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Hikarazine™ Z108 Enables Sensitive In Vivo Imaging of AAV-Mediated Luciferase Expression in the Mouse Brain

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INTRODUCTION

As part of our investigations into neurodegenerative disorders, we develop animal models of synucleinopathies to better understand the mechanisms underlying disease initiation and propagation, and to further investigate therapeutic compounds. In this context, we use viral vector–mediated gene delivery to drive the expression of pathogenic proteins, thereby recapitulating key molecular and cellular features of disease pathology in vivo.
To longitudinally monitor transgene expression, we rely on bioluminescence imaging (BLI), which offers high sensitivity and quantitative readouts in living animals. However, the performance of BLI is critically dependent on the physicochemical properties of the luciferase substrate, including its brightness, bioavailability, and tissue penetration.
Accordingly, the development of next-generation luciferase substrates with improved photon output and enhanced penetration through biological tissues represents a major determinant for increasing the sensitivity and reliability of in vivo imaging, particularly in deep brain structures. In this study, we assessed the performance of Hikarazine™ Z108, a novel luciferase substrate, for *in vivo* bioluminescence imaging of the mouse brain.

MATERIAL AND METHODS

In vivo experiments were performed using C57BL/6JRj mice. Three male C57BL/6Jr Tyr c/c mice (6 weeks old; Janvier Laboratories) were acclimatized for two weeks in the animal facility before injection. Mice received unilateral stereotaxic injections above the substantia nigra of 1 µL of AAV2/9-CMVie/SynP-luciferase-WPRE (0.86×10^{13} GCP/mL), obtained from the Neurodegenerative Diseases Institute Vector Core.
Luciferase production was then monitored by bioluminescence imaging 15- and 30-days post-injection using the Lumina III imaging system (PerkinElmer on Vivoptic Platform). A time course study was done to determine the best acquisition timing after substrate injection into the animal. Accordingly, 5 min after intraperitoneal injection of 54 nmol of Hikarazine™ Z108 (Synthelis Biotech), 3 animals were anaesthetised and placed into the imager for a 60-s acquisition across six consecutive exposures, with 1 or 2-minute intervals between acquisitions. Data were analyzed using Living Image software.

RESULTS AND DISCUSSION

At 15 and 30 days following intracerebral viral injection, a bioluminescent signal was detected after intraperitoneal administration of 54 nmol of Hikarazine™ Z108 (Figures 1A and 2A). In both cases, acquisition kinetics revealed persistent signal intensity up to 25 minutes post-injection (Figures 1B and 2B). These results demonstrate the high sensitivity of this novel substrate, as well as efficient brain biodistribution, yielding a stable signal over time and enabling robust bioluminescence imaging.

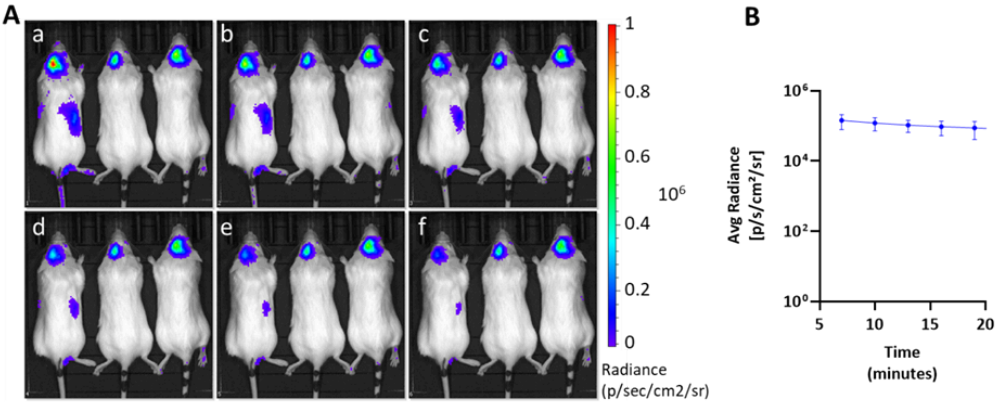

Figure 1: (A) Representative bioluminescence images 15 days after AAV injection. Mice received an intraperitoneal injection of HikarazineZ108 (54 nmols in 100 µL PBS), and were sedated 5 minutes later. Bioluminescence images (1 min, 4 ×4 binning) and photographs (100ms) were taken 7 min after substrate injection (a) and for 1 minute with 2-minute intervals (b, c, d, e, f). Bioluminescence signals were analyzed using Living Image software (Perkin Elmer) and plotted (B) as average radiance in photons/seconds/cm2/steradian.

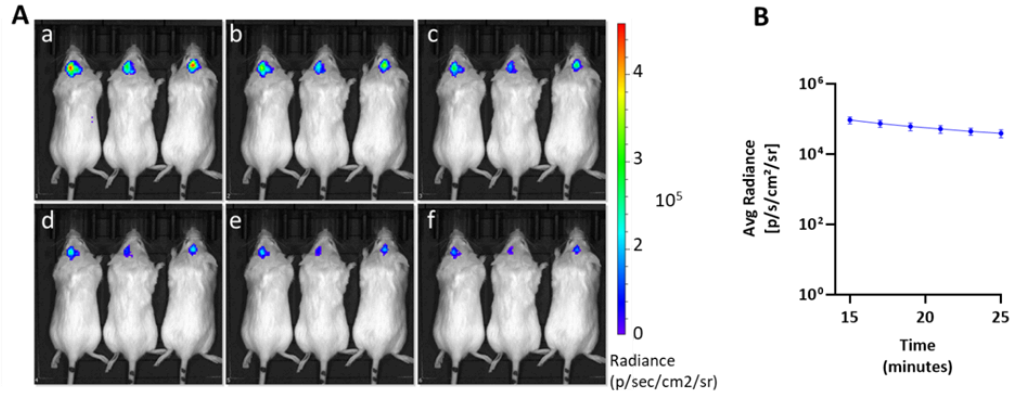

Figure 2: (A) Representative bioluminescence images 30 days after AAV injection. Mice received an intraperitoneal injection of HikarazineZ108 (54 nmol in 100 µL PBS), and were sedated 5 minutes later. Bioluminescence images (1 min, 4 ×4 binning) and photographs (100ms) were taken 15 min after substrate injection (a) and for 1 minute with 1-minute intervals (b, c, d, e, f). Bioluminescence signals were analyzed using Living Image software (Perkin Elmer) and plotted (B) as average radiance in photons/seconds/cm2/steradian.

CONCLUSIONS

In this study, we demonstrate that the novel substrate Hikarazine™ Z108 is well-suited for deep brain penetration. Specifically, in this model, the AAV is injected into the substantia nigra, a deep brain structure. The strong performance of this substrate, even at lower concentrations than those typically reported in the literature for competing compounds, supports its suitability for deep-tissue imaging applications requiring high sensitivity.

For more information, contact:
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