Abstract: Since there is no infrared fluorescence materials in the living body, infrared fluorescence labeling materials are very useful for making a diagnosis of a micro cancer. We have developed an infrared fluorescence endoscope (IRFE) and indocyanin green (ICG)-derivative as infrared fluorescence labeling materials to evaluate gastrointestinal neoplastic lesions. The study aims were to apply an IRFE and to demonstrate its usefulness in detecting cancerous tissue using an antibody coupled with ICG-derivative.

IRFE consisted of an infrared endoscope equipped with excitation (710-790nm) and barrier (810-920nm) filters and an intensified CCD camera. We have developed ICG N-hydroxy sulfo succinimide ester (ICG-sulfo-OSu) and 3-ICG-acyl-1, 3-thiazolidine-2-thione (ICG-ATT) as an infrared fluorescent-labeling reagent. ICG-derivative-labeled mouse anti-human carcinoembryonic antigen (CEA) antibody and MUC1 antibody were employed in this study. Moreover, we examined the ability of a reinforcement agent, octylglucoside, to intensity fluorescence from the labeled antibody. Biopsy specimens of gastric cancer were stained with anti-CEA antibody by the avidin-biotinylated peroxidase complex method. Among the positive specimens, freshly resected stomach from three cases were used for the infrared (IR) imaging analysis.

The incubation of freshly resected stomach specimens with ICG-anti-CEA antibody-complex resulted in positive staining of the tumor sites by IRFE, and the IR fluorescent images correlated well with the tumor sites. The immunohistochemical studies suggested that the intensity of IR fluorescence of ICG-ATT-MUC1 was stronger than that of ICG-sulfo-OSu. In tumor sections, the reinforcement agent intensified fluorescence, ever at low antibody concentrations.

Therefore, we conclude that an anti-CEA (and/or MUC1) antibody with affinity for cancerous lesions and labeled with ICG-derivative can be imaged with this IRFE.

Specific antibodies tagged with ICG-derivative with the reinforcement agent can label cancer cells and generate a strong enough fluorescent signal to detect small cancers when examined with an IR fluorescence endoscope. J. Med. Invest. 53 : 1-8, February, 2006

Keywords: infrared fluorescence, infrared fluorescence endoscopy, indocyanin green delivative, endoscopic diagnosis, bioendoscopy
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In vivo

In vivo experiments were performed on healthy adult rabbit brain tissue. The brain tissue samples were excised immediately after sacrificing the animals and then stored in ice-cold saline solution. The tissue was then sliced into small pieces and fixed in 4% paraformaldehyde solution for 24 hours. After fixation, the tissue was embedded in paraffin wax and sectioned into 5-μm-thick slices. The sections were then deparaffinized and rehydrated. Immunohistochemical staining was performed using primary antibodies against the target protein. The sections were then stained with a fluorescence-conjugated secondary antibody and counterstained with DAPI. The images were captured using a fluorescence microscope.
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Z

\[ \text{Chemical Structure} \]

\[ \text{Fluorescence Intensity} \]

\[ \text{Optical Density} \]

\[ \text{Graphs} \]
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Optical density

Wave length (nm)

700 800 900

A B

(64) FILTER

A B

(64) FILTER
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"In vivo" and "in vitro" experiments conducted by our group have revealed some interesting phenomena. As reported by several authors, certain substances exhibit unique properties under both conditions. This suggests the possibility of developing new therapeutic agents through an understanding of these phenomena. The observations made during these experiments provide valuable insights into the mechanisms underlying these phenomena.

In the "in vitro" studies, we observed that the substance X, which has been shown to be effective in treating certain conditions, also exhibited enhanced activity when administered in a specific manner. This finding is consistent with previous reports and further supports the potential therapeutic use of X.

On the other hand, the "in vivo" experiments showed a different pattern. While the substance Y was effective in controlling the disease when administered orally, it failed to produce the desired effects when given by injection. This discrepancy highlights the importance of considering the route of administration in designing therapeutic strategies.

Overall, these studies underscore the complexity of biological systems and the need for a comprehensive approach to drug development. Further research is necessary to fully understand the mechanisms at play and to optimize the therapeutic efficacy of these substances.

References


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