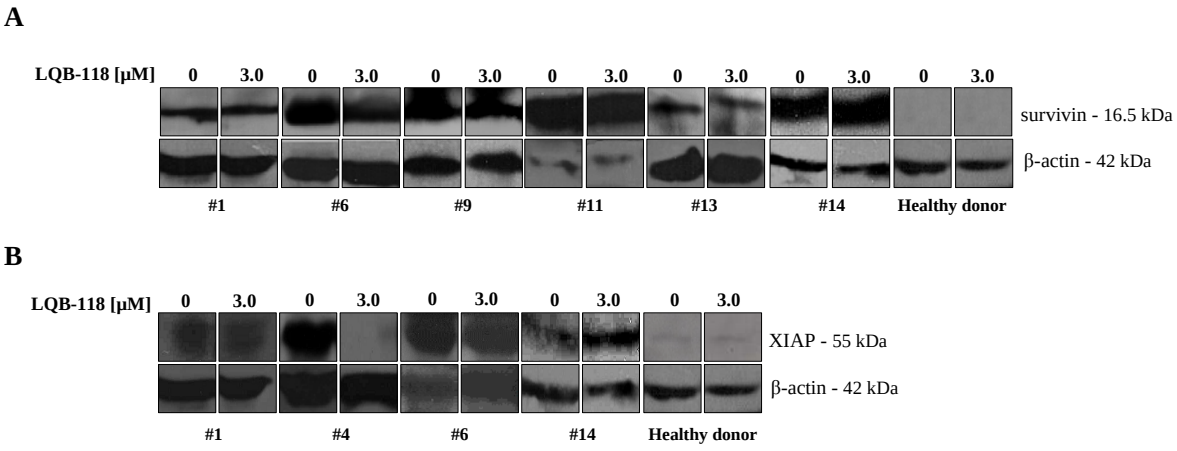


Fig. (9). Western blot analysis of survivin and XIAP levels in response to LQB-118 in cells of AML patients and healthy donor. Cells from patients and healthy donors were treated with LQB-118 3.0 μM for 24h. (**A**) shows survivin levels and (**B**) shows XIAP levels, after LQB-118 treatment. The protein expressions were normalized by β-actin.

that in six (#2, 3, 4, 6, 7 and 11) out of ten patients with hyperleucocytosis, the apoptosis index induced by LQB-118 was higher than 20% after 24h incubation. Another established significant prognostic variable in AML is the old age of patients, which is related to poor prognosis [3]. In our study, four patients (#6, 13, 14 and 15) were more than 60 years old. In this group, three (#6, 14 and 15) out four patients either had no remission or died during therapy induction. LQB-118 induced more than 20% of apoptosis after 24h incubation in two (#6 and 14) out of four patients older than 60 years.

In our study, eight (#1, 4, 7, 11, 12, 13, 15 and 17) patients presented normal karyotype. The cytogenetic abnormalities comprised nine cases including three (#2, 5 and 10) with t(15;17), two (#3 and 9) with t(8;21) and others with diverse alterations. Concerning the arbitrary high (> 20%) or low (< 20%) rate of apoptotic cells, no difference was observed between the group of patients exhibiting or not exhibiting normal karyotypes.

Although we had studied a very small number of samples from relapse and secondary AML patients, it was possible to observe that among these samples, there were some that exhibited an apoptosis rate above 20% either at 24h (two out of five) or at 48h (four out of six) incubation.

Altogether, our results indicate that LQB-118 was indistinctly effective in inducing apoptosis in AML cells from patients with normal as well as with a diversity of factors related to unfavorable response to chemotherapy.

3.11. LQB-118-induced Apoptosis in Cells from AML Patients Exhibiting a Multifactorial MDR Profile

It has been proposed that Pgp overexpression and/or its activity in AML are correlated with poor *in vivo* response to chemotherapeutic drugs [6]. Nevertheless, using an arbitrary cutoff point to discriminate between low (< 20%) and high (> 20%) index of apoptosis induced by LQB-118, we could observe that this compound was able to induce high apoptosis index in AML samples, independently of Pgp status (Table **1**). These results suggest that LQB-118 is effective in inducing apoptosis in cells exhibiting or not exhibiting MDR phenotype.

In relation to constitutive survivin and XIAP expressions analyzed by Western blot, a great variation was observed among the AML samples from patients. All patients exhibited the presence of both proteins when this feature was compared with healthy donor blood samples. It was possible to observe that there was a high index of apoptosis induced by LQB-118 even in samples exhibiting high levels of survivin and XIAP.

3.12. LQB-118-induced Modulation of Survivin and XIAP Expressions in AML Cells from Patients

Primary AML samples for survivin (n=6) and XIAP (n=4) expression levels were also analyzed by Western blot after 24h incubation with LQB-118 3.0 μM concentration. There was a decrease in survivin expression where the levels ranged from 0.09 to 3.63 fold (mean= 0.96) when quantified through the survivin/β actin densitometric ratio and compared with the basal expression in these samples (control). In this group, apoptosis was not observed in two samples (#9 and 13) despite the marked survivin reduction levels 0.09 and 0.23 fold). Only one AML sample (#14) displayed an increase in survivin expression after treatment with LQB-118 in which the survivin increase was equal to 3.6 fold and there were more than 20% apoptotic cells (Fig. **9A**; Table **1**).

Concerning XIAP modulation after treatment with LQB-118, it was observed that in four samples examined, there was a decrease in expression levels in three of them (#1, 4 and 6), (0.01 to 0.72 fold; mean=0.34) (Fig. **9B**; Table **1**). The fact that caught our attention was that in one sample (#4), there was a more significant decrease of XIAP (0.01 fold), and this correlated with the highest apoptosis rate observed after 24h incubation with LQB-118 treatment.

Healthy donor blood samples do not presented survivin expression either before or after 3.0 μM LQB-118 treatment (Fig. **9A**). The XIAP expression was not altered in same incubation conditions (Fig. **9B**).

4. DISCUSSION

The overall survival of AML patients is still unsatisfactory despite great progress in the new pharmacological arsenal [2, 13]. Therefore, there is a demand for new therapeutic approaches to improve the rate of cure in this disease.

We have previously established, in a preclinical study, that the new synthetic pterocarpanquinone LQB-118, may prove to be useful in the treatment of CML. We identified this compound as being capable of inducing high apoptosis rate in CML cells by inhibiting the IAPs survivin and XIAP. Furthermore, LQB-118 reduced the expression of Pgp in a significant way in a CML K562-Lucena resistant cell line. LQB-118 was also able to induce high apoptosis rate in cells from a group of CML patients presenting MDR phenotype [15].

In the present study, we addressed the issue of whether LQB-118 would be equally effective against AML cells exhibiting similar drug resistance profiles as CML samples, in spite of the fact that these myeloid diseases present different molecular pathogenesis. Therefore, this study tested the sensitivity of AML to LQB-118 compared to idarubicin and investigated a possible mode of action based on the inhibition of the IAPs survivin and XIAP.

Initially, LQB-118 capacity to reduce cellular viability and to induce apoptosis in AML cells was studied against the Kasumi-1 cell line that exhibits t(8;21), typical for AML sub-type M2. We demonstrated, through MTT assay, that LQB-118 exerted a cytotoxic effect on Kasumi-1 cells. Indeed, LQB-118 also induced a high apoptotic rate in these cells. Another evidence of apoptosis induction was caspase-3 activation and PARP cleavage after LQB-118 treatment. The same characteristic was also observed in our previous work [15] in which caspase-3 was activated by LQB-118 in CML cells. Together, these results demonstrate that the LQB-118 can induce apoptosis in myeloid cells regardless of their myeloid lineage type being acute or chronic. It should be pointed out that the LQB-118 concentration needed for AML cells was twice as much as that used against CML cells for the same purpose. However, it is also relevant to emphasize that despite this higher concentration, LQB-118 did not show significant cytotoxicity for peripheral blood mononuclear cells activated by the mitogen phytohemagglutinin, as previously shown (IC50 > 20.0 μM) [14].

Substantial differences could be observed between LQB-118 and idarubicin after triggering the apoptotic process. Following exposure to LQB-118, Kasumi-1 cells showed an important downregulation of survivin and XIAP detected by Western blot, this finding being parallel with the activation of caspase-3. This result contrasts with the effect observed after idarubicin exposure where the same assay demonstrated that this compound clearly upregulated survivin and XIAP. Such effect also agrees with the negligible effect observed by idarubicin in activating caspase-3. A recent study conducted by us also demonstrated that idarubicin was able to induce survivin expression in K562 cells, derived from CML, protecting these cells from idarubicin-induced apoptosis [23]. In the present study, similar to what was observed in that study, we also observed that there was G2/M arrest after treatment using the same concentrations of idarubicin (0.1 μM). It has been shown by other authors [29] that populations of the same neuroblastoma cell line, but in different cell cycle phases, presented distinct survivin expression patterns after synchronization, and the sensitivity to apoptosis induction was markedly different. The cells in G2/M phase could be more resistant than cells in other cell cycle phases,

like G1. This corroborates the suggestion that survivin over expression, concurrent with G2/M arrest, may facilitate checkpoint evasion and promote resistance to apoptosis. It also suggests that in an unsynchronized cellular population, the few cells that had not yet reached G2/M would be more susceptible to death. This population is identified as hypodiploid whose DNA fragmentation was evident after cytotoxic stimuli, such as that observed in the present study by treating the AML cell line, Kasumi-1, with idarubicin. On the other hand, LQB-118 3.0 and 6.0 μM concentrations did not cause arrest in any phase of the cell cycle, but 9.0 μM induced significant DNA fragmentation. These findings indicate that LQB-118 exerts its effect independent of the cell cycle phase, differently from idarubicin, corroborating the differences observed between the two drugs in other experiments such as caspase-3 activation and IAPs inhibition.

In order to evaluate whether survivin overexpression was associated with the apoptosis resistance observed in Kasumi-1 cells after treatment with idarubicin, we performed a siRNA treatment to downregulate survivin. Interestingly, we observed that LQB-118 was as efficient as the siRNA in downregulating survivin. Besides, siRNA was efficient in inhibiting survivin and in inducing apoptosis in idarubicin-resistant cells. These findings reinforce the hypothesis that survivin may offer resistance to the malignant clones in AML. The effect observed after survivin inhibition by LQB-118 was similar to a study using specific survivin inhibitors, including apoptosis induction [30].

Some authors have demonstrated that the NFκB signaling pathway is involved in the regulation of the expression of many target genes involved in apoptosis evasion or MDR, such as *survivin*, *XIAP*, and *ABCB1* [31]. In the present study, we showed that LQB-118 induced survivin and XIAP downregulation in both *in vitro* and *ex vivo* AML cells. Kasumi-1 cells treated with LQB-118 showed a stable or increased expression of IκBα, indicating a possible accumulation of this protein, and cytosolic NFκB localization. This suggests that IκB-NFκB complex remained in the cytoplasm in LQB-118 treated cells, probably preventing the induction of survivin and XIAP expressions. In contrast, idarubicin induced an overexpression of survivin and XIAP in Kasumi-1 cells. Consistent with our hypothesis, Kasumi-1 cells treated with idarubicin showed NFκB translocation to the nucleus and IκBα degradation. Thus, our data suggest different mechanisms of action for LQB-118 and idarubicin in regulating IAPs. Therefore, drugs that are capable of suppressing NFκB activation might have an important therapeutic potential in carcinogenesis inhibition. In addition, LQB-118 was able to induce apoptosis in cells exhibiting classical MDR phenotype, as was also observed previously [15]. These findings might indicate that there is a link between NFκB pathway and *ABCB1*. Other authors have shown previously that the inhibition of NFκB reduced *ABCB1* mRNA and Pgp expression in HCT15 colon cancer cells and increased the number of apoptotic cells in response to daunomycin treatment [32].

In the present study, we have shown that primary AML cells exhibit increased survivin expression as compared to control. Our data substantiate other observations showing that the expression of survivin is a frequent event as observed by Western blot where variable levels of this protein were found in cells from AML patients [33]. Another study has considered survivin to be an independent negative prognostic factor for overall survival in the intermediate cytogenetic risk AML patients [10]. Therefore, survivin has been studied not only for its role in cell proliferation but also for its important role in the regulation of apoptosis and prognostic factor in cancer patients [11, 34]. Moreover, IAPs have been found to be associated with other drug resistance mechanisms. Association of XIAP and MRP1, another ABC transporter protein, was detected in a resistant HL-60/ADR AML cell line. Simultaneous suppression of XIAP and MRP1 by antisense oligonucleotide overcame the drug resistance of this resistant cell line [34].

Based on the hypothesis that down regulation of IAPs might overcome chemoresistance of leukemic cells, we showed evidence that survivin and XIAP expressions were decreased dramatically after LQB-118 treatment. These results indicate that these IAPs may be targets for LQB-118 in AML cells. We also demonstrated that multifactorial drug resistance is very common in AML patients. Given that these different resistance pathways may be interconnected and can affect the response to chemotherapy, a novel drug to simultaneously affect two or more targets is highly desirable.

In summary, the compound LQB-118 activated the apoptotic cascade and inhibited IAPs expression. Interestingly, survivin and XIAP inhibition was concomitant to potential inhibition of NFκB activation, which suggests a functional relationship among survivin, XIAP and NFκB in the resistance mechanism of AML Kasumi-1 cells. These findings indicate that LQB-118 might be a promising therapeutic approach for AML patients through the inhibition of mechanisms that lead to the overexpression of the survivin antiapoptotic protein.

COMPETING INTEREST

The authors declare that they have no competing interest.

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REFERENCES

[1] Ferrara, F. Treatment of unfit patients with acute myeloid leukemia: a still open clinical challenge. *Clin. Lymphoma Myeloma Leuk*., **2011**, *11*(1), 10-16.

[2] Burnett, A.; Wetzler, M.; Lowenberg, B. Therapeutic advances in acute myeloid leukemia. *J. Clin. Oncol*., **2011**, *29*(5), 487-494.

[3] Smith, M. L.; Hills, R. K.; Grimwade, D. Independent prognostic variables in acute myeloid leukaemia. *Blood Rev*., **2011**, *25*(1), 39-51.

[4] Tiwari, A. K.; Sodani, K.; Dai, C. L.; Ashby, C. R., Jr.; Chen, Z. S. Revisiting the ABCs of multidrug resistance in cancer chemotherapy. *Curr. Pharm. Biotechnol*., **2011**, *12*(4), 570-594.

[5] Vasconcelos, F. C.; Gattass, C. R.; Rumjanek, V. M.; Maia, R. C. Pomolic acid-induced apoptosis in cells from patients with chronic myeloid leukemia exhibiting different drug resistance profile. *Invest. New Drugs*, **2007**, *25*(6), 525-533.

[6] Wuchter, C.; Leonid, K.; Ruppert, V.; Schrappe, M.; Buchner, T.; Schoch, C.; Haferlach, T.; Harbott, J.; Ratei, R.; Dorken, B.; Ludwig, W. D. Clinical significance of P-glycoprotein expression and function for response to induction chemotherapy, relapse rate and overall survival in acute leukemia. *Haematologica*, **2000**, *85*(7), 711-521.

[7] Ma, P.; Dong, X.; Swadley, C. L.; Gupte, A.; Leggas, M.; Ledebur, H. C.; Mumper, R. J. Development of idarubicin and doxorubicin solid lipid nanoparticles to overcome Pgp-mediated multiple drug resistance in leukemia. *J. Biomed. Nanotechnol*., **2009**, *5*(2), 151-161.

[8] Funato, T.; Satou, J.; Nishiyama, Y.; Fujimaki, S.; Miura, T.; Kaku, M.; Sasaki, T. *In vitro* leukemia cell models of Ara-C resistance. *Leuk. Res*., **2000**, *24*(6), 535-541.

[9] Prenkert, M.; Uggla, B.; Tina, E.; Tidefelt, U.; Strid, H. Rapid induction of P-glycoprotein mRNA and protein expression by

cytarabine in HL-60 cells. *Anticancer Res*., **2009**, *29*(10), 4071-4076.

[10] Adida, C.; Recher, C.; Raffoux, E.; Daniel, M. T.; Taksin, A. L.; Rousselot, P.; Sigaux, F.; Degos, L.; Altieri, D. C.; Dombret, H. Expression and prognostic significance of survivin in de novo acute myeloid leukaemia. *Br. J. Haematol.*, **2000**, *111*(1), 196-203.

[11] Tamm, I.; Kornblau, S. M.; Segall, H.; Krajewski, S.; Welsh, K.; Kitada, S.; Scudiero, D. A.; Tudor, G.; Qui, Y. H.; Monks, A.; Andreeff, M.; Reed, J. C. Expression and prognostic significance of IAP-family genes in human cancers and myeloid leukemias. *Clin. Cancer Res*., **2000**, *6*(5), 1796-1803.

[12] Marcucci, G.; Haferlach, T.; Dohner, H. Molecular genetics of adult acute myeloid leukemia: prognostic and therapeutic implications. *J. Clin. Oncol.*, **2011**, *29*(5), 475-486.

[13] Robak, T.; Wierzbowska, A. Current and emerging therapies for acute myeloid leukemia. *Clin Ther*., **2009**, *31* Pt 2, 2349-2370.

[14] Netto, C. D.; da Silva, A. J.; Salustiano, E. J.; Bacelar, T. S.; Rica, I. G.; Cavalcante, M. C.; Rumjanek, V. M.; Costa, P. R. New pterocarpanquinones: synthesis, antineoplasic activity on cultured human malignant cell lines and TNF-alpha modulation in human PBMC cells. *Bioorg. Med. Chem*., **2010**, *18*(4), 1610-1616.

[15] Maia, R. C.; Vasconcelos, F. C.; de Sa Bacelar, T.; Salustiano, E. J.; da Silva, L. F.; Pereira, D. L.; Moellman-Coelho, A.; Netto, C. D.; da Silva, A. J.; Rumjanek, V. M.; Costa, P. R. LQB-118, a pterocarpanquinone structurally related to lapachol [2-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthoquinone]: a novel class of agent with high apoptotic effect in chronic myeloid leukemia cells. *Invest. New Drugs*, **2011**, *29*(6), 1143-1155.

[16] Huang, Y.; Fang, Y.; Wu, J.; Dziadyk, J. M.; Zhu, X.; Sui, M.; Fan, W. Regulation of Vinca alkaloid-induced apoptosis by NF-kappaB/IkappaB pathway in human tumor cells. *Mol. Cancer Ther*., **2004**, *3*(3), 271-277.

[17] Braun, T.; Carvalho, G.; Fabre, C.; Grosjean, J.; Fenaux, P.; Kroemer, G. Targeting NF-kappaB in hematologic malignancies. *Cell Death Differ*., **2006**, *13*(5), 748-758.

[18] Yu, C.; Argyropoulos, G.; Zhang, Y.; Kastin, A. J.; Hsuchou, H.; Pan, W. Neuroinflammation activates Mdr1b efflux transport through NFkappaB: promoter analysis in BBB endothelia. *Cell Physiol. Biochem*., **2008**, *22*(5-6), 745-756.

[19] Fabre, C.; Grosjean, J.; Tailler, M.; Boehrer, S.; Ades, L.; Perfettini, J. L.; de Botton, S.; Fenaux, P.; Kroemer, G. A novel effect of DNA methyltransferase and histone deacetylase inhibitors: NFkappaB inhibition in malignant myeloblasts. *Cell Cycle*, **2008**, *7*(14), 2139-2145.

[20] Ahmad, R.; Liu, S.; Weisberg, E.; Nelson, E.; Galinsky, I.; Meyer, C.; Kufe, D.; Kharbanda, S.; Stone, R. Combining the FLT3 inhibitor PKC412 and the triterpenoid CDDO-Me synergistically induces apoptosis in acute myeloid leukemia with the internal tandem duplication mutation. *Mol. Cancer Res*., **2010**, *8*(7), 986-993.

[21] Vardiman, J. W.; Thiele, J.; Arber, D. A.; Brunning, R. D.; Borowitz, M. J.; Porwit, A.; Harris, N. L.; Le Beau, M. M.; Hellstrom-Lindberg, E.; Tefferi, A.; Bloomfield, C. D. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*, **2009**, *114*(5), 937-951.

[22] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **1983**, *65*(1-2), 55-63.

[23] Nestal de Moraes, G.; Silva, K. L.; Vasconcelos, F. C.; Maia, R. C. Survivin overexpression correlates with an apoptosis-resistant phenotype in chronic myeloid leukemia cells. *Oncol Rep*., **2011**, *25*(6), 1613-1619.

[24] Reis, F. R.; Vasconcelos, F. C.; Pereira, D. L.; Moellman-Coelho, A.; Silva, K. L.; Maia, R. C. Survivin and P-glycoprotein are associated and highly expressed in late phase chronic myeloid leukemia. *Oncol. Rep*., **2011**, *26(*2), 471-478.

[25] Nestal de Moraes, G.; Carvalho, E.; Maia, R. C.; Sternberg, C. Immunodetection of caspase-3 by Western blot using glutaraldehyde. *Anal. Biochem*., **2011**, *415*(2), 203-205.

[26] Vasconcelos, F. C.; Silva, K. L.; de Souza, P. S.; Silva, L. F.; Moellmann-Coelho, A.; Klumb, C. E.; Maia, R. C. Variation of MDR proteins expression and activity levels according to clinical status and evolution of CML patients. *Cytometry B Clin. Cytom*., **2011**, *80*(3), 158-166.

[27] Willmore, E.; Errington, F.; Tilby, M. J.; Austin, C. A. Formation and longevity of idarubicin-induced DNA topoisomerase II cleavable complexes in K562 human leukaemia cells. *Biochem. Pharmacol*., **2002**, *63*(10), 1807-1815.

[28] Abdel-Wahab, O.; Levine, R. L. Recent advances in the treatment of acute myeloid leukemia. *F1000 Med Rep*., **2010**, 2, 55.

[29] Pennati, M.; Folini, M.; Zaffaroni, N. Targeting survivin in cancer therapy: fulfilled promises and open questions. *Carcinogenesis*, **2007**, *28*(6), 1133-1139.

[30] Hansen, J. B.; Fisker, N.; Westergaard, M.; Kjaerulff, L. S.; Hansen, H. F.; Thrue, C. A.; Rosenbohm, C.; Wissenbach, M.; Orum, H.; Koch, T. SPC3042: a proapoptotic survivin inhibitor. *Mol. Cancer Ther*., **2008**, *7*(9), 2736-2745.

[31] Gilmore, T. D. Introduction to NF-kappaB: players, pathways, perspectives. *Oncogene*, **2006**, *25*(51), 6680-6684.

[32] Bentires-Alj, M.; Barbu, V.; Fillet, M.; Chariot, A.; Relic, B.; Jacobs, N.; Gielen, J.; Merville, M. P.; Bours, V. NF-kappaB transcription factor induces drug resistance through MDR1 expression in cancer cells. *Oncogene*, **2003**, *22*(1), 90-97.

[33] Wrzesien-Kus, A.; Smolewski, P.; Sobczak-Pluta, A.; Wierzbowska, A.; Robak, T. The inhibitor of apoptosis protein family and its antagonists in acute leukemias. *Apoptosis*, **2004**, *9*(6), 705-715.

[34] Wang, X.; Wang, C.; Qin, Y. W.; Yan, S. K.; Gao, Y. R. Simultaneous suppression of multidrug resistance and antiapoptotic cellular defense induces apoptosis in chemoresistant human acute myeloid leukemia cells. *Leuk. Res*., **2007**, *31*(7), 989-994.

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