

Ultra Violet –B Induced DNA Damage in Human Leucocyte Cells

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ABSTRACT The Single Cell Gel Electrophoresis (SCGE) Assay also known as Comet Assay is a rapid, simple and visual technique for assessing DNA damage in individual cells. Effect of UV-B was studied by irradiating human leucocytes. Irradiation of leucocyte increases the DNA instability, which can be assessed by Single Cell Gel Electrophoresis Assay. The blood samples were exposed to UV-B radiation for various time period and a correlation was found out between length of the comet tail and time period of exposure to UV radiation. The UV-B irradiation showed clear-cut dose effect relationship. It was observed that with the increase in time of exposure to radiation, the mean tail length also increased.

INTRODUCTION

The single cell gel electrophoresis or comet assay was first described by Ostling and Johanson (1984) and in more details by Ahuja and Saran (1999), Dhawan et al. (2001) and Collins (2004). Irradiation induces physical, chemical and molecular damage to tissues leading to genomic instability and can also cause cell death. Energy of ionizing radiation is transferred to the interacting cell's molecular system and results in tissue damage through different simultaneous pathways, including reactive oxygen species (R.O.S.), which when generated induce oxiditativ damage and genetic instability (Korenberg 1994). UV radiation causes damage at the molecular level to the fundamental building block of life, deoxyribonucleic acid (DNA). Over millions of years during evolution of living cells in the presence of UV radiations, cells have developed the mechanism to repair damaged DNA. In addition to this, living organisms are protected from excessive amount of UV radiations by Ozone layer. With the depletion of the Ozone layer and increase in life span of people, the role of UV radiation in the induction of age-related diseases of humans becomes a distinct possibility.

Ultra violet refers to all electromagnetic radiations with wavelength in the range of 200-400 nanometers. It can be divided into further sub groups based on their wavelength. UV-A wavelength range is from 315-400 nm and shorter UV-A wavelengths are considered possibly cancer-causing. UV-B radiation wavelength ranges between 290-320 nm and radiations below 290 nm comprise UV-C which cause damage at the molecular level to the DNA (Glasstone 1981).

Such short-wave UV is often used for germ killing purposes and cause damage to exposed cells. As early as 1928, Muller was the first to show that ionizing radiations were mutagens. However, the molecular processes involved in the transformation of radiation induced lesions into mutations are still not well known. Most mutations induced by ionizing radiations are large deletions (Sankaranaryanan 1991).

The aim of the present investigation was to find out UV-B induced damage in human leucocyte cells *in vitro*, with the help of single cell gel electrophoresis (SCGE) or comet assay.

MATERIALS AND METHODS

For the present study 3.5 ml of human blood sample was taken through venipuncture in the month of December 2005 from healthy male individual aged about 26 years. The consent of the donor was taken. This blood sample was distributed in seven microcentrifuge tubes each containing 500µl of blood. The tubes were labeled from 1 to 7. Blood in microcentrifuge tube labeled as No. 1 was taken as control and it was not exposed to UV-B radiation. The remaining samples were exposed to UV-B tube light (302 nm), a product of Philips (30 W, G30, Holland) used for cell culture laboratory sterilization. Microcentrifuge tubes 2 to 7 were exposed for time intervals of 5, 10, 15, 20, 25 and 30 minutes, respectively. After exposure to UV-B light, the cells were processed immediately for comet assay, thereby minimizing the introduction of strand breaks arising from DNA repair by the cell enzymes. The experiment was repeated after a month.

Alkaline Comet Assay

The irradiated and control samples were processed for the alkaline comet assay. The assay was performed according to the protocols given by Ahuja and Saran (1999). Samples were protected from direct sunlight during the processing for the comet assay. In brief, plain microscopic slides were precoated with 1% normal melting point agarose (NMPA) in double distilled water and allowed to solidify and dried at 37°C. It acts as an interface between the glass surface and sample layer. Following irradiation, the samples containing the human leucocyte cells were re-suspended in 0.5% low melting point agarose (LMPA) in phosphate buffered saline. For each slide 20µl of blood and 180µl of LMPA was used, layering was done and LMPA was allowed to gel at 4°C for 20-30 minutes. Third layer of LMPA was laid to fill any gap and have an uniformity of gel. Soon after gelling slides were transferred to horizontal coplin jar containing cold lysis buffer solution (2.5 M NaCl, 100mM EDTA, 10 mM Tris, pH-10) and allowed to lyse in dark for about two to three hours at 4°C. After completion of the lysis, the slides were placed on horizontal electrophoresis unit side by side and electrophoretic buffer (300mM NaOH, 200 mM EDTA, pH-13) was poured to submerge the slides completely so that the buffer level was about 3-5 mm above the agarose on the slides. Electrophoresis was carried out at 25 V or 1 V/cm and 300 mA of current. On completing the electrophoresis for 30 minutes, the slides were flooded with neutralizing buffer (0.4 M Tris) on staining tray after which slides were kept in fixing solution (0.9M Trichloroacetic acid, 0.1M Zinc sulphate, 0.5M Glycerol) for 10 minutes. Slides were stained using Silver-staining method (Ahuja and Saran 1999).

Acquisition and Analysis of Images

For comet visualization Olympus Trinocular Research microscope was used at 40X objective. The slides were coded prior to scoring to remove any personal bias. About 100 cells were scored from each slide. The microscope was pre calibrated using stage micrometer. Manual scoring was done by analyzing one comet at a time. Comets were typed into 0-4 types according to Collins (2004), type 0 being the undamaged DNA and type 4 as highly damaged. The comet tail length (defined as the distance from the center of mass of the head to distal end of the tail) was measured by the use of ocular micrometer. Migration length is directly related to fragment size and is expected to be proportional to the extent of DNA damage.

RESULTS

The experiment designed to measure DNA damage was performed with human leucocyte cell suspension and showed nuclear DNA damage in a exposure time-dependent manner by UV-B radiation. With the increase in time of exposure of UV-B radiation, the mean tail length also increased (Table 1, Fig. 1).

In the control sample, the comets showed a mean tail length of 13.26±4.64 µm. On increasing the exposure time to 5 minutes, the mean tail length reached to 28.02±5.42µm and with each increase of exposure time the mean comet tail length also increased and it went up to 88.72±2.83µm for exposure time of 30 minutes (Table 1). By using scoring method of Collins (2004), the DNA damage due to irradiations shows a linear dose-response from 5 to 30 minutes of exposure time (Fig. 2). Sufficient discrim-

Table 1: Mean ±SE (µm) of comet tail length in control and irradiated cells.

Sample No.	Time of exposure (minutes)	Comet tail length (µm) (Mean±SE)	TC	Cells with comet	Comet type					Arbitrary unit(AU)
					0	1	2	3	4	
1	0	13.26±4.64	100	28	72	10	10	5	3	57.0
	(control)									
2	5	28.02±5.42	100	66	34	48	9	9	0	93.0
3	10	34.60±5.77	100	72	28	25	19	20	8	155.0
4	15	43.71±4.83	100	79	21	25	13	25	16	192.0
5	20	56.63±4.11	100	92	8	17	23	32	20	239.0
6	25	62.18±3.56	100	100	0	10	33	44	13	260.0
7	30	88.72±2.83	100	100	0	5	14	31	50	326.0
Total			700	537						

TC- Total number of cells scored

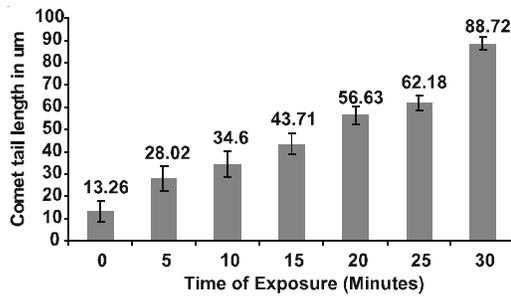


Fig. 1. DNA damage in human leukocyte cells irradiated with UV-B

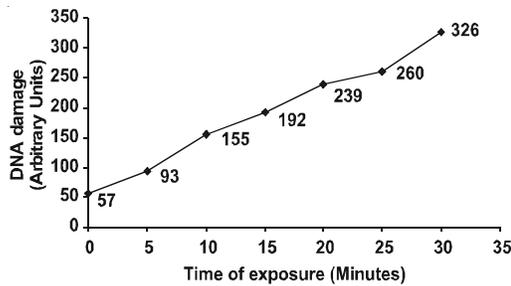


Fig. 2. UV-B irradiation responses for DNA damage in human leukocytes.

ination about the degree of damage in DNA according to comet appearance [Type 0 (no tail) to type 4 (almost all DNA in tail)] was given by Collins (2004). A total of 100 comets were scored from each sample and each comet assigned a value of 0 to 4 according to its type. The total score for the sample gel is given between 0 to 400 "arbitrary units". Most of the comets of unexposed samples belonged to type 0 and type 1 (Fig. 3). The frequency of type 2, 3 and 4 increased with respect to the increase in radiation exposure time to leukocytes, which further correspondingly effects the arbitrary unit, showing gradual increase form 57.0 in control sample to 326.0 in sample exposed to 30 minutes of radiations. Some baseline damage is always there which may be because of unwanted exposure to so many environmental mutagens in day to day life and partially may be due to processing error.

DISCUSSION

Gaps and breaks within the chromatid strands and DNA strands are commonly observed in radiation-induced lesion (Evans 1962; Yu 1971;

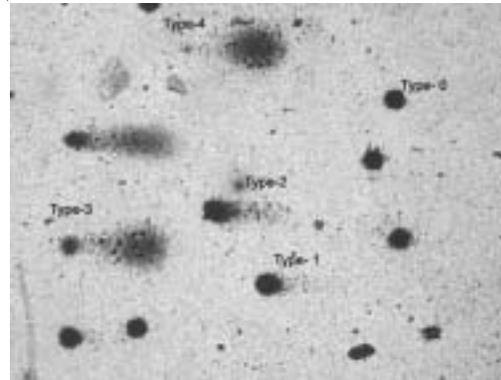


Fig. 3. Comets of different types.

Brecher 1977). DNA single-strand breaks taking place from alkali-labile lesions after exposure to UV radiations have been confirmed by many workers (Van Kuijik 1991; Fredrick 1993) as well as by the present experimental results which were based on alkaline version of the comet assay. Yadav and Seth (2000) reported increased level of both chromosome aberrations and sister chromatid exchanges in persons occupationally exposed to radiations.

The ionization effect of UV radiations, especially the ultraviolet B can be absorbed by nucleic acids, and may cause breaks and/ or perturbation of molecular structure (Shimmura and Tsubota 1997). These breaks are seen in the comet tails: as more the breaks, longer is the comet tail. Two explanations have been proposed in the literature about what the comet tail consist of, one is that it is a fragment DNA, the other is that the length of such a fragment is about 1 mm, but the length of the tail of a comet is few percentage points of it (Collins et al. 1997).

Patton et al. (1999) showed the lethal effect of near UV radiation peaking at 365nm, which killed the RPE (Retinal pigment epithelial) cells. Killing was dependent on the total energy dose of irradiation. They further reported that the UV-B exposed cells when allowed to repair DNA damage for 1 to 24 hours before analysis by comet assay, showed significant increase in mean percentage of tail DNA compared to those lysed immediately after irradiation.

In addition to photochemically-generated strand breaks, UV induces the formation of a variety of nucleobase photo-products that are subject to excision repair (Patton et al. 1999). The process produces new strand breaks that

are detected by the comet assay leading to a concomitant increase in mean percentage of tail DNA. Ichihashi et al. (2003) showed that the effects of UV-B radiation on DNA was mostly caused by formation of dimeric photoproducts between nearby pyrimidine bases on the same strand causing single strand breaks as shown in the increased comet tail length. In the present study, direct relationship was found between the time of exposure and DNA damage. In case of controls where no exposure was made, only minor basal damage took place which was indicated by the smaller comet tail length (13.26 μ m). The exposure time of 30 minutes gave a tail length of 88.72 μ m, indicating a highly damaged DNA. There was also a shift of type of comets (Collins 2004) as the exposure time to UV-B radiation increased. A corresponding move of the comets belonging to type 0 and type 1 in unexposed sample to type 3 and type 4 in samples exposed for 30 minutes was observed.

In the present experiment, some cells also showed the characteristic apoptotic comet tail structure at higher doses of radiation exposure. This is in agreement with the finding of Gniadecchi et al. (1998) and Caricchio (1998) who showed that UV-B radiation may also induce apoptosis in several cell systems in extreme cases.

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