

Effect of Radioactivity of Technetium-99m on the Autosterilization Process of non-sterile Tetrofosmin Kits

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ABSTRACT

Technetium-99m labeled radiopharmaceutical is commonly used in nuclear medicines as a diagnostic agent, by mixing the sterile kit with Tc-99m. Manufacturing of kits requires an aseptic facility which need to be well designed and maintained according to cGMP, since mostly kits can not be terminally sterilized. Radiopharmaceuticals as pharmaceuticals containing radionuclide is assumed to have an autosterilization property, but correlation between radioactivity and capability of killing microorganisms has to be studied so far. The aim of this study is to investigate the effect of radioactivity on the autosterilization process of radiopharmaceuticals. The study was carried out by adding Tc-99m of various radioactivity into non-sterile tetrofosmin kits, then the samples were tested for sterility. Sterile tetrofosmin kit and non-sterile kit with no Tc-99m added will be used as a negative control and positive control respectively. The sterility was tested using standard direct inoculation method, by inoculating samples in culture media for both bacteria and fungi and observing qualitatively within 14 days. The results showed that the samples with radioactivity of 1, 3 and 5 mCi changed the clarity of the media to turbid, conformed with the performance of positive controls but samples with radioactivity of 10 mCi and 20 mCi did not change the clarity of the media, conformed with the performance of negative control, indicating neither growth of bacteria nor fungi. It is concluded that Tc-99m behaves as an autosterilizing agent at certain radioactivity. Therefore the preparation of Tc-99m radiopharmaceutical can be considered as terminal sterilization rather than aseptic preparation.

Keywords: Tc-99m, tetrofosmin, autosterilization, radiopharmaceutical, radioactivity.

INTRODUCTION

Irradiation is an established method of sterilization for pharmaceutical products has been widely used for more than 40 years. Radiation sterilization has now become a commonly used method for sterilization of several active ingredients in drugs or drug delivery systems containing these substances without altering their physicochemical properties. It is currently applied to parenteral products that are sterilized by non-terminal sterilization processes. Radiation sterilization can be achieved with gamma rays, electron beams, and X-rays. Radiation sterilization may be performed using either gamma rays from a radioisotope source (usually cobalt-60) or electron-beam or X-ray irradiation, so far, gamma-ray irradiation is more commonly used. Radiation doses of 5 kGy and 25 kGy have been proven effective for electron beam and gamma ray respectively. The mechanism of sterilization is that radiation

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induces damage to the microbial cells through various biological pathways that may lead to cell death. Although DNA generally is considered the major subject of cellular damage, membrane damage also may contribute significantly to reproductive-cell death. One of the mechanism of actions is the effects of irradiation on water that lead to the formation of various radiolysis products of water, mainly are $\cdot\text{H}_2\text{O}$, H_2O_2 , and $\cdot\text{H}_2$. This peroxide and free radical species are known as strong oxidants which can destroy DNA of microbial cells and lead to cells death. Radical species can also modify the structure of peptide molecules [1-5].

The short half-lives of most radiopharmaceuticals used in nuclear medicine studies prohibit completion of the sterility testing before the release of radiopharmaceutical products. In addition, when the half-life of the radionuclide is very short (e.g., less than 20 minutes), administration of the radiopharmaceutical preparation to the patient is generally on-line with a validated production system. It is justifiable to dispense radioactive drug products before completion of the sterility test if the radiopharmaceutical is prepared by a validated aseptic process [6,7].

Radiopharmaceutical kits can not be sterilized terminally whether by heat or other methods because of its specific formulation, so it needs to be processed aseptically. Manufacturing of radiopharmaceutical kits follows cGMP regulation which has to be done in aseptic facility in a Class A room surrounded by Class B room, carried out by operators wearing specific gowning and of course everything has to be sterilized including container, packaging material, equipment and the cleanroom facility. To obtain cGMP conformed facility for aseptic manufacturing process, it needs commitment, high cost, and well-planning in the design, construction, maintainance and process [6,8].

Technetium-99m or Tc-99m as a gamma emitting radionuclide has possibility to act as sterilizing agent for the kit labeled with it, but so far how much radioactivity that give effect to killing microorganisms has not been investigated. A study has to be carried out regarding radioactivity-related effect of autosterilization in non-sterile kits added with Tc-99m. Sterility test according to the Standard can be done using 2 methods, a direct inoculation (immersion) and membrane filtration. Sample is aseptically added into culture media for growing bacteria and fungi, and incubating for at least 14 days in 30-35°C and 20-25°C respectively [9-13].

Tetrofosmin kit was used as a model in this study because this kit is widely used in nuclear medicine in the form of Tc99m tetrofosmin as diagnostic agent using Single Photon Emission Computed Tomography (SPECT) for myocardial perfusion imaging and detection of various cancers such as breast cancer, parathyroid adenomas, glioma and glioblastoma. Radiopharmaceuticals for breast cancer imaging can also reflect specific biological and functional lesion features such as perfusion, metabolic activity and receptor status. As reported, the uptake of Tc99m tetrofosmin into cancer cells is influenced by the expression of P-glycoprotein [14-21].

The direct inoculation method or immersion is also applied in an instrument available in the market. This instrument is a rapid and fully automatic system that has been approved by FDA, in which microbial detection is based on measurements of biochemical or physiological parameters that reflect growth in liquid medium, such as the detection of CO_2 production using colorimetric methods or by measuring changes in the headspace pressure. Another rapid sterility testing has also been developed elsewhere, which employs a combination of direct fluorescent labeling techniques and solid-phase laser scanning cytometry to rapidly enumerate viable microorganisms from aqueous samples. Besides, there are still several new innovation of sterility testing systems which has been validated [22-25].

The aim of this study is to prove that radiopharmaceuticals specifically Tc-99m radiopharmaceuticals can behave as autosterilizing agent at certain radioactivity. Therefore it

can be considered that preparation of Tc-99m radiopharmaceutical is equivalent with the product processed with terminal sterilization. Although kit manufacturing is carried out in an aseptic facility, but actually it is not necessary to treat it as strictly as other aseptically manufactured parenteral products.

EXPERIMENT

Chemicals and instrumentation

Fluid thioglycolate (FTG, Difco) was used as medium for bacteria, Tryptic Soy Broth (TSB, Bacto) was used as medium for fungi and Tryptic Soy Agar (TSA, Difco) was used for monitoring sterile environment in the form of agar plate. Bacteria used in this study was *Staphylococcus aureus* and the fungi used was *Aspergillus niger*. Demineralized water (demin water) was provided by purify tap water using water purification system (Merit). A series of 5 non-sterile tetrofosmin kits (PTRR-BATAN) was prepared, added with Tc-99m of various activity (PTRR-BATAN).

Instrumentation used for measuring radioactivity of Tc-99m was dose calibrator (Atomlab-300), samples for sterility testing was stored in 2 incubators with different temperature setting (Friocell).

Procedure

Preparation of media

An amount of 6.0 g of FTG was placed in an erlenmeyer flask, added with 200 mL of demin water, heated and allowed to dissolve, aliquoted 15 mL each into test tubes, put cotton cap, and autoclaved at 121°C for 20 min. The same amount of TSB was also prepared in the same way as FTG. An amount of 8.0 g of TSA was placed in an erlenmeyer flask, added with 200 mL of demin water, heated and allowed to dissolve, autoclaved at 121°C for 20 min and aseptically dispensed 20 mL each into sterile petri dish.

Prior to use, these media has to undergo growth promotion testing, to determine the suitability of the media used in sterility test and to ensure that the media can support the growth of microorganisms. One single colony of *S. aureus* was added to a test tube containing FTG broth and a test tube containing TSA, and one single colony of *A. niger* was added to a test tube containing TSB. All tubes and plates were incubated in suitable temperature for 5 days, and when turbidity occurred in fluid media or colony formed in agar media within those days, the media were valid and allowed to use. Tubes of FTG media and a half part of TSA plates were stored at 30-35°C while tubes of TSB and a half part of TSA plates were stored at 20-25°C for 5 days prior to use [6,9].

Preparation of work area

Clean room and laminar flow hood for conducting sterility test were cleaned with disinfectants (savlon followed by 70% alcohol), and allowed to stand for 3 h before use. Preparation of samples were started with various radioactivities of Tc-99m pertechnetate *i.e.* 1 mCi, 3 mCi, 5 mCi, 10 mCi and 20 mCi were added to a vial containing non-sterile tetrofosmin kit respectively. The vials were stored in lead-shielded area in a fume hood for 3-4 days to decay the radioactivity in order to minimize exposure of radioactivity onto the operator.

Sterility testing

Sterility test was carried out using direct inoculation method, in which samples and controls were added into several tubes containing fluid thioglycolate broth (FTG) and tryptic soy broth (TSB) respectively [9-13,26]. One mL of each sample was transferred aseptically into a pair of tubes containing TSB and FTG respectively and incubating the tubes at 20-25 °C for TSB and 30-35 °C for FTG for 2 weeks, within those days turbidity or clarity of the samples was observed everyday. Similar treatment was applied to a vial of sterile tetrofosmin kit and a vial of non-sterile tetrofosmin kit which will be used as negative and positive control respectively. When doing sterility test, a tube containing TSB inoculated with fungi, in this experiment *A. niger* was used and a tube containing FTG inoculated with aerob bacteria -*S. aureus*- were prepared and used as control for the whole experiments. The growth of bacteria as well as fungi was indicated when turbidity occurred in the tubes of respective media, whereas the sterile media remain clear. Media which became turbid indicates positive result (+), whereas media which remained clear indicates negative result (-). The experiment was repeated 3 times (n=3).

RESULT AND DISCUSSION

The culture media used in this study has been validated through growth promotion testing (fertility testing), in which turbidity of the media occurred indicating the growth of bacteria and fungi, as shown in Table 1.

Table 1. Growth promotion testing for culture media.

Microorganism	Aerob bacteria (<i>Staphylococcus aureus</i>)	Fungi (<i>Aspergillus niger</i>)
Temperature of incubation	30-35°C	20-25°C
Incubation period	5 days	5 days
Result (growth)	positive	positive

The sterilized autoclave culture media was stored in incubator with proper temperature, i.e 20-25 °C for TSB and TSA, and 30-35° C for FTG and TSA for 6 days, the result showed no turbidity occurred in fluid media, and no colony found in TSA plates (Table 2). Therefore, the media were approved to be used for sterility testing.

Table 2. Sterility observation of culture media before use.

Culture media	Sign of bacterial growth at 30-35°C	Sign of fungal growth at 20-25°C
FTG tubes	negative	n/a
TSB tubes	n/a	negative
TSA plates	negative	negative

Sterile kit which was added to both TSB and FTG media did not change the clarity of the media within 14 days observation, whereas that of non-sterile kit changed both media from clear to turbid one day after, thus both kits can be used as negative control and positive control respectively.

Three replications of non-sterile kits added with various activities of Tc-99m which were inoculated into TSB and FTG. Moreover, these kits were incubated at 20-25°C and 30-35°C respectively for 14 days showed significant differences. Those added with 1 mCi, 3 mCi and

5 mCi caused turbidity whereas those which added with 10 mCi and 20 mCi remained clear as the performance of negative control [26]. It indicated that radioactivity of Tc-99m starting from 10 mCi can sterilize the non-sterile kit, while radioactivity of 5 mCi or less did not affect the non-sterile kits, as can be seen in Table 3 and Figure 1. The turbidity of sample with 1 mCi of Tc-99m was unclear, but only a slight floating layer or fine particles observed, presumably because the volume of sample added into both media was too small.

Table 3. Effect of radioactivity on the autosterilization process of non sterile kits.

Sample tested	Bacterial growth	Fungal growth
Sterile kit (negative control)	-	-
Non-sterile kit (positive control)	+	+
Non-sterile kit + 1 mCi Tc-99m	+	+
Non-sterile kit + 3 mCi Tc-99m	+	+
Non-sterile kit + 5 mCi Tc-99m	+	+
Non-sterile kit + 10 mCi Tc-99m	-	-
Non-sterile kit + 20 mCi Tc-99m	-	-

Note : - : clear, indicated no microbial growth
+ : turbid, indicated microbial growth



Figure 1. The results observed at day 14th, in which tube #1 and #2 (from the left) were negative and positive control respectively, while tube # 3-7 were samples with 1, 3, 5, 10 and 20 mCi Tc-99m respectively

Preparation of Tc-99m labeled radiopharmaceuticals in the hospital usually involves more than 10 mCi of Tc-99m since the minimum injected dose for one patient is around 6-10 mCi. Addition of at least 10 mCi of Tc-99m to the kit can kill the microorganisms, so this phenomenon support the sterilization effort of the manufacturer, which use aseptic method in producing the kits. The drawback of this method is that the beginning of killing process on the microorganisms is not known, since the sterility test can only be done after the radioactivity has declined with regards to the safety of the operator.

Radiation sterilization on various products which is commonly used is based on gamma radiation from external source (Cobalt-60) with certain exposure dose rate and time of exposure. The effect of radiation is also related with distance between radiation source and the products being exposed, and the radiation dose which is commonly applied is 25 kGy [2]. In this study the distance between the radiation source and the product to be sterilized was

negligible since the radionuclide is intact in the molecule of Tc-99m tetrofosmin, therefore the radiation dose (radioactivity) is necessary to sterilize itself was much lower.

CONCLUSION

Tc-99m as a gamma emitting radionuclide at certain radioactivity was able to sterilize radiopharmaceutical products, and act as an autosterilization agent. This fact can be used to justify that the regulation of aseptic manufacturing process of radiopharmaceutical kits should not be as strict as that applied to non-terminally sterilized parenteral medicines.

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