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Cytogenetic alterations in human lymphocyte culture following exposure to radiofrequency field of mobile phone

Sabah F. El-Abd and Marwa Y.Eltoweissy

Sabah F. El-Abd
Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute, Sadat city, Egypt.

Marwa Y.Eltoweissy
Department of Zoology, Faculty of Science, Alexandria University, Egypt

ABSTRACT

The present study aims to address the possible cytogenetic effect of mobile phone on human lymphocyte culture. Human peripheral blood cultured from healthy, non-smoking donors exposed to 1950 MHz and safety limit (2w/kg) of absorption rate (SAR) mobile radiofrequency radiation for 5, 10, 15, 20, 25 and 30 min, then harvested after 24 hr after subjection. The alkaline comet assay, chromosomal aberrations and the micronucleus test were used, to check for changes, stress response and alterations in lymphocytes. The result indicated the presence of time-dependant cellular response to RF exposure of mobile phone kept in the standby position, through comet tail factor, DNA fragmentation, chromosomal aberrations and centromeric negative nuclei (MN) in human lymphocyte culture. This effect may be attributed to oxidative stress induced by mobile phone radiation.

Keywords: Mobile phone, Genetic alteration, Comet Assay, Chromosomal aberrations, Micronucleus.

INTRODUCTION

During the last decade, mobile phone use increased to almost 100% prevalence in many countries. The health effects of cell phone radiation exposure are a growing public concern. There is still ongoing discussion whether RF-EMF could induce biological relevant. Relatively short-term exposure to cell phone radiofrquency emission can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes (Tian, 2007). French *et al.,* (2001) reported that exposure to radiofrequency (RF) fields whose signals and intensities were similar to or typical of those of currently used mobile telephones, might affect heat-sensitive gene or protein expression. The significance of mobile phone radiation on male reproduction is a key element debate since several studies have suggested a relationship between mobile phone use and testicular function (Salama *et al.,* 2010. Moreover the induction of oxidative stress in these cells not only perturbs their capacity for fertilization but also contributes to sperm DNA damage, poor fertility, an increase incidence of miscarriage and morbidity in the offsprings, including childhood cancer. Statistical analysis of sperm head abnormality score showed that there was a significant difference in occurrence of dose dependant sperm head abnormality on the reproductive health (Otitoloju *et al.,* 2010).Using human endothelial cell lines have shown that mobile phone radiation induces statistically significance changes in the expression of several tens of proteins and that the response might be proteome-dependant (Nylund and Leszezynski, 2006).

For Correspondence
Sabah F. El-Abd
Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute, Sadat city, Egypt.

Genotoxic effect of long-term (52-78 weeks) exposure to radiofrequency (RF) electromagnetic fields can affect living tissues by energies that are much lower than those cause changes in the temperature of tissues.These temperature-insensitive response can influence the physiology of cells either in culture (Nylund and Leszezynski, 2004) or in organisms (Weisbrot *et al.,* 2002). On the other hand, no statistically significant effects of exposure were found, and there is no indication that emissions from mobile phones are associated with adverse effects on the human immune system (Tuschl *et al.,* 2006).

Using a cell phone for > or = 10 years approximately double the risk of being diagnosed with a brain tumor on the same side of head as that preferred for cell phone use. The data achieve statistical for glioma and acoustic neuroma but not for meningioma (Khurana *et al.,* 2009). Schwarz *et al.,* (2008) reported that UMTS exposure increased the CTF and induced centromere-negative micronuclei (MN) in human cultured fibroblasts in a dose and time-dependent way but not in lymphocytes. The present study aims to investigate the effect of mobile phone use on cytogenetic instability of human lymphocyte culture.

MATERIALS AND METHODS

Media

Human lymphocytes are easy to culture, and readily available. They do not divide and grow unless artificially stimulated this is usually activated by adding phytohaemagglutinin (PHA) to the cultures and results in a high mitotic yield. These lymphocyte cultures were used according to the method adopted by (Moorhead *et al.,* 1960). The culture medium consists of RPMI 1640 culture medium with L-glutamine (sigma) supplemented with 20% fetal calf serum; 1% peincillin (5000 IU/ml), streptomycin (5000mg/ml); and 0.005g**%** phytohaemagglutinine (PHA) (Biochrome)**.**

Applied techniques

a.Chromosomal aberrations

One ml heparinized whole blood from healthy nonsmoking donor was mixed with 5 ml culture medium and incubated at 37C°. Six cultures were exposed to 1950 MHz and safety limit (2w/kg) of absorption rate (SAR) mobile radiofrequency radiation at different intervals (5, 10, 15, 20, 25 and 30 min) after 24 hrs from culturing, and harvested on the next day. The exposure system consisted of GSM signal generator which generates 1950 MHz. The signal generator was located at about 20 cm near the culture flasks. Cells were arrested in metaphase by addition of colcimed. After 72 hrs, cells were centrifuged (1000rpm; 10 min) and the supernatants were discarded. The cell pellets were then resuspended in approximately 5 ml of prewarmed hypotonic solution(KCl) and incubated for 15 min. Tubes were then centrifuged at 1000 rpm for 10 min. The supernatant was removed and the pellets were thoroughly mixed with 5ml of cold fixative (1 part acetic acid to 3 parts methanol(added drop by drop; this step was repeated twice. The tubes were spanned at 900 rpm for 10 min. The cells pellets were resuspended in equal small volumes of fresh fixative to form a milky suspension. Slides were prepared by pipetting 4 drops of cells suspension on to clean**,** grease free slides and air dried. The slides were then stained with 10% Giemsa for 12 min; immersed in distilled water for washing; air dried for 3 days and mounted in DPX. For each treatment, at least 250 well spread metaphases were examined for chromosomal aberrations.

b. Total damage of DNA (double strand breaks)

Double strand breaks of DNA were detected according to Wlodek *et al.,* (1991). Human lymphocytes were isolated according to Boyum (1968) from whole blood by ficoll separating solution (Sigma). The cells were washed in a medium of TGD of DNA. The isolated lymphocytes were exposed to mobile radiofrequency radiation at different intervals (5, 10, 15, 20, 25 and 30 min). The viability of the cells was determined by trypan blue. From the stock of lymphocytes, 0.5ml (1x 10^6 lymphocytes) to 0.2 ml (4x10^5) were transferred to 1.5 ml ependorf tube and completed to 1 ml with medium. Viability of treated cells was measured**.** Treated cells were centrifuged for 1 min by Ependorf microcentrifuge. The pellets were suspended in 15 microliter medium and loaded directly in the well of gel**.**

Gel preparation

Gels were prepared with 1.5% electrophoretic grade agarose (BRL) and 0.2% polyvinylpyrolidine (PVP;Sigma). The agarose and PVP were boiled with tris borate EDTA buffer (1 x TBE buffer; 89mM Tris, 89mM boric acid, 2mM EDTA, pH 8.8). 0.5 micro-gram/ml ethidium bromide was added to gel at 40 C°, then gels were poured and allowed to solidify at room temperature for 1hr**.** before the samples were loaded. Treated human lymphocytes from 0.5 ml blood were loaded in wells. 15 micro-liter of lysing buffer (50mM NaCl, 1 mM Na2 EDTA, 1% SDS, pH 8.3) was added on the cells for 15 min. 5µl from 6X loading buffer was added on the lysis cells. Electrophoresis was performed for 2hrs at 50 volt using 1X TBE buffer as running buffer. Gel was photographed using a Polaroid camera while the DNA was visualized using a 312 nm UV light under a transilluminator**.**

Damage scoring

The intensity of intact, fragmented (released DNA) and RNA were measured as optical density by gel pro analyzer program**.**

c. Comet assay (single strand breaks of DNA)

DNA strand breaks were measured in the alkaline comet assay using the method described by and Singh *et al.,* (1988).The isolation and exposure of human lymphocytes to mobile radiofrequency radiation in this technique are similar to the steps of total genomic technique. Viability of the cells was determined by trypan-blue exclusion before using the cells for studies.

Examinations were done with a fluorescent microscope equipped with an excitation filter of 510 nm and barrier filter of 590 nm. The migration was evaluated by observing and measuring the nuclear DNA, where the rounded spot of DNA was considered

as a normal DNA spot, while the nuclear DNA, migrating towards the anode, appeared as comet spot and considered as damaged DNA spot. Five hundred spots of DNA were examined and classified into three types: (1) normal spots; round shape, (2) damaged spots; in which the length of the migrated fragments is less than or equal to the diameter of the basal nuclear DNA, and (3) strongly damaged spots; where the length of the comet was greater than the diameter of the basal nuclear DNA.

d. Micronucleus test (MN)

This technique was applied according to Mihael and Morely (1985) and modified by Suralles et al (1992), were cytocalasin B was added after 24 hrs from culturing, to stop cytoplasm division, while nucleus still divided, 100 micronuclei was evaluated per every exposure time.

RESULTS

1- Chromosomal aberrations

Six types of chromosomal aberrations were detected in human lymphocyte cultures exposed to mobile radiofrequency radiation (table1). These are: gap, break, fragment, dicentric, metacentric and ring chromosomes. A significant increase in the percentage of these aberrations was recorded after exposure to the mobile radiation in comparison with control group.

Table (1) Frequancy of chromosomal aberration of lymphocytes culture exposed to mobile radiation.

	% of Chromosomal aberrations						%Total
Exposure /min	Gap	break	frag	dicent.	metacent.	ring	Abnormality
0	0.8	0	0.2	0	0.1	0	1.1
5	1.0	0	1.0	0	1.0	0	2
10	2.0	1.0	1.0	0	1.0	0	5*
15	2.0	2.0	3.0	1.0	1.0	1.0	10**
20	4.0	2.0	3.0	1.0	1.0	1.0	12**
25	5.0	3.0	5.0	2.0	2.0	2.0	19**
30	3.0	4.0	8.0	2.0	2.0	5.0	24**

(*). Statistically significant at P<0.05
(**). Highly significant at p<0.001

Table (2): Comet tail length of blood lymphocytes exposed to mobile radiofrequency (RF).

Exposure/min	*Comet tail length (mean±SD)/mm*
0	0.9±.25
5	1.3±0.12
10	3.66±0.49
15	4.79±0.81
20	6.86±1.03
35	7.49±1.2**
30	8.93±1.63**

(**). Highly significant

2- DNA damage

DNA detection by comet assay in the exposed cells was estimated by measuring the DNA extent of the migration toward anode pole (Fig.1). The result indicates that the migration of DNA was increase with increase of exposure time, in direct relation manner and the faster migration was at 30 min. (table2). The frequency percent of DNA damaged lymphocytes in cells culture is illustrated in table (3). There was a significant increase in the percent of DNA damage frequency in calculated cells of mobile radiofrequency exposed cultures. Electrophoretic pattern of total DNA of lymphocytes revealed that the intensity of DNA fragmentation increased in exposed cells (Fig.2).

Table (3): Percentage of DNA damage in lymphocytes exposed to mobile radiofrequency.

Exposure/ min	Damage %/ 50 cells.
0	0.63±0.9
5	8.3±4. 2
10	17.91±6.5
15	27.67±8.7
20	38.44±9.9
35	53.10±11.4
30	70.18±7.8

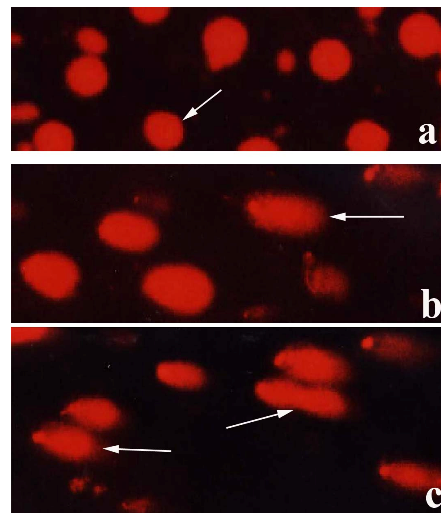

Fig.1. Photomicrograph showing single strand breaks (comet assay) of DNA of human lymphocyte cultures exposed to mobile phone radiation. (a) Normal DNA spots (no migration). (b) Damaged DNA spots (migration towards the anode). (c) Strong damaged DNA spots (more migration towards the anode).

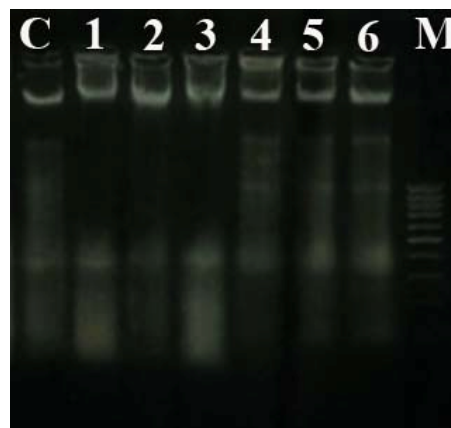

Fig. 2. Gel electrophoresis showing the effect of mobile phone radiation on total DNA in human lymphocyte cultures; lanes 1,2, 3, 4, 5, 6 represent human lymphocyte cultures treated for 5,10,15,20,25,30 min. respectively. C: control, M: marker.

3- Micronucleus test (MN)

Table (4) showed the frequency of micronucleus (acentric chromosome lagging in metaphase) in relation to the time of culture exposure. Significant increase in the percent of micronuclei started at 10 minute exposure with continuous and obvious

increase tell 30 minute exposure. The amount of micronuclei calculated in all phases of nucleus division (MI, MII, MIII and MIV).

Table (4): The frequency of micronuclei of human lymphocytes culture exposed to mobile phone radiofrequency.

Exposure/ min	% of total Micronucleus/ 50 cells.
0	2
5	4
10	10
15	16
20	20
25	22
30	29

DISCUSSION

The cytogenetic alterations observed in the present study estimated the changes and the accumulated dangerous effect of use of mobile phone. These results are in agreement with the investigators who studied the effect of mobile radiofrequency. Phillips *et al.,* (1998) reported that exposure of Molt-4 T-lymphoblastoid cells to the cell phone frequency of 836 MHz, consistently resulted in an observable variation of DNA damage. DNA strand breaks were reported in human diploid fibroblasts and cultured rat granulosa cells (Diem *et al.,* 2005) as well as in embryonic stem cell--derived neural progenitor cells (D'Ambrosio *et al.,* 2002) after RF field exposure. Gadhia *et al.,* (2003) reported that in individuals, who used digital mobile phones for at least 2 years, with uplink frequencies at 935-960 MHz, there was a significant increase in sister chromatide exchange, but there was no change in cell cycle progression .

Cell mutations such micronuclei (MN) formation and cellular aneuploidy were recorded following exposure to RF fields (Mazor *et al.,* 2008; Schwarz *et al.,* 2008). Tice *et al.,* (2002) found that exposure of lymphocytes for 24 h at an average SAR of 5.0 or 10.0 W/kg resulted in a significant and reproducible increase in the frequency of micronucleated cells.

Nylund and Leszezynski (2006) showed that gene and protein expression were altered, in two variants of human endothelial cell line: EA.hy926 and EA.hy926v1, in response to one hour mobile phone radiation exposure (900 MHz GSM signal) at an average specific absorption rate of 2.8 W/kg. Karien *et al.,* (2008) showed that the mobile phone radiation (radiofrequency modulated electromagnetic fields; RF-EMF) alters protein expression in human endothelial cell line. Long-term exposure of cells to mobile phone irradiation result in the activation of p38 as well as ERK (extracellular-signal-regulated kinase MAPKs (Joseph *et al.,* 2007**)**. On the other hand, Hung *et al.,*(2008**)** reported that RF exposure did not produce significant changes in cell numbers, cell cycle distribution, or level of DNA damage or global gene expression upon using 1763 MHZ RF radiation under 10 w/kg SAR for 24 h to Jurkat cells. Oxidative stress due to abnormal production of reactive oxygen species (ROS) is believed to be involved in the etiology of toxicities of radiation. These free radicals could cause membrane and macromolecule damage by 3 basic mechanisms: lipid peroxidation, DNA fragmentation, and protein oxidation. An association between human health and exposure to RF-EMR, with emphasis on a range of clinical conditions including childhood leukaemia, brain tumours, genotoxicity and neurodegenerative disease was reported (Hardell and Sage, 2008). While the cellular mechanisms underpinning these effects have not been completely resolved, it has been suggested that oxidative stress could be a key factor. A number of in vivo experiments have found mobile phone or simulated mobile phone radiation exposure can cause cell damage, reactive oxygen species (ROS) formation (which are the primary cause of DNA strand breaks), and cell death (Oral *et al.,* 2006; Panagopoulos *et al.,* 2007). In vitro experiments have found an association between RF exposure and ROS production, and then subsequent DNA single and double strand breaks (SSB and DSB) (Friedman *et al.,* 2007; Yao *et al.,* 2008). De Luliis *et al.,* (2009) reported that RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and, ultimately DNA fragmentation. Ozguner *et al.,*(2005) revealed that exposing rats to 900 MHz mobile phone caused an increase in tissue malondialdehyde (MDA, an index of lipid peroxidation), and a reduction in the activity of antioxidant enzymes, superoxide dismutase and catalase. In conclusion, the accumulating effect of exposure to low intensity pulsed radiofrequency emitted by a conventional mobile phone kept in the standby position could caused DNA damage, micronucleus frequency and chromosomal aberrations in human lymphocytes cultures. This effect may be due to increased free radical activity as a response to exposure.

REFERENCES

Boyum, A. Separation of leukocytes from blood and Bone marrow Scand. J. Clin. Lab. Invest. 1968; 21 (Suppl. 97): 77-89.

D'Ambrosio G, Massa R, Scarfi MR and Zeni O. Cytogenetic damage in human lymphocytes following GMSK phase modulated microwave exposure. Bioelectromagnetics. 2002; 23: 7-13.

De Luliis, G.N., Rhiannon J. Newey, Byuce V. King and R. John Aitken. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. Plos One, 2009; 4(7):e 6448.

Diem E, Schwarz V, Adlkofer F, Jahn O and Rüdiger HW. Non-thermal DNA breakage by mobile-phone radiation (1800 MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro. Mutat. Res. 2005; 583, 178-183.

Friedman J, Kraus S, Hauptman Y, Schiff Y and Seger R. Mechanism of a short-term ERK activation by electromagnetic fields at mobile phone frequency. Biochem. J. 2007; 405, 559-568.

Gadhia PK, Shah T, Mistry A, Pithawala M and Tamakuwala D. A Preliminary study to assess possible chromosomal damage among users of digital mobile phones. Electromag. Biol. Med. 2003; 22: 149-159.

French P.; Penny R.; Laaurence J. and McKenzie D. Mobile phones, heat shock proteins and cancer. Differentiation. 2001;67: 93-97.

Hardell L, Sage C. Biological effects from electromagnetic field exposure and public exposure standards. Biomed Pharmacother. 2008; 62:104–109.

Hung, T., Lee M., Oh E. Zhang, T., Seo, J., Park, W. Molecular responses of jurkat T-cell to 1763 MHZ radiofrequency radiation. International Journal of Radiation Biologgy. Int J Radiat Biol. 2008; 4(11):909-15.

Karien, A.; Heinavaara R., Nylund, R. , Leszczynski D. Mobile phone radiation might alter protein expression in human skin. BMC Genomics. 2008; 9:77.

Joseph F., Kraus,S., Hauptman,Y., Schiff, Y. , Seger, R. Mechanisms of short-term ERK activation by electromagnetic fields at mobile phone frequencies. Int. J. Radiat Biol, 2007; 83(4):212-20.

Khurana V, Teo C; Kundi M; Hardell L and Carlberg M. Cell phones and brain tumors: a review including the long-term epidemiological data. Sura Neurol. 2009; 72(3: 205-14.

Mazor R, Korenstein-Ilan A, Barbul A, Eshet Y, Shahadi A, Jerby E and Korenstein R. Increased levels of numerical chromosome aberrations after in vitro exposure of human peripheral blood lymphocytes to radio frequency electromagnetic fields for 72 hours. Radiat. Res. 2008; 169: 28-37.

Mihael F; and Morely H. Measurement of micronuclei in lymphocytes. Mut Res. 1985; 147: 29-36.

Moorhead P; Nowell P; Mellman W; Battips D and Hungerford D. Chromosomal preparation of leukocytes cultured from human blood. Exp. Cell Res. 1960; 20: 613-616.

Nylund R, Leszezynski D. Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome-and proteome-dependant. Proteomics. 2006; 6:4769-4780.

Nylund R. and Leszcznski D. Proteomic analysis of human endothelial cell line EA. Hy926 after exposure to GSM 900 radiation. Proteomics. 2004; 4: 1359-1365.

Oral B, Guney M, Ozguner F, Karahan N, Mungan T, Comlekci S and Cesur G. Endometrial apoptosis induced by a 900-MHz mobile phone: preventive effects of vitamins E and C. Adv. Ther. 2006; 23, 957-973.

Otitoloju A, Obe I, Adewole O, Otubanjo O and Osumkalu V. Preliminary study on the induction of sperm head abnormalities in mice, Mus musculus, exposed to radiofrequency radiations from global system for mobile communication base stations. Bull Environ Contam Toxicol. 2010; 84(1): 51-4.

Ozguner F, Altinbas A, Ozaydin M, Dogan A, Vural H, Kisioglu AN, Cesur G, Yildirim NG. Mobile phone-induced myocardial oxidative stress: protection by a novel antioxidant agent caffeic acid phenethyl ester. Toxicol Ind Health. 2005; 21(9):223-30.

Panagopoulos DJ, Chavdoula ED, Nezis IP and Margaritis LH. Cell death induced by GSM 900-MHz and DCS 1800-MHz mobile telephony radiation. Mutat. Res. 2007; 626, 69-78.

Phillips JL, Ivaschuk O, Ishida-Jones T, Jones RA, M. Campbell-Beachler M and, Haggren W. DNA damage in Molt-4 T-lymphoblastoid cells exposed to cellular telephone radio frequency fields in vitro. Bioelectrochem. Bioenerg. 1998; 45,103–110

Salama N, kishi,oto T, and Kanayama H. Effects of exposure to mobile phone on testicular functions and structure in adult rabbit. Int J Androl. 2010; 33(1): 88-94.

Schwarz C, Kratochvil E, Pilger A, Kuster N, Adlkofer F and Rÿdiger HW. Radio frequency electromagnetic fields (UMTS, 1,950 MHz) induce genotoxic effects in vitro in human fibroblasts but not in lymphocytes. Int. Arch. Occup. Environ. Health 2008; 81, 755-767.

Singh N; Mc Coy M; Tice R and Scneider E. A simple technique for quantitation of low level of DNA damage in individual cells. Exp Cell Res 1988; 175: 184-191.

Suralles J; Carbonell E; Marcos R; Degrass F; Ankoccta A and Tanzarella C. A collaborative study on the importance of the micronucleus test cultured human lymphocytes. Mutagenesis. 1982; 7: 407-10.

Tian,Z.,, Zou, S. Knapp,E.P. Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. Neurosol Lett. 2007; 22: 412(1):34-38.

Tice RR, Hook GG and Donner M. Genotoxicity of radio frequency signals. Investigation of DNA damage and micronuclei induction in cultured human blood cells. Bioelectromagnetics. 2002; 23,113-126.

Tuschl H, Navak W, Molla-Djafari H. In vitro effects oof GSM modulated radiofrequency field on human immune cells. Bioelectromagnetics. 2006; 27(3): 188-96.

Weisbrot D.; Lin H; Ye L.; Blank M. and Goodman R. Effect of mobile phone radiation on reproduction and development in Drosophila melanogaster. J. Cell. Biochem. 2002; 89: 48-55.

Wlodek, D., Banath, J. and Olive, PL. Comparison between pulsed-field and constant-field gel electrophoresis for measurement of DNA double-strand breaks in irradiated Chinese hamster ovary cells. Int. J. Radiat. Biol. 1991; 60(5):779-790.

Yao K, Wu W, Wang K, Shuang N, PanPan Y, YiBo Y, Juan Y and Li Xia S. Electromagnetic noise inhibits radio frequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. Mol. Vis. 2008; 14, 964-969.