Focal Points
Application Note FP-179

Low Light Imaging of Reactive Oxidative Species within Wound Sites Using Luminol

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Introduction

Luminol is a standard tool in forensic science. Typically, crime scenes are analyzed for blood samples by applying a solution of luminol and oxygen radicals onto surfaces. The iron-laden hemoglobin molecules present in blood serve as catalysts for the reaction which can then be captured by photograph. When combined with an oxidizing agent and a catalyst, luminol emits blue light (Fig 1).

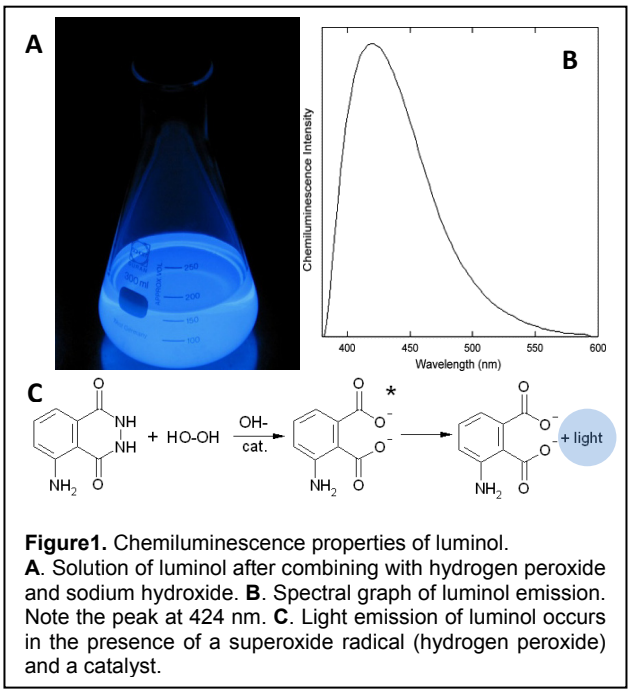

Figure1. Chemiluminescence properties of luminol. **A**. Solution of luminol after combining with hydrogen peroxide and sodium hydroxide. **B**. Spectral graph of luminol emission. Note the peak at 424 nm. **C**. Light emission of luminol occurs in the presence of a superoxide radical (hydrogen peroxide) and a catalyst.

For routine applications, as in forensic testing or in a laboratory setting, the luminol molecule is combined with a hydroxide salt to generate a dianion. When in the presence of a superoxide radical, the dianion molecule produces unstable organic peroxide. Upon relaxing to a ground state, the dianion emits a photon within the visible spectrum, primarily in the blue light range (peak emission is at 424 nm). The reaction typically lasts for a short time, on the order of seconds, as the luminol molecule is consumed in the generation of light.

In addition to forensics, the use of luminol extends into preclinical research and has been used when studying the generation of reactive oxidative species (ROS)[1]. Normal cellular respiration produces oxide radicals including superoxide anions, hypochlorite, hydroxyl anions and hydrogen peroxide. Additionally, ROS are generated during acute inflammation as well as in response to environmental stimulants. Therefore, inflammation can be studied using luminol due to the generation of substrate for the luminescent reaction to occur.

Recent studies have examined the use of luminol within in vivo models[2]. Specifically, the role of inflammatory cells has been under study due to the response of the immune system to stress. Typical inflammatory processes involve a cellular phase in which inflammatory cells are recruited to the site of trauma to mount a response. This response takes the form of an “oxidative burst” in which ROS are generated by a host of enzymes within the phagocytic cell to both destroy pathogens as well as to recruit and activate other inflammatory cells.

Luminol was used in vivo to monitor the generation of ROS, specifically due to the inflammatory response mounted by phagocytic cells. Two mice, a control and a mouse deficient in a factor that clears cells from the wound, were wounded at a specific time point to determine the degree of inflammation of a knockout mouse and imaged 12 hours later. The knockout mouse showed the greatest generation of luminescence, suggesting that this mouse has impairment in clearing of inflammatory cells.

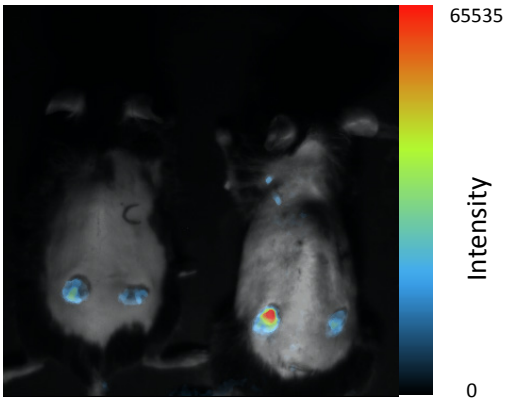

Figure 2. Luminescence at a site of local inflammation. Both mice were imaged 12 hours after wounding and immediately after luminol injection. Note that the intensity in the control mouse (left) was significantly lower than the experimental mouse (right).

Material & Methods

Experimental Protocol

Two mice were wounded at 12 hours prior to imaging. The mice were briefly anesthetized with xylazine/ketamine and injected with luminol intraperitoneally. The mice were then placed in the prone position on the iBox® Scientia™ Small animal Imaging System's (Fig 3) imaging stage and images were captured with a highly sensitive CCD camera immediately after injection of the luminol. Each mouse was imaged for 20 minutes at 4x4 binning (Fig 2). To ensure euthermia, the imaging stage/warming plate maintained the temperature of the mouse at 37°C.

Figure 3. iBox Scientia Small Animal Imaging System

Image Processing

Two images were acquired at each time point using a bright field (white light) and a luminescent channel. These images were then selected for overlay via the composite feature using VisionWorks®LS software. The luminescent image was pseudocolored with an intensity map. The pseudocolored luminescent image was then overlaid on the bright field image (Fig 4).

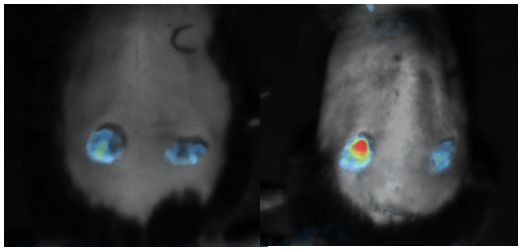

Figure 4. Luminescence at a site of local inflammation. Note the greater intensity of the experimental mouse lesions (right) as compared to control (left).

Results and Conclusion

A key biological catalyst in the generation of ROS in the inflammatory response is myeloperoxidase (MPO), an enzyme that converts hydrogen peroxide to hypochlorous acid (Fig 5) in addition to several other reactive species. This enzyme is present in phagocytic cells, inflammatory cells which engulf pathogens and cellular debris.

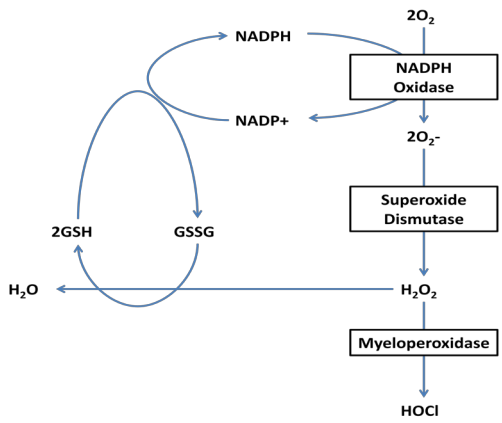

Figure 5. Generation of superoxide radicals within phagocytic cells. Oxidative bursts within sites of inflammation are the result of the action of key enzymes within phagocytic cells. A major enzyme used in production of ROS is myeloperoxidase (MPO), an enzyme that generates hypochlorous acid and other ROS for use in clearing of foreign material and cell signaling.

Acute wounding, either through mechanical or chemical means, generated intense signals upon exposure to luminol after 12 hours. The acute inflammatory process includes the recruitment of phacocytic cells, such as polymorphonuclear leukocytes or macrophages. These cells generate ROS during the phagocytic process to both destroy pathogens as well as to clear the wound of debris from damaged tissue. Key to wound resolution, however, is removal of inflammatory cells. Problematic wounds, such as diabetic ulcers, are impaired in wound resolution. One mechanism of wound resolution is via macrophage apoptosis by VEGF upregulating the expression of an important signal-transduction pathways[3].

The iBox Scientia is a powerful tool for imaging disease processes within an in vivo model. The Scientia couples a light-tight darkroom, a highly sensitive CCD camera and sophisticated optics for low-light imaging, ideal for luminescence related applications. Through the use of luminescence, real-time imaging of ROS generation by inflammatory cells can be observed evolving within the wound of a mouse.

[1]Liu, Wendy F., et al. Real-time in vivo detection of biomaterial-induced reactive oxygen species. Biomaterials 2011 Mar;32(7):1796-801.
[2]Gross, Shimon, et al. Bioluminescence imaging of myeloperoxidase activity in vivo. Nat Med. 2009 Apr; 15(4): 455-61
[3]Petreaca, M., M. Yao, C. Ware, and M. Martins-Green. VEGF promotes macrophage apoptosis through stimulation of tumor necrosis factor superfamily member 14 (Tnfsf14/LIGHT). *Wound Repair and Regeneration* 2008 Sep;16(5):602-614.