SPECIAL TESTS

TIIOS (Test for the Inhibition or Induction of Oxidative Stress)

Oxidative stress means a condition of increased oxidant production in cells which is characterized by the release of free radicals and resulting in cellular degeneration. It occurs when the production of damaging free radicals and other oxidative molecules exceeds the capacity of the body's antioxidant defenses to detoxify them. Oxidative stress is imposed on cells as a result of at least one of three factors: 1) an increase in oxidant generation, 2) a decrease in antioxidant protection, and 3) a failure to repair oxidative damage. It can also be the result of exposure to toxins or pathogens; inappropriate lifestyle factors; or even excessive exercise; or byproducts of normal metabolism. Consequently, a valid and rapid screening of pure substances, compounds, complex mixtures or biological extracts on their potential to detoxify oxygen radicals is a useful tool for determining their application as antioxidant, anti-inflammatory or oxygen radical scavenging active substances.

What is TIIOS and how does it work?

In general, TIIOS (**T**est for the **I**nhibition or **I**nduction of **O**xidative **S**tress) is a cell-based test assay which uses the formation of intracellular superoxide radicals of human monocytic/phagocytic cells (functional neutrophils) as a model to investigate the efficacy of biologically active substances for the detoxification of oxygen radicals. Upon stimulation with a phorbol ester (PMA), the cells produce superoxide radicals by an oxidative or respiratory burst. The efficacy of radical detoxification by a test substance can be measured and quantified by a decreased cleavage of a red tetrazolium dye, which yields a highly water-soluble yellow formazan dye upon cleavage.

An essential prerequisite for a successful and reproducable screening are the cells used for this assay. In contrast to freshly isolated human monocytes from different donors with a high range of individual variations, promyelocytic HL60 cells can be cultivated routinely as a stable cell line and aliquots can be chemically differentiated to functional neutrophils by use of dimethylsulfoxide.

In addition, TIIOS can be also be used for the screening of inflammatory substances or particles (e.g. nanoparticulate matter) by using instead of the phorbol ester and monitoring the production of oxygen radicals after addition to the cells.

What are the main advantages of TIIOS in comparison to other tests?

In comparison to other tests described in the literature, TIIOS in its present form has been designed as a unique test system which delivers a variety of informations on the test substance. The following list gives the main advantages of TIIOS:

- No individual variations and cell number limits as in the case of freshly isolated human monocytes of different donors; minor variations between cell batches
- Cells can be grown independently from the differentiation process as a permanent and stable cell line

- Only the optimised and highly standardised differentiation process yields highly activated functional neutrophils which are able to produce a maximum of oxygen radicals upon stimulation
- High signal spread in optical density, because a high number of activated cells is taken for a single test ($1x10^6$ cells/ml)
- TIIOS can be used as a cell-based assay as well as under cell-free conditions (KO_2 is taken as an exogen radical donor) in order to distinguish between a cell-related und cell-independent detoxification of oxygen radicals
- Highly improved and standardized process with a clearly defined test protocol and a variety of internal and external controls (e.g. special care is taken to exclude that the test substance itself does not cause a cleavage of the tetrazolium dye)
- Continuous monitoring of the cleavage and colour-change of the tetrazolium dye after addition of test substance for at least 90 to 120 minutes
- Since basal cell metabolism might be altered due to the addition of the test substance without any further stimulation, all test concentrations are done simultaneously with and without phorbol ester stimulation and then compared to each other
- In addition to oxidative burst, cell vitality of the functional neutrophils is determined in order to exclude wrong positive results due to cell death

Test principle of TIIOS

Human monocytic cell (differentiated and activated to functional neutrophil)

PMA activation
Inflammatory substances

Formation of superoxide anion (O_2^-) by oxidative burst ← **Anti-inflammatory substances**

Cleavage of tetrazolium dye to water-soluble formazan

Kinetic colorimetric measurement of formazan generation at 450 nm