Cytotoxicity of PVC tubes sterilized in ethylene oxide after gamma radiation exposure

CITOTOXICIDADE DE TUBOS DE PVC ESTERILIZADOS EM ÓXIDO DE ETILENO APÓS EXPOSIÇÃO À RADIAÇÃO GAMA

CITOTOXICIDAD DE TUBOS DE PVC ESTERILIZADOS EN ÓXIDO DE ETILENO LUEGO DE EXPOSICIÓN A LA RADIACIÓN GAMMA

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RESUMO

Materiais esterilizados em raios gama, ao serem re-esterilizados em óxido de etileno (EO), formam substâncias tóxicas? Esta questão norteou o objetivo deste estudo, que foi investigar o potencial efeito citotóxico do PVC esterilizado em radiação gama e re-esterilizado em EO pelo método da difusão em ágar em culturas celulares. Nove tubos de PVC foram submetidos à esterilização em radiação gama e re-esterilizados em EO. Os tubos foram divididos em um total de 81 unidades de análise, que foram testadas de forma a representar as superfícies internas, externas e massa de cada tubo. Concluiu-se que os materiais de PVC esterilizados em Radiação Gama e consecutivamente re-esterilizados em EO não são citotóxicos.

DESCRITORES

Esterilização
Raios gama
Óxido de etileno
Enfermagem

ABSTRACT

Do materials sterilized using gamma rays become toxic when re-sterilized in ethylene oxide? This question guided the objective of this study, which was to investigate the potential cytotoxic effect of PVC sterilized by gamma radiation and re-sterilized with EO by the agar diffusion method in cell cultures. Nine PVC tubes were subjected to gamma radiation sterilization and were re-sterilized in EO. The tubes were divided into a total of 81 units of analysis that were tested so as to represent the internal and external surfaces and mass of each tube. It was concluded that the PVC materials sterilized in gamma radiation and re-sterilized in EO are not cytotoxic.

DESCRIPTORS

Sterilization
Gamma rays
Ethylene oxide
Nursing

RESUMEN

Los materiales esterilizados con rayos gama, al ser re-esterilizados en óxido de etileno (EO), ¿forman substancias tóxicas? Esta pregunta orientó el objetivo del presente estudio, que fue investigar el potencial efecto citotóxico del PVC esterilizado en radiación gamma y re-esterilizado en EO por el método de difusión en agar en cultivos celulares. Nueve tubos de PVC fueron sometidos a esterilización por radiación gamma y re-esterilizados en EO. Se les aplicaron en total 81 unidades de análisis, las cuales fueron testeadas de manera tal de representar las superficies internas, externas y la masa de cada tubo. Se concluyó en que los materiales de PVC esterilizados con Radiación Gamma y, posteriormente, con EO, no son citotóxicos.

DESCRIPTORES

Esterilización
Rayos gamma
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Enfermería

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INTRODUCTION

In routine practice, central sterilization and supply departments follow the nursing policy of not using ethylene oxide (EO) to resterilize materials previously sterilized by gamma-irradiation. This practice dates from the 1967 publication of a letter warning of ethylene chlorohydrin (ETCH) formation in polyvinyl chloride (PVC) devices successively sterilized by gamma-irradiation and EO[1]. Although it did not present experimental data, the letter prompted several studies in the 1970s and 1990s. Four studies confirmed the incompatibility of the sterilization methods[1-4] and three refuted the hypothesis[5-7].

Because of controversies surrounding the compatibility of these sterilization methods, two publications, one from 1997[8] and the other from 2010[9], reviewed the available data. The first concluded that the number of studies was insufficient to answer the question and suggested that additional studies should be carried out. The second review analyzed the seven previous studies and concluded that the sensitivity of gas chromatography was too low to answer the experimental question. The review also concluded the results that demonstrated incompatibility were weakened because the studies either used environmental aeration or did not present the aeration method in sufficient detail. Environmental aeration is not accepted by the Association for the Advancement of Medical Instrumentation (AAMI) [10]; it has a lower efficiency than mechanical aeration, which requires temperature control. For example, EO sterilization of PVC materials requires mechanical aeration for 8 to 12 hours at 50 to 60°C[11], whereas the time required for environmental aeration is 7 days[11].

Two studies[6-7] that showed compatibility of the two methods employed cytotoxic tests of cell cultures to evaluate the effectiveness of the aeration method used in the sterilization process to remove EO residues and by-products[12]. In those methods, the sterilized samples were tested in living cells under conditions designed to model the effects that contact with material containing toxic residues would have in clinical practice. In this way, cytotoxicity tests may be able to resolve the question of incompatibility of these two sterilization methods[7,9].

Our initial hypothesis was based on the rationale that EO and ETCH are removed by the mechanical aeration process recommended by the AAMI. The absence of cytotoxic effects in cell cultures would eliminate the doubts of compatibility of the sterilizing methods. The objective of this study was to investigate the cytotoxic effects of PVC materials sterilized by gamma-irradiation and resterilized in EO with mechanical aeration.

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METHOD

The samples were non-sterile, flexible PVC tubes, (RWR®, São Bernardo do Campo, São Paulo, Brazil) 1.5 m long, with 1 cm external diameters and 0.6 cm internal diameters. The study sample size was calculated assuming a difference between experimental means of 50% of the standard deviation. A total of 74 analysis units were required for 95% reliability and 99% power. For safety reasons, we worked with 81 analysis units, making the power of the sample more than 99%.

The non-sterilized samples were packaged in plastic pouch and sent to the Institute of Nuclear Energy and Research (IPEN, São Paulo, Brazil) for sterilization by 25-kGy gamma-irradiation. After radiation, the samples were sent for EO sterilization using a mixture of 30% EO and 70% CO_2 at a concentration of 480–600 mg/l pressure of 0.40 kgf/cm^2, temperature of 50 ± 10°C, and relative humidity of 60 ± 25%. The chamber was loaded to 80% of maximum capacity. The initial aeration process took place in the sterilization chamber itself by means of three pulses of filtered air over approximately 30 minutes. After this step, the materials were transferred to a mechanical aeration chamber where they remained for 10 hours at 50°C with 25 air exchanges per hour. The equipment used is inspected annually, and the cycles are monitored with class 5 biological (Attest® 1294, 3M® *St. Paul*, *Minnesota, USA*) and chemical (Browne®, Waterside Road, UK) indicators.

After successive sterilization by gamma-irradiation and EO, assuming that the central, inner portion of the samples were the most difficult to aerate, 0.5-cm pieces were aseptically removed from these regions of each tube and used in the cytotoxicity tests. The cytotoxicity analysis was performed using an agar diffusion method that allowed qualitative evaluation of cytotoxicity[13]. In this test, semi-solid agar provided a substrate for cell monolayers and also allowed diffusion of chemical substances from the samples of sterile PVC tubing. The tests were performed in triplicate. The internal surface, external surface, and mass of each piece of tubing was tested.

To perform the cytotoxicity tests, 5 ml NCTC clone 929 cells were inoculated onto 60 × 15 mm Petri plates for cell culture (TTP®, Trasadingen, Switzerland) at a concentration of 3 × 10^5 cells/ml. The cultures were re-incubated for 48 hours at 37 ± 1°C, in atmosphere containing 5% CO_2. After this period, the monolayers were evaluated for confluence, and the culture media was replaced with an overlay of double-strength Eagle's media (BD® Franklin Lakes, New Jersey, USA) with 1.8% of a 0.01% neutral red vital dye solution. The agar used in this preparation was mixed (1:1) with Eagle's medium at 44°C [15]. After preparing the plates, three pieces of PVC tube, one each from the internal surface, external surface, and mass, were placed aseptically on the overlay medium. The cultures were incubated at 37 ± 1°C in an atmosphere containing 5% CO_2 for 24 hours.

Cytotoxicity was microscopically verified by changes in cell morphology around or under the samples and macroscopically by the presence of a colorless halo surrounding the sample[14]. Filter paper discs about 0.5 cm in diameter served as negative controls, and 0.5-cm diameter pieces of latex were positive controls. The test was considered valid if the positive control produced a halo of at least 0.5 cm and the negative control showed no biological reaction. After measuring the extent of any colorless halo in relation to the sample size, cytotoxicity was scored in accordance with the ISO 10993-5:2009 criteria for the agar diffusion test[13] as described in Table 1.

Table 1 – ISO 10993-5:2009 criteria for reactivity scoring of the agar diffusion assay

Grade	Reactivity	Reactivity zone description	Cytotoxicity
0	None	No detectable zone around or under specimen	-
1	Slight	Some malformed or degenerated cells under specimen	-
2	Mild	Zone limited to the area under specimen	-
3	Moderate	Zone extending specimen size up to 1.0 cm	+
4	Severe	Zone extending farther than 1.0 cm beyond specimen	+

RESULTS

Regardless of their original position, the samples did not cause cytotoxicity after successive sterilization by gamma-irradiation and EO. The results of the cytotoxicity tests are shown in Tables 2, 3, and 4, for the external, internal, and mass surfaces of the PVC tubing.

Table 2 – Cytotoxicity test results of the external surface of 27 study samples – São Paulo, 2010

	Replicate 1			Replicate 2			Replicate 3		
T	**Halo Diameter**	**Reactivity score**	**Cyto-toxicity**	**Halo diameter**	**Reactivity score**	**Cyto-toxicity**	**Halo Diameter**	**Reactivity score**	**Cyto-toxicity**
A	–	0	–	–	0	–	–	0	–
B	S	2	–	S	2	–	S	2	–
C	?	?	?	–	0	–	S	1	–
D	–	0	–	–	0	–	–	0	–
E	–	0	–	–	0	–	–	0	–
F	S	2	–	–	0	–	–	0	–
G	S	1	–	S	2	–	S	1	–
H	–	0	–	–	0	–	S	1	–
I	–	0	–	–	0	–	–	0	–

–, No halo formation/Negative cytotoxic effect; S, Limited effects under the sample area; ?, Results could not be scored because of irregular cell morphology

Table 3 – Cytotoxicity test results of the internal surface of 27 study samples – São Paulo, 2010

	Replicate 1			Replicate 2			Replicate 3		
T	**Halo Diameter**	**Reactivity score**	**Cyto-toxicity**	**Halo diameter**	**Reactivity score**	**Cyto-toxicity**	**Halo Diameter**	**Reactivity score**	**Cyto-toxicity**
A	–	0	–	S	1	–	–	0	–
B	S	2	–	S	2	–	–	0	–
C	?	?	?	–	0	–	S	2	–
D	–	0	–	–	0	–	–	0	–
E	–	0	–	–	0	–	S	2	–
F	–	0	–	–	0	–	–	0	–
G	S	2	–	S	2	–	S	2	–
H	S	1	–	S	2	–	–	0	–
I	S	2	–	S	1	–	–	0	–

–, No halo formation/Negative cytotoxic effect; S, Limited effects under the sample area; ?, Results could not be scored because of irregular cell morphology

Table 4 – Cytotoxicity test results of the mass of 27 study samples – São Paulo 2010

	Replicate 1			Replicate 2			Replicate 3		
T	**Halo Diameter**	**Reactivity score**	**Cyto-toxicity**	**Halo diameter**	**Reactivity score**	**Cyto-toxicity**	**Halo Diameter**	**Reactivity score**	**Cyto-toxicity**
A	–	0	–	–	0	–	–	0	-
B	–	0	–	–	0	–	–	0	-
C	?	?	?	–	0	–	–	0	-
D	–	0	–	–	0	–	–	0	-
E	–	0	–	–	0	–	–	0	-
F	–	0	–	–	0	–	–	0	-
G	–	0	–	–	0	–	–	0	-
H	–	0	–	–	0	–	–	0	-
I	–	0	–	–	0	–	–	0	-

–, No halo formation/Negative cytotoxic effect; S, Limited effects under the sample area; ?, Results could not be scored because of irregular cell morphology

DISCUSSION

The results of this study verify the safety of PVC materials previously sterilized by gamma-irradiation and re-sterilized in EO. However, there are three factors that can limit the practical application of these results. The first concerns the way in which aeration is conducted during sterilization by EO service providers. In 2006, a study described how aeration was carried out by EO service companies in the Southeastern Region of Brazil. The results revealed that 60% of companies use environmental aeration despite having equipment able to carry out mechanical aeration[16]. The second factor is concerned with the material characteristics of the sterilized objects, including the variety of raw materials and the shape. The present study utilized a simple form (tubes), but it can be challenging to control EO residues and by-products in items with complex shapes and/or large dimensions.

According to the AAMI[10], the minimum conditions recommended for chamber aeration for PVC are 8 hours at 60°C or 12 hours at 50°C. However, the recommendation does not consider the necessity of determining the minimum aeration requirements to confirm the effectiveness of different systems of mechanical aeration. Some devices require longer or shorter times of EO exposure because of their size and complexity. To account for these variables, additional studies that employ representative samples of each product to undergo EO sterilization are recommended to verify method compatibility.

The third factor is concerned with testing to assure the safety of devices sterilized with EO. Brazilian legislation[17] establishes tolerance limits for residual EO, ETCH, and ethylene glycol (ETG), which are the same values considered safe by the American Food and Drug Administration (FDA)[18]. These residues are quantified by gas chromatography and are the responsibility of EO sterilization service providers. In addition to the questionable effectiveness of gas chromatography used to assure the safety of EO sterilized materials, it is difficult to regulate the aeration processes carried out by EO sterilization service providers.

Healthcare providers that use sterilization services often do not receive evidence reports that assure the safety of materials returned for use[19]. It is essential that service companies provide data confirming the safety of sterilized materials, not only by the quantity of residues but by the elimination of cytotoxicity, certifying that the mechanical aeration used was sufficient to assure the safety of the sterilized material.

In addition to the problems discussed above, data published in 2006[20] revealed other unsafe practices in EO sterilization. These included insufficient time for appropriate reading of biological indicators used in sterilization process quality control, incomplete information on packing labels of materials sent for sterilization, compromised traceability of materials, and inadequate cleaning of materials by healthcare institutions that rendered the sterilization inefficient. These data show that unsafe use of materials sterilized by gamma ray sterilization may also be related to various cleaning and sterilization deficiencies.

CONCLUSION

Under the present study conditions, the PVC material sterilized in EO and consecutively re-sterilized in EO showed no toxicity. The extension of the results to clinical practice is dependent on confirmation of the effectiveness of the aeration process used to eliminate the cytotoxic effect in cultures.

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