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SHORT COMMUNICATION
DNA–protein crosslinks as a biomarker of exposure to solar radiation: a preliminary study in brick-kiln workers

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In India, fired clay bricks are produced in small-scale factories. There are 60 000 active brick kilns, providing employment to nearly 12 million people in different suboccupations. This industry is largely non-mechanized and operates from November to June. Almost all the workers are exposed to direct sunlight for 8–10 h a day. Cellular DNA–protein crosslinks (DPCs) are the biologically active nucleoprotein complexes formed between DNA and proteins. Ultraviolet light and γ-rays, and other suspected carcinogens in humans, induce DPC formation in blood cells. DPCs have therefore been identified as a biomarker for monitoring exposure to these hazardous agents. Here we report steady-state levels of DPCs in human peripheral lymphocytes from 46 brick-kiln workers exposed occupationally for 8–10 h a day to solar radiation in brickfields and 25 unexposed controls. A significant increase ($p < 0.05$) in DPC content and DPC coefficients in peripheral lymphocytes was observed in the brick-kiln workers compared with the controls. The data suggest that the DPC content of lymphocytes could be a possible biomarker of exposure to solar radiation. However, further work is necessary to confirm this.

Keywords: biomarker, DNA–protein crosslinks, human peripheral lymphocytes, brick-kiln workers, solar radiation.

Introduction

In India, fired clay brick production is a small-scale industrial process. There are about 60 000 active brick kilns spread all across the country, providing employment to nearly 12 million people in different suboccupations. This industry, which is largely non-mechanized, operates from November to June. The manual moulding of fresh bricks and the sun drying of freshly moulded bricks involve considerable sun exposure for the workers. Almost all the workers are exposed to direct sunlight for 8–10 h a day. A number of male workers prefer to work in minimal clothing because of the extreme heat, which, together with tropical climate, intensifies the harsh sun exposure.

Cellular DNA–protein crosslinks (DPCs) are the biologically active nucleoprotein complexes formed between DNA and proteins. DPC formation in blood cells is induced by certain suspected carcinogens in humans, including ultraviolet light, γ-rays, carmustine (BCNU) (Banjar *et al*. 1983, Kolomiitseva *et al*. 1994), chromium (Wedrychowski *et al*. 1985, Sugiyama *et al*. 1986), nickel (Patierno and Costa 1985), formaldehyde (Cosma *et al*. 1988), alkylating agents (Grunicxe *et al.*

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1973) and *cis*- or *trans*- platinum compounds (Banjar *et al*. 1984). DPCs are persistent in cells and are therefore sentinels of past exposure to the causative agents. The apparent low capacity of the cell to repair this type of DNA damage makes it a potentially important lesion to be used as a biomarker of exposure assessment, and it has been identified as a biomarker for monitoring exposure to the above-specified agents (Tsapakos *et al*. 1983, Taioli *et al*. 1995). The presence of DPCs can be detected long after removal of the crosslinking/effecting agent (Wedrychowski *et al*. 1985, Taioli *et al*. 1995). The significance of this lesion in cytotoxicity is currently not well understood. Nevertheless, DPCs may cause a loss of genetic material during DNA replication that may, for example, inactivate genes important for cell differentiation and growth (Zhitkovich and Costa 1992).

The aim of this study was to investigate DPCs as a biomarker of exposure to solar radiation. The steady-state levels of DPCs in human peripheral lymphocytes from workers occupationally exposed to solar radiation (i.e. workers in brick-kiln occupations at brickfields) were investigated and compared with levels in unexposed controls in order to look for further evidence in support of the suitability of this biomarker for the assessment of exposure to the causative agents.

Materials and methods

Subjects

A cross-section of 207 brick-kiln workers of all ages and either sex from local brick kilns were interviewed regarding their personal details, the nature of their job, health complaints, etc., using a structured questionnaire. A subsample of 46 subjects who volunteered to donate blood were used in the present study. A control group of 25 subjects of similar age, sex, body mass index and socioeconomic status, but who were not exposed to solar radiation, were also investigated. Control subjects were generally students, service personnel and shop owners. Informed consent was obtained from all the participants. Heparinized blood was collected from the brick-kiln workers and the healthy volunteers. Samples were transported at 4°C to the laboratory and processed.

DPC assay

Histopaque-1077, 3,4-diaminobenzoic acid and proteinase K were obtained from M/S Sigma-Aldrich (St Louis, Missouri, USA). The rest of the chemicals were of analytical grade. Lymphocytes were isolated over Histopaque, and approximately 1×10^6 cells were stored in 1% sodium dodecyl sulphate (SDS) and 10 mM Tris-HCl pH 7.5 at −20°C until analysis. DPCs were extracted using SDS and potassium salt. Protein-bound DNA was measured fluorimetrically after protein digestion with proteinase K and expressed as the DPC content and the DPC coefficient (Zhitkovich and Costa 1992, Taioli *et al*. 1995). DPC content was expressed as the amount of SDS-precipitable (protein-bound) DNA per million cells, and the DPC coefficient as the ratio of SDS-precipitable (protein-bound) DNA to total DNA (protein-free).

Statistical analysis

The significance of differences in the mean values of the parameters in the exposed and control subjects was analysed using the Student's *t*-test after ascertaining the homogeneity of variance. The significance of the proportions of subjects with different categories of DPC levels in the exposed and control groups was analysed using the χ^2 test.

Results and discussion

A total of 46 human lymphocyte samples from brick-kiln workers and 25 samples from the corresponding controls were analysed for DPC content and the DPC coefficient (Tables 1 and 2). In the normal population ($n = 25$), DPC content

Table 1. DPC values.

	Control		Exposed	
	Mean ± SE	Range	Mean ± SE	Range
DPC content (μg DNA/million cells)	0.8828 ± 0.17	0.05–3	4.13 ± 1.13*	0.26–40.85
DPC coefficient	0.0305 ± 0.0063	0.001–0.125	0.1176 ± 0.0320*	0.007–1.155

*$p < 0.0001$.

and the DPC coefficient ranged from 0.05–3 μg (mean ± SE, 0.8828 ± 0.17) and 0.001–0.125 (mean ± SE, 0.0305 ± 0.0063), respectively. In the solar radiation exposed group ($n = 46$), these parameters ranged from 0.26–40.85 μg (mean 4.13 ± 1.13) and 0.007–1.155 (mean 0.1176 ± 0.0320), respectively. Thus an increase of 3–4 order of magnitude was noted both in DPC content and DPC coefficient in the peripheral lymphocytes from the exposed group.

Analysis of the frequency distribution of the DPC coefficients revealed an interesting pattern. The frequency was divided into three categories: < 0.01, 0.01–0.1 and > 0.1. DPC coefficient values < 0.01 were frequent in the control subjects, occurring in 36% (nine out of 25), but were limited to 4.3% (two out of 46) of the exposed subjects. The frequency of subjects with DPC coefficient values between 0.01 and 0.1 was similar in both the controls and the exposed group. The frequency distribution of DPC coefficient values > 0.1 was low in the control subjects (4%; one out of 25), but was significantly higher in exposed subjects (20%; 10 out of 46) ($p < 0.05$). This analysis showed a preliminary relationship between the DPC coefficient and exposure to ultraviolet/γ-radiation through solar exposure in humans. Correlation coefficient analysis, however, failed to provide an association between the DPC coefficient and the duration in terms of the lifetime exposure (data not shown). This may be due to the small sample size.

Table 3 shows the profiles of the brick-kiln workers and corresponding controls. All were male with a similar body mass index. The frequency of smokers and vegetarians was higher in the exposed group, but there was no relationship with DPC content or the DPC coefficient. Thirty per cent of the exposed subjects reported clinical symptoms pertaining to the musculoskeletal, gastrointestinal and respiratory systems; however, no relationship could be established with the DPC content. No clinical symptoms were noted in the control subjects. There is a paucity of information in the literature on the relationship of DPC content with

Table 2. Frequency distribution of DPC coefficient values.

	Control		Exposed	
DPC coefficient value	*n*	%	*n*	%
< 0.01	9	36	2	4.3
0.01–0.1	15	60	34	73.9
> 0.1	1	4	10	21.8*

*$p < 0.05$.

Table 3. Profile of subjects studied.

	Control ($n = 25$)	Exposed to solar radiation ($n = 46$)
Age (mean ± SD)	30.1 ± 6.92	26.6 ± 8.7
Sex	Male	Male
Occupational exposure to sunlight	No	Yes (8–10 h for 1–20 y)
Body mass index (mean ± SD)	21.9 ± 3.81	20.2 ± 1.8
Smokers	8 (32%)	23 (50%)
Vegetarians	13 (52%)	5 (10.9%)

exposure to solar radiation. Some reports nevertheless indicate that ultraviolet and ionizing radiation could induce crosslinking of proteins to nucleic acids (Alexander and Moroson 1962, Banjar *et al.* 1983).

In conclusion, a significant increase ($p < 0.05$) in DPC content and the DPC coefficient was evident in brick-kiln workers compared with controls. The results of this study suggest that DPC content in human peripheral blood lymphocytes could be used as a biomarker of exposure to solar radiation, although other agents could be responsible. More work is however needed to confirm this observation and to establish a dose–response relationship between exposure to solar radiation and increases in DPC levels. An understanding of the physiological significance of DPC formation in radiation-induced toxicity will further clarify the suitability of this biomarker. Nevertheless, the use of this biochemical change as an exposure marker in screening, diagnosis or quantitative estimation of individual risk in tropical countries following exposure to solar radiation is appealing.

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