^{99m}Tc denaturated erythrocytes

1. Indications

Labelled ^{99m}Tc denaturated erythrocytes or labelled heat-damaged red blood cells (RBC) are prepared using a radiopharmaceutical kit Ultratag® RBC. ^{99m}Tc denaturated erythrocytes are used for spleen imaging studies.

The use of the Ultratag® RBC kit for labelled ^{99m}Tc denaturated erythrocytes is not registered. However it is described in SNM guidelines and several studies have shown the value of its use.

2. Preparation

The preparation of ^{99m}Tc denaturated erythrocytes could be adapted from the method described in the SmPC of the Ultratag® RBC kit. The steps for labelling the erythrocytes with ^{99m}Tc will need to be followed by a heat-treating step. The Ultratag® RBC kit consists of three separate components: reaction vial containing stannous chloride, syringe I containing a diluted sodium hypochlorite solution and syringe II containing a mixture of citric acid and dextrose. The preparation can be summarized in the following steps:

a. Fill a syringe with anticoagulant (ACD-A).
b. Collect blood using a needle with an inner diameter of 19 G. Needles with a smaller inner diameter will damage the cells. Make sure that the blood is well mixed with the anticoagulant.
c. Bring the anticoagulated blood into the reaction vial and mix gently.
d. Allow to react for 5 min.
e. Add the contents of Syringe I to the reaction vial, mix by gently inverting 4 to 5 times.
f. Add the contents of Syringe II to the reaction vial, mix by gently inverting 4 to 5 times.
g. Add the required activity of ^{99m}Tc pertechnetate to the reaction vial.
h. Mix by gently inverting 4 to 5 times and allow to react for 20 min with occasional mixing.
i. Incubate at 49,5°C for 15-20 min.
j. Draw the needed activity of ^{99m}Tc denaturated erythrocytes for the patient dose.

Special considerations:

- The heat-treating step causes spherocytosis and is critical in the preparation of ^{99m}Tc denaturated erythrocytes. Insufficient damage will result in a distribution in the blood pool with little or no spleen uptake. Excessive damage will cause liver uptake. The heat-treating step should be validated making sure that the right temperature and incubation time is established for the site specific method and apparatus.
- Working with blood can introduce risks to both the operator and the patient. The department labelling the cells should comply with all regulations. Adequate facilities,

equipment, procedures and training for operators should be present. Additional the risks for blood contamination should be recognised and precautionary measures should be implemented to minimise those risks.
- Excessive amounts of ^{99m}Tc, such as in the first eluate of a new generator, in the eluate of a generator with prolonged in-growth time or in an aged eluate can interfere with the labelling of ^{99m}Tc to the erythrocytes. It is therefore recommended to use fresh eluate (no older than 8 h) obtained from a generator which is eluted no longer than 24 h ago.

3. Quality control
- Before the blood cell labelling is started and throughout the procedure a check on the absence of blood clots needs to be performed.
- The labelling efficiency of the ^{99m}Tc denaturated erythrocytes should be determined after the heat-denaturation step. The labelling efficiency is defined as the total radioactivity measured in the cells as a percentage of the total radioactivity measured in both the cells and the plasma. The method is described in the SmPC for ^{99m}Tc erythrocytes and can be used for ^{99m}Tc denaturated erythrocytes as well. A labelling efficiency greater than 90% might be expected. The labelling efficiency depends on several aspects such as: hematocrit, volume of whole blood, stannous ion dose, cell damage, concentration of ligand and radionuclide, temperature and time during heat-denaturation step, operator inter-variability and drugs (see ‘interactions’).

Several other quality control tests have been described. Some of these tests are time consuming. The following tests could be performed periodically or for validation purposes:
- Millipore filtration test: this test the degree of haemolysis is a measure of the loss of reversible deformability (plasticity) of the erythrocytes. A known quantity of labelled denaturated cells is pushed through a 5 μm Millipore® filter. The degree of haemolysis can be determined.
- Mechanical fragility: with this test the mechanical fragility can be determined when damaging the cells by heating. The test is done by treating a erythrocyte sample in three different ways, being: suspension in 0,9% NaCl aqueous solution, haemolysis in distilled water and the heat-denaturation method. The samples are centrifuged and the supernatants are counted. The mechanical fragility can be calculated using the following formula:

$$Mechanical\ Fragility = \frac{[C - A]\ x\ 100}{B - A}$$

- where A = cpm in supernatant of blood placed in 0,9% NaCl aqueous solution, B = cpm in supernatant of blood placed in distilled water and C = cpm in supernatant of blood subject to heat-denaturation.
- Microscopic examination: inspection of the denaturated red blood cells. The biconcave shape of the erythrocytes should be transformed to spherical shaped spherocytes.

4. Interactions, contraindications & adverse reactions

Interactions

Considering the fact that the labelling of the ^{99m}Tc denaturated erythrocytes uses the same method as the in-vitro labelling of ^{99m}Tc erythrocytes, comparable interactions can be expected. The following drugs could interfere with the erythrocyte labelling.

- Aluminium
- Cefalosporines
- Daunorubicin
- Digoxin
- Doxorubicin
- Epirubicin
- Gentamicin
- Heparin
- Hydralazine
- Idarubicin
- Iodinated contrast media
- Methyldopa
- Mitoxantrone
- Prazosin
- Quinine
- Stannous overload
- Teflon material

- It is advised to do not use intravenous catheters or infusion sets made of Teflon material.
- When by accident an overload of stannous is used, a new procedure should be started.
- The labelling of erythrocytes with ^{99m}Tc should not be done within 24 h of the use of iodinated contrast media.
- The labelling of erythrocytes with ^{99m}Tc should be planned 9 days after a chemotherapy treatment with a "-ubicin" drug.
- The labelling of erythrocytes with ^{99m}Tc should be planned just before a chemotherapy treatment with mitoxantron (or 20 days after treatment).

Contraindications

There are no known contraindications.

Adverse reactions

There are no reported adverse reactions. However for ^{99m}Tc erythrocytes hypersensitivity and anaphylactic reactions have been reported. The frequency of adverse reactions is not known.

5. Biodistribution & pharmacokinetics

The heat-denaturating of the ^{99m}Tc erythrocytes creates a mixture of about 70-80% spherocytes and about 20-30% erythrocytes fragments. After injection of ^{99m}Tc denaturated erythrocytes about 60-75% of the activity is eliminated with a half-life of about 4-15 min and is accumulated in the spleen, reaching a plateau by 30 min. The rest of the activity accumulates in the liver and bone marrow.

6. Stability

It has been found that ^{99m}Tc denaturated erythrocytes can lose around 5% of label after a 1 h time period. No other studies are known regarding the stability of ^{99m}Tc denaturated erythrocytes. In practice, longer periods of time between blood being taken from a patient and the cells being re-injected have provided no evidence of problems. However it is recommended to re-inject as soon as possible, preferably within 1-2 h after labelling.

7. Literature

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