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Autosterilization Effect of Gamma Radiation in Non-sterile Radiopharmaceuticals

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Abstract

Gamma radiation is commonly used in sterilizing various products because of its microbial-killing property. Several radiopharmaceuticals are processed aseptically, which requires a well-managed GMP-based facility. This study aims to ensure that radiopharmaceutical can behave as an auto sterilizing agent since it contains radionuclide that emits gamma radiation. Sm-153-ethylenediaminetetramethylenephosphonate (Sm-153-EDTMP) and I-131-Hippuran were used as models in this study, in which various radioactivities of these products were added into non-sterile vials in the non-aseptic working area and tested for sterility using direct inoculation method. The result showed that samples containing 7 mCi of Sm-153-EDTMP and less than 2 mCi of I-131-orthoiodohippurate (I-131-Hippuran) changed the clarity of the media, but not for those containing higher radioactivity. The results showed that Sm-153-EDTMP and I-131-Hippuran at certain radioactivity can auto sterilize themselves, whereas the positive control sample and the products diluted with non-sterile water remained unsterile. This study showed that radiopharmaceuticals have auto sterilizing properties at relatively low radioactivity, depending on the products' bioburden. Therefore, the usual aseptic preparation of some radiopharmaceuticals can be considered terminal sterilization.

Keywords: Sm-153-EDTMP, I-131-Hippuran, gamma radiation, autosterilization, radiopharmaceutical, radioactivity

1. Introduction

Radioactive sterilization, e.g., gamma rays, electron beams, ultraviolet light, and X-rays, is commonly used in pharmaceutical industries on sterilizing active ingredients in drugs. This method is chosen since the physicochemical properties of active ingredients in drugs remain. Among all methods, gamma irradiation using a radioisotope source (e.g., cobalt-60 and cesium-137) is preferred since it penetrates lower in the object materials. So far, gamma-ray irradiation with a dose of 25 kGy is more commonly used in which one kGy is equivalent to one joule/gram of matter. The sterilization using radiation can damage or induce the rupture of microbial cells that will eventually kill the cell. Besides damaging DNA as the main target, the cell membrane can also be affected. The water irradiation will trigger the formation of water-derived radiolysis products, including H_2O, H_2O_2, and $^{\cdot}H_2$. In turn, strong oxidants formed, e.g., peroxide and free radical species, can damage microbial cells' DNA and lead to cell death [1-6].

Most radiopharmaceuticals used in nuclear medicine studies are short half-lives, allowing their release before sterility testing is completed. Furthermore, a short radionuclide half-life (e.g., under 20 minutes) allows radiopharmaceutical preparation on the patient to be administered online through a validated production system. If the radiopharmaceutical uses a validated aseptic process, the omission of radioactive drug products needs to be justified before completing the sterility test [7].

Because of their specific formulation, radiopharmaceutical kits cannot be terminally sterilized by heat or other methods and should be processed aseptically. Its manufacturing process requires compliance with cGMP regulation, i.e., conducted in a particular facility being aseptic within a Class A room inside Class B room. Operators who carry out the process must wear specific gowning. All equipment involved, such as containers, packaging material, equipment, and clean room, must be sterilized. Obtaining an aseptic manufacturing process that conforms to the cGMP facility is not easy and requires

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commitment, high budget, planning, and maintenance [8-9].

Gamma-emitting radionuclide Technetium-99m or Tc-99m is known to have the sterilizing capability on kits that are prepared to be labeled. A study to prove this characteristic was previously reported in which tetrofosmin kits were used as a model. Other studies also reported that the sterilization effect was dose-dependent [6, 10]. In an experiment using tetrofosmin kits, 10 mCi of Tc-99m was a minimum dose showing the sterilizing effect [10]. A similar study using other radiopharmaceuticals or radionuclides should be conducted to support this conclusion. Sterility tests according to pharmacopeia can be conducted through direct inoculation (immersion) and membrane filtration. The sample needs to be added aseptically in bacterial and fungi culture media to be incubated minimum 14 days at 30-35^{o}C and 20-25^{o}C, respectively [11-14].

FDA has already approved a rapid and automatic instrument for sterility testing that uses a direct inoculation method or immersion. The instrument works on the basis of biochemical or physiological growth parameters measured in a liquid medium. For instance, CO_2 production can be detected using colorimetric methods or measurement of headspace pressure changes. Another instrument also developed to test the sterility system using direct fluorescent labeling techniques and solid-phase laser scanning cytometry to count living microorganisms. Other validated innovations in sterility testing systems are also widely available [15-20].

This study aims to substantiate the argument that radiopharmaceuticals or radiolabeled compounds can behave as auto sterilizing agents at certain radioactivity. Samarium-153 (Sm-153) and iodium-131 (I-131) labeled radiopharmaceuticals in the form of Sm-153 ethylenediaminetetramethylenephosphonate (Sm-153-EDTMP) and I-131 orthoiodohippurate (I-131-Hippuran) were used as models. Although the best sterilizing method for pharmaceutical products is terminal sterilization, not all products can be treated this way, and some need to be processed aseptically. On the other hand, the aseptic process requires a lot of efforts, such as a well-designed facility and high-cost maintenance. By ensuring that gamma emitted radionuclide can act as an auto sterilizing agent, radiopharmaceutical preparation with filtering through a microbial filter instead of autoclaving is sufficient to produce a sterile product.

2. Material and Method

2.1. Materials

Materials used in this study were, i.e., Fluid thioglycolate (FTG, Difco), Tryptic Soy Broth (TSB, Bacto), Tryptic Soy Agar (TSA, Difco), cultures of *Staphylococcus aureus* and *Aspergillus niger*, radiopharmaceutical products (Sm-153-EDTMP and I-131-Hippuran (PTRR-Batan)), 0.22 µm Millipore filter (Millex-GS®), water for injection (Ikapharmindo), water purification system equipment (Merit), dose calibrator (Atomlab-300), glove box (Comecer), fume hood equipped with lead glass, disposable syringes, pipettes, and glasswares.

2.2. Methods

2.2.1.Preparation

We used fluid thioglycolate as bacterial growth medium, Tryptic Soy Broth as fungal medium growth, and Tryptic Soy Agar to monitor a sterile environment. The microorganisms studied were *Staphylococcus aureus* and *Aspergillus niger*. We used demineralized water (demin water) obtained by purifying the tap water through a water purification system.

Sm-153-EDTMP of various radioactivity was added into a series of clean but unsterilized vials without filtration, and a dose calibrator measured the radioactivity. The same treatment was applied to a sample of I-131-orthoiodohippurate (I-131-Hippuran). This preparation of samples was carried out inside a fume-hood equipped with lead glass to protect the operator from radiation exposure.

The same amount of Sm-153-EDTMP and I-131-Hippuran was added into a sterile vial through a 0.22 µm filter. The preparation was carried out inside an isolator (glove box) previously cleaned, sanitized, and stored for several days to decrease the radioactivity. Each filtered Sm-153-EDTMP and I-131-Hippuran were used as negative controls. Filtration of the samples through 0.22 µm porosity filter (Millex-GS®) is the main step in the aseptic process, which means sterilizing the products. A vial containing 2 mL of tap water was used as a positive control.

Various radioactivity of Sm-153-EDTMP, i.e., 7 mCi, 15 mCi, and 30 mCi was added into 3 vials that were previously prepared clean but not sterile. Afterward, the vials were stored in a fume-hood for ~ 2 weeks to decay the radioactivity. Various radioactivity of I-131-Hippuran i.e., 0.5 mCi, 1 mCi, 2 mCi, 5 mCi and 8 mCi was pipetted into 5 cleaned but non-sterile vials. The vials were then put into a lead-shield container and allowed for ~ 2 weeks in a fume-hood. The same radioactivities of Sm-153-EDTMP were also added into 3 vials, then added with 2 mL of non-sterile water, followed by the same treatment as the latter mentioned above.

In an Erlenmeyer flask, 6.0 g of FTG was added with 200 ml of demin water. The mixture was heated to dissolve. 15 ml of the mixture was put into test tubes covered with a cotton cap. The tubes were then sterilized using an autoclave at 121°C for 20 minutes. The same amount of TSB was prepared in a similar manner. Approximately 8.0 g of TSA

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was prepared in an Erlenmeyer flask with 200 ml of demin water. The mixture was heated and dissolved, sterilized using an autoclave at 121°C for 20 min, and 20 ml of it was placed aseptically into a sterile petri dish. Before the solution is utilized, these media must be tested for growth promotion to determine their suitability in sterility testing and ensure their ability to support microbial growth. *S. aureus* was added to test tubes with FTG and TSA. Meanwhile, *A. niger* was only added to test tubes with TSB. Afterward, all tubes and plates were incubated at a suitable temperature for 5 days. The validity of media obtained when turbidity occurred or colonies were formed; thus allowing the media to be used. Tubes containing FTG and a half of TSA plates were stored at 30-35°C and TSB tubes and a half TSA plates were stored at 20-25°C for 5 days before use [3,8,10,11].

Disinfection of the glove box used in sterility testing was done by cleaning it with savlon and 70% alcohol and left for 3 hours prior to use.

We carried out a sterility test using the direct inoculation method. Several tubes were added with samples and controls and FTG and TSB, respectively [11-14]. The sterility test was carried out in an isolator (glove box) which had been previously cleaned and sanitized, and during the test session sterile environment was monitored using TSA agar plates afterward; the environmental monitoring plates were incubated for 20-25°C up to 3 days, observed for microorganisms' growth within the days, alternatively in 30-35°C for 2 days [9].

We aseptically transferred 1 mL of each sample into tubes containing TSB and FTG, respectively. The TSB tubes were incubated at 20-25°C and FTG tubes at 30-35°C for 2 weeks. The turbidity or sample clarity was observed daily. A similar procedure was also done to a vial of sterile Sm-153-EDTMP and I-131-Hippuran as negative controls and a vial containing 1 mL of tap water as a positive control. The sterility test was done in a TSB tube with fungi *A. niger*. A tube containing FTG with bacteria *S. aureus* was also prepared and acted as a reference.

The growth of microorganisms was marked by turbidity in the media tubes, which indicated a positive result. The sterile media remained clear, indicating a negative result. The experiment was done in three replications.

3. Results and discussion

This study used a validated culture media through growth promotion testing (fertility testing) and turbidity test (Table 1). In this validation, culture media that have been added with 10-100 CFU of bacteria and fungi must produce colonies of bacteria and fungi within 5 days [14].

The autoclave-sterilized culture media was stored in a temperature-adjusted incubator, i.e., 20-25°C for TSB and TSA, and 30-35°C for FTG and TSA for 6 days. Turbidity was neither demonstrated in the fluid media nor in TSA plates (Table 2). Therefore, the media were approved and can be used for sterility testing.

Table 1. Growth promotion testing for culture media

Microorganism	**Aerobe bacteria (*Staphylococcus aureus*)**	**Fungi (*Aspergillus niger*)**
Temperature of incubation	30-35°C	20-25°C
Incubation period	5 days	5 days
Result (growth)	positive	positive

Table 2. Sterility observation of culture media before use

Culture media	**Sign of bacterial growth at**	**Sign of fungal growth at 20-25°C**
FTG tubes	negative	N/A
TSB tubes	N/A	negative
TSA plates	negative	negative

Furthermore, we have found that within 14 days of observation, the media's clarity did not change despite the addition of sterile samples to both the TSB and FTG media. By contrast, the non-sterile sample changes the two media from clear to turbid within a day of observation. This finding suggests that both samples can be used as negative and positive control, respectively.

Samples of I-131-Hippuran in non-sterile vials and inoculated into TSB and FTG showed no turbidity, similar to the negative control sample. In contrast, those with radioactivity of 0.5 and 1.0 mCi showed turbidity in both media, similar to the vial sample containing water as positive control (Table 3, Figures 1 and 2). These results indicate that radioactivity of I-131 from 2 mCi can sterilize itself. It is likely that I-131 which has higher energy than Tc-99m can act as an auto-sterilizing agent in much lower radioactivity [10].

Three replications of Sm-153-EDTMP samples with various radioactivity showed a significant difference. Those added with 7 mCi caused turbidity, while those with 15 mCi and 30 mCi remained clear and served as negative control (Figure 3 and 4, Table 4). On the other hand, the same series

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of samples diluted with non-sterile water showed turbidity (Table 4). Thus, the radioactivity of Sm-153 from 15 mCi can sterilize Sm-153-EDTMP, while radioactivity of 7 mCi cannot, and all samples with various radioactivity added with non-sterile water showed turbidity. The results showed that the level of radioactivity can auto sterilize to such an extent as long as the container is clean. However, the work is carried out in a fume-hood or regular work area. So the auto sterilization effect of radiopharmaceuticals depends on the level of radioactivity, and the cleanliness level of the medium since all the samples diluted with non-sterile water showed turbidity, as can be seen in Table 4.

Table 3. Sterility test on I-131-Hippuran with various radioactivity packed in unsterilized vial

Sample	**Bacterial growth (30-35°C, 14 d)**	**Fungal growth (20-25°C, 14 d)**
Sterile I-131-Hippuran (negative control)	-	-
Non-sterile water (positive control)	+	+
0.5 mCi of I-131-Hippuran	+	+
1 mCi of I-131-Hippuran	+	+
2 mCi of I-131-Hippuran	-	-
5 mCi of I-131-Hippuran	-	-
8 mCi of I-131-Hippuran	-	-

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

Table 4. Sterility test on Sm-153-EDTMP with various radioactivity packed in unsterilized vial, with and without dilution

Samples	**Diluted samples**		**Undiluted samples**	
	Bacterial growth (30-35°C, 14 d)	**Fungal growth (20-25°C, 14 d)**	**Bacterial growth (30-35°C, 14 d)**	**Fungal growth (20-25°C, 14 d)**
Sterile Sm-153-EDTMP (negative control)	-	-	-	-
Non-sterile water (positive control)	+	+	+	+
7 mCi of Sm-153-EDTMP	+	+	+	+
15 mCi of Sm-153-EDTMP	+	+	-	-
30 mCi of Sm-153-EDTMP	+	+	-	-

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

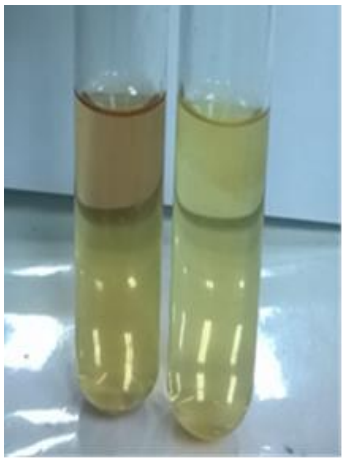

Figure 1. Performance of negative control (left) and positive control (right) in the I-131-Hippuran experiment in FTG media

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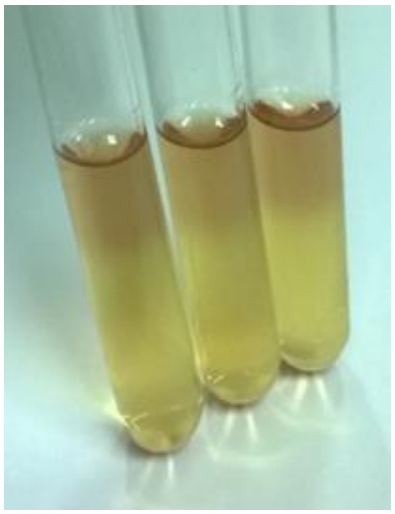

Figure 2. Performance of I-131-Hippuran samples of 2 mCi (left), 5 mCi (middle) and 8 mCi (right) in which all of them showed clear in FTG media

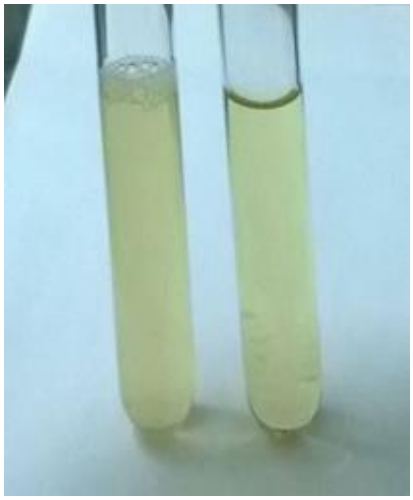

Figure 3. Performance of negative control (right) and positive control (left) in the Sm-153-EDTMP experiment in TSB media

Note : The turbidity which denotes insterility can not be shown clearly from these figures but it is always accompanied by foam at the surface of the media as shown clearly in these pictures above

Figure 4. Performance of Sm-153-EDTMP samples of 7 mCi, 15 mCi and 30 mCi, from which only one of them showed turbid (left) in TSB media

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Bioburden likely contributes to the effectiveness of this radiation auto sterilization process. The term bioburden informs the number and types of viable microorganisms present inside a product before sterilization. The radiation sterilization process will be more effective when the bioburden is low. As a requirement for common parenteral pharmaceutical products, the sterilization process and aseptic technique are to produce sterile products that conform to the sterility assurance level (SAL), which is usually smaller than one in one million units tested (10-6). SAL is derived from kinetic studies on the probability of living microorganisms on or inside a product after sterilization, known as microbial inactivation [4].

The mechanism of action can be direct or indirect. In the direct radiation effect, the ionizing radiation damages the DNA strands of microbial cells. In contrast, in the indirect radiation effect, the radiation interacts with water molecules, producing free radicals and peroxy radicals. These radicals can damage DNA and hamper cell reproduction, leading to microorganisms' death. Indeed, a liquid solution increases the microorganisms' sensitivity compared to a frozen state. In a frozen state, free radicals are immobile, preventing them from diffusing, thus preventing indirect radiation effects. Free radicals can be reactive to oxygen molecules, leading to the production of peroxy radicals that can damage biological cells. Thus, free water radicals yield is much lower in low water activity or dry conditions and microorganisms are becoming more resistant. Still, ionizing radiation becomes greater in wet material since gamma radiation's mechanism generates free hydroxyl radicals and is radiotoxic. Hydroxyl radicals are well-known as strong oxidants that can damage DNA strands and the chemical bond of molecules, either in microorganism cells or in other materials [4,5].

Both radionuclides, i.e., samarium-153 and iodium-131 which were used as models, have proven that radiopharmaceuticals labeled with gamma-emitting radionuclides can behave as an auto-sterilizing agent. One of the weaknesses of this method is the difficulty in identifying microorganisms at the beginning of the killing process. As per safety considerations, the sterility test can only be done after the radioactivity has declined.

Gamma irradiation technology has been widely used to sterilize food and medical devices effectively. However, a recent publication reported the side effect of using gamma radiation to sterilize allografts that, to some extent, can alter the physicochemical properties of sterilized material. Another publication also reported several animal studies that demonstrated that irradiated food consumption provoked genome instability and more likely mutagenic effects that could potentially induce cancer. These findings of the oncogenic potential of irradiated consumables strongly suggest that new, long-term, prospective clinical studies should be conducted soon to investigate whether irradiated food is safe for human consumption [4,5]. By understanding this phenomenon, it can be justified that radiopharmaceuticals commonly used in hospitals exhibit self-protection against microorganisms, so the aseptic manufacturing process of this product is not necessarily applied strictly because its auto sterilization property can be considered terminal sterilization.

4. Conclusion

Samarium-153 and Iodium-131, as gamma-emitting radionuclides labeled to pharmaceutical products at certain radioactivity, can sterilize themselves and act as an auto sterilization agent. Final filtration of radiopharmaceuticals in the manufacturing process inside a clean isolator and using sterilized glasswares and vials for packaging is sufficient to deliver sterile products. It is unnecessary to manufacture radiopharmaceuticals in a clean room with Class A classification, since the characteristic of gamma emitting radionuclide intact in the radiopharmaceutical can sterilize the product. The process can be considered a terminal sterilization process. This is a justification to loosen the existing regulation of the aseptic manufacturing process for radiopharmaceutical products in comparison with the one for aseptic non-radiopharmaceutical parenteral products.

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