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Short Communication
Synergistic Combination of Sulforaphane and Methylseleninic Acid in Inhibiting the Proliferation of MCF-7 Breast Cancer Cells

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Abstract

Background and Objective: Selenium has been reported to improve the anticancer activity of broccoli sprouts and florets. However, the anticancer properties of the combination of selenium and sulforaphane, the active anticancer compound in Broccoli have not been well investigated. This study was conducted to investigate the role of the combination of selenium and sulforaphane in inhibiting the proliferation of MCF-7 cells. **Materials and Methods:** Methylseleninic acid was used in this study as it can directly convert to monomethylated selenium for exhibiting its anticancer activity. The anti-proliferative properties of both sulforaphane and methylseleninic acid were analyzed separately and in combination with MCF-7 cells using WST assays. The combinations of sulforaphane and methylseleninic acid in ratios of 1:2 and 1:3 were analyzed using the Chou-Talalay method. **Results:** The 24 h IC_{50} values of sulforaphane and methylseleninic acid alone against MCF-7 cells were 24.55 and 182.35 μM, respectively. Furthermore, the results of the Chou-Talalay equation suggested that the combinations of sulforaphane and methylseleninic acid in ratios of 1:2 and 1:3 showed combination index values <1 at all tested concentrations. **Conclusion:** This study suggested that the combinations of sulforaphane and methylseleninic acid exert synergistic antiproliferative properties against the MCF-7 breast cancer cell line.

Key words: Sulforaphane, methylseleninic acid, drug combination, chou-talalay

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sulforaphane [1-isothiocyanato-4-(methylsulfinyl)butane] is a natural bioactive substance that can be found in broccoli, especially young sprouts. Broccoli-based preparations, which are a rich source of sulforaphane (SFN) are currently used in clinical studies investigating their efficacy in disease prevention and health preservation[1,2]. The SFN is a compound that can be derived from the hydrolysis of glucoraphanin. A previous study demonstrated that SFN could inhibit the proliferation of prostate cancer cells by inducing apoptosis and inhibiting protein expression associated with cell malignancies[3]. It has been reported that the ability of SFN to induce apoptosis is associated with its role in affecting the production of reactive oxygen species in cancer cells[4]. Further research has also reported that SFN not only cures but also prevents risk factors. In addition, regarding the anticancer properties of SFN, it has been reported that SFN decreases the size, complications and development of cells in the mammary gland of mice[5].

As an anticancer compound, SFN acts as a blocking and suppressing factor. The role of SFN as a blocking factor involves the inhibition of phase I of the enzyme metabolism process, which can convert pro-carcinogens into carcinogens and then induce enzyme metabolism resulting in the secretion of carcinogens[6]. Meanwhile as a suppressing factor, SFN inhibits progression of cancer cells. In addition, SFN induces apoptosis and inhibits the cell cycle that triggers the induction of other components involved in cancer progression, such as Bcl-2, caspases, p21, cyclins and CDKs. Furthermore, SFN is also capable of suppressing angiogenesis and metastasis through the regulation[7] of VEGF, HIF-1α, MMP-2 and MMP-9.

Several studies regarding the supplementation of Selenium (Se) have reported that Se could prevent and reduce the incidence of cancer. Se is an important element with important physiological functions[8-12] and broad pharmacological actions either alone or in combination as a cancer therapeutic agent for overt disease with hormonal therapy and well-established chemotherapeutic drugs[13,14], through varied mechanisms[15]. Moreover, it has also been extensively reported that Se increases the anti-cancer properties of broccoli[3,16-20]. However, the effects of the combination of SFN, the active anti-cancer compound from broccoli and MSA have not been well investigated. Therefore, this study was conducted to assess the anti-proliferative effect of the combination of SFN and MSA (SFN–MSA) against MCF-7 breast cancer cells.

MATERIALS AND METHODS

Cells and chemicals: The MCF-7 breast cancer cells were obtained from the American Type Culture Collection (ATCC), RPMI-1640 medium and MSA were purchased from Sigma (MO, USA), fetal bovine serum, penicillin and streptomycin were purchased from Gibco (NY, USA), SFN was purchased from LKT Laboratories (MN, USA) and WST-8 cell counting solution was purchased from Dojindo Laboratories (Tokyo, Japan).

Cell culture and treatment: The MCF-7 cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and anti-biotics (100 U mL^{-1} penicillin and 100 µg mL^{-1} streptomycin). Various concentrations of SFN, MSA, SFN-MSA (1:2) and SFN-MSA (1:3) were added to a 96-well plate and then incubated at 37°C for 24 h in a 5% CO_2 incubator. Then, 10 µL of WST-8 cell counting solution was added to each well and the solution was incubated again at 37°C for 3 h until the formation of a yellow formazan complex. WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] is a tetrazolium salt that produces a water-soluble compound. After adding 100 µL/well of 1 N HCl, the cell proliferation rate was determined by measuring the absorbance at a wavelength of 450 nm with a reference wavelength of 620 nm using a multiplate reader (TecanInfinite® 200 PRO, Switzerland).

Determination of combination index: The combination index (CI) was determined using the Chou-Talalay equation to measure the drug synergism based on multiple drug effects. The Chou-Talalay method used for assessing drug combinations is based on the median-effect resemblance, derived from the principle of mass action law, which is an integrated theory that provides a general relationship between the dynamics of first order and higher order and also for single entity and multiple entities. The general equation comprises most of the principles of biochemistry and biophysics, which include the Hill, Michaelis-Menten's, Scatchard and Henderson-Hasselbalch. The resulting CI theorem of Chou-Talalay provides a quantitative definition for additive effect (CI = 1), synergism (CI<1) and antagonism (CI>1) in drug combinations. This had been shown to be the simplest possible method for quantifying synergism or antagonism. The advantages of the Chou-Talalay method include resemblance, experimental design and the features of efficient data analysis, being economical and reducing the

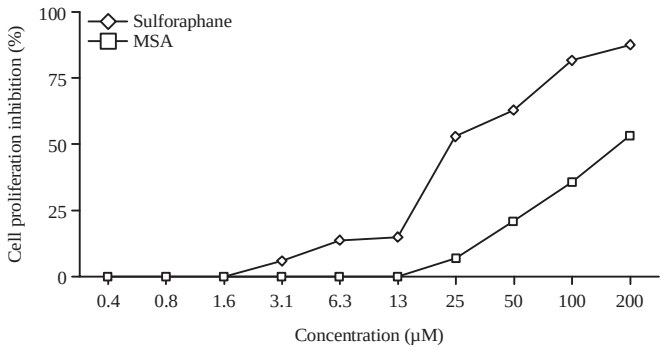

Fig. 1: Anti-proliferative activity of SFN and MSA against MCF-7

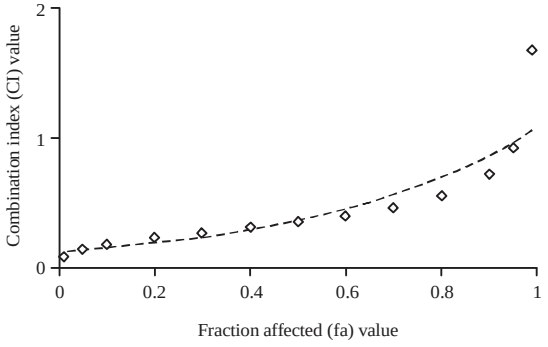

Fig. 2: CI value of SFN-MSA combination (1:2)

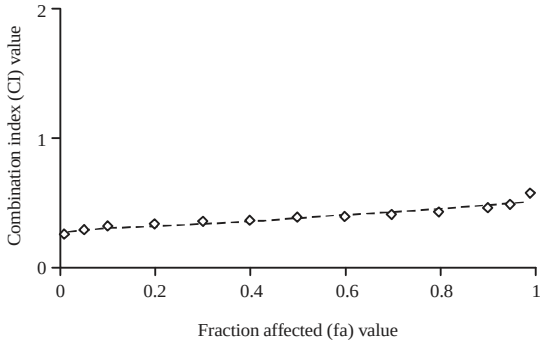

Fig. 3: CI value of SFN-MSA combination (1:3)

number of experimental animals or patients required for the clinical trial of drug combinations[21,22].

RESULTS

Figure 1 showed the results of the anti-proliferative properties of various concentrations of SFN and MSA. The IC_{50} values for SFN and MSA were 24.55 and 182.35 µM, respectively. The CI values were then determined using the Chou-Talalay method as the CI is the natural law-based general expression of pharmacologic drug interactions. The CI values for SFN–MSA (1:2) and SFN–MSA (1:3) were presented in Fig. 2 and 3, respectively.

As shown in the Fig. 2 and 3, the SFN–MSA (1:2) combination exerted a synergetic effect as long as the fraction affected (fa) value was <0.95. On the other hand, the SFN–MSA (1:3) combination was not dependent on the fraction affected (fa) value to exert a synergetic effect (CI<1). For the SFN-MSA (1:3) combination, the CI value at a maximum fa of 0.99 was 0.57. Overall, these data indicated that the use of the MSA-SFN combination treatment against the MCF-7 cell line did result in a synergistic anti-proliferative activity against MCF-7 breast cancer cells.

DISCUSSION

In the modern age of medication, a combination of drugs is considered as one of the most widely used options in cancer treatment. The primary objective of this particular option was to achieve a synergistic therapeutic effect, an effective dose, and, most importantly, reduce the toxicity rate. It is also anticipated that drug combinations could significantly reduce or suspend the induction of occurrence of drug resistance. The reduction of toxicity and resistance would also benefit the targeted outcomes of synergism[22]. Drug synergism emerges when the therapeutic effect of at least two or more drugs used in combination is larger than the effect of the drugs administered separately. Drug synergism may also occur when there is resistance to a drug that is delivered alone, whereas co-administration of the drug with a second compound prevents the mechanism of resistance, resulting in a synergistic response.

The ability of Se to prevent the growth of cancer cells has recently demonstrated notable promise in preclinical and clinical trials. In the present study, It investigated the effect of MSA in combination with SFN against the MCF-7 breast cancer cell line. This study selected a combination treatment

of MSA-SFN based on previously published data showing that pre-treatment with Se-containing compounds is essential for the inhibition of cancer cell proliferation and the induction of cell apoptosis by several different mechanisms[3]. Current results suggested that MSA did enhance the efficacy of SFN against MCF-7 breast cancer cells.

To author's knowledge, only one study has reported the anti-cancer properties of the combination of SFN and Se. Li *et al.*[23] reported the possible interaction and the synergistic effect of SFN and Se on the upregulation of the antioxidant enzyme TrxR-1, which may offer a better chemoprevention of cancer. Se is an essential mineral that is needed by the body as a cofactor for the production of active antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase (TrxR)[13]. TrxR itself is a pyridine nucleotide-disulphide oxidoreductase that contains Se in its chemical structure and commonly known as a selenoprotein. The ability of SFN to inhibit the growth of cancer cells is due to the fact that SFN could up regulate the gene expression level and the enzymatic activity that are involved in the elimination of carcinogens from the human body[24]. Thus, TrxR1 may be involved in the synergetic effect of the combination of SFN-Se in cancer cells.

In summary, in this study, it was demonstrated that the combination of SFN and MSA could synergistically inhibit the growth of MCF-7 breast cancer cells. Although the use of SFN–Se combination had been analyzed in human cancer prevention trials, the combinatorial effects of SFN and Se and its exact anticancer mechanisms still require further investigation.

CONCLUSION

This study has suggested that the use of SFN-MSA combination in ratios of 1:2 and 1:3 demonstrated a synergistic antiproliferative activity against the MCF-7 breast cancer cell line.

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