Effect of octreotide on cell-cycle kinetics and serum carcinoembryonic antigen level in hepatic metastases of colonic adenocarcinoma

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AIM: To study the inhibitory effect of somatostatin analogue (octreotide) on tumor growth.

METHODS: The influence of cell-cycle kinetics on hepatic metastases of BALB/c mice colonic adenocarcinoma (CT26) with octreotide treatment in vivo was investigated by flow cytometry. The serum carcinoembryonic antigen (CEA) levels were also determined.

RESULTS: The results showed that the proliferative index (PI) and the S-phase fraction in hepatic tumors of mice treated with octreotide decreased markedly and that the G0/G1 serum CEA phase fraction increased significantly in comparison with the control (P < 0.01). After administration of octreotide, the serum CEA levels were also lower than those in the control group. The incidence of liver metastases in the treated group was lower than that in the control. The body weight loss in the mice was slower and survival was longer in the treated group than in the control group. Furthermore, the changes in PI and the fraction distribution of S-phase or G1/G0-phase in cell cycle were closely related to the serum CEA levels.

CONCLUSION: Octreotide may be useful for inhibiting the hepatic metastases of colonic carcinoma.

Key words: Colonic neoplasms; Liver neoplasms/secondary; adenocarcinoma; Cytometry; Octreotide; Carcinoembryonic antigen/analysis

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INTRODUCTION

Somatostatin analogues (e.g. octreotide, RC-160 and others) are widely used to treat neuroendocrine tumors (such as carcinoids, vasoactive intestinal peptide (VIP)omas, insulinomas and glucagonomas), acute pancreatitis and fistulae of gastrointestinal tract[2,3]. Recently, these drugs have also been considered to have some therapeutic effect for non-endocrine gastrointestinal tumors[2,3]. In order to study the mechanism of octreotide and its anticancer effect, the proliferative index (PI) and the fraction distribution of cell cycle were investigated by flow cytometry. The changes in the serum carcinoembryonic antigen (CEA) level, incidence of hepatic metastases, body weight, and survival time were also determined. Furthermore, the relationship between the parameters of cell-cycle kinetics and the changes of serum CEA level were analyzed.

MATERIALS AND METHODS

Peptides, tumor cell line, and instrumentation
Octreotide was provided as a gift from Sandoz Company (Basel, Switzerland). The tumor cell line CT26, from the colonic adenocarcinoma of mice, was obtained from the Immunological Department of Second Military Medical University (Shanghai, China). The type of flow cytometry (FCM) apparatus used in this study was FACStar Plus, manufactured by Becton Dickinson Company (United States). The CEA ELISA kit was produced by Sanye Company of Shanghai Second Medical University.

Animals and treatment
Thirty-two male BALB/c mice, aged 5-7 wk, were used in this study. The mice were randomized into the following four groups: Group A (n = 10): Liver metastases treated with octreotide for tumor measurement and FCM analysis; group B (n = 10): Liver metastases used as control for tumor measurement and FCM analysis; group C (n = 6): Liver metastases treated with octreotide for survival time; group D (n = 6): Liver metastases used as control for survival time. The mice were placed under ether anesthesia, and a small incision was made in the left subcostal area. The spleen was injected with 1 × 10⁶ cultured CT26 in 100 μL of culture medium. The mice...
in groups A and C were administered daily subcutaneous injection of octreotide at a dose of 50 μg/kg daily, while those in groups B and D were injected with saline solution.

**Measurement and analysis of FCM**

The mice were weighed once a week. The animals in groups A and C were administered daily subcutaneous injection of octreotide at a dose of 50 μg/kg daily, while those in groups B and D were injected with saline solution.

- **RESULTS**

  **Change in body weight**
  
  The changes in the body weight of groups A and B are shown in Table 1. Compared to status before initiation of treatment, the weight in group A did not change significantly even 1 and 2 wk after treatment; however, the weight in group B decreased markedly after the second week. The difference in the rate of weight loss between groups A and B was statistically significant.

  **Change of hepatic metastases**
  
  Administration of octreotide for 3 wk significantly lowered the incidence of hepatic metastases and inhibited the growth of CT26 (Table 1). The incidence of grade II liver metastases in the control group decreased markedly, while that of grade III increased significantly in comparison with the control group (P < 0.01).

  **PI and phase fraction in tumor cell cycle**
  
  The PI and each phase fraction of tumor cell cycle, as determined by FCM, are shown in Table 2. After administration of octreotide, the PI and the S-phase fraction in hepatic tumors of mice decreased markedly, while the G0/G1-phase fraction increased significantly in comparison with the control group (P < 0.01).

  **Change of serum CEA and the relationship between CEA and cell cycle kinetics**
  
  After the third treatment session, the serum level of CEA was 89.60 μg/L ± 31.72 μg/L in group A and 140.50 μg/L ± 49.97 μg/L in group B (P < 0.05). Statistical regression analysis showed that the changes in the PI and the fraction distribution of S-phase and G0/G1-phase in cell cycle were closely related to the levels of serum CEA in the treated group (r = 0.6677, P < 0.05; r = 0.7071, P < 0.01; r = -0.6703, P < 0.05, respectively).

  **Evaluation of survival time**
  
  After 3 wk of octreotide treatment, the median survival time was 49.3 ± 13.0 d in group A and 34.2 ± 7.3 d in group B (P < 0.05).

  **DISCUSSION**
  
  Somatostatin analogue, designated as octreotide with eight amino acids, is long acting and much more potent than somatostatin in inhibiting insulin, glucagon, and growth hormone. Clinical studies have demonstrated that octreotide is an effective pharmaceutical agent in treating various endocrine tumors (e.g. carcinoids, insulinomas, VIPomas, etc.). In recent years, several investigators have described that somatostatin analogues can exert inhibitory effect in gastrointestinal “non-endocrine” tumors by means of suppressing the growth of tumors and prolonging the survival time[4,5].

  In view of cell-cycle kinetics, PI and S-phase fraction always reflect the proliferative state of tumor cells. This study demonstrated that octreotide could markedly decrease the PI and the S-phase fraction of hepatic tumors and significantly increase the G0/G1-phase fraction in comparison with the control. Administration of octreotide significantly lowered the incidence and inhibited hepatic metastases of CT26 colonic adenocarcinoma cell line. The animal’s body weight decreased slowly and the survival time of mice prolonged markedly. Therefore, octreotide can inhibit the proliferation of CT26 tumor cell line and block the G0/G1-phase cells of CT26 adenocarcinoma from entering the S-phase. These experimental results from the view of cellular kinetics suggest that octreotide has the antitumor effect.

  The possible mechanism of the antitumor action of somatostatin is still unknown. From the available data, the following are proposed as likely mechanisms: (1) The antitumor action of somatostatin could be mediated directly by specific receptors located on the tumor cell membrane. (2) Somatostatin analogues can suppress the release of gastrointestinal hormones (e.g. insulin, gastrin, cholecystokinin and others), interfere with the synthesis of autocrine or paracrine growth factors by tumor cells (such as epidermal growth factor, platelet derived growth factor, transforming growth factor and others). (3) Somatostatin also exerts an inhibitory effect on DNA synthesis in tumor cells. And (4) Furthermore, somatostatin analogues can also inhibit the angiogenesis of neoplasms[6-8].

  Our data suggested that octreotide can also influence serum CEA. The serum CEA levels in the group treated with octreotide decreased markedly and were positively related with PI and S-phase fraction of cell cycle and negatively related with G0/G1-phase fraction. CEA, an oncofetal glycoprotein, was first described in 1965. The present study shows that the CEA is not only a tumor associ-

## Table 1  Comparison of body weight and incidence of hepatic metastases

|         | Group A | Group B |
|---------|---------|---------|
| Body weight (x ± s, g) |       |         |
| 0       | 16.74 ± 0.94 | 16.96 ± 0.90 |
| 1st week | 16.55 ± 0.82 | 16.64 ± 0.80 |
| 2nd week | 16.11 ± 0.79 | 15.68 ± 0.68 |
| 3rd week | 15.35 ± 0.81 | 15.05 ± 0.87 |
| Weight decreasing rate (x ± s, %) |       |         |
| 1st week | 1.23 ± 0.82 | 1.74 ± 1.40 |
| 2nd week | 3.70 ± 1.12 | 7.48 ± 2.14 |
| 3rd week | 8.25 ± 2.65 | 11.27 ± 2.79 |
| Incidence of hepatic tumor |       |         |
| Grade 1 | 0       | 0       |
| Grade II | 70% (7/10) | 20% (2/10) |
| Grade III | 30% (3/10) | 80% (8/10) |

*P < 0.01 vs before experiment; **P < 0.05, ***P < 0.01, group A vs group B.

## Table 2  Changes in proliferative index and phase fractions in tumor cell cycle (x ± s)

|         | Group A | Group B |
|---------|---------|---------|
| PI      | 0.24 ± 0.06 | 0.31 ± 0.02 |
| S (%)   | 17.69 ± 5.11 | 24.54 ± 2.58 |
| G0/G1 (%) | 76.41 ± 3.31 | 69.38 ± 3.30 |
| G0/M (%) | 6.11 ± 1.71 | 5.67 ± 1.76 |

PI: Proliferative index. **P < 0.01, group A vs group B.**

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ogue, octreotide, can effectively inhibit the growth and development
of hepatic metastases. Recently, octreotide has been employed in the
 treatment of advanced gastrointestinal cancer patients refractory to
chemotherapy. Although the results of initial clinical trials of soma-

tostatin analogues were controversial, we believe that somatostatin
may be a useful agent in endocrinotherapy for gastrointestinal cancer.

REFERENCES

1 Liu R, Wang JM. The application of somatostatin in digestive diseases. Zhonghua
Shiyong Zazhi 1993; 13: 681-683.

2 Schally AV. Oncological applications of somatostatin analogues. Cancer Res 1988; 48:
6977-6985 [PMID: 2903792].

3 Saltz L, Trochanowski B, Buckley M, Heffernan B, Niedzwiecki D, Tao Y, Kelsen D.
Octreotide as an antineoplastic agent in the treatment of functional and nonfunctional
neuroendocrine tumors. Cancer 1993; 72: 244-248 [PMID: 8389666 DOI: 10.1002/10
97-0142(19930701)72:1<244::AID-CNCR2820720143>3.0.CO;2-Q].

4 Qin Y, Schally AV, Willems G. Treatment of liver metastases of human colon cancers
in nude mice with somatostatin analogue RC-160. Int J Cancer 1992; 52: 791-796.

5 Cascino S, Del Ferro E, Catalano G. A randomised trial of octreotide vs best support-
ive care only in advanced gastrointestinal cancer patients refractory to chemotherapy.
Br J Cancer 1995; 71: 97-101 [PMID: 7839058 DOI: 10.1038/bjc.1995.19].

6 Goustit N, Leof EB, Shipley GD, Moses HL. Growth factors and cancer. Cancer Res
1986; 46: 1015-1029 [PMID: 3002670].

7 van Eijck CH, Slooter GD, Hoefland LJ, Kort W, Jeekel J, Lamerts SW, Marquet RL.
Somatostatin receptor-dependent growth inhibition of liver metastases by octreotide.
Br J Surg 1994; 81: 1333-1337 [PMID: 7953484 DOI: 10.1002/bjs.1600810925].

8 Woltering EA, Barrie R, O’Donnico TM, Antc D, Unc T, Cramer A, Holmes D, Robert-
son J, Fassler J. Somatostatin analogues inhibit angiogenesis in the chick chorioallo-

tic membrane. J Surg Res 1991; 50: 245-251 [PMID: 1705618 DOI: 10.1016/0022-48
04/91/00186-P].

9 Williams AF. A year in the life of the immunoglobulin superfamily. Immuno
today 1987; 8: 298-303 [PMID: 25290835 DOI: 10.1016/0167-5699(87)90016-8].

10 Hostetter RR, Augustus LB, Mankarious R, Chi KF, Fan D, Toth C, Thomas P, Jessup
JM. Carcinoembryonic antigen as a selective enhancer of colorectal cancer metastasis.
J Natl Cancer Inst 1990; 82: 380-385 [PMID: 2304087 DOI: 10.1093/jnci/82.5.380].

11 Benchimol S, Fuls A, Jobly S, Beauchemin N, Shirata K, Stanners CP. Carcinoem-

bryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule.
Cell 1989; 57: 327-334 [PMID: 2702691 DOI: 10.1016/0022-8674(89)90907-7].

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