Bull. Environ. Contam. Toxicol. (1994) 52:687–690

Environmental Contamination and Toxicology

Detection of DNA Damaging Agents in Environmental Water Samples

D. Fairbairn, D. Meyers, K. O'Neill

Department of Microbiology, Brigham Young University, Provo, Utah 84602, USA

Received: 17 May 1993/Accepted: 15 August 1993

Industrial and chemical processes produce substances toxic to the environment and human health. Occasionally, such substances are simply introduced into the environment with or without detoxification. Chemical agents that damage DNA are potentially mutagenic and/or carcinogenic in humans (Cross *et al* 1987). DNA damage in the form of single or double strand breaks may share these dangerous mutagenic or carcinogenic properties (Imlay *et al* 1988). We have recently demonstrated the usefulness of laser scanning microscopy technology in the accurate detection of DNA single strand breaks (Fairbairn *et al* 1993) and double strand breaks (manuscript in preparation) using the single cell gel (SCG) assay. The SCG assay is a very sensitive, accurate, and reproducible method of detecting and measuring levels of DNA strand breaks in individual cells (Olive *et al* 1993, Vijayalaxmi *et al* 1992).

We demonstrate in this study the ability of the SCG assay to detect DNA damaging agents in a sensitive way in environmental water samples. We sampled water from various sources in Utah County and found that one sample in particular, which was taken from water being introduced into Utah Lake, contains some agent(s) capable of inducing DNA damage in the form of strand breaks or alkali labile sites in individual human cells.

MATERIALS AND METHODS

Samples of water were taken in 50 mL volumes from different sites in Utah Valley. One sample was taken from the outlet of a large pipe leading from an industrial area flowing into Provo River (a), two were taken from holding pools of waste water produced by large industrial companies that are subsequently released into Utah Lake (B and C), and the last sample was taken from industrial waste water flowing into Utah Lake (D). Deionized water was used as a control sample (E). All samples were filtered using a

Correspondence to: K. O'Neill

0.20 μm filter and diluted with stocks of phosphate buffered saline (PBS) to a final concentration of 1X PBS for treatment.

Raji cells (a human promyelocytic cell line) were maintained in RPMI 1640 plus 10% fetal bovine serum (Hyclone Labs, Logan UT) at exponential growth. A suspension of cells in 0.75% low melt point agarose was prepared at 37°C, and a very thin gel was prepared by spreading 300 μL on a fully frosted slide (50,000 cell total). The slides were placed in PBS prepared using different water samples for 60 minutes at 4°C to prevent DNA repair (Ward *et al* 1985). The single cell gel assay and laser scanning microscopic analysis was performed as previously described (Fairbairn *et al* 1993).

DNA damage has been shown to correlate with increased migration in the direction of electrophoresis (Gedik *et al* 1992, Fairbairn *et al* 1993). Measurements of comet length (x) and height (y) were taken and used during analysis. The ratio of y/x is useful in comparing relative damage levels; a control with no damage would have a y/x value nearing one, while the y/x value decreases with increased DNA damage. Statistical analysis was performed using the student's t-test. All experiments were performed blind.

RESULTS AND DISCUSSION

Figure 1 demonstrates the appearance of comets. DNA migrates from the head of the comet in the direction of electrophoresis in cells with induced damage. We initially screened the samples for substances toxic to the DNA of human cells by treating whole cells with PBS prepared using sample water. Cells were subjected to the SCG assay and individual comets were scored as described using laser scanning microscopy. The samples were diluted as one part water sample to two parts deionized water in the PBS preparation. The results of the initial screening displayed in Table 1 are averages of four experiments.

Table 1. Screening environmental water samples for DNA damaging agents.

Sample*	x (μm)	SD	difference from control (μm)	significance(p)
A (into river)	19.2	3.7	0.3	0.631
B (holding pool)	28.5	6.6	9.0	<0.001
C (holding pool)	20.0	3.6	0.4	0.479
D (into lake)	21.4	3.9	1.9	0.004
E (deion. water)	19.5	3.2	control	control

*25 comets were scored per sample in each of four trials.

The negative control comets (sample E) had an average length of

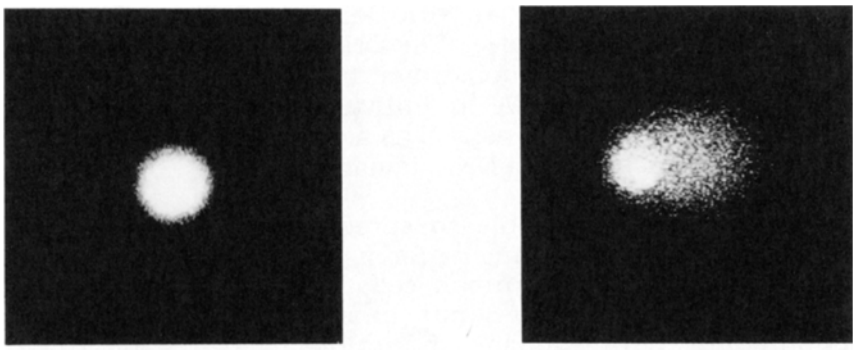

Figure 1. Comet images visualized using the LSM (A) Control cell DNA with no induced DNA damage (B) Raji cell with induced DNA damage. 25 μm = ⟷

19.52 μm, whereas the average comet length in sample B was 28.54 μm. We decided to further analyze sample B because it appeared to contain some agent capable of causing a significant increase in DNA migration under alkaline conditions which is representative of DNA damage.

We prepared three different dilutions of the sample and ran the SCG assay using these dilutions compared against a deionized water negative control. We included 1:2, 1:10 and 1:100 water sample dilutions in deionized water when preparing the PBS solutions for exposure. We wished to determine whether the agents responsible for the apparent increase in DNA damage in the above sample could be detected at the different dilutions. The experimental conditions used were identical to that used above. The results of four sets of experiments are given in Table 2.

Table 2. Effect of dilution on the ability to detect DNA damaging agents in an environmental water sample.

Sample*	y/x	SD	x (μm)	SD	diff. from cntrl(μm)	signif. (p)
control	0.92	0.06	21.1	3.8	control	control
1:100	0.91	0.07	21.6	3.3	0.5	0.453
1:10	0.76	0.10	26.7	5.0	5.6	<0.001
1:2	0.45	0.07	44.0	4.8	22.8	<0.001

*25 comets were scored per sample in each of four trials.

The comet characteristics were not significantly different for the 1:100 dilution. At this dilution, it was not possible to detect the DNA damaging agent(s). It was possible, however, to detect

significant DNA damage at dilutions less than 1:10 as demonstrated in the table above. The DNA damage induced by this environmental sample is demonstrated by both an increase in the length of migration of DNA in individual human cells when subjected to electrophoresis, as well as a decrease in the y/x value, which is also characteristic of DNA damage.

We found that it is possible to screen water samples being introduced into the environment for agents that are capable of inducing DNA damage in human cells using the single cell gel assay. Although we have not characterized the agent(s) responsible for DNA damage induction in the sample that demonstrated the most significant increases in DNA migration, we demonstrate in this study that it is possible to detect the DNA damaging agents. This assay is very sensitive and reproducible, and may prove to be useful in the detection of DNA damaging agents in environmental samples.

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