RESEARCH ARTICLE

Synergistic Effect of Ethaselen and Selenite Treatment against A549 Human Non-small Cell Lung Cancer Cells

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Abstract

    <u>Background</u>: In this study, we aimed to evaluate the growth inhibitory effect of the combination of ethaselen (BBSKE) and low fixed dose of selenite against A549 human non-small cell lung cancer cells *in vitro*. <u>Materials and Methods</u>: Growth inhibitory effects against A549 cells were determined by SRB assay. Combination index (CI) values were calculated based on Chou-Talalay median-effect analyses. Dose reduction index (DRI) values were applied to calculate dose reduction of selenite. Contents of free thiols and GSH were determined by DTNB assay and intracellular ROS levels by DCFH-DA fluorescence labeling. <u>Results</u>: Compared with BBSKE or selenite single treatment, the combined application of ethaselen and a low fixed dose of selenite shortened the onset time of sodium selenite, reduced IC_{50} values, and increased the maximum inhibition rates, suggesting a possible molecular mechanism of the synergism. Obvious synergistic effects were observed after different times of combination treatment, especially after 24 h. Compared with selenite single treatment, dosage of selenite could be remarkably reduced in combination therapy to gain the same inhibitory effect on cell proliferation. Compared with BBSKE single treatment, the content of free thiols and GSH were significantly reduced and ROS levels greatly elevated in the combination group. For the combination treatment, cell viability increased as greater concentrations of GSH were added. <u>Conclusions</u>: All these results indicate that the combination treatment of BBSKE and selenite showed synergism to inhibit A549 cell proliferation *in vitro*, and also reduced the selenite dosage to mitigate its toxicity which is very meaningful for combination chemotherapy of lung cancer. The synergism was probably caused by the accelerated exhaustion of intracellular reductive substances, such as free thiols and GSH, which ultimately leads to enhanced oxidative stress and apoptosis.

Keywords: Ethaselen - selenite - synergism - oxidative stress - A549 NSCLC cells

Introduction

    In 1969, one study showed that cancer mortality in American was negatively related to the content of selenium (Se) in plants. Based on this observation, Shamberger and Frost inferred that this essential trace element might play an important role in cancer prevention and therapy (Shamberger and Frost, 1969). Since then, a series of animal experiments (Combs et al., 1985; Medina and Morrison, 1988) and clinical trials (Li et al., 1993) confirmed the anti-tumor effect of Se-containing compounds, including sodium selenite, selenate and Se-enriched yeast. In the majority of the animal experiments, sodium selenite was used as the resource of Se (Shamberger and Frost, 1969), which was further employed as anti-tumor substance in clinical trials (An). Low concentration of selenium was reported to have antioxidant effect, influence cancer metabolism, enhance immunity, regulate cell cycle, and trigger apoptosis. However, high concentration of selenium had shown severe cytotoxicity (Shamberger and Frost, 1969). Whanger PD et al. have demonstrated that selenium at high

concentration led to reversible toxic reactions in animal models, such as loss of hair and nails (Whanger, 1992).

    The thioredoxin system is one of the most important systems maintaining the redox status *in vivo*. Previous studies have demonstrated the overexpression of thioredoxin reductase in patients suffering from many primary tumors including lung cancer, colon cancer, breast cancer, liver cancer, gastric carcinoma, pancreatic cancer and leukemia (Berggren et al., 1996; Han et al., 2002; Lincoln et al., 2003). Ethaselen (BBSKE) is a novel selenazole-based compound targeting thioredoxin reductase and now on phase II clinical trial in China. Our lab has previously reported the significant growth inhibition, apoptosis induction and cell cycle retarding effect of BBSKE against A549, MCF-7, PANC-1, and RKO cells *in vitro* (Shi et al., 2003; Zhao et al., 2006). BBSKE treatment in combination with cisplatin, fluorouracil or radiation therapy showed synergistic effect and attenuated toxicity in LoVo, BGC-823, and A549 cells (Li et al., 2008; Tan et al., 2010; Fu et al., 2011; Wang et al., 2011).

    Lung cancer is generally considered as a leading

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cause of cancer-related deaths, as its morbidity and mortality rates are still increasing sharply. An estimation of 1.6 million new lung cancer cases are diagnosed throughout the world (Deniz et al., 2014). Almost 80% of lung cancers fall within the non-small cell lung cancer (NSCLC) subgroup, of which the overall 5-year survival rate is only 15% (Liu et al., 2014). The high mortality rate of lung adenocarcinoma is partially attributed to its strong resistance to current therapeutics such as cisplatin-based chemoprevention and radiotherapy; and therefore, new therapeutic schedules are urgently needed. As the inhibitory mechanism of BBSKE against non-small cell lung cancer A549 cells has been clearly described, we here come up with a combination therapy of BBSKE and a relatively low concentration of selenite, which shows no obvious inhibitory effect by single treatment, against A549 cells *in vitro*, and provide a possible mechanism for the synergism effect.

Materials and Methods

Chemicals and drug treatment

Ethaselen (BBSKE) (PCT:080311) was designed and synthesized in our laboratory (State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing, China). BBSKE was dissolved in HPLC grade DMSO to make a 5 mM stock solution. Sodium Selenite and ROS detection kit were purchased from Macgene (Beijing, China). Glutathione reductase (GR), reduced glutathione (GSH) and DTNB were purchased from Sigma-Aldrich. NADPH was purchased from GenView. BCA Protein quantification kit was purchased from PPLYGEN (Beijing, China).

Cell culture

Human non-small cell lung cancer cell line A549 was provided by Cell Resource Center at Peking Union Medical College (Beijing, China). DMEM medium was purchased from Macgene. Fetal bovine was purchased from Excell Bio (Shanghai, China). A549 cells were cultured in DMEM medium supplemented with 10% fetal bovine and incubated at 37°C in 5% CO_2 atmosphere and medium was replaced every other day.

Cell viability assay

The SRB assay (Sulforhodamine B colorimetric assay) (Vichai and Kirtikara, 2006) was used to determine the inhibitory effect of BBSKE, selenite and combination treatment against A549 cells. Cells at exponential growth phase were detached from dishes, made into single-cell suspension and then planted into 96-well plates (Corning) at 8000 cells/well. After 24 h of incubation, cells were exposed to various concentrations of BBSKE (1, 2, 3, 4, 5, 6, 7, 8 μM), selenite (1, 2, 3, 4, 5, 6, 7, 8 μM) or a combination of BBSKE (1, 2, 3, 4, 5, 6, 7, 8 μM) and 0.5 μM selenite. After drug treatment for 24, 48 and 72 h, 100 μl/well pre-cold 10% (v/v) TCA was added and the plates were fixed at 4°C for 1 h. Plates were washed 4 times by using deionized water before they were blown dry. 100 μl/well SRB dye was then added and kept for 30 min at room temperature, followed by 4 times plate washing

by 1% (v/v) acetic acid. 200 μl/well destaining solution (20 mM Tris, pH 10.5) was added and the plates were placed on a shaker until the deposit was totally dissolved. The absorption values at 492 nm were determined on a Multiskan MK3 microplate reader (Thermo Fisher Scientific). All experiments were run in triplicate.

Chou-Talalay median-effect analyses

The median-effect analyses are based on the median-effect principle established by Chou and Talalay to calculate the combination index (CI) and dose reduction index (DRI) values for the treatment of BBSKE, selenite and the combination use. The mean values of three independent experiments were used. CI values reflect the ways of interaction between two drugs. CI<1 indicates synergism; CI=1 indicates an additive effect; and CI>1 indicates antagonism. DRI values reflect a measure of how many folds the dose of each drug in a synergistic combination may be reduced, at a given effect level, compared with the doses of each drug alone. DRI=1 indicates no dose reduction, whereas DRI>1 and <1 indicate favorable and unfavorable dose-reduction, respectively. The Chou-Talalay median-effect analyses were carried on CompuSyn software.

Determination of free thiols

A549 cells at exponential growth phase were planted into 60 mm dishes at 1.2×10^5 cells/ml. After 24 h of incubation, cells were exposed to 3 μM BBSKE, 0.5 μM selenite or a combination of both for 2, 4, 6, 8, 10 h respectively. Cells treated with equal volume of culture medium were set as blank control. 400 μl RIPA lysate was added and the protein concentration was determined by BCA assay. 50 μg protein were used for the free thiols determination and supplemented with 0.1 M sodium phosphate solution to make the total volume 50 μl. 150 μl DTNB-guanidine hydrochloride-sodium phosphate solution (guanidine hydrochloride 6M, DTNB 0.4mg/ml) was added. After an incubation of 0.5 h in dark, the absorption values at 405 nm were determined on a Multiskan MK3 microplate reader (Thermo Fisher Scientific). All experiments were run in triplicate.

Determination of GSH

A549 cells at exponential growth phase were planted into 60 mm dishes at 1.2×10^5 cells/ml. After 24 h of culture cells were exposed to 3 μM BBSKE, 0.5 μM selenite or a combination of both for 2, 4, 6, 8, 10 h respectively. Cells treated with equal volume of culture medium were set as blank control. 350 μl NP-40 lysate was added and the protein concentration was determined by BCA assay. GSH content was determined according to the previous description (Irfan et al., 2006). All experiments were run in triplicate.

Determination of intracellular ROS levels

A549 cells at exponential growth phase were planted into 60 mm dishes at 1.2×10^5 cells/ml. After 24 h of culture, cells were exposed to 3 μM BBSKE, 0.5 μM selenite or a combination of both for 3 h and 6 h. Cells treated with equal volume of culture medium were set as

blank control. DCFH-DA at a final concentration of 10 μM was added when chemical treatments were finished. After incubated at 37°C for 40 min, cells were detached from the dishes using 0.25% trypsin-EDTA. Cells were washed twice with pre-cold PBS and filtered through 0.22 μm membrane. Fluorescence at 340 nm was determined on flow cytometer (BD FACSCalibur). All experiments were run in triplicate.

Cell viability of combination treatment with additional supplementation of GSH

Cells at exponential growth phase were detached from dishes, made into single-cell suspension and then planted into 96-well plates (Corning) at 8000 cells/well. After 24
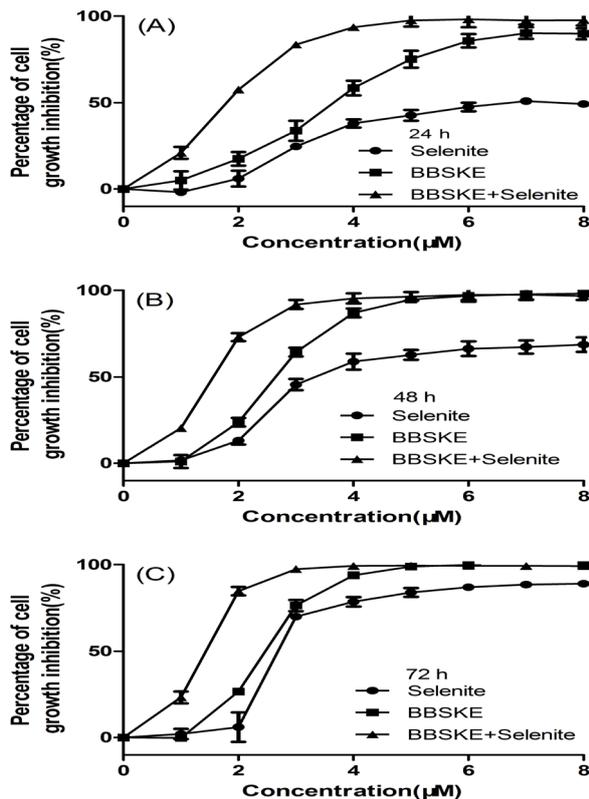

Figure 1. Cell Growth Inhibition Curves in A549 Cells After Treatment with BBSKE, Selenite or Combination of BBSKE and Selenite for 24, 48 and 72 h. For the combination use, the concentration of selenite was fixed at 0.5 μM, at which showed no growth inhibitory effect. The inhibitory effects on A549 cells were determined by the SRB assay. (A) A549 cells treated with BBSKE, selenite or combination of BBSKE and selenite for 24 h; (B) A549 cells treated with BBSKE, selenite or combination of BBSKE and selenite for 48 h; (C) A549 cells treated with BBSKE, selenite or combination of BBSKE and selenite for 72 h. Data were presented as the percentage of decreased values compared with untreated cells. Data are means±SD, n=3

h of incubation, cells were exposed to a combination of BBSKE (1, 2, 3, 4, 5, 6, 7, 8 μM) and 0.5 μM selenite, co-treated with 0.5, 2, 8 mM GSH, respectively. After drug treatment for 24 h, cell viability was determined by SRB assay as described above. All experiments were run in triplicate.

Statistical analyses

All data were shown as mean±SD. P values were calculated by using the Student's t test. $P<0.05$ were considered statistically significant.

Results

Growth inhibitory effect of single treatment with BBSKE or selenite against A549 cells

Exponentially growing A549 cells were exposed to indicated concentrations of BBSKE or selenite for 24, 48 and 72 h to determine the percentage of cell growth inhibition by SRB assay. As shown in Figure 1, Both BBSKE and selenite inhibited the growth of A549 cells in a time- and concentration- dependent manner. The inhibitory concentration 50 values (IC_{50}) and maximum inhibitory rates (MI) were shown in Table 1. The IC_{50} values of BBSKE were 3.60, 2.67 and 2.51 μM after 24, 48 and 72 h treatment, respectively. The IC_{50} values of selenite were 8.18, 4.29 and 2.57 μM after 24, 48 and 72 h treatment, respectively. In comparison between the growth inhibitory curves of these two compounds, BBSKE showed stronger inhibitory effect than selenite at the same concentration at any given time point, as its IC_{50} values were lower and MI values were higher.

Growth inhibitory effect of combination treatment with BBSKE and selenite against A549 cells

Exponentially growing A549 cells were exposed to a combination of various concentrations of BBSKE and a fixed concentration of selenite (0.5 μM, at which showed no inhibitory effect on A549 cell proliferation) for 24, 48 and 72 h. SRB assay was carried out to determine the percentage of cell growth inhibition. As shown in Figure 1, this combination treatment could inhibit A549 cell growth in a time- and concentration- dependent manner. The IC_{50} and MI values were listed in Table 1. The IC_{50} values for combination treatment were 1.70, 1.50 and 1.33 μM after 24, 48, and 72 h treatment, respectively, which were all lower than those of single treatment with BBSKE or selenite. These results indicated that the combination treatment was more powerful at inhibiting A549 cells proliferation.

Combination index analyses

Based on the law of large numbers theory and the

Table 1. IC50 and MI Values in A549 Cells Treated with BBSKE or Selenite alone, and Combination Treatment

Time of drug treatment (h)	Single treatment				Combined treatment	
	IC_{50} (μmol/L)		MI (%)		IC_{50} (μmol/L)	MI(%)
	BBSKE	selenite	BBSKE	selenite		
24	3.60±0.23	8.18±0.10	90.2	50.9	1.70±0.05	98.2
48	2.67±0.13	4.29±0.44	98.2	68.7	1.50±0.04	97.5
72	2.51±0.16	2.57±0.34	99.9	89.0	1.33±0.06	99.5

median-effect equation, the Chou-Talalay analysis is widely used to analyze the interaction ways between drugs (Chou, 2010). CI<1 indicates synergism, CI=1 indicates an additive effect and CI>1 indicates antagonism. The CI values for combination treatment of BBSKE and selenite against exponentially growing A549 cells for 24, 48 and 72 h were in Table 2. It came out that, after 24 h incubation, the CI values were less than 1 at any given concentration of BBSKE combined with 0.5 μM selenite. In addition, after 48 or 72 h incubation, the CI values were also lower than 1 in BBSKE ($< 6 \mu$M) combined with 0.5 μM selenite treatment, suggesting an overall strong synergistic effect in BBSKE and selenite co-treatment, especially at BBSKE low-dosage group (1-5 μM). The cell growth inhibition rate-CI plot further indicated that the most obvious synergism effect occurred after 24 h incubation (Figure 2), even at BBSKE high-dosage group (6-8 μM).

Dose-reduction index analyses

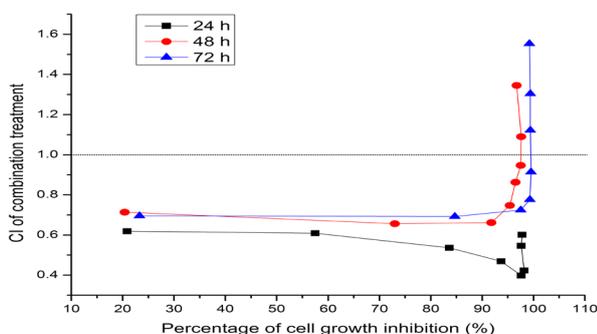Combination therapeutic is widely applied to many malignant diseases, such as cancers and AIDS. The main aim of combination therapeutic is to gain synergism effect, reduce the dose of specific toxic drugs, delay or minimize the possibility of drug resistance (Chou, 2005). We here analyzed the DRI of BBSKE and selenite when these two drugs were used in combination to inhibit A549 cell growth *in vitro*. As shown in Table 3, the DRI values of selenite were much bigger than 1 at any given BBSKE concentration and treatment time. As for BBSKE, the DRI values were bigger than 1 in the majority of the treatment groups, except for several BBSKE high-dosage groups (7, 8 μM for 48 h and 6, 7, 8 μM for 72 h), suggesting this combination therapy is capable of reducing the dose of both BBSKE and selenite. Moreover, the DRI values of selenite were much bigger than those of BBSKE, suggesting BBSKE co-treatment as an efficient strategy to reduce selenite dosage and mitigate its toxicity. As shown in Figure 3, the cell growth inhibition rate-DRI of selenite plot indicated the strongest dose-reduction effect of selenite occurred at 24 h treatment.
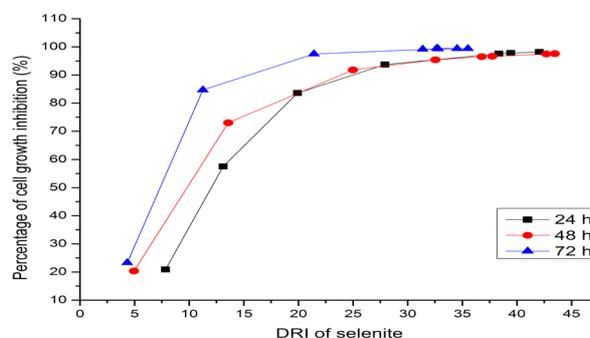

Figure 2. Cell Growth Inhibition Rate-CI Plot of Combination Plans for 24, 48 and 72 h in A549 Cells

Figure 3. Cell Growth Inhibition Rate-DRI of Selenite Plot in A549 Cells Treated with BBSKE and Selenite Simultaneously

Table 2. CI Analyses of Combination Treatment in A549 Cells

BBSKE concentration (μM)	24 h			48 h			72 h		
	Cell growth inhibition(%)	CI	Combined effect	Cell growth inhibition(%)	CI	Combined effect	Cell growth inhibition(%)	CI	Combined effect
1	20.9	0.62	S	20.4	0.71	S	23.3	0.70	S
2	57.5	0.61	S	73.0	0.66	S	84.7	0.69	S
3	83.6	0.54	S	91.8	0.66	S	97.5	0.72	S
4	93.7	0.47	S	95.4	0.75	S	99.3	0.78	S
5	97.6	0.40	S	96.5	0.86	S	99.5	0.91	S
6	98.2	0.42	S	97.5	0.95	S	99.4	1.12	A
7	97.6	0.55	S	97.6	1.09	A	99.4	1.30	A
8	97.8	0.60	S	96.7	1.35	A	99.2	1.55	A

*The combination index method is based on the theory described by Chou and Talalay(1984) and the computer software of Chou and Martin. CI<1 means synergism while CI>1 means antagonism

Table 3. DRI Analyses of Combination Treatment in A549 Cells

Concentration (μM)	24 h			48 h			72 h		
	Cell growth inhibition (%)	DRI BBSKE	Selenite	Cell growth inhibition (%)	DRI BBSKE	Selenite	Cell growth inhibition (%)	DRI BBSKE	Selenite
1	20.9	2.03	7.84	20.4	1.96	4.93	23.3	2.16	4.32
2	57.5	1.88	13.1	73.0	1.72	13.6	84.7	1.66	11.3
3	83.6	2.06	19.9	91.8	1.61	25.0	97.5	1.48	21.4
4	93.7	2.31	27.9	95.4	1.40	32.5	99.3	1.34	32.8
5	97.6	2.69	38.3	96.5	1.20	36.8	99.5	1.13	32.7
6	98.2	2.50	42.1	97.5	1.08	42.7	99.4	0.92	34.5
7	97.6	1.92	38.3	97.6	0.94	43.5	99.4	0.78	35.5
8	97.8	1.74	39.4	96.7	0.76	37.8	99.2	0.66	31.4

Synergistic Effect of Ethaselen and Selenite Treatment against A549 Human Non-small Cell Lung Cancer Cells
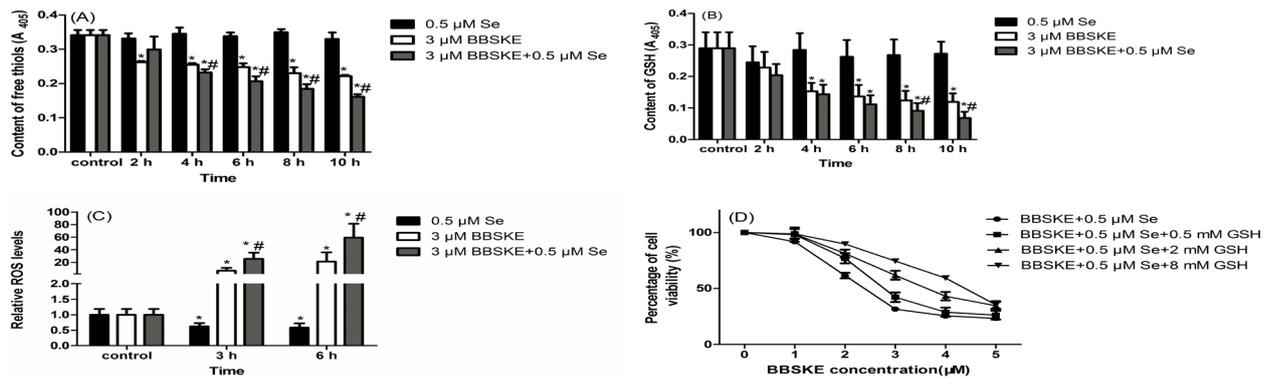

Figure 4. Content of free Thiols/GSH/ROS after Treatment with BBSKE, Selenite or Combination of BBSKE and Selenite for Different Time Periods and Cell Viability after Combination Treatment with Additions of Different Concentrations of GSH. (A) Content of free thiols in A549 cells treated with BBSKE, selenite, and combination of BBSKE and selenite for 2, 4, 6, 8 and 10 h; (B) Content of GSH in A549 cells treated with BBSKE, selenite, and combination of BBSKE and selenite for 2, 4, 6, 8 and 10 h; (C) ROS levels in A549 cells treated with BBSKE, selenite, and combination of BBSKE and selenite for 3 h and 6 h. ROS levels were presented as relative values compared to the control group; (D) A549 cell viability after combination treatment of BBSKE and selenite with addition of 0.5, 2 and 8 mM GSH for 24 h. *represents single treatment or combination treatment compared to control, $p<0.05$. # represents single treatment with BBSKE compared to combination treatment, $p<0.05$. Data are means±SD, n=3

Content reduction of free thiols and GSH after BBSKE, selenite alone and combination treatment in A549 cells

In order to investigate the potential mechanism involved in the synergism effect of this combination therapeutics, we determined the free thiols and GSH content in A549 cells after treatment with 3 μM BBSKE, 0.5 μM selenite or a combination of both for 2, 4, 6, 8, and 10 h, respectively. As shown in Figure 4 (A) and (B), treatment of selenite alone did not alter the content of free thiols and GSH at any given time point compared to the control group (add equal volume of medium instead of drugs as blank control). However, treatment of BBSKE alone or in combination with selenite was capable of reducing the free thiols content since 2 h incubation, as well as GSH content since 4 h incubation. Moreover, the combination group has revealed significantly higher reduction on free thiols/GSH content than BBSKE alone after 4 h/8 h or longer time, suggesting the exhaustion of these intracellular reductive substances as a potential mechanism of this combination therapeutics.

Intracellular ROS levels after BBSKE, selenite alone and combination treatment in A549 cells

To see if the exhaustion of reductive substances could lead to rapid oxidative stress, we further determined the intracellular ROS levels after treatment with 3 μM BBSKE, 0.5 μM selenite or a combination of both for 3 h and 6 h, respectively, using the widely applied DCFH-DA fluorescence labeling method. As Figure 4 (C) showed, different from selenite single treatment, BBSKE alone and combination treatment could both lead to rapid elevation of ROS levels compared to control group (add equal volume of medium instead of drugs as blank control). It should be noted that the combination treatment led to much more significantly ROS increase than BBSKE single treatment. Interestingly, selenite treatment alone reduced ROS slightly.

Cell viability of combination treatment with additional supplementation of GSH

We determined the cell viability with supplementation of different concentrations of GSH at the same time when cells were exposed to the combination treatment to see if added GSH could protect cells from death to some extent. As was seen in Figure 4 (D), after 24 h drugs exposure, GSH did increase A549 cell viability and as greater concentrations of GSH were added, cell viability increased more. It further proved that the synergism effect was partly due to GSH reduction.

Discussion

For several decades, the anti-tumor effect of selenium containing compounds has been widely studied. Previous reports have demonstrated that low concentration of selenium could slightly stimulate the growth of cells (Roy et al., 1994; Zeng, 2002; Ueno et al., 2007). However, high concentration of selenium can significantly inhibit cell proliferation and induce apoptosis *in vitro*, and lead to reversible toxic reactions *in vivo*. Considering the severe toxicity of selenite at high dosage, we have chosen a relatively low concentration of selenite (0.5 μM) in combination with BBSKE to study the synergistic inhibitory effect against A549 cell proliferation. As a result, this combination therapeutics have shown a significant synergism and dose reduction effect for selenite (Figure 2 and Figure 3), and 24 h treatment were proven to be the optimal time to maximize these effects. These findings suggested that the combination use of BBSKE could strongly mitigate the toxicity of selenite by reducing its effective dosage. Interestingly, the synergism (indicated by CI values) and dose reduction effect (indicated by DRI values) of BBSKE became very weak at BBSKE high-dosage group (> 6 μM) with long-time treatment (48 or 72 h). We concluded this effect as a result of the saturation of cell growth inhibition by high-dosage BBSKE. As BBSKE (> 6 μM) alone is sufficient to suppress A549 cell proliferation, CI/DRI analyses may become inaccurate and less sensitive.

Based on Chou-Talalay median-effect analysis, the

best synergism effect, indicated by the lowest CI value, occurred at 24 h treatment with 5 μM BBSKE combined with 0.5 μM selenite. However, the growth inhibitory rate under this condition was much close to 1, which prevented us from exploring its specific molecular mechanism. Instead, we decided to use 3 μM (approximately IC$_{50}$ value of treatment with BBSKE alone for 24 h) BBSKE and 0.5 μM selenite to perform our following mechanistic studies of the synergism effect.

Previous study has shown that selenite is metabolized to GSSeSG, during which process a depletion of GSH exists (Ganther, 1987). Wang L.H. et al have demonstrated that BBSKE reduces the GSH content in H22 hepatocellular carcinoma xenografts (Wang and Zeng, 2009). The direct reduction of GSH as well as the unavailability of many reductive substances caused by the inhibition of thioredoxin reductase by BBSKE both lead to elevated oxidative stress in A549 cells. Increase of intracellular reactive oxygen species (ROS) level leads to DNA breakage, lipid peroxidation, mitochondria membrane potential (MTP) decrease, permeability changes and cytochrome *c* release, which finally induce cell death or apoptosis. It has been verified that BBSKE can rapidly induce elevation of ROS levels in A549 cells (Xiong et al., 2014). Consistently, our results showed that BBSKE alone and a combination of BBSKE/selenite both could reduce the content of both free thiols and GSH and increase ROS levels. Moreover, the combination group had shown significantly higher reduction on free thiols/GSH content and greater elevation of ROS than BBSKE alone, suggesting oxidative stress might partially contribute to this synergism effect. Addition of GSH partially protected A549 cells from death further verifies that this synergism effect is partly due to intracellular redox imbalance. Considering that selenite alone did not significantly change the content of free thiols/GSH, our results indicated that low-dosage selenite might be able to facilitate the accumulation of BBSKE-induced oxidative stress. Interestingly, this low concentration of selenite reduced intracellular ROS levels slightly. We concluded this to the reason that selenite might be incorporated into Se-containing reductases, such as thioredoxin reductase, which helped reduce oxidative species. However, as the mechanisms of selenite metabolism and BBSKE-induced anti-tumor effect are still not completely unveiled, the detailed molecular underpinnings of this combination therapeutics might require some further studies.

Clinical trials of combination therapeutics are generally hard to be carried out due to the following reasons: (1) the gender, age, race, disease progression stages, and medication records of each individuals may have influence on the results; (2) whether it is ethically acceptable for subjects to take placebo or suboptimal combination therapeutics remains controversial; (3) it is not allowed to perform the toxic tolerance studies on specific drug in clinical trials, which is different from animal or *in vitro* experiments. Thus, it's scientifically necessary to carry out *in vitro* studies to investigate the best combination schedules (Chou, 2006). We here come up with an efficient combination use of a low-cost and widely used inorganic compound, sodium selenite with

BBSKE to inhibit A549 cell growth. This combination therapeutics of BBSKE and low-dosage selenite have shown significant synergism and dose reduction effect, providing new thoughts and basis for the treatment of lung cancer.

Acknowledgements

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