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Radiation-induced Degradation and Immune Toxicity Reduction of Endosulfan

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감마선 조사에 의한 endosulfan의 면역독성 저감

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Abstract

Endosulfan is an organochlorine pesticide that is widely used throughout the world for higher agricultural production. Its extreme toxicity, however, has caused health and environment concerns that have led to an interest in detoxification. In this study, the radiolytic degradation of endosulfan was investigated. Endosulfan in methanol solution (100 ppm) was irradiated at 0, 10, 30, and 50 kGy, and subsequent changes in immune toxicity and degradation of endosulfan were observed. The concentration of endosulfan that was used in this experiment did not affect the cell proliferation. The irradiation of endosulfan decreased the production of NO, indicating a decrease in the immune toxicity of endosulfan by irradiation. The concentration of endosulfan was significantly reduced by irradiation in a dose-dependent manner. The results suggest that gamma irradiation can degrade endosulfan and can reduce its immune toxicity.

Key words : endosulfan, irradiation, degradation, immune toxicity

Introduction

Endosulfan is an organochlorine pesticide and is widely used throughout the world for higher agricultural production. Endosulfan consists of α and β isomers (7:3) and is mainly used for the protection of cotton, vegetables, and fruits crops (1,2). At the same time, it is extremely toxic, especially to fish and aquatic invertebrates, whereupon it affects the central nervous system, kidney, liver, blood chemistry, and parathyroid gland and has reproductive, teratogenic, and mutagenic effects. These health and environmental concerns have led to an interest in detoxification of endosulfan in the environment (3).

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Endosulfan has been reported to be degraded by physical treatment, such as photo degradation (4) or biological treatment using microorganisms (3,5). Endosulfan could be degraded by attach on the sulfate group by oxidation and/or hydrolysis to form the toxic endosulfan sulfate and the non toxic endosulfan diol, respectively (6,7). Although these methods are effective in degradation, also, they have certain disadvantages including high cost and long treatment time. However, there is no report on the degration of endosulfan by another treatment, such as irradiation.

In addition to sterilization of foods, gamma irradiation has been applied for the destruction or reduction of toxic and undesirable materials or the modification of molecular structure (8-10). The objective of this study was to investigate the effect of gamma irradiation on the degradation of endosulfan and subsequent changes in immune toxicity using

a standard aqueous system.

Materials and methods

Sample preparation and gamma irradiation

Endosulfan (α + β isomers) was purchased from Sigma Co. (St. Louis, MO, USA). Due to water insoluble property, endosulfan was dissolved in methanol for a final concentration of 100 ppm of standard solution.

The samples were irradiated at 0, 10, 30, and 50 kGy in a cobalt 60 gamma irradiator (Nordion International, Ottawa, Ontario, Canada) at the Korea Atomic Energy Research Institute (Jeongeup, Korea). The source strength was approximately 11.1 PBq with a dose rate of 10 kGy/h at the location of the sample. Dosimetry was carried out using alanine dosimeters (Bruker Instruments, Rheinstetten, Germany) measured with a Bruker EMS 104 EPR Analyzer. The actual doses were within ±2% of the target dose.

GC-ECD Analysis

An Agilent Technologies 6890 Series gas chromatograph (Palo Alto, CA, USA) and an electron capture detector were used for the confirmation of endosulfan. A split inlet (split ratio, 50:1) was used to inject samples into a HP-1 capillary column (30 m × 0.25 mm × 0.25 μm), and the oven temperature was ramped (80℃ for 2 min, increased to 250℃ at 7℃/min, increased to 280℃ at 10℃/min and maintained for 15 min, and increased to 300℃ at 10℃/min and maintained for 10 min). Inlet and detector temperatures were 260 and 280℃, respectively. Nitrogen was the carrier gas at a constant flow of 1.0 mL/min. The obtained chromatogram was integrated using GC Chemstation software (Rev. A. 08.03, Agilent Technologies, Inc.).

RAW 264.7 cell proliferation assay

RAW 264.7 cells (5×10^5 cells/mL) were cultured in 48 well plates and treated with non-irradiated and gamma-irradiated endosulfan at dosages of 1, 5, and 10 μM. After 24 hr, the cultured supernatants were harvested and stored at 70℃ for nitric oxide production, and the cultured pellets were used for proliferation assay using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT; Sigma Chemical Co.) as described by Zhang et al. (11).

Detection of nitric oxide

Nitric oxide (NO) production was determined using Griess reagent (Sigma Chemical Co.). RAW 264.7 cell cultured supernatant and Griess reagent were mixed in equal volumes (100 μL) according to manufacturer's instructions (Sigma). The absorbance was measured at 595 nm using a plate reader (Zenyth 3100, Anthos Labtec Instruments GmbH, Salzburg, Austria). The absorbance values were then converted to concentration (μM) of NO using standard curves prepared with serial dilutions of $NaNO_2$ standards.

Statistical analysis

Statistical analysis was performed by one way analysis of variance (ANOVA). When significant differences were detected, the differences among the mean values were identified by Duncan's multiple range tests using SAS software at a confidence level of $P < 0.05$. Mean values and standard errors of the mean are reported.

Results and discussion

Degradation of endosulfan by gamma irradiation

The degradation rate of endosulfan in standard solution is shown in Fig. 1. A significant difference in degradation was observed by irradiation dose, especially α-endosulfan ($P < 0.05$). The degradation occurred in a dose dependent manner ($r^2 = 0.9981$). This result is similar to those reported previously, which found that α-endosulfan is photo degraded up to 67% by UV irradiation with a reaction time for 240 min at pH 5 (3). This reaction could be depicted by first order reaction kinetics, and the process was possibly due to ·OH and solvated electrons reacting with pesticide (12). Basfer et al. (13) reported that hydrolysis occurs at several reactive centers in a given organophosphorous pesticide molecules by radiation. It can also occur by homogeneous

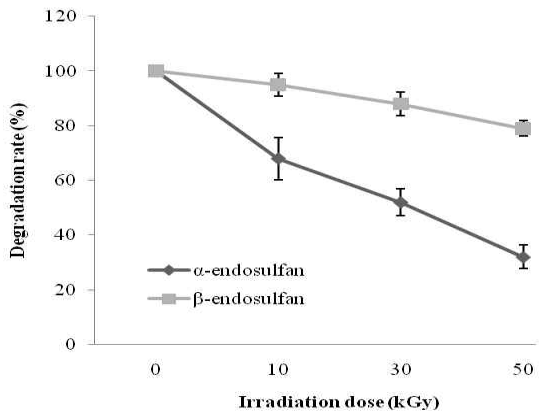

Fig. 1. Degradation of endosulfan by gamma irradiation.

mechanism, where H_2O and ·OH (H· catalysis is less common) act as nucleophiles (14). Water plays an important role in radiolytic degradation (15), since water is required for hydrophotolysis of pesticide by irradiation. The pH value of endosulfan decreased significantly upon an increase in the irradiation dose (data not shown). The pH value could affect the degradation efficiency (4,12). Zhang et al. (12) reported that the degradation value of diuron is enhanced in acid conditions. Based on the experimental results above, gamma irradiation is an effective method for decomposing pesticide.

Cell proliferation assay and detection of nitric oxide

The cytotoxicity of endosulfan in peritoneal macrophages and RAW 264.7 cells was assessed by MTT assay. The assay demonstrated that the concentration of endosulfan used in these experiments did not affect cell proliferation (> 95% cell viability, Fig. 2a). Immunotoxic effects of non-irradiated (0 kGy) and gamma-irradiated (10, 30 and 50 kGy) endosulfan were evaluated by measurement of NO in RAW 264.7 macrophage cells (Fig. 2b). Production of NO in the culture supernatants decreased in the irradiated endosulfan treated group when compared to the non-irradiated endosulfan-treated group (0 kGy). In particular, 50 kGy of irradiation on endosulfan significantly decreased NO production. However, NO production showed no difference after 10 kGy of irradiation. This result suggests that irradiation dose of 10 kGy is not sufficient to change the molecular structure of sample.

This result suggests that irradiated endosulfan decreased production of NO. NO has a wide and pervasive regulatory role in the inflammatory response in macrophage cells, and it was previously reported that macrophage-derived NO levels play an important role in the inflammatory response (17,18). Additionally, Han et al. (16) reported that endosulfan induces expression of iNOS and proinflammatory cytokines in macrophages. These inflammatory responses are mediated through the trans activation of NF-κB, which might provide endosulfan with the means to alter the immune response.

Barnes and Ware (19) found that the α-endosulfan was more toxic than either technical grade endosulfan or the β-endosulfan to house flies. Sutherland et al. (20) reported that α-endosulfan was significantly more toxic than the β -endosulfan in mammals. Results suggest that irradiation was disassemble of α-endosulfan and reducing its toxicity. However, very few studies have examined the mechanism of the structural changes and toxicity of endosulfan by irradiation. The components mainly responsible for this mechanism of structural changes and toxicity may be explained after further investigation.

In conclusion, we suggest that gamma irradiation is an effective method for degradation of endosulfan, especially α-endosulfan, and reducing its immune toxicity. Further research in real food systems is in progress.

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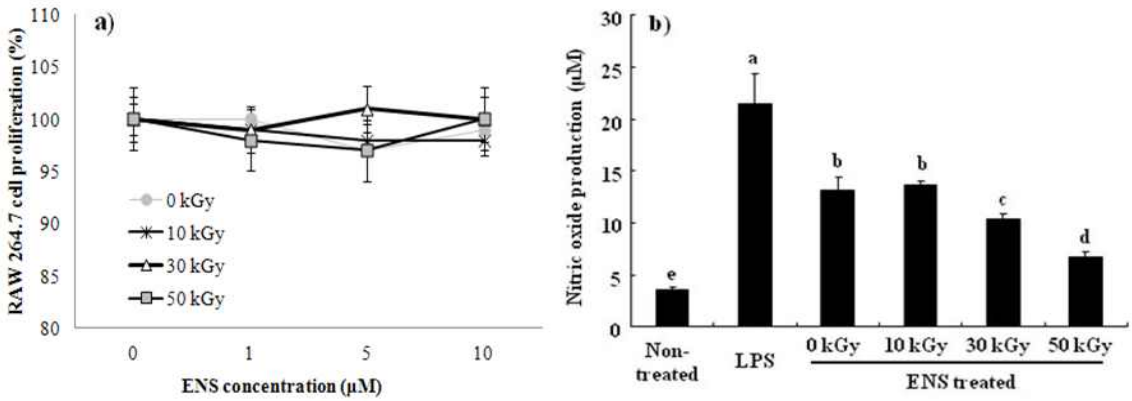

Fig. 2. Effect of gamma-irradiated endosulfan (ENS) on cell proliferation and nitric oxide.

(NO) production in RAW 264.7 macrophage cells. RAW 264.7 cells were treated with various concentrations (1, 5, 10 μM) of ENS for 24 hr, and LPS (1 μg/mL) was used as a control. The level of NO production was determined by Griess reagent. Cell viability was assessed by MTT assay. Each bar shows means ± S.D. (n = 3).

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