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計畫名稱：Scavenger Effect of Benzophenones on the Oxidative Processes of Menadione-induced Cytotoxicity

(英、中文) 雙苯基酮類化合物對新生老鼠肌母細胞自由基之抑制作用

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中文摘要：

關鍵詞：雙苯基酮類化合物、自由基、肌母細胞

雙苯基酮類化合物具有吸收紫外線之功能且已被工業及醫學界廣泛使用達 30 **年之久。由於雙苯基酮類化合物具有吸收紫外線之功能因而被廣泛使用於化妝品與防曬油，因此消費者長期暴露於雙苯基酮類化合物而不自知。然而雙苯基酮類化合物對於抗氧化作用如何至今仍未有學者深入探討；本研究即針對六種不同防曬油中具有雙苯基酮類化合物之抗氧化作用深入探討。**

初代之肌母細胞與 100 μM menadione **共同培養。**Menadione **所引發之細胞毒性則由** lucigenin **或** luminol **加以放大後之激光反應、**MTT **檢測，並同時探討各種不同雙苯基酮類化合物之抗氧化作用。**

結果顯示在肌母細胞與100 μM menadione**共同培養可引發**superoxide **及** hydrogen peroxide**之製造，並導致肌母細胞之氧化壓力；而此種氧化壓力可以藉由**superoxide dismutase (SOD)**及**catalase**加以清除。 而**catalase **對** menadione **引發之細胞毒性有顯著之保護作用，反之**SOD**對細胞毒性之保護作用並不明顯 雙苯基酮類化合物對於由**menadione**引發之肌母細胞細胞死亡有顯著之保護作用。此種保護作用在具有**ortho-dihydroxyl **結構且此種**ortho-dihydroxyl **結構位於同一苯環上時其保護效果更加明顯 雙苯基酮類化合物氧化壓力抗之細胞保護效果須要進一步研究釐清。**

英文摘要：

ABSTRACT

Benzophenone is an UV-absorbing agent that has been used in industry and medicine for more than 30 years. Consumers of cosmetics and sunscreen as UV-absorber are exposed to benzophenones on a daily basis owing to the widespread use of these compounds. However, the efficacy about these compounds on the scavenger effects of oxidative stress is still not well established. In the present study, we investigate the anti-oxidative capacity of six sunscreen benzophenone compounds.

Primary myoblasts culture was mixed in vitro with 100 μM menadione. The cytotoxic effect by menadione-induced oxidative stress was monitored by the lucigenin- or luminol-amplified chemiluminescence, MTT assay, and the anti-oxidative effects of various benzophenone compounds were evaluated.

The result showed that the adding of menadione can induce an oxidative stress on the myoblasts by superoxide and hydrogen peroxide production, which can be eradicated by superoxide dismutase (SOD) and catalase respectively in a dose dependent mode. The catalase has a protective effect on the cytotoxicity induced by menadion as measured by the MTT assay, while the SOD does not. The selected benzophenones also have significant scavenger effect on the menadione-induced cell death on the myoblasts. The ortho-dihydroxyl structure and more hydroxy groups in the same ring have the stronger scavenger effect on superoxide anion on myoblasts, and thus a stable penoxy radical may formed. The mechanism of this effect remains to be clarified.

KeyWords: benzophenones, free radicals, myoblasts.

INTRODUCTION

Oxygen-derived free radical injury has been associated with several cytopathic conditions.[1] This includes a decrease in cell redox capacity as observed in connection with cell aging and certain genetic diseases.[2-4] Oxidative stress has also been suggested to determine membrane alterations, including both lipid peroxidation and modifications of membrane fluidity accompanied by a parallel increase in the intracellular calcium ion concentration.[5] Quinones (e.g. phenanthrenequinone) and aromatic hydrocarbons are also prevalent as ubiquitous environmental pollutants formed by various combustion processes, e.g. cigarette smoke, automobile exhaust, diesel exhaust and urban air particulates. The industrial solvent and gasoline component benzene is thought to be carcinogenic by being metabolized to phenol and hydroquinone in the liver. The hydroquinone may then undergo activation by oxidation to benzoquinone by prostaglandin synthetase and/or myeloperoxidase in the bone marrow.[6] In particular, the naphthoquinone menadione was demonstrated to be reduced to a semiquinone radical, which forms superoxide anion radicals that induce a progressive impairment of several cellular processes.[4]

Hundreds of naturally occurred quinones have been isolated from biological tissue. Some quinones (e.g. ubiquinones) have important roles in the biochemistry of energy production and serve as vital link in electron transport. Other quionones have been attributed to a defense role as a result of their effectiveness at inhibiting the growth of bacteria, fungi, and parasites.[5] Phenolic compounds has been found among the more active inhibitor in the studies of anti-oxidants.[7-9] Benzophenone is an UV-absorbing agents that has been used in industry and medicine for more than 30 years. Consumers of cosmetics and sunscreen as UV-absorber are exposed to benzophenones on a daily basis owing to the widespread use of these compounds in many of the products on the market, such as lipsticks, hair sprays, hair dyes, shampoo and detergent bars and sunscreen lotions.[10] However, the efficacy and mechanism about these compounds on the scavenger effects of oxidative stress is still not well established. In the present study, we investigate the capacity of six sunscreen compounds to protect myoblasts from the oxidative stress induced by menadione.

MATERIALS AND METHODS

Culture of myoblasts

The method of enzymatic digestion of myogenic cells was similar to that of Bischoff with some modification.[11] Briefly, newborn Wistar-rat of both sexes were anesthetized by pentothal (25 mg/100gm, intra-peritoneal injection). The rat was prepared and disinfected. The skeletal muscles of hindlimbs were excised and rinsed several times with sterile normal saline solution. Muscle tissue was then minced into 1-mm fragments. The tissue fragments were trypsinize at 37^{o}C for two times for 30 minutes with 0.25 % trypsin (GIBCO BRL, Life Technologies Inc., Grand Island, NY, USA). After each digestion, the fragments were removed by low speed centrifugation (500 x g for 1 min.) and transferred to fresh medium for further dissociation. The supernatant containing the liberated cells was centrifuged again (1500 x g for 3 min.) to pellet the cells and large debris while leaving most of the myofibrils in suspension. The pellet was suspended in fresh medium and aliquots were transferred to culture dishes. Enrichment of the cell population for muscle is accomplished by pre-plating the cells at 37^{o}C for 30 minutes on a non-coated dish. This study received prior approval of the National Taiwan University Medical College's Animal Research Committee.

After the cells grew 80% confluence, primary myoblasts were resuspended in phenol-red free PBS at 1 x 10^5 cells per ml or cultured in 10% FBS-DMEM with 9.4 cm^2 culture dishes (Corning, NY, USA), or 96-well flat-bottomed plates with the density of 1.0 x 10^4 cells /cm^2. The culture dishes were incubated at 37^{o}C in an atmosphere supplemented with 5% CO_2 for 2 days and chemiluminescence was measured.

Menadione treatment

Fourty-eight hours after seeding, the culture medium was replaced with a phosphate buffered saline (PBS, supplemented with 1 mM $CaCl_2$ and $MgCl_2$, pH 7.3). Myoblasts were treated with various concentration of menadione [0, 10 μ M, 20 μ M, 100 μ M, 200 M, 2 mM, 20 mM, and 200mM menadione (2-methyl-1,4-naphthoquinone, Sigma) diluted in dimethyl sulphoxide (DMSO) by adding 200X stock solution] for 24 hours. Cells treated with equal amounts of vehicle alone were considered as controls.

In the pilot study, the minimal toxic dosage levels of menadione were first established using MTT assay.[12] MTT assay results showed that menadione at concentration of 100 μM showed its maximal toxicity to myoblasts. Therefore menadione level of 100 μM was tested in this experiment for various periods of incubation (0.5, 1.0, 3.0, 6.0, 9.0, 12, 18, 24 hours). To determine the effect of cytotoxicity on menadione-induced injury, assays were carried out as described below.

Colorimetric MTT (Tetrazolium) assay for cell viability[12]

The mitochondrial activity of the myoblasts after exposure to menadione was determined by colorometric assay which detects the conversion of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT, Sigma catalog no. M2128) to formazan. For the assay, 2.5 x 10^4 cells per well were incubated (5% CO_2, 37^{o}C) in the presence of menadione. After various time intervals the supernatant was removed, 100 μl per well of an MTT solution (1 mg /ml in test medium) was added and the wells were incubated at 37^{o}C for 4 h to allow the formation of formazan crystal. Again the supernatant was removed and acid-isopropanol (100 μl of 0.04 N HCl in isopropanol) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After a few minutes at

room temperature to ensure that all crystals were dissolved, the plates were read on Microelisa reader (Emax Science Corp, USA), using a test wavelength of 570 nm against a reference wavelength of 690 nm. Plates were normally read within 1 h after adding the isopropanol.

Determination of free radical production by luminol- and lucigenin-dependent chemiluminescence:

The measurement of luminol- and lucigenin-dependent chemiluminescence was similar to that described previously.[13] Briefly, primary myoblasts were resuspended and cultured in 10% FBS-DMEM with 9.4 cm^2 culture dishes (Corning, NY, USA) for 48 hours at the seeding density 6.25 x 10^4 myoblasts in 3.0 ml DMEM. After washing with PBS solution, myoblasts were incubated with menadione (100 μM). The chemiluminescence was then measured in an absolutely dark chamber of the Chemiluminescence Analyzing System (Tohoku Electronic Industrial Co., Sendai, Japan). At 100 second time point, 1.0 ml of 0.01 mM lucigenin (bis-N-methylacridinium nitrate, Sigma Co., U.S.A.) or 10^{-4} M luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma Co., U.S.A.) in PBS (pH = 7.4) was injected into the cell. The chemiluminescence in the sample was continuously measured for a total 600 seconds' time period. The total amount of chemiluminescence was calculated by integrating the area under the curve and subtracting it from the background level, which was equivalent to the dark average. The assay was performed in duplicate for each sample and was expressed as chemiluminescence counts/ 10 sec.

Scavenger effect of benzophenones, superoxide dismutase (SOD), and catalase

Eight different benzophenones were tested in this experiments. They are:
2,2'-Dihydroxybenzophenone (Aldrich, D11,020-5; M.W. =214.22),
2,4-Dihydroxybenzophenone (Fluka, 37610; M.W. =214.22),
4,4'-Dihydroxybenzophenone (Fluka, 37613; M.W. =214.22),
2,3,4-Trihydroxybenzophenone (Aldrich, 26,057-6; M.W. =230.22),
2,4,4'-Trihydroxybenzophenone (Aldrich, T6,570-6; M.W. =230.22),
2,2',4,4'-Tetrahydroxybenzophenone (Lancaster, 1567; M.W. =246.22),
2,3',4,4'-Tetrahydroxybenzophenone (Tokyo Kasei, T1371; M.W. =246.22),
3,4,5,2',3',4'-Hexahydroxybenzophenone (Janssen, 27.994.58; M.W. =278.22).

In the pilot study, benzophenones were found to have minimal effect on the viability to myoblasts culture when the concentrations were less than 25 μM (data not shown).

To test the scavenger effects of various antioxidant, superoxide dismutase (SOD, Sigma Corp., USA: 0.1 U/ml, 0.3 U/ml, 0.5 U/ml, 1.0 U/ml, and 5 U/ml), catalase (Sigma Corp., USA: 0.5 U/ml, 5 U/ml, 50 U/ml, and 500 U/ml), or different benzophenones (0.01 μM, 0.1 μM, 1.0 μM,10 μM, and 25 μM) was added 30 minutes before the menadione treatment. Then the scavenger effect of SOD, catalase, and different benzophenones on menadione-induced lucigenin-amplified chemiluminescence were determined. The benzophenones which have obvious quenching effect on the menadione-induced chemiluminescence were further examined. The scavenger effect on menadione-induced cytotoxicity were evaluated by MTT test as described before.

Statistical analysis

The data were evaluated by using analysis of variance (one way ANOVA) and student t test. Differences were considered significant if $P <$

0.05.

RESULTS

MTT is a pale yellow substrate that produces a dark blue formazan product when incubated with living cells. MTT ring is cleaved in active mitochondria, and the reaction occurs only in living cells.[12] Figures 1 and 2 show the effect of menadione on myoblasts viability measured by MTT assay. When cultured with menadione for 24 hours, even low concentration of menadione (10 μM) showed toxicity to the myoblasts; when the concentration of the myoblasts increased to 100 μM, the viability of osteoblasts nearly totally lost. In this study, we selected the concentration of 100 μM to produce the oxidative stress on the myoblasts. In the presence of 100 μM menadione, the viability of the myoblasts significantly decreased even after 0.5 hours′ culture; the viability of the myoblasts decreased significantly and reached to its maximal effect at 6 hours′ culture.

Luminol- and lucigenin-dependent chemiluminescence (CL):

The oxidative stress induced by menadione was shown by measurement of the chemiluminescence amplified by the lucigenin and luminol. In the control myoblasts without menadione treatment, the lucigenin or luminol-amplified chemiluminescence was quite low and near the backgrond level. After adding the menadione, the lucigenin or luminol-amplified chemiluminescences were significantly increased. The amplified chemiluminescence is more prominent by the lucigenin. The amplified chemiluminescence can be reduced by the pretreatment of the SOD and catalase in a dose-response pattern. After treatment with 100 μM menadione, the lucigenin-amplified CL levels increased significantly. The CL can be reduced significantly by SOD pretreatment (Table 1). There is a statistically significant difference existed in these measurements ($P<0.00001$ by ANOVA test). After treatment with 100 μM menadione, the luminol-amplified CL levels also increased significantly. The luminol-amplified CL can be reduced significantly by catalase pretreatment ($P<0.0001$) (Table 1). The SOD had a better scavenger effect on the myoblasts when the myoblasts is under the oxidative stress of menadione.

As shown in the above, the amplified chemiluminescence of myoblasts, is more prominent by the lucigenin and SOD had a better scavenger effect on the myoblasts. The scavenger effect of benzophenones on the menadione-induced oxidative stress was mainly evaluated by the lucigenin-induced chemiluminescence. Four of eight benzophenone compounds used in this study showed significant scavenger effect on the menadione-induced oxidative stress, they are 2,3,4-THBP, 2,3',4,4'-THBP, 3,4,5,2',3',4'-HHBP (Table 2). Further study on the cell viability was then focuses on these three compounds.

MTT assay for cell viability

After treatment with 100 μM menadione for 0.5 hr, the optic density (O.D.) of the MTT test in myoblasts can be decreased significantly. The scavenger effect of SOD on the menadion-induced oxidative stress on myoblasts was not significant ($P>0.05$). When myoblasts pretreated with 5 U/ml SOD then in 100 μM menadione for 0.5 hr, the viability was only 67.24% of the control; while if for 3.0 hr, the viability decreased to 24.87%. The scavenger effect of catalase on the menadion-induced oxidative stress on myoblasts was quite significant ($P<0.0001$). When myoblasts treated with 100 μM menadione for 0.5 hr, pretreatment of 500 U/ml catalase can increased the viability of myoblasts the viability to 100.10% of the control; while for 3.0 hr menadione treatment, catalase still can protect myoblasts from menadione induced oxidative stress.

The viability increased to 46.10% when pre-treated with 500 U/ml catalase. In this experimental condition, the catalase had a positive effect on the viability of the myoblasts in the oxidative stress of the menadione.

As shown in Table 3, the selected benzophenones also have significant scavenger effect on the menadione-induced (100 μM menadione) cell death on the myoblasts. In 0.5 hr's menadion treatment, benzophenone compound can increase the viability upto 92.70 - 100.12%; while in 3.0 hrs' menadione treatment, the viability increased to 39.42 - 41.70%. The differences existed in these measurements are a statistically significant (Table 3).

DISCUSSION

Reactive oxygen species can be generated either during the physiological metabolism of the cell or following the bio-transformation of several drugs and chemicals. Among these, quinones have been extensively investigated.[13,14] Menadione is a redox-cycling quinone which has been shown to lead to oxidative stress[5,15,16] followed by a progressive impairment of cellular processes which eventually lead to cytotoxicity.[4,13,17,18] In this study, when myoblasts cultured with menadione for 24 hours, even low concentration of menadione (10 μM) showed cytotoxicity and when the concentration increased to 100 μM, the viability of myoblasts nearly totally lost. We selected the concentration of 100 μM to produce the oxidative stress on the myoblasts. At 100 μM menadione, the viability of the myoblasts significantly decreased even after 0.5 hours′ culture; the viability of the myoblasts decreased significantly and reached to its maximal effect at 6 hours′ culture.

Many phenolic compounds such as flavonoids, capasacin, thymol, euhenol … etc. have scavenger effects on the oxygen free radicals. They exerted their scavenger effects through an active anti-oxidantion,[19] inhibition of xanthine oxidase[20] or prostaglandin cyclooxygenase.[21] Phenolic compounds has a wide applications in clinical medicine. Many non-steroid anti-inflammatory drug such ketoprofen, paclitaxel also have benzophenone or benzophenone-like chromophore.[22] In the present study, we use menadione-induced oxidative stress against the myoblasts as a model to evaluate the scavenger effect of benzophenones on the oxidative stress-induced cytotoxicity.

Chemiluminescence(CL) is defined as the light produced from chemical reactions. Cells that exhibit respiratory burst, such as phagocytes, produce chemiluminescnce. [23] The emitted light is very weak and needs the introduction of chemiluminigenic probes to increase the efficiency of light detection.[24] Luminol-dependent chemiluminescence is thought to reflect the production of hydrogen peroxide (H_2O_2) and singlet oxygen.[24] Lucigenin, on the other hand, was found to be insensitive to hypochlorite and hydroxyl radicals and to specifically measure superoxide radicals.[24] In this study, the oxidative stress induced by menadione was shown by the measurement of the chemiluminescence amplified by the lucigenin and luminol. After adding the menadione, the lucigenin- or luminol-amplified chemiluminescence was significantly increased. The amplified chemiluminescence is more prominent by the lucigenin. This is quite different to that observed in the osteoblasts, in which luminol-amplified chemiluminescence more prominent.[25] The reason for this difference is probably caused by more abundant mitochondria concentration within the myoblasts. The amplified chemiluminescence can be reduced by the pretreatment of the SOD and catalase in a dose-response pattern. After treatment with 100 μM menadione, the lucigenin-amplified CL levels increased significantly. The CL can be reduced significantly by SOD pretreatment (Table 1). The significant inhibition of lucigenin-derived chemiluminescence by superoxide dismutase indicates that superoxide has been involved in this assay system.[24,26] This was similar to that reported by Rembish and Trush that the lucigenin-induced CL was mainly used to quantitatively assess the modulation of mitochondrial superoxide generation of the mononuclear cells.[27] After treatment with 100 μM menadione, the luminol-amplified CL levels also increased significantly. The luminol-amplified CL can be reduced significantly by catalase pretreatment. The significant inhibition of luminol derived chemiluminescence by catalase indicates that H_2O_2 has been

involved in this assay system.[28] In our experiments, we added menadione to osteoblasts in the culture medium to mimic an environment of oxidative stress in vivo. Our data demonstrated that both superoxide and H_2O_2 were produced in this assay system. The SOD had a better scavenger effect on the myoblasts when the myoblasts is under the oxidative stress of menadione. In this study, four of eight benzophenone compounds used showed significant scavenger effect on the menadione-induced oxidative stress, they are 2,3,4-THBP, 2,3',4,4'-THBP, 3,4,5,2',3',4'-HHBP (Table 2). Different benzophenones have different scavenger effect on the oxidative free radicals.[20]

The conversion of the tetrazolium salt MTT into its formazan product can be cleaved in active mitochondria of living cells.[12,29]. Menadione can cause significant cellular damage in myoblasts, as evidence by the decreased cell viability measured with MTT assay. Since MTT is cleaved only by active mitochondria,[12] menadione-induced decrease of cell viability measured with MTT assay suggests that menadione may have damaged the mitochondria of myoblasts. As shown above, both SOD and catalase have a scavenger effect on the superoxide and H_2O_2 produced by menadione reaction. We performed MTT test to evaluate the scavenger effect of SOD and catalase on the viability of menadione treated myoblasts. The result showed that the scavenger effect of SOD on the myoblasts viability was not so obvious. Preatment of osteoblasts with catalase increased the amount of formazan formation in MTT test, suggesting a protective effect of catalase on mitochondria of myoblasts. Superoxide dismutase can remove the superoxide in the tissue, and the product of this reaction is H_2O_2. The increased concentration of H_2O_2 can inactivate CuZnSOD and FeSOD by removal of histidine.[28] Probably, the scavenger effect of SOD was then partially inhibited. Our data indicated that preincubation of myoblasts with catalase protect myoblasts from the cytotoxic effect induced by menadione. In this experimental condition, the catalase had a positive effect on the viability of the myoblasts in the oxidative stress of the menadione. The catalase can scavenge the end products of the reactive oxygen intermediates H_2O_2, while the end products of this reaction are H_2O and O_2. As shown in Table 3, the selected benzophenones also have significant scavenger effect on the menadione-induced (100 μM menadione) cell death on the myoblasts. The maximal scavenger effect of benzophenones is quite close that catalase (Table 3).

Menadione can induce cytotoxic effect on the myoblasts. From our study, it is quite obvious that reactive oxygen species, including superoxide, H_2O_2, and others, were produced immediately after the adding of menadione. As shown in our previous study, these reactive oxygen species may induce damage in cell membrane integrity, DNA fragmentation, and even apoptosis.[25] The naphthoquinone menadione was demonstrated to be reduced to a semiquinone radical, which forms superoxide anion radicals that induce a progressive impairment of several cellular processes.[4] As shown above, the selected benzophenones also have significant scavenger effect on the menadione-induced cell death on the myoblasts. It seems that the ortho-dihydroxyl structure and more hydroxy groups in the same ring may be contributing to the strong scavengering effect on superoxide anion on myoblasts, and thus a stable penoxy radical may formed.[30] The mechanism of this effect remains to be clarified.

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Table 1 Scavenger effect of superoxide dismutase (SOD) on the menadione-induced (100 μM menadione) oxidative stress on the myoblasts.

SOD (U/ml)	Chemiluminescence (counts/10sec) Mean (SD)	Catalase (U/ml)	Chemiluminescence (Counts/10 sec) Mean (SD)
0	2501.96 (SD: 115.12)	0	352.70 (SD: 21.72)
0.1	1917.91 (SD: 26.79)	0.5	314.55 (SD: 8.47)
0.3	673.06 (SD: 28.66)	5.0	272.92 (SD: 27.14)
0.5	428.12 (SD: 21.20)	50	197.29 (SD: 6.34)
1.0	316.22 (SD: 44.90)	500	142.18 (SD: 6.92)
5.0	137.31 (SD: 2.34)		

Note:

The differences existed between the measurement are statistically significant ($P<0.001$ by ANOVA test).

The differences existed between the measurements with SOD pretreatment (0.1, 0.3, 0.5, 1.0 and 5.0 U/ml) and without SOD pretreatment (menadione 100 μM only) are all statistically significant ($P<0.01$ by student-*t* test).

The differences existed between the measurements with catalase pretreatment (0.5, 5.0, 50, and 500 U/ml) and without catalase pretreatment (menadione 100 μM only) are all statistically significant ($P<0.01$ by student-*t* test).

Table 2 Scavenger effect of benzophenones on the menadione-induced (100 μM menadione) lucigenin-induced chemiluminescence on the myoblasts.

Compounds	% of control CL (%) Conc. Of BP (μM)			
	1	10	25	50
2,2'-DHBP	-	-	-	96.70 (SD: 4.03)
2,4-DHBP	-	-	-	-
4,4'-DHBP	-	-	-	-
2,3,4-THBP	76.90 (SD: 3.43)	51.74 (SD: 4.52)	29.07 (SD: 0.16)	21.48 (SD: 0.71)
2,4,4'-THBP	-	-	-	77.89 (SD: 0.96)
2,2',4,4'-THBP	-	-	-	-
2,3',4,4'-THBP	-	88.95 (SD: 1.96)	62.29 (SD: 1.57)	42.29 (SD: 1.19)
3,4,5,2',3',4'-HHBP	54.80 (SD: 1.20)	34.74 (SD: 0.77)	19.77 (SD: 0.52)	12.14 (SD: 0.83)

Note:
All values are presented in mean (SD: Standard. Deviation)
- : not significantly changed when compared with control chemiluminescence.
The values presented above means that there is a significant decrease in lucigenin-induced chemilunescence existed between the measurements with various benzophenones pretreatment and without benzophenones pretreatment (menadione 100 μM only) are all statistically significant ($P<0.01$ by student-*t* test.

Table 3 Scavenger effect of benzophenones on the menadione-induced (100 μM menadione) cell death on the myoblasts.

Compounds	Viability (% of control)				
	Conc. of BP (μM)				
	0	1	10	25	50
0.5 hr					
2,3,4-THBP	47.76 (SD: 2.03)	68.61 (SD: 1.94)	71.50 (SD: 2.03)	76.86 (SD:5.00)	92.70 (SD: 4.43)
2,3',4,4'-THBP	48.56 (SD:2.54)	-	65.51 (SD:0.75)	90.82 (SD:0.31)	100.12 (SD:1.39)
3,4,5,2',3',4'-HHBP	51.68 (SD:3.24)	67.91 (SD:1.99)	77.78 (SD:4.19)	83.48 (SD:3.40)	94.66 (SD:3.08)
3.0 hr					
2,3,4-THBP	28.70 (SD:0.61)	30.87 (SD:0.87)	34.06 (SD:1.09)	37.32 (SD:1.01)	39.42 (SD:1.28)
2,3',4,4'-THBP	30.13 (SD:0.44)	-	34.59 (SD:0.88)	36.73 (SD:1.72)	41.70 (SD:2.22)
3,4,5,2',3',4'-HHBP	33.33 (SD:0.64)	35.44 (SD:1.18)	36.67 (SD:0.79)	38.16 (SD:1.19)	41.14 (SD:1.22)

Note:
All values are presented in mean (SD: Standard. Deviation)
- : not significantly changed when compared with control chemiluminescence.
The values presented above means that there is a significant decrease in lucigenin-induced chemilunescence existed between the measurements with various benzophenones pretreatment and without benzophenones pretreatment (menadione 100 μM only) are all statistically significant ($P<0.01$ by student-*t* test.