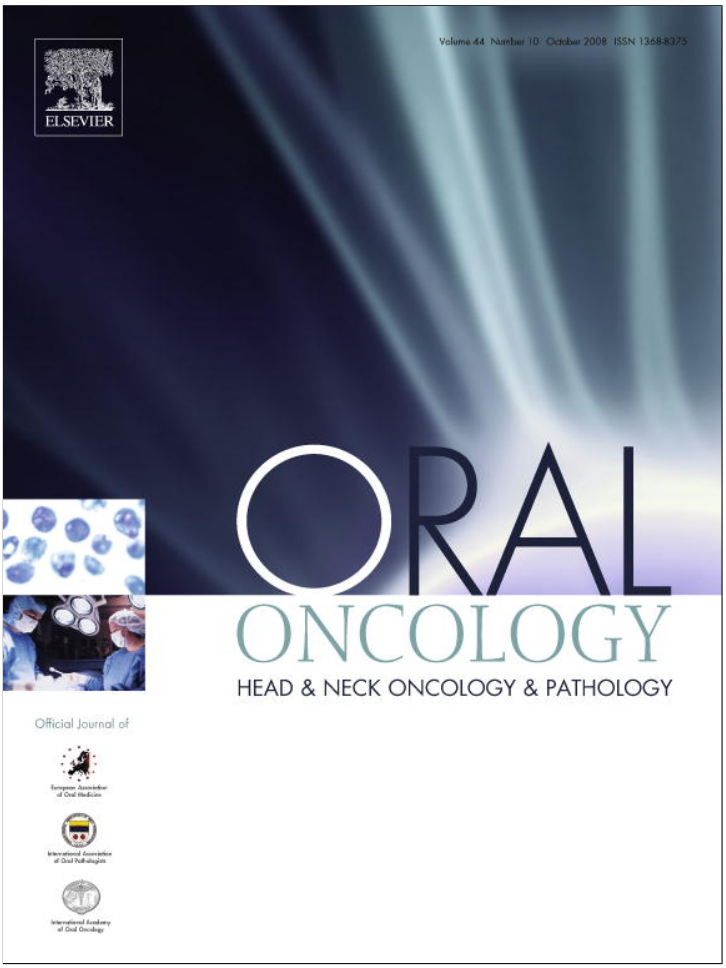

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ORAL ONCOLOGY

The inhibitory effect of a novel organoselenium compound BBSKE on the tongue cancer Tca8113 in vitro and in vivo ☆

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Summary The aim of this study was to evaluate the anti-cancer effect of a novel organoselenium compound BBSKE (1,2-[bis(1,2-Benzisoselenazolone-3(2H)-ketone)]ethane, BBSKE, PCT: CN02/00412) on cell growth and apoptosis, focusing on the protein activity of Thioredoxin Reductase (TrxR) and Caspase-3, in oral squamous cell carcinoma (OSCC) in vitro and in vivo. Oral squamous cancer cell line Tca8113 was treated with various concentrations of BBSKE. Growth and apoptosis as well as the protein activities were analyzed. Morphologic changes of Tca8113 cells after 24 h treatment of BBSKE were determined by fluorescence microscopy. The increase of Caspase-3 activity and decrease of Thioredoxin reductase (TrxR) activity were also measured. BBSKE induced a significant cell growth inhibition and elicited typical apoptotic morphologic changes (chromatic condensation, nucleus fragmentation). This phenomenon was accompanied by a change in protein activity of Thioredoxin reductase (TrxR) and Caspase-3. The anti-cancer effect of BBSKE was then studied in well-established Tca8113 xenografts in nude mice. In those tumors, anti-cancer effects were observed and significantly higher than the controls. Together, these results indicate that BBSKE can inhibit tongue cancer cell proliferation in vitro and in vivo, and induce apoptosis in Tca8113 cell lines partially via inhibiting the activity of TrxR and promoting the activity of Caspase-3.

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Introduction

Selenium (Se) is an essential trace element for mammals and is important for many cellular processes. Selenium is translationally incorporated in a small number of proteins under the form of selenocysteine, such as glutathione peroxidase, thioredoxin reductase.[1,2] Several of these selenoproteins display antioxidant activities. Others are involved in immune function, sperm motility, or production of thyroid hormones.[2–4] Numerous epidemiological data, as well as animal studies and supplementation trials indicate that Se compounds possess anti-cancer properties.[5–7] However, Se compounds can be either cytotoxic or possibly carcinogenic at higher concentrations. The extent between cancer therapy and toxic levels of selenium is extremely narrow, which inhibits the use of Se compounds in cancer therapy.[8,9] Therefore, many scholars try to find effective cancer-protecting Se compounds with low toxicity.

TrxR is a NADPH-dependent selenocysteine-containing flavoenzyme. It catalyzes the reduction of oxidized Trx. The Trx system (NADPH, TrxR/Trx) plays several key roles in DNA synthesis and activation of transcription factors that regulate cell growth.[25,26] Studies have shown that expressions or activities of Trx/TrxR system have been up-regulated in a variety of human primary tumors comparing to levels in its equivalent normal tissue.[27–30,41,42] It has been reported that the elevation of TrxR or/and Trx levels in many human primary cancers appears to contribute to the increase of cancer cell growth including an increase in the sensitivity of cells to other cytokines and growth factors, inhibition of the normal mechanism of programmed cell death and resistance to chemotherapy. Based on the fact that the Trx system in stimulating cancer cell growth and inhibition of apoptosis, TrxR offers a target for the development of drugs to treat and prevent cancer. BBSKE is a novel organoselenium compound synthesized by School of Pharmaceutical Sciences, Peking University, China.[10] The anti-tumor action of BBSKE is due to its action on Thioredoxin reductase (TrxR) which regulate the NF-κB dependent apoptosis pathway.[11–13] BBSKE, as a potent anti-tumor chemotherapeutic drug, showed a low toxicity (acute toxicity: LD50 value >5 g/kg body wt. p.o., repeated dose: 160 mg/kg is no toxicity dose for SD mice. p.o., Ames test and mutagenicity studies are negative) and an active action against a variety of human cancer cells (proliferation inhibition and apoptosis), including lung, gastric, hepatic, cervix, blood, prostate etc, which is probably related to TrxR inactivation and alterations in Bcl-2, Bax, and Caspase-3 expressions. Caspase-3 expressions in human cancer cell lines are different after exposure to BBSKE.[10–12]

Oral cancer is a public health priority and there are over 300,000 new cases diagnosed each year, with almost 130,000 deaths worldwide, annually.[14,15] Although many methods were used to treat oral cancer and significant advances in surgery, chemotherapy and radiotherapy over the past decades, the 5-year survival rate of oral cancer patients has improved only moderately, with a 5-year survival rate of 60% approximately.[16,17] Even though some region's tongue cancer can be diagnosed at an early stage, the prognosis is poor because of frequent recurrence and metastasis. Therefore, it is urgent to find new effective treatments to control this disease. BBSKE can inhibit a number of human cancers with low toxicity and may be clinically potential in tongue cancer therapy. In this study, our purpose is to investigate if BBSKE is effective in suppressing the growth of tongue cancer cells in vitro and in vivo, and if the TrxR activity is inhibited in Tca8113 tongue cancer cells treated with BBSKE. Furthermore, we try to elucidate the biological events of the novel potent anticancer agent in the induction of apoptosis, and to explore whether the molecular pathways of BBSKE on TrxR inactivation-induced apoptosis is dependent on Caspase-3 activity.

Materials and methods

Main reagents

The main reagents include RPMI 1640 (Invitrogen), fetal bovine serum (FBS, Sigma Chemical, St. Louis, MO), Annexin V-FITC apoptosis detection kit (BD Biosciences, San Jose, CA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT, Amresco Inc.) and dimethyl sulfoxide (DMSO, Amresco Inc.). NADPH, DTNB [5,5′-dithiobis (2-nitrobenzoic acid)] and Hoechst33342 were purchased from Sigma.

Cell culture

BBSKE is from research center of organoselenium Peking University, China. The human tongue cancer Tca8113 cell line was obtained from Shanghai Jiao Tong University College of Stomatology. Tca8113 cells were cultured in RPMI 1640 complete medium containing 100 μg/ml streptomycin, 100 units/ml penicillin and 10% heat-inactivated FBS, and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were digested with 0.25% trypsin—0.1% ethylenediaminetetraacetic acid solution.

Cell treatment

Cells were allowed to attach for 24 h before treatment with either DMSO (control) or BBSKE at indicated concentration and time. 20 mmol/l stock solution of BBSKE in DMSO was stored at −20°C in aliquots, and was diluted to proper concentrations with complete medium right before treatment. In control, complete medium with DMSO (concentration $\leqslant$ 0.1%) was added to the cell cultures, a possible influence on viability or growth behavior could not be monitored.

Cell proliferation

The inhibitory effects of BBSKE on Tca8113 cell growth were determined by the analysis of viable cells using the MTT assay.[18] Cells were trypsinized, counted, and diluted to the appropriate concentration. Then cells were seeded in 96-well plates (Corning, USA) at a density of 2×10^4 cells/well and allowed to attach overnight at 37 °C. Cells were placed in complete medium with several concentrations of BBSKE (control, 1, 5, 10, 15 μmol/l). After exposure of cells to BBSKE for 24 h, 20 μl of 5 mg/ml stock MTT solution was added to each well at a final concentration of 0.5 mg/ml and the cells were further incubated at 37 °C for 2 h. Supernatant was then aspirated carefully and formazan crystals

were dissolved with 150 μl DMSO. After a 10 min shake and incubation at 37 °C, the absorbance was determined at 570 nm in a microplate reader (BIO-Tek Elx808, USA). Independent experiments were repeated several times.

Apoptosis assay by Hoechst staining and Annexin V/PI staining

Tca8113 cells (1.5×10^6) were grown to confluence in 60 mm tissue culture dishes for 24 h, then incubated with 10 μmol/l BBSKE for 24 h, collected(both the suspended and attached cells), fixed on glass cover-slips with 4% paraformaldehyde, stained with 10 μg/ml of Hoechst33342, photographed with a fluorescence microscope (OLYMPUS BX60, Japan, 400×). Independent experiments were repeated several times, and the typical one is shown.

The above procedure was repeated. After treatment, cells were harvested by trypsinization and centrifugation and washed in ice-cold phosphate-buffered saline (PBS) once, then incubated in a binding buffer containing FITC-conjugated Annexin V and propidium iodide (BD Biosciences, San Jose, CA) at room temperature for 15 min in darkness, according to the manufacturer's protocol, photographed with a fluorescence microscope (OLYMPUS BX60, Japan, 400×).

Flow cytometric analysis for apoptosis

Cells were treated with BBSKE in 60 mm dish for 24 h. The complete medium containing 0.1% DMSO was used to serve as control for cell viability and apoptosis determinations. Cells were collected by trypsinization and centrifugation and washed in ice-cold phosphate-buffered saline (PBS) twice, then stained according to the instructions of the manufacturer using the Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, San Jose, CA). Flow analysis was done with a FACS Calibur instrument (BD Biosciences). Cell Quest Software was used for data acquisition and analysis. All measurements were done under the same instrument setting, analyzing 10,000 cells per sample. Annexin V-FITC-positive cells were scored as early apoptotic cells (the right and low quadrant); Annexin V-FITC-stained and propidium iodide-positive cells were scored as late apoptotic cells (the right and up quadrant); unstained Annexin V-FITC and propidium iodide negative cells were scored as viable or surviving cells (the left and low quadrant).

Measurement of Caspase-3 activity

Tca8113 cells were plated in 60 mm Dish (1.5×10^6cells/Dish) with growth medium for 24 h. The cells were then incubated with 5 μmol/l, 10 μmol/l, and 20 μmol/l BBSKE or vehicle (0.1% DMSO) under 10% FBS serum conditions for 24 h. Caspase-3 activity from the cell lysates was measured using the colorimetric CaspACE assay system (Promega, Madison, WI, USA) following the instructions provided by the manufacturer. The cells were harvested and washed twice in ice-cold PBS and resuspended in cell lysis buffer (provided by the manufacturer) at a concentration of 10^8 cells/ml. The cells were lysed by three cycles of freezing and thawing and incubated on ice for 15 min. The cell lysates were centrifuged at $15{,}000 \times g$ for 20 min at 4 °C and the supernatant fraction was collected and stored at −70 °C. To measure caspase activity, the concentration of protein in each sample was determined by dye binding method of Bradford. Eighty micrograms of total protein (in a volume of up to 20 μl) from each sample was added to each well of a 96-well plate. Then 32 μl of caspase assay buffer (provided by the manufacturer), 2 μl DMSO and 10 μl of a 100 mM DTT solution were added to each well. The final volume was adjusted to 98 μl and 2 μl of the Caspase-3 substrate DEVD-pNA was added to the reaction. The plate was incubated at 37 °C for 4 h. Absorbance at 405 nm was determined with a spectrophotometric microplate reader (BIO-Tek Elx808, USA). Independent experiments were repeated twice.

TrxR activity

TrxR activity was determined by an in vitro reduction of 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) to 5′-thionitrobenzoic acid (TNB) using a procedure adapted from those previously published.[19] The conversion of DTNB to TNB (a strongly yellow-colored product) was measured spectrophotometrically at 412 nm. After a 48 h-incubation with different concentrations of BBSKE, cells were lysed in lysis buffer (25 mmol/l Tris-HCl pH 7.4, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 137 mmol/l NaCl, and 10 mmol/l EDTA) and equal amounts of total cellular protein (10 μg) were added in the reaction mixture (100 mmol/l potassium phosphate pH 7.4, 0.3 mmol/l NADPH, 150 μmol/l DTNB). The final volume was 0.1 ml. The enzymatic reaction was followed by recording the increase in absorbance at 412 nm at 10 s intervals for 10 min. Independent experiments were repeated twice.

Tongue carcinoma model and treatment

The BALB/c nude mice were from the animal experiment center of Laboratory Animal center, Department of drug and biological product authentication, China. The care and treatment of experimental animals was in accordance with the institutional guidelines. Human tongue cancer Tca8113 cells were subcutaneously inoculated (2×10^6 cells/mouse) in the right flanks of 10 mice (5—6 weeks old). Two weeks later, the tumors had grown to 100—150 mm^3, and the mice were randomly divided into two groups (five mice/group). One group treated with BBSKE and the other with sodium carboxymethyl cellulose (CMC-Na) under the same conditions as control. The BBSKE group was treated with BBSKE by oral gavages at a dose of 120 mg/kg/day for 10 days consecutively. The first day of treatment was designated as day one. Tumor lengths and widths were measured with calipers every day until animal sacrifice, and the tumor volume was calculated using the formula V (mm^3) = length × $(\text{width})^2/2$, using width as the smaller dimension.[20] Animals were sacrificed by spinal cord dislocation and tumors were subsequently removed surgically and weighed.

Statistical analyses

All numerical data were expressed as the mean ± standard deviation. One-way analysis of variance was used for the statistical analysis of data. P values less than 0.05 were considered statistically significant.

Results

BBSKE inhibits proliferation and induces apoptosis of Tca8113 tongue cancer cells

The proliferation of Tca8113 cell was inhibited by BBSKE, with an IC50 of 7.5 μmol/l. It suggested that BBSKE could inhibit Tca8113 cell proliferation in a dose-dependent manner (Table. 1).

Table 1 The effect of BBSKE on the growth of Tca8113 cells

Dosage (μmol/l)	OD ($N = 6$)	Cell viability (%)	IC50
0	0.365 ± 0.034	100	7.5 μmol/l
1	0.406 ± 0.032	111	
5	0.300 ± 0.033*	82.2	
10	0.067 ± 0.036**	18.4	
15	0.018 ± 0.018**	4.9	

Effects of BBSKE on cell growth. Tca8113 cells (2×10^4/well) were plated in a 96-well plate and treated with different concentrations of BBSKE for 24 h. Cell growth was measured by MTT assay as described in materials and methods. Results shown are representative of four separate experiments.

* $P < 0.05$.

** $P < 0.01$.

Because changes in cell proliferation could be caused by alterations in either cell cycle progression or apoptosis, we examined whether the effects of BBSKE on cell growth were due partly to apoptosis. The ability of BBSKE to induce apoptosis was showed by typical morphologic changes of apoptosis as cytoplasmic and nuclear shrinkage and chromatin condensation or fragmentation (Fig. 1A and B). These hallmarks of apoptosis were not evident in the control (untreated) cells. After exposure to BBSKE 10 μmol/l for 24 h, Tca8113 cell membrane with green staining is shown as early apoptosis (Fig. 1C).

According to flow cytometric analysis, as the concentration of BBSKE increased, the percentages of apoptotic cells increased simultaneously. Moreover, the percentages of apoptotic cells (both PI and Annexin V-FITC-positive cells) in the experimental group were much higher than that in the control group. A dose-dependent relationship between BBSKE concentration and apoptosis rate was revealed (Fig. 2). After exposure of Tca8113 cells to BBSKE at the dosage of 10 μmol/l, 20 μmol/l for 24 h the apoptosis rates were 17.08%, 53.97%, respectively, significantly higher than that of the control group (4.99%).

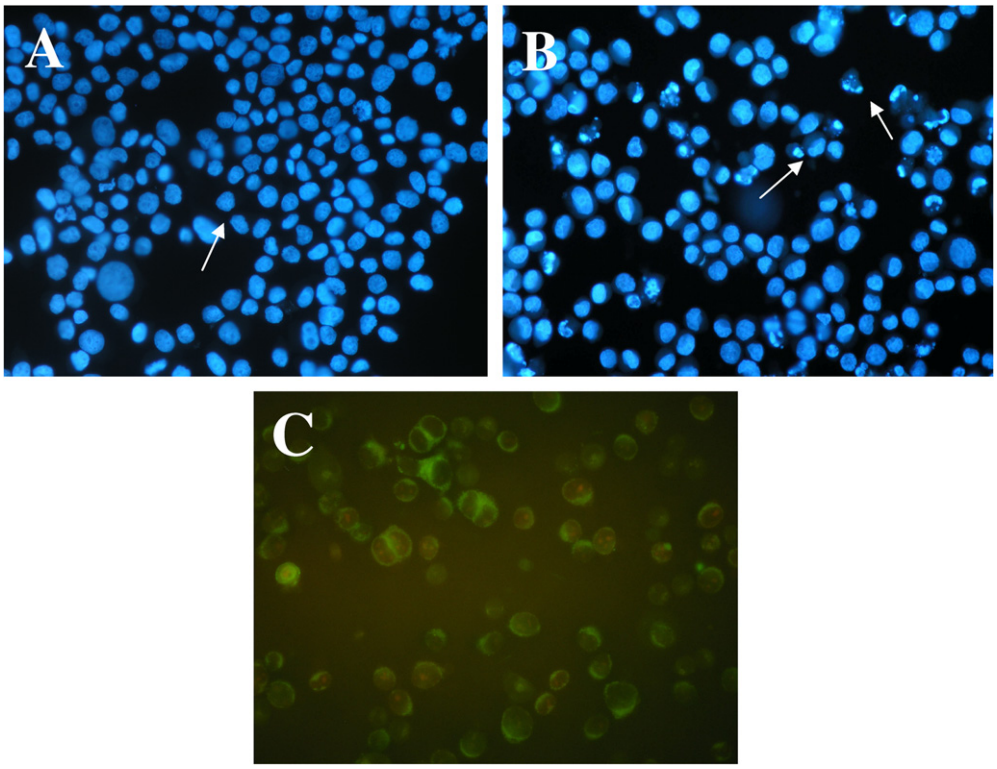

Figure 1 Apoptosis in Tca8113 cells induced by BBSKE. Cell apoptotic morphological examination with fluorescence microscope (×400). Apoptotic cells induced by 10 μmol/l BBSKE for 24 h were detected with Hoechst33342 staining (A, B) and Annexin V/PI staining (C). (A) Cells were treated with complete medium containing 0.1% DMSO as control. Control cells stained by Hoechst reveal the normal appearance of Tca8113 cell nuclei (arrowhead). (B) Cells were treated with 10 μmol/l BBSKE for 24 h. After exposure, evident apoptotic bodies could be noticed, as DNA fragments (arrowheads). (C) Cells were treated with 10 μmol/l BBSKE for 24 h. After exposure, the membrane was stained green as early apoptosis. Separate experiments were repeated several times and the typical one is shown.

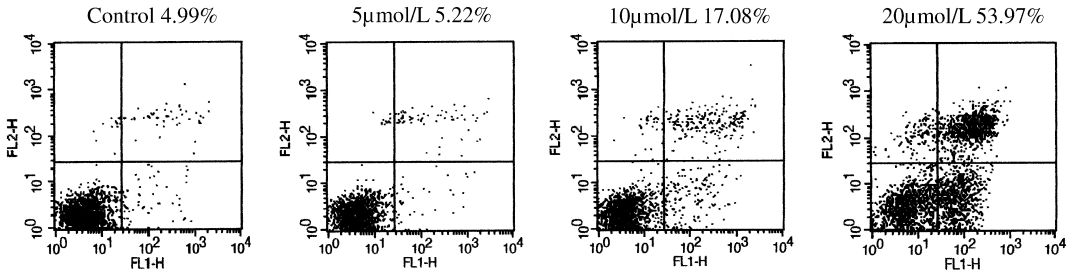

Figure 2 Effects of BBSKE on the apoptosis of Tca8113 cells. Cells (1.5×10^6 cells/Dish) treated with or without various dosages of BBSKE for 24 h, were assayed by Annexin V and propidium iodide staining method. Drug dosages and apoptotic proportions were labeled in each figure. After exposure of Tca8113 cells to BBSKE at the dosage of 10 μmol/l, 20 μmol/l for 24 h the apoptosis rates were 17.08%, 53.97%, respectively, significantly higher than that of the control group (4.99%).

Caspase-3 activity

The average expression of Caspase-3 activity from two separate experiments was determined by densitometry and is shown in the graph below the blots (Fig. 3). The optical density of 10 μmol/l and 20 μmol/lBBSKE-treated cells increased significantly versus the control (0.110 ± 0.037, 0.247 ± 0.043 versus 0.031 ± 0.019; $^*P < 0.05$ versus control). However, the optical density of cells treated with 5 μmol/l BBSKE did not increase (0.038 ± 0.008) versus the control (Fig. 3).

Effects of BBSKE on TrxR activity in Tca8113 cells

BBSKE could inhibit TrxR activity in a dose-dependent manner. As shown in Fig. 4, there is a significant decrease in the TrxR activity in Tca8113 cells after the treatment of 5 μmol/l, 10 μmol/l, 20 μmol/l BBSKE for 48 h. And the percentage of TrxR inactivation is, respectively, 35.5%, 48.2% or 69.1% at 5 μmol/l, 10 μmol/l or 20 μmol/l.

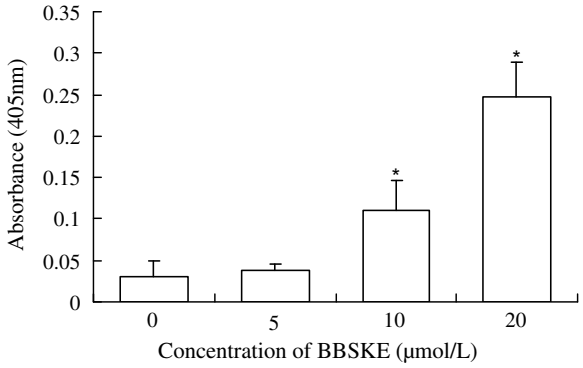

Figure 3 The effect of BBSKE on Caspase-3 activity in Tca8113 cells Caspase-3 activity was measured as described in 'Materials and methods'. The average expression of Caspase-3 activity from two separate experiments was determined by densitometry and is shown in the graph below the blots. Tca8113 cells were treated with BBSKE at different concentrations (0 μmol/l, 5 μmol/l, 10 μmol/l, 20 μmol/l) for 24 h, and their absorbance are 0.031 ± 0.019, 0.038 ± 0.008, 0.110 ± 0.037 ($^*P < 0.05$ versus control), 0.247 ± 0.043 ($^*P < 0.05$ versus control), respectively.

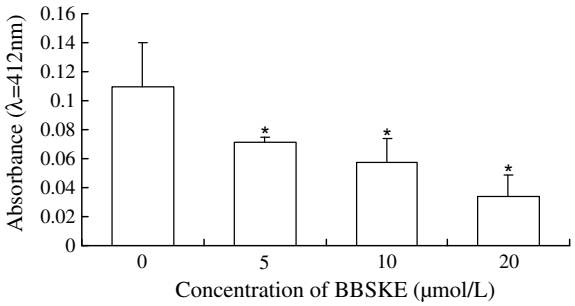

Figure 4 Effect of BBSKE on TrxR activity in Tca8113 tongue cancer cells. The average expression of TrxR activity from two separate experiments is shown. TrxR activity was measured using the DTNB assay after 48 h of treatment with 5 μmol/l, 10 μmol/l and 20 μmol/l BBSKE. The absorbances are 0.071 ± 0.004, 0.057 ± 0.017, 0.034 ± 0.015, respectively, significantly lower than the control (0.110 ± 0.030). The experiment repeated twice separately.

Inhibition of growth of established tongue carcinoma xenografts in nude mice

As shown in Fig. 5, 10 days after BBSKE treatment by oral gavages, there was statistically significant difference in tumor volumes between the BBSKE-treated mice (591.561 mm^3 ± 167.243) and the sodium carboxymethyl cellulose-treated mice (1193.868 mm^3 ± 239.623, $P < 0.05$). The tumors in mice treated with BBSKE showed statistically significant growth inhibition compared with the mice treated with sodium carboxymethyl cellulose ($P < 0.05$).

Discussion

Oral cancer is a public health priority and is estimated by WHO to be the eighth most common cancer worldwide. A principal clinical problem in tongue cancer is that there is no therapy has been shown to be able to enhance survival.[14–17] Because of this reason, innovative and nontoxic approaches to this disease are urgently required.

BBSKE, a novel organoselenium, is designed and synthesized to inhibit the activity of TrxR in several cancer cells[10–12] and it is considered to be a potential anti-cancer drug because of its low toxic effects (the maximum tolerance > 5000 mg/kg). It has lower toxic effects on normal hepatic cell line HC-7701 (IC50-24 h:53.83 μmol/l) than

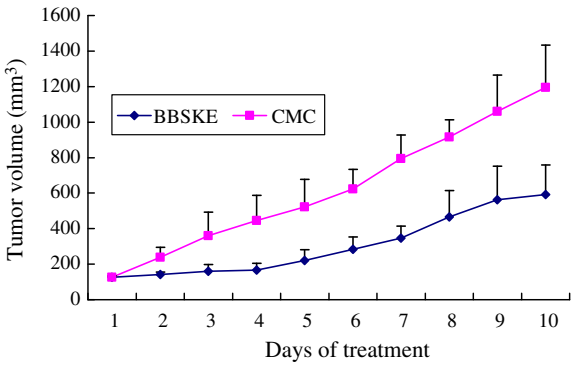

Figure 5 Inhibition of pre-existing tongue cancer xenograft growth by BBSKE. Xenografted BALC/c nude mice were treated from a tumor of volume 100—150 mm^3 until the animals were killed by daily oral gavages of BBSKE at a dosage of 120 mg/kg. The control group received oral gavages of sodium carboxymethyl cellulose according to the same conditions as that of the experimentally treated mice. The size of tumors after treatment with BBSKE and sodium carboxymethyl cellulose (CMC-Na) are shown. Data are presented as the mean ± SD, $n = 5$/group. There was statistically significant difference in tumor volumes between the BBSKE-treated mice (591.561 mm^3 ± 167.243 mm^3) and the carboxymethyl cellulose-treated mice (1193.868 mm^3 ± 239.623 mm^3, $P < 0.05$).

epithelial hepatoma cell line Bel-7402 (IC50-24 h:32.41 μmol/l) and human embryonic lung diploid cells CCC-HPF-1(IC50-24 h > 40 μmol/l)than human lung cancer cells A459(IC50-24 h:7 μmol/l).[12,13] Meanwhile, BBSKE has no effect of mutagenesis.[21] In animal experiments, Zeng et al. found that BBSKE has lower toxic effect than selenite and could also regulate immune activity by increasing relative weight of spleen, transforming activity of spleen lymphocytes, NK cell activity, lymphokine-activated killer (LAK)cells activity and percentage of CD_4^+, CD_8^+ T lymphocyte in vivo.[22—24]

Here, we investigated the effects of BBSKE on the TrxR activity and presented the data of TrxR activity in Tca8113 cells for the first time using DTNB reduction assay. Human TrxR system composed of TrxR, thioredoxin (Trx) and NADPH, is a ubiquitous intracellular oxido-reductase system with antioxidant and redox regulatory roles. It plays a key role in cellular thiol redox control and antioxidant defense, and is associated with cancer cell growth and anti-apoptosis process.[25,26] The TrxR system is found overexpressed in numerous tumors.[27—30] So a potential molecular target for therapeutic agents is found. Oxidative stress is considered as a key factor for DNA damage.[31,32] So the antioxidant TrxR system is regarded as a tumor preventing system.[32] The TrxR system is important for cancer cell proliferation and inhibition of apoptosis. Recent researches suggest that TrxR may function as a primary sensor for mutagenic H_2O_2 and initiate a signal pathway leading to the transcription of genes encoding antioxidative and anti-apoptosis proteins.[33] Tumor cell proliferation is crucially dependent on a constant deoxyribonucleotide supply, which in turn depends on an active Trx/TrxR system.[25,34,35] Moreover, this system provides reduced extracellular Trx as a growth factor and it protects the tumor cells from NK-lysin,[36] tumor necrosis factor-α[37] cytokine- and stress-induced apoptosis, and from immune cells.[38] Accordingly, it is not surprising that numerous cancer cells overexpress TrxR. Garth Powis has reviewed that a number of human primary cancers including cervical, gastric, colon, hepatoma, and lung cancer have a more than 50% of over-expressed Trx.[39] Thus, it is very important to control the activity of TrxR in cancer cells, which is probably related to the pathway of cancer treatment. Our results show that the activity of TrxR in Tca8113 tongue cancer cells treated with BBSKE is significantly inhibited, which is accord with the experiments in other cancer cells.[11,12] And it conformed that thioredoxin reductase (TrxR) is the molecular target of BBSKE for cancer therapy again.

Also, we observed its growth inhibition actions in Tca8113 tongue cancer cells with MTT assay and found that BBSKE caused a dose-dependent growth inhibition in Tca8113 tongue cancer cells. Similar findings were reported in other cancer cell lines.[10—12] Our results also demonstrate that BBSKE significantly inhibited tumor growth in nude mice xenograft model compared with the control.

The mechanisms that BBSKE inhibits tumor cell growth may be induction of S phase cell cycle arrest by regulating cell cycle regulatory proteins (cyclinA, cyclinE, P21, cyclinB1, cyclinD1, Cdk4 etc.), also may be induction of cell apoptosis by regulating the apoptosis regulatory proteins (Bax, NF-κB and Caspase-3).[11,12,35] In our present work, we adopted three methods to investigate apoptosis: Annexin V binding apoptosis assay, the Caspase-3 activity assay, and Hoechst 33342 and Annexin V/PI staining which are usually used to observe the typical morphological changes of apoptosis. We found that there was an induction of apoptosis in Tca8113 tongue cancer cells treated with BBSKE by these techniques. It has been known that apoptotic signaling eventually converge at the activity of the caspases, ultimate executioners of cell death.[40] In our study, the Caspase-3 activity was altered in favor of apoptosis, and the result suggests that BBSKE induces apoptosis through Caspase-3 pathway.

The nature of BBSKE in mediating the aforesaid responses in tongue cancer cells could make it a potential effect therapeutic agent against tongue cancer.

Over-expression of TrxR has been reported in many kinds of tumor tissues and found to be associated with the metastasis of prostate cancer. However, no previous work has directly compared the TrxR activity of human tongue cancer tissue and normal tongue tissue. Because BBSKE is a TrxR inhibitor, it is important to investigate whether TrxR is over-expressed in human tongue cancer tissue for the clinical apply of BBSKE.

In conclusion, our studies provide experimental evidence that BBSKE has an inhibition action on growth of Tca8113 tongue cancer cells in vitro and in vivo, which is probably related to TrxR inactivation, furthermore to proliferation inhibition and apoptosis with alteration in Caspase-3 activity.

Conflict of Interest Statement

None declared.

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