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Synthesis of novel phytosphingosine derivatives and their preliminary biological evaluation for enhancing radiation therapy

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Abstract—Eight D-*ribo*-phytosphingosine derivatives were synthesized from D-*ribo*-phytosphingosine and diverse acyl chlorides with *N*,*N*-diisopropylethylamine in tetrahydrofuran for 1 h at room temperature. Effect of these compounds on IR-induced cell death was evaluated on blood cancer cells (Jurkat). Among these, **3d** showed the highest enhancement of radiosensitizing effect.

Anticancer therapy is largely classified into surgery, radiation, and chemotherapy. Alkylating agents, antibiotics, antimetabolites, plant derivatives, and steroids are used in anticancer chemotherapy, for example, Cisplatin, Doxorubicin, Pentostatin, Taxol, and Dexamethasone. It is known that these anticancer drugs, however, have a limited activity against the common solid tumors due to side effects that can damage normal cells. In addition, chemoresistance or recurrence of solid tumors brings about serious problems in cancer therapy.[1] Therefore, radiation therapy is widely utilized for cancer treatment.[2,3] Radiation therapy, however, leads to some problems such as radioresistance of cancer cells and damage to normal cells, which result in decreasing radiation therapy efficiency. In this regard, considerable efforts have been made to develop radiosensitizers for increasing the radiation therapy efficiency and attempted to increase radiosensitivity in several solid tumors such as breast cancer, uterine cervical cancer, lung cancer, gastric cancer, and large intestine cancer using Taxol, 5-FU, and Cisplatin that are presently known as radiosensitizing agents.[4] These radiosensitizing agents, however, have a serious side effect and/or can be applied only to specific cancer cells.

Phytosphingosine (**1**, (2*S*,3*S*,4*R*)-2-aminooctadecan-1,3,4-triol) consists of a long-chain base with a 2-amino-1,3,4-triol and was found to be widely distributed in fungal, plants, and mammalian tissues such as brain, kidney, skin, liver, uterus, etc.[5,6] In addition to its structural feature of the long-chain base of sphingolipids in membranes, phytosphingosine (**1**) is associated with the heat stress response of yeast cells and induction of apoptosis in some cancer cells, and some of its derivatives exhibit important physiological activities such as high tumor inhibitory potency.[7]

Phytosphingosine is a plant-derived, cell membrane lipid metabolite. The precise physiological metabolism and the function of phytosphingosine or phytoceramide as an anticancer agent have not been known until recently. We previously reported the anticancer and radiosensitizing effects of phytosphingosine and a phytoceramide

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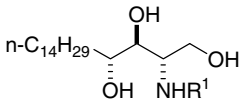


D-*ribo*-phytosphingosine, **1**, R^1 = H
D-*ribo*-phytoceramide, **2a**, C4PS, $R^1 = CO(CH_2)_2CH_3$
2b, C6PS, $R^1 = CO(CH_2)_4CH_3$
2c, C8PS, $R^1 = CO(CH_2)_6CH_3$
2d, C12PS, $R^1 = CO(CH_2)_{10}CH_3$

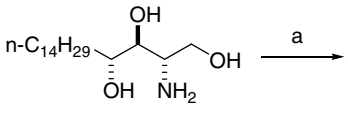
Figure 1. Structure of phytosphingosine and phytoceramide.

(Fig. 1) containing butanoyl (C4PS, **2a**), hexanoyl (C6PS, **2b**), octanoyl (C8PS, **2c**), and dodecanoyl (C12PS, **2d**) group on various cancer cells.[8] According to these results, phytosphingosine and its derivatives induced the apoptosis of uterine cervical cancer, breast cancer, blood cancer, and lung cancer cells without side effects, and exhibited a concentration- and post-treatment time-dependent increase. Moreover, as phytosphingosine and their derivatives were administered to various cancer cells in combination with ionizing radiation, the apoptotic rate of cancer cells was significantly enhanced compared to radiation or **1** and **2a–d** alone. Therefore, it seems that the administration of **1** and **2a–d** causes to improve the radiation therapy efficiency. In the current study, we describe the synthesis of novel eight phytosphingosine derivatives and their preliminary biological evaluation for enhancing radiation therapy. The biological properties of these new derivatives were evaluated in blood cancer cells (Jurkat).

Eight novel phytosphingosine derivatives **3a**–**h** were prepared in one step from D-*ribo*-phytosphingosine as shown in Scheme 1. The phytosphingosine derivatives were obtained by acylation of an amino group of phytosphingosine with acid scavanger, *N*,*N*-diisopropylethylamine (DIEA), at 0 °C to room temperature for 1 h in 35–78%[9] (structure analysis of **3a**, **3c** and **3d**: see Ref. 10). Acyl chlorides are commercially available (for **3a–d**) or obtained from diverse acid using thionyl chloride (for **3e**–**h**).[9,10]

The prepared phytosphingosine derivatives were evaluated in blood cancer cells for radiation enhancement effect with irradiation. Jurkat human T-cell lymphoma (Type II) was obtained from the American Type Culture Collection (Manassas, VA). Cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA), penicillin, and streptomycin at 37 °C in a humidified incubator with 5% CO_2. After cells were plated onto 60 mm dishes at a density of 2×10^6 cells/dish and exposed to 10 μM of phytosphingosine derivatives (**1** and **3a–h**) for 30 min, cells were exposed to γ-rays from a ^{137}Cs γ-ray source (Atomic Energy of Canada, Canada, located in Korea Institute of Radiological and Medical Sciences, Seoul, Korea) at a dose rate of 3.81 Gy/min. Cells were fixed with 4% *para*-formaldehyde for 30 min at room temperature and then washed once with phosphate-buffered saline (PBS). Hoechst 33258 (50 ng/mL) was added to the fixed cells, incubated for 30 min at room temperature, and then washed with PBS. Cells were mounted and examined by fluorescence microscopy. Apoptotic cells were identified by the condensation and fragmentation calculated from the ratio of apoptotic cells to total cells counted. At minimum, 500 cells were counted for each treatment. The radiation sensitizing enhancement ratio is defined as [value of combination with drug and ionizing radiation-induced cell death (%) − value of drug-induced cell death (%)/value of ionizing radiation-induced cell death (%)].

We examined whether the treatment of phytosphingosine derivatives in combination with ionizing radiation had a sensitizing effect on cell death in Jurkat human T-cell lymphoma or not. As shown in Figure 2, phytosphingosine (**1**) did not show synergistic effect on cell death when simultaneously treated with ionizing radiation. Instead, phytosphingosine treatment alone showed a significant cytotoxic effect. In addition, **3h** did not show ionizing radiation sensitizing effect. the treatment of **3h** also showed a significant cytotoxic effect. In the treatments of **3a**, **3c**, and **3d** in combination with ionizing radiation (IR), however, these phytosphingosine derivatives had synergistic effects on cell death. In comparison with control, the treatment of **3a** (11%), **3c** (12%), and **3d** (19%) have a synergistic effect on cell death in combination with ionizing radiation more than cell death ratio of IR plus cell death ratio of phytosphingosine derivatives. At this point, natural ceramide con-



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3a, $R^1 = -CH_2OBn$
3b, $R^1 = -CH_2COOCH_2CH_3$
3c, $R^1 = -(CH_2)_2COOCH_2CH_3$
3d, $R^1 = -(CH_2)_3COOCH_3$
3e, $R^1 = -(CH_2)_4COOCH_2CH_3$
3f, $R^1 = -(CH_2)_6COOCH_3$
3g, $R^1 = -(CH_2)_7COOCH_3$
3h, $R^1 = -(CH_2)_8COOCH_3$

Scheme 1. Reagents and conditions: (a) diverse acyl chlorides, DIEA, 0 °C to rt, 1 h for **3a**–**d** and diverse acyl chlorides from acid using $SOCl_2$, DIEA, 0 °C to rt, 1 h for **3e**–**h**.

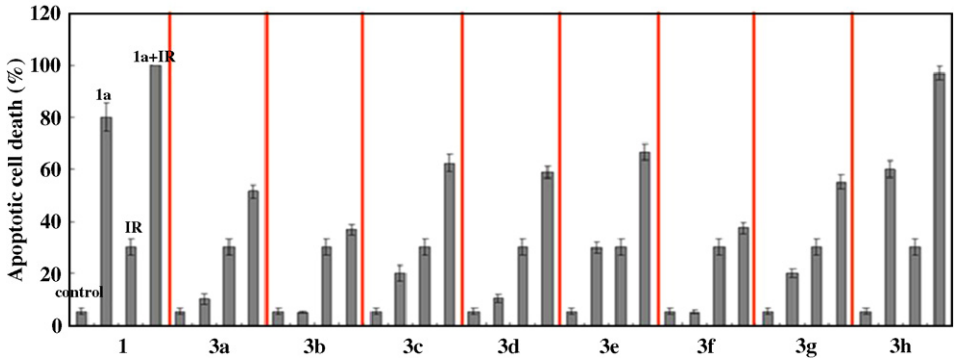

Figure 2. Effect of **1** and **3a–h** on IR induced cell death (IR: 10 Gy, treated concentration of **1** and **3a–h**: 10 μM, time: 48 h).

sists of a long chain sphingoid base activated at the nitrogen atom with an acyl chain. As this highly lipophilic chain is usually responsible for the low cell permeability of ceramide, shorter acyl chains are better (typically, two to eight carbon atoms).[6] The lipophilicity, therefore, is one of the most important factor for radiosensitizing effect in the modification base on phytosphingosine. To inclease cell permeability, it is important to decrease the lipophilicity of phytoceramide derivatives by introduction of ester group in terminal of diverse acyl chains. As the modification results, **3a**, **3c**, and **3d** with 6–8 atom chain will be lower lipophilicities than **2a–d**. Also compounds **3a**, **3c**, and **3d** showed higher enhancement of radiosensitizing effect than **2a–d**[8a] in Jurkat cell. All amide modification derivatives, moreover, could be decreased toxicity of themselves compared to **1** (Fig. 2) and comparison between **3c–d** and **2b–c** with 6–8 atom needs further study because **2b–c** showed diverse biological effect varying tumor cells.

The radiation sensitizing enhancement ratios of **1** and **3a–h** are summarized in Figure 3. Enhancement ratios (*Y* axis) were calculated by the equation as follows:

$$= \frac{\%\ \text{of}\ (\text{IR} + \text{compd}) - \%\ \text{of compd}}{\%\ \text{of IR}}$$

As shown in Figure 3, IR sensitizing enhancement ratio of **1** is <1. The radiation sensitizing enhancement ratios for 10 μM treatment of **3a**, **3c**, and **3d** were approximately 1.24, 1.28, or 1.45 times, respectively. But other derivatives did not show remarkable results in the enhancement of cell death with ionizing radiation.

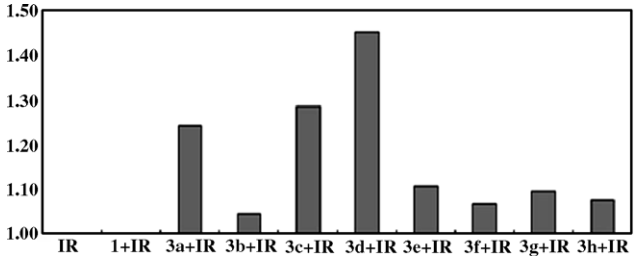

Figure 3. Enhancement ratios of phytosphingosine derivatives in radiation response in Jurkat cells.

In conclusion, eight novel phytosphingosine derivatives were synthesized from D-*ribo*-phytosphingosine with diverse acyl chlorides and evaluated in blood cancer cells. Our results demonstrate that the treatment of **3a**, **3c**, and **3d** in combination with ionizing radiation enhances the cell death of human cancer cells. Moreover, our results suggest that the combination treatment of novel phytosphingosine derivatives and ionizing radiation is potentially an effective way of treating cancers refractive to conventional radiation therapy.

Acknowledgments

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9. *General procedures*. Acyl chlorides for **3a**–**d** were purchased from Aldrich Co. To prepare acid chlorides for **3e–h**, diverse acids were converted to corresponding acid chlorides by dissolving it in 5.0–10.0 equiv of thionyl chloride ($SOCl_2$). The mixture was stirred at 40–50 °C for 3 h, followed by removal of extra thionyl chloride in vacuo. After the acid chloride was dissolved in THF, the mixture was added dropwise in THF solution containing phytosphingosine and DIEA at 0 °C. The reaction mixture was stirred at 0 °C for 15 min and continuously stirred at room temperature for 45 min. This solution was quenched by small amounts of water and dried with sodium sulfate. Flash column chromatography (80–100% EtOAc/hexane) gave a white solid **3a–h** in 35–88% yield.
10. *Structure analysis data*. Compound **3a**: ^{1}H NMR (300 MHz, CD_3OD) δ 7.42–7.30 (m, 5H), 4.63 (s, 2H), 4.25–4.20 (m, 1H), 4.00 (s, 2H), 3.79–3.74 (m, 2H), 3.58–3.52 (m, 2H), 1.71–1.69 (m, 1H), 1.56–1.53 (m, 1H), 1.43–1.23 (m, 24H), 0.91 (t, J = 6.6 Hz, 3H); ^{13}C NMR δ 170.6, 137.2, 128.1, 127.69, 127.74, 74.8, 73.1, 72.0, 68.8, 60.4, 51.8, 32.5, 31.6, 29.4 (m), 29.0, 25.4, 22.3, 13.0; MS (FAB) *m/z*: 466 (MH^+). HRMS (FAB) calcd for $C_{27}H_{48}NO_5$ (MH^+) 466.3532, found 466.3535. **3c**: ^{1}H NMR (300 MHz, CD_3OD) δ 7.85 (d, NH), 4.17–4.10 (m, 3H), 3.77–3.71 (m, 2H), 3.60–3.52 (m, 2H), 3.56–3.32 (m, 1H), 2.62–2.53 (m, 4H), 1.68–1.64 (m, 1H), 1.61–1.55 (m, 1H), 1.32–1.23 (m, 27H), 0.91 (t, J = 6.6 Hz, 3H); ^{13}C NMR δ 173.1, 172.7, 74.8, 71.9, 60.7, 60.3, 52.3, 32.0, 31.6, 30.2, 29.4 (m), 29.1, 29.0, 25.5, 22.3, 13.1, 13.0; MS (FAB) *m/z*: 446 (MH^+). HRMS (FAB) calcd for $C_{24}H_{48}NO_6$ (MH^+) 446.3482, found 446.3482. **3d**: ^{1}H NMR (300 MHz, CD_3OD) δ 7.82 (d, NH), 4.13–4.11 (m, 1H), 3.80–3.69 (m, 2H), 3.67 (s, 3H), 3.59–3.51 (m, 2H), 2.39 (t, J = 7.5 Hz, 2H), 2.29 (t, J = 7.5 Hz, 2H), 1.92 (qt, J = 7.5 Hz, 2H), 1.69–1.64 (m, 1H), 1.49–1.43 (m, 1H), 1.34–1.26 (m, 24H), 0.91 (t, J = 6.6 Hz, 3H); ^{13}C NMR δ 173.7, 173.6, 74.8, 72.0, 60.7, 34.8, 34.7, 32.6, 32.1, 31.7, 29.4 (m), 29.1, 25.6, 22.3, 20.8, 13.1; MS (FAB) m/z: 446 (MH^+). HRMS (FAB) calcd for $C_{27}H_{54}NO_6$ (MH^+) 446.3482, found 446.3475.