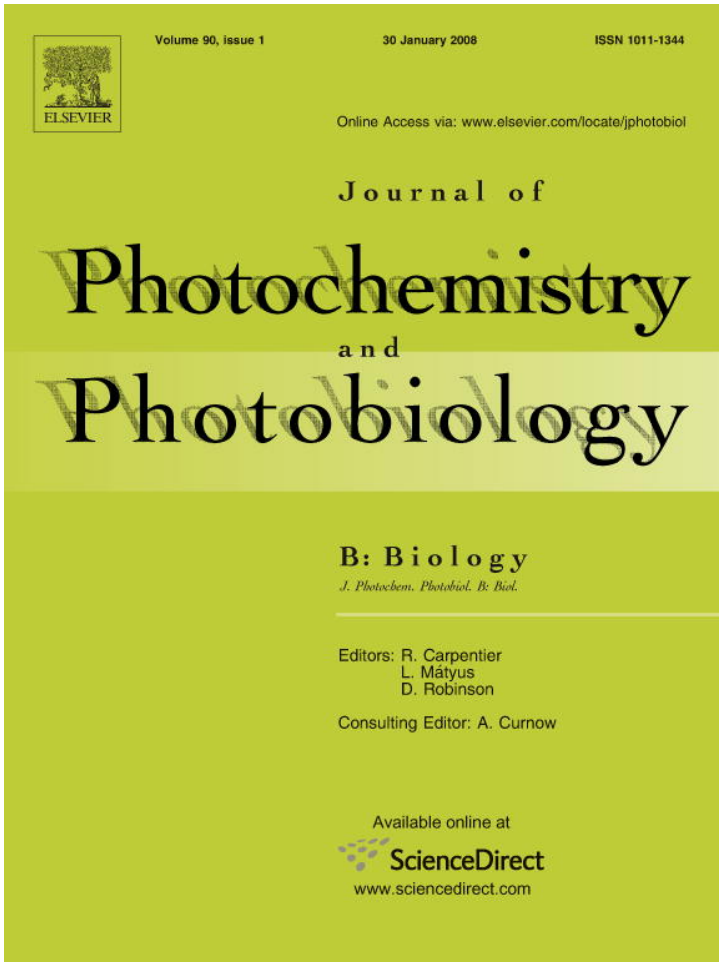

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Only complete rejoining of DNA strand breaks after UVC allows K562 cell proliferation and DMSO induction of erythropoiesis

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Abstract

DNA strand breaks are early intermediates of the repair of UVC-induced DNA damage, however, since they severely impair cellular activities, their presence should be limited in time. In this study, the effects of incomplete repair of UVC-induced DNA strand breaks are investigated on K562 cell growth and the induction of erythroid differentiation by addition of DMSO to the cell culture medium. The kinetics were followed after UV irradiation by single cell gel electrophoresis, and in total cell population by alkaline or neutral agarose gel electrophoresis. Shortly after exposure, an extensive fragmentation occurred in DNA; DNA double strand breaks were negatively correlated with recovery time for DNA integrity. DNA damage induced by UVC 9 J/m^2 rapidly triggered necrosis in a large fraction of irradiated K562 cells, and only 40% of treated cells resumed growth at a very low rate within 24 h of culture. The addition of DMSO to the culture medium of cells 15 min after UVC, when DNA strand break repair was not yet complete, produced apoptosis in >70% of surviving cells, as determined by TUNEL assay. Conversely, if DMSO was added when the resealing of DNA strand breaks was complete, surviving K562 cells retained full growth capacity, and their progeny underwent erythroid differentiation with normal levels of erythroid proteins, δ-aminolevulinic acid dehydrase and hemoglobin.

This study shows that the extent of DNA strand break repair influences cell proliferation and the DMSO induced erythroid program, and the same UVC dose can have opposite effects depending on cellular status.

Keywords: UVC; DNA repair intermediates; SSBs; DSBs; Differentiation; Apoptosis

1. Introduction

The consequence of DNA damage is quite diverse and may involve disturbed DNA metabolism, cell-cycle arrest, cell death and long-term effects such as carcinogenesis [1]. UV-induced lesions are representative of many other agents. With respect to DNA, cyclobutane pyrimidine dimers (CPDs) and the 6–4 photoproducts (6–4PPs) are the predominant lesions caused by short-wavelength (254 nm) UV light (UVC) [2], in humans, these DNA damages are removed by the nucleotide excision repair (NER). This system has been characterized and many aspects of this repair at the molecular level are understood, including the proteins involved in recognition, elimination and resynthesis [3,4].

DNA strand breakage is a major threat to genetic stability, the most common are single-strand breaks (SSBs), and double-strand breaks (DSBs). SSBs are present as intermediates during replication, in Okazaki fragments, and cell differentiation, these physiological SSBs are not harmful to cells if properly repaired. However, SSBs result as enzymatically produced intermediates in Nucleotide Excision Repair (NER) activated in response to the UV-induced CPDs and 6–4PPs UV. Replication forks stall or collapse at DNA lesions, and these events have often been associated with recombination and chromosomal rearrangements. Stalled forks generate SSBs that

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activate the replication checkpoint, which in turn functions to protect the stability of the fork until the replication can resume [5].

DSBs are some of the more detrimental DNA lesions as they disrupt both DNA strands. DSBs can be directly induced by extrinsic agents as reactive oxygen species, ionizing radiation, and chemicals that generate reactive oxygen species. Moreover, the majority of DSBs are produced enzymatically, and arise in DNA as intermediates during mitotic and meiotic recombination, DNA replication, transposition of certain mobile elements, transduction, transformation, and V (D) J recombination in the vertebrate immune system, respectively. The action of restriction endonucleases and topoisomerases also generates DSBs [6]. In eukaryotes, homologous recombination (HR) typically uses the sister chromatid or homolog as a template to repair DSBs in a largely error-free way, whereas non-homologous recombination (NHEJ) directly joins DNA ends in a template-independent manner that, depending on the exact DNA end structure, leads to error-prone DSB repair [4]. After UV irradiation, most cellular activities are temporarily suspended to repair damage to DNA.

Even if they are activated immediately, DNA repair pathways require time to restore original sequence and DNA structure, and during this period normal DNA replication and transcription are modified to cope with specific repair proteins and unscheduled DNA synthesis.

In order to investigate how the induction of a differentiative process could be affected by the presence of DNA strand breaks, we studied, in UVC-irradiated human K562 cells, the effects of SSBs and DSBs on the switch of the erythroid pathway by dimethyl sulfoxide (DMSO). This compound is a polar/apolar inducer and its target is the plasma membrane [7]. In murine erythroleukemia cells has been shown that a cation-dependent modulation of DMSO activity affected early steps of cell commitment and signal transduction. In Mel cells an early event was the activation of a Ca^{2+} Mg^{2+}-dependent endonuclease before commitment to erythroid differentiation by DMSO [8].

DMSO can act as a scavenger of some free radicals (OH radicals) [7], and SSBs and DSBs are induced on DNA early after its addition to murine erythroleukemia cells [9].

While terminal differentiation of myeloid cells may result in apoptosis, it has been shown that differentiating myeloid cells can become resistant to various apoptotic stimuli [10]. This indicates that cellular differentiation can affect apoptosis sensitivity. Here, we report studies on DMSO effects on proliferation and erythropoiesis induction, in UVC irradiated K562 cells, in relation to the kinetics of DNA strand breaks rejoining. We show that there is a precise time window after UVC irradiation of replicating cells in which DNA strand breaks are not yet repaired and DMSO addition leads to cell death.

The relationship between apoptosis and differentiation appears interesting, especially in the possible use of differentiation therapy in combination with irradiation.

2. Materials and methods

2.1. Cell culture and irradiation conditions

The human leukemia K562 cells are widely used as model system for studies on erythropoiesis [11]. These cells were cultured in the RPMI-1640 medium supplemented with 10% fetal bovine serum, penicillin and streptomycin (SIGMA) in a 5% CO_2 atmosphere at 37 °C. For UVC irradiation, exponentially growing cells were pelletted and rinsed with phosphate-buffered saline pH 7.0 (PBS), resuspended in 1 ml of PBS to a thin layer on a plastic dish (without lid) and irradiated with 254 nm UVC (Spectroline lamp) at 4.5 /m^2/s for 2 s (dose = intensity × time = 9 J/m^2) or 3 s (dose = 13.5 J/m^2), at 15 cm of height from the cell monolayer. In all subsequent steps, cells were always kept in the dark. Immediately after irradiation, K562 cells were rapidly pelletted and, within a few minutes (<5 min) of irradiation the cells were processed immediately, or were resuspended at the density of 5×10^4 cells/ml in 5 ml of their prewarmed medium for proliferation and differentiation studies. Cell viability was assessed by the Trypan blue exclusion method, by mixing (1 vol: 1 vol) Trypan blue (4% in PBS) and cells, and counting the number of cells that excluded the dye in a Burker chamber within 5 min of staining.

Erythroid differentiation was started by the addition of 1% DMSO to the culture medium of K562 cells, and the number of differentiated cells was assessed, after a week of cell incubation, using the benzidine test [12]. Hemoglobin was measured spectrophotometrically at 415 nm, in clear cell supernatants of lysed K562 cells that had been incubated for 7 days in a complete medium supplemented with 1% DMSO (induction medium).

Activity of δ-aminolevulinic acid dehydrase (ALA DH) was assessed in 1×10^7 cells, at day 4 of culture in the induction medium, and porphobilinogen was measured in cell lysates according to Sassa and Berstein [13]. The basal value of porphobilinogen in cells cultured without DMSO was subtracted from the value determined in the induced cells, and the obtained value was successively normalized for total proteins in the cell lysate. Protein content was assessed in the clear supernatant of lysed cells measuring OD at 280 nm.

2.2. Apoptosis detection by TUNEL

K562 cells were washed three times in PBS and adjusted to a density of 2×10^7 cells/ml. The cells were then transferred into a V-bottom 96-well microtiter plate (100 μl/well) and fixed in paraformaldehyde solution (4% in PBS, pH 7.4) for 1 h at room temperature, on a shaker to avoid extensive clumping of cells. After PBS washing, the cells were resuspended in a 100 μl/well of permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) and incubated for 2 min on ice. Finally, K562 cells were processed by the "In situ cell death detection kit, Fluorescein"

Boehringer Mannheim, Germany) [14]. Negative controls were incubated with the TUNEL label only, instead of the TUNEL reaction mixture. The observations were made with a Nikon Eclipse fluorescence microscope microscope equipped with an UV lamp. Fluorescent images were captured with an Optronic camera and digitalized with a PC program (Kontron KS300, Kontron Elektronik, Eching, Germany).

UVC-irradiated K562 cells were considered in necrosis when they showed altered cell morphology, positive Trypan blue stain and negative TUNEL staining.

2.3. Single cell gel electrophoresis (SCGE)

SSBs in irradiated cells were analyzed in alkaline conditions, at pH 10, according to Singh et al. [15]; 10 000 cells (in 20 μl) were used for each slide. After electrophoresis, the slides were first neutralized in sodium acetate 300 mM, 90% ethanol for 30 min, and then placed in absolute ethanol, for 15 h at 4 °C, to fix DNA. Finally, the slides were rehydrated in 70% ethanol for 15 min, and DNA was stained with 10 μg/ml DAPI for 3 h. To analyze DSBs, the irradiated K562 cells were lysed for 30 min in a cold solution of the neutral lysis buffer (2% SDS, 25 mM EDTA, 35 mM *N*-lauroyl sarcosine, 10 mM Tris at pH 8), in the dark. Slides were then dipped in PBS and electrophoresis was performed in 45 mM Tris–Borate, 1 mM EDTA, pH 8 at 25 V for 60 min, at 4 °C, in the dark, according to Angelis et al. [16]. Fixation and staining of DNA procedures were identical to those described for SSBs analysis. Images of a least 50 cells were captured by use of a Nikon Eclipse fluorescence microscope equipped with image analyzer Optronics. Only cells with an intact cell membrane were chosen for further analysis. Individual comet images were evaluated with the public image software Autocomet. To quantitate DNA damage in single cells, was evaluated the tail length (TL), that is head diameter subtracted from comet length.

2.4. DNA SSBs and DSBs in total cell lysates

SSBs analysis: aliquots of 1×10^6 UVC-irradiated K562 cells were pelletted and lysed for 2 h at 4 °C in 100 μl of the lysis solution (10 mM Tris at pH 10, 100 mM EDTA, 2.5 M NaCl). Then, 25 μl of the cell lysate was neutralized by the addition of HCl 0.5 M, and 9 μl of cell lysate was then withdrawn and added to 8 μl of loading solution (10 mM Tris HCl pH 6, 10 mM EDTA, 1% Triton X-100, 0.06% BBF, 5.4% glycerol). Thirteen microliters of this sample was finally loaded on a 0.7% agarose gel, in TAE 1X, 1 M urea, and ethidium bromide. The gel was run at 60 V for 1 h at 4 °C in the buffer TAE 1X, 1 M urea.

The presence of DSBs was monitored by analysis of genomic DNA in neutral conditions. After UVC irradiation, $1x10^6$ K562 cells were pelletted and were resuspended in 50 μl of RNase buffer (10 mM Tris HCl at pH 6; 300 mM NaCl; 5 mM EDTA, pH 8), and frozen at –80 °C for 15 min and thawed to obtain a rapid cellular lysis. RNase A (Sigma) 0.01 U was successively added, and thawed cell lysates were incubated for 30 min at 37 °C. Finally, 7 μl of the cell lysate was added to 8 μl of loading solution (10 mM Tris HCl; 1 mM EDTA; 1% Triton-X100; 0.05 BBF; glycerol 5.4%). Electrophoresis was then performed on a 0.7% agarose gel in the same conditions of the SSBs run. DNA markers: λ DNA Hind III digest (SIGMA). DNA fragments migration was calculated by the ratio of the band migration and the front of the gel.

2.5. Data analysis

All experiments were repeated at least three times, each point in duplicate, and data from repeated experiments were analysed for calculation of mean and standard deviation, correlation, and Student's *t*-test for paired values (Excel) ($p < 0.05$ was considered significant).

3. Results

3.1. UVC irradiation caused high mortality in K562 cells

In order to identify the dose of UVC light that was partially cytotoxic, K562 cells were irradiated with UVC light at 9 J/m^2 or 13.5 J/m^2. The higher dose was highly cytotoxic for K562 cells (Fig. 1a), as 60% cells rapidly went into necrosis as confirmed by the cell's positivity to Trypan blue dye and altered cell morphology. At the lower dose 9 J/m^2, only 40% of the irradiated cells survived treatment, after 24 h of incubation, while the remaining cells became necrotic. After a three day arrest in the cell cycle, the 9 J/m^2 UVC-survival cells slowly resumed growth, and at day 6 of incubation, they reached a cell density that was 19% of that of control cells (Fig. 1a). During the first 2–3 days of culture, the number of dead cells remained steady at about 60–40% of total cells, so apparently no further cell death was occurring during the lag phase. During the subsequent days of culture, the surviving cells started to proliferate while the number of Trypan-blue stained dead cells dropped successively to 5%, mainly for cellular lysis (Fig. 1b).

In Fig. 1c are reported the percentages of live and dead control cells. None of the K562 cells which had been irradiated at 13.5 J/m^2 UVC light appeared to resume growth in the subsequent 6 days of culture (Fig. 1a).

3.2. Kinetics of SSBs and DSBs rejoining

DNA damage was assessed in 9 J/m^2 or 13.5 J/m^2 UVC-irradiated K562 cells by the presence of the intermediates of DNA repair SSBs and DSBs. SCGE was used as it is a sensitive and powerful technique to study SSBs and DSBs. Immediately after irradiation (if not stated otherwise), the cells were processed by SCGE in alkaline conditions, to assess SSB formation. DNA fragmentation can be measured from migration of DNA from the nucleus (comets)

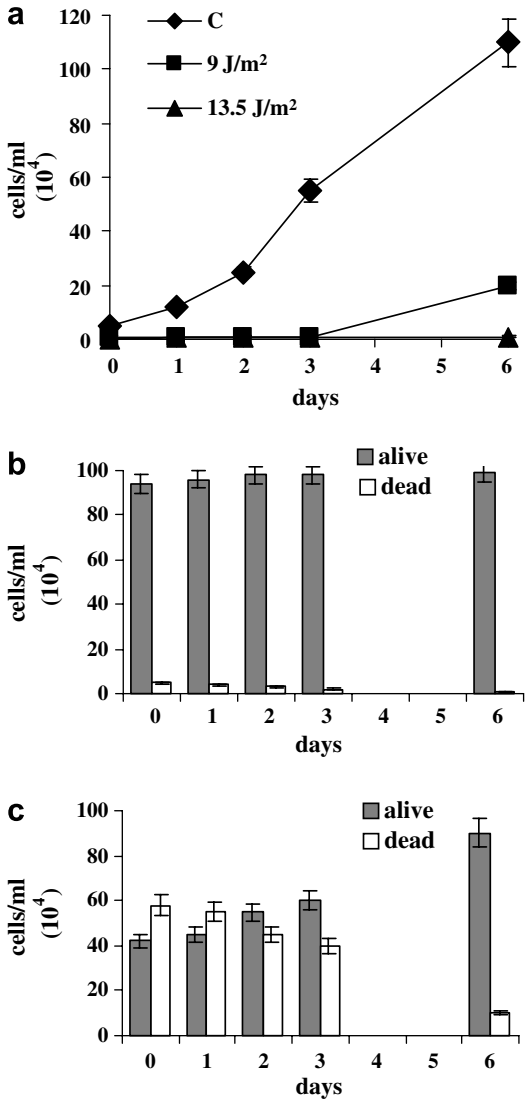

Fig. 1. K562 cells were irradiated at the indicated dose of UVC, then cultured for 6 days in RPMI-1640 plus 10% FBS. (a) cell density of K562 live cells: control, and irradiated with UVC at 9 or 13.5 J/m^2; (b) (conditions as in a) the percentage of 9 J/m^2 UVC-alive and dead cells; (c) (conditions as in a) the percentage of alive and dead control cells. SD are showed.

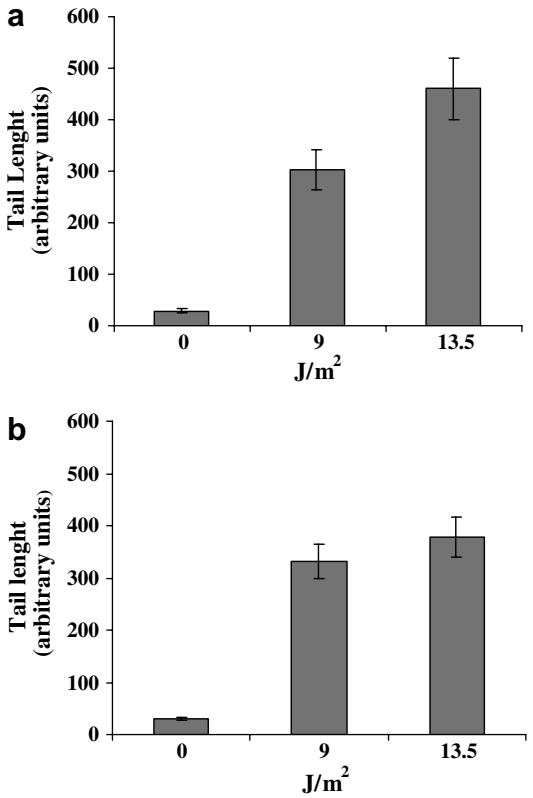

Fig. 2. UVC dose response effect on SSB and DSB formation in K562 cells. After irradiation with 9 J/m^2 or 13.5 J/m^2 UVC, the cells were processed for SCGE: (a) at pH 10, for analysis of SSBs into DNA; (b) or at pH 8, for analysis of DSBs.

by fluorescence microscopy. In control cells the DNA remained mainly in the nucleus. In the irradiated cells the fragmentation was caused by the DNA nicking activities occurring during initial events of recognition of UVC induced damage by DNA repair pathways. Tail length (TL), in control and irradiated cells (Fig 2a) is a parameter that evaluates the occurrence and extent of unrepaired DNA nicks in each cell. Within <5 min from irradiation, the DNA of irradiated cells appeared highly fragmented. A linear correlation was found between dose of UV light and values of TL; the correlation r coefficient was >0.98.

DSBs were monitored by means of SCGE at neutral pH (Fig. 2b). TL values for DSBs again showed a linear dose-relationship between UVC dose and DSBs; the r correlation coefficient for TL, measured in the cells irradiated at the 9 or 13.5 J/m^2 UV dose, was >0.98. However, we showed that DSBs were less frequently produced than SSBs. In order to monitor the time of persistence of DNA strand breaks into the DNA of irradiated cells, the kinetics of disappearance of SSBs and DSBs were studied next. K562 cells were first exposed to 9 J/m ^{2} UVC, and then incubated in a complete prewarmed medium for 0 min, 30 min, 60 min or 120 min at 37 °C for cell recovery. To obtain time 0, the cells were irradiated and then immediately processed for SCGE to prevent DNA repair. At the indicated time, the cells were immediately processed for SCGE in alkaline or neutral pH for SSBs and DSBs analysis, respectively. In Fig. 3a is reported the value of TL obtained from the comets of the K562 cells subjected to alkaline SCGE. At time 0, the DNA appeared highly fragmented, and this indicated that induction of DNA strand breaks is a very early event in the DNA repair processing of UVC damage. Instead, in the cells that recovered for 30 min, the comet's parameters diminished to a value that was 13% of that observed at time 0. This showed that, within 30 min from irradiation, the activated DNA repair rejoined the majority of SSBs. At 60 min and 120 min of cell recovery, the SSBs disappeared almost completely, and TL values were similar to those of unirradiated control cells. An inverse correlation was found between time of cell recovery and SSBs presence in cellular DNA, as r (TL) = −0.738.

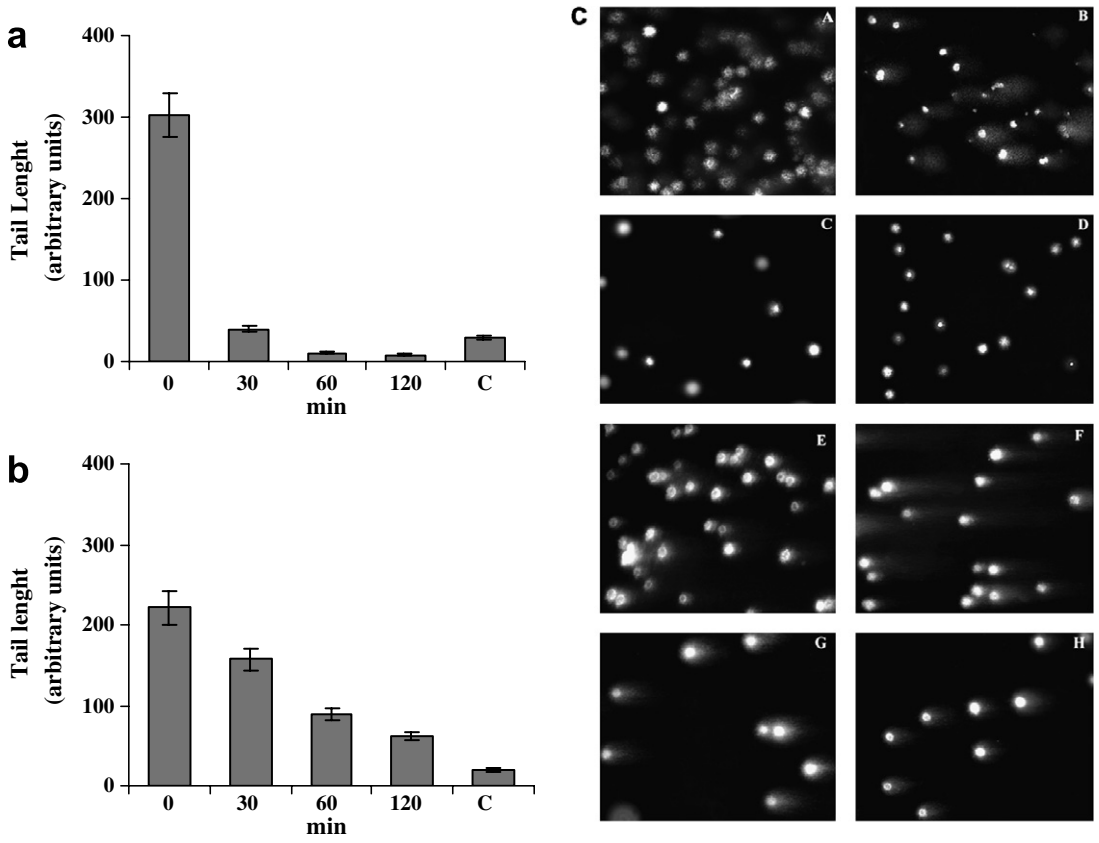

Fig. 3. K562 cells were irradiated with 9 J/m^2 UVC, recovered for DNA repair for the indicated times, then processed for SCGE in alkaline or neutral pH conditions. See text for statistic analysis. (a) SSBs: Tail Length values obtained by SCGE at pH 10 immediately after UVC irradiation (time 0), or at 30, 60, 120 min of DNA repair. c: non-irradiated control cells; (b) SCGE at pH 8, the DSBs were monitored in the same time conditions as a; (c) Comets obtained by SCGE at different times of DNA repair: SSBs detection: (A) Control cells; (B) at time 0 min; (C) at 30 min; (D) at 120 min. DSBs detection: (E) Control cells; (F), at time 0 min; (G) at 30 min; (H) at 120 min.

DSBs showed different kinetics of DNA repair (Fig. 3B). At time 0, all parameters tested showed the highest values. At 30 min, 60 min, and 120 min of recovery from irradiation, TL values respectively were 71%, 40%, and 28% of those obtained at time 0. An inverse correlation was found between time for repair and persistence of DSBs as r (TL) = − 0.93. However, the repair of DSBs was not completed in 120 min, differently from SSBs. In Fig. 3c are shown comets produced by cells processed by SCGE in alkaline or neutral conditions, respectively.

Time-course resealing of SSBs and DSBs was successively examined in total cell population, collected at different times of DNA repair from 9 J/m^2 UVC irradiation, as reported in Section 2.4. Genomic DNA was examined by 0.7% agarose gel electrophoresis. Fig. 4a shows that in alkaline conditions an extensive DNA fragmentation, due to the SSBs and DSBs, was observed at <5 min from 9 J/m^2 UVC irradiation of K562 cells, that corresponded to t0. DNA fragments appeared evenly distributed on gel among high, medium, and low mw. In cells allowed to repair for 15 min, a decrease of the low mw DNA fractions, and a general increase of medium and high mw DNA fractions (>23 kb), were observed. At 60 min or 120 min of DNA repair, the rejoining of SSBs seemed complete and

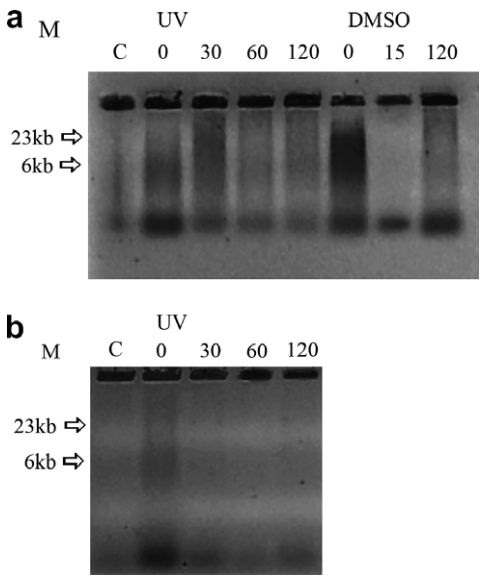

Fig. 4. Analysis of DNA fragmentation caused by SSBs and DSBs in genomic DNA irradiated with 9 J/m^2 UVC. (a) SSBs: from left. Line C, non-irradiated control cells; lines 2–4, times of DNA repair (min); line 5, DMSO added to non-irradiated cells; lines 6–7, DMSO was added to the irradiated cells after the indicated periods of DNA repair (min). (b) DSBs: from left. C, non-irradiated control cells; lines 2–4: times of DNA repair.

the DNA fragments were mainly of high molecular weight (>23 kb). DSB analysis in total cell population by neutral 0.7% agarose gel electrophoresis confirmed previous results obtained by SCGE in neutral conditions (Fig. 4b and 3b). Distance of migration of DNA fragments are reported (Table 1).

3.3. SSBs can affect K562 cell proliferation, and erythroid induction

After UVC irradiation, most cellular activities are temporarily suspended to repair damage to DNA. However, what would happen if new activities are stimulated in these cells before repair completion is not well documented. Thus, we considered of interest to study the effects of SSBs and DSBs on the activation of an inducible and multigenic cellular activity, such as erythroid differentiation, in multilineage K562 cells.

The erythroid pathway can be easily triggered in these cells by the addition of DMSO to their culture medium [11]. So 1% DMSO was added immediately after 9 J/m^2 UVC irradiation (time 0), or at different times of cell recovery from UVC irradiation, at 15 min or at 120 min incubation of cells in a fresh complete medium, at 37 °C. These time periods were chosen because DNA strand breaks were either still present (at 15 min) or were resealed (at 120 min) (Fig. 4a). K562 cells were then incubated, and their ability to differentiate into erythropoiesis and progress in cell growth during subsequent 6 days of culture. As expected, the addition of DMSO to non-irradiated cells caused an

Table 1
DNA fragments electrophoretic mobility

	Alkaline pH			Neutral pH		
	>23 kb	<23 kb	<23 kb	>23 kb	<23 kb	<23 kb
C			0.734	0.145		
UV 0	0.148		0.609		0.272	0.548
UV 30 min	0.148		0.589	0.088		0.533
UV 60 min	0.143		0.544	0.088		
UV 120 min	0.121		0.538	0.024		
DMSO 0			0.488			
DMSO 15 min	0.118					
DMSO 120 min	0.127	0.25				

Distance of migration was calculated from gel pictures showed in the Fig 4a (SSBs) and b (DSBs), respectively.

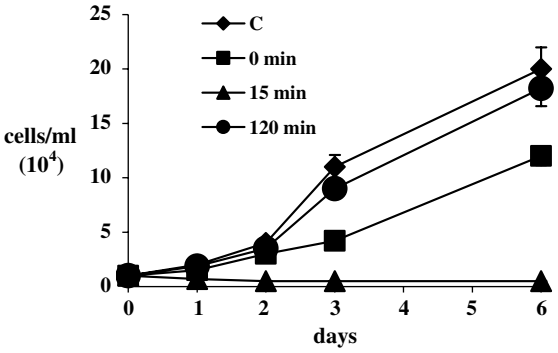

Fig. 5. Recovery of growth in irradiated K562 cells that were successively incubated in complete medium supplemented with 1% DMSO. Live cells are reported. C: control cells; 0: DMSO added immediately after irradiation with 9 J/m^2 UVC; 15 and 120 min: DMSO added at 15 min or 120 min of cell recovery. $p < 0.05$ (DMSO added 15 min after irradiation); $p = 0.2$ (DMSO added 120 min after irradiation) in reference to DMSO added immediately after UVC irradiation.

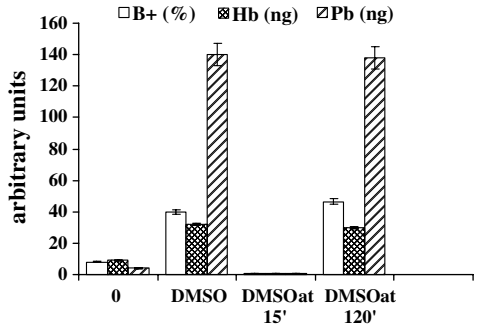

Fig. 6. Expression of erythroid markers. K562 cells were treated as reported in Fig. 5. Aliquots of cells were assayed for: (Pb), ALA DH activity; (Hb), Haemoglobin; (% B+), number of benzidine-positive cells.

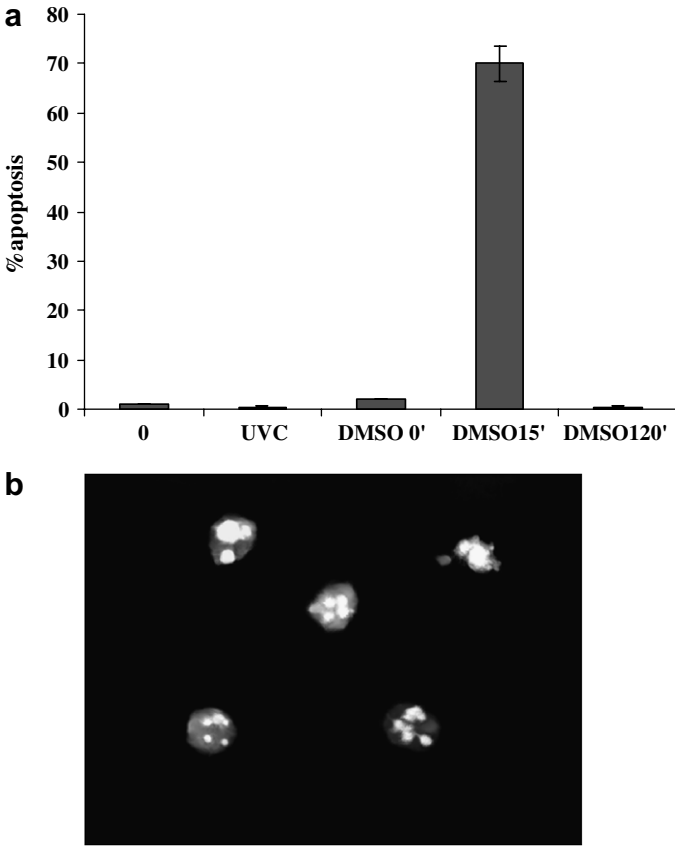

Fig. 7. Apoptosis by TUNEL. At indicated times after 9 J/m^2 UVC irradiation of K562 cells, 1% DMSO was added to the culture medium. (a) control; UVC irradiated; DMSO added at different time from irradiation: 0, 15 min or 120 min; (b) DMSO added at 15 min of cell recovery.

expected initial delay in the cell cycle [12,9], but then cell proliferation and differentiation progressed normally. When added 15 min after UVC irradiation, DMSO caused rapid and extensive cell death (Fig. 5). On the contrary, when the inducer was added to the cell culture medium after 120 min of incubation after UVC exposure, the UVC treated alive K562 cells were able to proliferate and differentiate like the induced non-irradiated control cells.

In Fig. 6 it can be seen that induction to erythroid differentiation progressed normally in the control and the surviving cell population. The markers of erythropoiesis were the number of benzidine-positive cells (% B+), the Hb amount (ng), and the porphobilinogen (ng) as product of ALA DH activity. UV 9 J/m^2 alone cannot switch erythropoiesis in K562 cells (data not shown).

Apoptosis analysis by fluorescence microscopy showed that the K562 cells irradiated with 9 J/m^2 UVC and either exposed to DMSO or recovered 120 min after UVC irradiation, were all negative in the TUNEL assay (Fig. 7a). Conversely, more than 70% of cells were in apoptosis when DMSO was added to the culture medium 15 min after UVC irradiation (Fig. 7a and b).

4. Discussion

In this report, it was analysed how the multilineage human leukemia K562 cells react to a DMSO differentiation stimulus when it is given in the state of ongoing damage repair. We found that DMSO enhanced DNA breakage and has apoptotic effect when UV damage is not yet repaired. Conversely, the inducer has a stimulatory effect on differentiation when DNA strand breaks are resealed as a sub-population of UVC irradiated K562 cells activated DNA repair and escape apoptosis, possibly due to the difference in the position of the cell cycle at the time of irradiation and less damaged DNA. That is not surprising as it has been previously shown that UVC irradiations of proliferating mouse and human erythroleukemia cells can be cytotoxic for a large subpopulation of cells, but do not significantly damage erythroid-specific proteins in the progeny of the alive induced cells that activated DNA repair [9].

Resealing of DNA strand breaks occurs very early during repair, and SSBs and DSBs removal can be achieved by overlapping steps of different pathways of repair [17]. CPD and 6–4 PP photoproducts can be bypassed and stay for a long time in proliferating cells [18,19]. These DNA breaks can potentially strongly affect nuclear activities until they are repaired [20], but normally their persistence is time-limited and depends on how many DNA fragments have to be resealed, cell type and tissue.

Erythroid differentiation is a very complex stepwise process that depends on activation of several erythroid-specific cellular activities, and on repression of many others, to sustain the change from the old cellular state into a new one (the erythrocyte). An increase of SSBs and DSBs is observed on DNA immediately after DMSO addition to the culture medium [21,22]. DNA breaks are produced by several inducers and appear functional to differentiation [21].

K562 cells were irradiated in the exponential growth phase, and the high fragmentation observed is due to physiological and induced different SSBs. Each type of DNA break activates a different signaling, nevertheless DNA strand breaks are dangerous to cells only if left open and unrepaired.

DMSO modulates the cellular surface potential and also it is a hydroxyl radical (HO.) scavenger, so it can affect sensible cellular ongoing processes. A cation-dependent modulation of the inducer activity affected early steps of commitment to erythropoiesis in Mel cells [7]. In U937 cells, induced to monocytes by DMSO, the resistance to peroxynitrite appears to involve an enhanced release of arachidonic acid that prevents the mitochondrial formation of H_2O_2 [23].

A differentiation pathway can be modulated by DMSO by means of different signaling. HL60 cells, induced to granulocytes by Retinoic acids (RA) express CD38 and accumulation of cyclic ADP ribose (cADPR), instead if induced by DMSO, CD38 is not expressed but an overexpression of concentrative cADPR-translocating nucleoside transporters allows cADPR accumulation [24]. In HL60 cells, the tumor suppressor PTEN, an antagonist of phosphatidylinositol 3-kinase (PI 3K), is upregulated by DMSO, while expression of PI 3 K is unaffected and Akt phosphorylation is decreased. The upregulation of PTEN in turn might be mediated by NF-kB activation, as the NF-kB- inhibitor PDTC potently inhibits the apoptotic response following DNA damage [25].

UVC irradiation or DMSO do not activate apoptosis in K562 cells, but DMSO added early after irradiation (15 min), but not at later time, caused a massive apoptosis. It appears that early events of repair of DNA damages and DMSO induction of erythropoiesis came into conflict when temporally overlapping, and as consequence of that signaling of apoptosis are rapidly triggered in K562 cells. Recent data have shown that UV light is a potent inducer of c-fos which is part of the heterodimeric transcription factor AP-1, a key regulator for many processes such as cell growth, differentiation, inflammation, and malignant transformation. Immediate and late induction of the JNC/fos pathway stimulates the repair of UVC induced lesions that protect against apoptosis. If this does not occur, cells do not recover from the transcription blockage, leading to activation of the death receptor pathway [26].

Apoptotic mechanisms play a relevant role in the control of erythropoiesis, and activation of death receptor responses during erythroid differentiation has recently been shown to play an important role in adjusting the rate of erythropoiesis, and are regulated at several different levels [27]. K562 cells are normally highly resistant to death receptor-mediated apoptosis TNF-α, FasL, and TRAIL, but when induced by hemin were potently sensitized to TRAIL by downregulation of the antiapoptotic protein

c-FLIP [28]. Bcl-2 regulated apoptotic pathway is triggered by imatinib through activation of several proapoptotic BH3-only proteins [29]. The differentiation – mediated sensitization to TRAIL occurs also in granulocytic differentiation by DMSO in HL60 cells [30].

The differential activity of DMSO on UVC irradiated K562 cells could relate to an interplay of pro- and antiapoptotic signals and proteins. In addition, attenuation of NER at global genome level is common in differentiating cells [31] as DNA repair scanning activities could interfere with the execution of local chromatin reorganization necessary to change the cellular state. The addition of DMSO early after irradiation seriously challenged the coordination of different systems of repair with differentiation pathway, and apoptosis was induced as proapoptotic signaling prevailed over antiapoptotic [27].

The relationship between irradiation, differentiation and apoptosis, deserves more attention, especially given the possible use of differentiation therapy in combination with irradiation.

5. Abbreviations

4',6' DAPI	4'-6'diamidino-2-phenylindole
6–4PPs	6–4 photoproducts
ALA DH	δ-aminolevulinic acid dehydrase
BBF	bromophenol blue
CPDs	cyclobutane pyrimidine dimers
DMSO	dimethyl sulfoxide
DSBs	double strand breaks
EDTA	ethylenediaminetetraacetic acid disodium salt
Hb	haemoglobin
HR	homologous recombination
NER	nucleotide excision repair
NHEJ	non-homologous end joining recombination
polδ/ε	DNA polymerase δ/ε
RNA pol II	RNA polymerase II
SCGE	single cell gel electrophoresis
SDS	lauryl sulphate sodium salt
SSBs	single-strand breaks
TL	tail length
Tris	Tris(hydroxymethyl)aminomethane
TUNEL	TdT-mediated dUTP nick end labeling

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