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World J Gastroenterol 2004;10(8):1132-1136
World Journal of Gastroenterology
 ISSN 1007-9327

• GASTRIC CANCER •

In vitro effects of recombinant human growth hormone on growth of human gastric cancer cell line BGC823 cells

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Received: 2002-08-10 **Accepted:** 2003-11-12

Abstract

AIM: To study the effects of recombinant human growth hormone (rhGH) on growth of human gastric cancer cell line *in vitro*.

METHODS: Experiment was divided into control group, rhGH group, oxaliplatin (L-OHP) group and rhGH+L-OHP group. Cell inhibitory rate, cell cycle, cell proliferation index (PI) and DNA inhibitory rate of human gastric cancer line BGC823, at different concentrations of rhGH treatment were studied by cell culture, MTT assay and flow cytometry.

RESULTS: The distinctly accelerated effects of rhGH on multiplication of BGC823 cell line were not found *in vitro*. There was no statistical significance between rhGH group and control group, or between rhGH+L-OHP group and L-OHP group ($P>0.05$). The cell growth curve did not rise. Cell inhibitory rate and cells arrested in G_0-G_1 phase were obviously increased. Meanwhile, cells in S phase and PI were distinctly decreased and DNA inhibitory rate was obviously increased in rhGH+L-OHP group in comparison with control group and rhGH group, respectively ($P<0.01$). Cell inhibitory rate showed an increasing trend and PI showed a decreasing trend in rhGH+L-OHP group compared with L-OHP group.

CONCLUSION: *In vitro* rhGH does not accelerate the multiplication of human gastric cancer cells. It may increase the therapeutic efficacy when it is used in combination with anticancer drugs.

Chen JY, Liang DM, Gan P, Zhang Y, Lin J. *In vitro* effects of recombinant human growth hormone on growth of human gastric cancer cell line BGC823 cells. *World J Gastroenterol* 2004; 10(8): 1132-1136
http://www.wjgnet.com/1007-9327/10/1132.asp

INTRODUCTION

Human growth hormone (rhGH) promotes protein synthesis and lipid mobilization and accelerates nitrogen balance. It is extensively applied in clinic for the adjustment of metabolic state in patients with severe trauma, burn or major operations. It was reported that recombinant human growth hormone (rhGH) was also applied in postoperative patients with tumor, but it is controversial whether rhGH accelerates the growth of tumor cells. In the present study we investigated the effects of rhGH on human gastric cancer cell line BGC823 *in vitro*, in order to clarify whether rhGH could be applied in postoperative patients with gastric cancer.

MATERIALS AND METHODS

Materials

Human gastric cancer cell line BGC823 was supplied by the cell bank of Shanghai Cell Biology Institute of Chinese Academy of Sciences. rhGH (Saizen) was supplied by Serono (Switzerland), one IU equals to 0.33 mg and the final concentration of rhGH was 50 ng/mL, 100 ng/mL, 200 ng/mL and 400 ng/mL respectively. Oxaliplatin (L-OHP) was selected as an anti-cancer drug supplied by Henrui Medical Company, Jiangsu, China, and its final concentration was 4 μg/ml. The main instruments were ELISA (EL340) and flow cytometer (EPICXU).

Methods

Experiment was divided into 4 groups: control group (I), anti-cancer drug group (II), rhGH group (III) and rhGH+anti-cancer drug group (IV) (Table 1).

Table 1 Experimental groups and concentrations of drugs

Groups	Names and concentrations of drugs
I	RPMI1640
II	L-OHP 4 μg/mL
III_a	rhGH 50 ng/mL
III_b	rhGH 100 ng/mL
III_c	rhGH 200 ng/mL
III_d	rhGH 400 ng/mL
IV_a	rhGH 50 ng/mL + L-OHP 4 μg/mL
IV_b	rhGH 100 ng/mL + L-OHP 4 μg/mL
IV_c	rhGH 200 ng/mL + L-OHP 4 μg/mL
IV_d	rhGH 400 ng/mL + L-OHP 4 μg/mL

BGC823 cells were placed in the medium containing 100 mL calf serum and RPMI1640, incubated at 37 ℃ in an atmosphere containing 50 mL CO_2 and 950 mL air. At logarithmic growth, the cells were digested by trypsin. Then the activity of the cells was examined (Via=99%) and the cells were counted in a hemocytometer using trypan blue exclusion. The density of single cell suspension was adjusted to 1×10^5/mL for use.

Single-cell suspension was added into a 96-well plate and 90 μL suspension was added to each well and there were 4 duplicate wells in each group. When the cells completely adhered to the wall of the well 4 h later, 10 μL test drugs was added into the wells (In rhGH+L-OHP group, the ratio of the volume of both drugs was 1:1). The cells were cultured for 1, 2, 3 and 4 d, respectively, at 37 ℃ in an atmosphere containing 50 mL CO_2 and 950 mL air. In addition, the cells were cultured as above for 48 h to examine cell inhibitory rates. A 10 μL

MTT (5 mg/mL) was added to each well 4 h prior to the ending of experiment. When the experiment ended, 100 μL triplicate liquid [100 g/L SDS-50 mL/L iso-butyl alcohol-0.012 mL/L HCL] was added into each well. Absorbent value of each well was examined at the wavelengths 570 nm and 630 nm by ELISA 12 h later.

A 10 mL cell suspension was placed and incubated in each well of 6-well plates and there were 3 duplicate wells in each group. When the cells completely adhered to the wall of wells 4 h later, the test drugs were added into each well as shown in Table 2.

Table 2 Scheme of drugs in each group

Group	I	II	III_a	III_b	III_c	III_d	IV_a	IV_b	IV_c	IV_d
L-OHP (μL)	/	50	/	/	/	/	50.0	50	50	50
rhGH (μL)	/	/	12.5	25	50	100	12.5	25	50	100
RPMI1640 (μL)	150	100	137.5	125	100	50	87.5	75	50	/

The cells were gathered after cultured for 48 h and washed with PBS. After fixed with 700 mL/L alcohol, the cells were kept at 4 ℃ overnight, then stained with fluorescence. Finally the cell cycle was examined at the wavelength 488 nm. Barlogie cell cycle assay was used[1].

Statistical analysis

Data were expressed as mean±SD and analyzed by variance analysis and *q* test. Statistical significance was considered at $P \leqslant 0.05$.

RESULTS

MTT colorimetric analysis

The inhibitory rate on gastric cancer cells was significantly higher in L-OHP group and rhGH+L-OHP group, compared with control group and rhGH group ($P<0.01$). The inhibitory rate was also higher in rhGH+L-OHP group than in L-OHP group, though the difference had no statistical significance ($P>0.05$). Between control group and rhGH group, the inhibitory rate did not change regularly with increase of the drug' s dose (Table 3).

Table 3 Effects of rhGH and L-OHP on BGC823 cells (n=4, mean±SD)

Groups	OD values	Survival rate (%)	Inhibitory rate (%)
I	0.863±0.172	100.00	0
II	0.425±0.086[b]	49.25[b]	50.75[b]
III_a	0.947±0.142	109.73	-9.73
III_b	0.894±0.220	103.59	-3.59
III_c	0.848±0.346	98.26	1.74
III_d	0.844±0.196	97.79	2.21
IV_a	0.338±0.240[b]	39.17[b]	60.83[b]
IV_b	0.318±0.038[b]	36.85[b]	63.15[b]
IV_c	0.318±0.018[b]	36.85[b]	63.15[b]
IV_d	0.306±0.200[b]	35.46[b]	64.54[b]

[b]$P<0.01$ *vs* control group or rhGH group.

Cell growth curve

Cell growth curve shows no obvious change between rhGH group and control group or between rhGH+L-OHP group and L-OHP group (Figures 1-2).

But it dropped sharply when rhGH+L-OHP and L-OHP groups were compared with the control group.

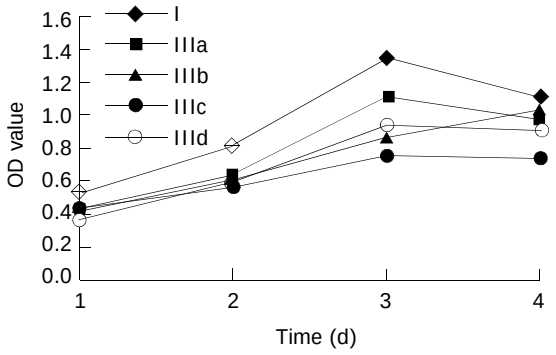

Figure 1 BGC823 cell growth curve.

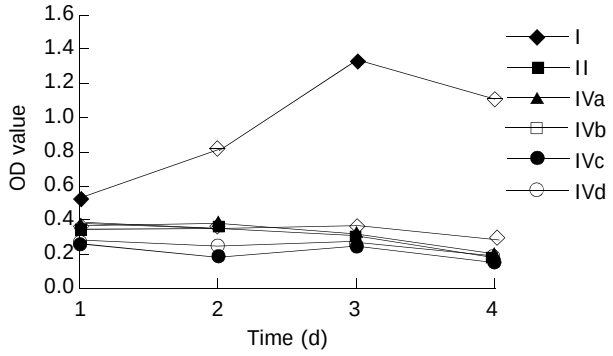

Figure 2 BGC823 cell growth curve.

Cell cycle

Cell cycle was obviously changed in L-OHP group and rhGH+L-OHP group. The number of cells in G_0-G_1 phase was obviously more in L-OHP group and rhGH+L-OHP group than in control group or rhGH group ($P<0.01$), but there was no significant difference in control group and rhGH group or in L-OHP group and rhGH+L-OHP group ($P>0.05$). The cells in S phase were fewer in L-OHP group and rhGH+L-OHP group than in control group and rhGH group ($P<0.01$), but there was no statistical significance between rhGH group and control group. The cells in G_2-M phase were significantly fewer in L-OHP group and rhGH+L-OHP group than those in control group or rhGH group ($P<0.01$). There was no statistical significance between L-OHP group and rhGH+L-OHP group or between control group and rhGH group. Further more, in S or G_2-M phase the cell number had no regular change in rhGH+L-OHP group (Table 4) (Figures 3-5).

Table 4 Percentages of BGC823 cells in various phases of cell cycle (n=3, mean±SD)

Group	G_0–G_1(%)	S (%)	G_2–M(%)
I	47.75±0.78	36.95±0.49	15.25±0.21
II	83.85±1.77[b]	7.85±0.64[b]	8.30±1.13[b]
III_a	53.03±4.31	32.17±7.47	14.73±3.40
III_b	52.33±4.55	32.63±6.19	14.73±1.88
III_c	52.33±4.94	33.50±8.17	14.17±3.29
III_d	51.27±5.94	33.67±8.89	15.07±3.07
IV_a	85.07±2.62[b]	6.67±3.88[b]	8.00±1.82[b]
IV_b	86.47±2.07[b]	5.47±1.88[b]	7.77±0.40[b]
IV_c	85.53±0.47[b]	7.87±4.99[b]	6.37±5.61[b]
IV_d	86.13±2.87[b]	4.20±1.61[b]	9.67±1.27[b]

[b]$P<0.01$ *vs* control group or rhGH group.

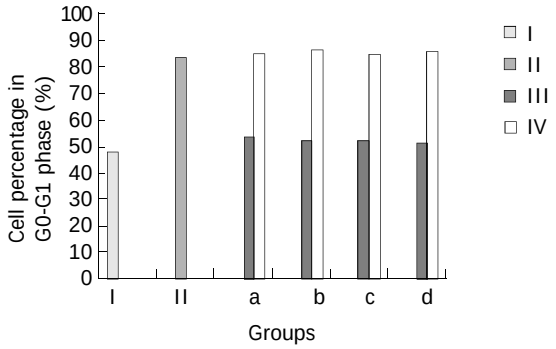

Figure 3 Percentages of cells in G0-G1 phase (%).

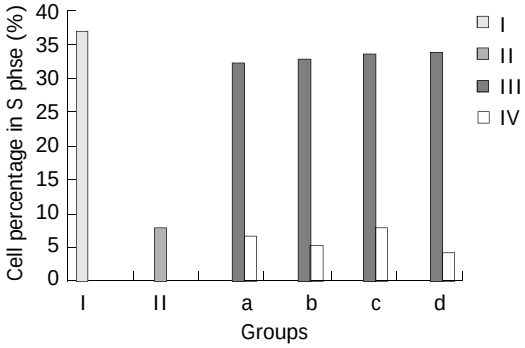

Figure 4 Percentage of cells in S phase (%).

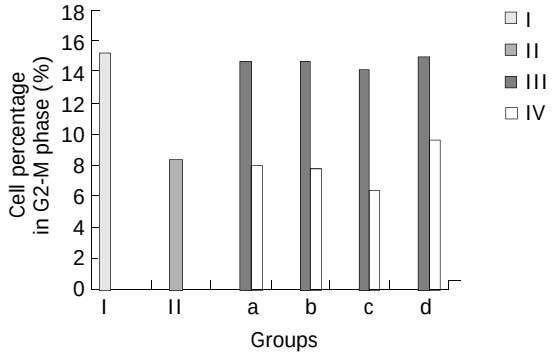

Figure 5 Percentage of cell in G2-M phase (%).

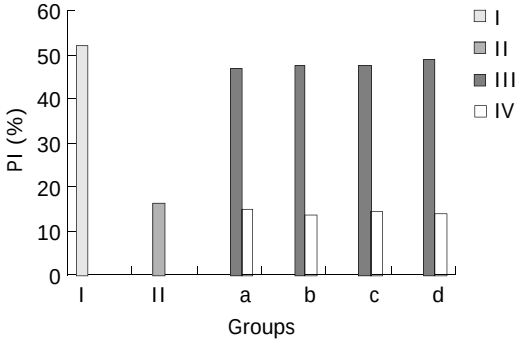

Figure 6 Cell PI (%).

Proliferation Index (PI)

PI was obviously reduced in L-OHP group compared with rhGH group or control group ($P<0.01$), but there was no statistical significance between rhGH+L-OHP group and L-OHP group, or between rhGH group and control group ($P>0.05$). PI showed a decreasing trend in rhGH+L-OHP group compared with L-OHP group (Table 5, Figure 6).

Table 5 Proliferation index in each group (n=3, mean±SD)

Group	PI
I	52.25±0.78
II	16.15±1.77[b]
III_a	46.97±4.31
III_b	47.67±4.55
III_c	47.67±4.94
III_d	48.73±5.94
IV_a	14.93±2.62[b]
IV_b	13.53±2.07[b]
IV_c	14.47±0.47[b]
IV_d	13.87±2.87[b]

[b]$P<0.01$ *vs* control group or rhGH group.

DNA inhibitory rate

DNA inhibitory rate was obviously increased in rhGH+L-OHP group (group IV) compared with rhGH group (group III) ($P<0.01$) (Table 6, Figure 7).

Table 6 DNA inhibitory rate in rhGH group and rhGH+L-OHP group (n=3, mean±SD)

Group	DNA inhibitory rate (%)
III_a	111.1±9.4
IV_a	178.1±6.8
III_b	109.5±9.8
IV_b	181.0±4.0
III_c	109.5±10.5
IV_c	178.2±1.9
III_d	107.2±12.4
IV_d	180.3±7.6

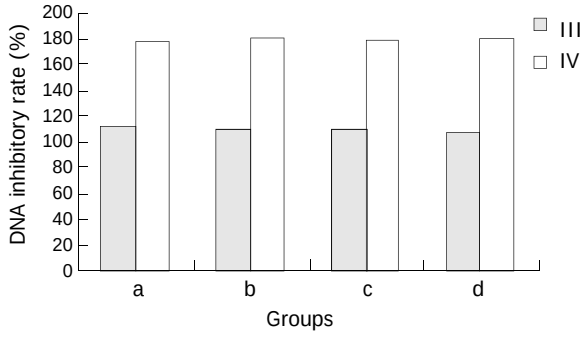

Figure 7 DNA inhibitory rate.

DISCUSSION

L-OHP is a new platinum compound that has the similar effects to cisplatin and carboplatin. The main mechanism of L-OHP is that it makes DNA broken by inserting platinum atom between two neighboring guanines or between guanine and adenosine in DNA, thus, DNA can not replicate or transcript. It is not a cell circle-specific anticancer drug. We determined its concentration *in vitro*, by referring to the maximal concentration in human plasma when the drug was used in pharmacological doses, and according to Limburg and Heckman formula. On the basis of this concentration, we devised the concentration gradient, and found the IC_{50} that L-OHP affected BGC823 cells was 4 μg/mL by sifting experiment of anti-cancer drugs *in vitro*. The majority of data reported that the concentration of rhGH *in vitro* was 50 ng/mL or 100 ng/mL[2]. Qi *et al*[3,4] devised the super-high concentration of 200 ng/mL according to its clinical

application. In addition, Estrov *et al*[5] suggested that rhGH could accelerate human leukemia cell proliferation at high concentrations (250-300 ng/mL), so we proposed this concentration. The duration of the drug treatment was 48 h according to the double proliferation time of BGC823 cells.

rhGH is secreted by pituitary gland, it can reverse many nutritional and metabolic abnormalities associated with severe catabolic states. It has been shown that rhGH can promote protein synthesis, improve nitrogen balance, accelerate wound healing[6-11], maintain host immune function and alleviate postoperation fatigue syndrome (POF)[12]. GH has become available for clinical use. It was reported that rhGH enhanced positive nitrogen balance in metabolic recuperation of postoperative patients with malignant tumor[13,14]. But it is still controversial whether rhGH should be used in postoperative tumor patients since hGH promotes the proliferation of normal cells, as well as tumor cells. Estrov *et al*[5] reported rhGH could increase the risk of human leukemia and solid tumor at a high concentration. Ogiliy-Stuart *et al*[15] thought rhGH was associated with the increasing risk for tumor, especially for colonic cancer, when it was used at a high concentration in patients with tumor. Akaza *et al*[16] found rhGH promoted carcinogenesis of chemically induced rat urinary bladder cancer. Ng *et al*[17] reported GH could increase the proportion of aneuploid cells in tumor-bearing rats. Some other studies showed that rhGH enhanced tumor growth[18-20]. However, Harrison[21] reported that rhGH did not prompt human pancreatic carcinoma growth. Tacke *et al*[22] thought that postoperative treatment with rhGH in a short term led to a faster recovery of the immune function, increased the activity of NK cells, helped clear away potential cancer cells, and inhibited the recurrence of tumor. Fiebig *et al*[23,24] also reported *in vitro* and *in vivo* rhGH did not promote tumor cells to proliferate. Bartlett *et al*[25] suggested GH inhibited tumor growth in protein-starved animals. Still, some other studies reported that rhGH did not increase tumor growth[26,27].

In the present study, the effects of rhGH on gastric tumor cells *in vitro* were investigated. The results showed that there was no apparent tumor growth stimulation. There were no distinct differences in survival rate, inhibitory rate, the number of cells in G_0-G_1 phase, S and G_2-M phase, PI and cell growth curve between rhGH group and control group or between rhGH+L-OHP group and L-OHP group (P>0.05). The results coincided with other clinical reports[26] and showed that it did not increase gastric cancer cell growth *in vitro* after rhGH was administered. In addition, the cell percentage in proliferation phase (S and G_2-M phase) was not obviously different between rhGH+L-OHP group and L-OHP group (P>0.05), demonstrating that rhGH did not stimulate tumor cell proliferation. The cell inhibitory rate was distinctly increased, the cell percentage of S and G_2-M phase and PI was decreased (P<0.05) and the cell growth curve was apparently dropped between rhGH+L-OHP group and control group, and between rhGH+L-OHP group and rhGH group. The cell inhibitory rate showed an increasing trend and PI showed a decreasing trend in rhGH+L-OHP group compared with L-OHP group (P>0.05). These results indicated rhGH could enhance L-OHP effect on tumor cells. It provides support for the practice that tumor patients should be treated by metabolic recuperation after operation or that in advanced cancer and inoperative patients the cachexia was improved, or in chemotherapeutic patients the adverse effect was alleviated by use of rhGH.

The mechanism of rhGH underlying tumor cell proliferation is unknown. Some reports showed GH could indirectly stimulate tumor cell proliferation by combining with IGF-1 receptors on the surface of tumor cells[28-32], but others showed that rhGH did not promote liver cancer cell proliferation because of reduced IGF-1 receptor expression[33,34]. Wennbo *et al*[35] found that the activation of prolactin receptors but not mammary tumor induced by growth hormone receptors in transgenic mice. The exact mechanism of rhGH underlying gastrointestinal tumor cell proliferation is still unknown.

In conclusion rhGH does not promote gastric cancer cell proliferation and fission, on the contrary, it can enhance anti-cancer effects of drugs on gastric cancer cells *in vitro*.

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Edited by Zhu LH and Wang XL **Proofread by** Xu FM