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RADIOIODINATED MALEIMIDES AND USE AS AGENTS FOR RADIOLABELING ANTIBODIES

Inventor: Prem C. Srivastava
105 Carson Lane
Oak Ridge, Tennessee 37830
U.S. Citizen

DISCLAIMER

RADIOIODINATED MALEIMIDES AND USE AS AGENTS FOR RADIOLABELING ANTIBODIES

This application is a continuation in part of the previously filed copending patent application SN 043,776. The invention relates to radiopharmaceuticals for labeling antibodies and more particularly to radioiodinated malemides capable of labeling thiol containing proteins and was developed pursuant to a contract with the U.S. Department of Energy.

BACKGROUND OF THE INVENTION

Cancer diagnosis and treatment is one of the most difficult problems facing clinical nuclear medicine today. This is due to the malignancy of some types of tumors, that is, their ability to spread to other parts of the body. By the time a primary tumor can be located it may already have metastasized establishing new colonies of tumor cells throughout the system. It is important to be able to locate these new colonies for it is these metastasis which usually prove to be fatal, not the primary tumor. Certain

antibodies can be found with their corresponding antigens at the tumor site. If these antibodies could be labeled with a gamma emmitting radionuclide it would allow the use of existing imagining instruments and techniques to trace the location of the new colonies.

Radiolabeling of antibodies with iodine-131 and iodine-123 has been attempted in the past using what is referred to as the Chloramine-T procedure or a modification of this method. It is believed that the phenyl group of the tyrosine residues in the protein contain the radiolabel. Drawbacks of this procedure include substrate exposure to chemicals during radioiodination and the possibility of non-specific radiolabeling and denaturation of the protein resulting in low yields of the radiolabeled antibody. Another major drawback is in vivo deiodination of the antibody and entrapment of free radioiodine in the stomach mucosa and decreased tissue half-life of the radiolabeled antibody. Therefore, there is a need to develop techniques that provide specific radiolabeling of proteins with a minimum of in vivo chemical degradation of the radiopharmaceutical.

SUMMARY OF THE INVENTION

In view of the above need, it is therefore an object of this invention to provide a radiopharmaceutical for labeling proteins that does not interfere with the biological activity of the protein.

A further object of this invention is the radiolabeling of antibodies that does not interfer with the biological activity of the antibodies.

Another object of this invention is to provide a radiopharmaceutical for labeling blood proteins that reacts with the thiol groups of the protein.

A further object of this invention is to provide an intermediate for preparing radiopharmaceuticals for blood labeling.

An additional object of this invention is to provide a kit that makes utilization of the radiopharmaceutical easy.

Upon further study of the specification and appended claims, further objects and advantages of this invention will become apparent to those skilled in the arts.

These objects are achieved by providing compounds of a maleimide having a radiohalogenated phenyl directly bound at the maleimide nitrogen site or a radiohalogenated alkylphenyl connected to the maleimide nitrogen by an alkyl chain. A preferred embodiment has an alkyl chain containing up to four carbons. The phenyl ring can be further substituted or unsubstituted.

The radiopharmaceuticals of this invention provide a valuable addition to the field of antibody and protein labeling. The compounds are easy and inexpensive to make and they also exhibit high specificity and are easy to detect using conventional imaging techniques. One particular advantage is that they bind to thiol sites which are ordinarily found in proteins but are not

necessarily required for biological activity thereby allowing the protein to be labeled and studied with its activity unimpaired.

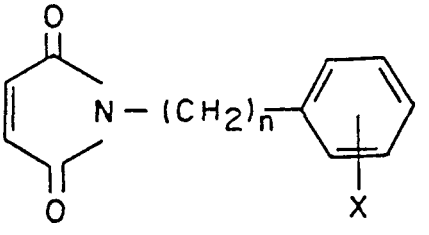
DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Previously protein labeling has been difficult when utilizing radiopharmaceuticals that react with nitrogen, which is often the active site of proteins. The new radiopharmaceuticals of this invention take advantage of the thiol-seeking properties of maleimides, that is, the tendancy to bind at the sulphur site, thereby freeing up the nitrogen site for further reaction. Maleimides have been previously used for studies of insulin in rat antibodies because of there thiol-seeking characteristics.

Another desirable characteristic of the radiopharmaceutical would be a stabilized radiolabel to prevent in vivo chemical decomposition that would remove the radiolabel from the labeling compound. In the past radiohalogens have been studied for protein labeling and methods to stabilize the radiohalogens have been found; specifically, the attachment of a radiohalogen to an unsaturated carbon such as a vinyl group or a phenyl group increases the stability of the halogen on the compound.

Therefore, a radiohalogen, such as iodine, bromine or fluorine, bound to an unsaturated carbon, such as a vinyl or phenyl, attached to a maleimide would be a theoretically suitable compound for labeling blood proteins, since it should attach the protein without disturbing its biological activity and hold the radiolabel on the compound during the process of radioimaging. The compounds

of this invention do precisely that. The radiohalogenated maleimides can be made by mixing in glacial acetic acid maleic anhydride and a radiohalogenated aniline or a phenylalkylamine in which the phenyl ring is radiohaloginated thereby forming a product which is mixed with sodium acetate and acetic anhydride and refluxed in benzene or another appropriate solvent to form the radiohalogenated maleimide. The radiohalogenated aniline or phenylakylamine can be made by persons of ordinary skill in the art. The radiopharmaceutical would have the following structure:



with X being the radiohalogen and, in the preferred embodiment, n being from 0 to 4. The phenyl ring can be further substituted in addition to the radiohalogen substitution.

The maleimide that is the preferred embodiment is labeled with radioiodine since the iodine product is most suitable for single photon emission computerized tomography that detects gamma radiation emmitted by the radioiodine.

A kit for preparing the radioiodinated maleimide is also provided so that the reaction time in radiation decay can be minimized when utilizing the radiopharmaceutical in a therapeutic or institutional setting. The kit provides an intact molecule

having a functional group that is readily substituted by a radioiodine or other radiohalogen to yield the desired product immediately prior to administration. It is a crystalline compound stable at room temperature and suitable for shipment to locations where it is needed for easy radiolabeling in good yield. The kit can also be used for radiolabeling with relatively short lived I-123 and I-131 radioisotopes. Should metallic radioisotopes be desired it would be possible to use other chelating functional groups to be subjected to similar substitution reactions.

Compounds of this invention can be used for antibody labeling for use in warm blooded animals such as mammals including humans. Administration can be by way of formation of the radiolabeled antibody by mixing the radiolabeled maleimide with the antibody and introduction of the antibody into the system in its radioiodinated state. Generally, this would be done by mixing the antibody with the radiohalogenated maleimide in a buffer and a solvent such as DMSO and extracting the unbound radioactive compound with ethyl ether and injecting the radiohalogenated antibody into the system.

If blood is to be labeled, either the above procedure can be used by first labeling a blood sample before injecting it into the body or by administering the radiohalogenated maleimide directly into the system of the mammal since tissue distributions studies in rats indicate preferential binding with the blood and the red blood cells. The actual preferred amounts of the active compound in a

specific case sufficient to provide a distinct image will vary depending on a number of factors and can be determined using conventional considerations by one of ordinary skill in the art.

ABSTRACT

A radiopharmaceutical for antibody labeling comprising an iodophenyl maleimide that attaches thiol sites of proteins thereby maintaining bioreactivity largely located at the nitrogen sites.