科技部補助專題研究計畫成果報告
期末報告

研究雙酚A誘發巨噬細胞的基因毒性與細胞毒性作用機轉

計 畫 類 別 ： 個別型計畫
計 畫 編 號 ： MOST 103-2314-B-040-018-
執 行 期 間 ： 103年08月01日至105年07月31日
執 行 單 位 ： 中山醫學大學牙醫學系（所）

計 畫 主 持 人： 黃富美
共 同 主 持 人： 關宇翔、張育超

中 華 民 國 105 年 10 月 31 日

中 文 摘 要 ： 雙酚A 已被證實具有內分泌干擾的功能。雙酚A 的製品在日常生活用品中相當常見，如奶瓶、罐頭的內附膜、食品和飲料包裝、牙齒粘合劑與填充劑、可微波使用的塑膠制品等等。巨噬細胞為初級免疫細胞之一，在人體對抗入侵的外來病原菌、癌細胞的清除、與正常人體的發育過程中都扮演著相當重要的角色。雙酚A以濃度依存性與時間依存性的方式引發RAW264.7 巨噬細胞的細胞毒性 ($p < 0.05$)。雙酚A以濃度依存性方式引發RAW264.7 巨噬細胞的細胞凋亡與壞死 ($p < 0.05$)。雙酚A以濃度依存性方式引發RAW264.7 巨噬細胞的DNA fragmentation 形成，進而導致細胞凋亡 ($p < 0.05$)。利用微小核形成與慧星實驗進行分析所得結果，發現雙酚A以濃度依存性方式引發RAW264.7 巨噬細胞的產生基因毒性 ($p < 0.05$)。再者，發現雙酚A以濃度依存性的方式，誘發細胞內超氧自由基的生成 ($p < 0.05$)。最後發現，BPA以濃度依存性的方式誘發caspase -3, -8, 與-9 的活性。綜合上述，BPA 經由DNA damage 與caspase 活化，進而導致細胞毒性與基因毒性。

中 文 關 鍵 詞 ： 雙酚A; RAW264.7 巨噬細胞； 細胞毒性；基因毒性

英 文 摘 要 ： Products of bisphenol A (BPA), which has been identified as an endocrine disruptor, are quite common in the daily necessities, such as baby bottles, coated cans for film, food, and beverage packaging, paper coatings, dental adhesives and fillers, and the microwave plastic products. Macrophage is one of the innate leukocytes, which plays an important role in defense against invasive pathogen in vivo, defense cancer cell scavenger, and normal human developmental processes. BPA demonstrated a cytotoxic effect to RAW264.7 cells in a concentration and time-dependent manner ($p < 0.05$). BPA was found to induce apoptosis and necrosis of cell death in a concentration-dependent manner ($p < 0.05$). BPA-induced cell apoptosis was demonstrated by the increase in DNA ladder formation. In addition, BPA exhibited genotoxicity via a dose-related increase in the numbers of micronucleus and DNA strand breaks ($p < 0.05$). Generation of reactive oxygen species was induced by BPA in concentration-dependent manner ($p < 0.05$). Furthermore, the activation of caspase-3, -8, and -9 were generated by BPA in a dose-dependent manner ($p<0.05$). These results indicated that cytotoxicity and genotoxicity induced by BPA in macrophages may be via DNA damage and caspase activation.

英 文 關 鍵 詞 ： Bisphenol A; RAW264.7 cells; cytotoxicity; genotoxicity; caspases

科技部補助專題研究計畫成果報告

（□期中進度報告/■期末報告）

研究雙酚 A 誘發巨噬細胞的基因毒性與細胞毒性作用機轉

計畫類別：■個別型計畫　□整合型計畫
計畫編號：MOST 103－ 2314 － B － 040 － 018 －
執行期間： 2014 年 08 月 01 日至 2016 年 07 月 31 日

執行機構及系所：中山醫學大學醫學系藥理學科

計畫主持人：黃富美
共同主持人：張育超, 關宇翔
計畫參與人員：

本計畫除繳交成果報告外，另含下列出國報告，共 _1__ 份：
□執行國際合作與移地研究心得報告
□出席國際學術會議心得報告

期末報告處理方式：
1. 公開方式：
□非列管計畫亦不具下列情形，立即公開查詢
■涉及專利或其他智慧財產權，□一年■二年後可公開查詢
2.「本研究」是否已有嚴重損及公共利益之發現：■否 □是
3.「本報告」是否建議提供政府單位施政參考 ■否 □是，____（請列舉提供之單位；本部不經審議，依勾選逕予轉送）

中　華　民　國 105 年 10 月 26 日

目錄……1
中文摘要及關鍵詞……2
英文摘要及關鍵詞……2
前言……3
研究方法……3
結果……6
討論……7

中文摘要

雙酚 A 已被證實具有內分泌干擾的功能。雙酚 A 的製品在日常生活用品中相當常見，如奶瓶、罐頭的內附膜、食品和飲料包裝、牙齒粘合劑與填充劑、可微波使用的塑膠制品等等。巨噬細胞為初級免疫細胞之一，在人體對抗入侵的外來病原菌、癌細胞的清除、與正常人體的發育過程中都扮演著相當重要的角色。雙酚 A 以濃度依存性與時間依存性的方式引發 RAW264.7 巨噬細胞的細胞毒性 ($p < 0.05$)。雙酚 A 以濃度依存性方式引發 RAW264.7 巨噬細胞的細胞凋亡與壞死 ($p < 0.05$)。雙酚 A 以濃度依存性方式引發 RAW264.7 巨噬細胞的 DNA fragmentation 形成，進而導致細胞凋亡 ($p < 0.05$)。利用微小核形成與慧星實驗進行分析所得結果，發現雙酚 A 以濃度依存性方式引發 RAW264.7 巨噬細胞的產生基因毒性 ($p < 0.05$)。再者，發現雙酚 A 以濃度依存性的方式，誘發細胞內超氧自由基的生成 ($p < 0.05$)。最後發現，BPA 以濃度依存性的方式誘發 caspase -3, -8, 與-9 的活性。綜合上述，BPA 經由 DNA damage 與 caspase 活化，進而導致細胞毒性與基因毒性。

關鍵字
雙酚 A; RAW264.7 巨噬細胞； 細胞毒性；基因毒性

Abstract

Products of bisphenol A (BPA), which has been identified as an endocrine disruptor, are quite common in the daily necessities, such as baby bottles, coated cans for film, food, and beverage packaging, paper coatings, dental adhesives and fillers, and the microwave plastic products. Macrophage is one of the innate leukocytes, which plays an important role in defense against invasive pathogen in vivo, defense cancer cell scavenger, and normal human developmental processes. BPA demonstrated a cytotoxic effect to RAW264.7 cells in a concentration and time-dependent manner ($p < 0.05$). BPA was found to induce apoptosis and necrosis of cell death in a concentration-dependent manner ($p < 0.05$). BPA-induced cell apoptosis was demonstrated by the increase in DNA ladder formation. In addition, BPA exhibited genotoxicity via a dose-related increase in the numbers of micronucleus and DNA strand breaks ($p < 0.05$). Generation of reactive oxygen species was induced by BPA in concentration-dependent manner ($p < 0.05$). Furthermore, the activation of caspase-3, -8, and -9 were generated by BPA in a dose-dependent manner ($p<0.05$). These results indicated that cytotoxicity and genotoxicity induced by BPA in macrophages may be via DNA damage and caspase activation.

Keywords
Bisphenol A; RAW264.7 cells; cytotoxicity; genotoxicity; caspases

1. Introduction

Bisphenol A (BPA), the high volume production chemical material, has been identified as an endocrine disruptor. Products of BPA are quite common in the daily necessities, such as baby bottles, coated cans for film, food, and beverage packaging, paper coatings, dental adhesives and fillers, and the microwave plastic products. Bisphenol A in the manufacturing process has demonstrated that dispersed into the water and air. BPA, an environmental hormone, is also a source of xenoestrogens. Dental materials, such as BisFMA, BisEMA, BisDMA, TEGDMA, HEMA, are composed by BPA. BPA release for dental material could influence health. Effects of BPA are similar to estrogen, such as promote the early arrival of female animals' puberty, lengthened animal estrus, and increase the occurrence of breast cancer. In addition, high doses of BPA may be fatal to animal. BPA was found to exhibit cytotoxicity and carcinogenesis in several various mammalian cells (Seachrist etc al, 2016).

In abnormal physiological conditions or various extracellular stimulators including DNA-damaging agents, growth factor deprivation, or death receptor activators, induces apoptosis, which is the sequential process of cell death. There are several features of apoptosis such as the exposure of phosphatidylserine (PS) on the cell surface and DNA damage. DNA damage contain two stages including chromatin loop domains are first cleavaged into DNA fragments followed by internucleosomal DNA cleavage into approximately 180 bp oligonucleosomal size. In addition, DNA damage also induced genotoxicity and cell cycle delay. Cysteinyl aspartatespecific proteinases (caspases) play the important roles in the PS exposure and DNA damage (Niida and Nakanishi, 2006). There are two processes called intrinsic and extrinsic pathways. Extrinsic pathway, activated by the death receptor stimulation, induced the intracellular molecular signaling pathway including caspase-8. Intrinsic pathway, activated by cytochrome c release via the disruption of mitochondria, leads the molecular signaling pathway including caspase-9. Caspases-8 and -9 result in the activation of their common downstream effector, caspases-3 (Suen et al., 2008).

Innate immune system, also called natural immunity, which have the non-specific first line defense against the invasive pathogens. Macrophage is one of the innate leukocytes, which plays an important role in defense against invasive pathogen in vivo, defense cancer cell scavenger, and normal human developmental processes (Kzhyshkowska et al., 2012). Up to now, the toxicological mechanisms of BPA in macrophages still remain to be elucidated. At present study, murine macrophage cell line RAW264.7 was used to estimate the effects of cytotoxicity, types of cell death, DNA damage, genotoxicity, and caspases activities stimulated by BPA.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Life Technologies (Grand Island, NY). BPA, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and phosphate buffered saline (PBS), were purchased from Sigma-Aldrich (St. Louis, MO). BPA was dissolved with DMSO and tested at concentrations of 3, 10, 30, 50, 100 μM. The final

percentage of DMSO was not higher than 0.5% (v/v).

2.2 Cytotoxicity assay

Dehydrogenase activity was measured by MTT colorimetric assay to monitor mammalian cell survival and proliferation. Cells were diluted in fresh complete medium and seeded in 96-well plates (2×10^4 cells/well). After overnight attachment, cells were treated with various concentrations of BPA for 12 hours or 24 hours, then 50 μL MTT dye was added to each well. Plates were incubated in a CO_2 incubator for 4 hours. Optical density was determined by eluting the dye with DMSO and the spectrophotometric absorbance measured at 550 nm using a microplate reader (Dynatech MR 4000; Dynatech, Boston, MA, USA)..

2.3 Detection of apoptosis and necrosis by flow cytometry

Apoptosis and necrosis were discriminated by Annexin V-FITC and propidium iodide (PI; Annexin V Assay Kits; BioVision, Mountain View, CA, USA). The processes of detection were according to manufacturer's instructions. Briefly, after incubation with serum-free medium and various concentrations of BPA for 24 hours, cells were collected by trypsination and washed once with ice-cold phosphate buffered saline, and 1×10^5 cells were incubated in 100 μL binding buffer containing Annexin V-FITC and PI for 30 minutes at room temperature in the dark. The samples were analyzed on a FACS flow cytometer (Becton, Dickinson, and Company). Data analysis was performed with CellQuest software (Becton, Dickinson, and Company), which allowed assessment of only specific populations, individualized by gates according to size granularity, and fluorescence parameters. Percentages of viable cells (annexin V−; PI–), early apoptotic cells (annexin V+; PI–), necrotic cells (annexin V−; PI+) and late apoptotic cells (annexin V+; PI+) were determined.

2.4 DNA Fragmentation Assay

Cells treated with various concentrations of BPA for 24 hr were washed with PBS and then incubated in 500 μL lysis buffer (50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 0.5% *N*-lauryl sarcosine sodium salt, 5 mg/mL proteinase K) at 37°C for 60 min. After centrifugation, cell lysates were treated with 0.1 mg/mL of RNase A and incubated at 50°C for 2 hr. DNA was extracted with chloroform:phenol:isoamylalcohol (24:25:1; v/v/v), and ethanol precipitated and resuspended in Tris-EDTA buffer (contain 1 mM EDTA, 10 mM Tris-HCl, pH 8.0). The 20 μg DNA extract and 100-bp DNA ladder marker were separated by electrophoresis on a 2% agarose gel, and visualized by ultraviolet transillumination after staining with 0.5% ethidium bromide.

2.5 Cytokinesis-block micronucleus assay

To assess the genotoxic effects of BPA in RAW264.7 cells, the cytokinesis-block micronucleus (MN) assay was performed as follows. 1×10^5 cells were plated out in 60-mm culture dishes. After 24 h, various concentrations of BPA and cytochalasin-B (Sigma Chemical Co.) were added. Following 1-h incubation period, cells were washed thrice with HBSS and further incubated in 3 mg /mL of cytochalasin-B for 17 h. Cells were then collected by trypsinization, resuspended in 75 mM

KCl for 1 min, fixed in 3 : 1 mixture of methanol/acetic acid and then spread on clean dry slides. The slides were then stained with 3% Giemsa (pH 6.4) solution. MN was analyzed microscopically in 1000 binucleated cells/slide of three parallel cultures (slides) per concentration. The scoring of MN was according to the following criteria: the diameter of the MN must be no larger than one-third of that of the main nuclei. They must be nonrefractile. The color must be the same as or brighter than that of the main nuclei. MN must be located within the cytoplasm but not in contact with the main nuclei. If there is any overlapping of the two main nuclei in binucleated cells, it was not scored.

2.6 Cell cycle analysis

As a parameter for cytotoxicity and mitotic activity, the cytokinesis-block proliferation index (CBPI) was determined in each treatment. The numbers of mononucleated, binucleated and polynucleated cells per 500 consecutive cells were counted for cell cycle kinetic analysis. Most often, a CBPI is used to indicate the average number of cell cycles in a given cell, and the calculation formula used is as following: CBPI = [M1 + 2M2 + 3(M3 + M4)]/N, where M1, M2, M3 and M4 correspond to the number of cells with one, two, three and four nuclei, and N is the total number of cells.

2.7 Alkaline single-cell gel electrophoresis

DNA damage in RAW264.7 cells was analyzed by using alkaline comet assay according to the method. 1×10^5 cells were cultured in 6-well plates and treated with BPA at various concentrations for 1 h. Slides were prepared in duplicate. 1×10^5 cells mixed with 1% low-melting-point agarose (Sigma Chemical Co.) were placed on a microscope slide that had been pre-coated with 1% normal-melting-point agarose (Sigma Chemical Co.), covered with coverslip and was kept on ice. After gently removing the coverslip, the slides were immediately submersed in lysis solution (2.5 mM NaCl, 100 mM EDTA, 10 mM Tris, 1% Tritron X-100, 200 mM NaOH, 34.1 mM N-lauroyl sarcosine and 10% DMSO, pH 10) at 4 °C for 1 h in the dark and then washed with cold PBS solution. Afterwards, the slides were placed in a horizontal electrophoresis tank filled
with electrophoresis buffer (300 mM NaOH and 1 mM EDTA) for 40 min at 4°C for DNA unwinding and denaturation. Electrophoresis was carried out for 30 min at 1.2 V/cm. All steps were performed in red light to reduce additional lightinduced DNA damage. After electrophoresis, the slides were washed thrice with a neutralization buffer (0.4 M Tris–HCl, pH 7.5), dried, stained with ethidium bromide (20 μg/mL) and then analysed by image analysis system Comet version 3 (Kinetic Imaging Ltd., Liverpool, UK). To quantify DNA damage, the following comet parameters were evaluated: percentage of DNA in the tail (tail DNA %; relative fluorescence intensity of tail), tail length (distance from head centre to the end of the tail; in lm) and tail moment (TM), which was calculated as tail length percentage of DNA in tail (in arbitrary units).

2.8 Fluorometric Assay for Caspase Activities

Caspase activity was assayed with a caspase fluorometric assay kit (Enzo Life Sciences, Plymouth Meeting, PA). Briefly, the 100 μg cell lysates from each sample was mixed with a reaction buffer

containing the caspase-3, -8, and -9 fluorogenic substrates, which are DEVD-AFC, IETD-AFC, and LEHD-AFC. The data were collected by using a fluorescence microplate reader (Molecular Devices, CA) at excitation emission wavelengths of 400/505 nm. To compare the caspase activity levels among various experimental groups, the fold of increases in caspase activity was determined by comparing the absorbance from each apoptotic sample with that from the corresponding untreated control.

2.9 Measurement of Reactive Oxygen Species Generation

The semiquantitative dichlorfluorescein-diacetate (DCFH-DA) fluorescence technique was used to detect the intracellular level of reactive oxygen species (ROS). . In a black 96-well plate, 5×10^5cells were grown for 24 h and then incubated with 20 lM DCFH-DA for 30 min in the dark. At the end of DCFH-DA incubation, cells were washed with PBS then treated with different concentrations of BPA for 1 h. After washing, the formation of fluorescent dichlorofluoroscein, which is the oxidized product of DCFH-DA in the presence of several ROS, principally hydroperoxide, was measured using the fluorescence microplate reader (Molecular Devices, CA) at excitation/emission wavelengths of 400/505 nm. Results were expressed as the fluorescence intensity.

2.10 Statistical analysis

All data were expressed as mean ± standard deviation from the mean (SD). Statistical analyses were performed using anova followed by the Bonferroni's t-test for multi-group comparisons test; $P < 0.05$ was considered significant for all tests.

3. Results

3.1. Cytotoxicity of BPA

The MTT assay is a colorimetric assay based on the ability of the viable cells to reduce a soluble yellow tetrazolium salt to blue formazan crystals. As shown in Fig. 1, BPA demonstrated a cytotoxic effect on CHO cells. Cytotoxicity was induced by BPA in a dose-dependent and a time-dependent manner ($p < 0.05$).

3.2. Effects of BPA on Necrosis and Apoptosis

To study the type of cell death caused by BPA, RAW264.7 cells were distinguished by annexin V-FITC and PI via flow cytometer (Fig. 2). The percentages of late apoptotic cells (up right phase; PI+, annexin V+), necrotic cells (up left phase; PI+, annexin V-), viable cells (low left phase; PI-, annexin V-), and early apoptotic cells (low right phase; PI-, annexin V +) were determined. BPA was found to induce both necrosis and total apoptosis in a concentration-dependent manner ($p<0.05$).

3.3 Effects of BPA on DNA Fragmentation

Chromatin condensation, a feature of apoptosis, was detected by DNA fragmentation. The

total DNA extracted from RAW264.7 without BPA stimulation did not show any sign of DNA fragmentation. The concentrations of BPA higher than 30 μM was found to display DNA ladder formation (Fig. 3).

3.4. Determination of DNA Damage and Cell Cycle Delay by MN Assay

The MN generated by BPA is shown in Table I. Our data demonstrated that there was a concentration increase in the numbers of MN ($p<0.05$). In addition, the cell cycle kinetics of RAW264.7 cells was significantly reduced by BPA in a concentration-dependent manner ($p<0.05$).

3.5 Assessment of DNA Damage by Comet Assay

As shown in Figure 3, BPA-induced DNA injury was measured by comet assay. Images of cells with increasing levels of DNA damage in comet assay are shown in Figure 4A. The concentrations of BPA higher than 30 μM, tail moment (Fig. 4B) and length (Fig. 4C) significantly increased DNA damage as compared with control groups in a concentration-dependent manner ($p <0.05$).

3.6 Effects of BPA on Generation of ROS

To examine whether BPA interfere with the assay for reactive oxygen species (ROS), BPA was administered to a reaction solution containing DCFH-DA, BPA did not directly interact with DCFH-DA. The levels of ROS induced by BPA in a concentration-dependent manner ($p <0.05$) (Fig 5).

3.7 Effects of BPA on Caspases Activity

Caspases activities were assessed by fluorometric assay kit. BPA was demonstrated to significantly upregulate caspase-3, -8, and -9 activities in a concentration-dependent manner ($p <0.05$) (Fig 6).

4. Discussion

BPA is usually used to make food-packaging materials and dental sealants and other applications in the worldwide. Therefore, human could be exposed routinely to BPA from various sources. Firstly, BPA is oxidized to BPA semiquinone, and then converted into BPA-3, 4-quinone by potassium nitrodisulfonate. BPA transfered to semiquinone derivatives is mediated by ultraviolet irradiation, which homolytic cleavage of the C-CH3 bond in the isopropyl group of BPA. However, BPA cannot be converted to semiquinones by ultraviolet irradiation via oral administration in biological systems. The formation of BPA semiquinones and related derivatives through binding GSH, which the enzymatic decomposition of BPA may cause cytotoxicity as a result of the formation of DNA-binding metabolites (Peltonen et al., 1986; Atkinson et al., 195). Macrophage is one of the innate important leukocytes, which plays the important role for defense against invasive pathogen in vivo, defense cancer cell scavenger, and normal human developmental processes (Kzhyshkowska et al., 2012). At present, we purposed the toxicological mechanisms of BPA in

RAW264.7 macrophages. RAW264.7 murine macrophage cell line has been used for immunocytotoxicity testing because of the cytotoxic response directly in the cell culture in the presence of many different chemical agents. Therefore, RAW264.7 cells were selected in the present study.

Our results have shown that BPA exhibited a dose- and time-dependent cytotoxicity to RAW 264.7 cells. Apoptosis and necrosis have long been considered as two distinct mechanisms of cell death. In this study, BPA was found to induce two modes of cell death. BPA is able to induce both modes of cell death but at different concentrations that apoptosis is induced at lower concentration and necrosis at higher concentration. An important purpose of this study is using comet and MN assay to predict BPA genotoxicity in RAW264.7 cells. Comet assay not only identifies reparable injuries or alkalilabel sites but also permits direct measurement of DNA strand breaking capacity of a tested agent. Conversely, MN assay detects injuries that survive at least one mitotic cycle and allows for measurement of chromosome breakage or chromosome loss. Therefore, the combination of both assays for the testing of genotoxins to understand the mechanisms underlying mutagenicity and to assess the lowest efficient dosage. In our study, both assays showed a significant DNA damage of RAW264.7 cells in a dose-dependent manner, which strongly suggested a positive relationship between primary DNA damage and chromosomal breakage or loss. At present, we found BPA is an agent able to exert genotoxic effects on mammalian cells. From CBPI value, the results showed that the prolongation of cell cycle as indicated by the decreased CBPI was observed when treated with BPA. As cell growth is strictly regulated by cell cycle progression, induced cell cycle disturbance by toxic chemicals will usually lead to growth retardation.

ROS and caspases play the important role in the apoptosis and DNA damage. At present study, we also found BPA-induced ROS generation and caspases activation, including caspase-3, -8, -9. In conclusion, BPA-induced cytotoxicity and genotoxicity via DNA damage, ROS generation, and caspase activation.

References

Atkinson A and Roy D. 1995. In vitro conversion of environmental estrogenic chemical bisphenol A to DNA binding metabolite(s). Biochem Biophys Res Commun 210: 424-433.

Kzhyshkowska J, Neyen C, Gordon S. 2012. Role of macrophage scavenger receptors in atherosclerosis. Immunobiol 217:492–502.

Niida H, Nakanishi M. 2006. DNA damage checkpoints in mammals. Mutagenesis 21:3–9.

Peltonen K, Zitting A, Koskinen H and Itkonen A. 1986. Free radicals from photodecomposition of bisphenol-A. Photochem Photobiol 43: 481-484.

Seachrist DD, Bonk KW, Ho SM, Prins GS, Soto AM, Keri RA. A review of the carcinogenic potential of bisphenol A. Reprod Toxicol. 2016; 59: 167-82.

Suen DF, Norris KL, Youle RJ. 2008. Mitochondrial dynamics and apoptosis. Genes Dev 22:1577–1590

Table 1 Both the calculated CBPI values and the frequency of MN expressed a dose-dependent tendency

BPA (μM)	MN	CPBI
0	6.89 ± 2.47	1.86 ± 0.04
3	10.25 ± 1.18	1.75 ± 0.06
10	21.15 ± 2.3	1.67 ± 0.06
30	22.7 ± 1.36	1.48 ± 0.02
50	27.08 ± 1.26	1.12 ± 0.03

Figure legends

Figure 1 BPA was found to induce cytotoxicity in RAW264.7 macrophages. Cytotoxicity was measured by MTT colorimetric assay after indicated times with 3-100 μM BPA. Values were expressed as a percentage of vehicle treated control group. Results are expressed as means ± SD (n=3). *p<0.05 compares with controls.

Figure 2 BPA was demonstrated to induce apoptosis and necrosis in RAW264.7 macrophages. Cells were treated with BPA at 3-100 μM for 2 hr. They were collected, stained by PI and annexin V, and analyzed by dual staining flow cytometry. Data are expressed as means ± SD (n=3-4). *p<0.05 compares with controls.

Figure 3 BPA was demonstrated to induce DNA fragmentation in RAW264.7 macrophages. Chromosomal DNA was isolated and analyzed by agarose gel electrophoresis and ethidium bromide staining.

Figure 4 BPA was found to induce DNA damage in RAW264.7 macrophages. DNA damage are quantification of tail moment and tail length, respectively; data are expressed as means ± SD (n=3). *p<0.05 compares with controls.

Figure 5 BPA was found to induce ROS generation in RAW264.7 macrophages. Values were expressed as a percentage of vehicle treated control group. Results are expressed as means ± SD (n=3). *p<0.05 compares with controls.

Figure 6 BPA was found to induce caspases activation and procaspases cleavage. The activation of caspase-3, -8, and -9 were induced by various concentrations of BPA. Data are expressed as means ± SD (n=4–5). *p<0.05 compares with controls.

Fig 1

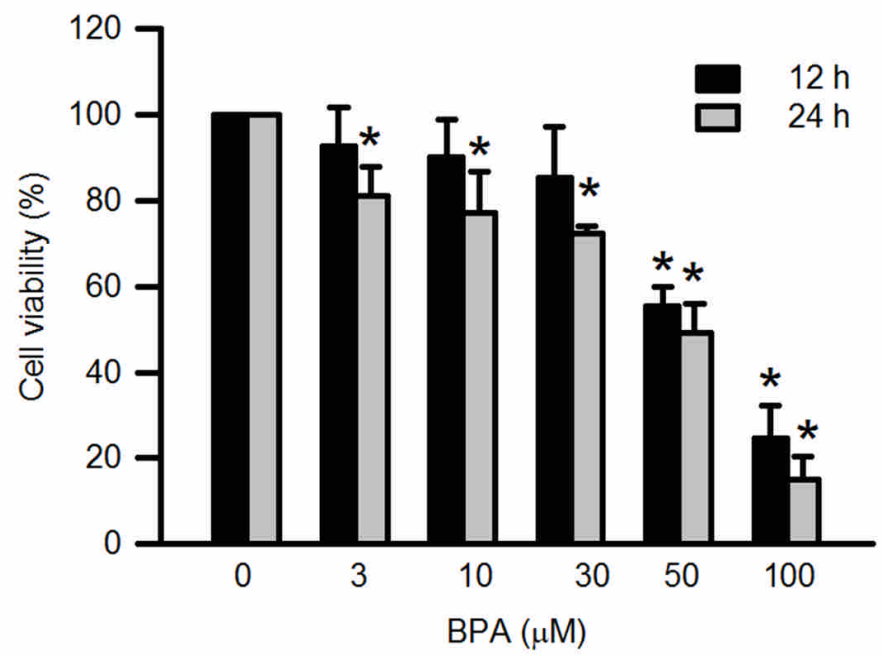

120
100
80
60
40
20
0
Cell viability (%)
12 h
24 h
*
0
3
10
30
50
100
BPA (μM)

Fig 2

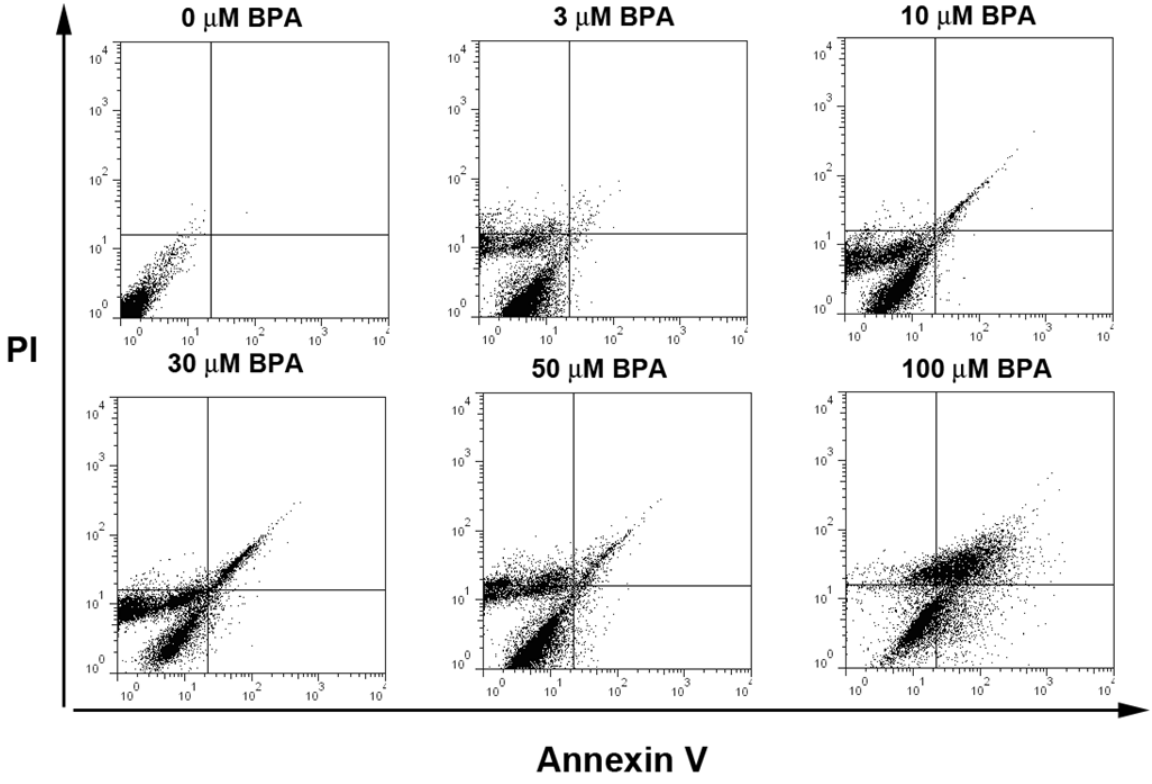

0 μM BPA
3 μM BPA
10 μM BPA
30 μM BPA
50 μM BPA
100 μM BPA
PI
Annexin V

Fig 3

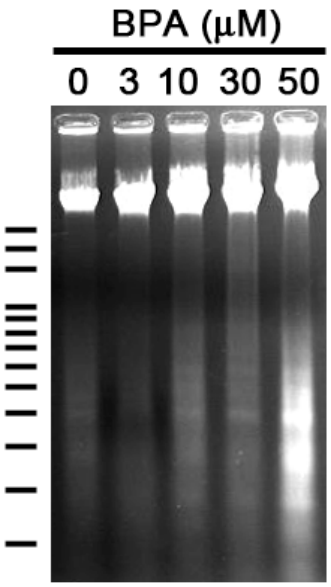
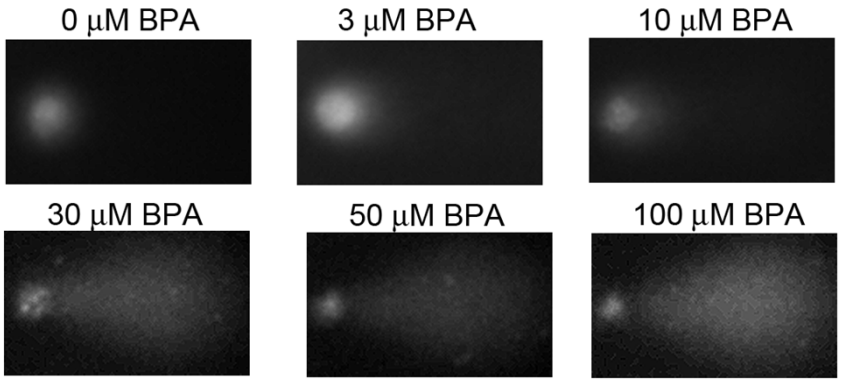
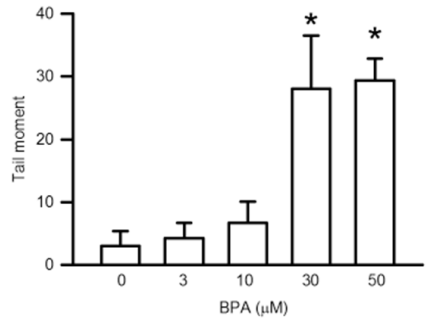

BPA (μM)
0 3 10 30 50

Fig 4


0 μM BPA
3 μM BPA
10 μM BPA
30 μM BPA
50 μM BPA
100 μM BPA


Tail moment
40
30
20
10
0
0 3 10 30 50
BPA (μM)
*
*

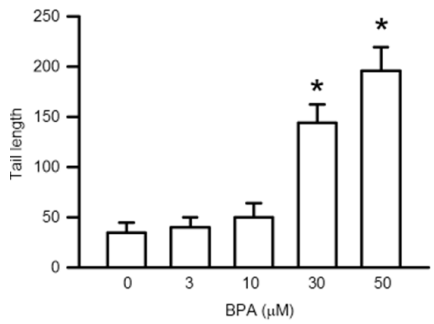

Tail length
250
200
150
100
50
0
0 3 10 30 50
BPA (μM)
*
*

Fig 5

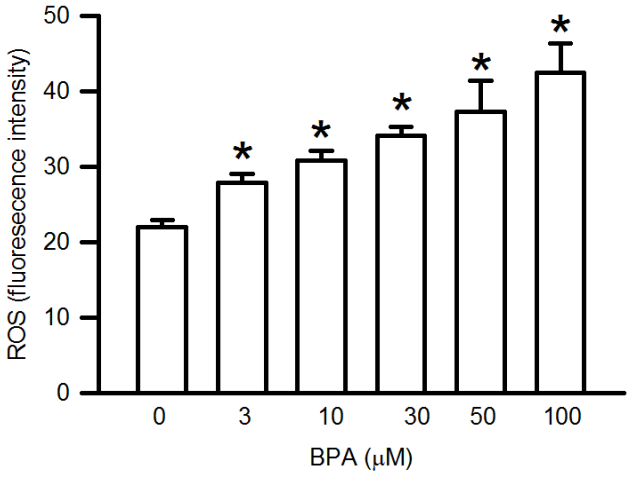
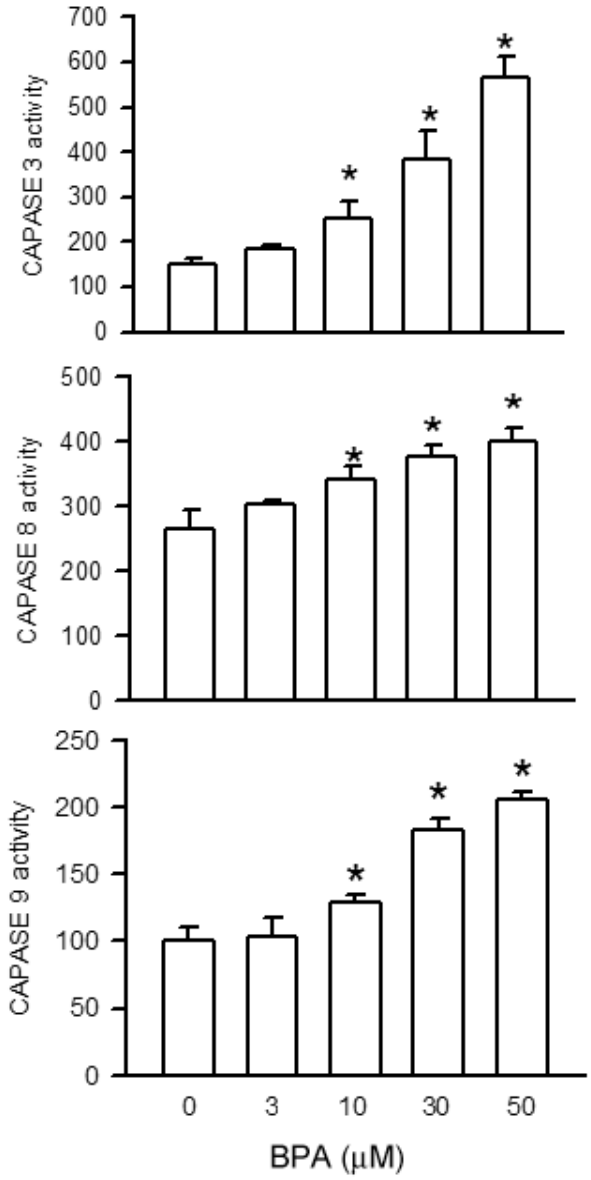

ROS (fluoresecence intensity)
50
40
30
20
10
0
*
*
*
*
*
0
3
10
30
50
100
BPA (μM)

Fig 6


CAPASE 3 activity
700
600
500
400
300
200
100
0
*
*
*
CAPASE 8 activity
500
400
300
200
100
0
*
*
*
CAPASE 9 activity
250
200
150
100
50
0
*
*
*
0
3
10
30
50
BPA (μM)

科技部補助計畫衍生研發成果推廣資料表

日期:2016/10/28

科技部補助計畫	計畫名稱: 研究雙酚A誘發巨噬細胞的基因毒性與細胞毒性作用機轉
	計畫主持人: 黃富美
	計畫編號: 103-2314-B-040-018- 學門領域: 牙醫學

無研發成果推廣資料

103年度專題研究計畫成果彙整表

計畫主持人：黃富美						**計畫編號：**103-2314-B-040-018-	
計畫名稱：研究雙酚A誘發巨噬細胞的基因毒性與細胞毒性作用機轉							
成果項目						量化	單位	質化 （說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等）
國內	學術性論文	期刊論文				0	篇	
		研討會論文				0		
		專書				0	本	
		專書論文				0	章	
		技術報告				0	篇	
		其他				0	篇	
	智慧財產權及成果	專利權	發明專利	申請中		0	件	
				已獲得		0		
			新型/設計專利			0		
		商標權				0		
		營業秘密				0		
		積體電路電路布局權				0		
		著作權				0		
		品種權				0		
		其他				0		
	技術移轉	件數				0	件	
		收入				0	千元	
國外	學術性論文	期刊論文				0	篇	
		研討會論文				1		#0874 Cytotoxicity and Genotoxicity of Bisphenol A in Macrophages IADR/AADR/CADR General Session & Exhibition (March 11-14, 2015) (Boston, Massachusetts) March 11-14, 2015, Boston, Massachusetts
		專書				0	本	
		專書論文				0	章	
		技術報告				0	篇	
		其他				0	篇	
	智慧財產權及成果	專利權	發明專利	申請中		0	件	
				已獲得		0		
			新型/設計專利			0		
		商標權				0		

		營業秘密	0		
		積體電路電路布局權	0		
		著作權	0		
		品種權	0		
		其他	0		
	技術移轉	件數	0	件	
		收入	0	千元	
參與計畫人力	本國籍	大專生	0	人次	
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
	非本國籍	大專生	0		
		碩士生	0		
		博士生	0		
		博士後研究員	0		
		專任助理	0		
其他成果 （無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。）					

科技部補助專題研究計畫成果自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否具有政策應用參考價值及具影響公共利益之重大發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估
   ■達成目標
   □未達成目標（請說明，以100字為限）
   　□實驗失敗
   　□因故實驗中斷
   　□其他原因
   說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）
   論文：□已發表　□未發表之文稿　■撰寫中　□無
   專利：□已獲得　□申請中　■無
   技轉：□已技轉　□洽談中　■無
   其他：（以200字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性，以500字為限）
   藉由此計畫，能有更深入的瞭解BPA 引發巨噬細胞的活化促發炎的機轉，進一步研究如何預防BPA對人體危害。發表論文於國際期刊，以提升台灣國際學術地位。

4. 主要發現
   本研究具有政策應用參考價值：■否　□是，建議提供機關
   （勾選「是」者，請列舉建議可提供施政參考之業務主管機關）
   本研究具影響公共利益之重大發現：■否　□是
   說明：（以150字為限）