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EDITORIAL:

ANALYSIS OF REACTIVE OXYGEN SPECIES

Hermann M. Bolt[1], Jan G. Hengstler[1], Joanna Stewart[1]

1 Leibniz-Institut für Arbeitsforschung an der TU Dortmund, Leibniz Research Centre for Working Environment and Human Factors (***IfADo***), Ardeystrasse 67, 44139 Dortmund, Germany

Corresponding author: e-mail: stewart@ifado.de

Reactive oxygen species play key roles as signalling molecules (Thannickal et al., 2000; Landar and Darley-Usmar, 2003; Pryor et al., 2006; Hengstler and Bolt, 2008) and are also involved in many pathogenic processes (Cederbaum et al., 2009; Sebai et al., 2009). Oxidative stress induction is a well-documented mechanism of many non-genotoxic carcinogens (Mátes et al., 2008; Hengstler and Bolt, 2007; Schug et al., 2008; Dewa et al., 2009; Binner et al., 2008). Particularly metal toxicity and carcinogenicity often involves generation of reactive oxygen species as a key mechanism (Beyersmann and Hartwig, 2008; Wang et al., 2009; Cervinková et al., 2009; Bolt and Hengstler, 2008; Verstraeten et al., 2008; Glahn et al., 2008; Pardo Andreu et al., 2009; Arivarasu et al., 2008).

Given the importance of oxidative stress numerous methods have been established and optimized to reliably quantify the levels of reactive oxygen species in tissues and cells (Dikalov et al., 2007; Borgmann 2009; Setsukinai et al., 2003; Yao et al., 2004; Tarpey and Fridovich, 2001; Tarpey et al., 2004). Some of the most frequently applied techniques include:

1. *Detection of 2-hydroxyethidium formation from dihydroethidium*

Dihydroethidium reacts with O_2^- to form 2-hydroxyethidium which can be quantified by HPLC (Zhao et al., 2003, 2005; Fink et al., 2004; Dikalov et al., 2007).

2. *DCFH-DA as a fluorescent probe to detect intracellular H_2O_2*

DCFH-DA (2',7'-dichlorofluorescein diacetate) can pass cell membranes and is cleaved by intracellular esterases to 2',7'-dichlorofluorescin (DCFH) and thereby trapped within the cells. A variety of reactive oxygen species oxidize DCFH to the fluorescent DCF (2',7'-dichlorofluorescein) (Bass et al., 1983; Khatri et al., 2004; Liu et al., 2003; Seshiah et al., 2002; Abdalla et al., 2005; Marchesi et al., 1999; Rota et al., 1999a, b; Wrona and Wardman, 2006; Kim et al., 2006). However, DCFH is easily autoxidized, resulting in unspecific fluorescence upon exposure to light (Hempel et al., 1999; Setsukinai et al., 2003). Therefore, research is ongoing to design more specific fluorescence probes (Setsukinai et al., 2003).

3. *Amplex red for detection of extracellular H_2O_2*

Amplex red (N-acetyl-3,7-dihydroxyphen-oxazine) is a nonfluorescent molecule that is oxidized to the fluorescent resorufin by horseradish peroxidase in the presence of H_2O_2 (Zhou et al., 1997). The amplex red technique can be applied to quantify H_2O_2 released by cells and tissues in cell-free supernatants.

4. Cytochrome c reduction to detect O_2^-

Ferricytochrome c is reduced to ferrocytochrome c in the presence of O_2^- (Landmesser et al., 2003; Guzik et al., 2002; Gongora et al., 2006). Ferrocytochrome c shows a different absorption spectrum compared to ferricytochrome and can easily be quantified spectrophotometrically. However, sensitivity of this technique is limited.

5. Chemiluminescence based methods

Exposure to chemiluminescent probes, such as lucigenin, to O_2^- leads to release of a photon from the probe, which can be detected luminometrically or by a scintillation counter (Li et al., 1998; Faulkner and Fridovich, 1993; Daiber et al., 2004). However, recently also rapid and easy to handle thermoluminescence based techniques have been established, where no extraction procedures are needed (Schumann et al., 2009). Using directly collected cell material the CCl_4 induced increase in thermoluminescence high temperature bands correlated well with the results of established biochemical methods.

6. Electron spin resonance (ESR) and electrochemical techniques

Free radicals may cause absorption of microwave radiation (Dikalov et al., 2007). Based on this principle ESR is a sensitive technique for studies of tissues. However, the technique is expensive and requires trained experts. Similarly, electrochemical methods where electrodes are applied to measure for instance H2O2 are technically challenging (Borgmann, 2009). However, an advantage of electrochemical methods is the possibility of real time quantification of reactive oxygen species in the microenvironment of cells. Recently, Tangkosakul et al. have presented a new technique for antioxidant activity in this years edition of the EXCLI J (Tangkosakul et al., 2009). This technique is based on lactate dehydrogenase oxidation and may find a place in the specific field of screening for compounds with antioxidative capacity. Presently, numerous studies are performed aimed at identification of compounds with antioxidative properties (Yang et al., 2009; Yu et al., 2009; Adam and Laufs, 2008; Helal and Helal, 2009). For this purpose easy to handle, quantitative, reproducible and cheap techniques are required that also allow high throughput analysis. All these requirements are fulfilled by the new LDH-based technique of Tangkosakul et al. (2009) which therefore may become a frequently applied method in screening for compounds with antioxidative properties.

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