Clinical Pharmacology & Biopharmaceutics

Iwase et al., Clinic Pharmacol Biopharmaceut 2014, S2
DOI: 10.4172/2167-065X.S2-004

Research Article Open Access

Biological Effects of the Plasticizer Tris (2-Ethylhexyl) Trimellitate

Harunobu Iwase[1], Shigeru Oiso[2], Hiroko Kariyazono[2] and Kazuo Nakamura[1]*

[1]*Department of Biopharmaceutics, Nihon Pharmaceutical University, Japan*
[2]*Division of Pharmaceutical Health Care and Sciences, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Nagasaki International University, Japan*

Abstract

Objectives: An alternative plasticizer, tris (2-ethylhexyl) trimellitate (TOTM), was developed from di-(2-ethylhexyl) phthalate (DEHP) for use in medical tubing. However, little is known about the biological effects of TOTM and thus its safety is not yet well-established. We investigated the leachability of TOTM from TOTM-plasticized Polyvinylchloride (PVC) feeding tubes. Furthermore, we studied whether TOTM influences the cell toxicity of human leukemia (HL)-60 cells, the cell proliferation of human breast cancer MCF-7 cells, and the binding affinity for human estrogen receptor α.

Methods: TOTM or DEHP-plasticized PVC feeding tubes were incubated with liquid nutriment containing soybean based salad oil. Thereafter, these solutions were mixed with extract solution and centrifuged, respectively. Obtained supernatant was served as extract sample. TOTM and DEHP were confirmed by high performance liquid chromatography (HPLC); molecular ion peaks corresponding to TOTM were detected by LC/mass spectrometry. The effects of these plasticizers on human leukemia (HL)-60 viability and breast cancer cell MCF-7 proliferations were investigated. Furthermore, the affinity of these plasticizers for binding human estrogen receptor (ER)-α was measured using an enzyme-linked immunosorbent assay (ELISA) kit.

Results: An existence of TOTM was confirmed in an extract sample by HPLC and LC/MS. Furthermore, TOTM decreased viability HL-60 cells while enhancing MCF-7 cell proliferation. However, these effects-as well as the activation of ER-α- were weaker by approximately 10-fold weaker for TOTM than DEHP.

Conclusion: The leaching of TOTM from TOTM-plasticized PVC feeding tubes is less toxic than DEHP, adverse effects may be associated with the production of ovarian estradiol via activation of a receptor-mediated signaling pathway. These findings nonetheless indicate that TOTM is a viable and safer alternative to DEHP for plasticizing medical devices for human use.

Keywords: TOTM; DEHP; Feeding tube; Soybean; LC; LC/mass spectrometry; MCF-7 cells; Human estrogen receptor α; Cytotoxicity

Introduction

Polyvinylchloride (PVC) is used in a wide range of medical devices because of its versatility. PVC is a relatively rigid and brittle polymer. Consequently, plasticizers, which are added to facilitate processing, make PVC flexible, resilient, and easier to handle. Many phthalates including di (2-ethylhexyl) phthalate (DEHP) are commonly used as plasticizers. Many plasticizer-containing PVC medical devices are used as intravenous fluid bags and tubing, blood and plasma bags, enteral feeding and dialysis equipment, catheters, and gloves. DEHP is not chemically bound to the PVC polymer and may leach when PVC medical devices are exposed to blood and drugs. The U.S. FDA Center for Devices and Radiological Health has published a review of the potential patient health risks associated with DEHP leaching from PVC medical devices [1]. Risk assessment of DEHP released from PVC medical devices is an important issue for medical patients. Several reports have demonstrated that plasticizers are easily released when such devices are used with lipophilic substances [2-5]. Furthermore, it has been reported that several pharmaceuticals, such as miconazole, cyclosporin, etoposide, chlordiazepoxide hydrochloride and tacrolimus, are adsorbed into the tubing of an intravenous administration set concurrent with the release of DEHP [6-10]. Some reports have demonstrated that the release of DEHP from PVC tubing is facilitated by surfactants, such as polysorbate 80 (Tween 80) and polyethylene castor oil (HCO60) that are used as solubilizers for water-insoluble drugs [4,11,12]. Subotic et al. [13] reported that DEHP leached from PVC tubes when DEHP-containing PVC nasogastric tubes were incubated with gastric juice or a feeding solution. Furthermore, Tanaka et al. [14] reported that the concentration of DEHP in the liquid nutriment, namely Ensure Liquid® solution, increased linearly over time and subsequently reached a plateau when this enteral nutrition passed through the PVC tube during enteral tube feeding in a temperature-dependent manner. In contrast, there have been no reports concerning the leachability of tris (2-ethylhexyl) trimellitate (TOTM) from TOTM- plasticized PVC feeding tubes during the incubation of the liquid nutriment.

Several reports have demonstrated that some phthalate esters including DEHP exhibit reproductive, developmental, and testicular toxicities [15-19], and the antiproliferative and adverse effects of DEHP on human keratinocytes [20]. With general levels of intake, reproductive risk appears to be minimal, whereas specific uses, such as long-term medical treatment, have presented potential problems [21]. The effect of DEHP on humans is not well understood, although DEHP intake from intravenous treatment or enteral tubing should be avoided. The Ministry of Health, Labour, and Welfare of Japan has classified DEHP as a chemical agent suspected of causing endocrine disruption and restricted oral tolerable daily intake (TDI) to 0.14 mg/(kg day) [22]. Moreover, data from AdvaMed and other significant studies have

***Corresponding author:** K Nakamura, Department of Biopharmaceutics, Nihon Pharmaceutical University, 10281 Komuro, Ina-Cho, Kitaadachi-gun, Saitama 362-0806, Japan, Tel: +81-48-721-1155; Fax: +81-48-721-7267; E-mail: vkazunaka@nichiyaku.ac.jp

Received March 05, 2014; **Accepted** March 20, 2014; **Published** March 22, 2014

Citation: Iwase H, Oiso S, Kariyazono H, Nakamura K (2014) Biological Effects of the Plasticizer Tris (2-Ethylhexyl) Trimellitate. Clinic Pharmacol Biopharmaceut S2: 004. doi:10.4172/2167-065X.S2-004

prompted the Center for Devices and Radiological Health (CDRH) and the U.S. Food and Drug Administration (FDA) to propose a parenteral tolerable intake of DEHP of 0.6 mg/kg/day [1]. Recently, PVC tubing that contains TOTM as an alternative plasticizer to DEHP has been developed. In 2006, the Japan Medical Devices Manufacturers Association reported a list of PVC-free and DEHP-free medical devices available in Japan [23]. Therefore, the number of medical devices using TOTM-plasticized PVC as an alternative to DEHP-plasticized PVC has increased compared with previous years. There are several reports concerning the leachability of TOTM compared with that of DEHP. Therefore, examining the possibility of TOTM leaching from PVC medical devices under various conditions is necessary.

The methyl thiazolyl tetrazolium (MTT) assay is a colorimetric assay that measures the activity of cellular enzymes by reducing tetrazolium dye to insoluble formazan [24,25]. The MTT assay is generally used to assess cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferative to resting status) of potential medicinal agents and toxic materials. The human leukemia HL-60 cell line has been used as a model system for studying leukemic cell differentiation [26,27]. This cell line can be induced to differentiate into granulocyte-like or monocyte-like cells by various agents, and differentiated cells lose their proliferative ability. Lee et al. [28] examined the pharmacological activity of DEHP by MTT assay using HL-60 cells.

It has been reported that at least two different estrogen receptors (ER) exist, namely, ERα and ERβ [29], and ERβ might modulate the estrogenic effects of ERα by forming heterodimers with ERα, thereby reducing the ERα-mediated response, resulting in the inhibition of ERα-mediated proliferation of breast cancer cells *in vitro* [30-32]. Endocrine disruptors (EDs), which are exogenous compounds such as xenoestrogens, including bisphenols and alkylphenols, some polychlorinated biphenyls, and phthalates, can induce estrogen-mediated signaling in an abnormal manner by binding to ERs [33,34]. Therefore, xenoestrogens, i.e., EDs, have been assessed as risk factors for human health problems including the immature reproduction system and adverse biological mechanisms in the environment [35-37]. Several reports have demonstrated that xenoestrogens bind to estrogen receptors and mimic estrogenic actions [38-42]. An estrogenic activity of some phthalate plasticizers was investigated using several *in vitro* tests, such as MCF-7 cell proliferation, ER binding in the rat uterus, and yeasts transfected with human ER gene construct [40-43]. The MCF-7 breast cancer cell line was derived from a pleural effusion taken from a metastatic breast cancer patient [44] and is an ideal model to study the mechanism of estrogenic action because these cells express functional wild-type estrogen receptors [45]. This cell line was used to develop an *in vitro* screening assay to detect xenoestrogens, such as polychlorinated biphenyls, bisphenol A, and phthalates [42,46,47]. Presently, TOTM has been used as a plasticizer in Japan instead of DEHP because of its suspected relevance as an ED as well as its obstruction of the function of testis and reproduction. In Japan, TOTM-plasticized PVC is commonly used in a wide range of medical-grade materials, such as feeding tubes. However, few studies have reported the possible patient risk of exposure to TOTM-containing medical devices.

In the present study, we investigated whether liquid nutriment that included soybean-containing salad oil can leach TOTM from TOTM-plasticized PVC feeding tubes. In addition, we measured HL-60 cell viability and MCF-7 cell proliferation to compare the cell toxicity of TOTM to that of DEHP as well as the endocrine-disrupting risks of these two plasticizers.

Materials and Methods

Chemicals and reagents

DEHP (Purity: >98%) and TOTM (Purity: >95%) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Dimethyl sulfoxide (DMSO), isopropanol, ethyl acetate, methanol, and acetonitrile were obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Roswell Park Memorial Institute (RPMI) 1640 medium, Dulbecco's modified Eagle's medium (DMEM), and fetal bovine serum (FBS) were obtained from Life Technologies Corporation (Carlsbad, CA, USA). Charcoal was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ensure Liquid was obtained from Abbott Laboratories (Abbott Park, IL, USA). Nisshin sarada oil was obtained from The Nisshin OilliO Group, Ltd. (Tokyo, Japan). TOTM-plasticized PVC feeding tubes (content of TOTM, approximately 30%) were obtained from Atomed Cebu, Inc. (Lapu-lapu, Philippines). Penicillin A and streptomycin were obtained from Meiji Seika Pharma Co., Ltd. (Tokyo, Japan). Tissue culture test plates were obtained from TPP AG (CH-8219, Trasadingen, Switzerland)

Cells

Human leukemia HL-60 cells were obtained from DS Pharma Biomedical Co., Ltd. (Osaka, Japan). The HL-60 cells were cultured in RPMI medium supplemented with antibiotics (100 U/mL of penicillin A and 100 U/mL of streptomycin) and 10% heat-inactivated FBS and maintained in a 37°C humidified incubator containing 5% CO_2. Human breast cancer MCF-7 cells were obtained from National Institute of Biomedical Innovation JCRB Cell Bank (Osaka, Japan). The MCF-7 cells were cultured in DMEM with 5% heat-inactivated FBS and maintained in a 37°C humidified incubator containing 5% CO_2.

Methodology Evaluation of TOTM release from TOTM-plasticized PVC feeding tubes

Nutrient samples were produced as follows: 240 mL of liquid nutriment (Ensure Liquid) was added to 10 mL of the soybean-containing salad oil, and this nutrient served as the test nutriment. In contrast, the liquid nutriment without salad oil served as the control nutrient. Ten centimeters of TOTM-plasticized PVC feeding tube (inside diameter ; 3 mm and outside diameter ; 4mm) were soaked in 30 mL of the test nutrient or the control nutrient, and incubated at 40°C for 24 h. Subsequently, 1 mL exudate was added to 5 mL extract solution (methanol : acetone = 1 : 1), and was mixed by vortex-mixer. Thereafter, this solution was centrifuged for 10 min at 2190 × g, and collected supernatant were served as extract sample.

Measurement of TOTM and DEHP

The existence of TOTM in extract sample was confirmed in high performance liquid chromatography (HPLC) and liquid chromatography/mass spectrometry (LC/MS) [46]. HPLC conditions of TOTM analysis were as follows: column, Waters XTerra Phenyl, 5 μm (4.6 mm i.d. × 250 mm length, Waters Co., Milford, MA, USA); mobile phase, 3:2:1 (v/v) acetonitrile:methanol : distilled water ; flow rate, 1 mL/min (HPLC), 0.5 mL/min (LC/MS); internal standard, TOTM or DEHP; detector, SPD-20AVP; column oven, CTO-20AVP; calculator, LC solution (Shimadzu Co., Kyoto, Japan). The LC/MS was performed using an LC/MS-2010 EV (Shimadzu Co.) equipped with an electrospray ionization source (ESI).

Cell viability assay

The MTT assay was performed to measure the mitochondrial-dependent reduction of tetrazolium dye to insoluble formazan as an indicator of cell viability. HL-60 cells were seeded in 96-well plates at a density of 2×10^4/mL (0.2 ml/well) and incubated for 24 h. Different concentrations of TOTM and DEHP were added to the plates. After incubation for 48 h, cells were washed with PBS twice, and 10 µL of MTT solution (5 mg/mL in PBS) were added to the plates that were incubated for an additional 4 h. Subsequently, 100 µL of acidic isopropanol was added to each well that was then mixed by vigorous pipetting to dissolve the precipitated formazan. UV-visible absorption was measured at 595 nm using an ELISA plate reader (ImmunoMini NJ-2300, Cosmo Bio Co., Ltd., Tokyo, Japan). The addition of ethanol instead of plasticizers to the HL-60 cells served as the control (plasticizer included 0 mM). Cell viability was examined in the absence and presence of various concentrations of TOTM and DEHP. Cell numbers were expressed as percentages, where the control with 0.1% ethanol alone was designated as 100%.

Cell proliferation assay

MCF-7 cells were grown and maintained in DMEM supplemented with 5% FBS. The MCF-7 cells were plated into 12-well tissue culture test plates at an initial concentration of 1,000 cells per well, and the medium was replaced by experimental medium (5% charcoal-dextran human serum supplemented with phenol red-free DMEM) after a 24-h incubation in the growth medium. The plasticizers were dissolved in ethanol, diluted with phenol-red free DMEM, and added to each well. The culture was continued for 72 h, and the absorbance was measured at 540 nm using an ELISA plate reader (Immuno Mini NJ-2300). Cell proliferation was examined in the absence (at 0 mM) and presence of various concentrations of TOTM and DEHP. The concentration of plasticizers at 0 mM served as the control. Cell numbers were expressed as percentages, where the control with 0.1% ethanol alone was designated as 100%.

Screening of TOTM and DEHP for ERα ligands

The formation of an ERα-TOTM or -DEHP complex to the co-activator peptide on the plate was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (EnBio RCAS for ERα, Tokyo, Japan) [48,49]. After immobilization of the coactivator (SRC1 peptide) to the wells of the plate, the plate was incubated at 25°C for 1 h with gentle shaking; ERα dilution standards and samples were added to the plate. After five washes, horseradish peroxidase (HRP)-conjugated anti-human ER antibody was added into each well and incubated at 37°C for 30 min. After five washes, 50 µL of tetramethylbenzene (TMB) substrate was added into each well, and the plate was incubated in the dark at 37°C for 15 min. After the addition of the stop solution, each well was read at an absorbance of 450 nm using an ELISA plate reader (ImmunoMini NJ-2300). The absorbance of the plate was measured in the absence (at 0 mM) and presence of various concentrations of TOTM and DEHP. The concentration of plasticizers at 0 mM served as the control. Ligand intensity was expressed as a percentage, where the control with 5% DMSO alone was designated as 100%.

Statistical analysis

All experiments were performed five times. Results are presented as the mean ± standard deviation (SD). Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Fisher's protected least-significant difference test. Parametric data were analyzed by two-way repeated measures ANOVA followed by Scheffe's *post hoc* test. Proportional differences were analyzed using the Fisher's exact chi-square analysis. Differences were considered to be significant at $P<0.05$. Stat View J-4.5 software (Abacus Concepts Inc., CA, USA) was employed for all calculations.

Results

HPLC analysis of plasticizers (DEHP and TOTM) standard products and Peak-1

As shown in Figure 1, the retention times of DEHP standard product (A) and TOTM standard product (B) in similar chromatographic conditions were approximately 6.9 min and 17.0 min, respectively. The test nutriment which immersed a TOTM-plasticized PVC feeding tube was concentrated, and this concentrate was analyzed by HPLC.

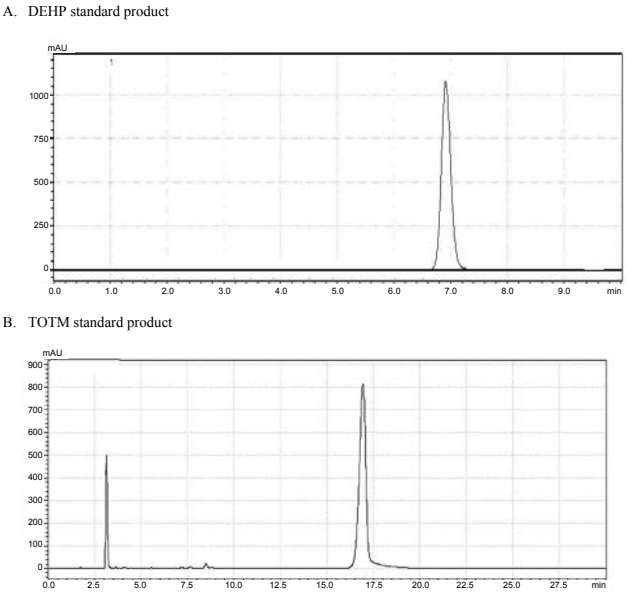

Figure 1: HPLC analysis of plasticizers. The retention times of DEHP standard product (A) and TOTM standard product (B) in similar chromatographic conditions were approximately 6.9 min and 17.0 min, respectively. TOTM: tris (2-ethylhexyl) trimellitate, DEHP: di (2-ethylhexyl) phthalate.

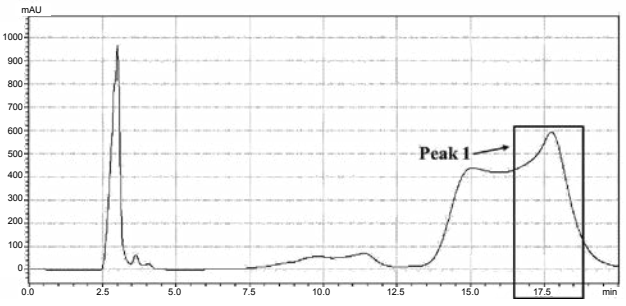

Figure 2: HPLC analysis of the extract of the test nutriment which used for immersion of a TOTM-plasticized PVC feeding tube.
The test nutriment which immersed a TOTM-plasticized PVC feeding tube was concentrated, and this concentrate was analyzed by high performance liquid chromatography (HPLC). This concentrate contained a peak showing the same retention time as TOTM standard product, and area of this peak was named Peak-1. TOTM: tris (2-ethylhexyl) trimellitate.

This concentrate contained a peak showing the same retention time as TOTM standard product, and area of this peak was named Peak-1 (Figure 2). The concentration of TOTM in the Peak-1 increased in a time-dependent manner. The cumulative amount of TOTM released from the PVC feeding tube up to 4h in the Peak-1 was less than 100 μg. On the other hand, the concentrate of the control nutrient did not show a TOTM peak.

Analysis of TOTM and Peak-1 by LC/MS

As shown in Figure 3A, the molecular ion peak of TOTM standard product was obtained at *m/z* 547 (M+) by LC/MS. The elementary analysis of TOTM generated C33H54O6 as the molecular formula (Figure 4). As shown in Figure 3B, a molecular ion peak similar to TOTM was observed in the Peak-1.

Effects of TOTM and DEHP on HL-60 cell viability

As shown in Figure 5, HL-60 cell viability at 1.0 mM TOTM was 78 ± 7%, and the value was significantly less than that of the control. In contrast, HL-60 cell viability at 0.1 mM DEHP decreased dramatically

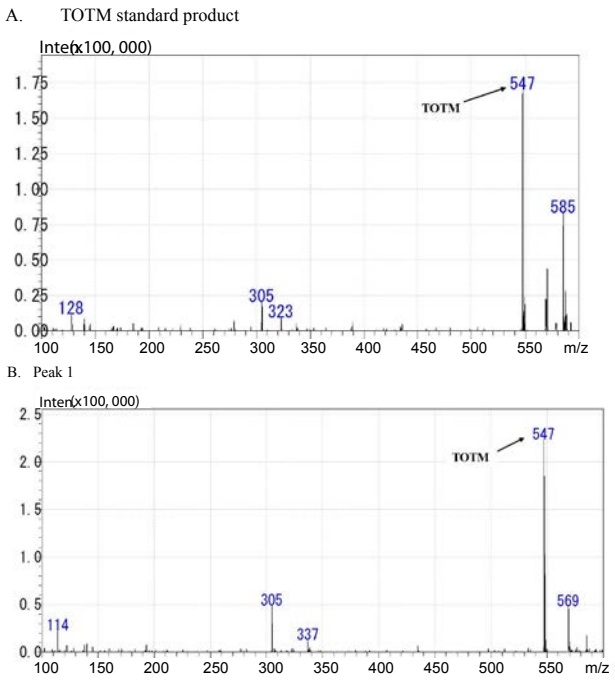

Figure 3: LC/MS analysis of plasticizers. The molecular ion peak of tris (2-ethylhexyl) trimellitate (TOTM) standard product was obtained at *m/z* 547 (M^+) by liquid chromatography/mass spectrometry (LC/MS). A molecular ion peak similar to TOTM was observed in the Peak-1.

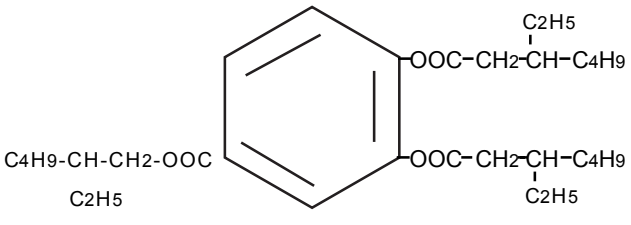

Figure 4: Structural formula of TOTM. Molecular Formula: $C_{33}H_{54}O_6$. Molecular Weight: 546.9

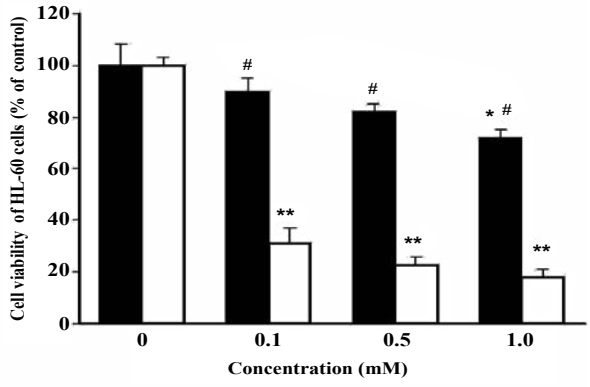

Figure 5: Inhibitory effects of plasticizers on HL-60 cell viability.
After incubating human leukemia HL-60 cells with various concentrations of tris (2-ethylhexyl) trimellitate (TOTM) and di (2-ethylhexyl) phthalate (DEHP) for 48 h, UV-visible absorption was measured at 450 nm using an ELISA plate reader. Cell viability was examined in the absence (at 0 mM) and presence of various concentrations of TOTM and DEHP. The concentration of plasticizers at 0 mM served as the control. Cell numbers were expressed as percentages, where the control with 0.1% ethanol alone was designated as 100%. Filled bars show data when various concentrations of TOTM were added to the plates (TOTM group, n = 5), and empty bars show data when various concentrations of DEHP were added to the plates (DEHP group, n = 5). *P<0.05, **P<0.01 versus the control; #P<0.01 versus the DEHP group. Data are given as the mean ± SD.

to 32 ± 8%. Cell viability at 1.0 mM DEHP was 21 ± 6%, and this value was significantly less than that at 1.0 mM TOTM.

Effects of TOTM and DEHP on MCF-7 cell proliferation

As shown in Figure 6, TOTM-treated MCF-7 cell proliferation increased in a concentration-dependent manner. MCF-7 cell proliferation at 1.0 mM TOTM was 155 ± 8%, and this value was significantly greater than that of the control. On the other hand, proliferative effects of DEHP on MCF-7 cells were observed at 0.1 mM concentration, and this value (151 ± 7%) was significantly greater than that of the control. Proliferative effects of DEHP on MCF-7 cells were approximately 10-fold greater compared with those of TOTM.

Stimulating action of plasticizers to ERα

As shown in Figure 7, the formation of ERα-TOTM complex to SRC1 peptide (% ligand intensity) at 1.0 mM TOTM was 212 ± 17%, and this value was significantly greater than that of the control. In contrast, the ligand intensity (%) induced by 0.1 mM DEHP treatment was significantly greater than that of the control, and this value (358 ± 28%) was significantly greater than that of the control. The ligand intensity (%) of DEHP increased in a concentration-dependent manner. Stimulating action of DEHP to ERα was approximately 10-fold greater compared with those of TOTM.

Discussion

First, we clarified the leachability of TOTM from TOTM-plasticized PVC feeding tubes when this feeding tube was incubated with liquid nutriment (Ensure Liquid) that included salad oil. In contrast, we did not observe TOTM in the extract of the control nutrient, namely the liquid nutriment without the salad oil. These phenomena suggest that salad oil leaches TOTM from the feeding tube. The concentration of TOTM in the extracts was confirmed by HPLC and LC/MS. There have been some reports concerning the leachability of TOTM from TOTM-plasticized PVC medical devices. According to Ito et al. [46], in the case of Prograf and Florid-F, there is a significant difference between

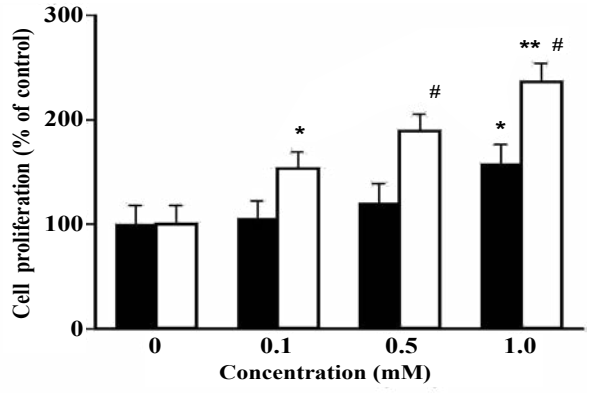

Figure 6: Proliferative effects of plasticizers on MCF-7 cells.
After incubating human breast cancer MCF-7 cells with various concentrations of tris (2-ethylhexyl) trimellitate (TOTM) and di (2-ethylhexyl) phthalate (DEHP) for 48 h, UV-visible absorption was measured at 450 nm using an ELISA plate reader. Cell viability was examined in the absence (at 0 mM) and presence of various concentrations of TOTM and DEHP. The concentration of plasticizers at 0 mM served as the control. Cell numbers were expressed as percentages, where the control with 0.1% ethanol alone was designated as 100%. Filled bars show data when various concentrations of TOTM were added to the plates (TOTM group, n = 5), and empty bars show data when various concentrations of DEHP were added to the plates (DEHP group, n = 5). *P<0.05, **P<0.01 versus the control; #P<0.01 versus the DEHP group. Data are given as the mean ± SD.

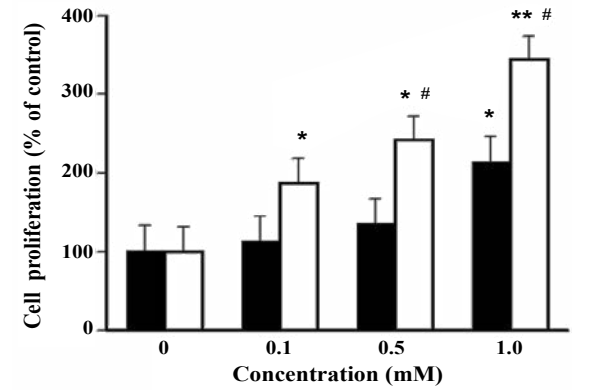

Figure 7: Formation of ERα-TOTM and -DEHP complexes to SRC1 peptide.
The formation of an ERα-plasticizer complex to the co-activator peptide on the plate was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit. Various concentrations of tris (2-ethylhexyl) trimellitate (TOTM) and di (2-ethylhexyl) phthalate (DEHP) were added to the wells and incubated for 1 h with gentle shaking. The absorbance at 450 nm of each well was read in a plate reader. Filled bars show data when various concentrations of TOTM were added to the plates (TOTM group, n = 5), and empty bars show data when various concentrations of DEHP were added to the plates (DEHP group, n = 5). Addition of dimethyl sulfoxide instead of plasticizers to the plate wells served as the control (0 mM). Ligand intensity was expressed as percentages, where the control with 5% DMSO alone was designated as 100%. *P<0.05, **P<0.01 versus the control; #P<0.05 versus the TOTM group. Data are given as the mean ± SD.

the concentration of TOTM released from gamma-ray-sterilized PVC/TOTM tubes and that from unsterilized control tubes. Flaminio et al. [50] measured plasma concentrations of TOTM and/or its metabolites and demonstrated that TOTM might leach from TOTM-containing dialysis tubes, although the amount of TOTM released from the TOTM-containing dialysis tubes was less than the amount of DEHP released from the DEHP-containing dialysis tubes.

Next, we investigated the anti-proliferative effects of TOTM and DEHP in HL-60 cells using the MTT assay and determined that 1.0 mM TOTM inhibited HL-60 cell viability. In contrast, the inhibitory effects of DEHP were 5-fold stronger than those of TOTM. Our results have been supported by the reports of Iwata et al. [26] and Lee et al. [28]. Other reports have indicated that DEHP causes a spectrum of toxic effects in developing and adult animals and in multiple organ systems including the liver, reproductive tract (i.e., testes, ovaries, and secondary sex organs) kidneys, lungs, and heart [2,15,51,52]. Furthermore, Latini et al. [53] indicated that DEHP has been shown to cause reproductive and developmental toxicity and is suspected to be an endocrine disruptor. More research may be necessary to clarify the toxic effects associated with TOTM exposure in humans.

We investigated the proliferative effect of plasticizers using MCF-7 cells. Many *in vitro* studies have reported the estrogenic effect of some phthalates by ERα-binding assays, proliferation assays using MCF-7 cells, and ERα-dependent transcription assays [38-42,54]. Moreover, these estrogenic responses at 10^{-5} M were suppressed by the ER antagonist, tamoxifen, suggesting that the estrogenic activities of phthalates might be induced by binding to ERα. In the present study, 1.0 mM TOTM-treated MCF-7 cells exhibited a significant increase in cell proliferation. Therefore, 1.0 mM TOTM may possess a proliferative effect in MCF-7 cells. In contrast, the proliferative effect of DEHP was approximately 10-fold stronger than that of TOTM because this effect was observed at 0.1 mM DEHP. Our results may be supported by Choi and Jeung [55]. We have considered that TOTM as well as DEHP may affect cell proliferation, apoptosis, and cell survival via estrogen-dependent signaling through ERα [37]. Furthermore, we investigated the endocrine-disrupting risks associated with these two plasticizers by measuring the affinity of plasticizers to ER proteins [56]. In our results, the estrogenic activity of TOTM was observed at 1.0 mM, and this effect is significantly weaker than that of DEHP. This is the first report concerning the estrogenic activity of TOTM via ERα activation. However, more research concerning the in vivo kinetics of TOTM and /or its metabolites are required to clarify the effects of TOTM in humans.

In our results, TOTM as a plasticizer demonstrated acute toxicity to HL-60 cells. However, acute toxicity of TOTM was approximately 10-fold weaker than that of DEHP. On the other hand, proliferative effects of TOTM on MCF-7 cells may be correlated to the activation of ERα. Furthermore, these effects of TOTM was approximately 10-fold weaker than that of DEHP. Several reports supported these effects of DEHP [39,41,45,57].

Conclusions

We have discovered that TOTM from PVC feeding tubes was leached by exposure to salad oil. Furthermore, it has been demonstrated that TOTM may possess cell toxicity and estrogenic activity through a receptor-mediated signaling pathway. However, these reactions of TOTM are significantly weaker compared with that of DEHP. Therefore, additional research may be necessary to confirm and extend these observations.

Acknowledgement

We would like to thank PhD. H. Saitou for the excellent technical assistance provided during the course of these studies.

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