PRELIMINARY REPORT

Effect of a seashell protein Haishengsu on cell growth and expression of apoptosis genes in leukemia K562 cells

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Abstract

Objectives: To investigate the effect of a seashell protein Haishengsu (HSS), an extract from a shellfish *Tegillarca granosa*, on cell growth and the expression of apoptosis genes in leukemia K562 cells.

Methods: Cultured K562 cells were treated with HSS at various concentrations (10-40mg/L). The cell cycle, cell growth and the expression of apoptosis suppressor gene bcl-2 and apoptosis promoting gene bax were evaluated.

Results: HSS, 20mg/L, inhibited cell cycle in the G₀/G₁ and S phases. HSS, 20mg/L, also inhibited the growth of K562 cells over time. Expression of bcl-2 gene in the HSS 20mg/L (58.8%±4.7%) and HSS 40mg/L group (26.6%±2.1%) were lower than in the control group (91.0±8.7%, $P<0.01$). Expression of bax gene in the HSS 20mg/L (77.7±3.6%) and 40mg/L group (90.6±3.7%) were higher than in the control group (10.9±6.6%, $P<0.01$).

Conclusion: HSS suppresses leukemia K562 cell growth by inhibiting the G₀/G₁ and S phases of the cell cycle. It also induces apoptosis in these leukemia cells by reducing the expression of apoptosis suppressor gene bcl-2, and increasing the expression of apoptosis promoting gene bax.

Further studies are required to investigate the clinical efficacy of HSS in leukemia.

Haishengsu (HSS) is a protein, mainly albumin with a molecular weight from 15 KDa to 23 KDa. It is extracted from a seashell, *Tegillarca granosa*, which has been widely used as a traditional Chinese medicine in mainland China for several decades. Previous studies have shown that HSS has a potent suppressive effect on several types of solid tumor cells *in vivo* and *in vitro*.^[1,2] The primary purpose of the present study was to investigate the effect of HSS on cell growth and the expression of apoptosis genes in K562 cells, an established cell line of chronic myeloid leukemia.³

Materials and Methods

Cultured K562 cells were obtained from the Institute of Hematology at Peking Union Medical College.

TABLE 1. Effect of HSS on K562 cell cycle.

	Concentration (mg/L)	G ₁	G ₀ /G ₁	S	G ₂ /M
Control	—	2.50	12.33	5.23	25.43
HSS	10	5.64 [#]	15.12 [*]	11.32 [#]	31.29 [*]
	20	20.51 [*]	10.35 [*]	29.41 [*]	26.35 [*]

Compared with control group * $P < 0.01$, # $P < 0.05$.

These cells were cultured at 37°C in RPMI1640 medium containing 10% fetal calf serum (Gibco BRL).

This study protocol involved three groups of cells: control group, HSS 10mg/L and HSS 20mg/L group. HSS was supplied by Qingdao Haisheng Oncology Hospitals (Qingdao, China). The K562 cells in each group were examined by flow cytometry (Beckman-XL, CA, USA) for cell cycle and growth.

Thiazolyl blue tetrazolium bromide (MTT) assay was used to assess cell proliferation. K562 cells were transferred to a 96-well plate. The cells were divided into control, placebo and five HSS groups with a treatment concentration of HSS at 0.1, 1, 10, 100 and 1000 mg/L, respectively. Cells in each well were incubated with 20μl (5g/L) of MTT (Sigma-Aldrich). At

TABLE 2. Inhibition of cell growth.

	Concentration (mg/L)	Survival rate (%)
Control group	0	100
HSS group	0.1	96.2
	1	86.1
	10	68.5
	100	23.7
	1000	6.5

six time points (0, 12, 24, 36, 48 and 72h), the optical density value of each well was measured on 492 nm emission with a microplate reader (Denley Dragon MK2, Finland). Cell inhibition rate was defined as following: $IR\% = (A_{\text{control}} - A_{\text{study}}) / A_{\text{control}} \times 100\%$.

An immunocytochemical kit (SP-20002, Zhongshan Golden Bridge, China) was used to detect bcl-2 and bax gene expression. The protocol involved the following groups: control, placebo and three HSS groups (10 mg/L, 20mg/L, 40mg/L). In each group, five visual fields were observed and the number of positive cells was calculated from 100 cells. The positive ratio was defined as the percentage of positive cells in every 100 cells counted in each visual field.

Data were expressed as means $\pm$ SD. SAS6.12 software was used for data analysis. Numerical data were analyzed with one-way ANOVA. Categorical data were analyzed with Chi-square test. $P < 0.05$ was considered statistically significant.

Results

In the HSS treated cells, the cell cycle was suppressed at G₀/G₁ and S phase (Table 1). There was inhibition of cell proliferation at the HSS concentration of 20mg/L (calculated from linear regression equation, IC₅₀ 20mg/L, Table 2).

In the control and placebo groups, the bcl-2 gene of the K562 cells was mainly expressed in the cytoplasm, the majority appeared as scattered small particles (Fig 1A and 1B). In the HSS groups, bcl-2 expression was absent (Fig 1C).

In the control and the placebo groups, there were no noticeable signs of bax expression (Fig 1D and Fig

FIGURE 1.

- A. Control group: Positive bcl-2 staining in K562 cells.
- B. Placebo group: Positive bcl-2 staining in K562 cells.
- C. HSS (20mg/L). Negative bcl-2 staining in K562 cells.
- D. Control group: Negative bax staining in K562 cells.
- E. Placebo group: Negative bax staining in K562 cells.
- F. HSS (20mg/L). Positive bax staining in K562 cells.

TABLE 3. Effect of HSS on the expression of bcl-2 and expression of bax.

	Concentration (mg/L)	Bcl-2 (%)	Bax (%)
Control group	—	91.0±8.7	10.9±6.6
Placebo group	—	91.3±3.6	9.3±9.4
HSS group	10	89.9±3.5	23.4±2.4
	20	58.8±4.7*	77.7±3.6*
	40	26.6±2.1*	90.6±3.7*

Compared with control and placebo groups * $P<0.05$

1E). However, bax expression was strongly positive in the HSS treated groups (Fig 1F).

The ratio of bcl-2 expression in the HSS treated groups was lower than in the control group (Table 3, $P<0.05$).

The ratio of bax expression in the K562 cells in the control and the placebo groups was lower than in the HSS 20 mg/L and HSS 40 mg/L groups ($P<0.05$, Table 3).

Discussion

HSS has been shown to block the G₀/G₁ phase of cell cycle of renal cancer, and the G₂-M phase of the lung cancer *in vitro*.² The present study is the first to demonstrate that HSS is able to suppress the growth of leukemia cells in the G₀/G₁ and the S phases of the cell cycle. It has also revealed that HSS facilitates the apoptosis of leukemia K562 cells.

Tumour cell proliferation and apoptosis determine the development and prognosis of cancer. When the apoptosis gene of tumour cell is inhibited, oncogenes will be activated, inducing unrestricted proliferation of tumor cells and invasion or metastasis of cancer. The Bcl-2 gene family is one of the most important apoptosis-regulating genes. These genes can be divided into apoptosis suppressor genes, such as bcl-2 and bcl-xl, and apoptosis promoting genes, such as bax and bak.⁴ In other words, Bcl-2 is the inhibiting gene for apoptosis, whereas bax genes are apoptosis trigger genes. The balance between bcl-2 and bax regulates apoptosis and the cell survival. The precise mechanisms of bcl-2 in suppressing apoptosis are not very clear, and such discussion is beyond the scope of this study. However, bcl-2 is also involved in the regu-

lation on the dynamics between cell proliferation and apoptosis.⁵

P16 and P21 are cell cycle protein kinase (CDK) inhibition proteins. They play an important role in cell cycle regulation, regulating G₁/S switch.⁶ P16 and P21 can prevent cells from entering S phase by influencing the activity of cell cycle protein kinase. In the present study, HSS diminished the expression of bcl-2 in the K562 cells and suppressed cell growth at the phase of G₀/G₁ and S. However, it is unclear how HSS could specifically inhibit the G₀/G₁ and S phases, and what role that bcl-2 suppression has played in this process.

In summary, this study has demonstrated that HSS has a suppressive effect on *in vitro* leukemia K562 cells. HSS seems to inhibit G₀/G₁ and S phases of the cell cycle. HSS also promotes K562 cell apoptosis by stimulating the expression of bax, an apoptosis promoting gene, and by reducing the apoptosis suppressor gene, bcl-2. Further studies are required to evaluate the clinical effect of HSS on leukemia.

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