Selenium-containing thioredoxin reductase inhibitor ethaselen sensitizes non-small cell lung cancer to radiotherapy

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It has been proposed that thioredoxin reductase (TR) is a mediator that allows non-small cell lung cancer (NSCLC) to develop resistance to irradiation; however, little is known regarding the detailed mechanisms of action. Thus, ethaselen {1, 2-[bis (1,2-benzisoselenazolone-3 (2*H*)-ketone)] ethane, BBSKE}, a novel organoselenium TR inhibitor, is currently being investigated in a phase I clinical trial in China. However, its radiosensitizing effect remains unexplored. In this study, we found that the activity of TR increased dramatically in both A549 and H1299 cells after radiation, and moreover, could be inhibited by pretreatment with BBSKE (5 μmol/l). As a TR inhibitor, BBSKE enhanced the efficacy of radiation therapy both *in vivo* and *in vitro* without observable toxicity. BBSKE was found to suppress irradiation-induced NF-κB activation dramatically when using A549 cells stably transfected with NF-κB luciferase reporter. These results show the critical role of TR in the radioresistance of NSCLC and suggest that BBSKE is a potentially promising agent for the treatment of patients with NSCLC clinically. *Anti-Cancer Drugs* 22:732–740 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Lung cancer is the leading cause of cancer-related death, with 159 390 estimated deaths in the year 2009 in the United States. Over 80% of these deaths are diagnosed as non-small cell lung cancer (NSCLC) [1]. Current treatment of NSCLC depends on the presentation stage at diagnosis. Standard treatment for NSCLC includes surgery, radiotherapy, chemotherapy, as well as other adjuvant methods, such as molecular-targeted therapy and immunotherapy [2].

Although radiotherapy has been widely used in clinical practice, when used alone it often fails to eliminate NSCLC, leading to radioresistance and the development of distant metastasis. Reactive oxygen species, among the biological effects of irradiation, play a central role in the cell-killing effect and signal transduction [3]. To maintain the balance of the redox state, cells are endogenously equipped with protective enzyme systems, such as superoxide dismutase, reduced glutathione, and the thioredoxin reductase (TR)/thioredoxin (Trx) system. The resulting upregulation of antioxidant molecules is thought to contribute to radioresistance [4–5]. Therefore, treatment with the aim to inhibit antioxidant molecules may offer an effective approach in enhancing cell killing after radiation.

The TR system, composed of Trx, NADPH, and TR, exists in all living cells (thoroughly reviewed by Tonissen and Trapani [6]). The relationship between the TR system and radiation is complex. It has been reported that after radiation the expression of TR increases dramatically [7], whereas the change in TR activity remains to be studied in depth. As a TR inhibitor [8], BBSKE has received a qualification certificate for phase I clinical trials in China. The combination of BBSKE and cisplatin has been reported to synergistically enhance the antitumor activity of cisplatin in nude mice bearing A549 xenografts [9]; however, its radiosensitizing effect has not yet been investigated.

In this study, we determined the level of TR activity change after radiation in both A549 and H1299 cells. As a TR inhibitor, the radiosensitizing effect of BBSKE was investigated both *in vivo* and *in vitro*. Furthermore, we showed the ability of BBSKE to suppress irradiation-induced NF-κB activation using the stably transfected A549-pNF-κB-TA-Luc cells.

Materials and methods

Chemicals and drug treatment

Ethaselen (BBSKE) (PCT: CN02-00412) was designed and synthesized in our laboratory (State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing, China). A stock

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solution of BBSKE was made with a concentration of 20 mmol/l in dimethyl sulfoxide; the agents were freshly diluted in culture medium Dulbecco's modified Eagle's medium (Gibco-BRL, Grand Island, New York, USA) for all cell experiments.

Cell culture

Parental A549, H1299, and H1666 cell lines were obtained from the cell center of Chinese Academy of Medical Sciences (Beijing, China) and the Lewis lung carcinoma cell line (LLC) was a gift from Wei Zhao (Beijing Institute of Cancer Research, Beijing, China). All the cell lines were cultured in DMEM supplemented with 10% fetal bovine serum and maintained at 37°C in a humidified atmosphere of 5% CO_2. Cells in the exponential growth phase were used for experiments.

Generation of the recombinant cell line A549-pNF-κB-TA-Luc and growth condition

Stable cell lines were generated by transfecting A549 cells with the pNF-κB-TA-Luc reporter construct (Beyotime, China) using the GeneJuice transfection reagent (Novagen, San Diego, California, USA) according to the protocol provided by the manufacturer. pNF-κB-TA-Luc reporter contains the luciferase gene sequences driven by an artificial promoter element with four NF-κB binding sites. Cells with genomic incorporation were selected on the basis of antibiotic resistance (Geneticin, Invitrogen, Carlsbad, California, USA). The newly generated cell line, A549-pN-κB-TA-Luc, was maintained in medium supplemented with Geneticin (800 ug/ml) and split (1 : 5) every 7 days. Medium was changed after a growth period of 4 days. The reporter response was tested by lipopolysaccharide stimulation.

Ionizing radiation modalities

Cells were cultured in phenol-red-free medium and irradiated with a ^{60}Co machine. The radiation was delivered as a single dose of 2–10 Gy in an appropriate field size at a dose rate of 0.86 Gy/min. Control cells were removed from the incubator and placed for the same period of time under the irradiation source, but without radiation treatment. In the combined treatment group, BBSKE (5 μmol/l) was added 24 h before irradiation.

Thioredoxin reductase enzymatic assay

TR activity was determined by a previously developed method [10] and performed as described [11]. In brief, cell extracts from log-phase cells were prepared by freeze–thaw lysis and quantified as described [12] and 20 μg of extracts were incubated with bovine insulin, NADPH, and Trx in 0.2 mol/l of HEPES (pH 7.6), for 20 min at 37°C. Reactions were terminated by the addition of of guanidine hydrochloride (6 mol/l)/0.4 mg/ml dithiobis (2-nitrobenzoic acid) prepared in 0.2 mol/l of Tris (pH 8.0). In each case, a corresponding experimental sample without Trx was used to correct for the basal level of TR activity (due to endogenous Trx and NADPH). At the same time, reactions without cell extracts and reactions with pure TR in place of cell extracts were also used as negative and positive controls, respectively. Triplicate samples were measured for enzymatic activity by spectrophotometric absorbance at 412 nm.

Clonogenic survival assay

Clonogenic survival is defined as the ability of cells to maintain cologenic capacity and form colonies. In brief, cells were exposed to different radiation doses (0, 2, 4, 6, 8, and 10 Gy). For the BBSKE and combination groups, BBSKE (5 μmol/l) was added 24 h before radiation. After irradiation, the media in all groups were aspirated and fresh media were added. After incubation for 14 days, the cells were fixed with methanol and stained with Giemsa. Colonies containing more than 50 cells were counted. The plating efficiency (PE) and survival fraction (SF) were calculated as follows: PE = (colony number/inoculating cell number) × 100%; SF = PE (tested group)/PE (0 Gy group) × 100%. A dose–survival curve was obtained for each experiment and used for calculating survival parameters. Parallel samples were set at each radiation dosage. The cell–survival curve was plotted with Origin7.5 software, using the equation: $SF = 1 - (1 - e^{-D/D_0})^N$. The multitarget single-hit model was applied to calculate the cellular radiosensitivity (mean lethal dose, D_0), the capacity for sublethal damage repair (quasithreshold dose, D_q), and the extrapolation number (N). The D_0 values were used to calculate the sensitizer enhancement ratios (SERs) [13].

Apoptosis analysis by flow cytometry

The treatment schedule for fluorescence-activated cell sorting incorporated four groups in the experiments (control, BBSKE-treated, irradiation-treated, and the combination group). In the combination group, BBSKE (5 μmol/l) was added 24 h before irradiation. After irradiation, the media in all groups were replaced with fresh media. The radiation-alone group and the combination group were exposed to γ-rays at 8 Gy and all the groups were harvested 48 h after irradiation. For detection of apoptotic cells, cells were trypsinized, counted, and washed twice with cold PBS. Cells used for apoptosis tests were stained with PI and Annexin V for 15 min in the dark and then analyzed by fluorescence-activated cell sorting using a flow cytometer EPICS XLMCL System II (Beckman Coulter, Inc, Fullerton, California, USA) according to the manufacturer's protocol (Biosea Biotechnology, China). Each test was performed three times [14].

Western blotting analysis

For the evaluation of TR1, whole-cell lysates were collected and protein concentrations were determined by the Bradford method. Protein (60 μg) was denatured and fractionated on 12% polyacrylamide gels containing SDS and then transferred to a polyvinylidene fluoride

membrane. The membrane was blocked in 5% milk in TBS-T, probed with 1 : 500 anti-TR1 (B-2) monoclonal primary antibody (Santa Cruz Biotechnology, Santa Cruz, California, USA), washed three times with Tris-buffered saline with Tween, and probed with 1 : 4000 goat anti-mouse horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology). Protein bands were visually detected by enhanced chemiluminescence (western blotting detection system; Applygen Technology, Beijing, China). Membranes were also probed with anti-actin antibody (Santa Cruz Biotechnology) to normalize sample difference.

Assay of NF-κB transcription/promoter activity

A total of 5×10^4 viable A549-pNF-κB-TA-Luc cells were seeded in each well of a 24-well plate. Cells were exposed to ^{60}Co radiation (8 Gy) after 24 h of seeding and harvested 3 or 6 h later. In the combination group, the cells were pretreated with BBSKE (5 μmol/l) for 24 h before irradiation. Luciferase activity was determined using the luciferase assay kit obtained from Promega (Madison, Wisconsin, USA).

In vivo antitumor activity of BBSKE with or without radiation

Five-week-old male C57BL/6 mice were used for all experiments. All experiments were performed in strict compliance with the ethical guidelines issued by the Ethics Committee of Peking University Health Science Center. LLC cells (2×10^6) were injected subcutaneously into the right hind leg of C57BL/6 mice. Tumor volume was determined by using caliper measurement of tumor length (L) and width (W) according to the formula: volume $= 0.5236 \times L \times W^2$ [15]. Treatment was initiated when tumors in each group achieved an average volume of 200 mm^3. Experiment groups consisted of control, BBSKE alone, radiation alone, and the combination of BBSKE and radiation. Each group contained 14 mice. In BBSKE-alone and the combination groups, BBSKE (36 mg/kg) was administered orally, every day for 14 days; mice in the control and radiation-alone groups were administrated with the vehicle (0.5% sodium carboxymethyl cellulose, CMC-Na). Tumors in legs were exposed to 2, 4, 6, 8, and 10 Gy of γ-radiation with a ^{60}Co irradiator at a rate of 0.86 Gy/min immediately after drug treatment, with the other parts of the body shielded with lead. Growth delay (GD) was calculated as the time required for treated tumors to achieve a six-fold increase in volume minus the corresponding time required for control tumors. The enhancement factor was then determined as $(GD_{combination} - GD_{BBSKE})/(GD_{radiation})$ [16]. After 14 days, five mice from each group were randomly chosen and killed; the blood samples were collected for biochemical analysis. In each group, the remaining mice were kept to complete the survival time experiments.

Statistical analysis

Unless otherwise stated, data are presented as mean ± SE. For comparisons between groups, we used analysis of variance. The significance of the difference between the means of two variables was determined by the paired Student's t-test. Comparisons among groups in the survival data were made using the log-rank test after Kaplan–Meier analysis. A probability value of $P < 0.05$ was considered as different and $P < 0.01$ as significantly different.

Results

Effect of BBSKE on TR activity after irradiation in A549 and H1299 cells

TR enzyme activity was evaluated 1, 3, 12, 24, and 48 h after a single irradiation dose of 8 Gy in both A549 and H1299 cells. Irradiation dramatically increased TR activity, with the maximum reached at 12 h after radiation, and returned to the normal level 48 h later (Fig. 1a and b). In A549 cells, BBSKE pretreatment decreased the TR activity by 40.0, 40.1, 59.4, 22.2, and 30.2% at different time points compared with radiation alone ($P < 0.01$; Fig. 1c), whereas the corresponding data in H1299 cells were 45.3, 36.8, 59.3, 24.8, and 35.6%, respectively ($P < 0.01$; Fig. 1d). These findings indicated that BBSKE suppressed irradiation-induced TR activity in both A549 and H1299 cells.

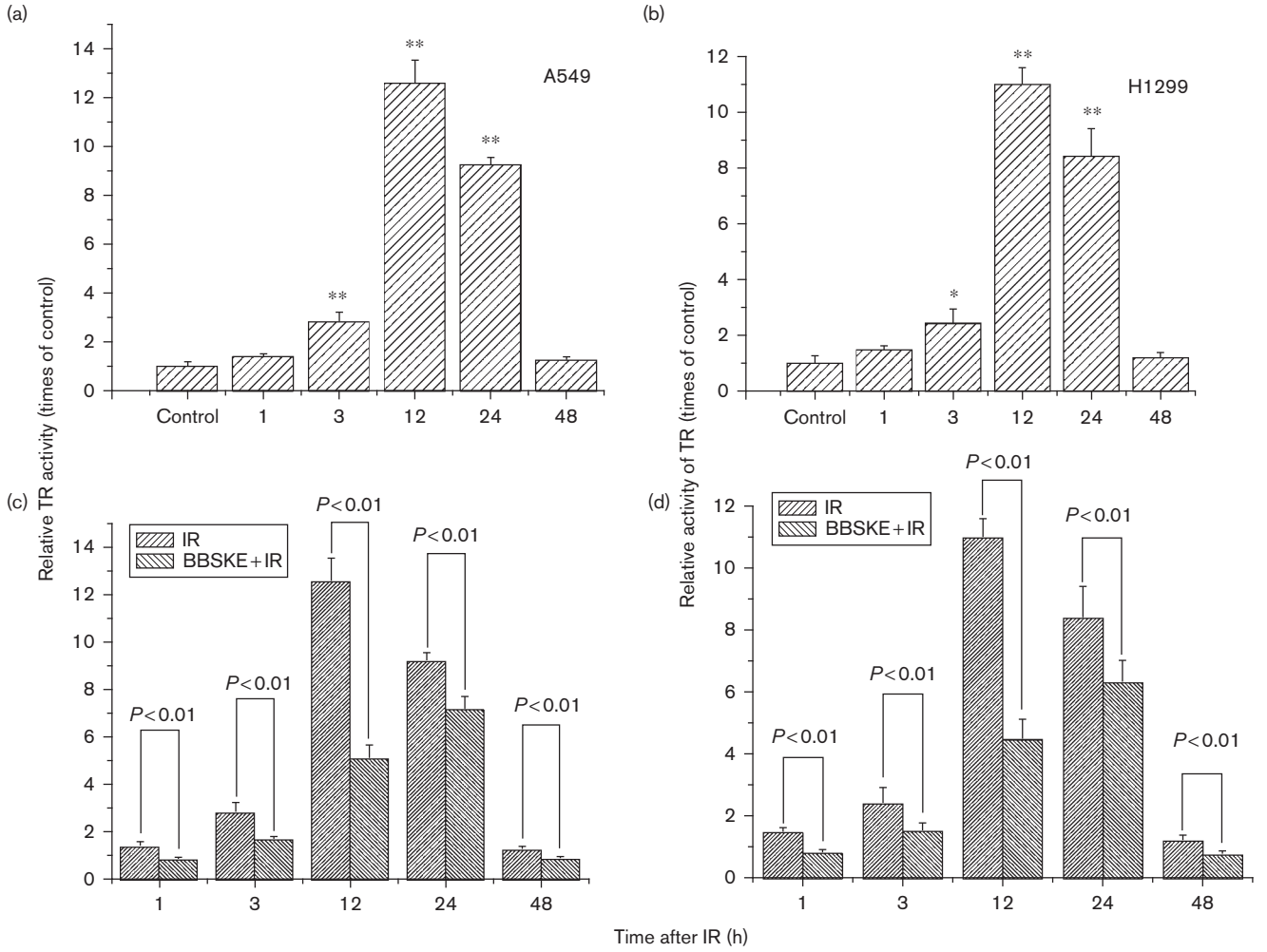
Radiosensitizing effect of BBSKE on A549, H1299, and H1666 cells

To study the radiosensitizing effect of BBSKE *in vitro*, a clonogenic survival assay was performed. The survival curves for control and BBSKE-treated cells after radiation are shown in Fig. 2. The radiobiological parameters of BBSKE-treated A549 cells were $D_0 = 0.983$, $D_q = 1.051$, and $N = 2.913$, whereas those of A549 cells irradiated only were $D_0 = 1.591$, $D_q = 1.125$, and $N = 2.029$. SER = D_0(control)/D_0(BBSKE) = 1.619. The radiobiological parameters of BBSKE-treated H1299 cells were $D_0 = 1.595$, $D_q = 0.687$, and $N = 1.538$; whereas those of H1299 cells irradiated only were $D_0 = 2.488$, $D_q = 1.451$, and $N = 1.792$. SER = D_0(control)/D_0(BBSKE) = 1.560. These results indicated that, for both A549 and H1299 cells, pretreatment with BBSKE significantly improved the biological effect of irradiation. To determine whether the radiosensitizing effect of BBSKE observed in the A549 and H1299 cells translated to other non-small cell lung cancer cell lines expressing a considerably lower level of TR1, we examined the radiosensitizing effect of BBSKE in H1666 cells (expression level of TR1; Fig. 3). The SER of BBSKE-treated H1666 cells was 0.936, indicating that BBSKE had no radiosensitizing effect on H1666 cells.

Apoptosis plays an important role in the radiosensitizing effect of BBSKE

The role of apoptosis in the sensitivity of A549 and H1299 cells to irradiation induced by BBSKE was detected by PI/AnnexinV double staining. As shown in

Fig. 1



BBSKE suppressed irradiation (IR)-induced activity of thioredoxin reductase (TR) in A549 cells and H1299 cell lines. At indicated times after IR, TR activity assay was performed as described in the Materials and methods, and the results were normalized to own cells untreated control (at indicated time points after IR, there were no difference of TR activity among each control group). (a) A549 after irradiation (IR), (b) H1299 after IR, (c) pretreatment with BBSKE (5 μmol/l) in A549, and (d) pretreatment with BBSKE (5 μmol/l) in H1299. Data are representative for three independent experiments. *$P<0.05$ compared with control, **$P<0.01$ compared with control or radiation group.

Fig. 4, when compared with the irradiation-only group, 5 μmol/l BBSKE increased the irradiation-induced apoptosis rate from 20.9 ± 2.1 to 47.8 ± 3.2% in A549 cells ($P < 0.01$; Fig. 4a) and from 25.4 ± 1.6 to 48.5 ± 3.8% in H1299 cells ($P < 0.01$; Fig. 4b).

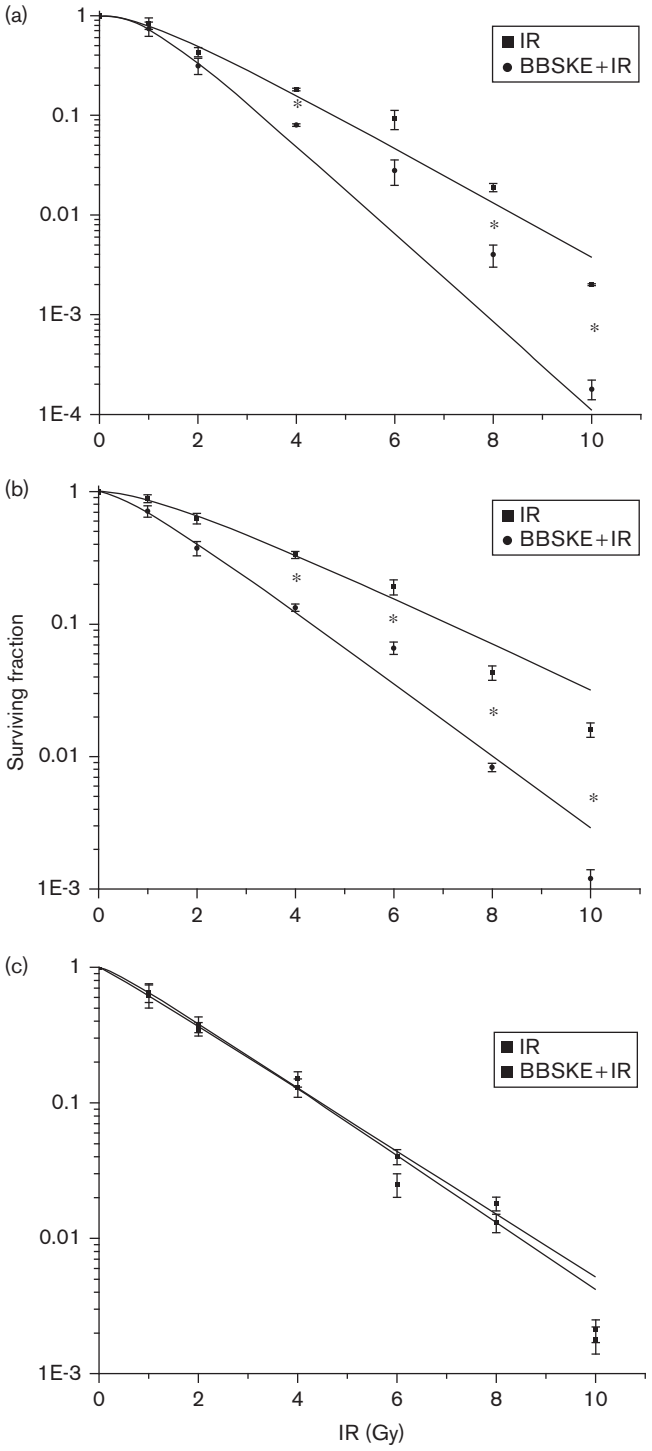
BBSKE inhibits irradiation-induced NF-κB activity

Radiation induces cell death after DNA damage [17]; however, numerous signaling pathways of tumors are also triggered to prevent cells from radiation-induced cell death, most of which have been shown to be connected with the NF-κB pathway [18]. Moreover, NF-κB has been shown to be a direct target of BBSKE in the A549 cell line [19]. Therefore, we decided to investigate the effects of BBSKE on irradiation-induced NF-κB activation. The A549 cells stably transfected with the NF-κB luciferase reporter (A549-pNF-κB-TA-Luc) were exposed to radiation (8 Gy) with or without pretreatment of 5 μmol/l BBSKE. The results indicated that BBSKE inhibited NF-κB transcriptional activity, which is consistent with the results reported by Lan LX [19]. Pretreatment with 5 μmol/l BBSKE significantly suppressed irradiation-induced NF-κB activation (as shown in Fig. 5).

Combination of radiation and BBSKE abridged the growth of lung tumor xenografts

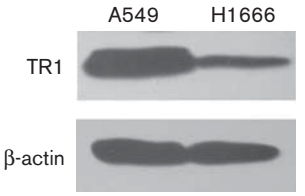
As shown in Fig. 6a, on tumor formation mice were treated with a single dose of irradiation at different dosage levels (0, 4, 8, 10, and 12 Gy). Growth rates of tumor decreased as the irradiation dose increased. As the

Fig. 2



Dose–survival curves of A549, H1299, and H1666 cells after irradiation with or without BBSKE administered 24 h before irradiation (a) A549, (b) H1299, and (c) H1666. In irradiation (IR) groups (■), cells were exposed to different doses of radiation. In the combination groups (●), cells were pretreated with BBSKE (5 μmol/l) for 24 h before IR. Experiments were performed at least three times, and the results are expressed as mean ± SE. Data were analyzed by pairwise comparisons. *Statistical differences (*$P<0.05$).

Fig. 3



Western blotting analysis of TR1 levels in A549 and H1666 cells. Total protein (60 μg) was separated on 12% SDS–polyacrylamide gels. Cellular expression of TR1 was detected by using anti-TR1 (B-2) monoclonal primary antibody (Santa Cruz Biotechnology) by western blotting procedures described in the Materials and methods. Beta-actin was used as a loading control.

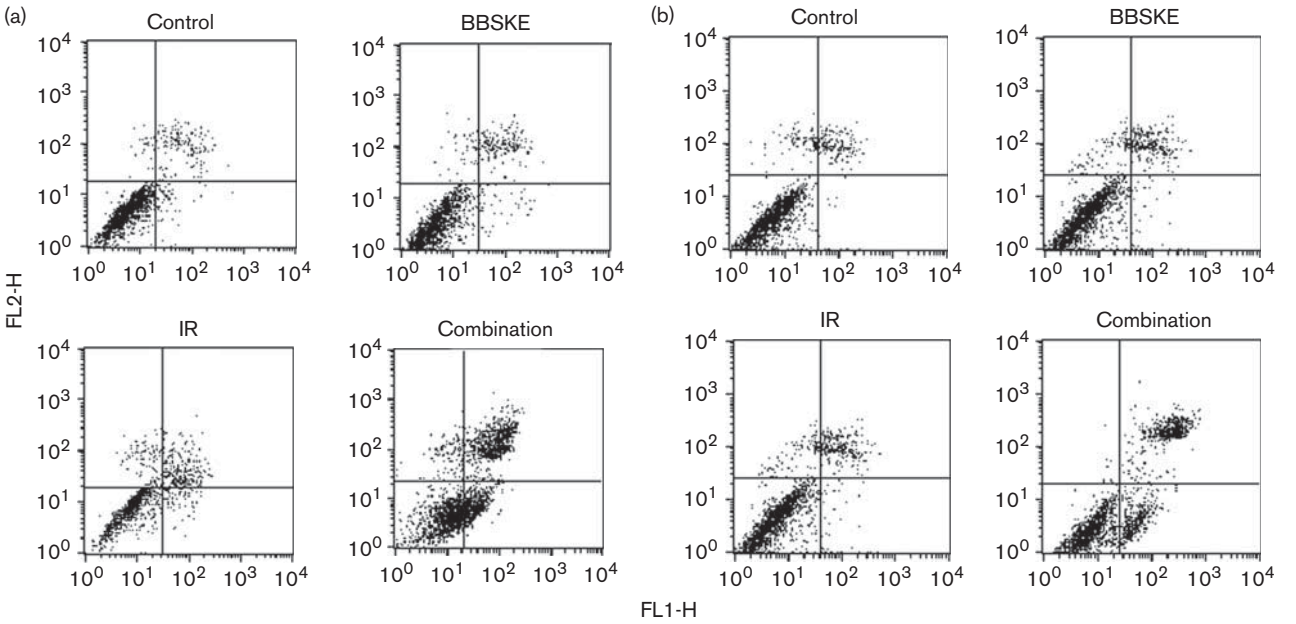
inhibition rate of 8 Gy was appropriate, thus, we used 8 Gy to observe the radiosensitizing effect of BBSKE.

To detect whether the BBSKE-induced radiosensitization of NSCLC cells observed *in vitro* might also be apparent *in vivo*, we injected LLC cells into C57 mice to elicit the formation of solid tumors. After tumors developed to around 200 mm^3, the mice were treated with BBSKE (36 mg/kg/day × 14 day), γ irradiation (8 Gy), or a combination. Tumor GDs induced by treatment with irradiation alone, BBSKE alone, or a combination treatment were observed at 2, 4, and 8 days, respectively. The enhancement factor for the effect of BBSKE on the efficiency of radiation was approximately 1.5 for LLC, indicating a synergistic effect (Fig. 6b).

Protective effect of BBSKE on C57 mice after irradiation

After irradiation, the general condition of mice in a single radiation group was abnormal (diarrhea was observed), whereas no difference was observed between the control group and combination treatment group. Body weight change is an important index to measure systemic toxicity. During the experiment, there was a tendency toward declining animal body weight in the BBSKE and combination treatment groups compared with control group, but it was not significant ($P > 0.05$), whereas the difference between the irradiation group and control group was highly significant ($P < 0.01$). At the end of the experiment, no difference of animal body weight was observed between control, BBSKE, and combination groups, but the body weight in the irradiation group was significantly decreased compared with the control or combination group ($P < 0.01$ vs. control, $P < 0.01$ vs. combination; Fig. 6c). The data indicate that BBSKE shows a protective effect against local irradiation. The serum levels of aspartate aminotransferase, alanine aminotransferase, urea nitrogen creatinine, complete blood cell counts, triglyceride, and total cholesterol showed no difference among groups (data not shown).

Fig. 4



Apoptosis rates of BBSKE, irradiation (IR), or combination in (a) A549 and (b) H1299 cells 48 h after radiation. (a) A549 cells were untreated (control), treated with BBSKE (5 μmol/l), IR (8 Gy), or IR combined with BBSKE for 24 h. (b) H1299 cells were untreated (control), treated with BBSKE (5 μmol/l), IR (8 Gy), or IR combined with BBSKE for 24 h.

Effect of BBSKE on the life span of C57 mice exposed to irradiation

Survival data and Kaplan–Meier plots for the control group, group treated with BBSKE, group receiving irradiation alone, and group treated with the combination of BBSKE and radiation are shown in Fig. 7. The survival plots of all treatment groups were different from that of the control group ($P < 0.05$). The mean survival time ± SE of controls was 18 ± 1 days after tumor implantation, compared with 33 ± 5 days for mice that received BBSKE and 27 ± 4 days for mice that received irradiation. In the combination group, there were five mice surviving more than 50 days, at which time the study was terminated, which was significantly different compared with controls ($P < 0.01$), BBSKE-treated group ($P < 0.05$), or irradiation-treated group ($P < 0.01$).

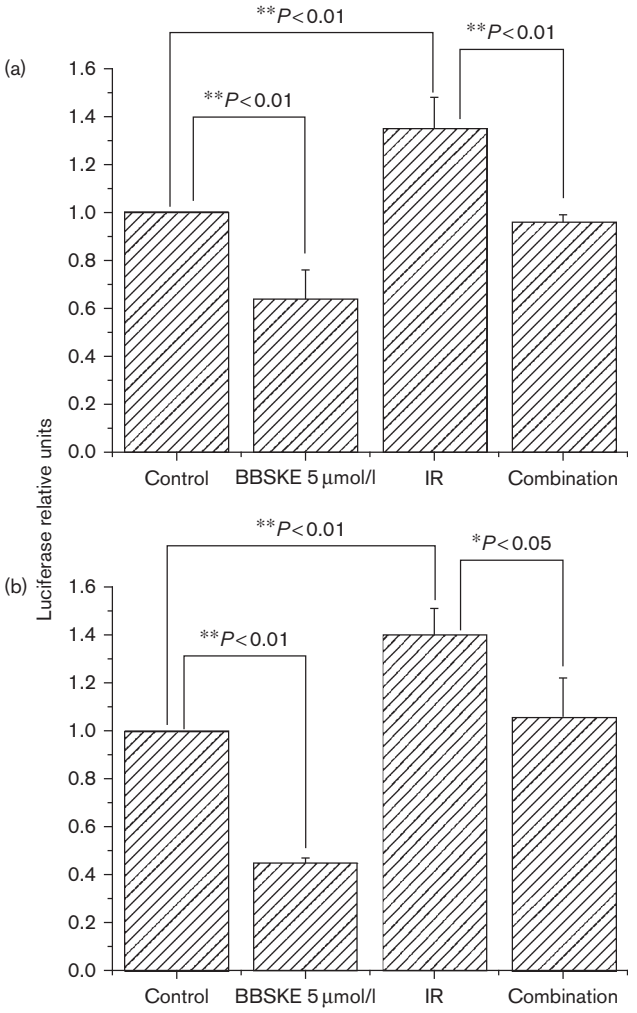
Discussion

Radiotherapy is an important choice for the clinical treatment of lung cancer; however, it usually shows serious side effects, such as immune system damage and marrow suppression in the hematopoietic system and gastrointestinal tract [20]. Another problem facing radiation therapy is that tumor cells develop radioresistance. Therefore, radiosensitizers have been developed to enhance the tumor cell killing while showing a reduced effect on normal tissue, a method that may partially solve the radioresistance problem. Cisplatin and carboplatin have been reported to present a radiosensitizing effect with the increasing extent of DNA double-strand breaks in several cancer cell lines [21–23]; however, additional toxicities were also observed [24]. Therefore, novel radiosensitizer development is needed and the proteins involved in cell signaling pathways and growth receptors are the primary targets [25].

It has been shown that the TR system is closely related to radioresistance. When cells are exposed to irradiation, TR has been reported to be a potential molecular target by regulating the activities of NF-κB and AP-1. Free radicals led by radiation are able to activate TR, which subsequently induces Trx to translocate from the cytoplasm into the nucleus. In the nucleus, the reduced form of Trx is able to regulate the DNA-binding activity of AP-1 and NF-κB, resulting in radioresistance [11,26,27]. Furthermore, using cell lines overexpressing the wild-type or dominant-negative form of TR, Smart *et al.* [28] tested the hypothesis that TR is essential in balancing the thiol redox status and in directly scavenging cytotoxic free radicals induced by irradiation. The study showed that Hela cells overexpressing the wild-type TR, but not the dominant-negative form, were more resistant to the lethal effects of irradiation, suggesting that TR is a clinically relevant target for novel radiosensitizing agents.

As a TR inhibitor in phase I clinical testing in China, BBSKE has been shown to exhibit antitumor effects and immune-regulating characteristics in several tumor models both *in vitro* and *in vivo* [8,29–33]. In A549 cells, the

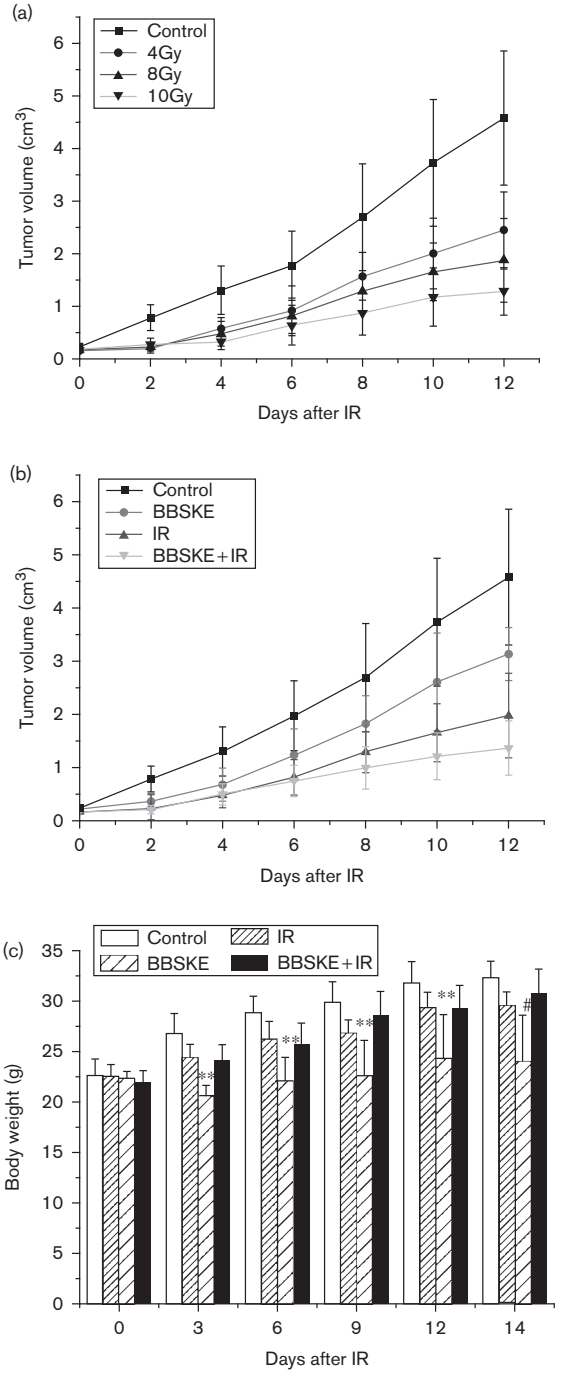
Fig. 5



BBSKE inhibited reporter gene expression in response to radiation. The A549 cells, which were stably transfected with the NF-κB luciferase reporter plasmid, were treated with irradiation (IR, 8 Gy) without or pretreated with BBSKE (5 μmol/l) for 24 h. (a) Three hours and (b) 6 h after IR, the relative NF-κB activity was measured by the luciferase assay. In the BBSKE group, cells were only treated with BBSKE (5 μmol/l) for 24 h. Results, presented as relative activity compared with untreated control cells, are given as mean ± SE from three assay wells. The experiment was repeated three times. $*P<0.05$ vs. irradiation group, $**P<0.01$ versus control or irradiation group.

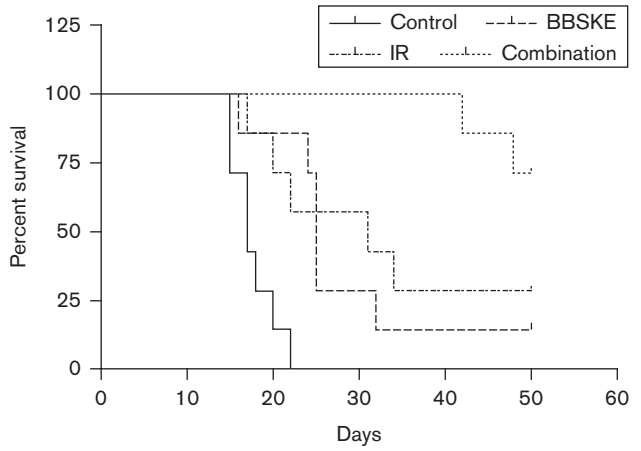
mechanism of the anticancer effect of BBSKE has been comprehensively shown, and is most likely induced by mitochondria-dependent apoptosis through suppression of the TR–Trx–NF-κB pathway [19]. As this pathway is also involved in radioresistance [11,26–28], we studied the radiosensitizing effect of BBSKE on NSCLC. A clonogenic assay is the classical method for evaluation of the kill effect of radiation. To study the radiosensitizing effect of BBSKE on NSCLC, two cell lines, A549 and H1299, were chosen. As shown in Fig. 2, the SER in both cell lines are greater than 1, which means that BBSKE exhibits a radiosensitizing effect. Furthermore, the

Fig. 6



Tumor volume and body weight changes after the treatment of BBSKE or irradiation (IR) and combination therapy on C57 mice inoculated with LLC. (a) Tumor volume changes of mice treatment with different doses of IR. (b) Tumor volume changes of mice treatment with control, BBSKE, IR, and the combination. BBSKE was dosed after injection with LLC cells, when tumor sizes were palpable (about 200 mm^3). BBSKE was administered orally continuously for 14 days, and IR was given once or combined with BBSKE. Tumors were measured daily and volume was calculated by the following formula: length × $width^2$ × 0.5236. (c) Body weight changes of C57 mice after treatment with control, BBSKE (continuously for 14 days), single-dose IR (8 Gy), or combination. Data are presented as mean ± SE. $*P<0.05$ vs. control, $**P<0.01$ vs. control, $^{\#}P<0.01$ vs. control and BBSKE or combination.

Fig. 7



BBSKE prolonged the life span of C57mice after irradiation (IR). Seven mice per group were monitored for survival. Differences in animal survival among groups were evaluated using the log-rank test. The survival time of the mice that received the combination of BBSKE and IR was significantly different from that of control, treated with BBSKE, or IR groups ($P<0.01$).

apoptosis rate was analyzed by flow cytometry, the coincidence between apoptosis rate and SF curves indicated that apoptosis might play an important role in the radiosensitizing effect of BBSKE in NSCLC (Fig. 4). Moreover, the radiosensitizing effect of BBSKE was observed in C57 mice bearing Lewis lung tumor xenografts. The above results indicate that BBSKE has the tendency to increase the curative effect of radiation therapy without obvious toxic effects, suggesting the great potential of BBSKE as an important radiosensitizer.

To study the mechanism of the radiosensitizing effect of BBSKE, the luciferase reporter method was performed. As the radiosensitizing effect of BBSKE was the most obvious at 8 Gy, this dosage level was chosen for further study. The results showed that NF-κB activity increased dramatically after radiation (8 Gy), which could be inhibited with pretreatment of BBSKE (5 μmol/l). To determine whether TR is involved in the enhancement of the cytotoxic response of BBSKE in combination with irradiation, we performed a cologenic survival assay on the H1666 cell line, which expresses a much lower basal TR level than the A549 cell line [34]. The results indicated that the radiosensitizing effect of BBSKE was not exhibited on the H1666 cells, indicating that TR is required for the BBSKE-mediated radiosensitization effect in NSCLC. Therefore, BBSKE-mediated inhibition of TR activity might contribute to its radiosensitizing effect by suppressing irradiation-induced NF-κB activation. As the majority of small molecule inhibitors are not specific, we have not excluded other possible targets for the radiosensitizing effect of BBSKE, such as protein disulfide isomerase and glutaredoxin in the Trx family.

Overall, this study has shown that the increase in TR activity after irradiation plays an important role in radioresistance and further supports the role of TR as a redox-sensitive factor responding to ionizing radiation [35]. BBSKE, a selenium-containing TR inhibitor, showed a radiosensitizing effect in NSCLC cell lines and tumor models. Furthermore, the identification of BBSKE as a radiosensitizer in NSCLC is also of considerable interest because of its affordability, ease of oral administration, and lack of toxicity in clinical use (unpublished works). All of these properties taken together make BBSKE a promising antitumor agent for the treatment of NSCLC by promoting the therapeutic activity of radiation.

Acknowledgements

Conflicts of interest

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