Can light be used to treat obesity and diabetes?

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ABSTRACT

This document is highly theoretical lab method paper. It may be used to perform thermogenesis from several cells in a living organism instead of only brown adipocytes. We perform thermogenesis from white adipocytes. The purpose is to uptake the blood glucose and lipids to produce heat and subsequent weight loss and to diminish blood glucose levels for people who suffering from diabetes and obesity. In fact thermogenesis consumes energy (i.e blood glucose and blood lipids). So we now describe a novel and original lab method based on thermogenin (called thermogenin-like system, UCP1-like system or TLS) that is involved in heat production and glucose/lipids capture by white adipocytes.

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In vitro assays

1 Use a cell line: white adipose cells in DMEM medium.

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Transfect cells by electroporation and with the following DNA constructs:

1) the light-driven
inward H+ pump PoXeR targeted to the inner mitochondrial membrane:

2) firefly luciferase

3) luciferin-regenerating
enzyme (LRE)

4) cysteine racemase

5) thioesterase

3

Measure oxygen consumption, ATP
levels and glucose uptake to estimate the thermogenic capacity of white adipose cells and subsequent the glucose and lipids uptake.

In vivo transfections

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The first step of this model is the in vivo transduction of adipose tissue with adeno-associated viral (AAV) vectors. The following transgenic DNA constructs are used in vivo:

1) the light-driven inward H+ pump PoXeR targeted to the inner mitochondrial membrane:

2) firefly luciferase

3) luciferin-regenerating enzyme (LRE)

4) cysteine racemase

5) thioesterase

5

PoXeR is a natural light-driven inward proton pump found in Parvularcula oceani, a deep-ocean marine bacterium. This pump controls the unusual directionality opposite to normal proton pumps and may lower the proton motive force when expressed in the inner mitochondrial membrane, producing heat similarly to thermogenin. A study shows that PoXeR can be expressed in mouse neural cells and it is functional in these cells. Another study shows that it is possible to construct photoenergetic mitochondria in cultured mammalian cells expressing another light-driven proton pump derived from Haloterrigena turkmenica. Taken together, these results suggest that PoXeR may be a good candidate for the TLS in vivo in mammals.

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The TLS may be used in normal cells to promote uncoupling-induced weight loss without significant
adverse effects. It may be possible to express PoXeR (and other transgenes of the TLS) under the control of a weak promoter to reduce the functionality of the system and subsequent weight loss. It may be also possible to express PoXeR (and other transgenes) under the control of a strong promoter to enhance weight loss without significant side effects. In addition, it is interesting to obtain a spatial control of the system in order to restrict transgenes expression to the adipose tissue. For this reason, we propose to use the following transgenic DNA constructs in vivo:

[Adiponectin promoter]-PoXeR targeted to the inner mitochondrial membrane

[Adiponectin promoter]-firefly luciferase

[Adiponectin promoter]-LRE

[Adiponectin promoter]-cysteine racemase

[Adiponectin promoter]-thioesterase

Injections

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Because the bioluminescence of firefly luciferase decreases over time, it is necessary either to continually bring the substrate (i.e firefly luciferin) by continuous infusion and/or to use a system that regenerates the firefly luciferin from oxyluciferin, the product of the reaction. A study shows that oxyluciferin is enzymatically regenerated into firefly luciferin by LRE in vitro.

This system can be improvable by using cysteine racemase and thioesterase. Finally, it is possible to
concentrate the substrate firefly luciferin in adipose tissue in vivo by using a suitable linker (firefly luciferin prodrug). Then, mice are injected with a firefly luciferin or a firefly luciferin prodrug so that the active firefly luciferin is mainly released in adipose tissue.

8 Now, the TLS needs to be sensitized to the light. In fact, all trans retinal is required for light-induced PoXeR activation in vivo.

All trans retinal may be not naturally present in adipose tissue, thus it is necessary to administer it by continuous infusion, it is not enzymatically regenerated in contrast to firefly luciferin. Because all trans retinal is hydrophobic it can be encapsulated in liposomes to reach the adipose tissue in vivo. Hydrophobic compounds have affinity to the phospholipid bilayer of liposomes.

Finally, few hours after all trans retinal injection, mice are injected with a firefly luciferin or a firefly luciferin prodrug.

Summary

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Firstly perform *in vitro* assays on white adipocytes cells by transfecting them with the previous enzymes. Evaluate oxygen consumption, ATP levels and glucose uptake with specific kits. If the *in vitro* assays are conclusive, continue with transduction of adipose tissue *in vivo* in mice.

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Then, use the previous transgenic DNA constructs for the *in vivo* transduction of adipose tissue with AAV vectors. In fact, a study indicates that AAV-mediated genetic engineering of white and brown adipose tissue is possible in adult mice.

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Then, use a continuous infusion of all trans retinal in mice. Few hours after all trans retinal injection, use a continuous infusion of firefly luciferin or firefly luciferin prodrug.

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Finally, estimate the weight loss, blood glucose levels or measure body temperature of mice using infrared imaging. Indeed, a study shows that it is possible to measure brown adipose tissue thermogenesis using infrared imaging in mice.