嘉南藥理科技大學專題研究計畫成果報告

計畫編號：CNBT94-02

計畫名稱： 不飽和負電性微脂粒 DOPG 氧化之質譜儀分析與巨噬細胞生長活性之影響

執行期間：94 年 1 月 1 日至 94 年 12 月 31 日

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計畫總主持人：郭榮華　　計畫主持人：

子計畫主持人：郭榮華, 鄭淨月

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ABSTRACT SUMMARY:
Dioleoylphosphatidylglycerol (DOPG) containing unsaturated sites is the target of air oxidation during preparation, storage, or in vivo use of anionic liposomes. We investigated the biological effect of air oxidation of DOPG on RAW 264.7 murine macrophage-like cells. These results point to a need for precaution in formulating DOPG liposomes for drug delivery and therapeutic purposes.

INTRODUCTION:
Dioleoylphosphatidylglycerol (DOPG), structurally classified as a component of membrane phospholipids (phosphatidylglycerol), has been extensively studied in different fields of biomedical science and technology such as drug carriers, membrane models, and pulmonary surfactant for respiratory distress syndrome [1]. Phosphatidylglycerol that contains unsaturated groups as part of its molecular structure is vulnerable to oxidation during the preparation, storage, or in vivo use of liposomes [2]. The accompanying change in membrane structure may then influence the stability and biological properties of commonly used liposome formulations that contain unsaturated chains [3]. The influence of the process of lipid peroxidation on cells and liposome stability has been widely investigated, but most studies have focused on phosphatidylcholines and phosphatidylglycerol with highly unsaturated sites [4]. There is, however, no information about how cells respond to the oxidation of DOPG containing only one double bond in each of its fatty chains. Because DOPG has been widely used in biomedical applications, we wanted to investigate whether the oxidation products of DOPG are harmful to macrophages that DOPG-containing liposomes will encounter in the body during drug delivery.

EXPERIMENTAL METHODS:
The effects of air oxidation of DOPG on cytotoxicity, cell membrane integrity, cell morphology, DNA fragmentation, DNA content, and zVAD-fmk inhibition were evaluated. We found that oxidized DOPG induced apoptosis in the RAW264.7 murine macrophage-like cell line.

RESULTS AND DISCUSSION:
The product of the air oxidation of DOPG was identified as the addition of one oxygen atom to one of the symmetrical fatty moieties of DOPG at m/z 814.77 (Figure 1). The treatment of DOPG with air oxidation produced dose-dependent cytotoxicity in macrophages (Figure 2). RAW 264.7 cells exposed to oxidized DOPG exhibited

morphological features of apoptosis, such as chromatin condensation and cell shrinkage (Figure 3). Typical apoptotic ladders were observed in DNA extracted from RAW 264.7 cells treated with oxidized DOPG (Figure 4). Flow cytometric analysis demonstrated an increase in the hypodiploid DNA population (sub-G1), indicating that DNA cleavage occurred after treatment with oxidized DOPG. In addition, we showed that pretreating RAW 264.7 cells with zVAD-fmk, a general caspase inhibitor, did not prevent apoptosis induced by oxidized DOPG, suggesting that apoptosis in macrophage cells follows a caspase-independent pathway (Table 1).

CONCLUSION:

In conclusion, the present study demonstrated that air oxidation of DOPG induced apoptosis in RAW 264.7 murine macrophage-like cells. These findings have important implications for precautions in formulating DOPG liposomes for drug delivery and therapeutic purposes.

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ACKNOWLEDGEMENTS:

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Figure 1

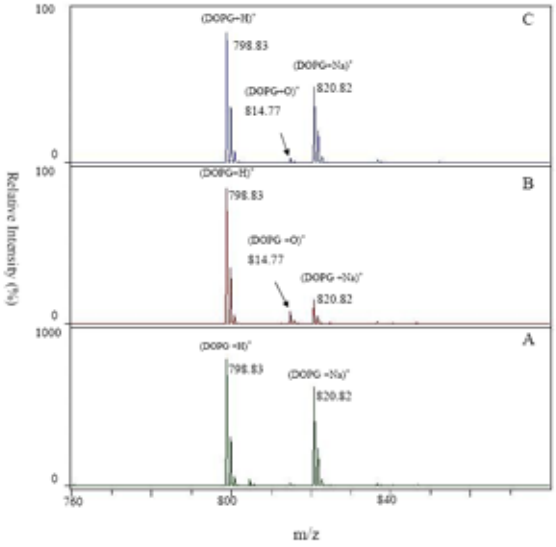

Figure 2

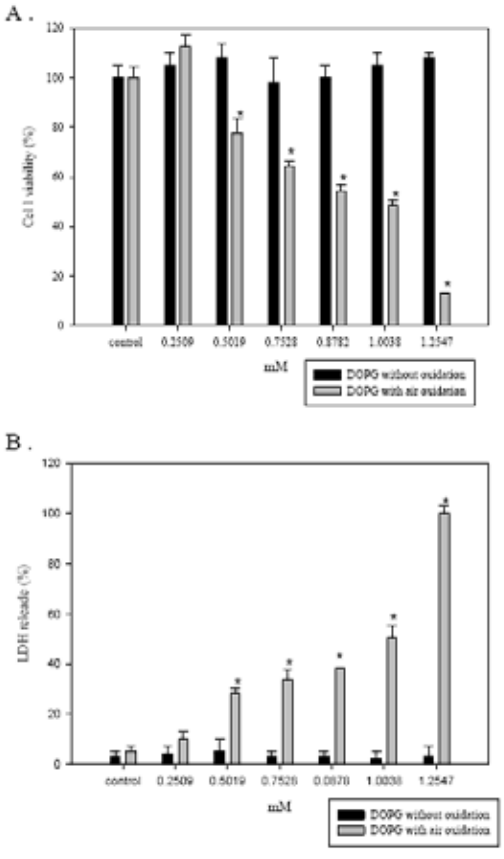

Figure 3

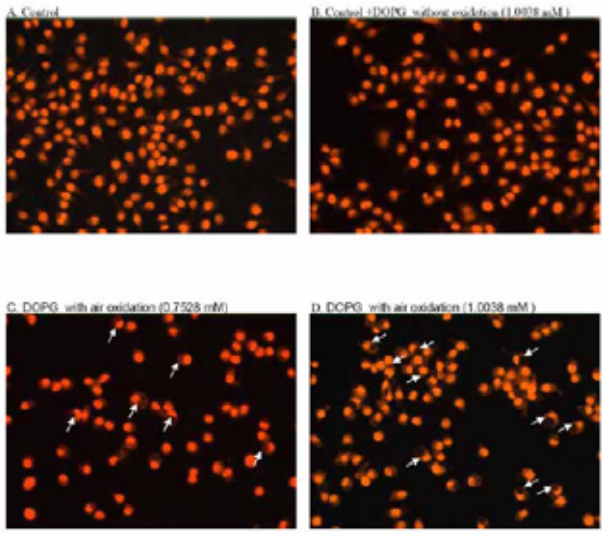

Figure 4

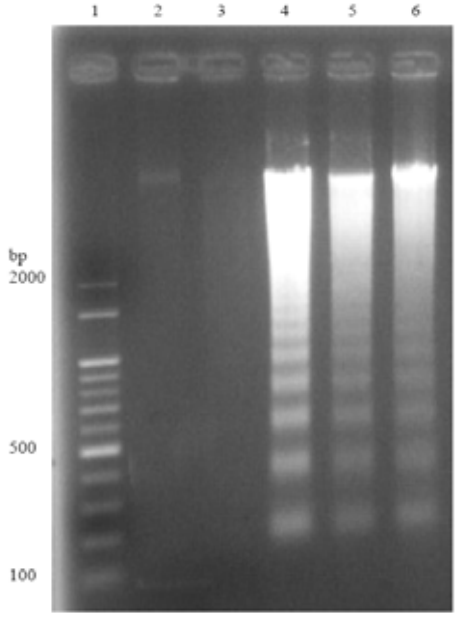

Table 1
Analysis of DNA content of RAW 264.7 cells after treatment with oxidized DOPG and effect of zVAD-fmk on inhibiting apoptosis

	[Sub-G1/M1] (%)
Untreated	4.53 ± 2.01
0.7528 mM oxidized DOPG	17.45 ± 2.23
0.8728 mM oxidized DOPG	21.58 ± 2.05
1.0038 mM oxidized DOPG	25.36 ± 1.92
20 μM C_2-ceramide	35.61 ± 2.52
20 μM C_2-ceramide + pre-incubation with 20 μM zVAD-fmk	3.57 ± 2.74
0.8728 mM oxidized DOPG + pre-incubation with 20 μM zVAD-fmk	22.57 ± 1.58
1.0038 mM oxidized DOPG + pre-incubation with 20 μM zVAD-fmk	25.42 ± 2.28

Results are given as means ± SD ($n = 6$).
M1 = sub-G1 + G0/G1 + S + G2/M.