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The Biliprotein C-Phycocyanin Modulates the DNA Damage Response in Lymphocytes from Nuclear Power Plant Workers

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1. Introduction

The biliprotein C-phycocyanin (C-PC) is a light-harvesting photoreceptor in cyanobacteria and in red algae (Rhodophyta and Cryptophyta) with applications as a natural colorant in nutritional industry and cosmetics (Prasanna et al., 2007) and as a fluorescent marker in medical and biological studies (Glazer, 1994; Sun et al., 2003). The protein is composed of two homologous subunits - α and β (Stec et al., 1999; Contreras-Martel et al., 2007), respectively with one and two phycocyanobilin chromophores, covalently attached to cysteine residues. The subunits form $\alpha\beta$ complexes which aggregate into $\alpha_3\beta_3$ trimers and $\alpha_6\beta_6$ hexamers, the latter being the functional unit of the protein. C-PC has been shown to display a variety of pharmacological activities, related to the antioxidant, anti-inflammatory, neuro- and hepato-protective, anti-tumour and wound-healing mechanisms (Romay et al., 2003; Ge et al., 2006; Li et al., 2005; Madhyastha et al., 2008). These properties have attracted attention to the compound as a possible radio-protective agent. It has been demonstrated that rats exposed to 5 Gy of X-rays and fed phycocyanin normalized their antioxidant system within 4 weeks after exposure (Karpov et al., 2000).

Recently, we studied the effects of C-PC in combination with ionizing radiation on lymphocytes, isolated from nuclear power plant workers, exposed to low doses of ionizing radiation (IR), and compared them with the effects on lymphocytes from nonexposed controls (Ivanova et al., 2010). We found that the biliprotein stimulated the expression of the antioxidant enzymes manganese superoxide dismutase (MnSOD), catalase and glutathione-S-transferase (GST) during the early radiation response of lymphocytes from workers, but not from controls. Since the biliprotein positively affects the antioxidant defense pathways, it might be of interest for the radioprotection of occupationally exposed people.

In this study we have further characterized the effects of C-PC on the early radiation response of lymphocytes from unexposed controls and from workers, exposed to low doses of radiation. We quantified the level of persisting radiation-induced DNA double-strand breaks (DSBs) in the presence and absence of C-PC. DSBs are the most dangerous type of DNA lesions, induced by several genotoxic agents, including gamma IR (γ -IR). The ability of cells to readily process DSBs is of vital importance for genomic integrity, as failure to repair these lesions results in

chromosomal breakage, fragmentation and translocation. Moreover, impaired or defective rejoining of radiation-induced DNA strand breaks usually correlates strongly with the individual susceptibility to cancer (Alapetite et al., 1999; Berwick & Vineis, 2000).

The amount of persisting DSBs in cells was determined by the comet assay (CA), a quick, simple and reliable method for analyzing DNA damage and repair that requires a small number of cells and can be performed on both freshly isolated and cryopreserved cells (Decordier et al., 2010). Due to its sensitivity, the method is preferred in human epidemiological studies related to biomonitoring (Möller et al., 2000; Touil et al., 2002). Additionally, the CA is able to provide information on different types of DNA damage/repair and detect cellular damage in a wide dose range of exposures from 0.05 to 10 Gy (Kalthur et al., 2008; Mohseni-Meybodi et al., 2009; Palyvoda et al., 2003). The experiments were performed on human lymphocytes, which, due to their radiosensitivity and circulation throughout the body, reflect the overall state of the organism and are the cellular type most frequently used for assessment of the systematic radiation response (Collins et al., 2008; Decordier et al., 2010). A major problem with CA is that its sensitivity often leads to detection of a high variation within a single individual. A reliable methodology should be able to detect differences between individuals, but should show a minimal intra-individual variation. Therefore, prior to the epidemiological experiment, in an attempt to achieve minimal intra-individual variation and a linear dose-response curve, we carefully tested a number of conditions. We attained a stable linear dose-response dependence of DNA lesions, persisting 2h after exposure in the dose range from 0.5 to 8 Gy gamma rays.

Our data indicated that C-PC might stimulate the repair of radiation-induced DNA lesions in lymphocytes from both occupationally exposed subjects and non-exposed controls. Moreover, the biliprotein seems to limit the manifestations of high radiosensitivity. Interestingly, we registered a pronounced lower genotoxicity of C-PC in lymphocytes from workers with cumulative doses higher than 20 mSv. Additionally, the effects of C-PC were age-dependent.

2. Experimental procedures

2.1 Subjects and sampling

The exposed group consisted of 44 workers aged between 26 and 62 years, employed at the “Kozloduy” Nuclear Power Plant (NPP), Bulgaria. Cumulative exposure to ionizing radiation (IR), estimated from personal dosimeter records, ranged from 0.32 to 330.77 mSv and represented the sum of the doses collected for the whole period of occupation in the “strictly controlled area”. The control group included 12 non-exposed subjects from the NPP administrative staff, aged between 42 and 58 years. In order to exclude external effect on the results of this study, we recorded information on the smoking habits, alcohol consumption, use of medications and previous diagnostic exposure to X-rays. The studied groups were homogenous on the aforementioned criteria and the statistical analysis found no significant effects due to any factor. The study was performed under the National Program “Genomics” of the Ministry of Health and Ministry of Education, Youth and Science of Bulgaria. Informed consent was obtained from all participants.

Blood (2 ml) drawn by venipuncture and collected in EDTA-coated tubes (Vakutainer, Benton Dickinson, Oxford, UK) was delivered to the laboratory and stored at 4°C for up to 24h before processing. The samples from the control and exposed subjects were handled concurrently and the assays were run on coded samples.

2.2 Isolation, treatment with C-PC and irradiation of lymphocytes from human peripheral blood

C-PC was extracted, purified and concentration-adjusted as previously described (Ivanova et al., 2010).

Peripheral blood mononuclear cells were isolated by density-gradient centrifugation (Lymphoflot, Biotest, Dreieich, Germany), suspended in 1 ml RPMI 1640 supplemented with 10% fetal calf serum (RPMI, Sigma, St Louis, MO, USA) and counted on haemocytometer. The lymphocytes from each subject were then split and subjected to four different treatments, using the conditions, described by Ivanova (Ivanova et al., 2010) as shown in Fig. 1a: **(A)** 4 hours of incubation with RPMI before lysis; **(B)** 2 hours of incubation in RPMI, followed by irradiation with 2 Gy (^{137}Cs gamma source, dose rate 2.07 Gy/min), incubation for another 2 hours and lysis; **(C)** 4 hours of incubation in RPMI, supplemented with 5 μM C-PC (RPMI-C-PC) before lysis; **(D)** 2 hours of incubation in RPMI supplemented with 5 μM C-PC, followed by irradiation (as described), incubation for another 2 hours and lysis. All above procedures were carried out at room temperature.

2.3 Single Cell Gel Electrophoresis (Comet assay)

The neutral comet assay was applied for analysis of radiation- and/or C-PC-induced strand breaks in DNA. Three comet test slides were prepared from each treatment, described in Section 2.2 and Fig. 1a. Lymphocytes (5×10^5 cells/ml) were suspended in low melting point agarose (final concentration 0.7% in phosphate buffered saline), dropped onto frosted glass slides which had been precoated with 0.5% normal melting point agarose, then refrigerated (4°C) for 15 min. To dissolve cellular proteins and lipids, the slides were immersed in lysis buffer (10 mM Tris, 100 mM EDTA, 2.5 M NaCl, 1% Triton X-100, pH 8.0) for 40 min at 4°C , and washed 3 times for 5 min in pre-cooled TBE buffer, pH 8.0. Electrophoresis was performed in TBE for 20 min at 0.5 V/cm². Finally, the slides were washed in ethanol and air-dried, stained with ethidium bromide (5 $\mu\text{g}/\text{ml}$) and analyzed under a fluorescence microscope (Olympus BX41). Double-strand breaks were analyzed by the parameter “tail moment” (TM), determined by the Comet Score 1.5 Software for fifty cells per slide. This parameter is the product of tail length and % DNA in the tail and is considered most informative when low levels of damage are present (Collins et al., 2008).

2.4 Statistical analysis

Distributions of variables were determined using Kolmogorov-Smirnoff test (Marques de Sá & Frias, 2007). Lilefors and Levene tests were used to determine the homogeneity of variance. The effects of different treatments (such as exposure to IR, C-PC treatment or the combination of C-PC treatment plus irradiation) were analyzed using one way ANOVA. Student t-test for dependent variables was carried out in order to compare every factor pair in each group. Results showing $p < 0.05$ were considered significant. As a null hypothesis it was presumed that there is no difference between groups.

3. Results

3.1 C-PC induces changes in DNA response to irradiation in non-irradiated subjects included in the control group

First we wanted to analyze whether the cells of each individual responded with an increase in DNA lesions to the different treatments. For this we determined the standard deviation

for the TM values which we had calculated from each triplet of comet test slides. Average TM values which increased for different treatments with more than two standard deviations, were considered elevated. Thus, as evident from Table 1, *in vitro* irradiation alone generates elevated levels of persisting DNA lesions in the lymphocytes from all (100%) of the non-exposed subjects included in the control group. 5 μ M C-PC by itself also causes an increase in the lesions in more than half (67%) of the cases suggesting that treatment with C-PC is toxic for more than half of the subjects, included in the control group. Notably, when incubated with C-PC prior to irradiation, the samples from only half of the subjects show levels of DNA lesions, higher than those of the non-treated samples. This means that C-PC treated cells do not accumulate additional lesions upon radiation exposure. Thus, despite the fact that C-PC shows some toxicity, it also seems to protect cells from additional radiation damage.

There was a significant increase in the median value of the parameter TM, upon irradiation of cells which were grown in the absence of C-PC (Fig. 1b, **B** vs. **A**, $t_{11}=6.4$). In contrast, for cells grown in C-PC supplemented medium, the median value slightly decreased upon irradiation (Fig. 1b, **D** vs. **C**, $t_{11}=2.36$). The lower median TM value, calculated for the combined treatment, C-PC plus irradiation (**D**), in comparison to the separate treatments with C-PC (**C**) or irradiation (**B**), suggested that the biliprotein exerted radio-protection. Residual damage calculations (**B** minus **A** vs. **D** minus **C**) confirmed these findings (data not shown).

The relatively high levels of data dispersion (wide confidence intervals), observed in all conditions (Fig. 1b) are consistent with high inter-individual variations in the cellular response of the control subjects. Notably, the cells irradiated after treatment with C-PC (**D**) showed a lower level of data scattering than that of treatments (**B**) or (**C**), which was comparable to the dispersion range of values found for the non-treated samples (**A**). This is evidently due to a reduction of the maximal TM values in (**D**). This observation suggests that the biliprotein limits the manifestations of high radiosensitivity.

3.2 C-PC induces changes in the DNA response of lymphocytes from workers with very low cumulative doses of radiation

The average annual exposure of 17 subjects with very low cumulative doses, ranging from 0.32 to 12.12 mSv, did not exceed 1 mSv/year - the public dose limit, mandated by ICRP (ICRP 60, 1990), and these workers were unified in a group with very low dose occupational exposure. Cumulative doses and data on the levels of DNA damage in the workers are summarized in Table 2. Similar to the non-exposed control group, the additional, *in vitro* irradiation of the cells generated a significant increase in the levels of persisting lesions (Fig. 1c, **B** vs. **A**, $t_{16}=3.63$) in the majority of cases (76%). Treatment of the cells with C-PC also generated elevated levels of unrepaired DNA strand breaks in the majority (82%) of the subjects (Fig. 1c, **C** vs. **A**, $t_{16}=3.11$). Notably, after irradiation, samples, which had been pre-incubated with C-PC, showed lower median levels of DNA breaks as well as a reduction in the number of the subjects with higher levels of persisting DNA lesions (59% of the subjects) when compared with the samples which were irradiated only (Fig. 1c, **D** vs. **B**, $t_{16}=2.68$) or incubated with C-PC without *in vitro* irradiation (Fig. 1c, **D** vs. **C**, $t_{16}=2.77$). This result was similar to the effect of the protein on the non-exposed control group and demonstrated its radio-protective effect on the subjects with a very low dose occupational exposure.

As seen in Fig. 1c, the exposure of the cells only to C-PC or to IR (**B** and **C**) elevated the median values of TM and extended the range of the data dispersion. This is consistent with the cellular toxicity of the two agents. The data dispersion towards the higher break

extremes was more drastic with C-PC (C) than with irradiation alone (B), although the median values of damage levels in C-PC treated cells (C) was lower than that of irradiated cells (B). Notably, in combination, C-PC and radiation (D) induced a well pronounced decrease in the median values of TM, which were brought down almost to the levels of the controls (A). Additionally, the combination of the two agents (D) narrowed the range of data dispersion, again bringing it close to that of controls (A). In conclusion, for this group we observed a beneficial effect of C-PC on lymphocytes treated prior to radiation exposure, despite the toxicity of the protein. This conclusion was further confirmed by residual damage calculations (B minus A vs. D minus C).

(a) Lymphocytes from each subject were treated as follows: **A** - 4 hours of incubation with RPMI before lysis (controls); **B** - 2 hours of incubation in RPMI, followed by irradiation, incubation for another 2 hours and lysis; **C** - 4 hours of incubation in RPMI, supplemented with 5μM C-PC; **D** - 2 hours of incubation in RPMI supplemented with 5μM C-PC, followed by irradiation, incubation for another 2 hours and lysis.
(b) TM for the different treatments of lymphocytes from non-exposed subjects
(c) TM for the different treatments of lymphocytes from workers with cumulative doses, ranging from 0.32 to 12.12 mSv
(d) TM for the different treatments of lymphocytes from workers with cumulative doses, ranging from 26.77 to 330.77 mSv
Whiskers represent non-outlier range, boxes: 25-75% confidence intervals (CI), (■) median value and (●) outlier values.

Fig. 1. Treatment patterns and their effects on subjects from different exposure groups

3.3 C-PC induces changes in the DNA response of lymphocytes from workers with higher cumulative doses of radiation

This group included 27 professionals with cumulative doses, ranging from 26.77 to 330.77 mSv. Data, summarized in Table 3, showed, that in this group, in comparison with the two previous groups (non-exposed controls and exposed to very low doses of radiation), which were characterized by high levels of radiation-induced DNA lesions in the majority of samples (100 and 76%, respectively), the number of workers with persisting DNA lesions, induced after the *in vitro* exposure of the cells to 2 Gy gamma rays or treatment with C-PC was reduced by half to 48% and 44%, respectively. This is consistent with improved repair capacity of the subjects included in this group, which is probably relevant to their chronic low dose radiation exposure, which may have acted as *in vivo* adaptive dose. C-PC showed the lowest cytotoxicity in this group of workers since the median TM values and the range of data scattering were similar to those in untreated samples (Fig. 1d, C vs. A). This is also consistent with a general robustness of the cellular DNA repair capacity of this group of subjects, which is evident from the similar TM median of treatments A and B (Fig. 1d) - a sign of possible protective adaptation to toxic exposures, developed in subjects with higher cumulative doses of radiation. Significant differences in the levels of persisting lesions were detected only between the cells, irradiated *in vitro* and those treated with C-PC (Fig. 1d, C vs. B, $t_{24}=2.44$). It is important to note, however, that regardless of the similarity of TM median values of B and A (Fig. 1d), irradiation of cells caused a definite increase in the data scattering towards the higher TM values, as compared to non-irradiated cells. Such an increase was not evident in the cells treated with C-PC only (Fig. 1d, C vs. A), rendering the C-PC treatment in this group less toxic to DNA than in the previous two groups (Fig. 1b and 1c). However, in contrast to the other two groups of subjects, C-PC treatment in this case did not cause a decrease in the amount of radiation-induced DSBs (Fig. 1d, D) - a finding that was confirmed by residual damage calculations (B minus A vs. D minus C).

3.4 The magnitude of the C-PC effect depends on the cumulative doses of exposure

We compared the TM values for each treatment among the three subject groups. As seen in Fig. 2, the only significant differences found were for treatment of cells with C-PC only (C), which showed that the protein was less toxic for workers with cumulative doses higher than 20 mSv (Fig. 2, group 3) and this effect contrasted with the toxicity registered for the controls and the group of professionals with very low dose radiation exposure (Fig. 2, groups 1 and 2). This indicates that chronic occupational exposure might stimulate the cellular defense mechanisms and induce resistance to DNA damage, caused by agents, such as C-PC. The workers with higher cumulative doses might also be more resistant to radiation-induced toxicity since in the same group (Fig. 1d, B) we registered lower median values of TM in the lymphocytes irradiated with 2 Gy gamma rays as compared to the TM values in the other two groups (Fig. 1b, B and 1c, B).

It is worth noting the differences between the control (Fig. 1b) and the two groups of workers (Fig. 1c and 1d) regarding the median values of the parameter TM. For both groups of professionals, we found lower median values of TM upon each of the exposures (C-PC, 2 Gy or the combination of the two agents) in comparison with the median TM values of the non-exposed controls (Group 1). This result suggests that workers possess lower levels of persisting DNA lesions than the controls, which is probably due to improved DNA repair capacity induced by the low dose professional exposure. This may also be relevant to radio-adaptive phenomena, mobilizing and activating repair of DNA damage in the groups of the professionals.

Fig. 2. TM in non-exposed controls (Group 1) and in subjects with cumulative doses, ranging from 0.32 to 12.12 mSv (Group 2) or from 26.77 to 330.77 mSv (Group 3) treated with 5 μ M C-PC. Vertical bars represent 95% CI.

3.5 Age dependence of the DNA response of lymphocytes treated with C-PC and/or irradiated with 2 Gy gamma rays

All individuals, non-exposed controls and occupationally irradiated workers, were divided into two groups. The first one included 24 subjects (3 controls and 21 occupationally exposed) of the age from 26 to 46 years. The second group consisted of 32 subjects, all of them older than 46 years (9 controls and 23 occupationally exposed). Comparison of all mean TM values in the first group showed significant differences between the levels of DNA damage in the non-treated samples and the *in vitro* irradiated lymphocytes in the presence and absence of C-PC (Fig. 3a, A vs. B and D, $t_{23}=2.35$ and $t_{23}=2.03$, respectively). We also observed a significant narrowing of the dispersion of the TM values for the cells, irradiated after pre-treatment with C-PC (Fig. 3a, D), indicating reduction of the inter-individual variability and unification of the radiation responses by C-PC. Notably, the dispersion was narrowed predominantly by reducing the non-outlier range from the top – indicating that C-PC, combined with radiation, selectively improves the repair capacity of cells which, in all other conditions (A, B and C) demonstrate impaired DNA repair mechanisms. The last observation suggested that the protein stimulated better the repair of the radiation-induced DNA lesions in lymphocytes of the susceptible individuals. This observation may be important for the maintenance of genomic integrity in this high-risk subgroup of the population.

Comparison of the TM values obtained for the older group (age 46-62 years, Fig. 3b) showed increased levels of persisting DNA lesions ($p=0.05$) in the cells irradiated *in vitro* (B, $t_{31}=3.54$), incubated with C-PC (C, $t_{31}=2.26$), or incubated with C-PC prior to radiation exposure (D, $t_{31}=2.93$), when compared to the control setting in this group (A). As with the younger group, the median values of the TM for treatment B, C and D were similar. However, the significant top-down reduction in the TM value scattering, described for the younger group after irradiation of the cells, pre-treated with C-PC (Fig.3a, D) , was not evident in this group of subjects.

Fig. 3. Effect of different treatment patterns on subjects, grouped according to age: (a) from 26 to 46 years and (b) from 47 to 62 years. Note that both groups included exposed subjects and non-exposed controls. Whiskers represent non-outlier range, boxes: 25-75% confidence intervals (CI), (■) median value and (●) outlier values.

No.	Age	TM (Ctrl.)	TM (2Gy)	TM (C-PC)	TM (C-PC + 2Gy)
1	50	1.85 ± 0.21	3.41 ± 0.32	6.16 ± 0.11	3.88 ± 0.19
2	48	3.10 ± 0.22	6.80 ± 0.10	6.86 ± 0.07	7.15 ± 0.16
3	50	3.86 ± 0.13	5.38 ± 0.18	2.38 ± 0.51	4.91 ± 0.29
4	45	7.71 ± 0.15	9.82 ± 0.13	6.65 ± 0.24	6.24 ± 0.10
5	58	7.23 ± 0.17	8.24 ± 0.13	8.36 ± 0.08	6.80 ± 0.16
6	50	3.23 ± 0.04	3.78 ± 0.09	3.93 ± 0.04	4.13 ± 0.04
7	47	5.38 ± 0.01	7.45 ± 0.04	4.52 ± 0.14	4.12 ± 0.06
8	53	4.44 ± 0.09	7.28 ± 0.05	5.84 ± 0.01	3.91 ± 0.05
9	49	1.61 ± 0.09	1.79 ± 0.02	2.07 ± 0.03	2.02 ± 0.09
10	42	5.21 ± 0.04	7.10 ± 0.12	5.32 ± 0.07	5.13 ± 0.07
11	54	4.62 ± 0.02	6.68 ± 0.04	6.39 ± 0.05	7.70 ± 0.05
12	42	5.46 ± 0.02	7.13 ± 0.41	5.28 ± 0.03	5.00 ± 0.26

Table 1. Levels of persisting DNA strand breaks determined by TM in lymphocytes of non-exposed controls treated like in Fig. 1a.

No.	Age	Cumulative dose (mSv)	TM (Ctrl.)	TM (2Gy)	TM (C-PC)	TM (C-PC + 2Gy)
1	33	0.32	3.32 ± 0.28	8.44 ± 0.17	11.29 ± 0.48	4.77 ± 0.51
2	32	1.15	2.82 ± 0.02	3.75 ± 0.08	3.08 ± 0.04	2.08 ± 0.10
3	37	1.33	4.36 ± 0.03	11.19 ± 0.13	8.78 ± 0.16	8.34 ± 0.05
4	35	1.67	4.15 ± 0.03	5.29 ± 0.08	4.45 ± 0.08	4.11 ± 0.17
5	32	1.79	3.46 ± 0.02	3.98 ± 0.07	3.69 ± 0.02	3.78 ± 0.05
6	38	1.80	2.35 ± 0.09	2.30 ± 0.04	2.97 ± 0.04	3.08 ± 0.08
7	55	2.29	4.22 ± 0.14	5.05 ± 0.44	7.77 ± 0.39	4.64 ± 0.18
8	55	2.29	3.62 ± 0.16	7.35 ± 0.12	7.96 ± 0.13	6.62 ± 0.08
9	55	2.59	1.24 ± 0.45	1.01 ± 0.18	1.16 ± 0.42	1.69 ± 0.73
10	55	3.50	5.47 ± 0.02	6.18 ± 0.04	6.58 ± 0.10	4.84 ± 0.03
11	26	4.71	3.62 ± 0.53	3.90 ± 0.31	5.67 ± 0.09	4.55 ± 0.15
12	46	6.55	4.50 ± 0.07	6.21 ± 0.09	6.15 ± 0.04	6.38 ± 0.04
13	52	8.37	4.14 ± 0.03	5.82 ± 0.06	4.39 ± 0.05	3.80 ± 0.10
14	26	9.32	5.57 ± 0.02	7.07 ± 0.03	7.11 ± 0.04	6.36 ± 0.06
15	44	9.71	2.14 ± 0.10	4.16 ± 0.09	4.21 ± 0.04	3.56 ± 0.08
16	30	10.06	1.86 ± 0.27	3.72 ± 0.26	0.86 ± 0.16	1.77 ± 0.09
17	49	12.12	1.08 ± 0.12	0.91 ± 0.20	0.82 ± 0.02	1.33 ± 0.27

Table 2. Levels of persisting DNA strand breaks (TM) in lymphocytes of workers with very low cumulative doses (0.32-12.12 mSv) treated like in Fig. 1a.

4. Discussion

Our study has addressed three main questions: (i) are there significant differences in the DNA strand break repair in lymphocytes of workers, chronically exposed to low doses of IR and of non-exposed controls; (ii) can the biliprotein C-PC modify the DNA repair capacity of lymphocytes and the early cellular radiation response; (iii) is the level of the chronic exposure of significance for the impact of the biliprotein on the repair of DNA lesions. To answer the questions we assessed the amount of unrepaired DNA lesions in lymphocytes by the neutral comet assay 2h after irradiation of the cells in the presence or absence of C-PC. The experiments were performed with freshly drawn human G₀ phase lymphocytes (Kaczmarek et al., 1987), which are known to utilize the NHEJ pathway for the repair of DSBs in DNA, supposedly the main lesions, detected by the neutral CA. NHEJ is a relatively fast process, which is completed within 2h after the exposure (Lankoff et al., 2006; Palyvoda et al., 2003). This was also confirmed by kinetics, utilizing the comet test in our laboratory (data not shown). With our setup we have detected changes in the cellular capacity to mend breaks in DNA generated after exposure to toxic agents, but not the differences in initial IR-induced lesions.

No.	Age	Cumulative dose (mSv)	TM (Ctrl.)	TM (2Gy)	TM (C-PC)	TM (C-PC + 2Gy)
1	38	26.77	0.90 ± 0.05	0.78 ± 0.11	3.46 ± 0.08	3.82 ± 0.04
2	44	28.47	2.05 ± 0.06	4.10 ± 0.10	1.79 ± 0.06	2.80 ± 0.15
3	53	31.14	4.65 ± 0.18	2.92 ± 0.09	2.44 ± 0.07	4.14 ± 0.28
4	54	31.43	7.82 ± 0.09	8.54 ± 0.09	6.23 ± 0.05	7.73 ± 0.18
5	48	36.39	3.47 ± 0.17	2.63 ± 0.20	0.94 ± 0.11	2.27 ± 0.11
6	56	37.06	1.61 ± 0.15	0.58 ± 0.05	0.76 ± 0.13	1.06 ± 0.06
7	48	38.05	5.76 ± 0.08	6.84 ± 0.12	6.33 ± 0.03	5.25 ± 0.16
8	32	38.72	3.74 ± 0.18	5.64 ± 0.26	4.11 ± 0.19	3.90 ± 0.05
9	45	44.66	1.44 ± 0.20	1.09 ± 0.08	1.30 ± 0.23	1.33 ± 0.30
10	50	53.19	0.81 ± 0.20	0.58 ± 0.12	0.67 ± 0.10	1.07 ± 0.01
11	36	56.07	3.97 ± 0.02	3.47 ± 0.16	2.90 ± 0.06	2.39 ± 0.10
12	49	67.76	4.06 ± 0.02	5.36 ± 0.07	5.17 ± 0.07	5.36 ± 0.03
13	41	70.46	5.36 ± 0.11	4.05 ± 0.13	3.88 ± 0.15	3.56 ± 0.06
14	50	73.21	2.54 ± 0.03	3.40 ± 0.12	3.04 ± 0.08	3.24 ± 0.05
15	45	84.57	4.34 ± 0.45	10.16 ± 0.54	6.16 ± 0.33	4.10 ± 0.15
16	54	85.80	1.04 ± 0.22	3.21 ± 0.14	1.43 ± 0.08	1.76 ± 0.08
17	48	87.48	3.95 ± 0.10	6.05 ± 0.03	4.66 ± 0.04	5.88 ± 0.07
18	56	88.28	3.60 ± 0.05	2.89 ± 0.09	5.00 ± 0.14	7.41 ± 0.05
19	50	95.02	2.35 ± 0.10	2.76 ± 0.17	2.19 ± 0.18	2.38 ± 0.12
20	52	95.14	2.10 ± 0.35	10.85 ± 0.96	11.05 ± 1.11	9.01 ± 1.64
21	62	116.82	2.95 ± 0.12	3.03 ± 0.09	2.98 ± 0.05	2.88 ± 0.17
22	42	125.17	2.82 ± 0.03	1.65 ± 0.21	0.91 ± 0.07	2.48 ± 0.32
23	48	126.39	2.55 ± 0.28	11.12 ± 0.41	2.76 ± 0.22	5.06 ± 0.11
24	41	130.23	1.22 ± 0.80	1.61 ± 0.38	3.45 ± 0.24	4.26 ± 0.32
25	41	180.61	3.37 ± 0.39	7.97 ± 0.34	4.03 ± 0.43	9.29 ± 0.37
26	40	246.94	8.21 ± 0.13	4.82 ± 0.14	3.79 ± 0.08	5.02 ± 0.02
27	48	330.77	2.42 ± 0.16	2.68 ± 0.15	2.52 ± 0.02	2.22 ± 0.11

Table 3. Levels of persisting DNA strand breaks (TM) in lymphocytes of workers with cumulative doses ranging from 26.77 to 330.77 mSv treated like in Fig. 1a.

The comet assay which we have used is an approach, applied in a number of studies to assess the repair capacity of occupationally exposed populations and recently reviewed by Decordier (Decordier et al. 2010). The CA method has also been applied in several small pilot studies, comparing the DNA repair capacity between cancer patients and healthy subjects (Alapetite et al., 1999; Leprat et al., 1998; Djuzenova, et al., 2006; Zhang et al., 2006). In these studies, the CA has demonstrated an impaired DNA lesion repair capacity in the lymphocytes of cancer

patients, when compared to that of the healthy controls. It has also been shown that CA could be a useful approach to study the radiosensitivity and individual risk of radiation therapy induced toxicity in cancer patients after *in vitro* challenging of the cells with ionizing radiation. The results of our work have indicated large inter-individual variations in the baseline endogenous levels of DNA lesions in lymphocytes from workers and non-exposed controls. Notably, the data dispersion covered a relatively large range of values and was registered in all three experimental groups, including the non-exposed control group. In the control group we observed the highest variation in persisting baseline levels of DNA lesions (Fig. 1b, A). *In vitro* irradiation with 2 Gy γ -rays or treatment with C-PC of the cells additionally enlarged the range of data dispersion, indicating significant differences in the individual susceptibility of the subjects to toxic exposures. Exposure to 2 Gy gamma rays exerted the strongest effects on data scattering in the group of professionals with cumulative doses higher than 20 mSv (Fig. 1d, B) whereas C-PC generated highest level of data dispersion in samples from the group of workers, exposed to very low doses of occupational IR (Fig. 1c, C).

The biliprotein elevated the number of persisting, unrepaired DNA lesions in the group of subjects with lower professional irradiation and in the control group. Such toxicity was not observed in the group of professionals with higher doses of occupational radiation exposure (Fig. 2). This effect might be attributed to induction of adaptive processes, due to the chronic exposure to low doses of radiation of the subjects in the last group. Studies of other laboratories on the repair capacity of nuclear power plant workers (Toili et al., 2002) or workers exposed to xenobiotics, lead or pesticides (Restreppo et al., 2000; Vodicka et al., 2004; Piperakis et al., 2009) have shown, similar to our results, that workers repair DNA damage more efficiently than the non-exposed controls. The authors attributed this phenomenon to adaptive response by the sub-chronic genotoxic exposures. The adaptive protection, shown in this study is also consistent with the conclusion of our previous study (Ivanova et al., 2010).

Notably, in combination with radiation exposure, C-PC exerted protective effects on lymphocytes of controls and of workers exposed to very low doses of radiation. For these groups, the lymphocytes, treated with C-PC and exposed to gamma rays, showed reduced levels of DNA lesions (in comparison to C-PC treatment alone), which suggested that the protein neutralized the genotoxic effects of IR and exerted radio-protection. It seems that the biliprotein selectively improved the DNA repair capacity of the individuals with higher radiosensitivity, which could be of particular interest for the radio-protection of high-risk subgroups of the population and workers.

The toxic effect of C-PC, registered in this study, is probably linked to the photosensitizing properties of the protein (Padula & Boiteaux, 1999; Zhang et al., 1999; Paul et al., 2006). It has been shown that visible light, absorbed by the tetrapyrrolic chromophores of phycobiliproteins, can generate reactive oxygen species (ROS), such as hydroxyl radical and singlet oxygen, which induce oxidative damage to DNA. The photosensitizing properties of C-PC may also contribute to the apoptotic effects of the protein in cancerogenic cell lines (Roy et al., 2007; Subhashini et al., 2004) and macrophages (Reddi et al., 2003). Apoptotic activity, however, is evident only for higher protein concentrations (>20 μ M) and longer incubation times of the cells with the protein (>24h). The trends in DNA damage, induced by C-PC, were found quite similar to those, induced by ionizing radiation or by hydrogen peroxide in the presence of transition metals (Epe, 1995; Padula & Boiteaux, 1999). Notably, for subjects with low cumulative doses of IR and for those from the control group, we registered higher levels of unrepaired DNA lesions both in cells, treated with C-PC and in those, irradiated with 2 Gy.

5. Conclusion

The data from this and from a previous study, carried out in our laboratory, which was focused on the effects of C-PC on the enzymatic anti-oxidant defense system of lymphocytes (Ivanova et al., 2010), suggest that C-phycocyanin can affect the anti-oxidant and repair mechanisms in lymphocytes, and modulate the early radiation response. They showed that the protein impacts the repair of deleterious forms of DNA damage, generated upon exposure of the cells to γ -rays, which is essential for the preservation of genomic integrity. Notably, the biliprotein might selectively improve the DNA repair capacity of the individuals with higher radiosensitivity, which could be of particular interest for the radio-protection of the high-risk subgroups of population and workers. The protein induced significantly less persisting DNA lesions in the group of workers with higher doses (>20 mSv, Fig. 2, group 3) in comparison with the other two groups, which is relevant to adaptive phenomena induced by the chronic occupational exposure of the subjects. However, the present study should be considered a pilot one and additional experiments are needed to decide whether the protein could be applied for the protection of occupationally exposed individuals (such as nuclear power plant workers, miners, some medical doctors and pilots), and subgroups of the population with higher susceptibility to the toxic effect of ionizing radiation.

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