DOI 10.1002/mnfr.200500045 Mol. Nutr. Food Res. 2005, *49*, 844 – 850

Xanthohumol kills B-chronic lymphocytic leukemia cells by an apoptotic mechanism

Sofie Lust[1]*, Barbara Vanhoecke[2]*, Ann Janssens[1], Jan Philippe[3], Marc Bracke[4] and Fritz Offner[1]

[1]Department of Hematology, Ghent University Hospital, Gent, Belgium
[2]Department of Gynaecology, Ghent University Hospital, Gent, Belgium
[3]Laboratory of Hematology, Ghent University Hospital, Gent, Belgium
[4]Laboratory of Experimental Cancerology, Department of Radiotherapy and Nuclear Medicine, Ghent University Hospital, Gent, Belgium

B-chronic lymphocytic leukemia (B-CLL) is an indolent lymphoid malignancy with variable prognosis. Adverse prognostic factors comprise treatment resistance, cytogenetics (11q- and 17p-), the presence of unmutated Ig genes, and the more comprehensive activation marker Zap70. In contrast to diminished sensitivity to chemotherapy, Zap70+ B-CLL cells retain their responsiveness to manipulation of signal transduction and monoclonals. Xanthohumol (XA) has recently been documented to have an impact on breast cancer cell growth and invasiveness *in vitro*. Based on these observations, lymphocytes from patients with B-CLL were cultured in the presence of XA *in vitro*. XA induced a dose-dependent killing of B-CLL cells at an $LD_{50}^{(24\ h)}$ of 24.4 ± 6.6 μM, independent of known adverse prognostic factors including functional loss of p53. Cell death was associated with poly (ADP)-ribose polymerase cleavage and annexin V positivity, suggestive of an apoptotic mechanism. Surprisingly, $p70^{S6K}$ phosphorylation was stimulated upon XA treatment. In conclusion, XA has an antitumor activity on B-CLL cells *in vitro*. The molecular mechanisms behind this pro-apoptotic effect deserve further investigation.

Keywords: B-chronic lymphocytic leukemia cells / Cell death / Phosphorylation / $p70^{S6K}$ / Xanthohumol

Received: April 1, 2005; revised: June 30, 2005; accepted: June 30, 2005

1 Introduction

B-chronic lymphocytic leukemia (B-CLL) is the most common form of leukemia in the Western world. Its clinical course is characterized by slow accumulation of mature nonfunctional lymphocytes in the blood, bone marrow, spleen, and lymph nodes [1–3]. The prognosis depends on lymphocyte doubling time, extent of disease, and intrinsic adverse characteristics such as cytogenetic abnormalities (11q-, 17p-, respectively, associated with ATM and p53 deletions [4]), activation markers (CD38+, Zap70+ [5–8]), and unmutated immunoglobulin gene mutation status [8, 9], making (often asymptomatic) survival range between less than 4 to over 15 years. There is currently no curative treatment for B-CLL, but an increasing number of products inducing durable objective responses can be used so far with little effect on the ultimate natural course of the disease [1, 10]. In treatment-resistant end stage, B-CLL leads to cachexia and progressive immune deficiency, and infection is the usual cause of death. B-CLL cells do not differentiate to plasma cells and can probably not re-enter germinal center reactions but retain many functional characteristics of normal B-cells *in vitro*, and their survival can therefore be influenced through B-cell receptor (BCR) stimulation (by sIgM crosslinking) [11], T-cell [12] and integrin (CXCR4-CCL12) interactions [13]. This makes manipulation of the transduction pathways of these signals attractive targets for treatment. The observation that B-CLL cells go readily into apoptosis *in vitro* over 7 days despite their excellent survival *in vivo*, suggests stimulation of a pro-survival pathway *in vivo* that can eventually be manipulated. The phenomenon of *in vitro* apoptosis has been explained by various well tested hypotheses: the concentration of serum albumin [14, 15], the presence of stromal interactions with bone marrow stromal cells [16] and so called

Correspondence: Professor Fritz Offner, Department of Hematology, Ghent University Hospital, De Pintelaan 185, B-9000 Gent, Belgium
E-mail: fritz.offner@ugent.be
Fax: +32-9-2402737

Abbreviations: AnnV, annexin V; **B-CLL**, B-chronic lymphocytic leukemia; **Erk**, extracellular-regulated protein kinase; **EtOH**, ethanol; **FBS**, fetal bovine serum; **MAPK**, mitogen-activated protein kinase; **M/U**, mutated *versus* unmutated; **PARP**, poly(ADP)-ribose polymerase; **PI**, propidium iodide; **XA**, Xanthohumol

* These authors have equally contributed to this work.

"splenic nurse cells" [17] keeping B-CLL cells alive through CXCR4/CCL12 interactions. From a signal transduction viewpoint albumin, integrin, and BCR ligation lead to activation of the serine/threonine kinase Akt, and BCR and CXCR4 ligation lead to variable induction of the mitogen-activated protein kinase (MAPK) pathway along p38MAPK and extracellular-regulated protein kinase (Erk) phosphorylation [11, 15, 18, 19].

Plant-derived flavonoids are known in other cell systems to interfere with survival in MAPK dependent ways. The hop-derived flavonoid xanthohumol (XA) has recently received attention as a chemopreventive agent in breast cancer models [20]. We have recently demonstrated that XA can induce cell death in the breast cancer cell line MCF7/6 [21] and the erythroleukemia cell line K562 (data not shown). This effect on cell death coincided with a prolonged stimulation of Erk phosphorylation. This prompted us to investigate the effect of XA on the *in vitro* survival and signaling of B-CLL cells of 16 patients.

2 Patients and methods

2.1 Patient characteristics

Blood samples were obtained from 16 patients with B-CLL after informed consent according to institutional guidelines. Patients demographics, time from diagnosis, and disease characteristics are summarized in Table 1. Seven patients had Rai stage 0, six stage 1, two stage 2, and one stage 3 disease. Ten patients had 5/5 and six 4/5 CLL scores, two were CD38+, six were Zap70+. Lymphocyte doubling time was shorter than 1 year in 5/14 patients, longer in 9/14, and unavailable in two: one patient required treatment because of large and increasing nodal mass in the absence of significant circulating lymphocytes and one because of too recent follow-up. Fluorescence *in situ* hybridization cytogenetics showed one patient with 17p-, four with trisomy 12, and four with deletion 11q-. Five patients had unmutated immunoglobulin genes, two were biallelic mutated *versus* unmutated (M/U), and nine had mutated immunoglobulin heavy chain genes.

Three patients never required any treatment, six were treated with chlorambucil for one episode at least 3 years previously, seven required several episodes of treatment (splenic or local radiation in five, repeated chlorambucil episodes in four, and fludarabine and alemtuzumab in two). No patients had received active treatment within the 4 wk prior to blood sampling.

2.2 Cell culture and chemicals

The lymphocytes were isolated using Lymphoprep (Nycomed Pharma, Denmark) and resuspended at a final concentration of $10–30 \times 10^6$ cells/mL in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Greiner Bio-one, Wemmel, Belgium), 100 IU/mL penicillin, 100 µg/mL streptomycin, and 0.56 µg/mL fungizone (Gibco BRL, Merelbeke, Belgium).

Table 1. Patients demographics, time from diagnosis, and disease characteristics

UPN *	Age (years)	Gender (M/F)	Follow-up (months)	Rai stage	Time diagnosis to treatment (months) **	ZAP-70	IgH mutation	LD_{50} (24h) (µM)	LD_{50} (48h) (µM)
UPN 01	75	M	33	1	17	pos	U	24.4	19.5
UPN 02	75	M	77	0	51.5	neg	M/U	23.3	20.4
UPN 03	69	M	69	0	na	neg	M	21.8	-
UPN 04	55	F	33	1	6	pos	U	22.2	-
UPN 05	56	M	96	0	85	neg	M	>50	9.2
UPN 06	72	M	35	1	10.5	neg	M	22.5	10.9
UPN 07	63	M	84	0	14	pos	U	40.8	20.8
UPN 08	63	M	79	1	na	neg	M	24	27.5
UPN 09	66	M	115	2	7	neg	M/U	28.2	20.9
UPN 10	41	M	177	2	125.5	pos	U	40.5	-
UPN 11	58	M	217	0	134.5	neg	M	28.3	21
UPN 12	54	F	161	1	0	pos	U	20.3	5.5
UPN 13	71	M	104	0	55	pos	M	19.4	9.8
UPN 14	63	M	1	1	na	neg	M	23.1	19.8
UPN 15	72	M	156	3	0	neg	M	23.8	20.9
UPN 16	66	F	52	0	12	neg	M	26.5	20.8

* UPN: unique patient number

** na: not available

XA was kindly provided by Professor De Keukeleire (Laboratory of Pharmacognosy and Phytochemistry, Ghent University, Belgium) and dissolved in 0.1% ethanol (EtOH) as a stock solution of 50 mM from which further dilutions were made.

Primary antibodies for Western blotting were rabbit polyclonal anti-Erk-1/2, rabbit polyclonal phospho-Erk-1/2, rabbit polyclonal anti-Akt, rabbit polyclonal anti-phospho-Akt, rabbit polyclonal anti-p38MAPK, and rabbit polyclonal anti-phospho-p38MAPK from Cell Signaling Technology (Beverly, USA), rabbit polyclonal anti-phospho-$p70^{S6K}$, rabbit anti-polyclonal $p70^{S6K}$, mouse monoclonal anti-α-tubulin from Sigma, rabbit polyclonal anti-phospho-JNK and rabbit polyclonal anti-JNK from Upstate (Charlottesville, US), and mouse monoclonal anti-poly(ADP)-ribose polymerase (PARP) from BD Pharmingen (California, USA). The secondary antirabbit and antimouse antibody was from Amersham Pharmacia Biotech (Amsterdam, The Netherlands).

2.3 Screening of cell death by flow cytometry

For flow cytometric analysis of cell death, B-CLL cells were washed three times with ice-cold PBS and 1×10^5 cells were stained with annexin V (AnnV)-FITC (Becton Dickinson, San Jose, US) and propidium iodide (PI) (Sigma). The percentage of AnnV-positive B-CLL cells in the lymphocyte gate was measured and results were expressed as percentage living (AnnV−, PI−), early apoptotic (AnnV+, PI–), and late apoptotic/dead cells (AnnV+, PI+). The percent of living cells was normalized to 100% living cells incubated in control medium with 0.1% EtOH. All measurements were made in duplicates and averaged. LD_{50} values were calculated by interpolation on a calculated best fitting curve linking values at 0, 10, and 25 μM XA.

2.4 Cytospin and May–Grunwald–Giemsa staining

Approximately 5×10^4 cells were spun onto a microscope slide for 5 min at $400 \times g$ under medium acceleration in a cytospin centrifuge. After air drying, slides were stained with May–Grunwald–Giemsa stain (Sigma) by a Mirastainer (Merck, Darmstadt, Germany) according to the manufacturer.

2.5 Western blotting

Cells were washed three times with ice-cold PBS before lysis. Cells were lysed with lysis buffer containing 1% Triton X-100, 1% Nonidet P-40, and the following protease inhibitors: aprotinin (10 μg/mL), leupeptin (10 μg/mL) (ICN Biomedicals, Asse-Relegem, Belgium), PMSF (1.72 mM), NaF (100 μM), $NaVO_3$ (500 μM), and $Na_4P_2O_7$ (500 μg/mL) (Sigma). Samples containing equal amounts of protein were prepared by mixing lysates and sample buffer (Laemmli with 5% β-mercaptoethanol) in appropriate amounts and boiling for 5 min. Proteins were separated on a 10% SDS-polyacrylamide gel and transferred onto NC membranes. Immunostaining of the blots was performed using the primary antibodies followed by the secondary antibody conjugated to horseradish peroxidase and detection by ECL reagent (Amersham). Quantitation of the autoradiograms was done using the Quantity One® software (BioRad, Nazareth, Belgium).

2.6 Statistics

Statistical evaluation of the data was performed with the Student's *t*-test.

3 Results

3.1 XA induces *in vitro* dose-dependent cell death in B-CLL cells

The evolution of mean normalized percentages of living, early, and late apoptotic/dead cells treated with 10 and 25 μM XA for the total population after 24 and 48 h is shown in Fig. 1. In conditions using 10% FBS, XA induces no cell death at 10 μM after 24 h (67.2 ± 17.4% viable cells, 10.8 ± 13.5% dead cells, average ± SD, $n = 16$) with a significant increase after 48 h (51.6 ± 22.2% viable cells, 22.4 ± 14.1% dead cells, $n = 14$) in comparison to control (after 24 h: 69 ± 14.9% viable cells and 13.8 ± 9.9% dead cells; after 48 h: 66.5 ± 17% viable cells and 17.5 ± 11.9% dead cells). Twenty five micromoles induced cell death of B-CLL cells after 24 h (34.5 ± 17.9% viable cells, 25.9 ± 16.1% dead cells, $n = 14$) and 48 h (19.4 ± 16.7% viable cells, 44.1 ± 17.8% dead cells, $n = 14$) in a flow cytometric pattern suggestive of apoptosis is shown in Fig. 2. The average $LD_{50\,(n\,=\,14)}$ for B-CLL cells was 24.4 ± 6.6 μM after 24 h treatment.

The influence of 25 μM XA on the morphologic phenotype of B-CLL cells is illustrated by a cytospin preparation stained by May–Grunwald–Giemsa in Fig. 3. Extensive vacuolization, shrinkage, and nuclear fragmentation suggestive of apoptosis can be noticed in the XA-treated cultures.

3.2 XA has equal cell killing potential in M/U B-CLL

Comparison of LD_{50} values for XA in B-CLL cells (combined after 24 and 48 h treatment) from patients with M/U Ig genes (20.7 ± 5.5 and 24.3 ± 11.6 μM), and from patients with *versus* without Zap70 expression (22.3 ± 11.3 *vs.* 21.8 ± 1.3 μM), showed no significant differences. In four

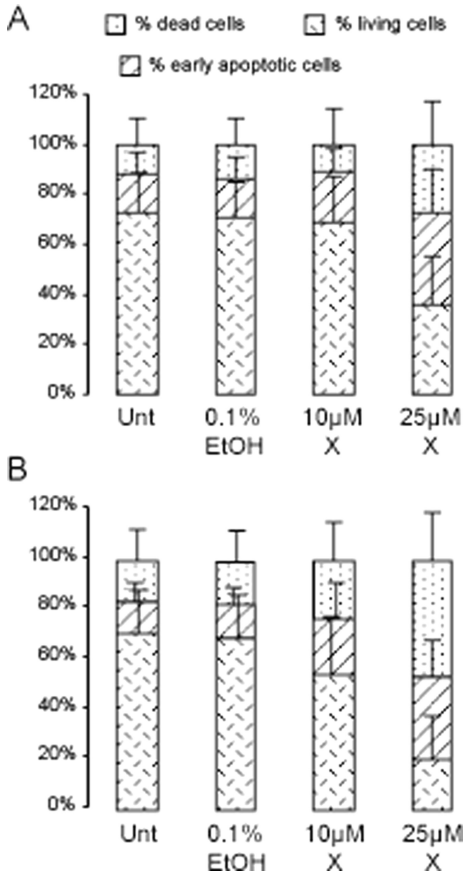

Figure 1. Flow cytometric evaluation of mean normalized percentages + SD of living, early, and late apoptotic/dead B-CLL cells untreated (unt), solvent-treated (0.1% EtOH) or treated with 10 and 25 μM XA for the total population (n = 16) after 24 h (A) and 48 h (B).

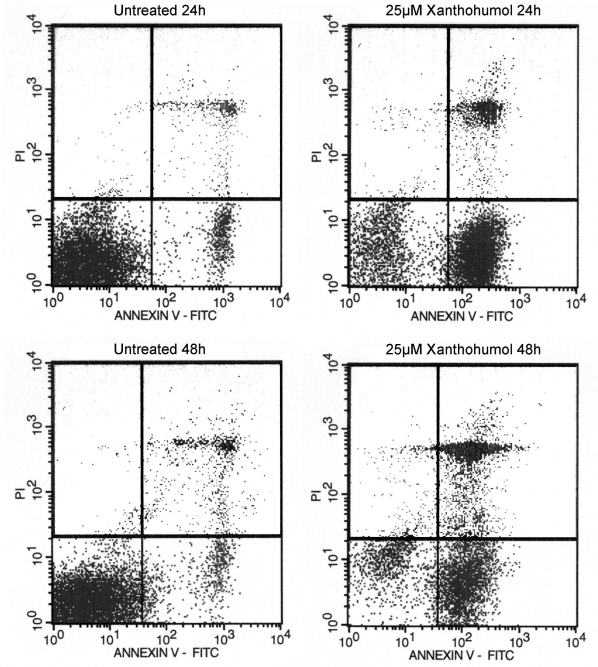

Figure 2. Flow cytometric analysis of cell death of B-CLL cells stained with AnnV-FITC and PI. Cells were treated for 24 h in absence (upper left panel: untreated 24 h) or presence (upper right panel: 25 μM XA 24 h) of 25 μM XA or for 48 h (lower left panel: untreated 48 h; lower right panel: 25 μM XA 24 h). The percentage of AnnV and PI positivity is indicated for B-CLL cells of one patient, representative for a population of 16 patients.

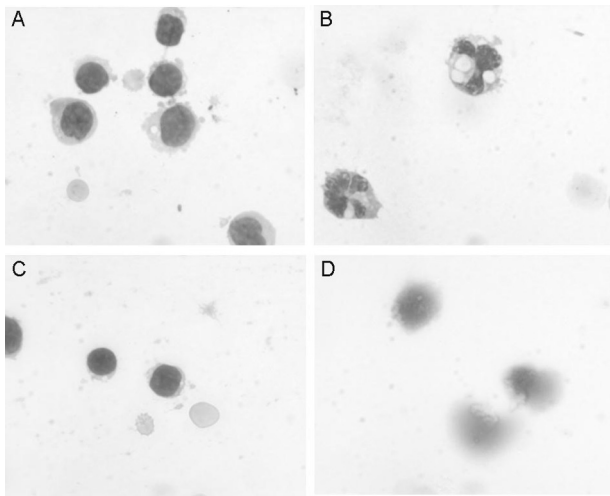

Figure 3. Cytospin preparations of B-CLL cells after 24 h treatment with (A) solvent (0.1% EtOH) or (B) 25 μM XA, showing extensive vacuolization and nuclear blebbing and after 48 h culture with (C) solvent (0.1% EtOH) or (D) 25 μM XA, where residual chromatin smears is disrupted from the nuclei.

patients with 11q-, LD_{50} was variable but in a similar range to others. In the single patient with 17p- deletion, lacking functional p53, no difference in LD_{50} was observed compared to the total average LD_{50} (20.8 μM compared to 24.4 μM after 48 h), suggesting a p53 independent cell killing.

Given the low number of patients studied and the very tight range in which all samples responded to XA, no difference was observed in XA response between patients who had never required treatment and those who continued to progress despite advanced treatment and adverse clinical prognostic characteristics.

3.3 XA induced cell death is associated with PARP cleavage

In previous experiments we found that XA-induced apoptosis of breast cancer and K562 cells, as evidenced by the cleavage of PARP. PARP (116 kDa), a nuclear substrate of caspases, is cleaved into two fragments, an *N*-terminal DNA-binding domain (±24 kDa) and a *C*-terminal catalytic

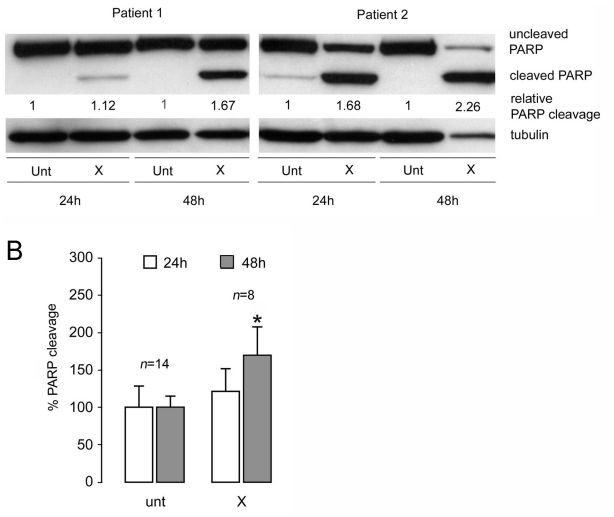

Figure 4. Effect of 25 μM XA on PARP cleavage in B-CLL cells. (A) PARP cleavage in B-CLL cells of two patients is demonstrated after 24 and 48 h treatment with 0.1% EtOH (unt) or 25 μM XA using a specific antibody recognizing the uncleaved (116 kDa) and the cleaved (±89 kDa) band of PARP. Antitubulin staining was performed as a control for loading and relative levels of PARP cleavage were calculated. Western blots of two patients is shown and is representative for the total population. (B) Relative levels of PARP cleavage measured in B-CLL samples after 24 h (n = 14) and 48 h (n = 8) treatment with 0.1% EtOH (unt) or 25 μM XA. Levels were normalized to tubulin expression and expressed as percentage of PARP cleavage (%) as determined by Western blotting. *P < 0.05 relative to levels in the corresponding controls.

domain (±89 kDa). In parallel, we were interested to examine the effect of XA on the cleavage of PARP in B-CLL cells. Cell lysates were used for Western blotting and blots were stained with an antibody against human PARP.

Under conditions using 10% FBS, 25 μM XA-induced PARP cleavage after 24 and 48 h as illustrated for two patients in Fig. 4A. The average cleavage of PARP for XA-treated B-CLL cells is 121 ± 30%, nonsignificantly different from solvent-treated cells (100 ± 28%) at 24 h (n = 14). However, after 48 h cleavage was significantly increased (170 ± 37%) compared to control (100 ± 15%) (n = 8) (p < 0.05) (Fig. 4B).

3.4 XA does not influence Akt, Erk, p38MAPK, JNK phosphorylation but induces p70^{S6K} phosphorylation

A variety of flavonoids is able to modulate cytoplasmic kinases either directly by binding to the ATP pocket or indirectly *via* the interaction with receptors. As demonstrated by our group on MCF7/6 and K562 cells, XA is able to stimulate the phosphorylation of Erk-1/2 (manuscript in preparation). Inspired by this, we performed a phosphorylation screening of a panel of kinases in order to reveal possible signaling molecules influenced by XA in B-CLL cells.

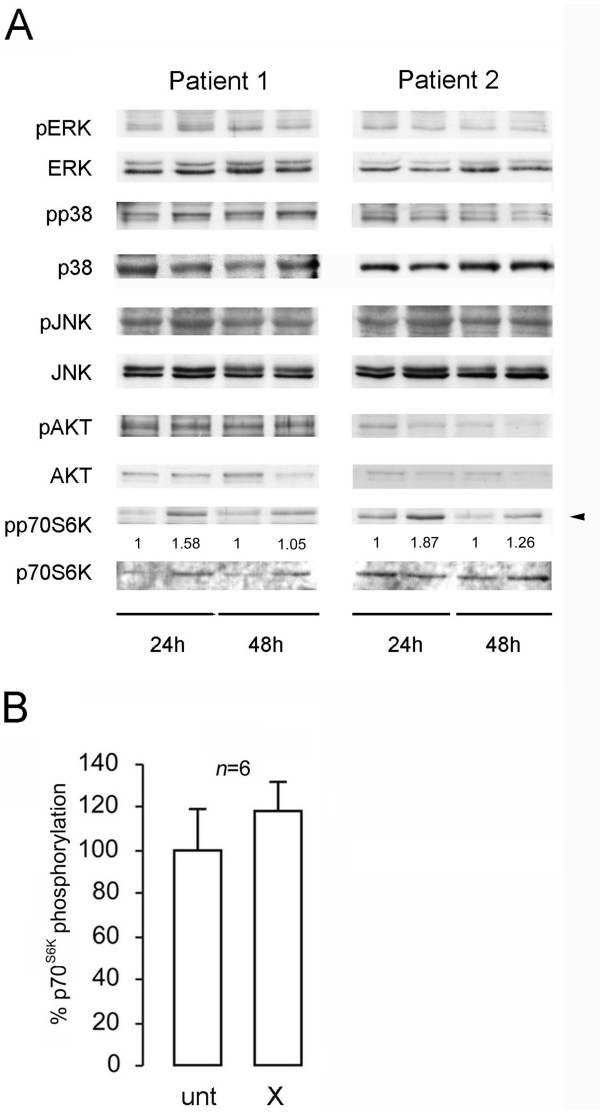

Figure 5. Effect of 25 μM XA on phosphorylation of kinases in B-CLL cells. (A) Phosphorylation of Erk, p38, Akt, JNK, p70S6K was evaluated in B-CLL cells of six patients after 24 h treatment with 0.1% EtOH (unt) or 25 μM XA using specific antibodies. Anti-Erk, p38, Akt, JNK, p70S6K staining was performed as a control for loading and relative levels of phosphorylation were calculated. Western blots of two patients is shown. No modification of phosphorylation levels of Erk, p38, Akt, or JNK could be observed. Stimulation of phosphorylation of p70^{S6K} was noted in four of the six B-CLL samples. (B) Relative levels of p70^{S6K} phosphorylation (normalized to unphosphorylated p70^{S6K} expression) and expressed as percentage of p70^{S6K} phosphorylation (%) as determined by Western blotting. Levels were determined after 24 h treatment in absence (unt) (n = 6) or presence of 25 μM XA (n = 6).

Patient samples were treated for 24 h ($n = 6$) with the vehicle (0.1% EtOH) or with 25 μM XA in serum-containing conditions. After treatment, lysates were made and Western blotting was performed. The effect of XA on the phosphorylation of Erk-1/2, p38MAPK, JNK, Akt, and $p70^{S6K}$ was evaluated. The results demonstrate that after 24 h phosphorylation of $p70^{S6K}$ is significantly stimulated in four of the six patient samples with an average of 58% compared to solvent-treated ($p < 0.05$), as shown in Fig. 5A for two patients. However, after 48 h, this stimulated phosphorylation diminishes. In two of the six patient samples neither a stimulation nor an inhibition of $p70^{S6K}$ could be noticed. Thus, over the total population ($n = 6$), the effect of XA on the phosphorylation of $p70^{S6K}$ is not significantly increased (Fig. 5B). The phosphorylation level of the other protein kinases, however, was not altered.

4 Discussion

This is the first reported study on the influence of XA on B-CLL cells. We demonstrate that XA is capable of inducing dose-dependent cell death in B-CLL cells. Moreover, although the studied population is very limited, this effect seems to be independent of unfavorable prognostic characteristics, previous treatment status, and cytogenetic anomalies known to be associated with chemotherapy resistance. This makes XA one of an increasingly long list of a potentially interesting biologicals that deserve further testing in treatment of B-CLL [22].

On the molecular level, XA-induced cell death of B-CLL cells is associated with PARP cleavage and transition through an AnnV+ stage suggesting an apoptotic mechanism of cell death. Furthermore, the mechanism of apoptosis seems to be caspase-3-independent (manuscript in preparation) and is currently subject of further study.

XA is a prenylated chalcone [20] that is structurally related to flavonoids [23] that have previously been reported to induce apoptosis *in vitro* in B-CLL cells. This is the case for the semisynthetic flavonoid flavopiridol [24], epigallocatechin gallate (EGCG) [22], and we have recently reported analogous results for citrus-derived flavonoids such as tangeretin and nobiletin (data not shown). Apoptosis is also induced in B-CLL cells by the semisynthetic aminoflavone PD98059 [25], which is structurally related and considered as a selective inhibitor of Erk-phosphorylation. The documented mechanisms of action implicated in the induction of apoptosis of B-CLL cells by flavonoids therefore vary widely. For flavopiridol, apoptosis is associated with an inhibition of iNOS expression, a marked down-regulation of NO production and a caspsase-3-dependent cleavage of $p27^{kip1}$ [26]. Nevertheless, structurally nonrelated compounds like roscovitin and olloumicine, well known to induce B-CCL cell death, also act *via* inhibition of Cdk [27–29]. Recently, we demonstrated that XA blocks cell cycle progression of MCF7/6 and K562 cells and that this is associated with an up-regulation of the expression levels of $p21^{CYP1}$, a Cdk2 inhibitor (data not shown). However, this result could not be reproduced with B-CLL cells. Furthermore, flavopiridol-induced apoptosis seems to be associated with activation of p38MAPK and suppression of Erk activity, thereby altering the balance between survival and cell death signals [30]. Also induction of apoptosis in B-CLL by PD98059 and tangeretin is associated with inhibition of Erk phosphorylation (data not shown). However, also other pathways may be implicated with B-CLL cell death. EGCG, for example, decreases vascular endothelial growth factor receptor phosphorylation and induces apoptosis in B-CLL cells *via* STAT signaling [22]. This wide range of potential pathways triggered by structural analogs prompted us to investigate the influence of XA on some major signal transduction kinases involved in cell growth and survival. To our surprise, XA did not affect the phosphorylation levels of Erk-1/2, p38MAPK, JNK, or Akt but stimulated prolonged $p70^{S6K}$ phosphorylation, although not in a statistically significant way. Since $p70^{S6K}$ stimulation is usually observed during protection against cell death rather than during apoptosis, it is intriguing to define its role in the context of XA-induced cell death and is therefore of interest for further research.

In conclusion, we observe a pro-apoptotic effect of the hop-derived chalcone XA *in vitro* in cells obtained from patients with B-CLL, irrespective of their clinical prognostic factors and grade of treatment resistance. Whether the stimulatory effect on the $p70^{S6K}$ pathway contributes to induction of B-CLL cell death is unclear and subject to further research. Although the precise molecular mechanism of action of XA on B-CLL cells is still unknown, its pro-apoptotic effect deserves further attention during development of cellular protocols for the cure of B-CLL.

We thank Jean Roels-Van Kerckvoorde and Joko Vanmeerhaeghe for technical assistance. Barbara Vanhoecke is a recipient of a predoctoral research grant offered by the Special Research Fund of the Ghent University (grant number B/00222/01).

5 References

[1] Byrd, J. C., Stilgenbauer, S., Flinn, I. W., *Hematology (Am. Soc. Hematol. Educ. Program)* 2004, *1*, 163 – 183.
[2] Ferrajoli, A., Keating, M. J., *Hematol. Oncol. Clin. North Am.* 2004, *18*, 881 – 893.
[3] Rai, K. R., Dohner, H., Keating, M. J., Montserrat, E., *Hematology (Am. Soc. Hematol. Educ. Program)* 2001, *1*, 140 – 156.
[4] Stilgenbauer, S., Bullinger, L., Lichter, P., Dohner, H., *Leukemia* 2002, *16*, 993 – 1007.

[5] Crespo, M., Bosch, F., Villamor, N., Bellosillo, B., *et al.*, *New Engl. J. Med.* 2003, *348*, 1764 – 1775.

[6] Hamblin, T. J., *New Engl. J. Med.* 2004, *351*, 856 – 857.

[7] Orchard, J. A., Ibbotson, R. E., Davis, Z., Wiestner, A., *et al.*, *Lancet* 2004, *363*, 105 – 111.

[8] Wiestner, A., Rosenwald, A., Barry, T. S., Wright, G., *et al.*, *Blood* 2003, *101*, 4944 – 4951.

[9] Damle, R. N., Wasil, T., Fais, F., Ghiotto, F., *et al.*, *Blood* 1999, *94*, 1840 – 1847.

[10] Kay, N. E., Hamblin, T. J., Jelinek, D. F., Dewald, G. W., *et al.*, *Hematology (Am. Soc. Hematol. Educ. Program)* 2002, *1*, 193 – 213.

[11] Bernal, A., Pastore, R. D., Asgary, Z., Keller, S. A., *et al.*, *Blood* 2001, *98*, 3050 – 3057.

[12] Schattner, E. J., *Lymphoma* 2000, *37*, 461 – 472.

[13] Mohle, R., Failenschmid, C., Bautz, F., Kanz, L., *Leukemia* 1999, *13*, 1954 – 1959.

[14] Wickremasinghe, R. G., Ganeshaguru, K., Jones, D. T., Lindsay, C., *et al.*, *Br. J. Haematol.* 2001, *114*, 608 – 615.

[15] Jones, D. T., Ganeshaguru, K., Anderson, R. J., Jackson, T. R., *et al.*, *Blood* 2003, *101*, 3174 – 3180.

[16] Lagneaux, L., Delforge, A., Bron, D., De Bruyn, C., *et al.*, *Blood* 1998, *91*, 2387 – 2396.

[17] Burger, J. A., Tsukada, N., Burger, M., Zvaifler, N. J., *et al.*, *Blood* 2000, *96*, 2655 – 2663.

[18] Till, K. J., Spiller, D. G., Harris, R. J., Chen, H., *et al.*, *Blood* 2005, *105*, 4813 – 4819.

[19] Kawauchi, K., Ogasawara, T., Yasuyama, M., *Int. J. Hematol.* 2002, *75*, 508 – 513.

[20] Gerhäuser, C., Alt, A., Heiss, E., Gamal-Eldeen, A., *et al.*, *Mol. Cancer Ther.* 2002, *1*, 959 – 969.

[21] Vanhoecke, B., Derycke, L., Van Marck, V., Depypere, H., *et al.*, *Int. J. Cancer.* 2005. DOI: 10.1002/ijc.21249.

[22] Lee, Y. K., Shanafelt, T. D., Bone, N. D., Strege, A. K., *et al.*, *Leukemia* 2005, *19*, 513 – 523.

[23] Stevens, J. F., Page, J. E., *Phytochemistry* 2004, *65*, 1317 – 1330.

[24] Byrd, J. C., Shinn, C., Waselenko, J. K., Fuchs, E. J., *et al.*, *Blood* 1998, *92*, 3804 – 3816.

[25] Thomas, A., Pepper, C., Hoy, T., Bentley, P., *Leuk. Lymphoma* 2004, *45*, 997 – 1008.

[26] Billard, C., Kern, C., Tang, R., Ajchenbaum-Cymbalista, F., *et al.*, *Leukemia* 2003, *17*, 2435 – 2443.

[27] Decker, T., Hipp, S., Hahntow, I., Schneller, F., *et al.*, *Br. J. Haematol.* 2004, *125*, 141 – 148.

[28] Alvi, A. J., Austen, B., Weston, V. J., Fegan, C., *et al.*, *Blood* 2005, *105*, 4484 – 4491.

[29] Hahntow, I. N., Schneller, F., Oelsner, M., Weick, K., *et al.*, *Leukemia* 2004, *18*, 747 – 755.

[30] Pepper, C., Thomas, A., Fegan, C., Hoy, T., *et al.*, *Leuk. Lymphoma* 2003, *44*, 337 – 342.

