5. INVESTIGATION OF THE EFFECT OF OESTROGENIC AGENTS ON DNA DAMAGE IN THE MALE GERM LINE

L.E. Bennetts[1], *D.E. Sawyer*[1] *and R.J. Aitken*[2]
[1]Discipline of Biological Sciences and [2]ARC Centre of Excellence in Biotechnology and Development, [2]Hunter Institute of Medical Research, The University of Newcastle, NSW 2308, Australia

There is evidence to suggest that paternal exposures to certain environmental toxicants may result in germ line mutations and adverse reproductive outcomes. Oestrogenic agents have been linked with various types of DNA damage, particularly damage induced via oxidative mechanisms. However, at present the effect of oestrogenic agents on DNA in the male germ line is poorly characterised. In this study, we have investigated the susceptibility of GC-2 cells to DNA damage resulting from oestrogenic agent exposure. GC-2 cells were treated with diethylstilbestrol (DES; 0–1 μM) over a 1-h period. Alternatively, GC-2 cells were glutathione depleted for 12 h with 100 μM buthionine sulphoximine (BSO), or treated with vehicle alone, and subsequently treated with either 500 μM $4OHE_2$ or 1 mM H_2O_2 for 1 h. Cell vitalities were determined by trypan blue assay after treatment. DNA was purified, and damage assessed in an 8.8-kb fragment of the β-globin gene and a 10.4-kb fragment of mitochondrial DNA by the quantitative polymerase chain reaction assay (QPCR). Double-strand breaks in DNA were assessed by pulsed-field gel electrophoresis. Redox activity was also monitored in some experiments using lucigenin-dependent chemiluminescence. No treatment was found to significantly affect cell vitality. QPCR analysis did not detect DNA damage in either the nuclear or mitochondrial DNA fragments of cells treated with DES. However, in cells treated with BSO and $4OHE_2$, a significant ($P<0.05$) increase in damage in the β-globin gene was detected (0.39 lesions/10 kb). In this DNA fragment, damage was also significantly increased in H_2O_2 treated ($P<0.05$; 0.38 lesions/10 kb) and H_2O_2 + BSO treated cells ($P<0.05$; 0.48 lesions/10 kb). In the mitochondrial DNA fragment, significant increases in DNA damage were observed in cells treated with BSO ($P<0.05$; 0.20 lesions/10 kb), $4OHE_2$ ($P<0.01$; 0.30 lesions/10 kb); BSO + $4OHE_2$ ($P<0.05$; 0.29 lesions/10 kb), H_2O_2 ($P<0.01$; 1.3 lesions/10 kb), and H_2O_2 + BSO ($P<0.01$; 2.5 lesions/10 kb). Pulsed-field gel electrophoresis showed no difference in the occurrence of double strand breaks in the DNA of these samples. Lucigenin-dependent chemiluminesecence of GC-2 cell suspensions was not altered as a result of either DES or $4OHE_2$ treatment. These results indicate a susceptibility of GC-2 cells to DNA damage as a result of $4OHE_2$ exposure, particularly under conditions of oxidative stress. As DNA double-strand breakage and redox cycling were not found to be enhanced as a consequence of $4OHE_2$ exposure, it appears likely that DNA adducts are the predominant type of lesion formed.

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