

Oxidative DNA Damage and a Novel method for its Detection The Biotrin OxyDNA Test

Introduction

Oxygen, while essential to life, is also a major toxic risk to living organisms and its activity is closely controlled. Many macromolecules, such as proteins, fats, carbohydrates and nucleic acids are easily oxidised, compromising their function and possibly leading to cell death or mutagenesis¹. In response to this, organisms have a range of defence systems against oxidation plus mechanisms to repair or remove damaged molecules. However, no systems are perfect and oxidative damage may accumulate and may be involved in ageing, carcinogenesis and many chronic diseases¹. Oxidative DNA damage can result from radiation, or the toxic effects of endogenous metabolites, natural toxins or xenobiotics.

The importance of monitoring oxidative DNA damage is clear, but previous methods for detecting it have been laborious and have slowed the development of this field. Biotrin has developed a sensitive, specific and convenient method for detecting oxidative DNA damage - the Biotrin OxyDNA Test - that will expand the possibilities of research in this field². The following review will present the role of oxidative DNA damage in biology and disease processes and its detection using the Biotrin OxyDNA Test.

The Production of Oxidative DNA Damage

Molecular oxygen is not particularly dangerous, but a range of situations such as exposure to radiation or oxidising agents, can lead to the production of reactive oxygen species (ROS). These can react with the DNA bases (adenine, cytosine, guanine and thymine) producing over 20 different adducts, however, the main one is 8-oxo-deoxyguanine^{+ 3,4}.

In defence, cells contain anti-oxidants and antioxidant enzymes plus DNA repair systems. However, these systems are not perfect and DNA extracted from apparently healthy cells contains a low level of oxidised DNA bases.

Effects of DNA damage

DNA damage can be directly lethal to the cell, but more insidious and, maybe more serious, is the incomplete or incorrect repair of DNA lesions leading to mutagenesis or carcinogenesis. The association of 8-oxoguanine formation with mutation makes it a particularly important biomarker of oxidative DNA damage³

+ Guanine forms together with deoxyribose the DNA base guanosine. Upon oxidation, guanine forms 8 oxo 2 deoxy guanine (8-oxoguanine) which forms part of the base 8 oxo 2 deoxy guanosine. The oxidised guanine can tautomerise to the 8-OH guanine (8 OH 2 deoxy guanosine). Depending on the author, and the area of interest, any of the above terms may be used. For simplicity, in this presentation, the term 8-oxoguanine will be used.

Oxidative DNA Damage in Disease

Increased oxidative DNA damage is found in many disease conditions¹ and tends to accumulate during life, leading to speculation that it could be involved in ageing and degenerative diseases of old age. For example, neural tissue from subjects with Alzheimer's disease contains increased levels of oxidative DNA damage².

More specific links with disease are found in inflammatory diseases such as arthritis and other autoimmune diseases⁵. This could be the result of increased secretion of reactive oxygen species by inflammatory cells.

The assay of oxidative DNA could be used to study the effect of intervention on disease processes, for example, increasing the levels of anti-oxidants in the diet lead to decreased levels of oxidised DNA in circulating lymphocytes⁶.

Oxidative DNA Damage and Testing for Drug Toxicity

It is important to avoid drugs that have the potential to induce DNA damage. For example, Thalidomide has been shown to cause oxidative DNA damage in rabbit fetuses⁷.

The Biotrin OxyDNA Test.

- The Biotrin OxyDNA Test is a fluorescent-labelled protein binding method for the direct detection of oxidative DNA damage *in-situ* (see figure 1). It is based on the use of a naturally occurring binding protein with a high specificity and avidity for 8-oxoguanine².

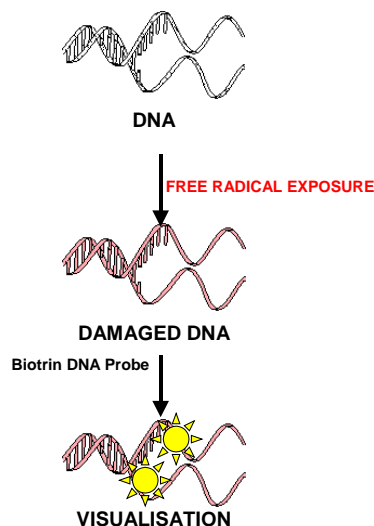


Figure 1: Principle of the Biotrin OxyDNA Test

- By binding to the oxidised DNA via the 8-oxoguanine adduct, the Biotrin OxyDNA Test provides a very sensitive and specific test for oxidative DNA damage. It also provides information on the mechanism of DNA damage.

- The Biotrin OxyDNA Test is a simple method allowing rapid screening of cell cultures and cell suspensions for oxidative DNA damage. The cells are fixed and permeabilised before being incubated with the Biotrin OxyDNA Test probe. After washing, the cells can be studied using the FACS techniques. Increasing levels of DNA damage is associated with increasing fluorescence. This is shown in figure 2 for lymphocytes exposed to methylene blue plus light.
- The ease of use and rapid results obtained with the Biotrin OxyDNA Test offer the potential for studying oxidative DNA damage in a wide range of medical conditions plus simplifying the *in-vitro* testing of potential pharmaceuticals for genotoxicity.
- So far, the Biotrin OxyDNA Test method has been used to study oxidative DNA damage in a variety of cells and tissues such as:
 - Radiation.
 - Neurodegenerative disease.
 - Ischaemia-reperfusion injury⁵
 - Nutrient deficiency
 - Stem cell toxicity
 - Fertility studies

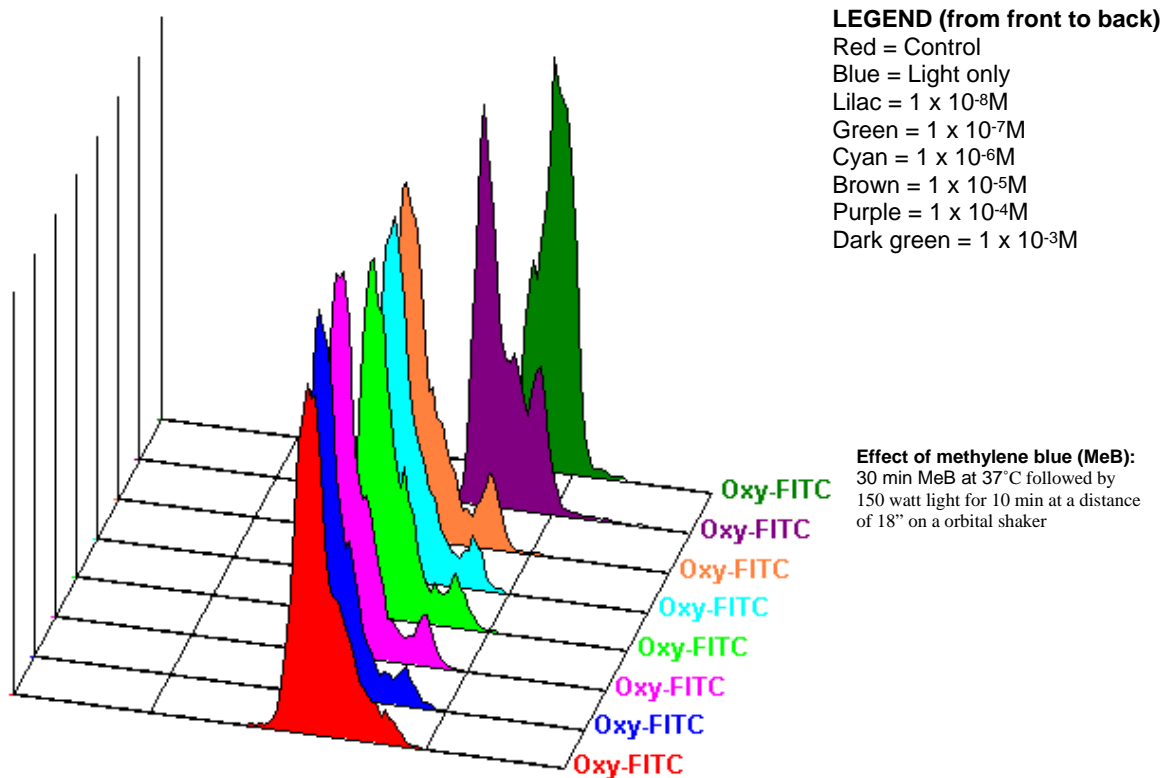


Figure 2. Oxidative DNA Damage in Lymphocytes Exposed to Methylene Blue Plus Light

Conclusions

Oxidative DNA damage is an important step in the development of many medical conditions plus being a major risk in the development of new pharmaceuticals. The development of new, easier methods such as the Biotrin OxyDNA Test will enable its study to be performed more widely and rapidly with the possibility of identifying new disease processes and intervention measures.



References and Further Reading

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