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Red wine polyphenols cause growth inhibition and apoptosis in acute lymphoblastic leukaemia cells by inducing a redox-sensitive up-regulation of p73 and down-regulation of UHRF1

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ABSTRACT

Several epidemiological studies suggest that a diet rich in fruits and vegetables, which contain high levels of polyphenols, is associated with a reduced risk of cancer. The aim of the present study was to determine whether a red wine polyphenolic extract (RWPs, a rich source of polyphenols; 2.9 g/L) affects the proliferation of human lymphoblastic leukaemia cells (Jurkat cells) and, if so, to determine the underlying mechanism. Cell proliferation and viability were determined by the MTS and trypan blue exclusion assays, respectively. Cell cycle analysis, apoptosis activity and oxidative stress levels were assessed by flow cytometry, and the expression of p73, UHRF1 and active caspase-3 by Western blot analysis. RWPs inhibited the proliferation of Jurkat cells and induced G0/G1 cell cycle arrest in a concentration-dependent manner. Moreover, RWPs triggered apoptosis, which is associated with an increased expression level of the pro-apoptotic protein p73 and the active caspase-3. RWPs induced apoptosis confirmed by DNA fragmentation analysis, and this effect was associated with down-regulation of the antiapoptotic protein UHRF1. Furthermore RWPs significantly increased the formation of reactive oxygen species (ROS). Intracellular scavengers of superoxide anions (MnTMPyP, MnTBAP, PEG-SOD) prevented the RWPs-induced formation of ROS and apoptosis, while native extracellular superoxide dismutase (SOD) was without effect. In addition, the effect of RWPs on the expression levels of p73, active caspase-3 and UHRF1 was also prevented by MnTMPyP. Thus, these findings indicate that RWPs induce apoptosis in Jurkat cells by a redox-sensitive mechanism involving the intracellular formation of superoxide anions and consequently the up-regulation of p73 and down-regulation of UHRF1.

1. Introduction

Cancer is one of the main causes of death worldwide, claiming over 6 million people each year. Chemoprevention is a promising strategy which uses natural and/or synthetic compounds to reduce the risk of cancer.[1] Epidemiological studies suggest that diets particularly those rich in vegetables and fruits have such chemopreventive activities.[2,3] The beneficial effects of these diets are attributable, at least partly, to polyphenols, which are present at high levels in vegetables and

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fruits. Indeed, several types and sources of polyphenols including green tea catechins have been shown to have anticancer properties in both cancer cell lines and several animal models of tumourigenesis including carcinogenic chemical-induced cancers and grafted cancer cells.[4–9]

Grapes and grape-derived products such as red wine are an abundant source of polyphenols and represent an important dietary component. Epidemiological studies have indicated an inverse correlation between regular and moderate consumption of red wine and the incidence of coronary heart disease and cancer.[10,11] The beneficial effect of grape-derived polyphenols on the cardiovascular system involves several mechanisms, including improvement of the lipid profile, anti-atherosclerotic, anti-hypertensive and anti-inflammatory effects.[12–14] The anticancer properties of grape-derived products have predominantly focused on resveratrol, an antifungal phytoalexin of RWPs found in grapes and grape-derived products. Resveratrol has been shown to exhibit cancer chemopreventive properties, in part, by arresting cell cycle progression and by triggering cell death through a pro-apoptotic pathway.[15–18] However, red wine contains, besides resveratrol, several hundreds of different polyphenols, including anthocyanins and procyanidins, which have been suggested to mediate the protective effects of red wine on the endothelial function.[19,20] Therefore, the aim of the present study was to determine whether RWPs inhibit the proliferation of a human leukaemia cell line, the Jurkat cells, and, if so, to determine the underlying mechanisms.

2. Materials and methods

2.1. Preparation of a red wine polyphenolic extract

RWPs, a dry powder from French red wine (Corbières A.O.C.), were provided by Dr. M. Moutounet (Institut National de la Recherche Agronomique, Montpellier, France) and analysed by Pr. P.L. Teissedre (Faculté d'Oenologie de Bordeaux, Université Victor Ségalen, Bordeaux, France). The extract was prepared and analysed as previously described.[21,22] Briefly, phenolic compounds were adsorbed on a preparative column, then alcohol was desorbed, the alcoholic eluent was gently evaporated and the concentrated residue was lyophilised and finely sprayed to obtain the phenolic extract, which contained 471 mg/g of total phenolic compounds expressed as gallic acid. The phenolic levels in the red wine extract were measured by HPLC. The extract contained 8.6 mg/g catechin, dimers (B1: 6.9 mg/g; B2: 8.0 mg/g; B3: 20.7 mg/g and B4: 0.7 mg/g), anthocyanins (malvidin-3-glucoside: 11.7 mg/g; peonidin-3-glucoside: 0.06 mg/g and cyanidin-3-glucoside: 0.06 mg/g), phenolic acids (gallic acid: 5.0 mg/g; caffeic acid: 2.5 mg/g and caftaric acid: 12.mg/g) and stilbenes (trans-resveratrol: 0.4 mg/g; trans-piceid: 0.9 mg/g).

2.2. Cell line and culture conditions

The Jurkat cell line was maintained in a humidified incubator with 5% CO_2 at 37 °C. Cells were cultured in RPMI-1640 with 10% (v/v) fetal bovine serum (Biowhitaker, Lonza, Belgium), 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma St. Louis, MO).

2.3. Trypan blue exclusion assay

The effect of RWPs on proliferation of Jurkat cells was determined by trypan blue exclusion assay. Jurkat cells were seeded at a density of 10^5 cells/mL in 6-well plates and cultured for 24 h before the addition of either the vehicle (ethanol 0.03%) or RWPs. The cells were collected every 24-h period and resuspended in 0.4% trypan blue (Sigma–Aldrich, St-Quentin Fallavier, France). The number of cells was determined using a haemocytometer.

2.4. MTS assay

Cytotoxicity of RWPs on Jurkat cells was assessed using the MTS assay. Briefly, Jurkat cells (2×10^4) were cultured in complete RPMI-1640 medium in the presence of the vehicle for RWPs (ethanol 0.03%) or RWPs for 24 h in triplicate in 96-well plates. Thereafter, MTS reagent (20 μL per well of Aqueous One® Reagent, Promega, United States of America) was added to each well before incubation of the plate for 2 h. The absorbance was measured at 490 nm using a multiwell ELISA plate reader. The percentage of living cell was calculated as a ratio of the OD value of each sample to the OD value of the vehicle.

2.5. Cell cycle analysis

Cellular DNA content was measured by flow cytometry. Cells (2×10^4) were exposed to either vehicle (ethanol 0.03%) or RWPs for 24 h in 6-well plates. Thereafter, the cells were collected, washed, fixed in 70% ethanol and incubated with RNase (Sigma–Aldrich). After fixation, the cells were washed with PBS

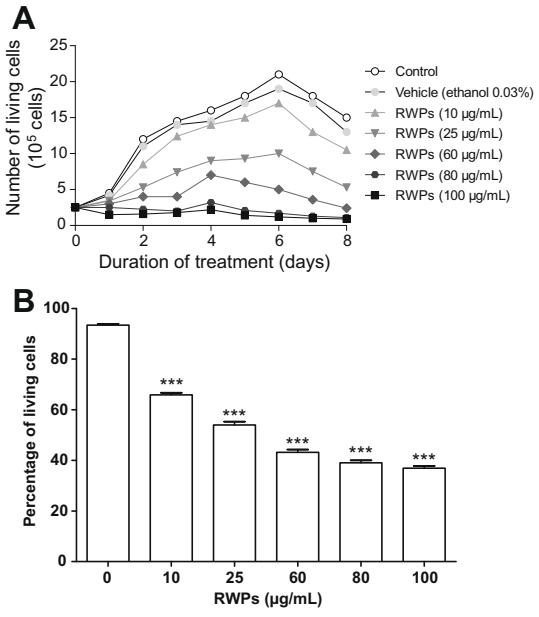

Fig. 1 – RWPs concentration-dependently decrease cell viability of a human leukaemia cell line, the Jurkat cells. Cells were exposed to either vehicle (0.03% ethanol) or RWPs for 24 h. Thereafter, the number of living cells was determined using (A) the trypan blue exclusion assay and (B) the MTS assay. Values are shown as means ± SEM; $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus control.

and stained with the DNA fluorochrome propidium iodide (50 μg/mL, Sigma–Aldrich) for 30 min at room temperature. Propidium iodide fluorescence was measured by flow cytometry (FACScan, BD Biosciences, USA). A minimum of 20,000 cells were acquired per sample, and the data were analysed using the Modfit software. The percentage of cells in G0/G1, S and G2/M was determined from DNA content histograms.

2.6. *Apoptosis analysis*

Annexin V-FITC apoptosis kit (BD Pharmingen, USA) was used to detect early and late apoptosis. Annexin V has a strong affinity for phosphatidylserine, which is externalised in membranes of apoptotic cells. Briefly, cells were exposed to either vehicle (ethanol 0.03%) or RWPs for 24 h before being washed in PBS and resuspended in binding buffer. Thereafter, 5 μL of V-FITC annexin and 10 μL propidium iodide (final concentration of 50 μg/mL) were added to each sample before being incubated in the dark for 20 min. The cells were then subjected to FACS analysis. At least 10,000 events were recorded and represented as dot plots.

2.7. *Assessment of DNA fragmentation*

The percentage of subdiploid cells was determined by staining cells with propidium iodide as described previously.[23] The cells

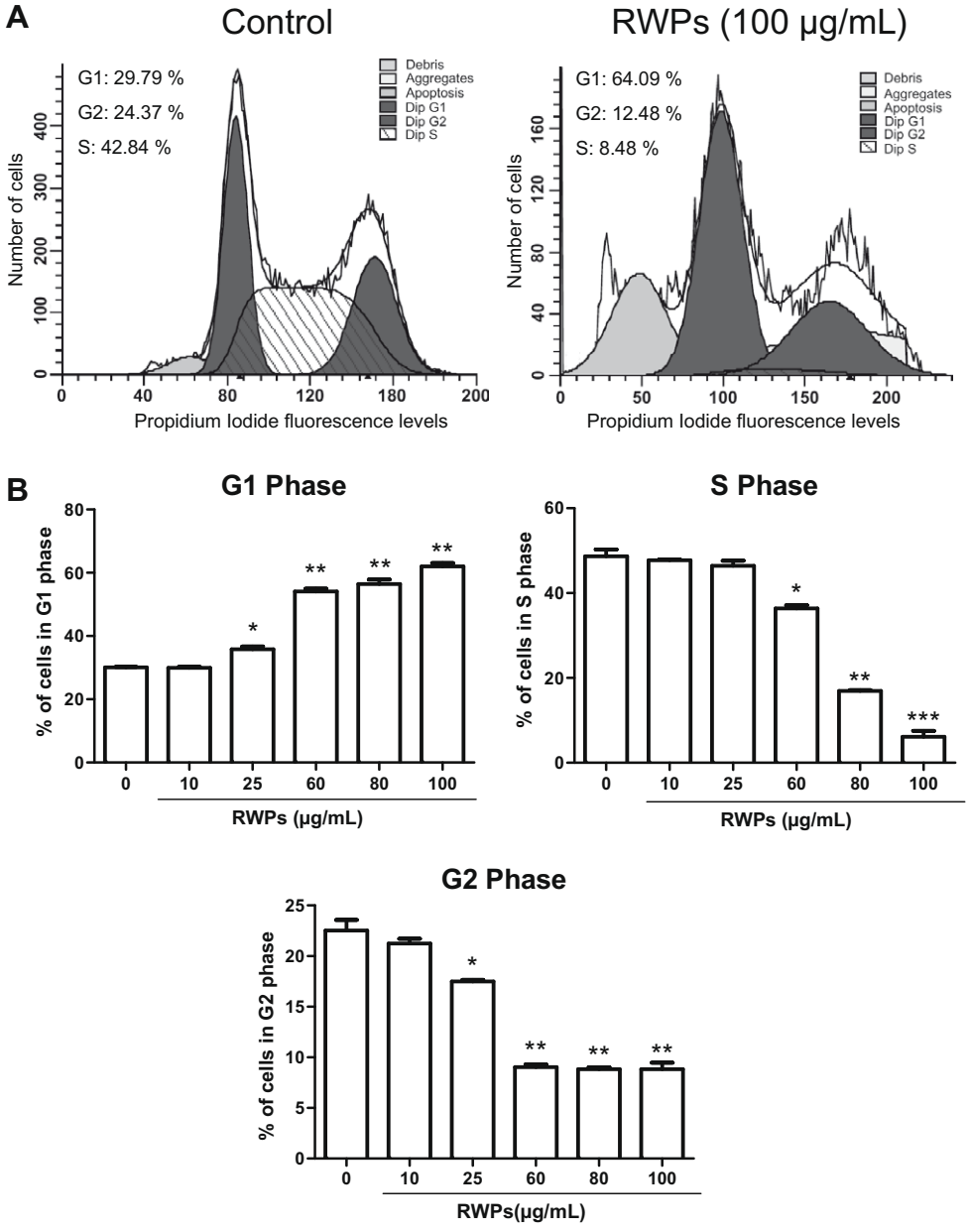

Fig. 2 – RWPs arrest Jurkat cells in the G0/G1 phase of the cell cycle. Cells were exposed to either vehicle (0.03% ethanol) or RWPs for 24 h. Cell cycle was than analysed by flow cytometry using the DNA fluorochrome propidium iodide. (A) Representative flow cytometry analysis of the cell cycle, and (B) corresponding cumulative data. Values are shown as means ± SEM; $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus respective control.

were exposed to either vehicle (ethanol 0.03%) or RWPs for 24 h. Thereafter, the cells were centrifuged at 200*g* for 5 min and resuspended in 500 μL of propidium iodide solution (50 μg/mL propidium iodide, 0.1% Triton-X-100 and 0.1% sodium citrate in PBS) for 20 min at room temperature in the dark. Samples were gently vortexed before being assessed by flow cytometry on the FL-3 channel FACSCalibre, and were analysed using Flow Jo software (version 8.84, Three Star, Inc., USA).

2.8. Determination of reactive oxygen species formation using dihydroethidine

Dihydroethidine, a redox-sensitive fluorescent probe, is rapidly oxidised to ethidium (a red fluorescent compound) by superoxide anions.[24] Ethidium is trapped in the nucleus by intercalating into DNA, leading to an increase of ethidium fluorescence. Cells were exposed to either vehicle (0.03% ethanol) or RWPs for 30 min [corresponding to the early formation of reactive oxygen species (ROS)] and for 24 h (corresponding to the sustained formation of ROS) at 37 °C in 5% CO_2-humidified atmosphere. Dihydroethidine (2.5 μM) was added to each well, and the plate was incubated for 15 min in a 5% CO_2-humidified atmosphere at 37 °C. The cells were then immediately analysed using a FACSCalibre flow cytometer (BD Biosciences). Data were acquired and analysed using the Cell Quest software (BD Biosciences). Histograms of 10,000 events were analysed per experiment. In some experiments, an antioxidant compound was added to cells 30 min before the addition of RWPs.

2.9. Western blot analysis

Cells were exposed to either vehicle (ethanol 0.03%) or RWPs for 24 h. Thereafter, the cells were washed with PBS, resuspended in RIPA buffer (25 mM Tris, pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate and 0.1% SDS; Sigma–Aldrich, USA) containing protease inhibitors and were incubated on ice for 15 min. Cell suspensions were sonicated three times for 30 s, and then were centrifuged at 10,000*g* for 16 min. The supernatant was collected and the protein

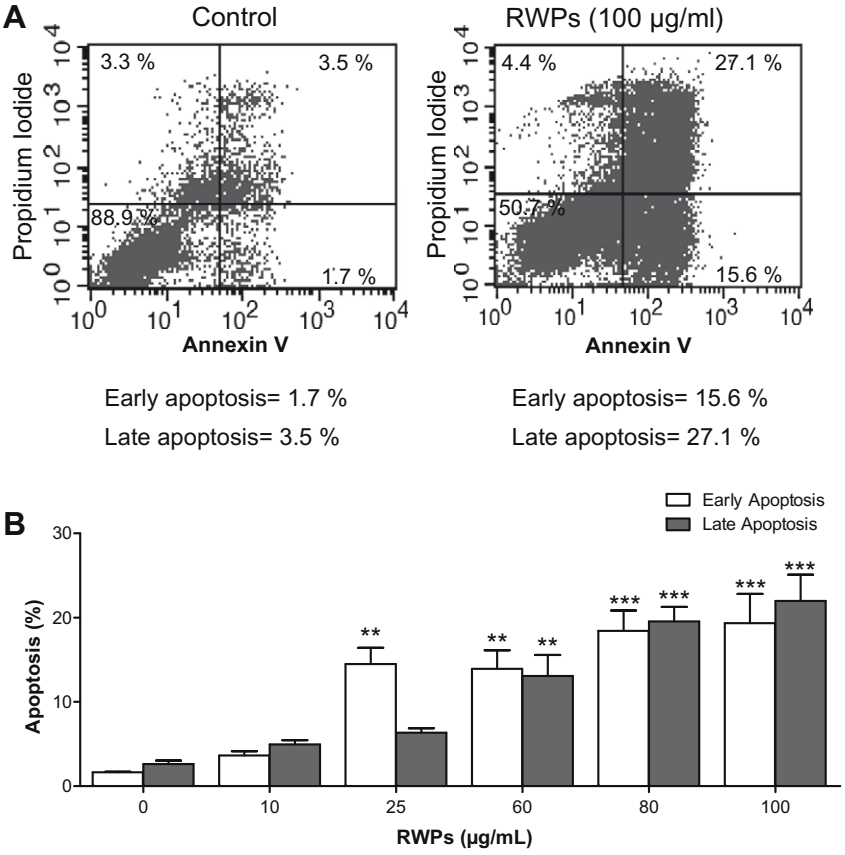

Fig. 3 – RWPs induce apoptosis in Jurkat cells. Cells were exposed to either vehicle (0.03% ethanol) or RWPs for 24 h before being processed for annexin V-FITC/propidium iodide double staining and subjected to FACS analysis. (A) Representative flow cytometry analysis of cells. The lower left quadrant shows cells which are negative for both annexin V-FITC and propidium iodide; the lower right shows annexin V-positive cells, which are in the early stage of apoptosis; upper left shows propidium iodide-positive cells, which are dead, and the upper right shows annexin V- and propidium iodide-positive cells, which are in the stage of late apoptosis. (B) Corresponding cumulative data. Values are shown as means ± SEM; $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus control.

concentration was determined by the Bradford method. Proteins (30 μg) were separated using a 10% SDS–polyacrylamide gel. Following electrophoresis, the proteins were transferred onto a PVDF membrane (Millipore, USA), which was probed with appropriate primary antibodies (p73, UHRF1, human active caspase-3 and ß-tubulin) overnight at 4 °C. The membrane was then incubated for 1 h with the corresponding horseradish peroxidase-linked secondary antibody diluted 1:10,000 in TBS containing 0.05% Tween and 0.5% non-fat dry milk. Immunoreactive bands were detected using the ECL chemiluminescent solution (Amersham™). The membrane was stripped subsequently and reprobed with an anti-tubulin antibody.

2.10. Statistical analysis

All values are expressed as mean ± SEM of three determinations. Statistical evaluation was performed using Student's paired *t*-test or ANOVA followed by Bonferroni *post hoc* test where appropriate. Values of $p < 0.05$ were considered as significant. Levels of significance were indicated as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

3. Results

3.1. RWPs decrease cell viability of Jurkat cells

The effect of RWPs on the proliferation of Jurkat cells was assessed using the trypan blue exclusion assay. As shown in Fig. 1A, RWPs significantly decreased cell viability, after one day and up to 8 days of treatment, in a concentration-dependent manner with an inhibitory effect observed at concentrations greater than 10 μg/mL. Cell viability determined at day 2 indicated that RWPs at 60 μg/mL reduced cell viability by 66.7% whereas 81.2% and 87.5% were observed in response to 80 and 100 μg/mL, respectively (Fig. 1A). The cytotoxic effect of RWPs was further assessed using the MTS assay, which is based on the reduction of MTS by living cells. RWPs decreased the percentage of living cells in a concentration-dependent manner with a signifi-

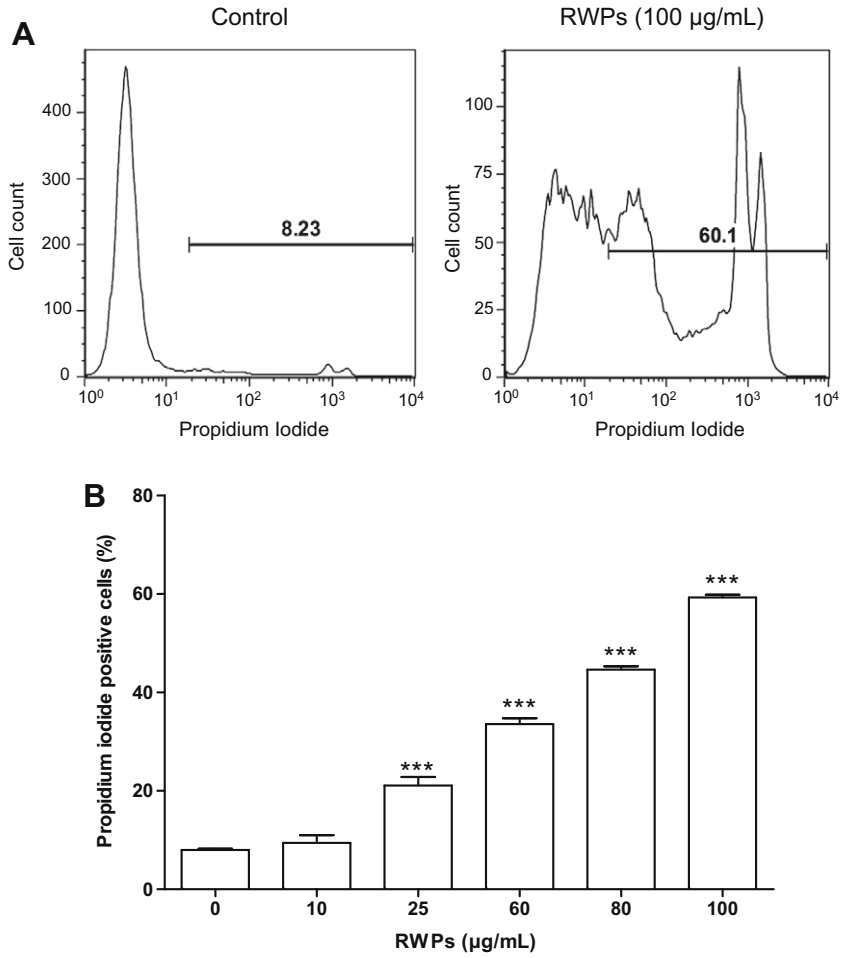

Fig. 4 – RWPs induce DNA fragmentation in Jurkat cells. Cells were exposed to either vehicle (0.03% ethanol) or RWPs for 24 h before being processed for propidium iodide staining and subjected to FACS analysis. (A) Representative flow cytometry analysis of DNA fragmentation in Jurkat cells. The M1 region from 20 to 10,000 corresponds to propidium iodide (PI) positive cells. (B) corresponding cumulative data. Values are shown as means ± SEM; $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus control.

cant inhibitory effect observed with all concentrations (Fig. 1B).

3.2. RWPs cause G0/G1 cell cycle arrest of Jurkat cells

Analysis of the cell cycle of Jurkat cells by flow cytometry indicated the presence of about 29.8% cells in the G1 phase, 42.8% in the S phase and 24.4% in the G2 phase peak (Fig. 2). A small sub G peak mostly corresponding to dead cells was also identified (Fig. 2). Treatment with RWPs significantly increased the percentage of cells in sub G and G1 phases, and decreased those in S and G2 phases in a concentration-dependent manner indicating a G0/G1 cell cycle arrest (Fig. 2).

3.3. RWPs induce an intracellular superoxide anion-dependent apoptosis and DNA fragmentation in Jurkat cells

Phosphatidylserine residues, which are normally located in the internal phospholipid layer, are actively translocated to the external layer in apoptotic cells and they can be detected by annexin V staining. As indicated in Fig. 3, RWPs increased concentration-dependently both early (annexin V-positive cells) and late (annexin V and propidium iodide-positive cells) apoptosis with a statistically significant effect observed at concentrations of or greater than 25 μg/mL. In addition, RWPs induced DNA fragmentation as indicated by the concentration-dependent increase of the percentage of subdiploid cells; this effect was significant at concentrations greater than 25 μg/mL (Fig. 4).

Previous studies have shown that in contrast to the widely antioxidant properties of polyphenols including resveratrol, the growth inhibitory effect of polyphenols on cancer cells is dependent on a pro-oxidant mechanism.[25–27] Therefore, experiments were performed to determine whether the RWPs-induced DNA fragmentation and apoptosis is mediated through the intracellular formation of ROS. The data indicate that treatment of cells with a membrane permeant analogue of superoxide dismutase (SOD) including MnTMPyP, MnTBAP and PEG-SOD markedly reduced both the early and late apop-

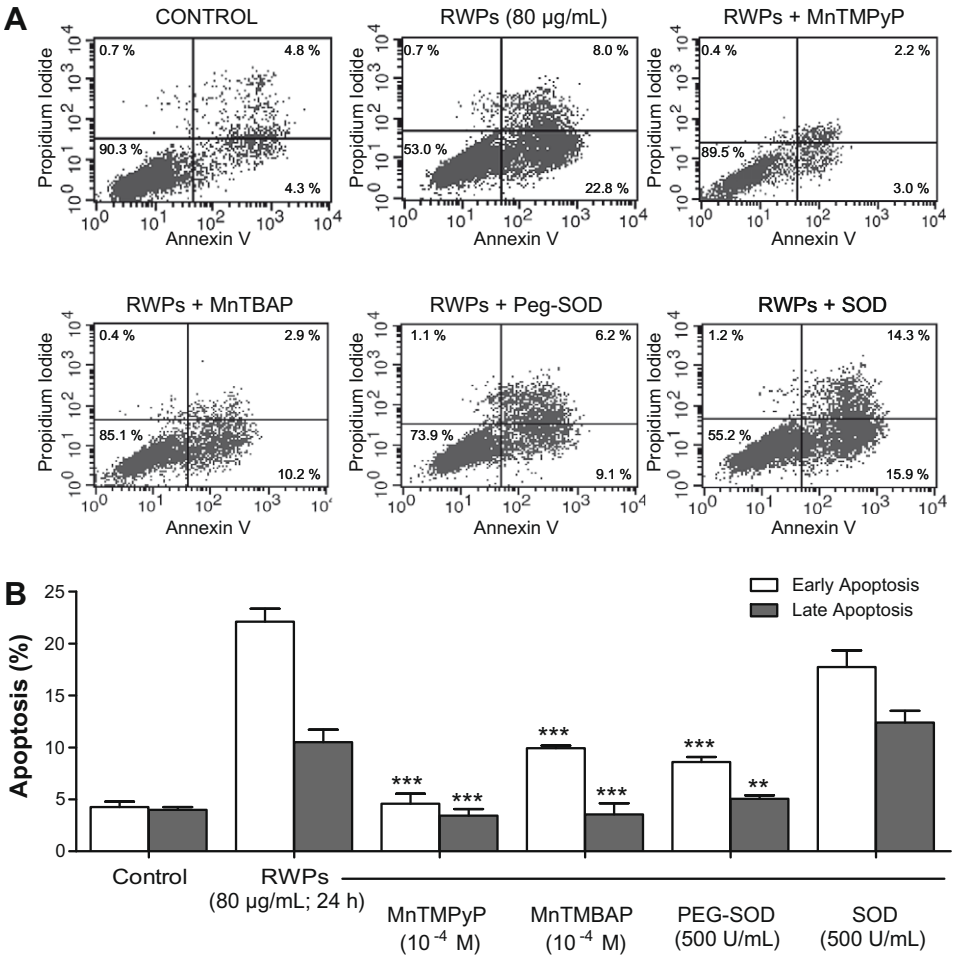

Fig. 5 – The RWPs-induced apoptosis in Jurkat cells requires the intracellular formation of superoxide anions. Cells were exposed to an antioxidant for 30 min before the addition of either vehicle (0.03% ethanol) or RWPs for 24 h. Thereafter, cells were processed for annexin V-FITC/propidium iodide double staining and subjected to FACS analysis. (A) Representative flow cytometry analysis of cells. The lower left quadrant shows cells which are negative for both annexin V-FITC and propidium iodide; the lower right shows annexin V-positive cells, which are in the early stage of apoptosis; upper left shows propidium iodide-positive cells, which are dead, and the upper right shows annexin V- and propidium iodide-positive cells, which are in the stage of late apoptosis. (B) Corresponding cumulative data. Values are shown as means ± SEM; $n = 4$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus RWPs treatment.

tosis induced by RWPs (80 μg/mL) whereas native SOD, which is unable to cross membranes, was without effect (Fig. 5). Thus, these data indicate that the RWPs-induced apoptosis of Jurkat cells is dependent on an intracellular pro-oxidant response involving superoxide anions.

3.4. *RWPs induce the intracellular formation of superoxide anions*

Next, direct evidence that RWPs stimulate the formation of reactive oxygen species was obtained using dihydroethidine. Indeed, exposure of cells to RWPs strongly increased the ethidium fluorescence both after a 30-min and a 24-h treatment period (Fig. 6 and data not shown). The stimulatory effect of RWPs after a 24-h treatment period was significant at concentrations of or greater than 25 μg/mL (Fig. 6). Next, the role of superoxide anions in the pro-oxidant response to RWPs was determined. Treatment of cells with MnTMPyP, MnTBAP or PEG-SOD markedly reduced the stimulatory effect of RWPs whereas native SOD was without effect (Fig. 7). Thus, RWPs triggers a sustained intracellular formation of superoxide anions in Jurkat cells.

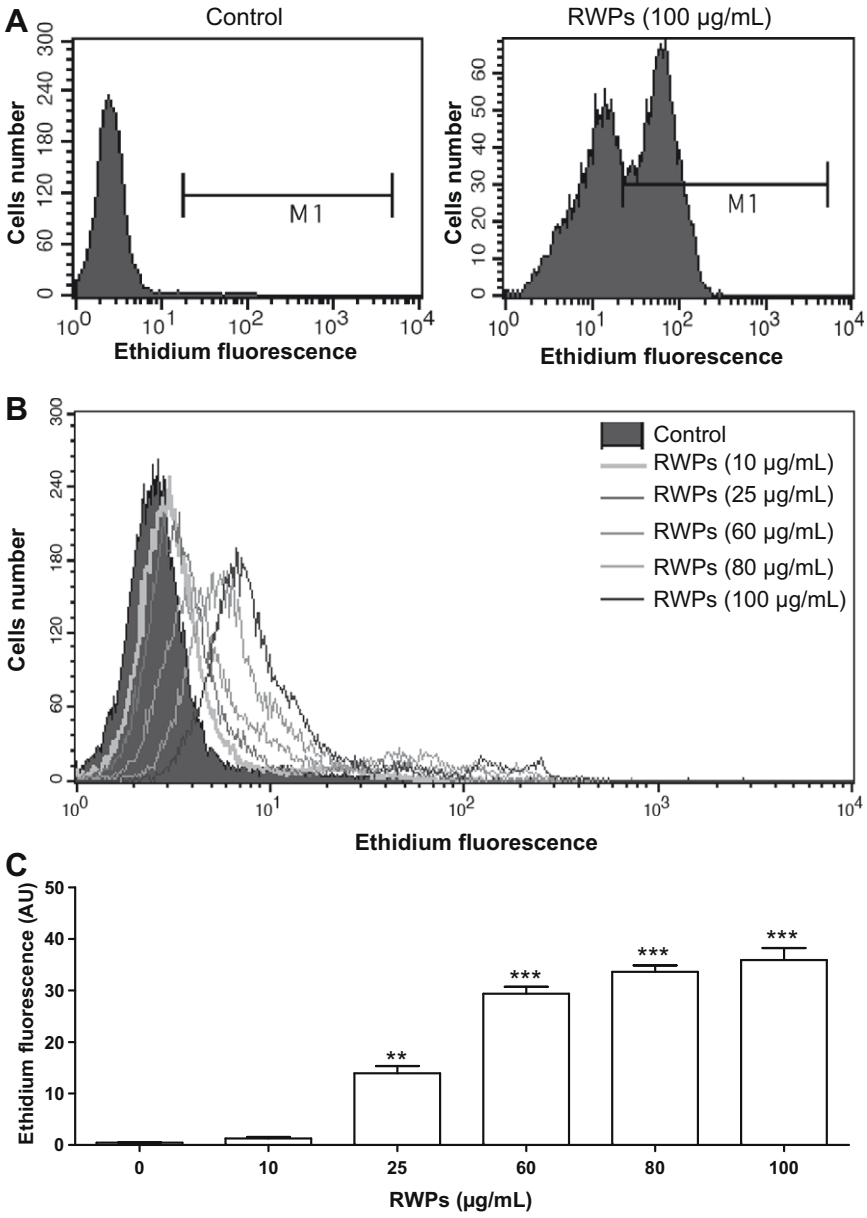

Fig. 6 – RWPs cause the formation of reactive oxygen species in Jurkat cells. Cells were exposed to either vehicle (0.03% ethanol) or RWPs for 24 h before the addition of the redox-sensitive fluorescent probe dihydroethidine (2.5 μM) for 15 min. Reactive oxygen species production was measured as ethidium fluorescence by FACS. (A) Representative flow cytometry analysis of cells; the M1 marker indicates ethidium-positive cells. (B) Overlay showing the concentration-dependent increases in ethidium-positive cells by RWPs. (C), Corresponding cumulative data. Values are shown as means ± SEM; $n = 4$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus control.

3.5. *RWPs cause a pro-oxidant-mediated increase of the expressions of p73 and active caspase-3 and down-regulation of UHRF1*

Next, we determined by immunoblot analysis whether the anti-proliferative and pro-apoptotic effects of RWPs on Jurkat cells are associated with changes in the expression of proteins involved in the control of the cell cycle and apoptosis. As shown in Fig. 8A, RWPs concentration-dependently increased the expression of p73, a tumour suppressor protein involved in G1 cell cycle arrest. RWPs also down-regulated the expression of UHRF1, a protein involved in cell proliferation and G1/S transition.[28–31] In addition, RWPs also upregulated the expression of active caspase-3, a key executor and effector protein of apoptosis (Fig. 8A).[32] Moreover, the role of intracellular superoxide anions in the RWPs-induced changes in protein expression was also examined. Treatment of cells with MnTMPyP abolished the RWPs-induced up-regulation of p73 and active caspase-3, and the down-regulation of UHRF1 (Fig. 8B).

4. Discussion

The present findings indicate that RWPs very effectively inhibited the proliferation and promoted apoptosis in a human T cell acute lymphoblastic leukaemia cell line, the Jurkat cell line. This anticancer activity of RWPs is dependent on a pro-oxidant response involving the intracellular formation

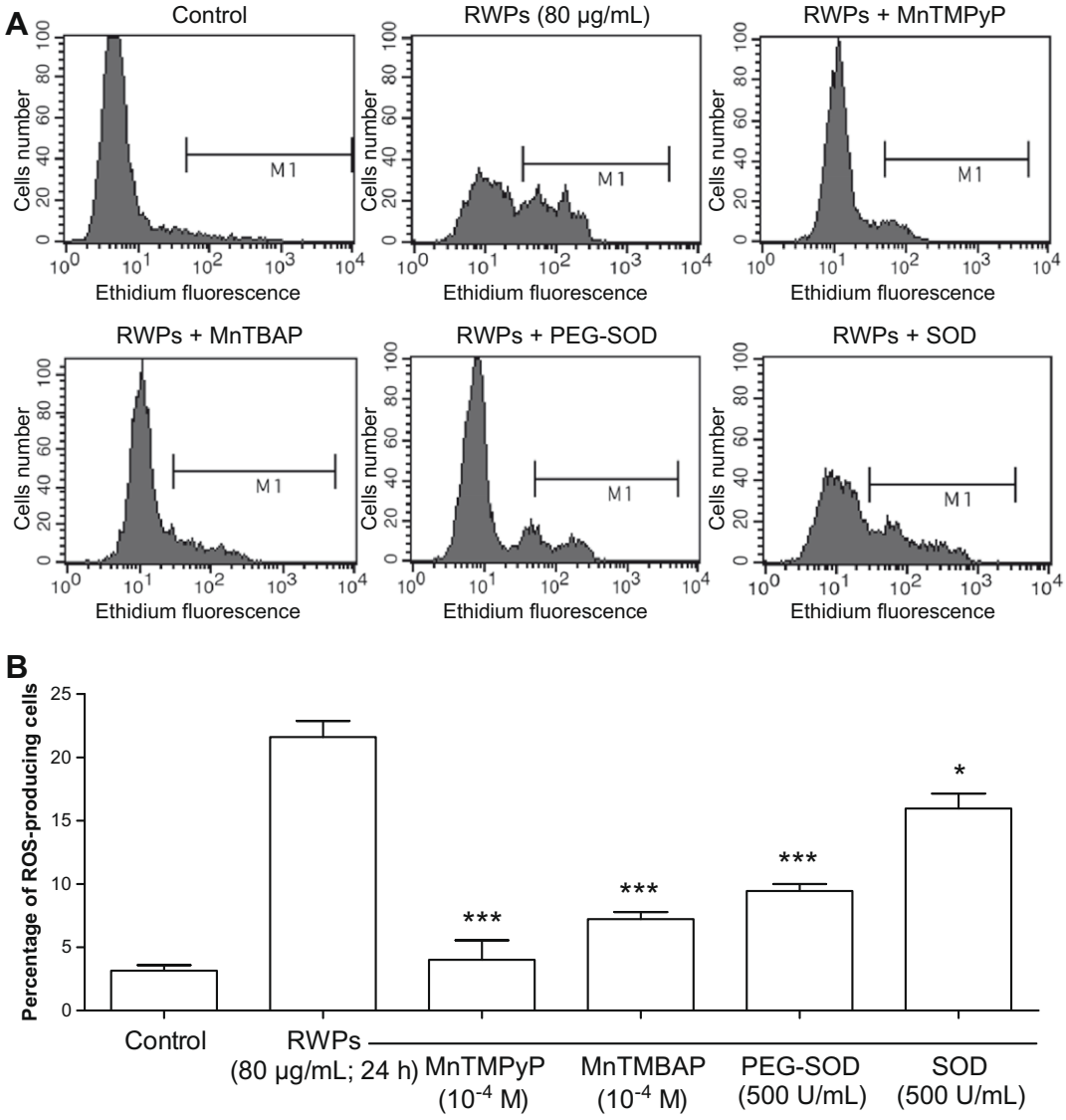

Fig. 7 – The RWPs-induced pro-oxidant response in Jurkat cells involves superoxide anions. Cells were exposed to an antioxidant for 30 min before the addition of either vehicle (0.03% ethanol) or RWPs for 24 h. Thereafter, the redox-sensitive fluorescent probe dihydroethidine (2.5 µM) was added for 15 min. Reactive oxygen species production was measured as ethidium fluorescence by FACS. (A) Representative flow cytometry analysis of cells; the M1 marker indicates ethidium-positive cells. (B) Corresponding cumulative data. Values are shown as means ± SEM; $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus RWPs treatment.

of superoxide anions, which subsequently regulates the expression of major proteins regulating the cell cycle. In particular, a modulation of the expression of the cell cycle check point regulators p73 and UHRF1 was observed. As a conse-

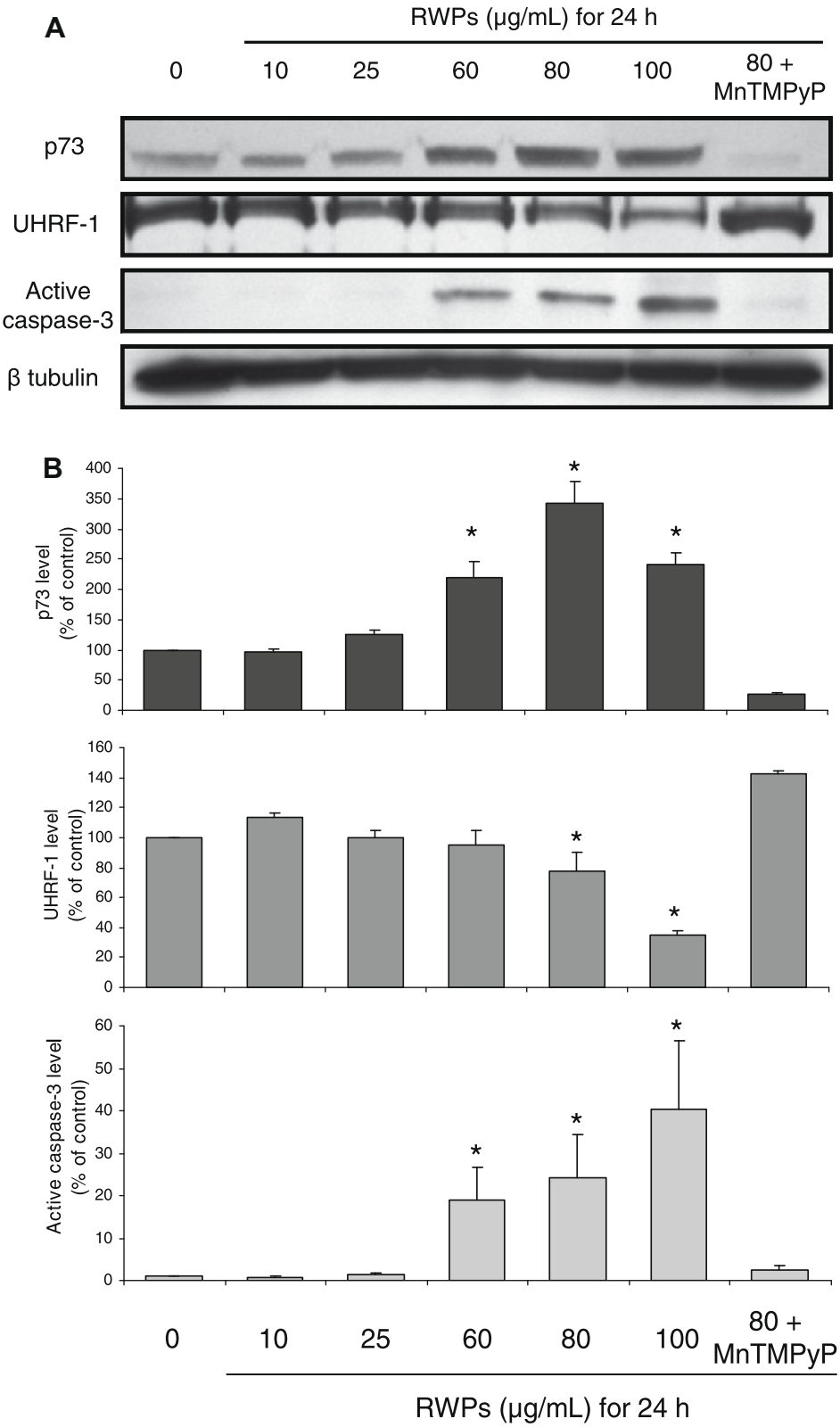

Fig. 8 – RWPs cause the redox-sensitive up-regulation of p73 and active caspase-3 and the down-regulation of UHRF1. Cells were exposed to either vehicle (0.03% ethanol) or RWPs for 24 h. Thereafter, the level of p73, UHRF1 and active caspase-3 was determined by Western blot analysis. (A) Representative immunoblots, and (B) corresponding cumulative data. Values are shown as means ± SEM; $n = 3$. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus control.

quence RWPs-induced pro-oxidant response mediates the up-regulation of active caspase-3, a major effector of the apoptotic pathway. Altogether, these results highlight the potential of grape-derived polyphenols as chemopreventive agents against leukaemia.

Previous studies have shown that grape-derived polyphenols have anticancer activity in a great variety of cancer cells including breast cancer cells, lung cancer cells, colon cancer cells, prostate cancer cells, glioma cells, ovarian cancer cells and oral squamous carcinoma cells.[33–38]The present findings further extents this list since RWPs very effectively inhibited proliferation and promoted apoptosis in a human T cell leukaemia cell line, as indicated by proliferation and apoptosis studies. In addition to Jurkat cells, we have also assessed the cytotoxic effect of RWPs on primary leukaemia cells isolated from a single patient of the University Hospital of Strasbourg (France). These experiments indicated that RWPs decreased the percentage of living cells in a concentration-dependent manner as assessed using the MTS assay (values were 92%, 54% and 36% of living cells, respectively, for control and cells treated with either 25 or 60 μg/mL of RWPs). In addition, the RWPs treatment increased the percentage of apoptotic cells (values were 9.6%, 41.3% and 44.8%, respectively, for control and cells treated with either 25 or 60 μg/mL of RWPs). Thus, these results indicate that RWPs efficiently induced cytotoxic effects on leukaemia cells associated to the induction of apoptosis.

Next, the cell cycle was analysed by flow cytometry to determine the effect of RWPs on cell cycle. These investigations showed that RWPs prevented the progression of Jurkat cells into the S phase indicating that RWPs target crucial events involved in the transition from G1 phase into the S phase of the cell cycle. Previous studies have indicated that several proteins such as those of the p53 family play a key control role in the G1/S transition.[39] Indeed, the p53 tumour suppressor gene has been shown to stimulate the expression of several proteins involved in cell cycle arrest and apoptosis including p21, an inhibitor of the cyclin-dependent kinase 2 and 4, thus preventing cells to pass through to the next stage of cell division. Moreover, introduction of the wild-type p53 gene using an adenoviral vector in pancreatic cancer cells with p53 mutations, effectively induced apoptosis and inhibited cell growth.[40] However, more than 60% of human leukaemic T-cells including the Jurkat cells exhibit resistance to the p53-dependent apoptotic program due to mutations in the p53 tumour suppressor gene.[41] Besides p53, p73 is a p53-related nuclear transcription factor, which can bind to the p53-responsive elements, and transactivate an overlapping set of p53-target genes implicated in G1 cell cycle arrest and apoptotic cell death.[42,43] Of interest, p73 can induce apoptosis in cancer cells exhibiting resistance to the p53-dependent apoptotic program.[43,44] The present findings indicate that p73 is likely to be a mediator of the anticancer activity of RWPs since p73 was markedly upregulated at concentrations inducing growth arrest and apoptosis in Jurkat cells. In addition, UHRF1, a member of a subfamily of RING-finger type E3 ubiquitin ligases, is known to bind to specific DNA sequences, and to recruit a DNA methyltransferase 1 (DNMT 1) to regulate gene expression.[45] UHRF1 plays a major role in the G1/S transition by regulating topoisomerase II alpha and retinoblastoma gene expression, and functions in the p53-dependent DNA damage checkpoint.[30,46] The present findings indicate that RWPs markedly down-regulated UHRF1 in Jurkat cells. Altogether, these findings indicate that the anti-proliferative and pro-apoptotic effects of RWPs involve an up-regulation of the cell cycle inhibitor p73 and a down-regulation of the cell cycle promoter UHRF1. Consequently, RWPs induces apoptosis, as indicated by annexin V staining and DNA fragmentation, and the expression of active caspase-3.

A major novel finding of the present study is that the anticancer effects of RWPs require an intracellular pro-oxidant response involving superoxide anions. Indeed, membrane permeant analogues of superoxide dismutase markedly prevented the pro-apoptotic effect of RWPs whereas native superoxide dismutase, which is unable to cross membranes, was inactive. Such a concept is further supported by the fact that MnTMPyP abolished the effect of RWPs on the expression of p73, UHRF1 and active caspase-3. Moreover, direct evidence that RWPs caused a sustained formation of superoxide anions in Jurkat cells was obtained using a redox-sensitive fluorescent probe. Previous studies have also shown that resveratrol at subapoptotic concentrations induced senescence-like growth arrest in HCT 116 colon carcinoma cells that required a pro-oxidant step.[27]A pro-oxidant response has also been shown to be mediated during the (–)-epigallocatechin-3-gallate-induced apoptosis in pancreatic cancer cells and the human B lymphoblastoid cell line Ramos.[47,48] Moreover, hydrogen peroxide caused an increase in the expression of p53 and p73 that leads to apoptosis in cervical carcinoma cells.[49] Although the source of the RWPs-induced pro-oxidant response in Jurkat cells remains unclear; previous studies have shown that resveratrol and hyroxylated analogues increased the generation of reactive oxygen species involving superoxide anions and hydrogen peroxide. These intracellular reactive oxygen species are predominantly originating from the mitochondrial respiratory chain and reduced oxidative defence subsequently to the down-regulation of mitochondrial superoxide dismutase, MnSOD.[27,50] In conclusion, the present findings indicate that RWPs very effectively caused apoptosis of the T-cell-derived lymphoblastic leukae-

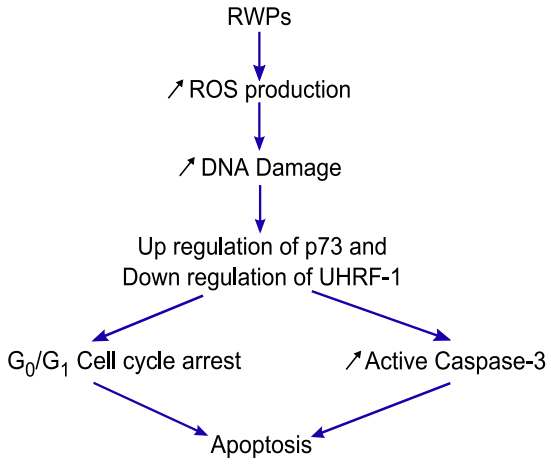

Fig. 9 – Proposed mechanism of the red wine polyphenol-induced apoptosis in Jurkat cells.

mia cells, which is accompanied by growth arrest at an earlier phase of the cell cycle. These responses require a pro-oxidant response involving the intracellular formation of superoxide anions, which, in turn, regulates the expression of key regulators of the G1/S transition of cell cycle and apoptosis namely p73 and UHRF1 (Fig. 9).

Conflict of interest statement

None declared.

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