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Live-cell imaging approaches for the investigation of xenobiotic-induced oxidant stress☆,☆☆

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ABSTRACT

Background: Oxidant stress is arguably a universal feature in toxicology. Research studies on the role of oxidant stress induced by xenobiotic exposures have typically relied on the identification of damaged biomolecules using a variety of conventional biochemical and molecular techniques. However, there is increasing evidence that low-level exposure to a variety of toxicants dysregulates cellular physiology by interfering with redox-dependent processes.
Scope of review: The study of events involved in redox toxicology requires methodology capable of detecting transient modifications at relatively low signal strength. This article reviews the advantages of live-cell imaging for redox toxicology studies.
Major conclusions: Toxicological studies with xenobiotics of supra-physiological reactivity require careful consideration when using fluorogenic sensors in order to avoid potential artifacts and false negatives. Fortunately, experiments conducted for the purpose of validating the use of these sensors in toxicological applications often yield unexpected insights into the mechanisms through which xenobiotic exposure induces oxidant stress.
General significance: Live-cell imaging using a new generation of small molecule and genetically encoded fluorophores with excellent sensitivity and specificity affords unprecedented spatiotemporal resolution that is optimal for redox toxicology studies. "This article is part of a Special Issue entitled Air Pollution , edited by Wenjun Ding, Andy Ghio and Weidong Wu".

1. Introduction

Oxidant stress is one of the most commonly cited mechanistic features of the adverse action of xenobiotics. Methodological approaches of varying efficiency have shown a wide variety of oxidant events associated with the toxicological effects of environmental contaminants [1,2], pharmaceutical agents [3,4] and natural toxins [5,6]. In addition, the field of redox biology continues to elucidate physiological redox processes that represent previously unappreciated targets for toxicological disruption.

As with any analytical endpoint, the detectability of xenobiotic oxidant stress is a function of the magnitude, duration and specificity of the signal that the effect of interest produces, as well as the sensitivity, processing speed and resolution of the method used to detect it. Experimentally, it naturally follows that more sensitive methods allow the detection of oxidant effects induced by lower levels of exposure that may more closely approximate real-world exposure scenarios. This review will focus on imaging methods available for toxicological research that employ a new generation of fluorogenic small molecule and genetically encoded sensors that, when combined with light microscopy, enable monitoring of reactive species and markers of intracellular oxidant stress in living cells with high specificity and sensitivity, offering unparalleled spatiotemporal resolution that is optimal for the study of xenobiotic-induced oxidant stress.

2. Redox toxicology

The term “oxidant stress” is often used non-specifically in the toxicological literature to refer to a wide variety of chemical reactions involving electrophilic attack on biomolecules, as well as disparate biological

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outcomes. "Oxidant stress" is used to refer to the generation of supra-physiological levels of reactive oxygen or nitrogen species (ROS, RNS), free radical-mediated damage to macromolecules, oxidation of glutathione and other intracellular "antioxidants", and the activation of signaling cascades, most notably the KEAP1/Nrf2 pathway.

In practical terms and for the purposes of this review, oxidant stress is defined as an increase in the concentration of oxidants or oxidized biomolecules in the cell relative to a homeostatic baseline condition. Fundamentally, this stress is understood to be the result of an accumulation of oxidants or oxidant damage at a rate that exceeds the homeostatic capacity of the cell to dissipate or repair it. In toxicology, this damage may be caused directly by the xenobiotic compound itself [7–11], and/or secondarily by oxidant species produced by cellular processes [12–16], or by endogenous oxidant species that are of cellular origin [17–20]. Although the concept is evolving [21], a diminished capacity of defense and repair processes in the cell, as occurs in aging [22,23], may also be considered a form of oxidant stress.

Exposure to a broad range of structurally disparate environmental and therapeutic agents has been reported to induce oxidative modification of cellular biomolecules [3,16,24–36]. A significant fraction of these xenobiotics or their metabolites have been reported to generate reactive oxygen species through redox cycling [37–40], by perturbing energy metabolism [41–45], or by altering redox-dependent processes in the cell [46–48], including the induction of "reductive stress", a situation in which limited availability of electron acceptors leads to the reduction of oxygen to form ROS [49]. Collectively, the study of the adverse outcomes induced by the reductive or oxidative effects of xenobiotic exposure can thus be referred to as redox toxicology.

3. Methodological approaches to redox toxicology

Given the widespread relevance of oxidant stress endpoints to the toxicology of xenobiotics, there is a constant need for improved methodologies for the detection of oxidant species and stress markers. Analytically, the study of oxidative stress in vitro has most commonly been based on the quantification of concentrations of reactive species [50], such as superoxide [51], hydrogen peroxide [52], hypochlorite [53], free radicals [54], and peroxynitrite [55].

Alternatively, in vitro evidence of oxidant stress has also been based on the measurement of levels of oxidized biomolecules, principally oxidized and adducted proteins [56–65], lipids [66–68], and nucleic acids [69–72]. Another commonly employed approach relies on the measurement of the concentrations and form of intracellular antioxidants [73] such as glutathione (GSH) [74–76], ascorbate [77,78], tocopherols [79,80], carotenoids [79,81,82], and the energy intermediates NADPH and NADH [83,84].

More recently, functional measurements based on the effects of oxidant stress on cells have been developed. For instance, oxidant stress has been shown to activate intracellular signaling through multiple mechanisms, including the activation of kinases and the loss of phosphatase activity [85–88]. The state of activation of signaling intermediates in oxidant-sensitive pathways (e.g., KEAP1/NrF2) is increasingly used as a marker of oxidant stress [89–91]. Similarly, induction of transcriptional expression of hemoxygenase 1 (HO-1) [92–95] and NADPH quinone oxidoreductase 1 (NQO1) [96,97], both of which are genes regulated by the transcription factor NrF2, is gaining in acceptance as a relatively specific marker of the cellular response to oxidant stress. A genomic approach that builds on this concept monitors profiles of gene expression known to be involved in the sensing, signaling and response to oxidant stress [98–100].

A major limitation inherent in all of the aforementioned biochemical and analytical assays used for the study of oxidant stress is that they consume the sample, requiring the use of extraction procedures to isolate the oxidized biomolecules, proteins or mRNA of interest, which precludes repeated collection of data points from the same cells undergoing a response over time. Moreover, disrupting compartments within the cell inevitably removes physical and kinetic constraints that normally modulate the equilibration of redox pairs and regulate interaction between enzymes and their substrates, thus potentially leading to loss of information and, more pernicious, the introduction of artifacts [101].

4. Advantages of live-cell imaging for redox toxicology studies

The generation of reactive oxygen species and the cellular responses induced by oxidant stress are often short-lived events that can be difficult to capture. This may be especially true in cases where the duration of the oxidant event is insufficient to result in the death of the cell but instead initiates adverse cellular responses, a scenario that is often of concern in toxicology. The ability to monitor events by collecting readouts at close time intervals in the same cells is therefore essential to capturing transient oxidative events. Examples include a sharp increase in the concentration of an ROS of interest such as H_2O_2, or a change in the relative concentration of glutathione (GSH) and its oxidized derivative (GSSG). The transient and localized nature of biochemical redox reactions places unprecedented demands on the spatial and temporal resolution required to study them under physiological and toxicological conditions.

Live-cell imaging is a light microscopy technique for monitoring physiological parameters in living cells in real time [102,103]. Environmental control hardware is used to maintain physiological temperature, pH and humidity levels to support cell viability throughout the period of data collection [103]. By preserving the integrity of the cell throughout the experiment, live-cell imaging avoids many of the limitations of extractive techniques, including loss of spatial information and the introduction of methodological artifacts. Since cellular compartments are not disturbed, live-cell imaging shows the effects of oxidant stress as they unfold in time and space relative to a physiological, baseline or resting state established during a suitable period just prior to the introduction of the exposure agent. This approach obviates the need to consider artifacts or aberrations introduced by sample extraction and analysis as potential alternative explanations for the effects observed.

In addition to capturing fleeting events, the high temporal resolution of live-cell microscopy systems can provide valuable insights on kinetic indices of interest, which can produce evidence of cause and effect by establishing the existence of a time lag or temporal difference between events that may appear to occur simultaneously when observed by other techniques. Properly equipped live-cell imaging systems are capable of temporal resolution that is impractical to replicate using non-imaging analytical approaches, making this approach in many cases the gold standard for the study of oxidative events associated with xenobiotic exposure. Similarly, given the intrinsic spatial resolution of microscopy techniques for examining subcellular sites and structures, live-cell imaging is an optimal technique for identifying cellular organelles that are targeted by oxidant stress, or compartments that are involved in the production of reactive species. Spatiotemporal information afforded by live-cell microscopy can contribute comprehensively towards a mechanistic understanding of the role of oxidant events in cellular responses to a physiological or xenobiotic stimulus.

5. Fluorogenic sensors for live-cell imaging of redox toxicology

While the sensitivity and dynamic range of a live-cell assay are characteristics determined by the performance specifications of the components of the imaging system (e.g., light source, quality of the optics, detector sensitivity), specificity, selectivity and dynamicity are properties imparted entirely by the sensor molecule that reports the event of interest. Thus, the potential of live-cell microscopy is largely dependent on the availability and performance of sensor molecules that can report the endpoint of interest with suitable efficiency.

Most of the sensors used in live-cell microscopy are fluorogenic. These sensors are designed to respond to a specific physicochemical

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