News Press Research Release

Title

Shining Light specifically Destroys Microbes: Development of New Antibody-guided Photo-Antimicrobial Targeting Therapy (PAT^2)

Key Points

- Multidrug-resistant bacteria are a worldwide problem and new anti-microbial therapies are needed.
- Antibody therapy offers various advantages due to its specificity but its use in clinical practice is still limited due to its low efficacy.
- In this study, we succeeded in developing a near-infrared photon anti-microbial target therapy by using an antibody to guide near-infrared light to target and specifically destroy *Candida* species. The antibody used was egg yolk immunoglobulin (IgY), which is inexpensive and can be mass-produced.
- This research offers a new modality for the treatment of infectious diseases and is expected to open a new way for applying antibodies in clinical studies.

Summary 1

A research group led by Hirotoshi Yasui (first author), a fourth-year doctoral student in the Department of Respiratory Medicine, Nagoya University Graduate School of Medicine, and Kazuhide Sato (corresponding author), Assistant Professor, S-YLC, Department of Respiratory Medicine, Nagoya University, 907 Project of Advanced Imaging Analysis Center/Medical Engineering Collaboration Unit (Unit B3: Research Unit for Creation of New Fields for Young Scientists), JST Consortium for Human Resource Development in Science and Technology, Graduate School of Advanced Studies, Nagoya University, Associate Professor Yoshiyuki Nakagawa, Assistant Professor Tomohiro Akashi, and the research group led by Dr. Koji Umeda, Dr. Shofiqur Rahman, and Dr. Van Sa Nguyen of EW Nutrition Japan Co. has successfully developed a near-infrared photo-antimicrobial targeting therapy (PAT^2) for *Candida albicans*[*1] as a preclinical study.

Antibiotic resistance is a worldwide problem and there is a need to develop new anti-microbial therapies. Antibody therapy is attractive due to its specificity but expensive and its therapeutic effect is limited in many cases. Egg yolk immunoglobulin (IgY), obtained from immunized avian eggs, can be produced at relatively low cost in large quantities. However, like other polyclonal antibodies, the anti-microbial effect of IgY is limited if used alone and there is a need to ingest large amounts for a long period of time. To overcome this drawback, we developed a new near-infrared light photoimmunotherapy (NIR-PIT) method using IgY antibody as a guide for the light to target and kill only pathogens while leaving tissues unharmed.

NIR-PIT is a new method of cancer treatment first reported by Dr. Hisataka Kobayashi and his colleagues at the National Cancer Center (NCI/NIH) in 2011. A complex consisting of an antibody specifically recognizing proteins expressed by cancer cells and a photosensitive substance IRDye700Dx (IR700)[*2] is synthesized, and when the complex is bound to the target proteins on the cell surface and exposed to near-infrared light around 690 nm, it causes damages to the target cells.

In this study, we applied NIR-PIT to the treatment of infectious diseases and developed a PAT[2] that selectively destroys target pathogens. The use of IgY antibody, which is available in unlimited amount at a relatively low cost, makes it possible to apply this method to treat infectious diseases, which often require a larger amount of antibodies than cancer treatment.

This research was supported by JST Grant-in-Aid for Human Resource Development in Science and Technology, " Program for Developing Next-generation Researchers (Japan Science and Technology Agency)", the program for promoting the enhancement of research universities, Toukai Foundation for Technology, GSK Japan Research Foundation, etc. and was published in the scientific journal "ADVANCED THERAPEUTICS" on January 5th,2021 published by Wiley-VCH.

In addition, this research won the Uehara Infection/Chemotherapy Research Award from the Japanese Society of Chemotherapy and the Grand Prize at the Fungal Disease Forum 2020 Annual Meeting.

Summary 2

Research Background

Since the discovery of MRSA in 1963, many resistant bacteria have emerged and because of that fewer new anti-microbial agents have been developed in recent years. Therefore the need for new effective anti-microbial therapies has become an urgent issue. Antibody therapy for infectious diseases has been attracting attention again recently due to advances in antibody development technology. However, antibody therapy is expensive and still limited against certain pathogens when used alone.

Egg yolk immunoglobulin (IgY) is an immunoglobulin found in the egg yolk of birds and reptiles that induces immunity in the offspring. It is attracting attention as a new candidate for anti-microbial therapy because it can be stably produced in large quantities by immunizing the parent birds, and its effectiveness against various microorganisms has been reported. However, like other polyclonal antibodies, it needs to be used in large quantities for a long time to see effects. In order to solve this problem, our research group attempted using near-infrared photoimmunotherapy (NIR-PIT) to enhance the anti-microbial effect of IgY antibodies.

NIR-PIT is a new cancer treatment method reported in 2011 by Dr. Hisataka Kobayashi and Co at the National Cancer Center (NCI/NIH). NIR-PIT is a molecularly targeted cancer therapy that utilizes a

conjugate of the light-absorbing phthalocyanine dye IRDye700Dx (IR700), which is activated by near-infrared light irradiation, and a monoclonal antibody. After the antibody-IR700 conjugate reacts with the cancer cell, IR700 is activated by near-infrared light irradiation at 690nm, which destroys the cell membrane of the cancer cells to which the antibody-IR700 conjugate is bound. It is currently being applied clinically and was approved in Japan in September 2020 for the treatment of "unresectable locally advanced or locally recurrent head and neck cancer.

Our group attempted to apply NIR-PIT to the treatment of infectious diseases using IgY, which is inexpensive and available in large quantities, and to develop a near-infrared photo-antimicrobial targeting therapy (PAT^2) that selectively destroys only the target microorganisms.

Research Results

We prepared CA-IgY-IR700 by synthesizing a complex consisting of anti- *Candida albicans* IgY (CA-IgY) and IR700. CA-IgY-IR700 was tested against multiple strains of *C. albicans* and related *Candida* species. Microscopic observation showed that the *Candida* cells were punctured and strongly deformed and destroyed. The number of live fungal cells significantly decreased in the test group. In the infected mouse ulcer model, CA-IgY-IR700 treatment resulted in decreased number of *C. albicans* and lower inflammation scores. More importantly, the ulcer healing process in CA-IgY-IR700 treatment group was similar to that in the non-infected mouse group (Figure 1).

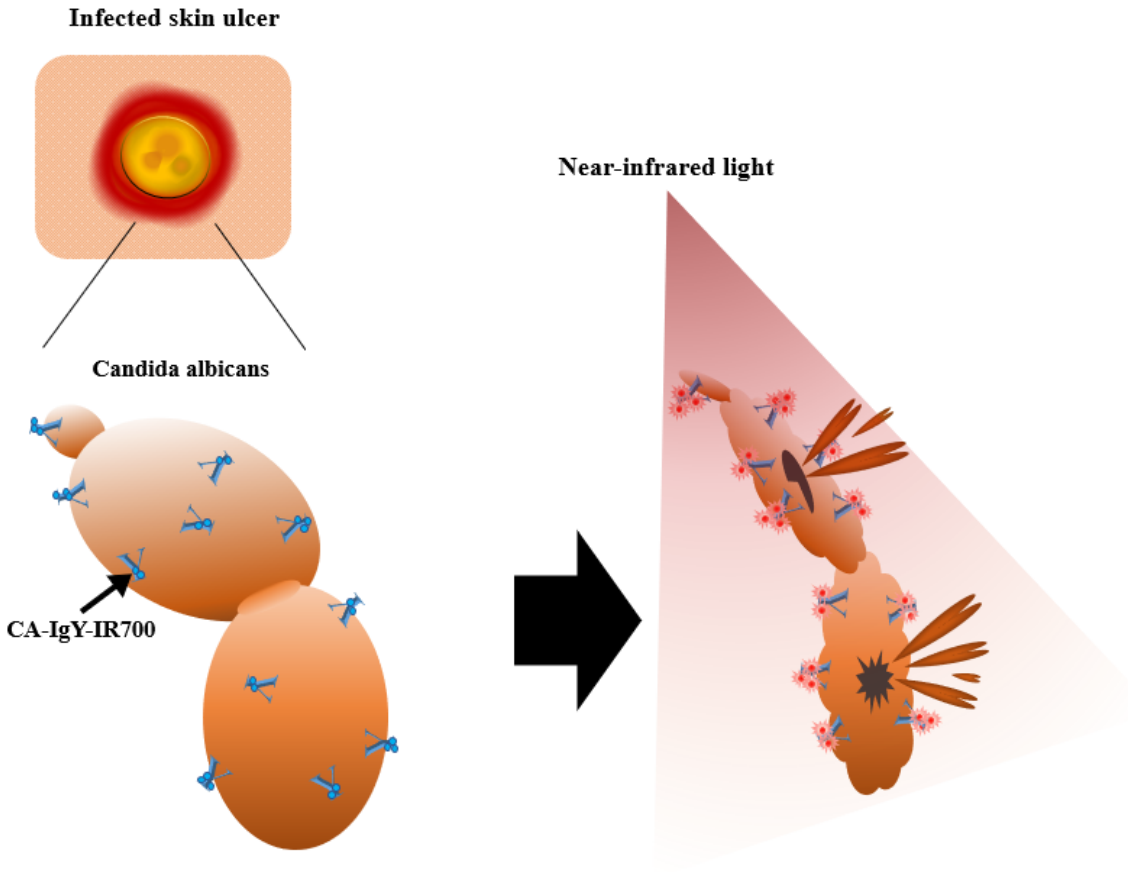

Figure 1. Overview of IgY-photo-antimicrobial targeting therapy (IgY-PAT[2]) for *C. albicans*.

Research Summary and Future Perspective

Near-infrared light anti-microbial targeting therapy using CA-IgY was found to be effective against *C. albicans* both in in-vitro and in animal experiments. This is the first time the concept of near-infrared light PAT[2] has been developed, which is expected to open a new way for applying antibodies in clinical studies and for treatment of infectious diseases especially the ones caused by antibiotic resistant microbes. Further studies on applying near-infrared light PAT[2] against different pathogenic microorganisms and infection models will be conducted in the near future.

*1 *Candida albicans*: A fungus with pathogenic properties. 1 *Candida albicans* is a pathogenic fungus that is commonly found on the surface of the human body, but can cause candidiasis when a person is unwell.

*2 IRDye700DX: A water-soluble photosensitive substance with a silicon phthalocyanine skeleton that absorbs light at wavelengths near 690 nm and emits fluorescence at 700 nm.

Publication

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