

RESEARCH ARTICLE

Preparation of Selenium-enriched *Bifidobacterium Longum* and its Effect on Tumor Growth and Immune Function of Tumor-Bearing Mice

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Abstract

In this study, we demonstrated selenium (Se) accumulation in *Bifidobacterium longum* strain (*B. longum*) and evaluated the effect of Se-enriched *B. longum* (Se-*B. longum*) on tumor growth and immune function in tumor-bearing mice. Analysis using high-performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) revealed that more than 99% of Se in Se-*B. longum* was organic, the main component of which was selenomethionine (SeMet). In the *in vivo* experiments, tumor-bearing mice (n=8) were orally administrated with different doses of Se-*B. longum* alone or combined with cyclophosphamide (CTX). The results showed that the middle and high dose of Se-*B. longum* significantly inhibited tumor growth. When Se-*B. longum* and CTX were combined, the antitumor effect was significantly enhanced and the survival time of tumor-bearing mice (n=12) was prolonged. Furthermore, compared with CTX alone, the combination of Se-*B. longum* and CTX stimulated the activity of natural killer (NK) cells and T lymphocytes, increasing the levels of interleukin-2 (IL-2) and tumor necrosis factor- α (TNF- α), and the leukocyte count of H22 tumor-bearing mice (n=12).

Keywords: *Bifidobacterium longum* - chemotherapy - selenium - selenium speciation - tumor

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Introduction

Bifidobacterium longum (*B. longum*) is a lactic acid-producing bacterium that grows naturally in the human gastrointestinal tract and defends the host against viral infection (Otles et al., 2003; Picard et al., 2005). As an anaerobe, *B. longum* can selectively germinate and proliferate in the hypoxic regions of solid tumors (Yazawa et al., 2000). Previous studies showed that *B. longum* had anticancer effects on many types of tumors, such as colon, breast and liver cancers (Singh et al., 1997; Hamaji et al., 2007).

Selenium (Se), an essential micronutrient for the health of both humans and animals, exhibits its biological effect in many ways. Epidemiological data suggest that there is an inverse relationship between Se status and the risk of tumorigenesis (Karimi et al., 2012; Yadav et al., 2012), and recent studies indicated that Se compounds inhibited the proliferation and invasion of tumor cells in a variety of tumor models (Menter et al., 2000; Zeng et al., 2008; Sanmartin et al., 2012). Some groups used mouse models to study the role of Se in cancer cells and their results showed that Se exhibited antitumor activity in several tumor-bearing mouse models (Chen et al.,

2007; Sanmartin et al., 2012). Although the mechanisms of the antitumor activities of Se are not fully understood, proposed mechanisms include antioxidant protection, metabolic alteration of some carcinogens, stimulation of the immune system, inhibition of tumor cell proliferation and induction of cell apoptosis (Jiang et al., 2001; Yazdi et al., 2012).

The bioavailability and toxicity of Se are closely correlated with its chemical form. Several studies have suggested that organic Se is usually less toxic and more bioavailable than inorganic Se (Brown et al., 2000). Recently, research showed that some microorganisms were capable of accumulating large amounts of Se and transforming inorganic Se into organic Se (Suhajda et al., 2000; Yang et al., 2009; Jin et al., 2012). Of these microorganisms, Se-enriched yeast has been studied extensively and is now commercially available as a nutritional Se supplement (Suhajda et al., 2000). In addition, *Lactobacillus* was also found to have the ability to transform inorganic Se into organic compounds (Yang et al., 2009). In our previous study, Se was enriched to transformed *B. longum* carrying pBV22210-endostatin (*B. longum*-En). Its biochemical characteristics, effects on pathogenic bacteria, macrophage activity and activity

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in H22 tumor mouse models were investigated (Li et al., 2010). In the present study, we used *B. longum* strain without transporting genes to accumulate Se, determined the Se species in Se-enriched *B. longum* (Se-*B. longum*) and investigated the effect of Se-*B. longum* on solid tumor growth, survival time and immune function of tumor-bearing mice when treated alone or combined with cyclophosphamide (CTX).

Materials and Methods

Bacterial strain and reagents

B. longum NQ-1501 was obtained from the Inner Mongolia Shuangqi Medical Industry Corporation (Inner Mongolia, China). Sodium selenite was purchased from Shanghai LuYuan Fine Chemical Factory (Shanghai, China). SeMet, SeCA and SeCys were purchased from Sigma (USA). SeUr was purchased from Fluka (USA). Sodium dodecyl sulfate (SDS) and DL-dithiothreitol (DTT) were purchased from Beijing XinKe Co. Ltd. (Beijing, China). Trypsin and proteinase K were purchased from Merck (Germany). Selenium Yeast Tablets was purchased from Mudanjiang Lingtai Pharmaceuticals Co. Ltd. (Mudanjiang, China). CTX was purchased from Shanghai Lianhua Pharmaceutical Co. Ltd. (Shanghai, China).

Animals and tumors

Male and female nude mice and male Kunming mice (20±2g) were obtained from the Experimental Animal Center of the Chinese Academy of Medical Sciences (Beijing, China). The animals were maintained in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication 80-23, revised 1996) and the experimental protocols were approved by Nanjing University Animal Care and Use Committee. All efforts were made to minimize the number of animals used and their suffering. Human hepatoma cells BEL7402 and murine hepatoma cells H22 were supplied by the Chinese Academy of Medical Sciences (Beijing, China). The BEL7402 tumor models were established by the subcutaneous injection of BEL7402 tumor cells into the left flank of nude mice. The H22 tumor models were established by the subcutaneous injection of H22 tumor cells into the left flank of Kunming mice.

Preparation of selenium-enriched *B. longum* and total selenium determination by hydride generation-atomic fluorescence spectrometry (HG-AFS)

B. longum cultured overnight in TPY medium was inoculated into fresh medium supplemented with sodium selenite at concentrations of 5 µg ml⁻¹, 10 µg ml⁻¹, 15 µg ml⁻¹, 20 µg ml⁻¹ and 25 µg ml⁻¹, respectively, and then cultured at 38°C for 10 h. The numbers of viable bacteria were counted according to the Manufacturing and Determining Regulations of Oral Live Bifidobacterium Preparatum in Regulations for Biological Products of the People's Republic of China. Se-enriched *B. longum* was then harvested by centrifugation at 8000g for 20 min and washed twice with distilled water. Finally, all samples

were powdered and freeze-dried. The Se concentration of the tested samples was determined by HG-AFS as previously reported (Mazej et al., 2004).

Selenium species determination by HPLC-ICP-MS

The identification of Se speciation in Se-*B. longum* was carried out by the Institute of High Energy Physics, Chinese Academy of Sciences (Beijing, China). In brief, 5 ml 30 mmol l⁻¹ Tris-HCl (pH 7.2) buffer solution containing 10 mg lysozyme was added to 15 mg powdered Se-*B. longum* and incubated at 37°C for 12h, and then sonicated for 30 min. Next, SDS and DTT were added, and trypsin and proteinase K were added three times every 12h. The hydrolysate was analyzed by HPLC-ICP-MS after centrifugation and filtration. The identification and quantification of Se species were carried out by comparing the retention times of standards and using the standard addition method.

Antitumor activity of Se-*B. longum* alone or combined with a chemotherapeutic drug

To evaluate the antitumor activity of Se-*B. longum*, BEL7402 tumor-bearing nude mice were divided into seven groups (n=8). Negative control mice were administered 13% defatted milk. Six treatment groups received a high dose of Se-*B. longum* (3×10¹⁰ bacteria kg⁻¹, i.g., days 1-15), middle dose of Se-*B. longum* (1.5×10¹⁰ bacteria kg⁻¹, i.g., days 1-15), low dose of Se-*B. longum* (0.75×10¹⁰ bacteria kg⁻¹, i.g., days 1-15), Selenium Yeast Tablets (52 µg kg⁻¹, i.g., days 1-15), *B. longum* (1.5×10¹⁰ bacteria kg⁻¹, i.g., days 1-15) or CTX (100 mg kg⁻¹, i.p., days 1, 7), respectively.

To evaluate the antitumor activity of Se-*B. longum* combined with CTX, BEL7402 tumor-bearing nude mice were divided into seven groups (n=8). Mice in each group received a high dose of Se-*B. longum* plus CTX (30 mg kg⁻¹), middle dose of Se-*B. longum* plus CTX (30 mg kg⁻¹), low dose of Se-*B. longum* plus CTX (30 mg kg⁻¹), middle dose of Se-*B. longum*, CTX (100 mg kg⁻¹), CTX (30 mg kg⁻¹) or 13% defatted milk, respectively. Se-*B. longum* cells and 13% defatted milk were administered (i.g.) for 15 continuous days, whereas CTX was administered (i.p.) on day 1 and 7. Twenty-four hours after the last administration, the animals were sacrificed and tumors were excised and weighed. The inhibition rate (IR) of tumor growth was determined by the following formula: $IR = (\text{tumor weight of control group} - \text{tumor weight of treatment group}) / \text{tumor weight of control group} \times 100\%$

Effects of Se-*B. longum* on survival time of H22 tumor-bearing mice

Kunming mice were weighed and randomly divided into six groups (n=12) 24h after hypodermic inoculation (day 0) of H22 cells. The negative control group received 13% defatted milk. Five treatment groups received a high dose of Se-*B. longum* (3×10¹⁰ bacteria kg⁻¹) plus CTX (100 mg kg⁻¹), middle dose of Se-*B. longum* (1.5×10¹⁰ bacteria kg⁻¹) plus CTX (100 mg kg⁻¹), low dose of Se-*B. longum* (0.75×10¹⁰ bacteria kg⁻¹) plus CTX (100 mg kg⁻¹), middle dose of Se-*B. longum* (1.5×10¹⁰ bacteria kg⁻¹) or CTX (100 mg kg⁻¹), respectively. Se-*B. longum* cells and 13%

defatted milk were administered (i.g.) for 15 continuous days, whereas CTX was administered (i.p.) on days 1 and 7. Survival time was recorded up to day 60. All mice were sacrificed on day 61 and the survival data were analyzed.

Effects of Se-B. longum on NK cell killing activity, T lymphocyte transformation index and levels of IL-2, TNF- α and leukocytes

H22 tumor-bearing Kunming mice were divided into six groups (n=12). The mice in each group received a high dose of Se-*B. longum* plus CTX, middle dose of Se-*B. longum* plus CTX, low dose of Se-*B. longum* plus CTX, middle dose of Se-*B. longum*, CTX or 13% defatted milk, respectively, as mentioned in the survival experiment above. Twenty-four hours after the last administration, blood samples were drawn from the orbit for subsequent experiments. The mice were then killed and the spleens were excised. Spleen cells were obtained as effector cells and incubated in RPMI 1640 with 10% fetal bovine serum at a concentration of 5×10^6 cells ml^{-1} . Yac-1 mouse lymphoma sensitive to NK cells were obtained as target cells and incubated in RPMI 1640 with 10% fetal bovine serum at a concentration of 2.5×10^5 cells ml^{-1} . Then 100 μl effector cells and 100 μl target cells were thoroughly mixed and inoculated into a 96-well plate at 37°C, 5% CO_2 . Effector cells and target cells were also inoculated into a 96-well plate as a control. After 24h of culture, 10 μl MTT (5 mg ml^{-1}) was added to each well. At 4h after culture, 150 μl DMSO was added to each well. The $\text{OD}_{540\text{nm}}$ value was then measured. The killing activity of NK cells was determined by the following formula: $(\text{OD}_{540\text{nm}} \text{ of effector cells} + \text{OD}_{540\text{nm}} \text{ of target cells} - \text{OD}_{540\text{nm}} \text{ of mixture}) / (\text{OD}_{540\text{nm}} \text{ of effector cells} + \text{OD}_{540\text{nm}} \text{ of target cell}) \times 100\%$

To assay the transformation index of T lymphocytes, spleen cells (100 μl well $^{-1}$) were added to 10 μl concanavalin A (Con A, 5 μg ml^{-1} , final concentration) and 90 μl RPMI 1640 with 10% fetal bovine serum, and then inoculated into a 96-well plate at 37°C, 5% CO_2 . Wells without Con A were used as negative controls. After 72h of culture, the $\text{OD}_{540\text{nm}}$ value was measured using the MTT method. The transformation index of T lymphocytes was calculated using the formula: Transformation index = $\text{OD}_{540\text{nm}}$ of wells with Con A / $\text{OD}_{540\text{nm}}$ of wells without Con A.

To assay the levels of IL-2 and TNF- α , the blood samples from H22 tumor-bearing mice from the different treatment groups mentioned above were placed at room temperature for 1 h. Serum was obtained following centrifugation at 3000 g for 10 min. Levels of IL-2 and TNF- α in the serum of tumor-bearing mice were determined by the ELISA method according to the manufacturer's instructions. To assay the levels of leukocytes, anticoagulant was added to the blood samples and the leukocyte count in blood was determined using a blood cell counter.

Statistical analysis

The results are presented as mean \pm SD values. The data were analyzed for statistical significance using the Student's t-test; comparisons among multiple groups

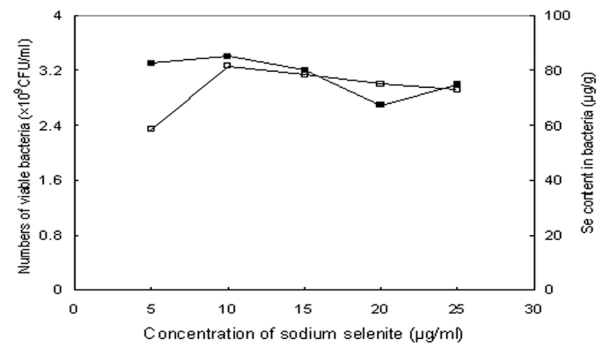


Figure 1. Selenium Accumulation by *B. longum* Cultured in Medium with Different Concentrations of Sodium Selenite. (■) The numbers of viable *B. longum*. (□) Total Se content in *B. longum*. The concentration of Se was determined by HG-AFS

were performed using the Student-Newman-Keuls q-test. Survival analysis was performed using SPSS 15.0. *p* values less than 0.05 were considered statistically significant.

Results and Discussion

Selenium accumulation by B. longum

B. longum was cultured in medium supplemented with different concentrations of sodium selenite at 38°C for 10 h. As shown in Figure 1, at a sodium selenite concentration of 10 μg ml^{-1} , Se accumulation by *B. longum* was highest and the number of viable bacteria was also highest. The number of viable bacteria was $(3.4 \pm 0.06) \times 10^9$ CFU ml^{-1} and the total Se content in *B. longum* was 81.56 ± 1.8 μg g^{-1} . In our previous study, *B. longum*-En was used to enrich Se and the results showed that both the number of bacteria and Se content reached peak values when the concentration of sodium selenite was 10 μg ml^{-1} (Li et al., 2010). Considering that Se accumulation by *B. longum* without pBV22210-endostatin may be different, the same assay was performed in the present study. The results suggested that the most suitable concentration of sodium selenite to enrich Se in the *B. longum* strain without transporting genes was also 10 μg ml^{-1} . Therefore, Se-*B. longum* obtained from the optimal culture condition (10 μg ml^{-1} sodium selenite) was used in subsequent experiments.

Identification of selenium speciation in Se-B. longum

To determine Se speciation in Se-*B. longum*, standard solutions containing inorganic Se (IV) and Se (VI), organic selenomethionine (SeMet), selenocysteine (SeCys), selenourea (SeUr) and selenocystamine (SeCA) were used. Figure 2A shows the chromatogram of a mixture of Se standards with different retention times. The HPLC-ICP-MS chromatogram of Se-*B. longum* is shown in Figure 2B. A peak which matched the retention time of SeMet standard was detected, suggesting that SeMet was the major Se species in the Se-*B. longum* sample. Based on the HPLC-ICP-MS data, the percentage of inorganic Se (IV) and Se (VI) in the total amount of Se in Se-*B. longum* was only 0.144%, whereas the percentage of SeMet was 59.55%.

Organic forms of Se are more bioavailable and

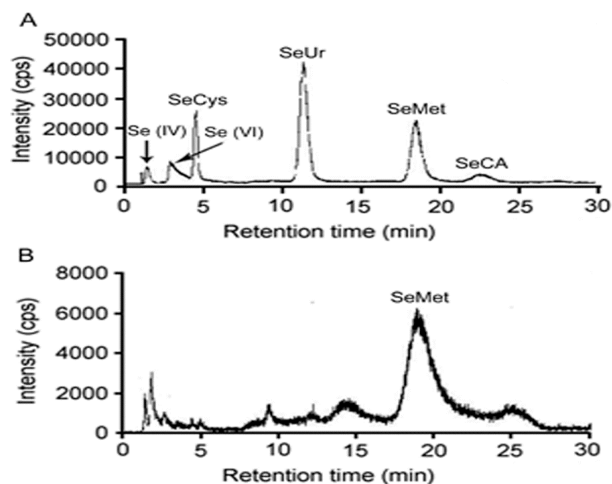


Figure 2. Chromatographic Profiles Obtained by HPLC-ICP-MS of (A) Mixture of Se Standards and (B) Enzymatic Extraction of Se-*B. longum*. Isotope ^{80}Se was Monitored

maintain higher post-supplementation levels than the inorganic forms are (Daniels 1996; Wang et al., 1997). Our previous work showed that *B. longum*-En accumulated Se and the percentage of organic Se was >96% of the total Se content (Li et al., 2010). However, the primary form of organic Se was not analyzed. In the present study, we showed that *B. longum* efficiently bioaccumulated Se when the inorganic compound sodium selenite was added to the culture medium, and more than 99% of Se was converted to organic forms. These results indicated that *B. longum* is an ideal bacterium to accumulate and transform Se. Se exerts its biologic functions largely through its presence in selenoproteins and is known to be incorporated into proteins as SeCys and SeMet. In commercial Se-enriched yeast, SeMet is the major selenocompound (Kotrebai et al., 1999). Calomme et al. (1995) showed that Se was generally incorporated as SeCys into protein of *L. delbrueckii* subsp. *Bulgaricus*. Zhang et al. (2009) reported that SeMet was the major selenocompound in protein of Se-enriched *B. animalis*. In this study, we detected SeMet in Se-*B. longum*, whereas SeCys was not detected. A possible reason for this may be that there is no genetic code UGA in the genome of *B. longum*, while SeCys is a genetically encoded amino acid translated into proteins by reading of the UGA codon (Johansson et al., 2005). Therefore, Se was mainly incorporated into protein of *B. longum* as SeMet.

Suppression of BEL7402 tumor growth by treatment with Se-*B. longum* alone or combined with CTX

BEL7402 tumor-bearing nude mice were used to examine the antitumor effect of Se-*B. longum*. The mean tumor weights in the different treatment groups are shown in Figure 3. Compared with the control group, Se-*B. longum* (middle and high dose) and CTX significantly inhibited tumor growth shown by tumor weight by 54.5% ($p < 0.01$), 42.5% ($p < 0.05$) and 83.9% ($p < 0.01$), respectively. The inhibitory effects of Se-*B. longum* (low dose), *B. longum* and Selenium Yeast Tablets on tumors were not obvious and there were no statistical differences between these groups and the control group (Figure 3A).

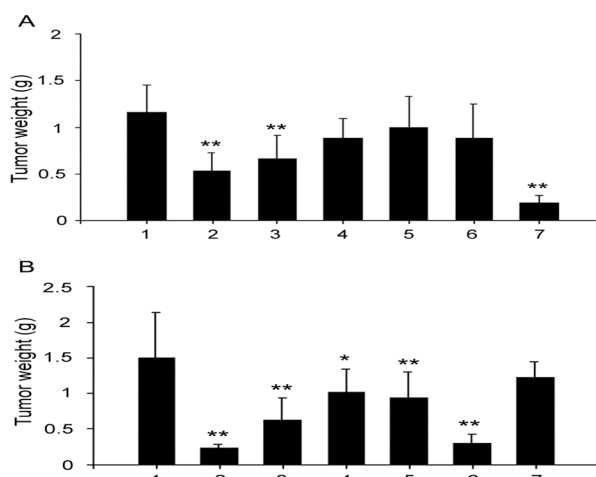


Figure 3. The Inhibitory Effects of Se-*B. longum* Alone or Combined with CTX on BEL7402 Tumor-Bearing Mice. Tumor weight was measured for each mouse. (A) The average tumor weights excised from tumor-bearing mice treated with different doses of Se-*B. longum*. Bars 1-7 are 13% defatted milk, high dose of Se-*B. longum*, middle dose of Se-*B. longum*, low dose of Se-*B. longum*, *B. longum*, Selenium Yeast Tablets and the CTX (100 mg kg⁻¹) group, respectively. (B) The average tumor weight excised from tumor-bearing mice treated with Se-*B. longum* combined with CTX (30 mg kg⁻¹). Bars 1-7 are 13% defatted milk, high dose of Se-*B. longum* plus CTX, middle dose of Se-*B. longum* plus CTX, low dose of Se-*B. longum* plus CTX, middle dose of Se-*B. longum* plus CTX (100 mg kg⁻¹) and the CTX (30 mg kg⁻¹) group, respectively. * $p < 0.05$, ** $p < 0.01$ compared with the negative control group treated with 13% defatted milk

These results suggested that accumulation of Se enhanced the antitumor activity of *B. longum* and Se-*B. longum* inhibited tumor growth in a dose-dependent manner.

Chemotherapy is still the major approach in current cancer therapy, therefore we further evaluated the antitumor activity of Se-*B. longum* combined with a low dose of CTX (30 mg kg⁻¹). As shown in Figure 3B, the inhibition rate (IR) of CTX (30 mg kg⁻¹) alone was only 18.0% and there was no significant difference between the IR in this group and that in the control group ($p > 0.05$). However, the IR of Se-*B. longum* (high dose) plus CTX, Se-*B. longum* (middle dose) plus CTX, and Se-*B. longum* (low dose) plus CTX group was 84.7% ($p < 0.01$), 58.0% ($p < 0.01$), and 34.7% ($p < 0.05$), respectively. The IR of CTX (100 mg kg⁻¹) alone was 79.7% ($p < 0.01$). These results demonstrated that CTX (30 mg kg⁻¹) was ineffective when used alone; however, the combined use of this low dose of CTX and different doses of Se-*B. longum* had a marked effect on tumor inhibition. Furthermore, when CTX (30 mg kg⁻¹) was combined with high dose of Se-*B. longum*, the IR was even higher than that of high dose of CTX (100 mg kg⁻¹) alone, suggesting that the combined use of Se-*B. longum* could reduce the dosage of chemotherapeutic drugs, while enhancing the antitumor effect. As SeMet was the predominant form of Se in Se-*B. longum*, the action of Se-*B. longum* as a promoter of CTX chemotherapy may be associated with the anticancer activity of SeMet. It was reported that SeMet induced apoptosis of tumor cells through the Akt/mTOR signal transduction pathway and induced cell-growth arrest

Table 1. Effects of Se-*B. Longum* on Immune Function of H22 Tumor-Bearing Mice

Groups	Killing activity of NK cell (%)	Transformation index of T lymphocyte	IL-2 (pg ml ⁻¹)	TNF-α (pg ml ⁻¹)	leukocyte (10 ⁹ L ⁻¹)
13% defatted milk	38.5±10.1	1.08±0.03	96.3±27.4	130.7±26.9	10.3±2.99
Se- <i>B. longum</i> (high dose)+CTX	46.1±7.77*†	1.73±0.07***†	196.2±16.8***†	223.6±18.2***†	13.1±4.32*†
Se- <i>B. longum</i> (middle dose)+CTX	41.5±12.1	1.48±0.14***†	174.6±23.6***†	184.8±18.2***†	11.1±2.16††
Se- <i>B. longum</i> (low dose)+CTX	27.7±12.4	1.13±0.08*†	105.8±31.3††	146.4±19.9††	8.56±2.57
Se- <i>B. longum</i> (middle dose)	38.5±13.2	1.11±0.06	158.6±19.5***†	172.5±23.6***†	10.6±3.27
CTX	32.1±12.6	1.07±0.04	87.7±17.1	73.5±13.6**	7.94±2.76*

* $p < 0.05$, ** $p < 0.01$ compared with 13% defatted milk control group; † $p < 0.05$, †† $p < 0.01$ compared with CTX group

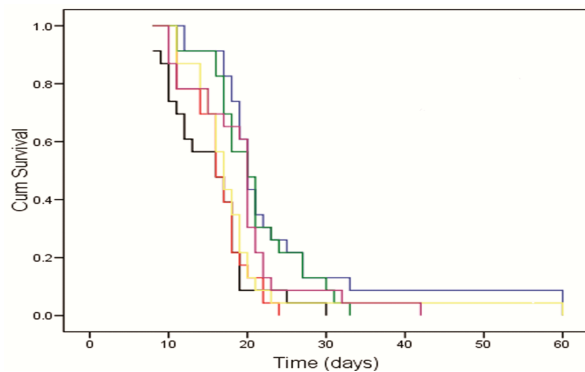


Figure 4. Kaplan-Meier Curves of the Survival of H22 Tumor-Bearing Mice. Mice in each group were treated with 13% defatted milk (black line), high dose of *Se-B. longum* plus CTX (blue line), middle dose of *Se-B. longum* plus CTX (green line), low dose of *Se-B. longum* plus CTX (yellow line), middle dose of *Se-B. longum* (red line) and CTX (purple line), respectively

through sustained ERK phosphorylation (Cao et al., 2004; Goulet et al., 2005). With regard to other chemotherapeutic drugs, Cao et al. (2004) reported that SeMet significantly augmented the antitumor activity and the cure rate of several chemotherapeutic agents.

Treatment with *Se-B. longum* prolonged the survival time of tumor-bearing mice

The survival rate of H22 tumor-bearing Kunming mice (n=12) following different treatments is represented in the Kaplan-Meier survival curve in Figure 4. The mean survival times of mice in 13% defatted milk, *Se-B. longum* (high dose) plus CTX, *Se-B. longum* (middle dose) plus CTX, *Se-B. longum* (low dose) plus CTX, *Se-B. longum* (middle dose) and the CTX group were 15, 24, 21, 19, 16 and 19 days, respectively. Compared with the control group, *Se-B. longum* (high dose) plus CTX and *Se-B. longum* (middle dose) plus CTX significantly prolonged the survival time by 59.4% ($p < 0.01$) and 38.6% ($p < 0.01$), respectively. These results suggested that although CTX significantly inhibited tumor growth, the survival time of mice treated with CTX alone was short. However, when *Se-B. longum* (high dose or middle dose) was combined with CTX, the survival time of mice was significantly prolonged.

Effect of *Se-B. longum* on immune function of tumor-bearing mice

To determine the effect of *Se-B. longum* on immune function of H22 tumor-bearing mice, NK cell killing

activity, T lymphocyte transformation index, and levels of IL-2, TNF-α and leukocytes were assayed. As shown in Table 1, the NK cell killing activity of H22 tumor-bearing mice (n=12) treated with high dose of *Se-B. longum* plus CTX was significantly improved compared with the negative control ($p < 0.05$) and CTX group ($p < 0.05$). The transformation index of T lymphocytes of tumor-bearing mice treated with the three doses of *Se-B. longum* plus CTX was significantly improved compared with the control and CTX group. The activities of IL-2 and TNF-α were markedly enhanced by the three doses of *Se-B. longum* plus CTX compared with the control and CTX group in tumor-bearing mice. However, there was no statistical difference between the low dose of *Se-B. longum* plus CTX group and the control group. In conclusion, compared with the CTX alone group, combined use of *Se-B. longum* improved immune function of tumor-bearing mice.

Leucopenia, chemotherapy-induced myelotoxicity, is one of the major toxic effects of chemotherapy (Crawford et al., 2004). In our study, CTX alone induced leucopenia of H22 tumor-bearing mice, however, when the middle or high dose of *Se-B. longum* was combined with CTX, the leukocyte count significantly increased. This decrease in toxicity induced by CTX may be related to the protective effect of SeMet. Laffon et al. (2010) reported that SeMet protected against bleomycin-induced DNA damage and repaired this damage in human leukocytes. Dos Santos et al. (2008) showed that SeMet treatment reduced DNA damage and cytotoxicity induced by doxorubicin in human lymphocytes.

In conclusion, our results show that *B. longum* is capable of accumulating Se and incorporating most of the Se as SeMet in intracellular proteins. *In vivo* studies in mice indicated that *Se-B. longum* significantly inhibited tumor growth. When *Se-B. longum* was combined with CTX, the antitumor effect was significantly enhanced, the survival time of tumor-bearing mice was extended and the activities of some immune cells and factors were improved in tumor-bearing mice. We expect that *Se-B. longum* will be further explored and applied in humans as a promising approach in cancer therapy.

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