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Activation of deoxycytidine kinase by UV-C-irradiation in chronic lymphocytic leukemia B-lymphocytes

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Abstract

Deoxycytidine kinase (dCK), a key enzyme of the deoxynucleoside salvage pathway, might have a preponderant role in DNA synthesis in resting chronic lymphocytic leukemia B-lymphocytes. In these cells, two important enzymes in deoxynucleoside triphosphate production, ribonucleotide reductase and thymidine kinase (TK), both cell-cycle regulated, are indeed very weakly expressed. This study investigated the regulation of dCK activity in response to UV-C light, a condition which causes DNA lesions and DNA repair synthesis. We observed that activity of dCK in B-CLL cells was upregulated up to 3-fold, 30 min after irradiation with 30 J/m^2 UV-C, whereas TK activity was unchanged. Activation of dCK by UV-C light was caused neither by a change in concentration of a low molecular weight metabolite nor by an increase in the amount of dCK protein. Activation of dCK by UV-C was mimicked by H_2O_2, markedly counteracted by *N*-acetylcysteine, a general antioxidant, and completely abolished by the growth factor receptor inhibitor suramin. Taken together, these results indicate that dCK activity is upregulated by UV-C light through a postranslational modification that may be initiated at the cell surface through oxidative mechanisms. Suramin also suppressed the increase in DNA repair synthesis elicited by UV-C irradiation, suggesting that upregulation of dCK activity could contribute to the normal completion of DNA repair synthesis elicited by UV light.

Keywords: *N*-Acetylcysteine; 2-Chloro-2′-deoxyadenosine; DNA repair; H_2O_2; Suramin; Thymidine kinase

1. Introduction

Several DNA repair processes, among them nucleotide-excision repair, comprise, after removal of the damaged site, a step of new DNA synthesis. The latter uses the complementary strand as template [1]. DNA repair synthesis is evidenced by incorporation of labelled dThd into DNA repair patches in the presence of the ribonucleotide reductase inhibitor HU, utilized to prevent replicative DNA synthesis [2]. This process, also termed unscheduled DNA synthesis, is markedly enhanced in response to UV-C irradiation or DNA alkylation [1,3–7]. Because upregulation of DNA repair synthesis requires an adequate supply of dNTPs, enzymes involved in nucleotide biosynthesis might play an important role during DNA repair. Elevation of the activities of several enzymes involved in dNTP formation, namely dCK (E.C. 2.7.1.74), thymidylate synthase and TK, has already been shown following ionizing radiation in various cancer-derived cells [8,9]. Increased activity of ribonucleotide reductase has also been reported after DNA alkylation [10]. A rise of dCK activity, which can supply all the dNTPs required for DNA synthesis, might be particularly important for upregulation of DNA repair in resting cells, in which ribonucleotide reductase and TK, both cell-cycle regulated enzymes, are weakly expressed. In the present study, we investigated the effect of UV-C light on two enzymes involved in dNTP synthesis, dCK and TK. UV light given in the germicidal range (254 nm, close to the DNA absorption peak) causes DNA lesions that are susceptible to removal

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Abbreviations: CdA, 2-chloro-2′-deoxyadenosine; CLL, chronic lymphocytic leukemia; dCK, deoxycytidine kinase; dCyd, deoxycytidine; dNTP, deoxynucleoside triphosphate; dThd, thymidine; HU, hydroxyurea; NAC, *N*-acetylcysteine; TK, thymidine kinase.

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by nucleotide-excision repair [1]. Experiments were performed in lymphocytes isolated from B-CLL patients, firstly, because these cells are resting and, secondly, because they retain a DNA repair capacity that comprises such a resynthesis step and hence requires the availability of dNTPs [3,6]. The results show that dCK activity is upregulated by UV light. The mode of activation of dCK as well as its potential contribution to DNA repair synthesis were investigated.

2. Materials and methods

2.1. Chemicals

[^{3}H-Methyl]dThd (84 Ci/mmol) and [5-^{3}H]dCyd (24 Ci/mmol) were from Amersham International. HU, cycloheximide and suramin were from Sigma–Aldrich. CdA was synthesized and supplied by Prof. L. Ghosez (Laboratory of Organic Chemistry, Louvain-la-Neuve). Purified recombinant dCK, prepared by Dr. J. Talianadis, was kindly provided by Dr. M. Sasvari-Szekely (Semmelweis University of Medicine, Budapest, Hungary). Ficoll-plaque Plus (density: 1.077) and Sephadex-G25 were from Pharmacia Biotech. FCS and penicillin/streptomycin were purchased from BioWhittaker Europe, RPMI-1640 was from Life-Technologies and all tissue culture reagents were from Gibco. Actinomycin D was from Roche Diagnostics. Other chemicals, materials and reagents were from Acros, Merck, or Sigma, and were of the highest quality available.

2.2. Lymphocyte isolation and cell culture

Freshly obtained peripheral blood from B-CLL patients was fractionated by Ficoll-Paque sedimentation. Mononuclear cells were washed and resuspended in RPMI-1640 supplemented with 10% FCS and 1% penicillin-streptomycin. Cells were counted, diluted to indicated concentration in RPMI, and incubated at 37° in 5% CO_2 in air. All patients had a confirmed diagnosis of B-CLL by cytological and immunological studies, and were free of any cytotoxic therapy for at least 6 months. Only patients with lymphocyte counts over 30,000/μL were selected. The continuous cell line EHEB was purchased from DSMZ-German Collection of Microorganisms and Cell Culture. This cell line was cultured in the same medium than mononuclear cells, but without antibiotics, and routinely tested for *Mycoplasma* contamination.

2.3. UV-C irradiation

Cells, incubated in 50-mm dishes, were irradiated with an UVGL-25 lamp (UVP, Inc.) delivering its bulk at 254 nM. Radiation intensity was calibrated by an UVX-25 UV meter (UVP, Inc.).

2.4. Preparation of cell extracts and enzyme assays

After various times of incubation, cells were washed twice in PBS, resuspended in 0.15 mL of 50 mM Tris–HCl buffer, pH 7.6, containing 5 mM benzamidine, 0.5 mM phenylmethylsulfonylfluoride, 2 mM dithiothreitol, 20% glycerol and 0.5% Nonidet P-40, and disrupted by three freeze-thawings. After centrifugation, the supernatants were used immediately, or frozen at −20° and stored for subsequent enzyme activity assays. It was verified that storage of extracts for a week at −20° did not modify enzyme activity compared to that of freshly prepared extracts. When indicated, cellular extracts (0.8 mL) were filtered on a Sephadex G-25 fine column (0.9 cm × 15 cm) equilibrated in the lysis buffer minus glycerol and Nonidet P-40. TK and dCK activities were measured as described by Arner *et al.* [11] with 10 μM [^{3}H]dThd or 10 μM [5-^{3}H]dCyd (∼1,000 cpm/pmol), in 100 μL of a reaction mixture containing 50 mM Tris–HCl, pH 7.6, 2 mM dithiothreitol, 5 mM $MgCl_2$, 5 mM ATP, 10 mM NaF and 0.03–0.04 mg of cellular protein. The enzyme assays were carried out at 37° and 10 μL aliquots were taken after several appropriate time intervals, to yield a linear reaction rate (within 40 min). The protein content of cell extracts was measured by the method of Bradford [12], using bovine serum albumin as the standard. It was verified that the addition of 1 mM dThd to the assay (to inhibit TK2) did not modify the level of dCK activity. It was also verified that the level of dCK activity was not influenced by the presence of HU in the cell suspension.

2.5. Nucleotide analysis

To verify that all metabolites have been removed from the cell extract by filtration on Sephadex G-25, neutralised perchloric extracts of the lysate were analyzed by high-performance liquid chromatography, as described by Hartwick and Brown [13], and modified by Vincent *et al.* [14].

2.6. Western blot analysis

Cell extracts were prepared as described for the enzyme assays. Electrophoretic separation, Western blotting, and immunostaining were performed as described by Sasvari-Szekely *et al.* [15], using rabbit antibody raised against the C-terminal peptide of human dCK [16], manufactured by Eurogentec. Briefly, proteins were separated on 12% SDS-polyacrylamide gels, transferred during 2 hr to nitrocellulose membranes, and probed at room temperature with the rabbit dCK antibody at 1:2,000 dilution. After removal of specific antibody, the blot was incubated with horseradish peroxydase-conjugated antirabbit IgG (Sigma) at 1:10,000 dilution. Immunocomplexes were visualised by the enhanced chemiluminescence reaction.

2.7. Incorporation of precursors into DNA

B-CLL cells were resuspended at a concentration of 3×10^6/mL, and pre-incubated for 30 min in the presence of 1 mM HU in order to inhibit residual semi-conservative DNA synthesis [6]. Thereafter, they were incubated for 3 hr in the presence of [^{3}H]dThd or [^{3}H]dCyd, both at a concentration of 5 μCi/mL. Radioactivity incorporated into DNA was measured using the Millipore Multiscreen Assay System (Millipore), as previously described [6].

2.8. Statistical analysis

Differences in enzyme activity or precursor incorporation between control and treated cells were analysed for statistical significance by the two-tailed Student's *t*-test for paired or unpaired samples, at a level of significance of $P = 0.05$. All means were calculated from experiments performed with lymphocytes isolated from at least three different patients.

3. Results

3.1. Increase of dCK activity by UV-C light

B-CLL cells were exposed or not to UV light and assayed for dCK and TK activity at various time points. Before irradiation, dCK activity was 156 ± 36 pmol/min/mg protein (mean ± SEM of three separate experiments). It remained at this level throughout the incubation in unirradiated cells. Within 1 hr after UV-C irradiation (10 J/m^2), dCK activity increased by 2- to 3-fold, and remained high for at least 3 hr thereafter (Fig. 1A). In contrast, TK activity, of which the initial level was 8.2 ± 0.4 pmol/min/mg protein (mean ± SEM of three separate experiments), was not influenced by UV light under the same conditions (Fig. 1B). As depicted in Fig. 2, the effect of UV-C on dCK activity was roughly dose-dependent and already significant 30 min after irradiation.

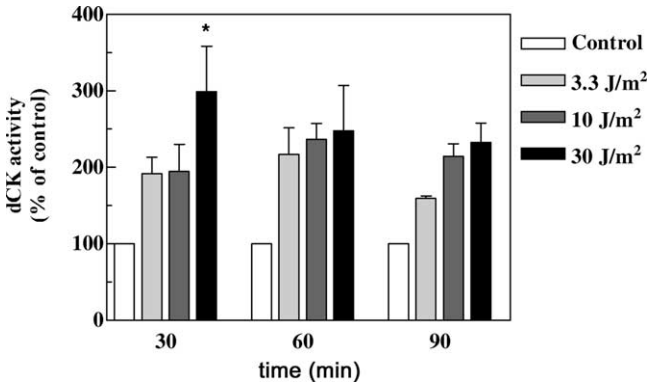

Fig. 2. Dose effect of UV irradiation on dCK activity. B-CLL lymphocytes were exposed to UV at the indicated doses and incubated for the indicated times before the measurement of dCK activity. Results are means ± SEM of three separate experiments. Activity of dCK in control unirradiated cells at time 0 min was 148 ± 66 pmol/min/mg protein. $^*P = 0.01$.

The maximal stimulation (299 ± 59% of control, $P < 0.01$) of dCK activity was recorded 30 min after 30 J/m^2 UV-C. Some activation of dCK was occasionally already detectable 15 min after irradiation, but not in a reproducible way. The initial level of activity of dCK, as well as that reached after UV radiation, was variable, but not correlated with disease stage or lymphocytosis. At all time points and UV doses tested (until 3 hr and up to 30 J/m^2), TK activity was not modified (not shown).

3.2. Mechanism of dCK activation by UV-C light

Dialysis or filtration on Sephadex G-25 of the lysates used for the assay of dCK did not modify its activity, either under basal conditions, or after activation by UV-C light (not shown). As verified by HPLC, both procedures were effective in removing low molecular weight metabolites. This indicated that the activation of dCK by UV light can not be explained by a change in the concentration of small metabolites, such as the allosteric inhibitor dCTP, but is rather due to a stable change of the protein itself. Activation of dCK observed 60 min after 30 J/m^2 UV-C was not

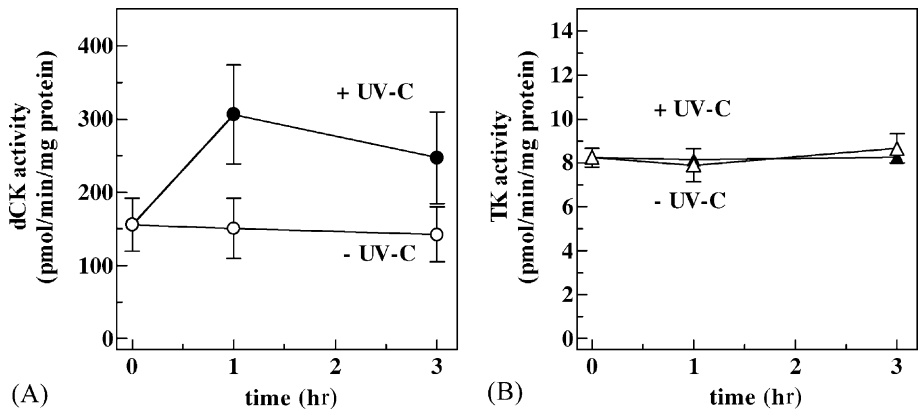

Fig. 1. Effect of UV-C irradiation on dCK (A) and TK (B) activities. B-CLL lymphocytes were exposed (●, ▲) or not (○, Δ) to UV (10 J/m^2) and incubated thereafter for 1 or 3 hr before determination of enzyme activities. Results are means ± SEM of three separate experiments.

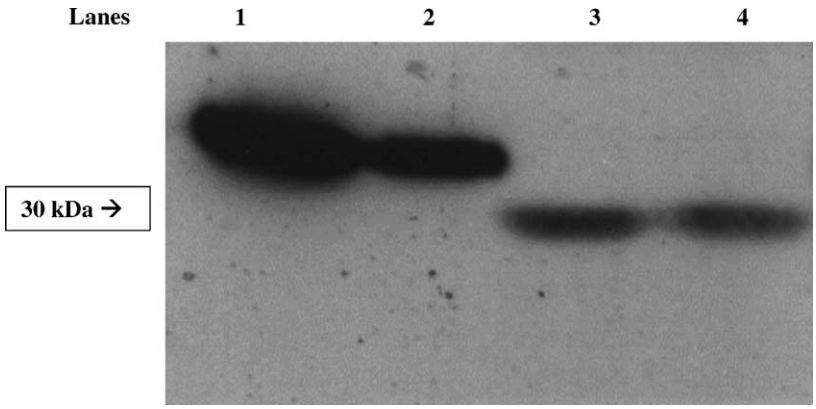

Fig. 3. dCK protein level after UV irradiation. B-CLL lymphocytes were irradiated or not with UV-C (30 J/m^2) and incubated for 1 hr. The dCK protein level was determined, as described in Section 2, by immunostaining with a polyclonal anti-dCK antibody, using 80 μg of protein of the lysate per line. Recombinant human dCK was used as reference. Results shown are representative of four experiments with similar results. Lane 1, recombinant dCK (20 ng); lane 2, recombinant dCK (10 ng); lane 3, control unirradiated cells; lane 4, UV-C irradiated cells. Recombinant dCK has a higher MW because of a His-tag fused to dCK.

reduced by 0.01 μg/mL actinomycin D or 10 μM cycloheximide, which are known to induce at these concentrations a significant inhibition of RNA and protein synthesis, respectively (results not shown). Furthermore, as shown in Fig. 3, UV-C did not modify the level of dCK protein in B-CLL cells, as determined by immunoblot of the cytosolic proteins, although the activity of the enzyme was increased by more than 2-fold over control in the same sample. Taken together, these results indicate that activation of dCK by UV light occurs through a post-translational modification of the enzyme.

3.3. Effect of suramin on dCK activation

During the last decade, it has become apparent that several growth factor receptors are involved in the UV-response [17–19]. Suramin, a synthetic polysulfonated urea derivative, that has been shown to abrogate receptor activation in response to growth factors [20,21], has also been shown to inhibit the UV-C-induced signal transduction pathway [17,22–26]. We, therefore, investigated the effect of suramin on the activation of dCK by UV-C light. After a 30-min incubation with increasing concentrations of the drug, B-CLL lymphocytes were irradiated, and analysed for dCK activity 45 min later. As shown in Fig. 4, suramin had no effect on dCK activity in unirradiated cells, but completely abrogated the activation of dCK induced by UV-C irradiation.

Because of its strongly anionic nature, suramin is membrane impermeant [27]. The inhibition of UV-induced dCK activation by suramin is thus likely to take place at the cell surface level. However, because suramin can be internalized via endocytosis, it is not excluded that it may act at a more distal intracellular level, such as a putative signalling cascade. To clarify this, we tested the effect of suramin on the activation of dCK induced by conditions assumed to be independent of growth factor receptor activation at the cell surface, but still thought to rely on a post-translational modification of dCK. Previous reports have shown that in normal and leukemic lymphocytes, dCK activity can be increased by various agents, including etoposide, aphidicolin and the deoxynucleoside analogue CdA [15,28–30]. As illustrated in Fig. 5, these three agents also induced an activation of dCK in B-CLL lymphocytes, which was quantitatively similar to that achieved by UV-C irradiation. However, as also illustrated in Fig. 5, suramin did not prevent activation of dCK by these agents. These results, together with the fact that suramin had no effect on unstimulated dCK, suggest that suramin interacts with a process specifically elicited by UV light, possibly growth factor receptor activation at the cell surface.

It has been shown that H_2O_2 can mimic the effect of UV on growth factor receptors. On the other hand, NAC—a general antioxidant—may inhibit these effects of UV. We observed that incubation of B-CLL cells with H_2O_2 upregulated dCK activity approximately 2-fold (Fig. 6A). Conversely, preincubation of cells with 10 or 40 mM NAC,

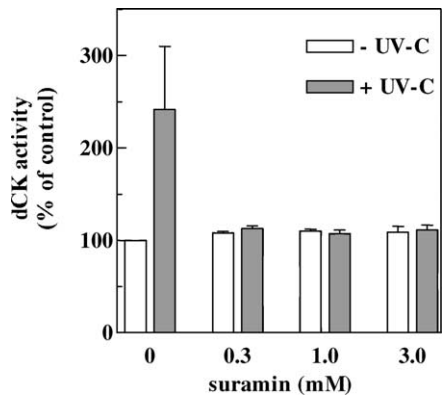

Fig. 4. Effect of suramin on dCK activation by UV-C. B-CLL lymphocytes were preincubated during 30 min with the indicated concentrations of suramin, exposed or not to UV (30 J/m^2), and incubated for 45 min before determination of dCK activity. Results are means ± SEM of three separate experiments. Activity of dCK in control cells was 132 ± 30 pmol/min/mg protein.

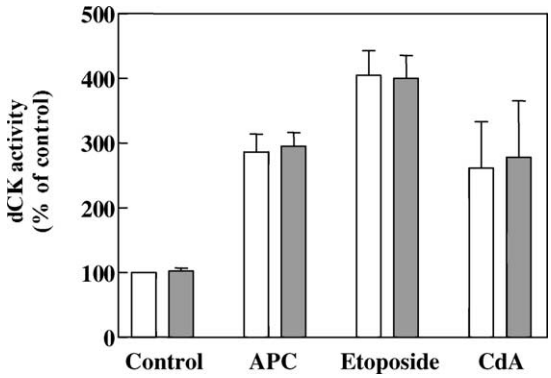

Fig. 5. Effect of suramin on upregulation of dCK activity by aphidicolin (APC), etoposide, or CdA. B-CLL lymphocytes were preincubated with (■) or without (□) 0.3 mM suramin during 30 min, and exposed for additional 2 hr either to 10 μM APC, 100 μM etoposide, or 1 μM CdA, prior to determination of dCK activity. Results are means ± SEM of three separate experiments. Activity of dCK in control cells was 84 ± 12 pmol/min/mg protein.

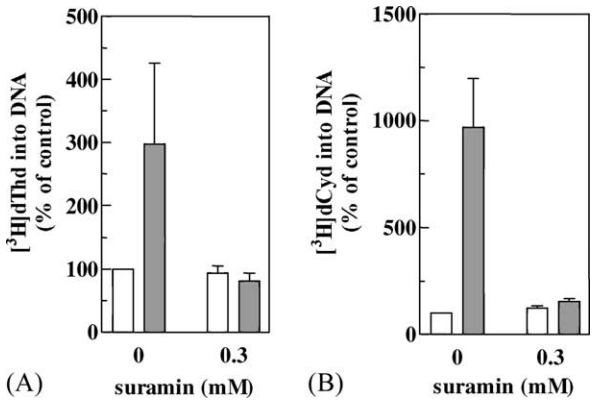

Fig. 7. Effect of suramin on DNA repair synthesis triggered by UV-C irradiation. B-CLL lymphocytes were preincubated or not with 0.3 mM suramin during 30 min, exposed (■) or not (□) to UV (30 J/m^2) and incubated for additional 3 hr with [^{3}H]dThd (A) or [^{3}H]dCyd (B) (5 μCi/mL). Results are means ± SEM of three separate experiments. In unirradiated samples incubated without suramin, incorporation of dCyd and dThd into DNA was 250 ± 76 and 278 ± 45 dpm/10^6 cells, respectively.

reduced the effect of UV light on dCK activity (Fig. 6B). In a separate experiment, we observed that the effect of UV was completely abrogated at 100 mM NAC (not shown). These results support the hypothesis that dCK might be activated by an event initiated at the cell surface involving oxidation processes.

3.4. Effect of suramin on DNA repair synthesis

Unscheduled DNA synthesis, i.e. DNA synthesis not devoted to replication, can be assessed by measuring [^{3}H]dThd incorporation into DNA in the presence of HU. This process occurs in unstimulated B-CLL cells *in vitro*, and is markedly stimulated by DNA damaging conditions, such as UV-C irradiation [3,5,31]. Others and we have shown that this stimulation was exclusively linked to DNA repair and not to a re-entry into the cell cycle [6,31]. The lack of dCK activation by UV-C in the presence of suramin led us to examine the contribution of this activation to the UV-induced DNA repair. B-CLL lymphocytes, preincubated for 30 min with 1 mM HU, in the absence or in the presence of suramin, were irradiated or not with 30 J/m^2 UV-C, and thereafter, allowed to perform repair in the presence of [^{3}H]dThd during 3 hr. As shown in Fig. 7A, suramin, which had no effect on basal DNA repair synthesis, completely prevented the UV-stimulated incorporation of [^{3}H]dThd. The effect of UV and suramin were also investigated on [^{3}H]dCyd incorporation into DNA. As illustrated in Fig. 7B, the increase of DNA labelling following UV irradiation is markedly higher with dCyd as labelled precursor than with dThd. Still, suramin, which had no effect in unirradiated cells, completely abolished the UV-induced incorporation of dCyd. This result shows that suramin abrogated both upregulation of DNA repair and dCK activation. We also tested the effect of suramin on DNA repair synthesis elicited by masfosfamide

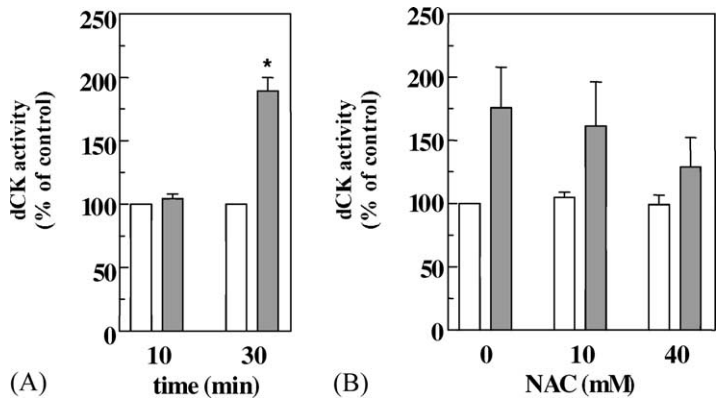

Fig. 6. (A) Effect of H_2O_2 on activity of dCK. B-CLL lymphocytes were incubated with (■) or without (□) 0.2 mM H_2O_2 for the indicated times before measurement of dCK activity. Activity of dCK in control cells was 75 ± 7 pmol/min/mg protein. $^*P < 0.01$. (B) Effect of NAC on activition of dCK by UV irradiation. B-CLL lymphocytes were preincubated during 60 min with NAC at the indicated concentrations, UV-irradiated at 30 J/m^2 (■) or not (□), and incubated thereafter during 30 min before measurement of dCK activity. Activity of dCK in control cells was 74 ± 4 pmol/min/mg protein.

which is thought to achieve this effect through direct DNA alkylation [6]. The increase of DNA repair synthesis, measured by [^{3}H]dCyd incorporation into DNA after a 16-hr incubation with 10 μM mafosfamide, was not suppressed by suramin (results not shown). This indicates that suramin did not directly influence the activity of the enzymes implicated in this form of repair. It also shows that mafosfamide and UV light activate DNA repair by different pathways.

4. Discussion

In this report, we show that the activity of dCK is significantly upregulated by UV-C irradiation in B-CLL cells. This effect is roughly dose-dependent within the range of UV doses tested. In comparison, the activity of TK is much lower than that of dCK, and unsensitive to the effect of UV light. TK assay with dThd as substrate measures both the cytosolic TK1 and the mitochondrial TK2. However, since B-CLL cells are predominantly resting, the major part of the measured TK activity, as shown by Arner *et al.* [11], is due to TK2, which is not S-phase dependent.

Activation of dCK could not be explained by allosteric effects of a low molecular weight metabolite, as verified by dialysis and gel-filtration experiments. It also did not require new synthesis of protein, as demonstrated by the lack of effect of transcription and translation inhibitors, and by Western blot (Fig. 3). Taken together, these results indicate that activation of dCK by UV-C could be the result of a post-translational modification. That dCK may be regulated by post-translational modification has already been suggested by studies showing that it is activated by CdA or aphidicolin, without elevation of either the amount of its protein, or its mRNA level [15,29]. By contrast, activation of dCK by γ-irradiation in colon cancer cell lines correlated with an increase of dCK protein [9].

Post-translational activation of dCK by UV light might result from phosphorylation of the enzyme. Indeed, earlier studies have shown inactivation of partially purified dCK by protein phosphatase, as well as the inhibition of this process by sodium(meta)vanadate, a phosphatase inhibitor [29]. Regulation of dCK activity by a phosphorylation/dephosphorylation process was also indicated by the ability of NaF, another inhibitor of protein phosphatases, to elevate dCK activity in intact human tonsillar lymphocytes [32]. Phosphorylation of dCK can be performed *in vitro* by protein kinase C [33], but has never been demonstrated in intact cells. Nevertheless, bryostatin-1, an activator of protein kinase C, can upregulate dCK activity. Still, the time required to produce this effect (48–72 hr) contrasts with the shorter interval (30 min) needed for UV-C to achieve maximal activation of dCK [34].

Signal transduction pathways leading to activation of dCK remain to be discovered. Treatment of mammalian cells with UV causes a series of phosphorylation events leading to the activation of several transcription factors. A major part of this response is not initiated within the nucleus, but at the cell surface, and is mediated by the ligand-independent activation of growth factor receptors [17–19,35]. Activation by UV light of these receptor tyrosine kinases would be due to inactivation of receptor-directed protein tyrosine phosphatases, through oxidation of an essential cysteine residue in the active site of the latter enzymes [36,37]. Suramin, which has been shown to block the activity of several growth factors by interfering with the binding to their receptors, has also been shown to abolish UV-induced receptor tyrosine kinase activation [17]. Our observation that suramin prevented the activation of dCK by UV-C suggests that the latter effect might also be initiated by UV-activation of growth factor receptors. However, we can not completely exclude an intracellular action of suramin. Indeed, although suramin is membrane impermeable because of its charge, it can be internalized via the process of adsorptive endocytosis [38]. Nevertheless, the fact that suramin did not affect the basal activity of dCK (Fig. 4) allows to preclude a direct inhibitory effect of suramin on dCK. Furthermore, suramin did not affect activation of dCK by agents that are not thought to achieve this effect by a membrane-linked process (Fig. 5). These include CdA, which needs to be phosphorylated intracellularly before activating dCK, and two inhibitors of DNA synthesis, aphidicolin and etoposide [15,29,30]. Activation of dCK by UV-C should thus occur upstream of that induced by these agents. Other elements strengthen the assumption that the activation of dCK by UV-C is initiated at the membrane level, possibly by activation of a tyrosine kinase receptor. Firstly, we observed that activation of dCK by UV was reduced by NAC, an antioxidant which counteracts inactivation of receptor-directed tyrosine phosphatases provoked by UV. In addition, dCK activity was significantly upregulated, up to the level achieved by UV light, by addition of external H_2O_2 (Fig. 6). The latter has been shown to mimick the effect of UV light on surface receptors through the inhibitory oxidation of receptor-bound protein phosphatase [26,36,37,39]. UV light may trigger several signal transduction pathways, including the mitogen-activated protein kinase pathway [40]. Still, the pathway leading to activation of dCK remains speculative and its identification needs further investigations.

The inhibitory effect of suramin on the activation of dCK by UV light gave us the opportunity to study the potential contribution of this enzyme to DNA repair synthesis. UV-C provokes in resting cells an increase in dThd incorporation into DNA, attributable to DNA repair synthesis [6]. This increase was also recorded when dCyd was used as a precursor, as previously shown by Xu *et al.* [41] in dividing CCRF-CEM cells. We observed that the increase in DNA labelling induced by UV-C was completely abrogated by suramin, whether dCyd or dThd was used as precursor (Fig. 7). This observation is apparently not due to a direct effect of suramin on enzymes involved in DNA synthesis.

Indeed, suramin affected neither basal DNA repair synthesis in unirradiated B-CLL lymphocytes, nor replicative DNA synthesis in proliferating EHEB cells, a cell line [42] derived from lymphocytes from a B-CLL patient (results not shown). In addition, we did not find an effect of suramin on DNA repair synthesis elicited by mafosfamide which provokes DNA lesions by alkylation of DNA. Suramin, thus, specifically abolishes upregulation of DNA repair synthesis induced by UV-C. Activation of growth factor receptors by UV may, thus, play a role in the process leading to upregulation of DNA repair synthesis, and activation of dCK and upregulation of DNA repair might be related. Activation of dCK could be a prerequisite for the normal completion of DNA repair synthesis in B-CLL lymphocytes, because it allows a proper availability of DNA precursors. Indeed, among the enzymes likely to provide B-CLL lymphocytes with dNTPs, dCK seems the only one able to fulfil this role upon UV-C irradiation. TK, with a 40-fold lower activity than dCK, was indeed not influenced by UVC. Ribonucleotide reductase also does not appear to play a role in DNA repair synthesis in our model, because our experiments were done in the presence of HU. The critical role of dNTP supply in DNA repair is substantiated by the observation that dNTP pool depletion prevents completion of repair in non-cycling cells following treatment with DNA-damaging agents [43].

The increase of dCK activity by UV-C, together with an unchanged activity of TK, may also provide an explanation for our observation that DNA labelling is much more stimulated by UV irradiation when [^{3}H]dCyd, rather than [^{3}H]dThd, is used as precursor (Cf Fig. 7A and B). Indeed, dCK, which phosphorylates not only dCyd, but also deoxyadenosine and deoxyguanosine [44], can also provide dTMP from dCMP by the successive actions of dCMP deaminase and dTMP synthase. As a result of dCK activation, the rate of formation of dTMP by the latter pathway will be faster after UV-C, which would lead to a decline of specific radioactivity of [^{3}H]dTTP formed from [^{3}H]dThd and to an underestimation of the extent of stimulation of DNA synthesis. On the contrary, the specific radioactivity of dCTP formed from [^{3}H]dCyd is not expected to decrease after UV-C. Also emphasising the importance of the dCMP pathway, it has been shown in human tonsillar lymphocytes that 50–75% of dCyd is converted into dTTP via dCMP deaminase [45].

In conclusion, UV light adds to the list of agents that can upregulate dCK activity. This includes conditions able to either block DNA synthesis and/or cause DNA damage such as etoposide, aphidicolin, nucleoside analogues (CdA, fludarabine, ara-C), as well as γ-radiation. Upregulation of dCK activity has been shown to be due either to increased protein synthesis (γ-radiation) [9], or to a stable postranslational modification of the enzyme (CdA, aphidicolin, etoposide) [15,28,29]. In addition, we show that dCK activity might be under the control of growth factor receptors. Other enzymes of nucleotide biosynthesis, such as TK and ribonucleotide reductase, have been shown to be induced in response to DNA-damaging conditions in other models [8–10,46]. Enzymes involved in dNTP synthesis might, thus, play a role in DNA repair. The relative contribution of salvage and *de novo* pathways in DNA repair may be different according to cell type and forms of cellular aggression. In B-CLL cells, dCK may be the sole provider of dNTPs to be used in DNA repair and hence a limiting factor. The contribution of enzymes involved in dNTP synthesis in processes aimed at overcoming DNA damages renders them potential targets for cancer chemotherapy.

Acknowledgments

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