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A Comparison of Tc-99m Labeled Radiopharmaceuticals for Imaging of the Central Nervous System

by:

Kara L. Duncan, Pharm.D.

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A COMPARISON OF Tc-99m LABELED RADIONUCLIDEALS FOR IMAGING OF THE CENTRAL NERVOUS SYSTEM

STATEMENT OF OBJECTIVES

The objective of this correspondence lesson is to enhance the reader's knowledge of the history of imaging of the central nervous system (CNS) and to review the progression of CNS imaging and the various Tc-99m labeled compounds which have been used for this purpose. This lesson includes an overview of the anatomy of the brain, a brief review of the history of CNS imaging, and a review of the two classes of Tc-99m labeled compounds which are used in CNS imaging today. A detailed discussion of the two newest agents is also included.

Upon successful completion of this material, the reader should be able to:

1. describe the characteristics and function of the blood-brain barrier.
2. recognize the differences between diffusible and nondiffusible radiotracers used in CNS imaging.
3. identify the drawbacks to the various radiotracers which have historically been used for CNS imaging.
4. cite the characteristics, benefits, and drawbacks of the diffusible radiotracers which are used for CNS imaging.
5. discuss the characteristics, preparation, and uses of the two most recently released agents for CNS imaging.
COURSE OUTLINE

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II. THE CENTRAL NERVOUS SYSTEM (CNS)
   A. Brain anatomy
   B. Blood-brain barrier

III. CNS IMAGING
   A. History
   B. Agents

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   A. Tc-99m sodium pertechnetate
   B. Tc-99m pentetate (DTPA)
   C. Tc-99m gluceptate (GH)

V. DIFFUSIBLE AGENTS
   A. I-123 iofetamine (IMP)
   B. Tc-99m exametazime (HMPAO)
   C. Tc-99m bicisate (ECD)

VI. TC-99m EXAMETAZIME (HMPAO)
   A. Chemistry
   B. Preparation
   C. Stabilized Tc-99m exametazime (HMPAO)
   D. Pharmacokinetics
   E. Uses

VII. TC-99m BICISATE (ECD)
   A. Chemistry
   B. Preparation
   C. Pharmacokinetics
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VIII. CONCLUSION

A COMPARISON OF Tc-99m LABELED RADIOPHARMACEUTICALS FOR IMAGING OF THE CENTRAL NERVOUS SYSTEM

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INTRODUCTION

The use of nuclear medicine as a tool for diagnosing pathology of the brain has become a more widely-used technique, thanks, in part, to developments in instrumentation and radiopharmaceuticals. The diversity of uses for the radiolabeled compounds has expanded from simple blood flow agents which circulate only within the vasculature, to cerebral perfusion agents which map regional blood flow within the brain. These advancements have increased the ability of these studies to provide detailed information on a wide variety of diseases of the brain. This article covers the historical basis of central nervous system (CNS) imaging using Tc-99m labeled compounds with a focus on two of the most recent agents developed for cerebral perfusion imaging, Tc-99m exametazime [hexamethylpropyleneamine oxime (HMPAO)] and Tc-99m bicisate [ethyl cysteinate dimer (ECD)].

THE CENTRAL NERVOUS SYSTEM

Brain

The brain is the central component to all human life and is an important factor in many different physiological problems occurring in humans. The brain itself is separated into two symmetric hemispheres, each containing four lobes; frontal, parietal, temporal and occipital. The outer cerebral cortex is composed of a layer of unmyelinated neurons called grey matter. This covers tracts of myelinated neural fibers which are called white matter as well as aggregates of neural cells termed basal ganglia. It is estimated that the human brain contains ten to fourteen billion neurons, the functional units of the brain which deliver messages in
the form of electrical impulses to and from the brain. The brain requires a constant supply of oxygen to maintain survival of the nerve cells. The blood flow to the brain is supplied by two main blood vessels, the carotid and vertebral arteries. The brain receives almost 20% of the total cardiac output of the heart and consumes approximately 20% of the body’s oxygen supply when at a resting state.

The Blood-Brain Barrier

The utility of the newer cerebral perfusion agents is their ability to "look into" the brain, thanks to their physical characteristics, which allow them to cross an intact blood-brain barrier. The blood-brain barrier is basically a phenomenon which involves the structural and functional characteristics of capillaries in the brain. Systemic capillaries allow small molecules to pass from the plasma to the surrounding extracellular fluid through small clefts between the endothelial cells. In the brain, however, there is a lack of these clefts, i.e., there is a "tight junction" between the cells of the capillary endothelium. This phenomenon results in a virtually uninterrupted barrier between the capillaries and the extracellular space. Some compounds enter the brain easily through passive diffusion, whereas others are selectively allowed entrance or effectively excluded from transport (1).

The blood-brain barrier functions to provide a protective mechanism for the brain. Larger sized compounds such as glucose, the brain's primary energy source, and essential amino acids are selectively allowed into the brain only through active transport. The cells must expend energy in order to move these compounds across their membranes and into the brain. The barrier prevents movement of substances such as plasma proteins into the brain which would result in edema since there is no lymphatic supply to maintain osmotic concentrations. Toxic substances are also excluded and rapid concentration changes within the brain are limited. However, if the blood-brain barrier is compromised, whether through trauma or physiologic damage, the brain capillaries revert to the same permeability as capillaries elsewhere in the body and the protective mechanism for the brain is lost (2).

In determining whether or not a radiopharmaceutical will be able to permeate the blood-brain barrier, one must look at the physical properties of the compound in question. Based on the principle of the blood-brain barrier, radiopharmaceuticals are divided into two main categories, nondiffusible and diffusible. Nondiffusible tracers are unable to penetrate the blood-brain barrier and will not localize in brain tissue under normal circumstances. Imaging is done using conventional planar techniques, obtaining both flow studies and static delayed images. Abnormal uptake is noted as a "hot spot" when there is some type of breakdown to this protective barrier. Nondiffusible tracers share the characteristics of being hydrophilic, polar, larger in size and usually bound to proteins in the circulation. In contrast, diffusible tracers are able to cross the intact blood-brain barrier and move into the brain tissues. This provides a relatively good indication of the blood flow to the brain tissue. These agents are imaged using single photon emission computed tomography (SPECT) resulting in improved resolution and more detailed images. Abnormalities in this type of procedure are indicated by "cold spots" or areas of decreased perfusion. The characteristics of these tracers are opposite those of the nondiffusible tracers in that they are hydrophobic, non-polar, generally small (less than 500 daltons) and not bound to any components in the blood stream (3). Table 1 is a listing of both the diffusible and nondiffusible tracers which are currently used in nuclear medicine imaging of the brain.

Table 1. Tc-99m Labeled Compounds for Brain Imaging

<table>
<thead>
<tr>
<th>NONDIFFUSIBLE TRACERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc-99m Sodium pertechnetate</td>
</tr>
<tr>
<td>Tc-99m Diethylenetriaminepentaacetic acid (DTPA)</td>
</tr>
<tr>
<td>Tc-99m Gluceptate (GH)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DIFFUSIBLE TRACERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc-99m Exametazine (HMPAO)</td>
</tr>
<tr>
<td>Tc-99m Bicisate (ECD)</td>
</tr>
</tbody>
</table>

CNS IMAGING

History

The advent of brain imaging dates back to the late 1940s where cerebral tumors were detected using compounds such as P-32 sodium phosphate, I-131 diiodofluorescein and I-131 human serum albumin. At the time, patients were not "imaged" using a camera as they are today. Instead, the radiation was detected by some external source and this information was extrapolated to make a diagnosis. Due to the exclusive beta emissions of P-32, the tumor had to be surgically exposed to detect the emissions since beta emissions are unable to travel long distances through tissue. The iodine-labeled agents could be detected externally using
Geiger-Mueller detectors since they have characteristic gamma rays in addition to beta emissions, however the physical characteristics of I-131 are not ideal for routine use (4). Following these methods, there were no major advances in brain imaging until the advent of the Mo-99/Tc-99m generator system. This brought about widespread availability of a compound with a short half-life (6 hours), ideal imaging energy (140 keV), decay strictly by gamma emissions, and production in sterile form for administration with minimal manipulations. Generator-produced Tc-99m went on to be one of the most widely used agents for many types of imaging procedures including CNS imaging. It has been estimated that, today, approximately 85% of all procedures performed in nuclear medicine departments worldwide utilize Tc-99m labeled tracers (5).

Table 2. Indications for Imaging with Nondiffusible Tracers

<table>
<thead>
<tr>
<th>Indications</th>
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</thead>
<tbody>
<tr>
<td>Brain death</td>
</tr>
<tr>
<td>Inflammatory disease (i.e., Herpes)</td>
</tr>
<tr>
<td>Brain abscess</td>
</tr>
<tr>
<td>Stroke</td>
</tr>
<tr>
<td>Atherosclerotic disease</td>
</tr>
<tr>
<td>Transient ischemic attacks</td>
</tr>
<tr>
<td>Arteriovenous malformations</td>
</tr>
<tr>
<td>Aneurysms</td>
</tr>
<tr>
<td>Tumors</td>
</tr>
<tr>
<td>Trauma</td>
</tr>
</tbody>
</table>

Agents

With the clinical utility of Tc-99m pertechnetate, there also came problems. Long imaging times and interference from normal sites of uptake made imaging time-consuming and images difficult to interpret. This led to the search for new agents which would improve imaging time and provide a clearer image of the brain. With time, other agents were developed for use in imaging other organ systems by combining Tc-99m with a variety of compounds to form water-soluble complexes which localize in different regions of the body. Fortunately, some of the properties of these agents possess have proven to be useful in improving the data obtained in brain imaging. Two of these agents, Tc-99m pentetate [diethylenetriaminepentaacetic acid (DTPA)] and Tc-99m gluepcate [glucoheptonate (GH)] are also utilized as renal imaging agents due to their rapid elimination through the kidneys (6). These agents, like Tc-99m pertechnetate, are nondiffusible tracers which do not penetrate the intact blood-brain barrier. The rapid blood pool clearance due to renal excretion leads to improved target-to-background ratios when imaging. As a result, these agents are more popular for conventional planar imaging. Table 2 lists the diagnoses which can be evaluated using the nondiffusible radiopharmaceuticals.

As the science of cerebral imaging progressed, new agents were developed which, unlike previous agents, were able to penetrate the intact blood-brain barrier and localize in the tissues of the brain. In order to do functional brain imaging, the radiopharmaceutical must be able to cross the blood-brain barrier, distribute in proportion to regional cerebral blood flow (rCBF) and remain in the brain for a sufficient period of time to allow completion of SPECT imaging (7). In addition, the agent should have ideal imaging characteristics such as are found with Tc-99m. Initially, radiopharmaceuticals such as the inert gas Xe-133 and I-123 labeled amines were used for rCBF determination. Xe-133, while able to diffuse into the brain relative to blood flow, clears rapidly from the tissues and has a low photon energy (81 keV) which decreases the accuracy of this tracer at deeper depths of the brain. I-123 labeled amines were the first tracers to be synthesized and have ideal kinetics for brain imaging. Unfortunately, problems with production limit their use. The difficulties in imaging with these nuclides led to the development of Tc-99m labeled compounds for SPECT imaging. The first Tc-99m labeled agent, Tc-99m exametazime was introduced in 1988 and remained the only Tc-99m labeled agent used in SPECT brain imaging until the development and release of Tc-99m bicisate in 1995. Table 3 lists the common indications for this class of brain imaging agents.

Table 3. Indications for Imaging with Diffusible Tracers

<table>
<thead>
<tr>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrovascular disease</td>
</tr>
<tr>
<td>Brain death</td>
</tr>
<tr>
<td>Epilepsy</td>
</tr>
<tr>
<td>Dementia</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
</tr>
<tr>
<td>Closed head injury</td>
</tr>
<tr>
<td>Tumor</td>
</tr>
</tbody>
</table>

NONDIFFUSIBLE AGENTS

Tc-99m sodium pertechnetate

Tc-99m sodium pertechnetate was the first agent used for conventional planar imaging. Its low cost,
availability, ideal energy (140 keV) and short half-life (6 hours) makes this a very useful agent for imaging. However, it is not without some negative aspects.

Following administration of Tc-99m pertechnetate, its utility as a "flow" agent to monitor the movement of blood through the body makes it useful in determining whether or not blood is reaching the brain. Unfortunately, in looking for defects in the blood-brain barrier, one must look for unusual retention in the brain tissues itself. Tc-99m pertechnetate is slow to clear from the blood pool. Due to this, although the radiopharmaceutical may have breached the blood-brain barrier and entered the brain, it is difficult to differentiate from any Tc-99m which is still in the blood circulation. As a result, static imaging must be delayed for three to four hours to have enough clearance from the blood by the body's excretory mechanisms for one to clearly see any abnormal distribution. In addition, Tc-99m pertechnetate has certain distinct areas in the body where it will normally localize. These include the gastrointestinal mucosa, the salivary glands, the thyroid gland and the choroid plexus, a mass of blood vessels which lie in the lateral ventricles of the brain. It is the uptake in the choroid plexus which may interfere with brain imaging. To prevent this, the patient must be predosed with potassium perchlorate (an agent which blocks uptake in the choroid plexus by saturating the Tc-99m pertechnetate binding sites) at a dose of 200 to 400 mg orally approximately 30 minutes prior to the injection of the radiopharmaceutical. As a result of these drawbacks, although Tc-99m pertechnetate has certain distinct areas in the body where it will normally localize.

**Tc-99m pentetate**

As more and more kit formulations were developed for use in imaging other organ systems, their properties which made them useful in other parts of the body often had beneficial results when used in brain imaging. Tc-99m pentetate is one such agent. Tc-99m pentetate is utilized as a renal imaging agent but can be used for brain imaging in lesions with excessive neovascularity or an altered blood-brain barrier. Following intravenous (i.v.) administration, Tc-99m pentetate is rapidly distributed throughout the extracellular space with less than 5% bound to plasma proteins, then rapidly cleared through the kidneys by glomerular filtration. This results in a high target to nontarget ratio in the brain. Clearance of Tc-99m pentetate is rapid with 80% to 85% of the injected dose removed from the plasma within one hour. Approximately 90% is eliminated in the urine within 12 hours of injection. In addition, the rapid clearance from the blood and higher target-to-nontarget ratios allow for imaging to be completed at one to one and one-half hours post injection as compared to three to four hours for Tc-99m pertechnetate. Figure 1 shows the chemical structure of Tc-99m pentetate.

![Figure 1: Tc-99m Diethylenetriaminepentaacetic acid (DTPA)](attachment:image)

**Tc-99m gluceptate**

Like Tc-99m pentetate, Tc-99m gluceptate is a renal imaging agent that also can be used for brain imaging. Its structure is pictured in Figure 2. Following i.v. administration, it is cleared rapidly by the kidneys, with less than 15% of the injected dose in the blood at one hour. Urinary excretion is 40% at one hour, by both glomerular filtration and tubular secretion. Like Tc-99m pentetate, Tc-99m gluceptate concentrates in cerebral lesions in cases where there is breakdown of the blood-brain barrier. Due to the high clearance rate, imaging with Tc-99m gluceptate provides a higher target-to-nontarget ratio and allows for imaging at one hour postinjection. In a study by Rollo, it was shown that Tc-99m gluceptate is taken up by all lesions that normally would accumulate Tc-99m pertechnetate or Tc-99m pentetate and also localizes in some lesions which do not concentrate the other two agents (8). Like Tc-99m pentetate, imaging with Tc-99m gluceptate does not require predosing with perchlorate to block salivary and choroid plexus activity.

![Figure 2: Calcium gluceptate](attachment:image)
DIFFUSIBLE AGENTS

Cerebral blood flow can be measured quantitatively by studying the clearance of the inert gas Xe-133 which is rapidly removed from the brain. This requires very sensitive instrumentation which can measure the rapid clearance rate. The use of other radiotracers with slower clearance from the brain allows for an estimate of rCBF. These tracers do not completely satisfy the requirements for computing rCBF but they mimic rCBF closely enough to have clinical use. In addition, when imaging the CNS using SPECT, exact quantification of the rCBF is generally not needed. The clinical utility of nuclear brain imaging is reflected in the variations in tracer uptake and retention which can be shown visually on the computed images which are obtained.

I-123 Iofetamine

The first diffusible brain perfusion tracer was a synthesized I-123 labeled amine, namely I-123 iofetamine (isopropyl iodoamphetamine (IMP)). Amines are chemicals which have a variety of roles in brain function. It is thought that certain neurologic disorders are associated with some degree of alteration in amine kinetics or function (2). The kinetics of this compound are the most ideal for cerebral imaging and remain so today. The problem with this agent was found in its production. I-123 is a cyclotron-produced agent with a 159 keV photon and has a 13 hour half-life. Until recently, I-123 iofetamine was available directly from the manufacturer as a pretagged, ready-to-administer compound. The commercially-available I-123 may also contain the contaminant I-124 as a result of the specific method of production. This contaminant has a much longer half-life (100 hrs) and higher energy photons (603 keV to 3.0 MeV) which may distort the images acquired by interfering with the characteristic 159 keV photopeak of I-123. In addition, the higher photon energy results in a higher radiation exposure to the patient which limits the dose which can be given. The cost involved with a cyclotron-produced agent and difficulty in obtaining the agent due to production problems also limit its use. Since this agent had to be specially ordered direct from the manufacturer, its use in an emergency situation was severely restricted (2,7). As a result, with the development of Tc-99m labeled agents, I-123 labeled amines are no longer commercially available for brain imaging.

Tc-99m EXAMETAZIME

Chemistry

Tc-99m labeled exametazime was the first of a new generation of brain imaging agents for SPECT imaging. Released commercially in December 1988, Tc-99m exametazime is a neutral, lipid-soluble derivative of an early Tc-99m complex named propyleneamine oxime (PnAO). Tc-99m PnAO was found to have poor
retention in the brain, so synthesis of a wide variety of derivatives was undertaken to find a ligand which had a sufficient retention time to allow for SPECT imaging. Tc-99m exametazime proved to have these qualities as well as the ideal imaging characteristics of Tc-99m. The structure of Tc-99m exametazime is shown in Figure 3.

![Figure 3: Exametazime](image)

**Preparation**

Tc-99m exametazime is easily prepared from a lyophilized reagent kit by simply adding Tc-99m pertechnetate directly to the vial. It does not require any unusual manipulations when compared to other Tc-99m labeled kits. The main limitations with preparation of Tc-99m exametazime concern the Tc-99m eluate which is used. Tc-99m exametazime can exist in two chemical forms, a primary lipophilic compound and a secondary compound which is less lipophilic and therefore has suboptimal uptake and shorter retention in the brain. Under normal circumstances, Tc-99m exametazime has limited stability in aqueous solutions (3,9,10). Following first order kinetics, the primary complex will slowly convert to the secondary complex and free pertechnetate at a rate of approximately 12% per hour, which limits the time between preparation and administration to patients (11). There are many factors which may influence the conversion from the primary to secondary form. The pH of the product following reconstitution is ≥ 9, and it has been shown that the rate of decomposition increases when the pH reaches this level. When the pH falls into the 4 to 8 range, there is no significant detrimental effect on Tc-99m exametazime stability (11). Excess tin in the formulation increases the rate of conversion to the less lipophilic species suggesting that decomposition is accelerated by redox reactions (9).

Ideally, when preparing Tc-99m exametazime, steps should be taken to minimize the formation of the secondary complex. To help with this, certain restrictions on the generator eluate of Tc-99m should be followed. The manufacturer recommends that elutions must come from a generator which has been eluted within the previous 24 hours. In addition, the eluate must be used within two hours. During generator ingrowth and eluate aging, a large portion of the Tc-99m which had built up will have decayed to Tc-99 which competes with Tc-99m in the redox reaction required for chelation to occur. Due to the fact that there is very little tin present in the commercially available kit, excessive amounts of Tc-99 will lead to reduction of the Tc-99 instead of Tc-99m (12). Piera et al. have shown that radiochemical purity is dependent on the specific activity of the Tc-99m which is used. Elutions from a generator which had not been eluted in the previous 24 hours were thought to have a low-specific activity and generally produced low radiochemical purity results. When labeling was performed with Tc-99m from a generator which had been eluted one to four hours previously, radiochemical purity was consistently greater than 90% (13). According to package insert instructions, a maximum of 30 mCi of Tc-99m in a volume of approximately 5 ml should be added to the kit (3,12,14). However, studies from Ballinger et al. (10) and Piera et al. (13) demonstrated that the use of high specific activity Tc-99m permits the use of at least 3000 MBq (81 mCi) to label a single exametazime kit without adverse effects on radiochemical purity and image quality.

Following reconstitution and patient dose preparation, the dose must be administered within 30 minutes to avoid excessive conversion to the secondary, less lipophilic species. In this time, quality control of the product should be done prior to administration to the patient. The quality control method for Tc-99m exametazime is a somewhat complex 3-strip miniature thin layer chromatography system in which the migration of the primary lipophilic Tc-99m exametazime complex, the secondary (less lipophilic) component, free pertechnetate (TcO$_4^-$), and hydrolyzed reduced (HR) technetium can be determined. Table 4 shows the various components required to perform quality control of Tc-99m exametazime. TcO$_4^-$ is localized at the solvent front in the ITLC-SG / 0.9% saline system while the product and other impurities remain at the origin. HR determination is made using Whatman 31ET strips with 50% acetonitrile, with the HR portion isolated at the origin of the strip while the other substances migrate to the solvent front. The third strip uses ITLC-SG with methyl ethyl ketone as a solvent. The bound primary complex is found along with free pertechnetate at the solvent front. The radiochemical purity of Tc-99m exametazime in the
Table 4
Quality Control of Tc-99m Labeled Diffusible Tracers

<table>
<thead>
<tr>
<th>SUPPORT</th>
<th>SOLVENT</th>
<th>ORIGIN</th>
<th>SOLVENT FRONT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITLC-SG</td>
<td>MEK</td>
<td>2º and HR</td>
<td>1º and free TcO4</td>
</tr>
<tr>
<td>ITLC-SG</td>
<td>0.9% NaCl</td>
<td>1º, 2º and HR</td>
<td>free TcO4</td>
</tr>
<tr>
<td>Whatman</td>
<td>Acetonitrile</td>
<td>HR</td>
<td>1º, 2º and free TcO4</td>
</tr>
</tbody>
</table>

1º= primary lipophilic compound
2º= secondary, less lipophilic compound

Tc-99m BICISATE

<table>
<thead>
<tr>
<th>SUPPORT</th>
<th>SOLVENT</th>
<th>ORIGIN</th>
<th>SOLVENT FRONT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baker Silica Gel</td>
<td>Ethyl acetate</td>
<td>Te EDTA</td>
<td>Tc-99m bicisate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>free TcO4</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>HR</td>
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<td></td>
<td></td>
<td>Te ECM</td>
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<td></td>
<td></td>
<td>Te-(IV) ECD</td>
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lipophilic form is calculated using a combination of the results of these three systems. The radiochemical purity results should be 80% or greater of the primary lipophilic component (11,15,16). The tedious nature of this quality control method has led to the search for a simplified, one strip method for determining radiochemical purity. The development of a procedure using Whatman 17 paper with an ethyl acetate solvent has reduced the time to determine quality control results from up to 15 minutes to less than one minute (11,15,16,17). A single drop of Tc-99m exametazime is spotted and allowed to migrate. The strip is cut 2.5 cm from the bottom of the strip. The primary lipophilic compound will migrate to the solvent front while all other impurities will remain at the origin. The two sections are counted; the net activity of the top portion of the strip is divided by the total activity of both portions to give the total percentage of the primary lipophilic component (15,17).

Stabilizing Tc-99m exametazime formulation

In 1995, a new variant of Tc-99m exametazime was released. The original lyophylized form of exametazime was not changed, however, a separate stabilizer was included with the kit as a post reconstitution additive. Upon chemical analysis, it was found that three major factors contributed to the instability of the primary lipophilic compound; (1) a high pH in the range of 9 to 9.8 following reconstitution, (2) the formation of radiolytic intermediates such as hydroxy radicals and (3) an excess of stannous ions which act to reduce the Tc-99m from +7 to +5 for binding. To counteract this, it was determined that a phosphate buffer and methylene blue combination would limit the conversion to a less lipophilic species. The methylene blue is thought to contribute by (1) acting as a free radical scavenger and (2) causing oxidation of excessive stannous ions. The phosphate buffer lowers the pH of the product to a point just below neutral where the rate of radiolytic decomposition is at its lowest. Although the addition of phosphate buffers enhance decomposition, the combination of the buffer and methylene blue together significantly reduces decomposition (18). As a result, stabilized Tc-99m exametazime can be injected up to four hours post reconstitution. When using the stabilizer, the usual considerations for use of generator eluate still apply, with the exception of the time limit following elution. When stabilizing this agent, use of elutions from generators "milked" within 30 minutes result in the highest yield of the primary complex (11). Since the compound exametazime itself has not been altered in any way, the choice to stabilize the product can be made on case-by-case basis depending on how and when it is to be used.

Pharmacokinetics

Administration of Tc-99m exametazime results in maximum uptake in the brain within one minute of intravenous administration. This is approximately 5%
of the injected dose. Tc-99m exametazime is a "technetium essential" compound in that its lipophilicity allows it to diffuse freely across the blood-brain barrier, but it requires complexation to Tc-99m for accumulation in the brain (3). It is believed that once inside the brain, the compound undergoes in vivo conversion to a hydrophilic product by reacting with glutathione, trapping it in the brain (19). Of the initial activity taken up in the brain, approximately 15% will wash out within two minutes, after which there is minimal loss of activity over the next 24 hours other than that which is lost by physical decay (3). There is no redistribution of the compound between gray and white matter during the first hour after administration (3,20). Approximately 12% of the injected dose remains in the blood pool due to interaction with glutathione present in red blood cells. The blood activity has been determined to contribute only 1.7% of the total counts when imaging at 1 hour post injection, thus having little adverse affect on the resultant images obtained (3). The remainder of the dose is distributed throughout the body, especially in the muscle and soft tissue. Thirty percent of the injected dose localizes in the gastrointestinal tract immediately after injection and half of this is eliminated through the intestinal tract within 48 hours. Up to 40% of the initial dose is excreted through the kidneys over this same time period.

Uses

The package insert states that Tc-99m exametazime is used as an adjunct in the detection of altered regional cerebral perfusion in stroke. Many studies have been performed to examine the benefit of Tc-99m exametazime imaging in patients with a variety of other neurologic diseases as well. Cerebrovascular disease results in damage to the neurons of the brain due to an ischemic event, whether transient or for extended periods. This results in significant changes in blood perfusion resulting in alterations in glucose and oxygen metabolism. These changes can be detected using radionuclide imaging (5). Changes in perfusion can often be detected using Tc-99m exametazime when there is no definitive anatomical abnormality found using conventional methods such as computed tomography (CT) or magnetic resonance imaging (MRI) (21). Tc-99m exametazime imaging produces a positive correlation with ischemic damage under a variety of conditions (5,20,21,22). The use of Tc-99m exametazime in patients with epilepsy has also been studied. There is an increase in activity in the seizure focus during the ictal stage when the patient is actively seizing, and a decrease in activity during the interictal phase. Since it is easier to detect a "hot spot" than a "cold spot," the limitation of Tc-99m exametazime in the study of seizures has been the inability to keep a prepared dose on hand waiting for the patient to seize. It has been shown that a "true ictal" study is difficult to obtain with Tc-99m exametazime; administration is delayed from 5 to 20 minutes while the dose is being prepared (23). Bedside reconstitution when the patient begins to seize has been used with some success. In a study by Newton et al., it was shown that ictal studies were superior in accurately locating the seizure focus when compared to postictal and interictal examinations (23). With the introduction of the stabilized form of Tc-99m exametazime, the limitation for imaging patients with seizure disorder should be eliminated. Clinical experience with Tc-99m exametazime has also been documented in patients with Alzheimer's disease (5,24), a variety of psychiatric disorders (5), brain death, (5,25,26) and trauma (6). Clinical use of the new stabilized version may in time lead to additional indications for the use of Tc-99m exametazime.

Tc-99m BICISATE

Chemistry

Tc-99m bicisate is the second Tc-99m labeled tracer released for SPECT imaging of the brain. Commercially available in 1995, bicisate is a neutral, lipid-soluble member of a class of agents which are derivatives of diamine dithiol. Members of this group have been shown to have high initial cerebral extraction as well as rapid clearance (27,28). The synthesis of bicisate has produced a compound which has excellent brain uptake and is retained in the brain for a sufficient time to allow SPECT imaging. In addition, it has the ideal imaging characteristics of Tc-99m. The structure of Tc-99m bicisate is shown in Figure 4.

![Figure 4: Tc-99m Bicisate](image)

Preparation

Like Tc-99m exametazime, Tc-99m bicisate is prepared from a lyophilized reagent kit which contains the active ingredient as well as stannous chloride for reduction of the Tc-99m which is added in order for complexation to occur (Vial A). In addition, a second vial contains a buffer solution which is also needed in
bicisate is slightly more time-consuming and requires 
more manipulation than Tc-99m exametazime, however, 
it is still a simple procedure. Up to 100 mCi of Tc-99m 
is added to the buffer solution in Vial B in a 
volume of 2 ml. Vial A containing the bicisate is 
reconstituted in 3.0 ml of normal saline, then 1.0 ml of 
this solution is drawn up and added to Vial B within 30 
seconds. This mixture should be allowed to incubate 
for 30 minutes at room temperature. Tc-99m bicisate 
does not have the restraints on the generator elution 
used for reconstitution as does Tc-99m exametazime. 
According to product labeling, Tc-99m bicisate is stable 
for 6 hours after reconstitution (29).

Quality control of Tc-99m bicisate is performed 
using a single Baker silica gel plate and an ethyl acetate 
solvent system. One drop of the compounded product 
is placed at the origin and allowed to dry for 5 to 10 
minutes. The plate is then placed in the developing 
chamber and allowed to develop until the solvent front 
reaches a point 7 cm from the bottom of the plate. This 
will take approximately 15 minutes. The plate is 
allowed to dry and cut at a point 4.5 cm from the 
bottom. The top portion of the plate should contain Tc-99m 
bicisate, while the bottom portion contains the 
radiochemical impurities. There are five possible 
impurities which can be present in a Tc-99m bicisate 
kit. These include free TCO₄⁻, hydrolyzed reduced Tc, 
Tc-99m EDTA (an intermediate in the formation of 
Tc-99m bicisate), Tc-99m ECM (the labeled monoacid 
monoester of ECD which may be present in the original 
vial), and Tc-99m (IV) ECD which is formed when 
there are excess levels of stannous ion in the kit (30). 
This method of quality control does not quantify the 
amount of each impurity which is present. Like Tc-99m 
exametazime, there is a rapid single strip quality 
control method which has been developed to replace the 
recommended method. This procedure reduces the time 
to perform radiochemical purity analysis from 40 to 60 
minutes (when using the traditional method) to one to 
two minutes. The rapid procedure uses the same 
solvent system and support as that used for Tc-99m 
exametazime. A single drop of the final product is 
spotted on Whatman 17 paper and developed in an ethyl 
acetate system. Once the solvent migrates to the top, 
the strip is cut in half and each portion measured. Tc-99m 
bicisate will be present on the top half, while the 
impurities are located near the origin. The percentage 
of Tc-99m bicisate is determined by dividing the 
activity of the top portion of the strip by the total 
activity of the strip. Like the Tc-99m exametazime 
procedure, this method does not quantify the specific 
radiochemical impurities (17,31).

Pharmacokinetics
Following i.v. administration of Tc-99m bicisate, 
this compound enters the brain by passive diffusion and 
reaches its maximum brain uptake within five minutes. 
Approximately 6% of the injected dose is retained in the 
brain (29,32). Once inside the brain, the complex is 
rapidly trapped due to enzymatic metabolism of the 
ilipophilic species to polar metabolites (32,33). Unlike 
Tc-99m exametazime which experiences a rapid 
washout of the original brain uptake, Tc-99m bicisate 
is retained in the brain with little change for at least 20 
minutes after injection (32,34). Initial washout from 
the brain is estimated to be 12% to 14% in the first 
hour compared to 15% washout of Tc-99m 
exametazime in two minutes (3,32,35). Like Tc-99m 
exametazime, there is little redistribution within the 
brain. Blood clearance of Tc-99m bicisate is much 
more rapid than that of Tc-99m exametazime with 
statistically significant differences up to one hour post-
injection (36). This rapid clearance is also noted from 
tissues throughout the body, including the lungs and 
facial structures. This results in a much higher brain-
to-background ratio and, therefore, SPECT images 
which some consider to be of higher quality and "easier 
to read." (22,34,36,37) Tc-99m bicisate is mainly 
excreted by the kidneys with 50% of the injected dose 
excreted in two hours and 74% by 24 hours, both of 
which are significantly higher than Tc-99m 
exametazime. Only 12.5% of the dose is eliminated 
through the gastrointestinal tract (29).

Uses
Since its introduction, Tc-99m bicisate has been 
studied in a wide variety of pathologic conditions which 
affect the brain. Studies have shown that Tc-99m 
bicisate produces good results in normal volunteers with 
no underlying pathology (32,34,36). Currently, Tc-99m 
bicisate is indicated as an adjunct to CT or MRI 
imaging to localize the site of a stroke in patients who 
have already been diagnosed with stroke using these 
other modalities. Matsuda et al. concluded that SPECT 
imaging using Tc-99m bicisate provided excellent 
contrast between background brain structures and the 
area where the stroke had occurred (22). In addition, 
another study in subacute strokes comparing Tc-99m 
bicisate and I-123 IMP has shown that Tc-99m bicisate 
shows high diagnostic accuracy with more intense, 
better delineation of the defects in perfusion (38). 
Holoman found that 97% of patients with clinical 
evidence of chronic stroke which was supported by CT 
findings were also found to have focal abnormalities 
detected during a Tc-99m ECD study (32). Comparisons with I-123 IMP and Tc-99m exametazime
have shown that Tc-99m bicisate produces results equal
to or better than these older agents (39,40). Use of Tc-
99m bicisate in patients with epilepsy has also been
shown to be a promising indication for this agent. As
stated earlier, a seizure focus should show decreased
tracer uptake in the interictal period and increased
uptake during the seizure or ictal phase. Grunwald et
al. demonstrated that Tc-99m bicisate followed this
model, and in their patients the tracer had a lesser
degree of washout from the ictal focus when compared
to the total brain activity, thus further enhancing the
area (41). Also, the increased stability of this agent
allows it to be prepared and kept on hand in order for
it to be “injected” in the patients as they are seizing,
thus creating a true ictal study. Tc-99m bicisate has
also undergone early clinical studies in patients with
Alzheimer’s disease (42) and Parkinson’s disease (43).
As this agent becomes more widely available and
further studies are undertaken, its use in a wider variety
of cerebral pathologies should become evident.

CONCLUSION

The release of the newest Tc-99m labeled diffusible
tracer for brain imaging has brought a second player
into the market which was previously dominated by a
single agent, Tc-99m exametazime. Tc-99m
exametazime has now become the standard to which all
new agents are compared. Tc-99m bicisate has some
characteristics which are superior to those of Tc-99m
exametazime. However, since this agent is new on the
market, comparisons with Tc-99m exametazime are still
being carried out to determine its benefit and place in
the nuclear medicine community. The addition of a
stabilized form of Tc-99m exametazime has also
heightened the competitiveness between the two agents.
The use of Tc-99m bicisate has been compared to Tc-
99m exametazime in a variety of conditions, with early
clinical results which look promising. With increasing
use, the choice of which agent to use in different
situations will become clearer. The addition of this
second agent allows practitioners a greater choice when
diagnosing pathology involving the brain and provides
another agent with which physicians and nuclear
pharmacists must become familiar, in an effort to
provide the greatest service to the nuclear medicine
community.

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QUESTIONS

1. What percentage of the total cardiac output is received directly by the brain?
   a. 10%
   b. 20%
   c. 30%
   d. 50%

2. What is the blood-brain barrier?
   a. A protective reservoir of fluid surrounding the brain which prevents movement of substances into the brain
   b. A physiological phenomenon involving the selective permeability of the capillaries which surround the brain
   c. The outer covering of the brain which is made up of unmyelinated neurons
   d. A collection of blood vessels in the lateral ventricles of the brain

3. What is the function of the blood-brain barrier?
   a. Limit toxic substances from entering the brain
   b. Maintain osmotic concentrations within the brain
   c. Prevent large concentration changes within the brain
   d. All of the above

4. Which of the following is a characteristic of nondiffusible tracers for CNS imaging?
   a. Hydrophobic
   b. Non-polar
   c. Small size
   d. Protein bound in circulation

5. What is the maximum size for compounds which act as diffusible tracers?
   a. 50 daltons
   b. 100 daltons
   c. 500 daltons
   d. 1500 daltons

6. Which is not an indication for radionuclide imaging of the brain?
   a. Alzheimer's type dementia
   b. Mental retardation
   c. Transient ischemic attacks
   d. Trauma

7. Which of the usual sites of Tc-99m pertechnetate uptake interferes with brain perfusion imaging?
   a. intestinal mucosa
   b. salivary glands
   c. thyroid gland
   d. choroid plexus

8. Which nonradioactive agent is given to prevent uptake of Tc-99m pertechnetate in sites which may interfere with brain imaging?
   a. Lugol's solution
   b. Sincalide
   c. Potassium perchlorate
   d. Bethanechol

9. Tc-99m pentoate and Tc-99m gluceptate are also used for what other type of imaging?
   a. Renal imaging
   b. Gastrointestinal imaging
   c. Liver imaging
   d. Lung imaging

10. What percentage of Tc-99m pentoate is removed from the bloodstream at 1 hour post injection?
    a. 5 to 10%
    b. 30 to 40%
    c. 80 to 85%
    d. Greater than 95%

11. Tc-99m gluceptate is ideal for which cerebral lesions?
    a. Any lesion that takes up Tc-99m pertechnetate or Tc-99m pentoate
    b. Lesions which have a decrease in blood flow
    c. Lesions which do not disrupt the blood brain barrier
    d. All of the above

12. Why is Xe-133 not routinely used to measure regional cerebral blood flow?
    a. Measurement requires sensitive equipment to adequately quantify flow
    b. Quantification of CBF is usually not necessary in order to obtain an adequate amount of information
    c. Xe-133 clearance from the brain is very rapid
    d. All of the above

13. Why were 1-123 labeled amines used for brain imaging?
    a. Readily available for use
    b. Low cost
    c. Amines are thought to play a role in many neurologic disorders
    d. Low radiation exposure to the patient
14. What diastereoisomeric form of Tc-99m HMPAO (exametazime) is found in the commercially available preparation?
   a. "d,1" form
   b. "meso" form
   c. a mixture of the "d,1" and "meso" forms
   d. Exametazime does not exist in diastereoisomeric forms

15. What was one of the initial benefits of Tc-99m bicisate over Tc-99m exametazime when first released?
   a. Easier preparation
   b. Extended stability of the final preparation
   c. Greater number of indications for use
   d. More favorable physical characteristics

16. Which of the following is NOT acceptable for preparation of Tc-99m exametazime?
   a. An elution which is less than 2 hours old
   b. An elution which is not the first elution from a newly received generator
   c. An elution which is from a generator which has been milked within the previous 24 hours.
   d. 100 mCi of Tc-99m in a volume of 0.5ml

17. How much time may elapse between reconstitution and patient injection when using the stabilized form of Tc-99m exametazime?
   a. 30 minutes
   b. 1 hour
   c. 4 hours
   d. 6 hours

18. What is the minimum acceptable radiochemical purity for Tc-99m exametazime?
   a. 95%
   b. 92%
   c. 90%
   d. 80%

19. Which of the following is used as a stabilizing agent in the new stabilized form of Tc-99m exametazime?
   a. Ascorbic acid
   b. Methylene blue
   c. Glutathione
   d. Gentisic acid

20. How is Tc-99m exametazime retained in the brain?
   a. Reaction with glutathione to form polar metabolites
   b. Binding to amino acids within brain tissues
   c. Conversion to a hydrophilic form through enzymatic metabolism
   d. Binding to receptor sites on brain tissues

21. Which of the following is true regarding the renal excretion of Tc-99m exametazime?
   a. 30% of the dose excreted immediately following injection
   b. 40% of the injected dose excreted within 4 hours
   c. 50% of the injected dose excreted within 48 hours
   d. 74% of the injected dose excreted within 24 hours

22. What is the contents of Vial B used in the preparation of Tc-99m bicisate?
   a. Buffer solution
   b. Ethyl cysteinate dimer in lyophilized form
   c. Stannous chloride
   d. Methylene blue

23. What percent of the injected dose of Tc-99m bicisate is retained in the brain?
   a. 15%
   b. 10%
   c. 6%
   d. 1%

24. What contributes to the idea that Tc-99m bicisate images are "easier to read" than Tc-99m exametazime images?
   a. Superior affinity of Tc-99m bicisate for brain lesions
   b. Longer retention time of Tc-99m bicisate in the lesion itself
   c. Greater percent the injected dose of Tc-99m bicisate taken up in the lesion
   d. Faster rate of washout of Tc-99m bicisate from tissues other than the brain

25. Which of the following is true regarding the use of diffusible tracers in seizure patients?
   a. There is increased uptake of the tracer in the seizure focus during the ictal phase
   b. There is decreased uptake of the tracer in the seizure focus during interictal phase
   c. It is easier and more accurate to detect a seizure focus when the tracer is injected during the ictal phase as compared to the interictal phase
   d. All of the above