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ORIGINAL PAPER

Diethylamino-curcumin mimic with trizolyl benzene enhances TRAIL-mediated cell death on human glioblastoma cells

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Abstract

Backgrounds: Glioblastoma multiforme is one of the most aggressive human malignant brain tumors. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is known as the death ligand, which induces preferential apoptosis of transformed cancer cells. In this study, we demonstrated that the newly synthesized diethylamino-curcumin mimic with trizolyl benzene (YM-4) enhances cytotoxicity in combination with TRAIL in human glioblastoma cells.
Methods: We synthesized diethylamino-curcumin mimic with trizolyl benzene (YM-4) and investigated possible apoptotic cell signaling by co-treatment with YM-4 and TRAIL on human glioblastoma cells.
Results: Caspase-8, 9, and 3 and poly (ADP-ribose) polymerase were more efficiently cleaved with co-treatment of YM-4 and TRAIL than treatment with each alone in human glioblastoma cells. Co-treatment with YM-4 and TRAIL significantly increased the expression of Bax and Smac/Diablo and also inhibited the expression of the X-linked inhibitor of apoptosis protein and Survivin in human glioblastoma cells.
Conclusion: These results demonstrated that YM-4 can be an anticancer candidate that can be effective on human glioblastoma cells in combination with TRAIL.

Keywords: Curcumin mimic, TRAIL, Apoptosis, Human glioblastoma cells

Introduction

Glioblastoma multiforme (GBM) is the most common subtype of primary human brain tumors. It is characterized by aggressive biologic behaviors, such as rapid growth, widespread invasiveness, and vigorous neo-angiogenesis[1]. Despite a standard complete surgical resection followed by concurrent chemoradiation therapy, most patients suffer relapses and have a dismal prognosis, with median survival of less than a year[1]. Multiple chemotherapeutic agents have been extensively studied to prolong patient survival; but only temozolomide in combination with radiotherapy has demonstrated a survival benefit[2]. However, the use of oral alkylating temozolomide resulted in hematologic and non-hematologic toxicities in a significant number of patients[2]. Therefore, an effective and safe chemotherapeutic agent is required for the treatment of GBM.

Curcumin (diferuloyl methane) is a lipophilic phenolic substance that originates from the dried rhizome of turmeric (*Curcuma longa* L) plant. Curcumin is known to possess versatile biological properties such as anti-inflammatory[3], antioxidant[4], antiviral[5], antimicrobial[6], and wound-healing[7]. Moreover, pre-clinical studies have demonstrated the anticancer potential of curcum-

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in in various human cancers such as those of colon, breast, kidney, hepatocellular, lymphoid, melanoma, and prostate[8-10]. Curcumin lacks significant toxicities in both animal and human studies[11]; hence, it has received a lot of attention as a potential anticancer drug candidate. However, there are major obstacles in the drug development because of its low bioavailability due to poor aqueous solubility and high first-pass metabolism[12]. Based on the biochemical properties of curcumin, various synthetic analogs have been investigated for their potential pharmacological anticancer activities.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily of cytokines, is an attractive anticancer agent that induces apoptosis of tumor cells, with minimal harm to normal cells[13]. In our previous work, we synthesized diethylamino-curcumin mimic with trizolyl benzene (YM-4) and found that it augments TRAIL-mediated cell death of human glioblastoma cells[14]. In this study, we further investigated possible apoptotic cell signaling by co-treatment with YM-4 and TRAIL on human glioblastoma cells. Our results indicated that co-treatment with curcumin mimic and TRAIL significantly increased the activity of caspas-3, 8, and 9 and the expression of Bax and Smac/Diablo as well as inhibited the expression of X-linked inhibitor of apoptosis protein (XIAP) and Survivin.

Materials & Methods

Cell culture

CRT-MG human gliobalstoma cells were maintained in RPMI 1640 medium containing 10% FBS (Gibco, MA, USA), 10 mM Hepes (pH 7.2), and 1 mM Earle's balanced salt solution supplemented with 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin, as described previously[15].

Reagent

Human recombinant TRAIL was a generous gift from Dr. Eunil Lee (Korea University, Seoul, Republic of Korea). Temozolomide was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Detection of cell death

To confirm the cell death, an lactate dehydrogenase activity in the culture medium was measured by an lactate dehydrogenase release assay kit (Thermo Fisher Scientific, MA, USA), according to the protocol. Briefly, following treatment, the culture medium was centrifuged at 400 × g and room temperature for 5 min, and then 100 μL supernatant was collected and mixed with 100 μL 2,4-dinitrophenylhydrazine. After incubation at 37°C for 30 min. The OD was analyzed on a microplate reader (Thermo Multiskan MK3; Thermo Fisher Scientific, MA, USA). Total lactate dehydrogenase activity was determined by lysing control cells with 1% Triton X-100 for 30 min. Lactate dehydrogenase release was expressed as the percentage of total lactate dehydrogenase activity.

Western blot analysis

The protein extract was centrifuged to remove cell debris. The total protein concentration in the resulting supernatant was quantified using the Bradford method (Bio-Rad Laboratories, CA, USA). Samples of 50 μg protein were then separated using 10% Tris-HCl SDS-PAGE (Bio-Rad Laboratories) and transferred to a nitrocellulose transfer membrane (Bio-Rad Laboratories, CA, USA). Caspase-3, 8, 9, PARP, Bcl-2, Bax, XIAP, Survivin and Smac/Diablo were each detected using specific monoclonal antibodies (Cell Signaling Technology, MA, USA) diluted 1 : 500, followed by a rabbit anti-mouse secondary antibody (Cell Signaling Technology). Proteins were visualized using the ECL technique (Thermo Fisher Scientific), as described previously[16].

Statistical analysis

Data are presented as means ± SD. Levels of significance for comparisons between samples were determined using Student's t-test distribution.

Results

Diethylamino-curcumin mimic with trizolyl benzene (YM-4) Synthesis

We selected one curcumin mimic candidate that highlighted moderate cytotoxicity in a dose-dependent manner. We obtained a synthetic intermediate [3] with the aldol reaction of 4-(diethylamino)benzaldehyde [1] with 3-azidoacetophenone [2] in the presence of a basic catalyst (40% KOH) in ethanol at room temperature for 10 h[17]. Then, we synthesized a curcumin derivative [4] by Huisgen 1,3-cycloaddition reaction between phenyl acetylene and an intermediate [3] with $CuSO_4$ and sodium ascorbate in a solution mixture of chloroform, ethanol, and water (5 : 3 : 1) at room temperature for 5 h. Hereinafter, we renamed this final curcumin mimic [4] as "YM-4" which was called as "11c (benzenetriazolyl chalcone)" in our previous study[14] (Figure 1).

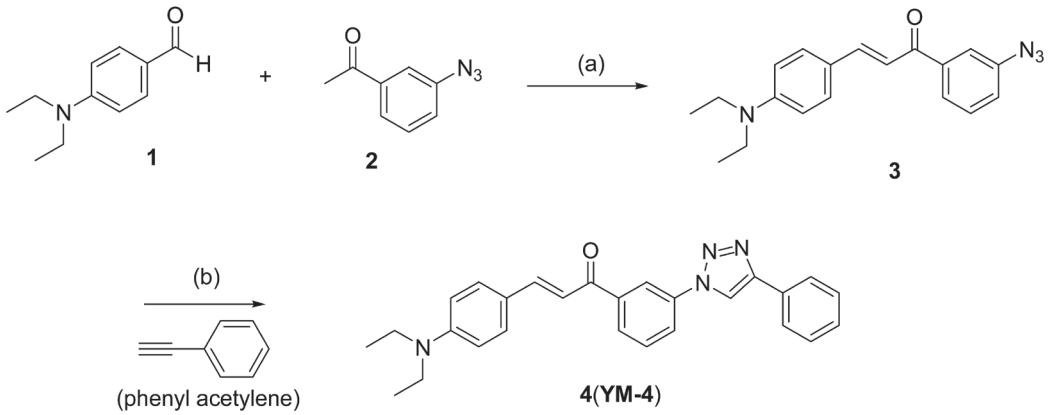

Figure 1. Synthesis and structure of a curcumin mimic with trizolyl benzene, YM-4. Reagents and conditions: (a) 40% KOH, EtOH, RT, 10 h; (b) sodium ascorbate (2.5 equiv), $CuSO_4$ (1 equiv), chloroform/EtOH/H_2O = 5 : 3 : 1, RT, 5 h; isolated yields for 3, 58%; YM-4, 94%.

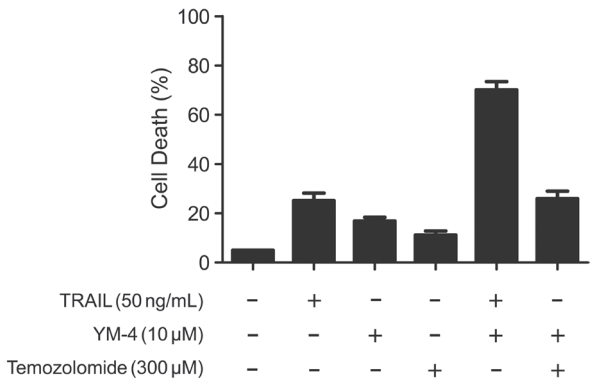

Figure 2. YM-4 enhances TRAIL-mediated cell death on human glioblastoma cells. Cells were treated with TRAIL (50 ng/mL) and/or YM-4 (10 μM) for 24 h. Cell death was assessed using a lactate dehydrogenase release assay.

YM-4 enhances TRAIL-mediated cell death on human glioblastoma cells

Human CRT-MG glioblastoma cells were stimulated with TRAIL, YM-4 or temozolomide for 24 h, and cell death was measured by lactate dehydrogenase release assay. Currently temozolomide is used as an anti-glioblastoma drug in clinical onco-chemotherapy. Treatment with TRAIL (50 ng/mL) induced cell death (upto ~25%) of CRT-MG cells. Treatment with YM-4 (10 μM) induced cell death (upto~17%) of CRT-MG cells. Treatment with temozolomide (300 μM) induced cell death (upto~10%) of CRT-MG cells. Temozolomide-mediated cell death was not significantly augmented by co-treatment with YM-4, whereas TRAIL-mediated cell death was significantly augmented by co-treatment with YM-4 (Figure 2).

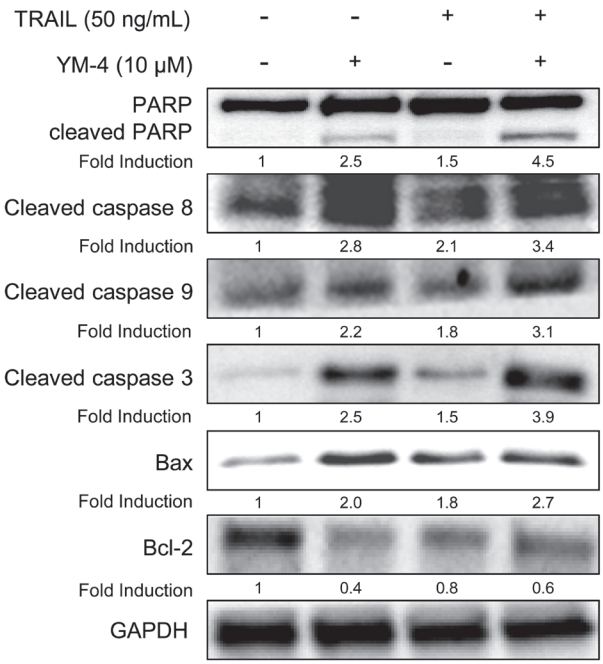

Figure 3. Co-treatment with YM-4 and TRAIL induces activation of Caspase, PARP and Bax. Cells were treated with TRAIL (50 ng/mL) and/or YM-4 (10 μM) for 12 h and an immunoblot analysis was then performed.

Co-treatment with YM-4 and TRAIL enhances apoptotic cell signaling in human glioblastoma cells

TRAIL is known to induce apoptotic cell death through caspase-dependent mechanisms[15]. We hypothesized that treatment with YM-4 might sensitizes TRAIL-resistant glioblastoma cells by modulating apoptosis-related protein activity. To demonstrate our hypothesis,

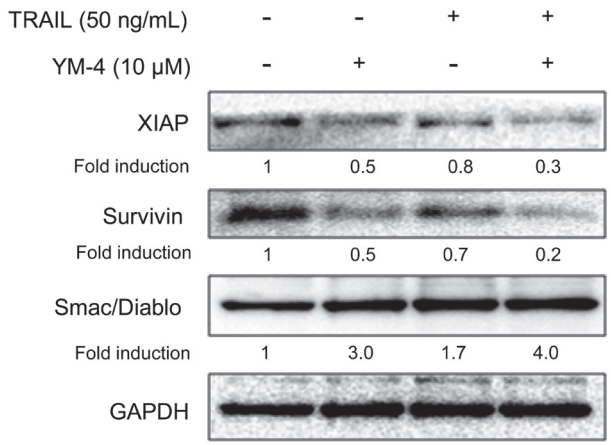

Figure 4. Co-treatment with YM-4 and TRAIL induces the down-regulation of XIAP and Survivin, and up-regulation of Smac/Diablo. Cells were treated with TRAIL (50 ng/mL) and/or YM-4 (10 μM) for 12 h and an immunoblot analysis was then performed.

CRT-MG cells were treated with YM-4 or/and TRAIL for 12 h. Then, we analyzed the activity of caspase-8, 9, and 3 and poly (ADP-ribose) polymerase (PARP). We observed that co-treatment with YM-4 and TRAIL enhanced the activity of caspase-8, 9, and 3 and PARP compared to treatment with each alone (Figure 3). Additionally, co-treatment with YM-4 and TRAIL enhanced the expression of Bax and Smac/Diablo and downregulated the expression of XIAP and Survivin (Figures 3 and 4). Co-treatment with YM-4 and TRAIL did not show a synergistic effect on the expression of Bcl-2 (Figure 3).

Discussion

TRAIL is an effective chemotherapeutic agent to treat various types of cancers. However, several cancer cells have developed resistance toward TRAIL, which is a limitation[18]. For resistance cancer cells, TRAIL is required a chemotherapy sensitizer. In this study, YM-4 is a synthesized as a novel curcumin mimic originating from Huisgen 1,3-cycloaddition reaction between azido chalcone intermediate [3] and benzene alkyne. To determine whether YM-4 is a sensitizer of TRAIL, human glioblastoma cell were treated with YM-4 and TRAIL. This co-treatment effectively induced cell death in human glioblastoma cells compare to treatment with each alone. Additionally, YM-4 induced cell death in human glioblastoma cells more effectively than temozolomide, and co-treatment with YM-4 and TRAIL more effectively induced cell death than co-teratment with temozolomide and TRAIL (Figure 2). Temozolomide, a second-generation imidazotetrazine prodrug, has high bioavailability and tolerability and transports well across the blood-brain barrier, providing modest antitumor activity against glioblastoma[19]. Since 2005, surgical resection followed by radiotherapy plus daily temozolomide has been widely recognized as the standard treatment regimen for patients with newly diagnosed glioblastoma[1,19].

To access the mechanisms involved in the induction of cell death by co-treatment with YM-4 and TRAIL, we analyzed the activity or expression of apoptosis-related proteins in human glioblastoma cells. Because caspase is a family of protease enzymes that play essential roles in apoptosis, we analyzed caspase activities. A western blot analysis indicated that caspase-8, 9, and 3 and PARP were more efficiently cleaved with co-treatment with YM-4 and TRAIL than treatment with each alone (Figure 3). YM-4 enhanced TRAIL-mediated cleavage of caspase-8 and 9, and the activated caspase-8 and 9 enhanced cleavage of caspase-3. Activated caspase-3 induced apoptotic cell death via PARP activation. Additionally, we analyzed Bcl family as well as Smac/Diablo, XIAP, and Survivin expression. The release of Bax and Smac/Diablo and consequent inhibition of XIAP is essential for the induction of apoptosis. Bax and Smac/Diablo are released into the cytosol to relieve caspase suppression and activate executor caspases[20]. XIAP, an endogenous caspase inhibitor, blocks initiator and executioner caspases activation by binding to caspases[19]. Survivin belongs to the family of inhibitor of apoptosis proteins. Survivin inhibits Bax-mediated apoptosis, leading to negative regulation of apoptosis[21]. In this study, Bax and Smac/Diablo expression were more upregulated with co-treatment with YM-4 and TRAIL than that by treatment with each alone. XIAP and Survivin expression were downregulated by co-treatment with YM-4 and TRAIL than that by treatment with each alone (Figures 3, 4). These results suggested that YM-4 increases pro-apoptotic protein expression and suppresses anti-apoptotic protein expression, thereby enhancing TRAIL-mediated apoptosis

Collectively, YM-4 promoted TRAIL-mediated apoptosis by increasing the cleavage of caspase-8, 9, and 3 and PARP, and upregulating Bax and Samc/Diablo expression as well as by downregulating XIAP and Survivin expression in human glioblastoma cells.

Conclusion

This study demonstrated that YM-4 can be an anticancer candidate that can be effective on human glioblastoma cells in combination with TRAIL.

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Conflict of Interest Yongchel Ahn, Seokjoon Lee, Cheon-Soo Park, Hyuk Jai Jang, Ji Hwan Lee, Byong-Gon Park, Yoon-Sun Park, Woon-Seob Shin & Daeho Kwon declares that they have no conflict of interest.

Human and animal rights The article does not contain any studies with human and animal and this study was performed following institutional and national guidelines.

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