The International Comet Assay Workshop

Diana Anderson\textsuperscript{1,3} and Michael J. Plewa\textsuperscript{2}
\textsuperscript{1}BIBRA International, Woodmansterne Road, Carshalton, Surrey SM5 4DS, UK and \textsuperscript{2}Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

The Comet (single cell gel electrophoresis) assay primarily measures DNA strand breakage in single cells (Singh et al., 1988). Briefly, cells are suspended in low melting point agarose on a microscope slide. The slides are put in lysing buffer to allow the DNA to unwind and then in electrophoresis buffer. During electrophoresis the broken DNA moves towards the anode forming a Comet tail, with the greater the extent of damage, the greater the tail. Assays can be conducted under neutral or alkaline (>pH 13) conditions. Double-strand breaks are measured under neutral conditions and single-strand breaks under alkaline conditions, where abasic sites and other alkali-labile sites or intermediates in base or nucleotide excision repair can also be detected. There are several good review articles addressing the assay (McKelvey-Martin et al., 1993; Fairbairn et al., 1995; Tice, 1995).

Introduction

An International Comet Assay Workshop held at the Croydon Park Hotel, 8–10 May, 1997 was attended by 87 delegates. There were 33 oral presentations, with discussions after each presentation, 19 poster presentations with summaries and discussions and a panel discussion. The meeting was convened to determine new advances in the different topic areas which were addressed. The topic areas were divided into four main sections; clinical applications, human monitoring, somatic and underlying mechanisms/new developments. Most of the assays to be described were conducted under alkaline conditions. Some were conducted under neutral conditions, e.g. some of the sperm assays. Details of the assays, such as exposure conditions, can be obtained from the authors.

Tice opened the meeting with an overview of the assay. He spoke of the neutral and alkaline techniques and how the numbers of researchers and range of applications were increasing exponentially. Only small samples of body fluids and cells are required for the assay and technical advancements involve the ability to score images in dried gels.

Clinical applications

Houlbrook et al. reported the time course of DNA damage and repair in lymphocytes from 24 patients with malignant melanoma, following treatment with dacarbazine and tamoxifen. The lymphocytes were stably stored at -70°C. They were able to correlate the percent DNA in the Comet tails at 4 h after treatment with nausea and vomiting and at 24 h with neutropenia.

Everett et al. demonstrated that the lower levels of DNA strand breaks in patients with gastric epithelial mucosal cells infected with Helicobacter pylori could be explained by a greater flux of DNA damage and repair. Helicobacter pylori eradication returned Comet levels towards normal, suggesting a possible role for this organism in gastric cancer prevention. That this was incomplete after 6 weeks probably reflected the persistence of chronic gastritis after this time. The lower Comet levels in the gastric antrum were almost certainly linked to the predominance of gastritis in this area in H. pylori infection.

Șardaș et al. applied the Comet assay to cancer patients with Hodgkin's lymphoma and bone metastases, before and after they had completed their radiation therapy. Cells from peripheral blood samples were classified as normal, stretched (stitching of attached strands of DNA around the cell without migration) or migrated. Irrespective of irradiation therapy, all patients had different grades of damage, but cells were mainly stretched and after irradiation there was a highly significant increase in migrated cells, indicating increased DNA damage.

Gutiérrez et al. suggested that the Comet assay was sensitive enough to detect genetic damage induced by exposure to ¹³¹I. However, a high variability was found between patients and the responses were less clear than those obtained with the micronucleus assay. Although cancer patients received a dose 10 times higher than hyperthyroid patients, responses were similar in both types of patients, possibly because the latter had an intact thyroid gland which retained ¹³¹I, acting as an internal radiation source, and the cancer patients also suffered treatment-related thyroid ablation.

Plappert and Fiedler examined whether changes in the repair capacity after in vitro irradiation of blood cells could be used as a biomarker for radiation exposure. Individuals chronically exposed to irradiation exhibited a decreased repair capacity. Since this was not the case after acute exposure, it was considered that decreased repair capacity after in vitro irradiation might be a useful effect biomarker for chronic exposure.

Pool-Zobel et al. used the Comet assay with and without exonuclease III to determine single-strand breaks (SSB) and oxidized pyrimidines in colon cells of biopsies and in human tumour cell lines (HT29 and HT29 clone 19A, a butyrate-differentiated subclone). Human primary colon differentiated cells contained more DNA SSB and oxidized bases than the clone 19A or HT29 stem cells and the clone had more genetic damage than the stem cells.

Leprat et al. suggested that a DNA repair defect may be involved in initiation of thyroid carcinogenesis. This is because the response of lymphocytes from 13 patients with thyroid cancers after radiotherapy for a primary cancer, when compared with seven normal healthy donors, two individuals without thyroid tumours and four patients with spontaneous tumours, showed delayed repair kinetics and a greater percentage (85%) of residual damage after irradiation with 2 and 5 Gy in vitro. Such a defect may allow identification of individuals at risk.

\textsuperscript{a}To whom correspondence should be addressed. Tel: +44 181 652 1000; Fax: +44 181 661 7029; Email: danderson@bibra.co.uk
Human monitoring

Šrám et al. collected blood samples from hospitalized pregnancies in 1995–1996, 211 from Teplice (an area polluted by the combustion of brown coal in Northern Bohemia) and 117 from Prachatice (control, Southern Bohemia). Venous and cord blood were analysed. Percentage tail (%T), tail lengths (TL), tail moments (TM), concentrations of the pollutants (SO$_2$, NO$_x$) during the previous 30 and 90 days, district, smoking habit, education and metabolic genotype of glutathione S-transferase MI (GSTMI) were evaluated by multiple regression analysis. No effect of GSTMI was observed but Comet parameters were affected by the length of gestation and birth weight. Thirty days exposure to SO$_2$ affected %T in mothers and children and TM in mothers whilst 90 days exposure to SO$_2$ affected TL and TM in mothers.

Frenzilli et al., in a follow-up to a previously published study, examined 90 smokers who showed increased means of Comet tail lengths when compared with appropriate controls. After 1 year of follow-up, Comet lengths were shorter in those who completely quit smoking compared with those who relapsed into smoking, irrespective of the number of cigarettes previously smoked. Besides the genotoxins in cigarette smoke, smoking-induced reactions such as inflammation might induce DNA damage. Other pathological responses, such as allergy, asthma and autoimmune conditions, may also represent important confounding factors in the Comet assay when used for human monitoring studies, but no effect of age or sex was found in this study.

Cebulska-Wasilewska et al. showed that a strong correlation existed between DNA damage as measured in the Comet assay and chromosomal damage detected in human lymphocytes exposed in vitro to various types of radiation (X-rays and fast neutrons) and to three different structurally related chemicals. The best correlation was observed between the percentage of aberrant cells and cells with the highest degree of DNA damage. In addition, radiation dose–response curves for both cytogenetic and DNA damage allowed estimation of the absorbed dose in people accidentally exposed to radiation.

Frenzilli et al. reported that the Comet assay was useful in discriminating 'healthy' children from 60 children exposed to Chernobyl fallout, some of whom had developed thyroid tumours. The radionuclide-exposed children had higher levels of damage in their lymphocytes before therapy and some of them after surgical and iodine therapy. Such a difference was also revealed by a possible adaptive response in children with cancer after in vitro treatment with bleomycin. The children are still living in irradiated areas and are chronically exposed to contaminated food. The presence of clastogenic effects in the exposed children was also examined and such effects may also contribute to modulation of the adaptive response.

Wojewódzka et al. examined 49 professionals likely to be at risk from low doses of ionizing radiation. Confounding factors such as age, smoking habit, the use of therapeutic drugs, work-related exposure to hazardous agents and diagnostic X-rays were registered. Forty matched controls were also examined. Individuals were divided according to risk of exposure, smoking habit and gender and groups compared for mean tail moments and percentage of DNA in the tail without enzyme treatment and with endonuclease III and formamidopyrimidine glycosylase (FPG) treatment-in test groups. There was a significant difference between the control and hazard groups without enzyme treatment only and higher damage was found for men than women, but there was no relation of DNA damage to age and smoking habit. The level of oxidation damage was the same in control and hazard groups.

Lebailly et al. examined lymphocytes from farmers occupationally exposed to selected pesticides [the herbicide isoproturon (n = 12), the fungicide mixture cyprodinil + epoxiconazol (n = 14) and a fungicide (chlorothalonil–insecticide mixture (n = 8)]. There was no relationship between the DNA damage level observed and exposure parameters. There was no effect of age, but a slight effect of smoking.

Rojas et al. reported that in Mexico City there is an uneven distribution of air pollutants since the mountains which surround the city limit free air circulation. The wind blowing from the south sweeps all the ozone precursors to the southwest, whilst particulates and hydrocarbons remain concentrated in the north. Leukocytes and nasal and buccal epithelial cells were examined in the Comet assay in young adults. There were increases in DNA migration in leukocytes in nasal epithelial cells but not in buccal cells in individuals in the south of the city. Respiratory symptoms were also more evident in people from the south.

Collins et al. reported that long- or short-term antioxidant supplementation to individuals has been shown to decrease the endogenous oxidation of bases in their lymphocyte DNA, to increase the resistance of lymphocyte DNA to in vitro oxidation by H$_2$O$_2$ and to enhance the recovery of lymphocytes from oxidative damage. This last effect is probably not due to a stimulation of DNA repair, but a protection against the atmospheric oxygen to which lymphocytes are exposed in culture. Oxidative DNA damage has been demonstrated in subjects suffering from diabetes and ankylosing spondylitis and elevated strand breaks were seen in tyre factory workers compared with administrative or laboratory workers.

Andreoli et al. reported that increased damage was observed in white blood cells of gasoline station attendants exposed to 3 mg/m$^3$ benzene compared to matched controls. No concurrent increase in cytogenetic damage was found in the same population. In subsequent in vitro experiments in white blood cells, hydroquinone (HQ) and other benzene metabolites produced DNA damage in the Comet assay. In mitogen-stimulated cells the DNA damage capacity was decreased. The repair inhibitor cytosine arabinoside partially restored the sensitivity of stimulated cells to HQ.

Mračková et al. examined 24 coke-oven workers exposed to high concentrations of polycyclic aromatic hydrocarbons and an unexposed control group of the same socio-economic status and age. The GSTMI genotype and NAT-2 genotype were examined. Higher levels of DNA damage in the Comet assay were found in the factory control than in the city control and in exposed groups than in non-smokers. However, higher levels of chromosomal aberrations and sister chromatid exchanges were found.

Niedźwiedź et al. presented comparisons between DNA damage induced in the Comet assay after treatment of lymphocytes with aromatic amines at 4 and 37°C. Contrary to expectations, greater responses were seen at 4 than at 37°C. In lymphocytes treated with neutrons and γ-rays the kinetics of repair were also examined at 4 and 37°C and it was shown that significant repair takes place in the first hour after treatment.

Cebulska-Wasilewska examined four individuals suspected of being accidentally exposed to γ-radiation. To estimate absorbed doses, the lymphocytes were analysed for the presence
of unstable chromosomal aberrations and DNA damage in the Comet assay. A linear quadratic dose–response relationship was obtained after X-irradiation and an almost linear one after irradiation with neutrons. Damage was also found in the Comet assay. Using a calibration coefficient, all absorbed doses were determined to be <1 Gy. Only in one case was the possibility of radiation exposure excluded.

Dagliastant and Şardaş examined chronic arsenic poisoning from individuals exposed to contaminated well water. Thirty individuals (17 smokers and 13 non-smokers) were compared with 20 controls (10 smokers and 10 non-smokers). Blood and hair samples and a detailed questionnaire were taken. The results indicated that arsenic exposure alone did not cause direct DNA damage but had a co-mutagenic effect and smoking potentiated the effect.

**Somatic and germ cells in vitro and in vivo**

**Human lymphocytes**

De Boeck et al., in a study on the genotoxic potential associated with cobalt-containing dusts, used human lymphocytes and discovered that a cobalt alloy with tungsten carbide induced greater DNA damage in vitro than did cobalt alone. This observation of co-mutagenicity was important in that workers often have occupational exposure to tungsten carbide and cobalt, which is associated with interstitial lung fibrosis (‘cobalt lung’). The authors suggest the use of the Comet assay as a part of human biomonitoring to identify individuals that are at high risk of ‘cobalt lung’.

Frenzilli et al. presented a validation study employing 15 agents known to induce different types of damage. They monitored clastogenic damage, DNA damage using the Comet assay and cell toxicity in human lymphocytes treated in vitro. Their study confirmed the high sensitivity of the Comet assay. The microtubule poison griseofulvin induced cell lethality but did not induce an increase in DNA damage. Ascorbic acid and glucose also gave negative results. Results such as presented here are important in defining the sensitivity and specificity of the Comet assay.

Bushfort et al. investigated the DNA repair capacities in the Comet and monoclonal antibody based-assays in normal and malignant human lymphocytes. By measuring the initial DNA strand breaks and the kinetics of DNA repair after pulse alklylation they noted a wide range of repair capacity. After blocking base excision repair using methoxyamine, normal and malignant lymphocytes were similar in their level of Comet formation. However, in malignant human lymphocytes there was a higher level of variation, which suggests the loss of stringent control of DNA repair.

**Animal models**

Štětina et al. uncovered an intriguing relationship between the consumption of cholesterol and the enhancement of DNA damage in endothelial cells of the aorta of rats. This damage was amplified after endonuclease III treatment, indicating that oxidative lesions were enhanced in association with cholesterol consumption. Liver cells and lymphocytes were also assayed using Comet analysis and only responses in the lymphocytes agreed with the response seen in aortal endothelium cells. These authors suggested that Comet analysis of human lymphocytes might be a good biomarker for increased risk of atherogenesis.

Valverde et al. studied the impact of the inhalation of cadmium (Cd) and lead (Pb) on DNA damage in lung, kidney, liver, bone marrow, brain, leukocytes, testis and nasal epithelial cells in CD-1 mice. Their data indicated that Cd was more genotoxic than Pb and that inhalation with both metals showed a synergistic effect. With Cd inhalation, bone marrow cells were the most sensitive to acute exposure, while leukocytes expressed the most DNA damage after chronic treatment. With acute Pb exposure lung cells were the most damaged, however, after chronic Pb treatment the brain cells expressed the most Comet formation. A synergistic response in liver and kidney cells was seen when both metals were inhaled by the mice. Thus the study demonstrates that there is organ specificity for DNA damage governed by the length of treatment and the specific metal that the animal has inhaled and also the importance of evaluating synergistic and antagonistic reactions.

Møller et al. measured the uptake of insoluble dyes, yellow pigment-12 and DNA orange, by freshly isolated rat hepatocytes and induction of DNA damage demonstrated that insoluble compounds can induce Comet formation. They indicated that standard genotoxicity analysis of these agents was limited due to their insobility in aqueous solutions. The effect of incubation times was investigated and the longer the incubation time, the greater the strand breakage.

Agurell et al. studied the effects induced by carbon tetra-chloride (CCL4), ethylnitrosourea and methyl methanesulfonate in NMRI mice. Linear concentration–response curves were seen in liver cells when assayed by the Comet technique. CCL4 caused some DNA damage, but only after exceedingly high doses. The data indicate that direct acting genotoxins can be differentiated from indirect acting toxic agents.

Dixon et al. applied the Comet assay to the marine invertebrate Mytilus edulis for use as an environmental monitor of ocean pollution. They conducted baseline studies on induction of DNA damage in isolated gill cells. The levels of DNA migration were high in the exposed organism but a homogenous response was obtained after in vitro exposure. The mollusc may be more useful in an in vitro Comet assay in screening of agents destined for release or disposal into the marine environment.

Belpaeme and Kirsch-Volders examined a planar polychlorinated biphenyl (PCB77) in vivo in fish using the Comet and micronucleus assays in erythrocytes of brown trout exposed to different concentrations of PCB77 in the water. No positive responses were found. In parallel, PCB77 was also tested in human lymphocytes in vitro in the same assays. Again no positive responses were found.

**Human cell lines**

Marples et al. reported a significant correlation between the extent of double-strand break repair and radiosensitivity (ratio of initial and residual slopes) among seven human cervical carcinoma cell lines of differing radiosensitivity. This approach of taking a ratio of slopes assesses damage from a range of multiple dose points and minimizes the variability between experiments. By this analysis of the data a significant correlation was resolved between DNA damage repair capacity and radiosensitivity expressed as the surviving fraction at 2 Gy.

Hambly et al., using a human colon adenocarcinoma cell line (Caco-2), assayed the aqueous fraction from human faeces (faecal water) from 35 healthy non-smoking volunteers (23 from England and 12 from Sweden). Of the samples, 31% induced a high level of DNA damage as measured by the Comet assay, while 54% of the samples were refractory. The remaining samples expressed intermediate levels of DNA...
damage. Lower responses were shown in the Swedish samples. These human faecal water studies may be useful in investigations on the mechanisms underlying the dietary aetiology of colon cancer. Research using these types of human samples will be continued to elucidate possible causative genotoxic agents. Such analysis may identify individuals at risk of colon cancer who otherwise appear healthy.

Ollikainen et al. studied oxidative resistance of human transformed mesothelial cells (Met5A) and transformed bronchial epithelial cells (BEAS2B) using the Comet assay and lytic cell injury by lactate dehydrogenase (LDH) release after exposure to hydrogen peroxide or menadione. The data from the Comet assay did not correlate with the data generated from the LDH release experiments. The authors argued that the Comet assay provided a highly discriminating method for comparing the potential sensitivities of different pulmonary cell types to oxidants. The lines Met5A and BEAS2B provide a suitable model to study the genotoxicity of compounds to human airway epithelial cells and mesothelial cells under in vitro conditions.

McKelvey-Martin employed Raji lymphoblastoid human cells and exposure to X-rays or H2O2 and investigated the protective effects of 60 μM ascorbic acid and 30 μM α-tocopherol. The level of DNA damage as measured by Comet analysis was found to be significantly reduced after the 24 h supplementation period. The findings obtained are consistent with the concept that ascorbic acid and α-tocopherol can protect against oxidatively induced DNA damage.

Sperm Comet analysis
Singh described a novel method of preparing human sperm DNA and found a linear dose–response relationship for double-strand breaks from 0.125 to 1 Gy X-rays under neutral gel electrophoresis. He also discovered that no DNA repair of the double-strand breaks occurred after 2 h incubation at 37°C.

Haines et al. found similar effects for the induction of double-strand breaks in human and mouse sperm after 100 Gy X-irradiation. However, when these treated sperm cells were analysed under alkaline electrophoresis a dose–response relationship was only observed for the murine cells. Haines also discussed possibly that the high level of radio-resistance of spermatozoa as compared with somatic cells is related to the structure of spermatogonial DNA.

Underlying mechanisms/new developments
Oxidative DNA damage detected with the Comet assay
Speit and Denny reported how DNA damage induced by reactive oxygen species plays an important role in induction of mutations, cancer and other degenerative diseases in humans. They conducted a Comet assay on leukocytes obtained from volunteers exposed to 100% O2 at 2.5 atm for three 20 min periods. After treatment with FPG, significant oxidative DNA damage was found using the Comet assay. However, the DNA damage was detected only after the first treatment and not in further treatments on following days, which demonstrates that the initial oxidative DNA damage is repaired rapidly. This suggests that antioxidant defenses are increased rapidly after oxidative stress.

Green presented a novel cell-free Comet assay method where the damaging treatment was applied after the lysing step in the Comet procedure. This allowed the opportunity to test for DNA protection by enzymes such as superoxide dismutase, which will not enter the cell. Using this assay, glutathione, which is a major protective agent within the cell, acts as a DNA damaging prooxidant and superoxide dismutase is protective.

Ward et al. conducted experiments employing laser excitation of methylene blue to generate singlet oxygen and induce 8-hydroxyguanine DNA lesions. Using two cell cultures that express different levels of FPG, it can be determined if there is a relationship between DNA damage as seen in the Comet assay and the level of FPG protein. The use of purified repair enzymes in the Comet assay is a powerful tool and can be used to understand the mechanisms underlying the repair of complex and varied DNA damage.

Dusinska et al. examined alveolar macrophages and pneumocytes treated with paraquat. Pneumocytes expressed a 10 times higher background of endogenous DNA damage than alveolar cells. Pneumocytes were also more sensitive to oxidative stress in general, which might contribute to the high risk of tumourgenicity associated with these cells.

DNA repair in the Comet assay
Collins et al. reported on modifications of the Comet assay to detect DNA repair and how it can be used to follow rejoining of strand breaks. With the inclusion of suitable lesion-specific endonucleases, specific DNA lesions can be detected, such as peroxynitrite dimers, oxidized pyridines, 8-hydroxyguanine and ring-opened purines. Another approach is to measure repair by examining the capacity of a cell extract to perform repair on a defined DNA substrate. Yet another way is to incubate cells with DNA synthesis inhibitors to accumulate incomplete repair sites as DNA strand breaks.

Bock et al., working with UVA radiation at 365 nm, detected DNA strand breaks in a variety of different human cell types. Human lymphocytes isolated from peripheral blood were less sensitive than a cell line of lymphocytes. Lymphocytes were also more sensitive than epithelial cells. DNA repair studies revealed an incision step in the mechanism.

Hellbig et al. examined CHO V79 and rainbow trout RTG-2 cell cultures for their sensitivity in the Comet assay. From their results there was longer lesion persistence in RTG-2 cells than V79 cells and they conclude that the Comet assay would have the most sensitivity for in vitro genotoxicity testing in cell lines that express a low or impaired DNA repair capacity. Calibration of the Comet assay and possible mechanisms Mackay et al., in a study on the effect of unwinding time in mouse skin keratinocytes treated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), discovered that DNA unwinding time is a critical parameter of the Comet assay and that it may require optimization for each tissue/cell type used in experiments. Their data also suggest that agents that alter the structure of chromatin may increase Comet tail length and alter head DNA content in the absence of induction of DNA lesions. Thus there may be two discrete classes of chemical interaction with chromosomal DNA that yield identical Comet assay results.

Plewia et al. used cultured CHO AS52 cells that contain a transgenic target gene, gpt, to calibrate the induction of forward mutation, Mutation formation and whole cell clastogenicity after ethyl methanesulphonate, 2-acetoxyacetylaminofluorouene or UV radiation. These three genetic end points were detected in cells that were treated under identical conditions. Cell toxicity was measured by vital dye analysis and by cloning efficiency. Forward mutation was measured by resistance to 6-thioguanine,
whole cell clastogenicity was analysed by flow cytometry and the alkaline Comet assay was used to determine acute DNA damage. In general, the forward mutation test was somewhat more sensitive, but all three end points show a high degree of agreement in their dose–response relationships. These data define the genotoxic impact and biological relevance of the Comet assay in terms of forward mutation and whole cell clastogenicity.

Slamenová et al. conducted calibration of the Comet assay against the DNA unwinding and alkaline elution methods. The Comet assay was sensitive to low levels of MNNG-induced DNA damage in mammalian cells but its resolution was easily saturated when compared with DNA unwinding or alkaline elution. The kinetics of DNA rejoining demonstrated that the Comet assay could recognize the sites of incomplete repair. The combination of the DNA repair inhibitors cytosine arabinose and hydroxyurea were effective in increasing the sensitivity of the Comet assay at very low concentrations of MNNG (<2 μg/ml).

Henderson et al. carried out a small validation study of the Comet assay to discriminate between genotoxins and cytotoxins. The Comet assay detects DNA damage due to the genotoxic impact of agents or it can respond to DNA degradation due to necrosis or apoptosis. The latter could introduce a false positive response due to cytotoxicity. In this study toxicity was measured by trypan blue exclusion and total cell counts. The results indicated that the maximum concentration of test agent should produce viabilities >70% in order to avoid cytotoxicity-induced false positive responses.

Gábelová et al. evaluated the transformation of polycyclic aromatic hydrocarbon– or N-heterocyclic aromatic hydrocarbon–DNA adducts into DNA strand breaks as measured by the Comet assay. Under in vitro conditions strand breaks were probably not induced by the adducts themselves but by secondary processes such as DNA repair. The use of DNA repair inhibitors or specific DNA glycosylases increased the response in the Comet assay. In assessing the genotoxicity of xenobiotics with the Comet assay, the authors suggest the use of DNA repair inhibitors or glycosylases in order to avoid false negative results.

The International Comet Assay Workshop

**Panel discussion: where are we now?**

The wealth of information presented in the Workshop was witness to the fact that the Comet assay now plays an important role in many areas of science.

Tice requested that all papers should include information on cell viability, gel concentration, cell density, lysing solution (pH and ingredients of lysing solution), alkali unwinding conditions (pH and duration), electrophoresis conditions (pH, voltage, amperage and duration), neutralization, stain imaging conditions, scoring, cell selection criteria and any other factors which might affect responses.

In considering whether the assay would attain regulatory status, McKay pointed out for this end point as for other genetic end points. However, some validation has begun. McKay and Henderson both stressed that it is very important to take great care when selecting populations in the human biomonitoring field to avoid confounding factors (see below).

Speit thought the assay useful for examining target tissues in vivo and for in vitro testing and research purposes. Green concurred with this opinion and felt that the assay could provide answers to specific scientific questions. Plewa felt that it was a useful assay for measuring antimutagenesis and modulating effects and this was confirmed by other workers.

Tice thought the assay useful not only for measuring DNA
damage, but also for repair studies after different sampling times in vitro and in vivo. Site-specific repair enzymes were also available for inclusion in the assay. It was felt that the totality of the data might also be helpful for risk assessment purposes.

Various other points raised

The researchers using the Comet assay determine what is a positive response in their system depending on the system (cells or animals) and the statistical methods used. It is known that different cells have different responses, e.g. human sperm have higher background levels in the alkaline assay than human lymphocytes.

The biological significance of the test is not as yet firmly defined. The Comet assay is capable of detecting DNA strand breaks, alkali-labile sites and incomplete excision repair sites in individual cells. Depending on whether the neutral or alkaline assay is used, double-strand or single-strand breaks are detected. Most laboratories use the alkaline assay and a larger range of effects are detected with this assay. A positive response in the assay means that the above events have been detected.

The assay has been used for many applications, primarily the four different areas addressed in this meeting, but there will no doubt be other future applications, since it is a very versatile assay.

The confounding factors to be taken into consideration for this assay when used for human monitoring are the same as those taken into account for other human monitoring studies, i.e. age, gender, smoking, drinking, etc. It is known, for example, that smoking can produce a positive response in this assay and age can increase responses. Exercise can also affect responses, so controls and exposed individuals need to be in the same physiological state at the time of sampling.

Acknowledgements

We acknowledge support for the Workshop from Glaxo R&D Ltd, Unilever Research, Zeneca plc, Kinetic Imaging Ltd, Perceptive Instruments and Flowgen Instruments Ltd.

References

and alkali sites in rat hepatocytes exposed to DNA orange and yellow pigment 12.
Mračková, G., Topinka, J., Peterka, V., Piščík, T., Gajdošová, D. and Šram, R.
Effect of genotype on DNA damage analysed by the Comet assay of coke-oven workers.
Niedźwiedź, W., Nowak, D. and Cebulska-Wasilewska, A. Influence of temperature and repair on DNA damage assessed by the Comet assay.
Ollikainen, T., Kinnula, V.L. and Linnaimaa, K. DNA single strand breaks as indices of oxidant effects on human pleural mesothelial and bronchial epithelial cells.
Plappert, U.G. and Fiedner, T.M. Can DNA repair capacity be used as a valuable biomarker for radiation exposure?
Pool-Zobel, B.L., Abrahamse, S.L., Oberreuter, D. and Rechkmerner, G.
Endogenous DNA breaks and oxidised DNA bases in human cells derived from colon biopsies and in human colon tumour cell lines.
Rojas, E., López, M.C., Valverde, M., Martínez-Lomelí, I., Sanchez, J., Lopez, I., Fortoul, T.I. and Ostrosky-Wegman, P. Single strand break in leukocytes, buccal and nasal epithelial cells from individuals exposed to different levels of air pollution in Mexico City.
Šardas, S., Ural, Y., Kuşoğlu, C. and Esat Karakaya, A. Single cell electrophoresis (Comet assay) technique for detection of DNA damage induced by UV-radiation in cancer patients.
Singh, N.P. Sperm effects in the Comet assay.
Slamenová, J., Gabelová, A., Horváthová, E., Farkasová, T. and Ružeková, L.
Validation of Comet assay against DNA unwinding technique and alkaline elution of DNA.
Speit, G. and Denong, C. Biological significance of oxidative damage detected with the Comet assay.
Šram, R.J., Podrazilová, K., Dejmek, J., Mračková, G., Piščík, T. and Topinka, J.
The sensitivity of Comet assay in human population studies.
Tice, R. Overview.
Valverde, M., Lopez, M.C., Sordo, M., Lopez, I., Sanchez, J., Fortoul, T.I., Ostrosky-Wegman, P. and Rojas, E. Evaluation of genotoxicity and morphological changes by inhalation of cadmium and lead in CD-1 male mice.
Ward, T.H. and Marples, B. A comparison of radiation-induced DNA damage in tumour cells measured using two Comet analysis systems.

Received on June 4, 1997; accepted on September 1, 1997