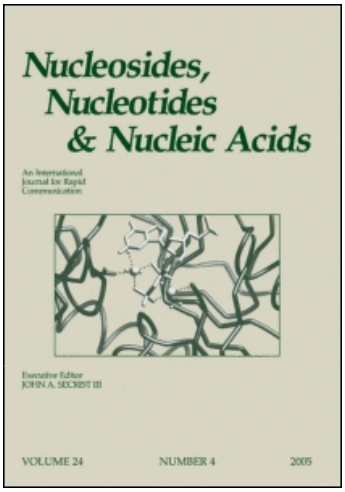

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ENHANCED ACTIVITY OF DEOXYCYTIDINE KINASE AFTER PULSED LOW DOSE RATE AND SINGLE DOSE GAMMA IRRADIATION

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□ *In both pulsed low dose rate (LDR) and single high dose radiation schedules, gemcitabine pretreatment sensitizes tumor cells to radiation. These radiosensitizing effects could be the result of decreased DNA repair. In this study, the effect of irradiation on the deoxycytidine kinase (dCK) needed for DNA repair was investigated. The activity of dCK, a deoxynucleoside analogue-activating enzyme was increased upon irradiation in both schedules. No change in dCK protein expression was observed that indicates a post-translational regulation. The benefit of this increased activity induced by irradiation should be further investigated in combination with deoxynucleoside analogues activated by this enzyme.*

Keywords Radiation; Deoxycytidine kinase

INTRODUCTION

Radiation can be given at various schedules (e.g., pulsed low dose rate (LDR) and single dose). As reviewed in Pauwels et al.,[1] gemcitabine (dFdC) pretreatment has been shown to result in significant radiosensitization in both schedules that is most likely due to decreased DNA repair.[2]

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Deoxycytidine (dCK) and thymidine kinase (TK) are essential in the synthesis of deoxynucleotides required for DNA repair and involved in deoxynucleoside analogue activation. Strikingly, dCK also activates deoxynucleoside analogues such as gemcitabine (dFdC). In vivo, a good correlation between dCK levels and gemcitabine sensitivity in various murine tumors and human tumor xenografts was observed.[3] Exposure of human lymphocytes to 0.5–2 Gy dosage of γ-radiation showed an increase in dCK activity.[4] Since LDR also resulted in an increase in activity of both TK and dCK,[5] we investigated whether single dose radiation would lead to a similar effect and timecourse. We also investigated whether this increase in dCK activity after irradiation of the human squamous lung carcinoma cell line SW-1573 was due to enhanced protein synthesis or post-translational modification which might be related to the phosphorylation status of dCK.

MATERIALS AND METHODS

The human squamous lung carcinoma cell line SW-1573 was grown in Leibowitz-15 medium as described previously.[5] Cells were irradiated in late exponential growth (with 50–65% of the cells in G1-phase, 30–40% in S-phase, 7–15% in G2-phase as determined by BrdU incorporation).

LDR irradiation was simulated by giving the cells small irradiation pulses as described previously.[5] Irradiation was performed followed by a resting period of 4 minutes, 52 seconds resulting in a mean dose rate of 1 Gy/h.

Single high dose irradiation was at a dose rate of 0.6 Gy/min, with 2 ^{137}Cs sources one above and one below the position of the cell culture dishes. Radioactive assays were performed for measuring dCK activity as earlier described using $^{[3H]}$-CdA[3] as the substrate. CdA is a very specific dCK substrate and not a substrate for TK2, for example.

Western blotting by rabbit polyclonal antibodies directed to human dCK (dilution 1:5000) was performed as decribed previously.[6]

RESULTS AND DISCUSSION

Both pulsed LDR and single high dose rate irradiation of SW1573 increased activities of dCK (Figure 1). The increase after LDR was not only dose but also time dependent. At the single dose irradiation (4 Gy) this time dependency was investigated more in detail (Figure 1B) with a plateau between 2 and 5 hours. The activity of dCK returned to control levels after about 10 hours, possibly because cells were recovered from the effects of radiation.

For dCK the increase in activity was not associated with an increase in protein, indicating a posttranslational regulation. This upregulation of

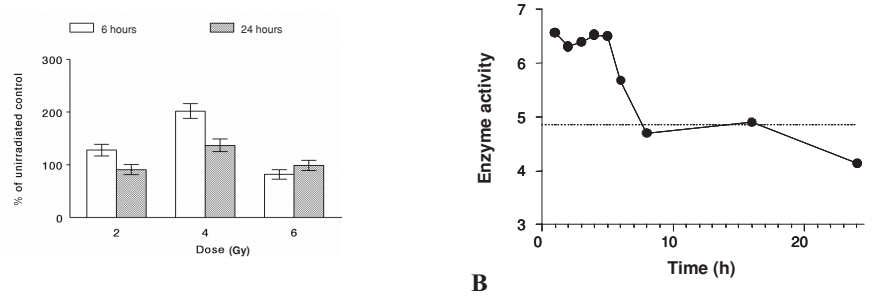

FIGURE 1 A: dCK activity 6 and 24 h after pulsed LDR irradiation of SW-1573 cells. Enzyme activities are expressed as percentages of unirradiated controls. Error bars represent SEM of 3 experiments. B: dCK activity in time after single high dose (4 Gy) irradiation of SW-1573 cells. Enzyme activity is expressed in nmol/h/mg protein. The dashed line represents the enzyme activity in unirradiated control cells.

activity per ng protein after irradiation (Figure 2) is most likely due to phosphorylation. This elevation could be an adaptive response to irradiation and explain the observed increased survival in the initial part of the SW1573 cell survival curve. In literature, there is controversy whether phosphorylated dCK is active or inactive. Wang and Kucera[7] proposed that PKC mediated phosphorylation of dCK resulted in a less active form, but this hypothesis was not confirmed by others.[8] However, cell permeable inhibitors of PKC did not modify dCK activity in intact cells.[9] Data obtained with protein phosphatase clearly indicate that the phosphorylated form is more active than the dephosphorylated form.[9,10] Thus the hypothesis of a role of PKC in the activation or phosphorylation of dCK is very speculative. We hypothesize that irradiation induces DNA damage, initially leading to increase of dNTP pools by increasing activity of deoxynucleoside kinases in which PKC might be involved.

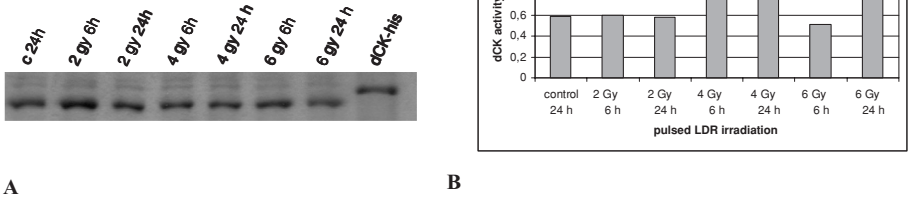

FIGURE 2 Western blot (A) of dCK and specific dCK activity per ng dCK protein (B) after pulsed LDR irradiation in SW1573 cells, harvested at different time points. Unirradiated cells at 24 hours (c24h) were used as controls, dCK with a his-tag (dCK-his) was used as a positive control to calculate the amount of dCK protein.

Since antitumor agents activated by dCK such as gemcitabine are used in combination with radiotherapy in the clinic it might be advantageous to apply these agents during the period of increased enzyme activity induced by radiotherapy treatment instead of before.

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