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Thiamine prevents X-ray induction of genetic changes in human lymphocytes *in vitro*

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The effects of thiamine (vitamin B1) on the level of spontaneous or radiation-induced genetic changes in human lymphocytes *in vitro* were studied. Cultured lymphocytes were exposed to increasing concentrations of thiamine (0–500 μg/ml) and irradiated with X-rays. The DNA damage was estimated as the frequency of micronuclei and apoptotic or necrotic morphological changes in fixed cells.

The results show that thiamine alone did not induce genetic changes. A significant decrease in the fraction of apoptotic and necrotic cells was observed in lymphocytes irradiated in the presence of vitamin B1 at concentrations between 1–100 μg/ml compared to those irradiated in the absence of thiamine. Vitamin B1 at 1 and 10 μg/ml decreased also the extent of radiation-induced formation of micronuclei. Vitamin B1 had no effect on radiation-induced cytotoxicity as measured by nuclear division index. The results indicate that vitamin B1 protects human cells from radiation-induced genetic changes.

Oxidative stress influences DNA and other biomolecules damage *via* oxidative changes to their chemical structure. These changes are believed to increase the risk of cancer, heart disease and aging processes (Beckman & Ames, 1998). It has been demonstrated that antioxidants such as ascorbic acid, tocopherols or flavonoids give protection against oxidative damage and several degenerative diseases, including cancer (Ames *et al.,* 1993; Collins, 1999). It has also been shown that antioxidant vitamins can protect human cells *in*

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Abbreviations: CBL, binucleated lymphocytes; MN, micronuclei; NDI, nuclear division index.

vitro (Konopacka *et al.*, 2001; Jaruga *et al.*, 2002; Dusińska *et al.*, 2003) and animal cell also *in vivo* (Sarma & Kesavan, 1993; Konopacka *et al.*, 1998) against the genetic damage induced by ionizing radiation. On the other hand, some reports have indicated toxicity of some vitamins, mainly A and D (Draper, 1998). Also vitamin C at high concentrations shows detrimental properties and induces DNA strand breaks in human cells *in vitro* (Singh, 1997; Konopacka *et al.*, 2002). Little is known about whether B group vitamins give protection against DNA damage in human cells.

The water-soluble vitamin B1 (thiamine) is commonly included in multivitamin preparations. It is believed to be non-toxic for humans because its excess is excreted in the urine. It has been shown that thiamine is an antioxidant that scavenges hydroxyl radicals (Hu *et al.*, 1995). It seems to be of interest to estimate the radioprotective properties of this vitamin and its influence on the level of radiation-induced genetic damage in human cells. In the present study the effects of thiamine on the extent of spontaneous as well as radiation-induced DNA damage was measured in cultured human lymphocytes. The DNA damage was estimated using the cytokinesis-block micronucleus test which is a useful method for assessing radiation-induced chromosome damage in peripheral blood lymphocytes (Fenech & Morley, 1985). This system can also be used to estimate cell proliferation, apoptosis and necrosis (Fenech *et al.*, 1999).

MATERIAL AND METHODS

Cell culture and thiamine treatment. The study was performed on cultures of human peripheral blood lymphocytes obtained from three healthy donors. Whole blood cultures were prepared by adding 0.5 ml of blood to 4.5 ml of Dulbecco's modified Eagle's medium (DMEM, Sigma, U.S.A.) complemented with 15% fetal calf serum (ICN, Biomedicals) and antibiotics. Cultures in plastic dishes were kept at 37°C in a humidified 5% CO_2 atmosphere. Vitamin B1 (thiaminum hydrochloricum, Pliva, Kraków, Poland) was dissolved in medium and added to cultures one hour before irradiation. It was tested at a final concentration in the range between 1–500 μg/ml. Non-irradiated cultures were also treated with vitamin B1 and kept parallel. The thiamine range concentration used was based on a preliminary experiment. Bleomycin at 10 μg/ml was used as positive control for genotoxicity. It was added to cultures prior to mitotic stimulation.

Micronucleus assay. The micronucleus test was performed according to a described procedure (Fenech & Morley, 1985). Briefly, lymphocytes were stimulated with 5 μg/ml of phytohaemagglutinin (Lectin, Sigma, U.S.A.), immediately after irradiation. Cytochalasin B (Sigma, U.S.A., 6 μg/ml) was added 44 h later, to accumulate cells that had divided once. After incubation for 72 h, the cultures were harvested and fixed in three changes of methanol/acetic acid (3:1, v/v). The cells were spread onto glass slides (two slides for each culture), dried and stained with May-Grunwald and Giemsa dyes. Experiments were repeated on the samples of blood obtained from each donor and the results were summarized. Micronuclei (MN) were scored in 1000 binucleated cells per slide. The data are expressed as the number of MN per 1000 binucleated cells as well as the frequency of binucleated cells containing one or more MN (MN-CBL). For cell cycle analysis, 400 cells per treatment group were scored for the presence of one, two or more than two nuclei and the nuclear division index (NDI) was calculated as follows:

$$NDI = [1N + (2 \times 2N) + (4 \times >2N)] / 400$$

where 1N is number of cells with one nucleus, 2N – with two nuclei, and >2N – with more than two nuclei.

The fraction of apoptotic-like and necrotic cells were also counted on the same slides.

The Student's *t*-test was used to determine the statistical significance of differences in the extent of DNA damage between the tested groups.

Irradiation. Irradiation was performed using X-rays (Clinac 600, 6 MV, Varian) with a dose of 2 Gy (1 Gy/min) at room temperature.

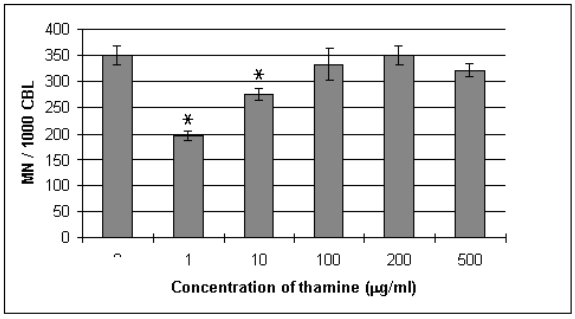

Figure 1. Effect of thiamine on the number of radiation-induced micronuclei in human lymphocytes.

Bars show the mean ±S.D. $^*P < 0.01$ compared with control irradiated in the absence of thiamine.

RESULTS AND DISCUSSION

In the present study the ability of thiamine to modulate the level of spontaneous and X-irradiation-induced genetic changes was tested in cultured human lymphocytes. The effects of thiamine on the spontaneous frequency of micronuclei in cultured human lymphocytes are presented in Table 1. The background level of micronuclei obtained from three donors was 22.0. Addition of thiamine at concentrations ranging between 1–500 μg/ml did not cause any measurable changes (Table 1). Bleomycin used as a positive control for genotoxicity increased the number of micronuclei over 4-fold.

Data presented in Fig. 1 show the effect of thiamine on the level of radiation-induced micronuclei in human lymphocytes *in vitro*. In the cultures irradiated in the presence of thiamine, a protective effect was observed at the concentrations of 1 and 10 μg/ml. When the concentration of thiamine was increased to values above 100 (up 500 μg/ml) the frequency of radiation-induced micronuclei was the same as in cultures that were irradiated in the absence of thiamine. The observed protective effect of thiamine can be explained by its antioxidant properties since it is an effective scavenger of hydroxyl radicals (Hu *et al.*, 1995). At concentrations higher than 200 μg/ml vitamin B1 did not influence the level of radiation-induced genetic changes. It has been shown that thiamine at low concentrations inhibited microsomal lipid peroxidation induced *in vitro* by $FeCl_3$ and ascorbate

Table 1. The frequency of micronuclei (MN) in human lymphocytes incubated *in vitro* with the indicated concentrations of thiamine

Treatment	Concentration (μg/ml)	MN/1000 CBL
Negative control	0	22.0 ± 2.99
Thiamine	1	20.5 ± 1.47
Thiamine	10	23.0 ± 1.25
Thiamine	100	20.2 ± 2.60
Thiamine	200	21.0 ± 0.71
Thiamine	500	22.5 ± 1.87
Bleomycin[a]	10	104.8 ± 6.76

[a]Bleomycin is a positive control of genotoxicity. CBL, binucleated lymphocytes. Data represent mean ± S.D.

whereas at high concentrations it stimulated this process (Hu *et al.*, 1995). These findings as well as the results from the present study indicating the lack of a radioprotective effect of thiamine used at high concentrations suggest that supplementation of large amounts of this vitamin may not be desirable.

In order to assess the effect of thiamine on the proliferative capacity of irradiated human lymphocytes the nuclear division index (NDI) was calculated. The results presented in Table 2 show a cell cycle delay in lymphocytes exposed to X-radiation at a dose of 2 Gy. The NDI decreased from 1.73 in untreated control cells to 1.47 in irradiated ones. Vitamin B1 did not influence the cell cycle kinetics either in non-irradiated or irradiated cells.

We also tested whether thiamine could reduce the frequency of apoptotic and necrotic cells. Figure 2 presents the fraction of apoptotic and necrotic morphological changes in lymphocytes induced by thiamine alone (Fig. 2a) or by thiamine and X-radiation (Fig. 2b). The results demonstrate that in untreated control the levels of necrotic and apoptotic cells were less than 12 and 1.5%, respectively. Vitamin B1 at concentrations between 1–500 μg/ml did not influence the background level of necrotic and apoptotic cells. In lymphocytes irradiated only (Fig. 2b) the fraction of necrotic cells increased about 3-fold in comparison with non-irradiated ones. Thiamine present at concentrations from 1 to 500 μg/ml during irradiation significantly decreased the level of cells with apoptotic morphology. Vitamin B1 used at concentrations from 1 to 100 μg/ml reduced the fraction of necrotic cells in comparison with the cells irradiated without the vitamin.

In conclusion, the present study demonstrates that vitamin B1 significantly decreases the formation of micronuclei and apoptotic and necrotic cells induced by X-radiation in human lymphocytes *in vitro*.

It has recently been shown that in aerated medium vitamin B1 protects cells against radiation but in an air free environment it shows a cytostatic effect. Because cancer cells are anaerobic, whereas oxygen is present in normal tissues, it seems possible that thiamine can specifically lead to the elimination of cancer cells during irradiation but at the same time protect the normal ones against radiation damage (Heinrich & Getoff, 2002). The results of this study, where thiamine protected human lymphocytes against *in vitro* radiation-induced genetic changes leading to cell death, indicate that this vitamin may be effective in protecting normal cells during radiotherapy.

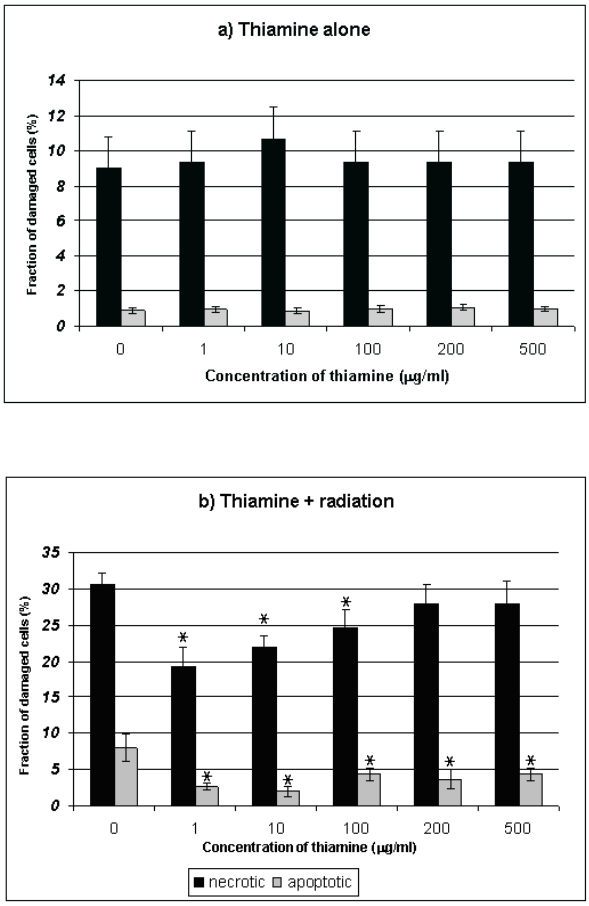

Figure 2. Effect of thiamine and X-radiation on the number of lymphocytes exhibiting necrotic and apoptotic morphological changes.

Bars show the means ±S.D. *$P < 0.01$ compared with control irradiated in the absence of thiamine.

Table 2. Effect of thiamine and X-radiation (2 Gy) on the nuclear division index (NDI) in cultured human lymphocytes

Treatment	Thiamine concentration (μg/ml)	NDI (%)
Control	0	1.73 ± 0.16
Thiamine	500	1.75 ± 0.14
Radiation	0	1.47 ± 0.18
Radiation + thiamine	1	1.46 ± 0.19
Radiation + thiamine	10	1.57 ± 0.24
Radiation + thiamine	100	1.48 ± 0.20
Radiation + thiamine	200	1.41 ± 0.22
Radiation + thiamine	500	1.40 ± 0.19

Data represent mean ±S.D.

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