BIOMEDICAL AND ENVIRONMENTAL SCIENCES **18**, 117-123 (2005)

Assessment of Human DNA Repair (NER) Capacity With DNA Repair Rate (DRR) by Comet Assay[1]

WEI ZHENG[*], JI-LIANG HE[*,#,2], LI-FEN JIN[*], JIAN-LIN LOU[*], AND BAO-HONG WANG[*]

[*]*Institute of Occupational and Environmental Health, Medical College of Zhejiang University, Hangzhou 310006, Zhejiang, China;* [#]*Medical College of Jiaxing University, Jiaxing 314001, Zhejiang, China*

Objective Alkaline comet assay was used to evaluate DNA repair (nucleotide excision repair, NER) capacity of human fresh lymphocytes from 12 young healthy non-smokers (6 males and 6 females). **Methods** Lymphocytes were exposed to UV-C (254 nm) at the dose rate of 1.5 J/m^2/sec. Novobiocin (NOV) and aphidicolin (APC), DNA repair inhibitors, were utilized to imitate the deficiency of DNA repair capacity at the incision and ligation steps of NER. Lymphocytes from each donor were divided into three grougs: UVC group, UVC plus NOV group, and UVC plus APC group. DNA single strand breaks were detected in UVC irradiated cells incubated for 0, 30, 60, 90, 120, 180, and 240 min after UVC irradiation. DNA repair rate (DRR) served as an indicator of DNA repair capacity. **Results** The results indicated that the maximum DNA damage (i.e. maximum tail length) in the UVC group mainly appeared at 90 min. The ranges of DRRs in the UVC group were 62.84%-98.71%. Average DRR value was 81.84%. The DRR difference between males and females was not significant (P<0.05). However, the average DRR value in the UVC plus NOV group and the UVC plus APC group was 52.98% and 39.57% respectively, which were significantly lower than that in the UVC group (P<0.01). **Conclusion** The comet assay is a rapid, simple and sensitive screening test to assess individual DNA repair (NER) capacity. It is suggested that the time to detect DNA single strand breaks in comet assay should include 0 (before UV irradiation), 90 and 240 min after exposure to 1.5 J·m^{-2} UVC at least. The DRR, as an indicator, can represent the individual DNA repair capacity in comet assay.

Key words: DNA repair capacity; Comet assay; UVC; Novobiocin; Aphidicolin

INTRODUCTION

DNA repair is a defense system to protect the integrity of genome. Deficiencies in this system can result in the development of cancer. Inter-individual variability in human response to carcinogens have been studied repeatedly. More attention has been paid to heritable gene polymorphisms associated with carcinogen metabolism. Another potentially important source of inter-individual variability in relation to the development of cancer is DNA repair capacity, including genetic instability syndromes, such as ataxia-telangiectasia (A-T), Fanconi anemia, Bloom's syndrome and xeroderma pigmentosum (XP). Besides these, individuals differ in their capacity of repairing DNA damage induced by both exogenous agents and endogenous reactions, such as oxidation[1]. Therefore, the epidemiology of DNA repair capacity and its effect on cancer susceptibility in humans are an important area of' investigation. A number of epidemiological studies have been conducted to compare the difference of DNA repair capacity between patients with cancer and healthy controls to assess the role of repair in the development of human cancer[1-4]. The high DNA repair capacity protects psoriasis patients against chemically induced basal cell carcinoma[5]. DNA repair processes are classified into several pathways: base excision repair (BER), nucleotide excision repair (NER), homologus recombinational repair (HRR), non-homologus end-joining (NHEJ) and mismatch repair[2]. Among them, nucleotide excision repair (NER) is the most important and versatile one involved in the removal of a variety of structurally different DNA lesions induced by physical and chemical carcinogens in environment[3].

So far, five kinds of' techniques have been used to estimate DNA repair capacity[1]. But there is a need for a reliable, robust and sensitive assay for global DNA repair, suitable for human lymphocyte

[1]This work was supported by International Cooperation Foundation of Science-Technique Bureau of Zhejiang Province (No. 012104), 2001-2002.

[2]Correspondence should be addressed to Ji-Liang HE, Tel: 86-571-87217188. Fax: 86-571-87217184. E-mail: he_jiliang@hotmail.com

Biographical note of the first author: Wei ZHENG, male, born in 1977, post graduate student for Ph. D. in Institute of Occupational and Environmental Health, Medical College of Zhejiang University.

0895-3988/2005
CN 11-2816/Q

samples in molecular epidemiological investigations. The comet assay or single cell gel electrophoresis is considered as a simple and sensitive assay, which is faster than cytogenetic assays and has been used as an alternative to unprogrammed DNA synthesis (UDS) for monitoring excision repair in human cells exposed to UV radiation[1,6-10]. The comet assay detects all types of DNA damage, including frank SSB, DSB and alkali-labile lesions[9]. In DNA repair capacity assays, the damage is delivered in the form of a "pulse" of carcinogen (e.g.: γ-rays, UV radiation, Benzo[a]pyrene Diol Epoxide(BPDE), and H_2O_2) applied to cell culture or to fresh or cryopreserved lymphocytes[1]. Although UV induces two major types of damage in DNA, i.e., cyclobutane pyrimidine dimers (CPD) and 6-4 photoproducts (6-4 PP), the damages themselves do not result in DNA breakage directly. NER processes produce DNA breaks as intermediates so that DNA repair capacity can be detected with comet assay by measuring the dynamic damage of lymphocytes in different durations of repair after UVC exposure[11-12]. Comet assay usually uses comet tail length (TL) as the indicator of DNA migration[12-16].

The aims of our study were to determine the time to observe the maximum DNA single strand breaks after UVC exposure and the time to exhibit DNA single strand breaks adjacent to the pre-exposure level during the process of NER, and to establish a model for assessing the DNA repair capacity of different individuals with the alkaline comet assay. Some chemicals can inhibit DNA excision repair at different steps of DNA repair processes. Novobiocin (NOV), an inhibitor of the ATPase subunit of topoisomerase II, acts at the incision step of NER to diminish markedly repair-specific DNA cleavage. Aphidicolin (APC), a DNA synthesis inhibitor, acts at the ligation step of NER to block the rejoining of DNA strands[17-20]. These two chemicals were used to determine the reliability of comet assay in assessing the DNA repair capacity in this study.

MATERIALS AND METHODS

Separation of Peripheral Blood Lymphocytes

Heparinized venous blood was collected from 12 healthy non-smokers (25-27 years old, 6 males and 6 females), who had never experienced photosensitivity or other unusual reaction consequent on the sun exposure during their life. Lymphocytes were isolated from the whole blood on histopaque gradients (Histopaque 1077, Sigma). The cells were washed two times with PBS, then suspended in PRMI 1640 medium (GIBCO, containing 20% fetal calf serum, antibiotics, PHA10 μg/mL) and incubated at 37℃ for 20 h in order to get G_1 lymphocytes. After UVC exposure, cells were tested for viability using the trypan blue dry exclusion technique at each time point of the study. Only those cell samples whose viability was over 95%, were measured by comet assay.

Treatment of UVC and DNA Repair Inhibitors

Novobiocin (NOV) and aphidicolin (APC) were from Sigma. NOV was dissolved in dimethylsulfoxide (DMSO)[20]. A stock solution of APC (2 mg/mL in ethanol)was stored at 4℃ and further diluted in Hank's solution[18-19]. NOV (500 μmol) was added to the cell suspension one hour before UV irradiation. APC (1 μmol) was immediately supplemented with the cell suspension after UV irradiation[21,22].

The UVC (254 nm, 1.5 $J \cdot m^{-2}$) exposure was radiated with a UV germicidal lamp (Waldmann ST 204 with Philips bulb). The energy flux was measured with a short-wave ultraviolet intensity meter (UVP, USA). UV irradiated cells were then suspended in RPMI 1640 medium and incubated for varying durations: 30 min, 60 min, 90 min, 120 min, 180 min, and 240 min respectively. After incubation the lymphocytes were washed in pH 7.4 phosphate buffered saline (PBS). All lymphocytes from each donor were divided into three groups: UVC group, UVC plus APC, and UVC plus NOV.

Single-cell Gel Electrophoresis

DNA single strand breaks in all kinds of irradiated cells were measured with comet assay. The alkaline (pH>13) SCG assay was performed by a modified method of Singh *et al.*[23-24]. The solution containing 0.65% normal melting agarose (NMA) and 0.65% low melting agarose (LMA) was prepared in Ca^{2+}, Mg^{2+} free PBS. After different incubation durations, cells with or without NOV and APC treatment were suspended in LMA, and 85 μL was pipetted onto a frosted glass microscope slide pre-coated with an 100 μL layer of 0.65% NMA. The third layer of 85 μL of 0.65% LMA was added finally. Then the slides were immersed in ice-cold freshly prepared lysis solution (1% N-lauroylsarcosine sodium salt, 2.5 mol/L NaCl, 100 mmol/L Na_2EDTA, 10 mmol/L Tris-HC1, 1% Triton X-100 and 10% DMSO, pH=l0) to lyse the cell proteins and allow DNA unfolding. After at least 1 h at 4℃ in the dark, the slides were covered with fresh buffer (1 mmol/L Na_2EDTA, 300 nmol/L NaOH, pH>13) in a horizontal electrophoresis unit. The slides were allowed to sit in this buffer for 20 min for DNA unwinding. Then, the DNA was electrophoresed at 20 V (1.09 V/cm) and 300

mA for 20 min. Both unwinding and electrophoresis were performed at an ambient temperature of 4℃. The slides were washed gently to remove alkali, deterged in a neutralization buffer (0.4 mol/L Tris-HC1, pH=7.5) and placed in methanol for 3 min, then stained with 50 μL ethidium bromide (2 μg/mL). All steps described above were conducted under yellow light or in the dark, to prevent additional DNA damage. The pictures of 50 cells per treatment sample (25 cells/slide) were taken individually under a fluorescence microscope (Olympus, BX51) and digital camera (Olympus, DP50) at 400 × magnification. Nuclear width and the extent of migration of DNA fragments were analyzed using Image-Pro Plus program (Media Cybernetics, Inc. USA).

Statistical Analysis

Statistical analyses were performed using the SPSS 11.0. Mean tail length (MTL) of 50 cells was calculated as the representatives of DNA migration. Because absolute values of DNA migration were not able to express directly the DNA repair capacity among 12 donors due to the difference of individual responsiveness to UV, DNA repair capacity was represented with DNA repair rate (DRR). The numerator indicates repaired DNA and the denominator indicates damaged DNA at 90 min of incubation. The formula is:

$$\text{DRR}=\frac{\text{Repaired DNA}}{\text{Damaged DNA}}\times 100\% \quad \text{or}$$

$$\text{DRR}=\frac{\text{MTL}_{90}-\text{MTL}_{240}}{\text{MTL}_{90}-\text{MTL}_{0}}\times 100\%$$

Paired sample *t*-test was performed to compare the DRR difference between UVC group and UVC plus NOV or APC groups. One-way ANOVA was used to evaluate DRR differences between males and females. Independent sample *t*-test was utilized to compare DNA migration difference at 90 min or 240 min of incubation among three groups.

RESULTS

DNA Damage After UVC Exposure

Table 1 shows MTL values at 0-240 min of incubation in the UVC group. It was found that the maximum MTLs of 11 donors appeared at 90 min of incubation. Only the maximum MTL of donor 5 presented at 120 min of incubation, which was 2.96 μm close to MTL value (2.93 μm) at 90 min of incubation. The range of MTL values at 90 min of incubation for 12 donors was 2.93-6.33 μm. The average MTL at 90 min of incubation was 4.77 μm, which was the biggest among 7 average values. At 240 min of incubation, the average MTL was 2.43 μm, which was close to the level (1.93 μm) of UVC pre-exposure.

TABLE 1

MTL (μm) at 0, 30, 60, 90, 120, 180, and 240 min of Incubation in UV Group

Donor	Sex	Pre-irradiate $\bar{x}\pm s$	30 min $\bar{x}\pm s$	60 min $\bar{x}\pm s$	90 min $\bar{x}\pm s$	120 min $\bar{x}\pm s$	180 min $\bar{x}\pm s$	240 min $\bar{x}\pm s$
1	M	2.27±0.11	2.66±0.16	2.71±0.08	6.22±0.49*	3.21±0.19	3.48±0.48	3.17±0.33
2	M	1.99±0.12	---	3.37±0.19	6.33±0.70*	4.11±0.39	4.72±0.65	3.13±0.25
3	M	2.09±0.15	2.41±0.14	4.03±0.53	5.19±0.56*	3.39±0.40	3.14±0.45	2.13±0.18
4	M	1.41±0.11	0.89±0.05	1.96±0.14	3.40±0.38*	2.29±0.17	2.46±0.16	1.94±0.37
5	M	1.40±0.07	2.01±0.11	2.03±0.13	2.93±0.31	2.96±0.22*	1.84±0.15	1.77±0.11
6	M	1.63±0.15	1.99±0.22	2.19±0.12	4.87±0.26*	1.85±0.11	2.09±0.15	1.79±0.15
7	F	2.62±0.15	2.96±0.20	4.09±0.44	5.38±0.66*	4.98±0.46	4.55±0.45	2.95±0.16
8	F	2.64±0.18	2.88±0.21	3.72±0.46	5.63±0.58*	4.75±0.55	4.68±0.69	2.86±0.28
9	F	1.77±0.15	1.92±0.11	3.24±0.17	4.38±0.50*	3.87±0.52	3.50±0.41	2.74±0.23
10	F	2.28±0.16	2.66±0.25	2.45±0.17	3.96±0.35*	3.78±0.32	3.81±0.31	2.64±0.28
11	F	1.37±0.08	1.72±0.13	2.83±0.38	4.27±0.47*	3.65±0.49	1.89±0.19	1.60±0.38
12	F	1.71±0.08	2.05±0.11	1.91±0.08	4.64±0.52*	3.12±0.21	2.37±0.22	2.47±0.22
$\bar{x}\pm s$		1.93±0.45	2.20±0.60	2.88±0.80	4.77±1.05	3.50±0.91	3.21±1.08	2.43±0.56

Note. - Data deletion. *The maximum MTL.

DNA Damage of Three Treatment Groups

MTLs at 0, 90, 240 min of incubation in three groups are shown in Table 2. MTL at 90 min of incubation in the UVC plus NOV group was 3.16 μm, which was significantly lower than that (4.77 μm) in the UVC group (P<0.01). On the other hand, MTL at 240 min of incubation in the UVC plus APC group was 3.43 μm, which was significantly higher than that (2.43 μm) in the UVC group (P<0.01).

Fig. 1 shows the mean values of MTLs at various incubation time points in three groups respectively. Moreover, all peak values of average MTL in three groups appeared at 90 min of incubation.

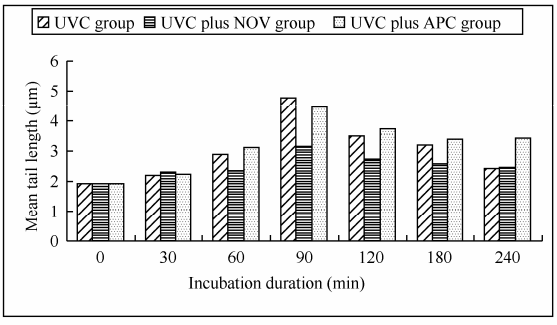

FIG. 1. Mean value of MTLs in 12 donors.

DRR of Three Groups

DRR values of three groups are shown in Table 2. In the UVC group, the range of DRR values was 62.84% to 98.71%, and DRR value of 11 donors was more than 70%. The range of DRR values in the UVC plus NOV group was 7.95% to 71.28%, only one DRR value was more than 70%, 6 DRR values were lower than 60%. In the UVC plus APC group, the range of DRR values was 18.89 to 71%, also only one DRR value was more than 70% and 11 DRR values were lower than 60%. It was discovered that average DRR in the UV group was 81.84% which was significantly higher than those (52.98% and 39.57%) in the UVC plus NOV or APC groups (P<0.01). Table 3 denotes the average DRR values of males and females in the UV group, which were 82.32% and 81.37% respectively. The results showed no significant difference between males and females (P>0.05)

TABLE 3

DRR (%) of Males and Females in UVC Group

Order	DRR	
	Male	Female
1	77.21	88.04
2	73.73	92.64
3	98.71	62.84
4	73.37	78.57
5	75.82	92.07
6	95.06	74.06
$\bar{x} \pm s$	82.32±11.43	81.37±11.75

Note. Male vs Female; Independent Sample t-test (95% confidence): Homogeneity of variance P>0.05.

DISCUSSION

The ability to repair DNA lesions is strongly associated with the risk of cancer and other diseases, as it is a ubiquitous defense mechanism essential for cell survival and cell cycle control. Inter-individual variation in DNA repair capacity has been observed in several *in vitro* lymphocyte assays. The differences in DNA repair capacity among individuals reflect genetic differences. DNA repair capacity in different subpopulations of lymphocytes from the same individual has a similar repair capacity, and the inter-individual variations in repair capacity was are significantly smaller than the variations among individuals[2,6]. For this reason, detecting DNA repair capacity of human peripheral lymphocytes can express individual DNA repair capacity. So far, five categories of assays of DNA repair capacity have been developed, but in most assays currently used, it is not possible to make a distinction between DNA damage and repair, and there are some shortages. For example, the measurement of cell survival (usually by cloning efficiency) was too long and too expensive to apply for large-scale studies, the variability of UDS was around 20%, depending on the growing potential of cultured cells, and the variability of the host cell reactivation assay (HCR) differed greatly from one laboratory to another[1-3].

Recently, the comet assay has been used to measure the DNA repair capacity of human lymphocytes from different individuals, as a rapid screening assay[1-3]. However, two key issues in comet assay should be solved for measuring DNA repair capacity of human lymphocytes. One is the time after exposure to mutagens, e.g the time of "UV" to detect DNA strand breaks which may reflect the situation of incision step or legation, step in NER. UV could induce 6-4 photoproducts and cyclobutane pyrimidine dimers (CPD), which are also repaired by NER. There are two important steps, i.e. DNA incision step and ligation step during the process of NER[3,25-26]. DNA strand breaks induced by UV usually emerge at the incision step of NER. Most frank breaks are repaired immediately after exposure. But enzymatically generated breaks were observed only when excision of damaged DNA bases exceeded the rate of DNA strand rejoining, and this disequilibrium became apparent about an hour after UV exposure[9,12,14,24]. DNA damage generally first reaches a maximum and then decreases over time to their pre-exposure situation. In healthy cells, repair of lesions was complete

TABLE 2

MTLs (μm) at 0, 90, 240 min of Incubation and MTL-DRRs (%) in Three Groups

Donor	Sex	MTL at 0 min			MTL at 90 min			MTL at 240 min			MTL-DRR		
		UVC	UVC+NOV	UVC+APC	UVC	UVC+NOV	UVC+APC	UVC	UVC+NOV	UVC+APC	UVC	UVC+NOV	UVC+APC
1	M	2.27	2.27	2.27	6.22	4.15	3.17	3.17	2.81	3.00	77.21	71.28	18.89
2	M	1.99	1.99	1.99	6.33	3.82	5.90	3.13	2.57	3.89	73.73	68.31	51.41
3	M	2.09	2.09	2.09	5.19	3.01	3.90	2.13	2.42	2.92	98.71	64.13	54.14
4	M	1.41	1.41	1.41	3.40	2.15	2.68	1.94	1.81	2.34	73.37	45.95	26.77
5	M	1.40	1.40	1.40	2.93	2.50	3.24	1.77	1.93	2.74	75.82	51.82	27.17
6	M	1.63	1.63	1.63	4.87	2.25	4.01	1.79	1.85	3.22	95.06	64.52	33.19
7	F	2.62	2.62	2.62	5.38	3.50	6.08	2.95	3.43	5.37	88.04	7.95	20.52
8	F	2.64	2.64	2.64	5.63	3.54	6.47	2.86	3.22	4.72	92.64	35.56	45.69
9	F	1.77	1.77	1.77	4.38	3.66	5.60	2.74	2.70	4.00	62.84	50.79	41.77
10	F	2.28	2.28	2.28	3.96	3.01	4.60	2.64	2.73	3.49	78.57	38.36	47.84
11	F	1.37	1.37	1.37	4.27	3.61	4.11	1.60	2.07	3.11	92.07	68.75	36.50
12	F	1.71	1.71	1.71	4.64	2.72	3.90	2.47	2.03	2.345	74.06	68.32	71.00
$\bar{x} \pm s$		1.93±0.45	1.93±0.45	1.93±0.45	4.77±1.05	3.16±0.65**	4.47±1.26	2.43±0.56	2.46±0.54	3.43±0.92**	81.84±11.06	52.98±18.86**	39.57±15.40**

Note. **Comparing with the UVC group, $P<0.01$.

after 4 h of incubation as indicated by the absence of comet formation[12]. However, in our experiment, comet tail lengths from 12 donors approximately reached the maximum value at 90 min of incubation after UVC exposure at the dose rate of 1.5 $J \cdot m^{-2}$, then underwent a time-dependent decrease close to pre-exposure levels at 240 min of incubation after exposure, supporting the assumption that this change was related to the predominance of ligation step in the DNA repair process. It was found that the time of peak MTL value after UVC exposure in our experiment was different from 60 min and 10 min reported by Tuck and Mohankumar, respectively. The reason for the difference may be related to the UVC energy flux used. The latter was 4 $J \cdot m^{-2}$ and 30 $J \cdot m^{-2}$ respectively[12, 27].

Another important issue is how to evaluate the individual DNA repair capacity according to the detected DNA strand breaks. Comet assay can only detect the DNA strand breaks induced by UVC. DNA damage, i.e. tail length, cannot be directly utilized to assess the individual DNA repair capacity. In this experiment with UVC as a mutagen, the DRR served as an indicator to assess the individual DNA repair capacity according to MTLs at 0, 90, and 240 min of incubation after UVC exposure, because the DNA strand breaks at 0, 90, and 240 min after UVC exposure were the basic, maximal and residual values of DNA damage in the DNA repair process respectively. DRR is only a relative value, because the ratio of repaired damage to maximum DNA damage reflects the proportion of repaired DNA damage during the repairing period of 4 h, regardless of the absolute value of DNA damage.

The results of the experiment showed that most DRR values (11/12) were >70% in spite of a very significant difference in MTL values among 12 donors. Also there was no significant difference in DNA repairing speed among the 12 donors, the peak values (11/12) were found at 90 min of incubation, so DRR values suggested the absence of visible differences among the donors as expected on the basis of their phenotype (healthy persons). Therefore, DRR seemed to be a more reliable indicator of individual DNA repair capacity than direct values of DNA damage such as MTL. Results of our study showed no significant difference in DNA repair capacities between males and females.

In the experiment with DNA repair inhibitors, DNA repair inhibitors NOV and APC were used to mimic the low DNA repair capacity. The MTL values at 90 min of incubation after UVC exposure in the UVC plus NOV group were significantly lower than those in the UVC group. The MTL values at 240 min of incubation after UVC exposure in the UVC plus APC group were significantly higher than those in the UVC group. The situation indicated that NOV inhibited the incision process of NER and APC inhibited the ligation process of NER. It should be emphasized that the DRR values in both the UVC plus NOV and the UVC plus APC groups were significantly diminished, compared with the DRR values in the UVC group. The DRR values used in the experiment displayed that the DNA repair (NER) capacity of human lymphocytes was inhibited by NOV and APC.

In conclusion, the comet assay is a rapid, simple and sensitive screening test to measure individual DNA repair capacity, the time to detect DNA single strand breaks in comet assay should include 0 min (before UV irradiation), 90 min and 240 min after 1.5 $J \cdot m^{-2}$ of UVC irradiation at least, and the DRR as an indicator may express the individual DNA repair capacity in comet assay.

REFERENCES

1. Berwick, M. and Vineis, P. (2000). Markers of DNA repair and susceptibility to cancer in human: an epidemiologic review. *J. Natl. Cancer Inst*. **92**, 874-897.
2. Mohrenweiser, H. W. and Jones, I. M. (1998). Variation in DNA repair is a factor in cancer susceptibility: a paradigm for the promises and perils of individual and population risk estimation. *Mutat. Res*. **400**, 15-24.
3. Benhamou, S. and Sarasin, A. (2000). Variability in nucleotide excision repair and cancer risk: a review. *Mutat. Res*. **462**, 149-158.
4. Grossman, L. (1997). Epidemiology of ultraviolet-DNA repair capacity and human cancer. *Environ. Health Perspect* **105**, 927-930.
5. Dybdahl, M., Frentz, G., Vogel, U., Wallin, H., and Nexo, B. A. (1999). Low DNA repair is a risk factor in skin carcinogenesis: a study of basal cell carcinoma in psoriasis patients. *Mutat. Res*. **433**, 15-22.
6. Schmezer, P., Rajaee-Behbahani, N., Risch, A., Thiel, S., Rittgen, W., Drings, P., Dienemann, H., Kayser, K.W., Schuiz, V., and Bartsch, H. (2001). Rapid screening assay for mutagen sensitivity and DNA repair capacity in human peripheral blood lymphocytes. *Mutagenesis*. **16**, 25-30.
7. Collins, A. R., Dusinská, M., Horváthová, E., Munro, E., Savio, M., and Stetina, R. (2001). Inter-individual differences in repair of DNA base oxidation, measured *in vitro* with the comet assay. *Mutagenesis* **16**, 297-301.
8. Olive, P. L. (1999). Review DNA damage and repair in individual cells: application of the comet assay in radiobiology. *Int. J. Radiat. Biol*. **75**, 395-405.
9. Reinhardt-Poulin, P., Mclean, J. R., Deslauriers, Y., Gorman W., Cabat, S., and Rouabhia, M. (2000). The use of silver-stained "Comets" to visualize DNA damage and repair in normal and Xeroderma pigmentosum fibroblasts after exposure to simulated solar radiation. *Photochem. Photobiol*. **71** , 422-425.
10. Gedik, C. M., Ewen, S. W. B., and Collins, A. R. (1992). Single-cell gel electrophoresis applied to the analysis of UVC damage and its repair in human cells. *Int. J. Radiat. Biol*. **62**, 313-320.
11. Karmakar, P. and Natarajan, A. T. (2000). Characteristics of UV-induced repair patches relative to the nuclear skeleton in human fibroblasts. *Mutagenesis* **15**, 115-120.
12. Tuck, A., Smith, S., and Larcom, L. (2000). Chronic

lymphocytic leukemia lymphocytes lack the capacity to repair UVC-induced lesions. *Mutat. Res*. **459**, 73-80.

13. Visvaridis, E. E., Tassiou, A. M., and Piperakis, S. M. (1997). Study of DNA damage induction and repair capacity of fresh and cryopreserved lymphocytes exposed to H_2O_2 and γ-irradiation with the alkaline comet assay. *Mutat. Res*. **383**, 71-80.
14. Myllyperkiő, M. H., Koski, T. R. A., Vilpo, L. M., and Vilpo, J. A. (2000). Kinetics of excision repair of UV induced DNA damage, measured using comet assay. *Mutat. Res*. **448**, 1-9.
15. Udumudi, A., Jaiswal, M., Rajeswari, N., Desai, N., Jain, S., Balakrishna, N., Rao ,K. V., and Ahuja, Y. R. (1998). Risk assessment in cervical dysplasia patients by single cell gel electrophoresis assay: a study of DNA damage and repair. *Mutat. Res*. **412**, 195-205.
16. Jatoszyfiski, P., Kujawski, M., Swierczek, M.C., Markowska, J., and Szyfter, K. (1997). Bleomycin induced DNA damage and its removal in lymphocytes of breast cancer patients studied by comet assay. *Mutat. Res*. **385**, 223-233.
17. Bock, C., Dittmar, H., Gemeinhardt, H., Bauer, E., and Greulich, K. O. (1998). Comet assay detects cold repair of UV-A damages in a human B-lymphoblast cell line. *Mutat. Res*. **408**, 111-120.
18. Speit, G. and Hartmann, A. (1995). The contribution of excision repair to the DNA effects seen in the alkaline single cell gel test (comet assay). *Mutagenesis* **10**, 555-559.
19. Gedik, C. M. and Collins, A. R. (1991). The mode of action of 1-13-D-arabinofurenosylcytosine in inhibiting DNA repair; new evidence using a sensitive assay for repair DNA synthesis and ligation in permeable cells. *Mutat. Res*. **254**, 231-237.
20. Thielmann, H. W., Popanda, O., Gersbach, H., and Gilberg, F. (1993). Various inhibitors of DNA topoisomerases diminish repair-specific DNA incision in UV-irradiated human fibroblasts. *Carcinogenesis* **14**, 2341-2351.
21. Kishi, K. (1988). Suppressive effect of novobiocin on the frequency of chromosome-type aberrations induced by ara C in the G 1 phase of human lymphocytes. *Mutat. Res*. **109,** 113-109.
22. Meenakshi, R. N., Pai, S. A., Shinde, S. R., and Ghosh, S. N. (1998). Reduced DNA Repair Capacity in Breast Cancer Patients and Unaffected Individuals from Breast Cancer Families. *Cancer Genet. Cytogenet*. **102**, 65-73.
23. Singh, N. P., McCoy, M. T., Tice, R. R., and Schneider, E. L. (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res*. **175**, 184-191.
24. Frenzilli, G., Bosco, E., and Barale, R. (2000). Validation of single cell gel assay in human leukocytes with 18 reference compounds. *Mutat. Res*. **468** , 93-108.
25. Dittmann, K. H., Dikomey, E., Mayer, C., and Rodemann, H. P. (2000). The Bowman-Birk protease inhibitor enhances clonogenic cell survival of ionizing radiation-treated nucleotide exmslon repair competent cells but not of xeroderma pigmentosum cells. *Int. J. Radiat. Biol*. **76**, 223-229.
26. Bernstein, C., Bernstein, H., Payne, C. M., and Garewal, H. (2002). DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. *Mutat. Res*. **511**, 145-178.
27. Mohankumar, M. N., Janani, S., Prabhu, B. K., Kumar, P. R, and Jeevanram, R. K. (2002). DNA damage and intergrity of UV-induced DNA repair in lymphocytes of smokers analysed by the comet assay. *Mutat. Res*. **520**, 179-187.

(*Received May* 20, 2004 *Accepted November* 10, 2004)