Increased selectivity in inflammatory site identification via labelling of IgG with $N\text{-succinimidyl-4-}^{125}\text{I} \text{iodobenzoate}$

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Summary

Human nonspecific polyclonal IgG was labelled with $^{125}\text{I}$ through direct and indirect labelling methods using chloramine-T and a nonphenolic radioiodinated intermediate $N\text{-succinimidyl-4-}^{125}\text{I} \text{iodobenzoate (}^{125}\text{I-SIB)}$, respectively. Tissue distribution of radioiodinated IgG was assessed in normal and induced inflammation mice. Although, radioiodinated IgG accumulated in the inflammatory area, results showed decreased thyroid and stomach activity and improved inflammatory thigh-to-normal tissue ratios with the indirect labelling method ($^{125}\text{I-IB-IgG)}$ compared with the direct labelling method ($^{125}\text{I-IgG)}$, indicating reduced in vivo deiodination. These results indicate that the $^{125}\text{I-SIB}$ is probably a preferable approach for labelling antibodies with iodine radioisotopes. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: $N\text{-succinimidyl-4-iodobenzoate;}$ turpentine; in vivo deiodination; human nonspecific polyclonal IgG

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Introduction

Radioiodinated monoclonal antibodies through direct and indirect labelling methods have been used for imaging, therapy, and research purposes. The major problem in labelling methods is in vivo deiodination of labelled IgG leading to increased thyroid and stomach activity. Direct labeling methods involve mild oxidation of iodine at slightly alkaline pH, using oxidizing agents such as chloramine-T, in which $^{125}$I covalently attaches ortho to hydroxyl group of tyrosine residues in protein. Exposure of some antibodies to the oxidizing and reducing agents used in this method may disrupt structural integrity, and diminish the immunoreactivity of the antibody. Furthermore, following intravenous administration of radioiodinated IgG, in vivo deiodination occurs due to structural similarity of the iodotyrosines and thyroid hormones. Deiodination increases activity in normal tissues such as thyroid and stomach, and hence reduces the target tissue to background tissue ratio. Indirect labelling methods involves radioiodination of a secondary molecule, typically, an active ester, which then reacts with N-terminal and lysine amino groups of antibodies. Secondary molecules with meta or para substitution of radioiodine, with diminished similarity to thyroid hormones, are believed to be suitable reagents for iodination of antibodies.

We have already reported synthesizing of $^{125}$I-SIB with substitution of the radioiodine in the para position of the aromatic ring. Due to accumulation of human nonspecific polyclonal IgG in infectious and inflammatory sites, human nonspecific polyclonal IgG was used as an antibody model, and inflammation was induced in Balb/c mice as a target model. IgG was radioiodinated through direct and indirect labelling methods with $^{125}$I. Normal and inflammation bearing mice were used to evaluate the tissue distribution of radioiodinated IgG [$^{125}$I-IB-IgG and $^{125}$I-IgG].

Results and discussion

Radioiodination of antibodies through direct methods is an efficient and widely used procedure for direct substitution of iodine into the tyrosyl residues of proteins. Disadvantages that are associated with this method are protein damage, reduction of immunoreactivity, and in vivo
deiodination.5 Deiodination occurs via deiodinases, enzymes that may not distinguish radioiodinated tyrosines from thyroxin. Attaching iodine to proteins in a manner that minimizes this similarity is believed to decrease the extent of in vivo deiodination.6 In the indirect labelling methods, protein is not directly exposed to the radioiodine solution or to the agents used in the iodination reaction.7,8 IgG reacts under mild conditions with the secondary molecule that has previously been labelled with radioiodine and purified.

The purpose of this study was to develop a method for radioiodination of antibodies with decreased in vivo deiodination. The 125I-SIB, synthesized in 80% radiochemical yield via our previously reported method,3 was used to decrease structural similarity to thyroid hormones. SIB reagent was radioiodinated with 125I, which is a cheap, widely available, and easily detectable radionuclide, as the tracer to follow the distribution of the antibody in mice.9 To evaluate the efficiency of 125I-SIB in labelling of antibodies, IgG was also radioiodinated via the direct method using Chloramine-T as oxidizing agent. Normal and induced inflammation mice received IgG labelled with 125I using the 125I-SIB reagent and the chloramine-T method.

Results of the tissue distribution in normal and induced inflammation mice at 4, 24, and 48 h post injection of radioiodinated IgG are shown in Figures 1 and 2. 125I-IB-IgG resulted in a significant decrease in the thyroid uptake. Radioactivity was also decreased in stomach and intestine. As shown in Figure 2, both 125I-IB-IgG and 125I-IgG accumulated in inflammatory left thigh. There was significant difference between the radioactivity of left and right thigh after injection of both 125I-IB-IgG and 125I-IgG. The inflammatory thigh-to-normal tissue ratios was higher following injection of 125I-IB-IgG compared with 125I-IgG. Differences were most striking for thyroid and stomach. The best inflammatory thigh-to-normal tissue ratios was obtained at 24 h post administration of labelled IgG. Based on results, radioiodination of antibodies via 125I-SIB decreased thyroid and stomach uptake, suggesting a lower rate of deiodination compared to IgG labelled using chloramine-T. Increased inflammatory thigh uptake with improved inflammatory thigh-to-normal tissue ratios was also noted in indirect method. In summary, these results suggest that the 125I-SIB reagent is an alternative method to direct labelling methods of antibodies.
Figure 1. Histograms showing tissue distribution at 4, 24, and 48 h post injection of $^{125}$I-IgG and $^{125}$I-IB-IgG in normal mice. Tissue distribution was expressed as percent of injected dose per gram (% ID/g).
Figure 2. Histograms showing tissue distribution at 4, 24, and 48 h post injection of $^{125}$I-IgG and $^{125}$I-IB-IgG in induced inflammation mice. Tissue distribution was expressed as percent of injected dose per gram (% ID/g).

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Experimental

$^{125}$I as Na$^{125}$I with the radioactivity concentration of 100 $\mu$Ci/µl in NaOH was purchased from Amersham. IgG was prepared from human plasma. Radioactive samples were measured using a NaI gamma counter.

Radioiodination of IgG

1. Direct labelling method: IgG was iodinated by chloramine-T method. Briefly, 100 $\mu$g of IgG was incubated with 2 mCi of Na$^{125}$I and 25 $\mu$g of chloramine-T for 1 min. Free radioactive iodine was separated by Sephadex G-50 column chromatography. The specific activity of $^{125}$I-IgG was estimated to be between 5 and 10 $\mu$Ci/µg.

2. Indirect labelling method using $^{125}$I-SIB: Compound $^{125}$I-SIB was synthesized according to our previously reported method. IgG (100 $\mu$g) in 200 $\mu$l of 0.1 M borate buffer, pH 8.5, was added to the dried iodinated ester and the reaction mixture agitated for 30 min at 0°C. The reaction was terminated by addition 0.2 M glycine in 0.1 M borate buffer, pH 8.5, for 5 min at 0°C. The $^{125}$I-IB-IgG was separated from the other products of the conjugation reaction by sephadex G-50 column chromatography. The specific activity of $^{125}$I-IB-IgG was estimated to be 10–15 $\mu$Ci/µg (Scheme 1).

Animal studies

An animal model was developed injecting 40 $\mu$l of turpentine in the posterior left thigh of Balb/c mice weighing approximately 25 g. Mice
were left for 48 h in normal condition to develop the inflammation foci. 100 µCi/0.1 ml/20 µg radioiodinated IgG/mouse was injected through tail vein into mice. Six mice from each group were killed with ether for determination of tissue radioactivity at times 4, 24, and 48 h post-administration of radiolabels. Selected organs (blood, spleen, liver, stomach, intestine, kidney, left thigh, right thigh, and thyroid) were removed and placed into pre-weighed tubes and radioactivity was measured. The percent of injected dose per gram tissue (% ID/g tissue) was determined. The total injected dose was calculated by measuring the radioactivity of syringes before and after injection to each animal.

Statistical significance

All values were expressed as mean ± standard deviation (Mean ± SD) and data were compared using student’s t-test. Statistical significant was defined as $P < 0.05$.

Conclusion

It is clear that radioiodinated monoclonal antibodies are becoming valuable resources in diagnosis, therapy, and research of human diseases. Therefore, development of new chemical methods to increase stability towards deiodination and decrease accumulation of radioiodine in thyroid and stomach are desirable. The indirect labelling method described has proved a valuable approach to improving the quality of $^{125}$I-labelled IgG for such applications.

References