EVALUATION OF N-(P-[^{125}I]IODOPHENYL)MALEIMIDE FOR LABELING MONOCLONAL ANTIBODIES P. C. Srivastava, F. F. Knapp, Jr., J. F. Allred and †D. J. Buchsbaum
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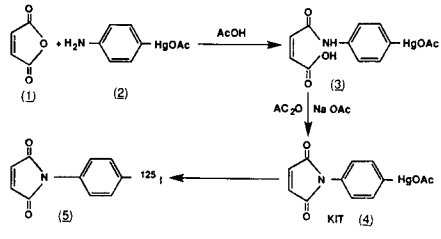
Radiolabeling of monoclonal antibodies (MoAb) with iodine-123 and iodine-131 is attractive for diagnosis and radioimmunotherapy of cancer. Some of the number of radioiodination procedures reviewed recently (1) include oxidative (2) and Bolton Hunter (3) techniques for radiolabeling antibodies. These techniques involving direct exposure of the antibody with the oxidizing agent and other harsh conditions allow the labeling of tyrosine phenolic groups or amine functional groups of the protein. The major disadvantages of these procedures include the possibility of denaturation resulting in low yields and in vivo deiodination resulting in decreased tissue half-life of the radiolabeled MoAb. The rate of deiodination of radioiodinated proteins often depends upon the method of iodination (4). The investigations for labeling MoAbs to inhibit in vivo deiodination have been pursued in our laboratory.

The N-substituted maleimides have natural affinity to react with protein-thiols forming a thoether linkage under mild conditions (5). The opportunity for site-specific protein radioiodination led us to design and synthesize N-(p-[^{125}I]iodophenyl)maleimide (IPM, 5) in which iodine is stabilized on the phenyl ring attached to the maleimide moiety with retention of thiol-binding reactivity (6,7). IPM has been prepared via Na[^{125}I] or [^{125}I]ICl iodination of the mercury acetate kit (4) as shown in Scheme I.

For a comparative study, MoAb specific to melanoma and colon tumors was radiolabeled by the ICl method (8) or by conjugation of IPM by incubating MoAb with IPM for 30 min at 37°C. The efficiency of incorporation of ^{125}I in the MoAb by ICl or IPM procedures was 19% (Sp. act. 0.14 mCi/mg) and 43% (sp. act. 5 μCi/mg), respectively. In vivo tissue distribution following i.p. administration of ^{125}I-labeled MoAb (ICl) or ^{125}I-labeled MoAb (IPM) preparations was conducted in nude mice (3 animals/group) implanted with colon carcinoma. The tumor uptake of radiolabeled MoAb preparations at various times after injection is shown in Fig. 1. The ICl preparation showed a maximum tumor uptake of 3.6% at 12 h after injection, which declined to 2.7% at 6 days. The IPM preparation showed a maximum tumor uptake of 1.5% at 12 h after injection, which declined to 0.5% at 6 days. However, the ICl preparation showed substantially greater uptake in the thyroid with values of 2.07% i.d./g at 6 h after injection reaching a maximum of 4.25% i.d./g after 6 days. In contrast, the IPM preparation showed a constant level of uptake in thyroid of 0.13- 0.17% i.d./g (Fig. 2).



Scheme I

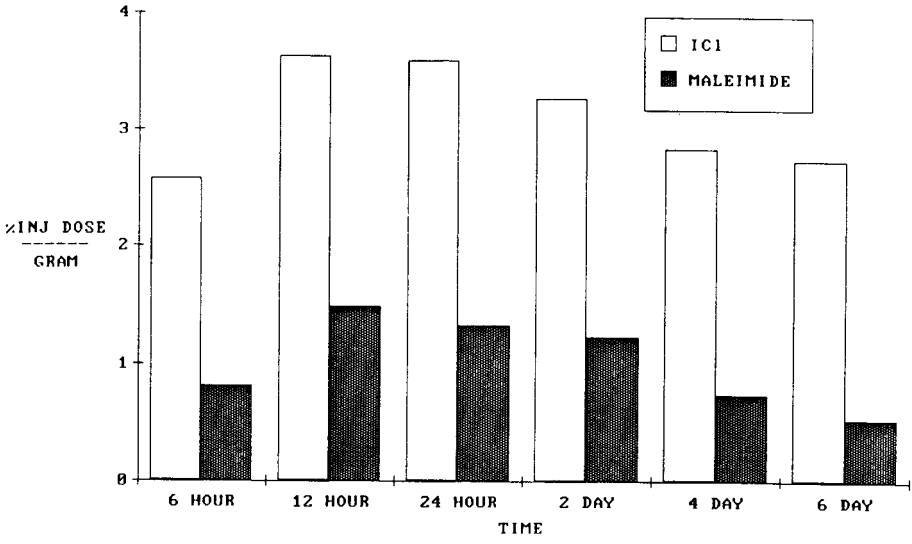


Fig. 1. Tumor uptake of radioactivity following intravenous administration of [^{125}I]MoAb labeled by ICl or maleimide (IPM) techniques into nude mice bearing colon tumor.

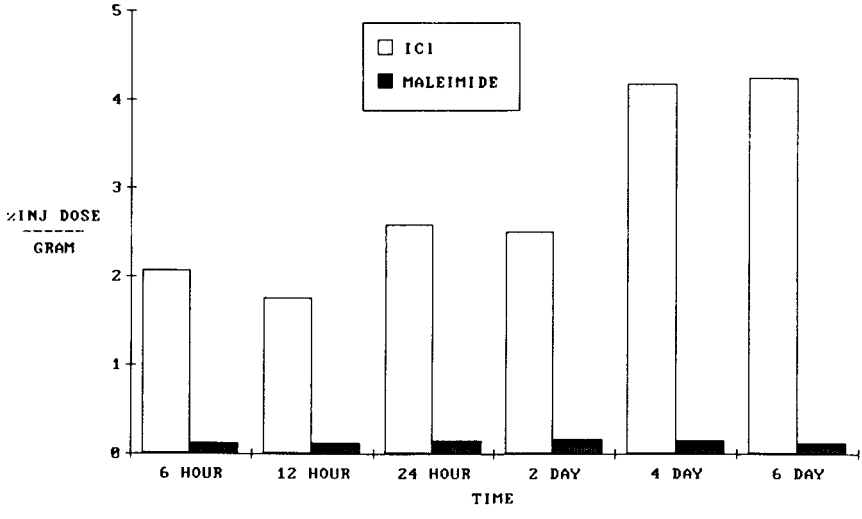


Fig. 2. Thyroid uptake of radioactivity following intravenous administration of [^{125}I]MoAb labeled by ICl or maleimide (IPM) techniques into nude mice bearing colon tumor.

The results of these preliminary studies demonstrate that the IPM labeled MoAb showed markedly less uptake in thyroid indicating insignificant in vivo deiodination than conventionally (ICl) labeled MoAb, while retaining tumor uptake in vivo. Studies are in progress to optimize IPM radioiodination conditions and to improve the tumor localization.

Acknowledgements:

Research supported by the Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

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