

Video Article

Bioluminescence Imaging of Heme Oxygenase-1 Upregulation in the Gua Sha Procedure

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Abstract

Gua Sha is a traditional Chinese folk therapy that employs skin scraping to cause subcutaneous microvascular blood extravasation and bruises. The protocol for bioluminescent optical imaging of HO-1-*luciferase* transgenic mice reported in this manuscript provides a rapid *in vivo* assay of the upregulation of the heme oxygenase-1 (HO-1) gene expression in response to the Gua Sha procedure. HO-1 has long been known to provide cytoprotection against oxidative stress. The upregulation of HO-1, assessed by the bioluminescence output, is thought to represent an antioxidative response to circulating hemoglobin products released by Gua Sha. Gua Sha was administered by repeated strokes of a smooth spoon edge over lubricated skin on the back or other targeted body part of the transgenic mouse until petechiae (splinter hemorrhages) or ecchymosis (bruises) indicative of extravasation of blood from subcutaneous capillaries was observed. After Gua Sha, bioluminescence imaging sessions were carried out daily for several days to follow the dynamics of HO-1 expression in multiple internal organs.

Protocol

1. Female HO-1-*luciferase* transgenic mice (age of 4-6 weeks) can be purchased from Taconic Farms, Inc. Upon arrival, mice are placed in an animal housing facility for at least 4 days to allow accommodation.
2. Preparation for bioluminescence imaging:
 1. Weigh each HO-1 mouse. Animal weight is required for calculating the dosage of luciferin.
 2. Hair removal: Use a cotton swap to apply hair remover Nair® over the abdomen and/or back of the animal. Waiting for 5 to 10 seconds. Use a clean cotton swap to wipe off hair. Dip a piece of tissue in distilled water to wipe clean remaining hair.
 3. Place a non-fluorescent black paper (Strathmore Artagain® black paper) on the imaging platform of an IVIS 100 station to reduce background noise.
 4. Prepare luciferin (from Xenogen Corporation) solution at concentration of 7.5 mg/ml (dissolved in sterile H₂O). The dose of luciferin is 65.5 mg/kg body weight. Thus, a 20 gram mouse requires intraperitoneal injection of 0.175 ml luciferin solution. To achieve this, a single injection volume of luciferin solution (0.175ml) is preloaded to a syringe with a 26-gauge needle
3. Gua Sha procedure: Gua Sha is applied only once before running the bioluminescence imaging protocol. Since Gua Sha is not painful, the mouse needs only to be briefly anesthetized by isoflurane to stay calm. Gua Sha procedure includes the following steps:
 1. Apply cooking oil or distilled water to lubricate skin areas targeted by Gua Sha a few times during the Gua Sha procedure.
 2. Repetitively scrape the hair-free region of the back of the mouse in gentle but firm force using a ceramic soup spoon or a plastic spoon.
 3. The scraping continues until the back skin turns red, which is a sign of subcutaneous blood extravasation, usually achieved within 2 to 3 minutes.
4. Bioluminescence imaging may be started immediately or several hours after Gua Sha as HO-1 upregulation is built up slowly over a few hours. The procedure for bioluminescence imaging is:
 1. Anesthetize the mouse in an anesthesia induction chamber filled with a mixture of isoflurane (1.5%) and medical grade air.
 2. Once the mouse is anesthetized, move the mouse to the imaging chamber on the IVIS 100 optical imaging station. Position the mouse in a supine position (abdomen up). The imaging chamber is continuously infused with 1.5% of isoflurane. The imaging platform is heated at 37°C to keep the mouse warm.
 3. Set the imaging acquisition at "medium binning" and set exposure time to be 30 seconds. Start to acquire images. Set the machine to repeat the imaging acquisition every three minutes, either manually or automatically. After the acquisition of the first image, a region of interest (ROI) is drawn to cover the abdominal, chest, and head area. This ROI is then copied and pasted to the following images using "Living Image®", software accompanying the IVIS 100 station.
 4. Signal intensity is measured in photons per second. Mark time for the signal intensity to reach its peak value and keep on imaging the mouse for about 5 to 10 minutes after the peak time.
 5. When the imaging acquisition at the supine position is done, flip the mouse over and lay it in the prone position (back up). Continue imaging the animal for 5 to 10 minutes with the ROI now drawn to cover the back and the head area in the use of the software "Living Image®". The choice of imaging the prone position after the supine position is arbitrary. One can choose to start with the prone

position, depending on the primary organs of interest. Another option is to image the supine and prone positions at separate experiments.

- When the imaging acquisition at the prone position is finished, turn off isoflurane and move mouse from the imaging chamber to the induction chamber to recover. The induction chamber is now infused with medical air only and the mouse usually wakes up in less than one minute.
 - Save imaging data for post-processing.
 - After the initial Imaging study, additional imaging acquisitions are carried out for several days after Gua Sha. One sample schedule is repeated imaging at the 12th hour, 24th hour, 36th hour, 48th hour, 72nd hour and 120th hour but the exact timing of the imaging schedule is flexible. Additional imaging sessions may be added if a longer follow-up is needed. Before imaging, repeat the hair removing procedure if the hair has grown back.
5. For the control group, repeat all procedure but skip Gua Sha (step 3). If the same group of mice is used for both control and Gua Sha, the control experiment should be carried out preferably before the Gua sha procedure or at least one month afterwards.

Representative Results:

The bioluminescence images in figure 1 show *in vivo* upregulation of HO-1 in response to Gua Sha. The graph in Figure 2 shows the quantitative temporal change over 120 hours in optical flux (photons/sec) from the whole body of the same mouse related to Gua Sha...

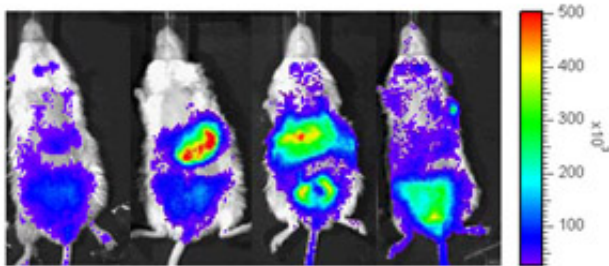


Figure 1. From left to right, representative images of the front view (supine) of same mouse before Gua Sha, at 18 hours, 36 hours and 120 hours post Gua Sha, respectively. After Gua Sha, one observes the progress of significant signal intensity increase in multiple organs which encompass regions of the gastrointestinal tract, the genital tract, the liver, kidneys (from the back view, not shown), and others. Please [click here](#) for a larger version of figure 1.

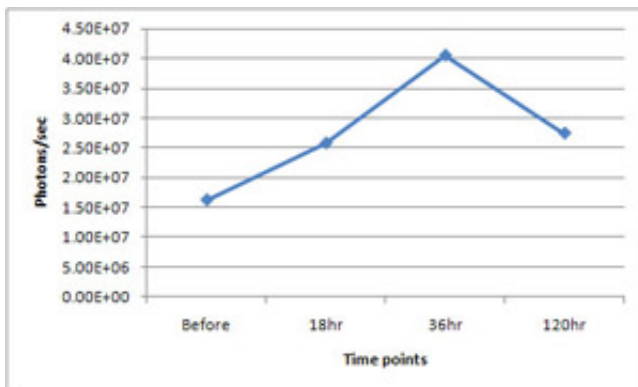


Figure 2. Quantitative change of flux (photons/sec) from the whole body tracked over 120 hours following Gua Sha in the same mouse of Figure 1. Please [click here](#) to see a larger version of figure 2.

Discussion

Transcription of HO-1, an inducible form of heme oxygenase, is upregulated by many factors including heme, hydrogen peroxide, UV irradiation, hypoxia, and physical stresses. Whole body imaging such as the reported HO-1 bioluminescence protocol provides a quick snapshot of systemic HO-1 expression in multiple organs. In small animals, bioluminescence imaging allows high-sensitivity *in-vivo* real-time quantitative longitudinal optical assessment of alterations in systemic gene expression, as shown. Bioluminescence molecular imaging lowers the cost and increases the throughput of gene expression assays in small animals, making it practical to rapidly achieve the statistical power needed for investigation of complex systems biology hypothesis and to assess for systemic effects of proposed pharmaceutical and other therapeutic interventions. One limitation is low spatial resolution of the anatomy. Newer optical imaging systems that have tomographic capability would alleviate the problem. Image acquisition and registration by micro-CT or small animal MRI would help to properly identify the anatomy. Other limitations are low light penetration through tissue and the need for transgenic animals. These essentially preclude translation to larger animals or human use, but are not a significant drawback for small animal systems biology or preclinical therapeutic hypothesis testing.

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