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Nanoparticle-emitted Light Attenuates Amyloid- β -induced Superoxide and Inflammation in Astrocytes

Brittani L Bungart, MS^a, Li Dong, MS^a, Daniel Sobek, PhD^b, Grace Y. Sun, PhD^c, Gang Yao, PhD^a, and James C-M. Lee, PhD^a

^aDepartment of Biological Engineering, University of Missouri, Columbia, Missouri 65211

^bZymera, Inc., San Jose, California 95138

^cDepartment of Biochemistry, University of Missouri, Columbia, Missouri 65211

Abstract

Alzheimer's disease (AD) is the sixth leading cause of age-related death with no effective intervention yet available. Our previous studies have demonstrated the potential efficacy of Low Level Laser Therapy (LLLT) in AD cell models by mitigating amyloid- β peptide (A β)-induced oxidative stress and inflammation. However, the penetration depth of light is still the major challenge for implementing LLLT in animal models and in the clinical settings. In this study, we present the potential of applying Bioluminescence Resonance Energy Transfer to Quantum Dots (BRET-Qdots) as an alternative near infrared (NIR) light source for LLLT. Our results show that BRET-Qdot-emitted NIR suppresses A β -induced oxidative stress and inflammatory responses in primary rat astrocytes. These data provide a proof of concept for a nanomedicine platform for LLLT.

Keywords

Alzheimer's disease; light nanomedicine; inflammation

Background

Many diseases, including AD, are characterized with aberrant cellular activities resulting from increased oxidative stress and inflammation (1, 2). In AD, A β when aggregated to its oligomeric form has the most cytotoxic effects (3). Toxic A β increases oxidative stress and triggers inflammatory responses in glial cells through increased expression of inflammatory proteins, such as interleukin-1 β (IL-1 β) and inducible nitric oxide synthase (iNOS) (4).

Low level light with wavelengths ranging from 632.8 to 904 nm have been reported to attenuate inflammation and oxidative stress in a number of models (5, 6). For example, we have previously demonstrated that a low level energy laser at 632.8 nm attenuated oxidative stress and inflammation in primary astrocytes induced by A β (5). However, the use of laser

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Corresponding author: James C-M. Lee, 240 Agricultural Engineering, Columbia, Missouri 65211; Fax: 573-882-1115; Phone: 573-884-3686; LeeJam@missouri.edu.

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or LED light as a clinical therapy for the treatment of AD brains is limited due to the low penetration of light in tissues (7).

In this study, we demonstrate a new light delivery method by using a recently engineered nanoparticle, BRET-Qdot. This light delivery nanoparticle has been used in a number of *in vivo* applications (8) and exploits the Föster resonance energy transfer from an engineered enzyme, *Renilla Reniformis* luciferase (Luc8) (9). When activated with its substrate, coelenterazine-h (coel-h), Luc8 is known to emit at 480 nm to a CdSe or CdTe quantum dot, which then re-emits the light at 800 nm. A typical BRET-Qdot construct incorporates 3 to 6 Luc8 molecules conjugated to the surface of CdTe quantum dots (Figure 1). Conceptually, this light delivery approach could potentially resolve difficulties in providing light from external sources to the central areas of the brain.

Methods

To test the efficacy of BRET-Qdots (Zymera, San Jose, CA, USA) as proposed, we used primary cortical rat astrocytes (Invitrogen, Grand Island, NY, USA) as in our previous study (5). The maximum fluency and duration of the BRET-Qdots' emitted light was observed using a PIXIS 512 CCD camera (Princeton Instruments, Trenton, NJ, USA) in conjunction with a cold mirror with a 0° AOI (Edmund Optics Inc., Barrington, NJ, USA) to reflect >90% visible light. Then, the BRET-Qdots were used as the pretreatment prior to a 5 μ M A β (American Peptide, Sunnyvale, CA, USA) exposure to astrocyte, followed by the characterizations of superoxide anion accumulation using fluorescence microscopy of dihydroethidium (DHE), and the expressions of IL-1 β and iNOS using Western blot analysis. Detailed methodological information can be found in the Supplementary Materials.

Results

BRET-Qdot with coel-h attenuates A β -induced superoxide anion production

We test if the superoxide anion induced by A β can be reduced by 800 nm-emitting BRET-Qdots which preceded A β treatment. Figure 2 shows that exposing astrocytes to 5 μ M A β for 2 h increased superoxide anion production by 40% as compared to the untreated control. Light pre-treatment using 66.7 pM BRET-Qdots activated with 2.5 μ M coel-h reduces A β -induced superoxide anion production to untreated control levels. Either BRET-Qdots or coel-h individually reduces superoxide anion production on average compared to A β -treated cultures, but not statistically significant.

BRET-Qdot with coel-h pretreatment attenuates A β -induced inflammatory marker expression

Similarly to the superoxide anion experiment, we investigate if the light pretreatment with 66.7 pM BRET-Qdot activated with 2.5 μ M coel-h reduce the inflammation contributed by 5 μ M A β as measured by IL-1 β and iNOS expression levels (Figure 3). Incubating astrocytes with 5 μ M A β for 18 h increased IL-1 β and iNOS expression by 30 and 50%, respectively. BRET-Qdot-based light pre-treatment lowered A β -induced expression of IL-1 β to that of the untreated control, while A β -induced iNOS expression was reduced to below untreated control levels. Neither BRET-Qdot nor coel-h alone showed the same reduction in IL-1 β or iNOS expression.

Discussion

Our previous study with astrocytes showed that application of 632.8 nm laser caused a decrease in oxidative stress and inflammation (5). However, red to NIR light cannot be

transmitted through the scalp to the brain more than a few centimeters (7). This makes non-invasive LLLT for AD nearly impossible.

In this present study, we demonstrated the ability of a light-generating nanoparticle to attenuate A β -induced oxidative stress and inflammatory responses in astrocytes. The application of this light delivery method based on light-generating nanoparticles demonstrated a novel method for applying LLLT.

Some studies on the application of quantum dots in biological systems may consider the possible toxic effects of quantum dots due to their heavy metal content, nanoparticulate nature, or coating composition. Nevertheless, investigations of quantum dots *in vivo* have shown retention of the material in tissues from days to weeks with no major complications (10). Here we also show that exposing astrocytes to BRET-Qdots with coel-h for 2 and 12 h did not significantly lower cell viability as compared with control. (see Supplementary Materials, Fig. S2)

Results here show the advantages of light-generating nanoparticles as a concept to be further developed for *in vivo* light-delivery for LLLT. CdSe/CdTe quantum dots may be used further in animal models as non-toxic, NIR-emitting nanoparticles become available. In addition, even though this study only covered A β -induced inflammation and oxidative stress, as studied in the AD pathology, the LLLT delivered by light-generating BRET-dots may also be applied to other cellular systems and diseases involving oxidative stress and inflammation, such as diabetes (6). A light-generating nanoparticle-based therapy could potentially create the ability to offer LLLT in an *in vivo* setting to reduce the onset of these other pernicious illnesses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
BRET-Qdot light delivery system to cultured tissues for protective, anti-inflammatory effects and anti-oxidative benefits

Figure 2.

BRET-Qdot with coel-h pretreatment on Aβ-induced superoxide anions production. **(A)** Aβ treatment increased the fluorescent intensity of DHE by 40% as compared to control. The pretreatment of BRET-Qdot (66.7 pM) and coel-h (2.5 μM) suppressed the Aβ-induced increase in superoxide anion production back to that of control. Both the BRET-Qdot and Coel-h alone pretreatment lowered Aβ-induced superoxide anion, but not with a statistical significance. **(B)** Fluorescence images representing each experimental group. Each experiment was repeated three times. ** = $P < 0.05$ as compared to control. ◆◆ = $P < 0.05$ as compared to 5 μM Aβ. Data of each treatment group was normalized by the control for each independent experiment prior to statistical analysis.

Figure 3.

Inflammation induced by A β is attenuated by BRET-Qdot with Coel-h pretreatment. Western blots representing the expression of (A) iNOS and (B) IL-1 β . A β increased iNOS by 50% as compared to control, while the pretreatment of BRET-Qdot and coel-h reduced these levels to 20% below control. Neither the pretreatment of BRET-Qdot or coel-h alone prevent A β -induced iNOS production. Similarly, A β increased IL-1 β by 30% as compared to control which was suppressed by the BRET-Qdot with coel-h pretreatment. Each experiment was repeated three times. ** = $P < 0.05$ and *** = $P < 0.001$ as compared to control. ◆◆ = $P < 0.05$ and ◆◆◆ = $P < 0.001$ as compared to 5 μ M A β . Data of each treatment group was normalized by the control for each independent experiment prior to statistical analysis.