Noninvasive Imaging Of Hemorrhage-Induced Global Ischemia With A Transgenic Mouse Expressing Luciferase Coupled To Hypoxia-Inducible Factor (HIF1α)

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Abstract

Hemorrhagic shock leads to global ischemia, but available blood is distributed unevenly to the body's organs and it is generally accepted that blood is shunted to those organs that maintain critical functions. The organs that become most ischemic and their role in hemorrhagic shock, however, have not been determined. We used the recently described, genetically engineered mouse FVB.129S6-Gt(ROSA)26Sortm1(HIF1α/luc) Kael/J* (Jackson Laboratories) which has a luciferase gene fused to the region of HIF1α that binds to von Hippel-Lindau protein in an oxygen-dependent manner generating a reporter that can be used to monitor oxygen availability in intact tissues. Thus more light would be emitted from ischemic tissue. To determine if this mouse could be used to identify organs affected by hemorrhagic shock, 40 per cent of the calculated blood volume was removed via the submaxillary vein, and the mice were injected with luciferin, anesthetized with isoflurane, and imaged in the Xenogen IVIS 100 Imaging system as a function of time after hemorrhage. The ventral surface of these mice exhibited increased light emitted from the region of the intestine that was most prominent at 3-6 h after hemorrhage but still evident at 72 h. This technique should allow more detailed studies of those tissues most affected by hemorrhagic shock.

Introduction

Following hemorrhage, delivery of blood to organs and tissue is compromised, resulting in hypoxia to some organs but those tissues most effected by hypoxia are not known. Safran et al. have recently described a transgenic mouse that has been engineered to express the firefly luciferase bioluminescent reporter fused to a region of HIF that is sufficient for oxygen-dependent degradation. This mouse is designed for use in monitoring hypoxic tissues and evaluating therapeutic agents that stabilize HIF1α. We asked if it could be used to monitor hypoxia induced by hemorrhage

Methods

Male mice (25-30 g) of the FVB.129S6-Gt(ROSA)26Sortm1(HIF1A/luc)Kael/J strain (Catalog #006206) were obtained from the Jackson Laboratories, Bar Harbor, ME. Mice were anesthetized with 2% isoflurane and about forty percent of the calculated blood volume was withdrawn over a 30 second period by inserting the Medipoint Lancet (Medipoint , Mineola, NY) into the submaxillary vein. Submaxillary veins of sham animals were punctured with the lancet but bleeding stopped by the application of pressure from sterile gauze. For bioluminescence imaging, mice were anesthetized with isoflurane/air and injected with 100 µl of luciferin (dissolved in phosphate buffered saline into the peritoneal cavity. Five min after luciferin injection, mice were imaged for 1-5 minutes. Photons emitted from specific regions will be quantified using the LivingImage software (Xenogen) and luciferase activity acquired as photons emitted per second. Organs (liver, lung, kidney, spleen, duodenum, jejunum, ileum, stomach, brain, salivary gland, skeletal muscle, and testes) were removed 5 min following injection of luciferin to produce a higher resolution view of the light coming from organs as a function of luciferase production concomitant with HIFα induction.

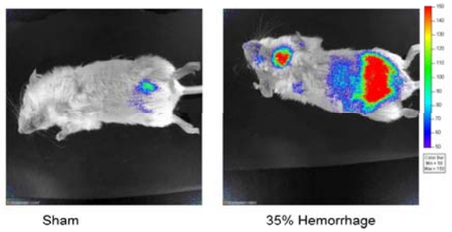

Figure 1. Noninvasive imaging of the FVB.129S6-Gt(ROSA)26Sortm1 (HIF1A/luc)Kael/J following 35% hemorrhage. The intestine appears to be most effected at 3 h post hemorrhage. The throat region is probably giving off light because it became ischemic as a result of hemorrhage via the submaxillary vein.

Figure 2. Noninvasive imaging of the FVB.129S6-Gt(ROSA)26Sortm1 (HIF1A/luc)Kael/J following 40% hemorrhage as a function of time after hemorrhage. The same mouse was followed for 96 h before (prehemorrhage) and following hemorrhage (posthemorrhage). At each time was mouse was anesthetized with isofluorance, injected IP with luciferin and imaged at 5 min following injection. The principal source of luciferase by whole body imaging is the intestinal region.

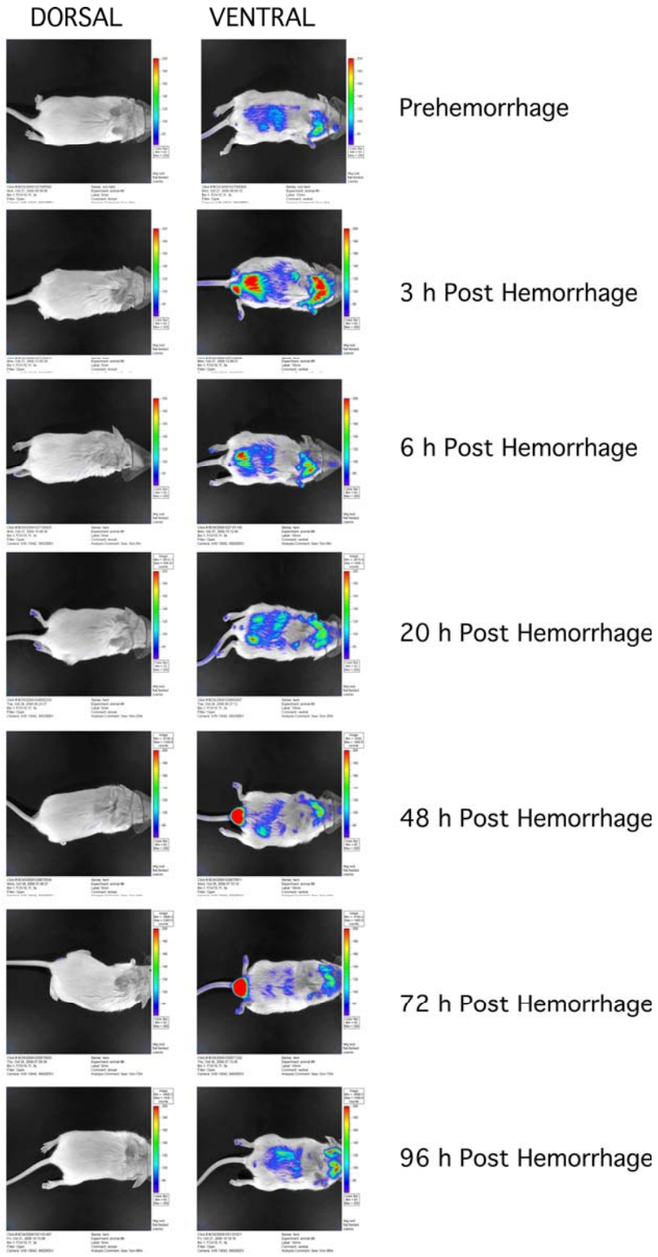

Figure 2

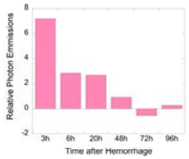

Figure 3. Quantification of light emanating from the intestinal region by luminescent imaging. The region of interest (ROI, intestine) was demarcated and applied to each time point in Figure 2. Bars represent average number of photons/ROI at each time.

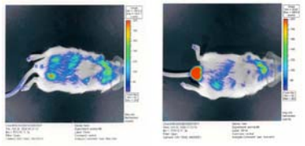

Figure 4. The testes as a site of HIF1a/luciferase activity in mice. The same mouse imaged on different days. On the left, the testes are retracted into the body. On the right the testes have descended and express high levels of luciferase activity. This has recently been reported by Lysiak et al. Hypoxia Inducible Factor-1α is Constitutively Expressed in Murine Leydig Cells and Regulates 3β-Hydroxysteroid Dehydrogenase Type I Promoter Activity. J Androl. 2009 Mar-Apr;30(2):146-56.

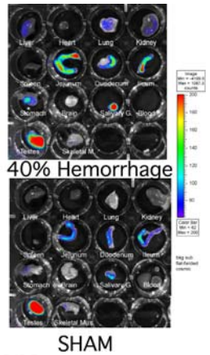

Figure 5. Evaluation of light emanating from isolated organs of hemorrhaged and sham HIF1α/luciferase mice. Organs were immediately removed from mice following in vivo imaging and placed in wells of a 24-well multiplate and reimaged. In addition to high photo emissions from components of the intestine, testes and salivary gland, liver, lung, kidney, and stomach also produced light indicating some hypoxia in these organs.

Conclusions

1. The HIF1α/luciferase mouse provides a useful model for studying hypoxia associated with ischemia of hemorrhage. The high signal/noise allows detection of HIF1α activity at very low levels.
2. In vivo imaging is problematic as many effected organs are not evident from in vivo imaging.
3. Even imaging of organs may not accurately reflect the degree of hypoxia as photons emitted from highly pigmented organs may be reduced requiring homogenization of tissue prior to luciferase determination.

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