Synergistic Effect of Vitamin C on DNA Damage Induced by Cadmium

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Abstract. Salts of divalent cadmium are well-known human mutagens and carcinogens. In the present work, the ability of vitamin C to modulate genotoxic effects of cadmium chloride on human lymphocytes was assessed using single cell gel electrophoresis (comet assay). Vitamin C at 20 and 100 μmol/l and cadmium at 5, 30 and 150 μmol/l significantly increased the tail moment of lymphocytes. Vitamin C also increased the tail moment of cells exposed to cadmium. This effect was concentration-dependent: the higher the vitamin C concentration the greater the tail moment. The combined effects of cadmium and vitamin C were more pronounced at all concentrations tested than the sum of the effects of the compounds applied separately ($p < 0.05$), so cadmium and vitamin C can be considered to have synergistic effects. The results obtained can be partly explained by the participation of cadmium in the Fenton reaction and reduction of its oxidized form by vitamin C.

Key words: DNA damage — Cadmium — Vitamin C — Comet assay

Introduction

Cadmium can be carcinogenic to humans and experimental animals, and it may induce several types of tumors after various modes of injection (IARC 1993). The mechanism(s) underlying the observed carcinogenicity of this metal is not fully understood. Cadmium can be also genotoxic *in vitro*. Divalent cadmium ions were shown to induce DNA strand breaks (Lopez-Ortal et al. 1999), oxidative DNA damage (Mikhailova et al. 1997), chromosomal aberrations (Han et al. 1992), and inhibition of cell growth (Bakka and Dignernes 1984). It was suggested that genotoxic action of cadmium might be linked with its ability to decrease intracellular glutathione content, which in turn increased free radical levels (Yang et al. 1996).

Vitamin C (ascorbic acid) is a water-soluble dietary antioxidant that plays an important role in controlling the oxidative stress (Panayiotidis and Collins 1997).

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It can protect DNA against damage induced by reactive oxygen species (Duthie et al. 1996). Vitamin C is also a powerful reductant and may react with copper or iron in the cell producing free radicals (Benzie and Strain 1999). Vitamin C is able to cause DNA damage *in vitro* at concentrations achievable *in vivo via* supplementation (Crott and Fenech 1999). These properties reflect a dual, anti- and pro-oxidative, nature of vitamin C and it is important to establish the mode of its action in the presence of a specific substance under particular cellular conditions (Poulsen et al. 1998).

DNA damage induced by chemicals, including metals, appears primarily in the form of alterations of the phosphate backbone, sugar or base modifications, which are substrates for DNA repair mechanisms. The single cell gel electrophoresis (comet assay) is a sensitive test to investigate DNA damage and repair (Singh et al. 1988; Collins et al. 1997; Anderson et al. 1998). In the present work, the ability of cadmium to induce DNA damage in human lymphocytes in the presence of vitamin C was investigated using the comet assay. Although both cadmium and vitamin C are known to be able to cause DNA damage, vitamin C may decrease DNA damage induced by many mutagens (Duthie et al. 1996). Therefore, it seems important to determine the influence of vitamin C on DNA-damaging potential of cadmium.

Materials and Methods

Blood was obtained from young, healthy, non-smoking donors. Peripheral blood lymphocytes were isolated by centrifugation in a density gradient (Hunt 1987). The viability of the cells was measured by trypan blue exclusion, and it was constantly found to be about 99 %. The final concentration of the lymphocytes was adjusted to 1–3 $\times$ 10^5 cells/ml by adding RPMI 1640 medium to the single cell suspension.

Cadmium chloride and sodium ascorbate were derived from stock solutions and were added to the suspension of lymphocytes to give the desired concentrations. The cells were incubated with the chemicals for 1 h at 37 ℃.

Cell viability was determined by the Trypan blue exclusion analysis. Cells were incubated with cadmium chloride or sodium ascorbate at 10, 20, 100, 200, 500 and 1000 μmol/l for 1 h at 37 ℃, washed and resuspended. An equal volume of 0.4 % Trypan blue reagent was added, and the percentage of viable cells was evaluated under a field microscope. Assays were performed in triplicate.

The comet assay was performed under alkaline conditions, essentially according to the procedure of Singh et al. (1988) with modifications (Klaude et al. 1996). Comets were observed at 200 $\times$ magnification in a Labophot-2 fluorescence microscope (Nikon, Japan) with a video camera attached, equipped with a UV filter and connected to a personal computer-based image analysis system Comet v. 3.0 (Kinetic Imaging, Liverpool, UK). Fifty images were randomly selected from each sample and the comet tail moment (a product of DNA fraction in tail and tail length) was measured. Two parallel tests with aliquots of the same sample of cells were performed for a total of 100 cells and the mean comet tail moment was calculated. The comet tail moment is positively correlated with the level of DNA

breakage in a cell (Singh et al. 1988). The mean value of the tail moment in a particular sample was taken as the index of DNA damage in this sample.

All the values in this study were expressed as mean ± SEM from two separate experiments. The data were analyzed using STATISTICA (StatSoft, Tulsa, OK, USA) statistical package. If no significant differences between variations were found, as assessed by Snedecor-Fisher test, the differences between means were evaluated by applying Student *t*-test. Otherwise, Cochran-Cox test was used.

All chemicals were of analytical grade and were purchased from Sigma Chemical Co. (St. Louis, MO).

Results and Discussion

The results of the viability testing of human lymphocytes after incubation with cadmium chloride and vitamin C at various concentrations are displayed in Figure 1. There was a concentration-dependent decrease in cell viability in the presence of cadmium, but at the highest concentration tested, 150 μmol/l, nearly 90 % of the cells remained viable. Vitamin C did not change significantly the viability of the lymphocytes.

Table 1 shows the mean comet tail moments for the lymphocytes exposed for 1 h to cadmium in the presence or absence of vitamin C. Cadmium significantly increased the tail moment of the lymphocytes in a dose-dependent manner. Vitamin C also increased the tail moment of the lymphocytes exposed to cadmium. This effect was concentration-dependent: the higher the vitamin C concentration the greater the tail moment.

To study the nature of the interaction of cadmium and vitamin C we compared the magnitudes of the tail moment of the cells exposed to cadmium and vitamin C in combination with the sums of the magnitude of the tail moments of cells exposed to both compounds separately (Table 1). The combined effect of cadmium

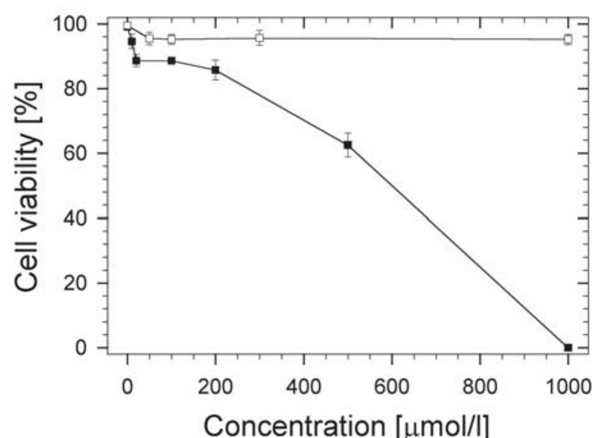

Figure 1. Effects of cadmium chloride (■) and sodium ascorbate (□) at indicated concentrations on human lymphocytes viability measured by the trypan blue exclusion method. Each point represents the mean of three experiments; error bars indicate standard deviation.

Table 1. Mean tail moment[a] of human lymphocytes exposed for 1 h at 37 ℃ to cadmium and vitamin C. The calculated sums of tail moments of lymphocytes exposed separately to cadmium and vitamin C at particular concentrations are given in the brackets.

Cadmium concentration (μmol/l)	Vitamin C concentration (μmol/l)		
	0	20	100
0	6.07 ± 0.59	12.51 ± 1.70	18.89 ± 3.14
5	10.07 ± 2.30	17.2 ± 1.9 (16.5 ± 2.9)	25.4 ± 2.9 (22.9 ± 3.9)
30	15.68 ± 2.12	22.4 ± 2.4 (22.1 ± 2.8)	34.6 ± 3.6 (28.5 ± 3.8)[b]
150	23.04 ± 2.50	34.7 ± 7.0 (29.5 ± 3.0)	51.0 ± 3.7 (35.9 ± 4.4)[c]

[a]mean ± S.E.M., $n = 100$; [b]$p < 0.05$, [c]$p < 0.01$, relative to calculated sum of effects of cadmium and vitamin C acting separately

and vitamin C was stronger at all concentrations than the sum of the effects of the compounds applied separately ($p < 0.05$), so a synergistic induction of DNA damage by cadmium and vitamin C can be assumed.

The most basic way of viewing the data from the comet assay is the distribution of cells according to the percentages of DNA in the tail, which is positively correlated with the comet moment (Ashby et al. 1995). Figure 2 shows the distribution of lymphocytes according to their comet tail moments after treatment with cadmium with and without vitamin C. It can be seen that increasing the concentration of cadmium was associated with an increase in the fraction of lymphocytes with greater comet tail moments compared with the unexposed control. These comets contained more DNA in their tails, which indicated greater DNA damage in the treated cells. The action of vitamin C at both 20 and 100 μmol/l apparently increased the fraction of lymphocytes with a greater tail moment.

Our results obtained confirm that the comet assay is a highly sensitive technique to analyse DNA damage induced by cadmium (Hartmann and Speit 1996).

The increase in the comet tail length of the cadmium-exposed lymphocytes may be caused by DNA strand breaks induction by cadmium, which in turn can be attributed to interference of this metal with the repair of DNA lesions caused by it. It is well known that cadmium at low concentrations affects the incision step of the repair process (Hartwig et al. 1996; Dally and Hartwig 1997).

The results of some studies suggest that vitamin C may have protective action against cadmium toxicity (Fox 1975; Ray et al. 1981; Nagyova et al. 1994), but on the other hand vitamin C was shown to be able to cause DNA damage in the presence of cadmium (Littlefield and Hass 1995; Mikhailova et al. 1997). This can be due to a Fenton-like reaction in which vitamin C in the presence of cadmium generates hydroxyl radicals, which in turn may induce damage to DNA. DNA damage induced by vitamin C has also been observed by other investigators (Galloway and Painter 1979; Singh 1997). The new information brought by the results of this

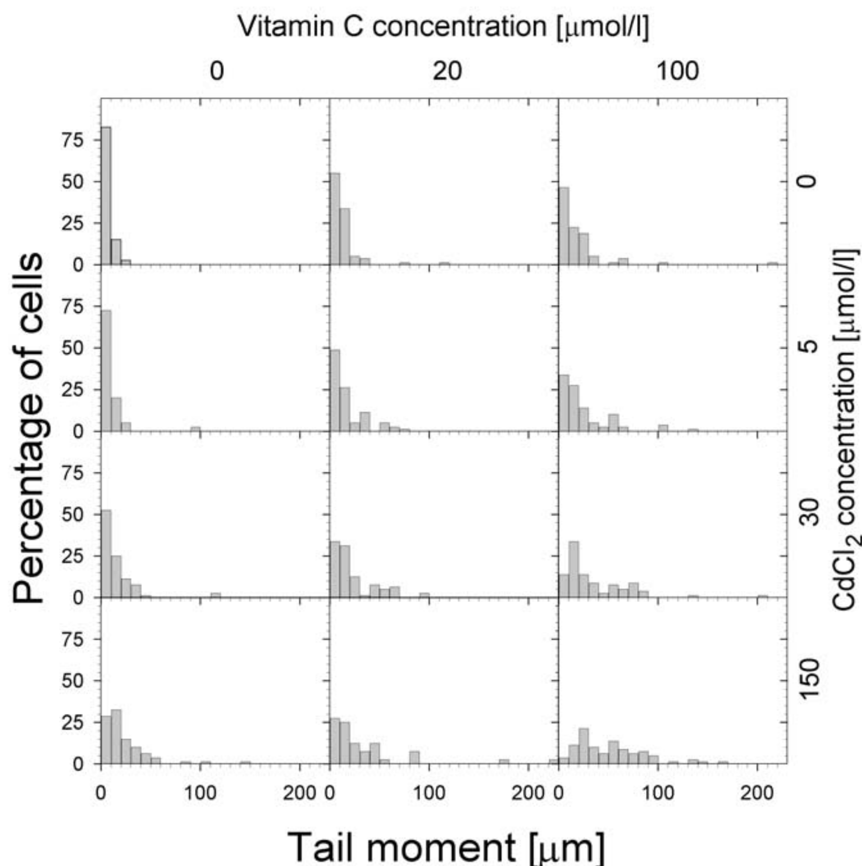

Figure 2. Histograms of the distribution of comet tail moments in human lymphocytes treated for 1 h at 37 °C with cadmium chloride and vitamin C at indicated concentrations. The number of cells scored for each treatment was 100.

research is that the effect of vitamin C was synergistic with that of cadmium. The mechanism underlying the observed effect is the Fenton reaction and disturbances of DNA repair processes, but further studies are needed to elucidate their exact mechanism.

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