P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wcjd@public.bta.net.cn www.wjgnet.com

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• GASTRIC CANCER •

Methionine-dependence and combination chemotherapy on human gastric cancer cells *in vitro*

Wei-Xin Cao, Jing-Min Ou, Xu-Feng Fei, Zheng-Gang Zhu, Hao-Ran Yin, Min Yan, Yan-Zhen Lin

Wei-Xin Cao, Jing-Min Ou, Xu-Feng Fei, Department of Clinical Nutrition, Shanghai Institute of Digestive Surgery, Ruijin Hospital, Shanghai Second Medical University, Shanghai 200025, China
Zheng-Gang Zhu, Hao-Ran Yin, Min Yan, Yan-Zhen Lin, Department of Surgery, Shanghai Institute of Digestive Surgery, Ruijin Hospital, Shanghai Second Medical University, Shanghai 200025, China
Supported by the Science Foundation of Ministry of Health of China, No.96-2-296
Correspondence to: Dr. Wei-Xin Cao, Department of Clinical Nutrition, Ruijin Hospital, Shanghai Second Medical University, 197 Ruijiner Road, Shanghai 200025, China. fische@citiz.net
Telephone: +86-21-64370045 Ext.661688 Fax: +86-21-64333548
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Abstract

AIM: **To elucidate whether human primary gastric cancer and gastric mucosa epithelial cells *in vitro* can grow normally in a methionine (Met) depleted environment, i.e. Met-dependence, and whether Met-depleting status can enhance the killing effect of chemotherapy on gastric cancer cells.**

METHODS: **Fresh human gastric cancer and mucosal tissues were managed to form monocellular suspensions, which were then cultured in the Met-free but homocysteine-containing (Met^-Hcy^+) medium, with different chemotherapeutic drugs. The proliferation of the cells was examined by cell counter, flow cytometry (FCM) and microcytotoxicity assay (MTT).**

RESULTS: **The growth of human primary gastric cancer cells in Met^-Hcy^+ was suppressed, manifested by the decrease of total cell counts [1.46±0.42 ($\times10^9\cdot L^{-1}$) in Met^-Hcy^+ *vs* 1.64±0.44 ($\times10^9\cdot L^{-1}$) in Met^+Hcy^-, $P<0.01$], the decline in the percentage of G_0G_1 phase cells (0.69±0.24 in Met^-Hcy^+ *vs* 0.80±0.18 in Met^+Hcy^-, $P<0.01$) and the increase of S cells (0.24±0.20 in Met^-Hcy^+ *vs* 0.17±0.16 in Met^+Hcy^-, $P<0.01$); however, gastric mucosal cells grew normally. If Met^-Hcy^+ medium was used in combination with chemotherapeutic drugs, the number of surviving gastric cancer cells dropped significantly.**

CONCLUSION: **Human primary gastric cancer cells *in vitro* are Met-dependent; however, gastric mucosal cells have not shown the same characteristics. Met^-Hcy^+ environment may strengthen the killing effect of chemotherapy on human primary gastric cancer cells.**

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INTRODUCTION

Gastric cancer is common in China and abroad[1-20], and chemotherapy is still a main method for advanced cancer so far[21-30]. Up to now, quite a few studies have elucidated the property of methionine (Met) dependence of cancer cells[31-34]. If that property was utilized in combination with specific chemotherapeutic drugs, the proliferation of tumor cells would be suppressed. This study intended to culture the human primary gastric cancer cells in diverse environments to judge whether the gastric cancer cells were Met-dependent and whether Met^-Hcy^+ could enhance the effect of chemotherpy.

MATERIALS AND METHODS

Study on Met-dependence

Human primary gastric cancer cells were collected from gastric cancer tissues of 37 advanced gastric cancer patients in our hospital, and human gastric mucosal epithelial cells were simultaneously obtained in 31 patients. Based on the Met-free RPMI-1640 medium, dialyzed bovine serum, and other ingredients necessary for cell growth, Met-free but homocysteine-containing (Met^-Hcy^+) medium and Met-containing but Hcy-depleting (Met^+Hcy^-) medium were prepared[31]. Fresh gastric cancer and mucosa tissues were obtained during operations. After being clipped into pieces and digested, the samples were made into monocellular suspensions, which were then shared out equally in the Met^-Hcy^+ medium (test group) and Met^+Hcy^- medium (control group) separately. The cell concentration was adjusted to $5\times10^8\cdot L^{-1}$. Seeded in the bottles the inside of which was covered with mouse tail collagen, the cells were incubated at 37℃ in an atmosphere containing $50mL\cdot L^{-1}$ CO_2. After being cultured continuously for 96h, the cells were digested by protease to monocellular suspensions, which were then examined by cell counter and phase-contrast microscope and submitted to FCM study for cell kinetics.

Study on Met^-Hcy^+ environment in combination with chemotherapeutic drugs

Human primary gastric cancer cells were sampled from gastric cancer tissues of 40 advanced gastric cancer patients. The methods and conditions of gastric cancer sampling, treatment, and culture were the same as mentioned above. Human primary gastric cancer cells were inoculated in 96-well plates, in which any one of adriamycin (ADM), cisplatin (DDP), 5-fluorouracil (5-FU), mitomycin C (MMC) and methotrexate (MTX) was then added. The concentrations of those drugs were 0.4, 3.0, 10.0, 3.0 and $4.0mg\cdot L^{-1}$ respectively. Each drug group was designed to be repeated for 3 times, and the concentration of 0 was the empty control. Tetrazolium salt was added after the cells were incubated at 37℃ in a $50mL\cdot L^{-1}$ CO_2 atmosphere for 48h. The cell suspensions were centrifuged, added with dimethyl sulfoxide (DMSO), shaken and aspirated until crystals dissolved completely. Microcytotoxicity assay (MTT) was applied, and the cells were colorimetrically analyzed by Bio-tek Instruments at 490nm to obtain the A (absorbance) value, which reflected the amount of surviving cells.

Statistical analysis

Paired *t* test was used.

RESULTS

Human primary gastric cancer cell cycle and amount

After a 96-h culture, the proliferation of human primary gastric cancer cells in the test group was affected, showing bad adherence and cytolysis; however, the cancer cells in the control group did not show

the similar phenomenon. The percentage of cells in G_0G_1 phases in the test group was significantly lower than that in the control group while the percentage of S phase cells in test group was obviously higher than that in the control group. There was no statistical significance between the percentage of G_2M cells in the test group and that in the control group. The result of cell counting was that human primary gastric cancer cells in the test group were apparently less than those in the control group (Table 1).

Table 1 The distribution of human primary gastric cancer cell cycle and the amount of cells ($\bar{x}\pm s$, n = 37)

Groups	Fractions in different phases			Amount of cells ($\times10^9\cdot L^{-1}$)
	G_0G_1	S	G_2M	
Met^+Hcy^-	0.80±0.18	0.17±0.16	0.04±0.06	1.64±0.44
Met^-Hcy^+	0.69±0.24[b]	0.24±0.20[b]	0.07±0.13	1.46±0.42[b]

[b]P<0.01, *vs* Met^+Hcy^- group.

Human gastric mucosa cell cycle and amount

Gastric mucosa epithelial cells grew well both in the test group and in the control group. There was no obvious difference in the percentages in various phases in cell cycle between the two groups. The amount of cells in the test group was slightly higher than that in the control group but without statistical significance (Table 2).

Table 2 The distribution of human gastric mucosa epithelial cell cycle and the amount of cells ($\bar{x}\pm s$, n = 31)

Groups	Fractions in different phases			Amount of cells ($\times10^9\cdot L^{-1}$)
	G_0G_1	S	G_2M	
Met^+Hcy^-	0.90±0.13	0.07±0.11	0.02±0.02	1.70±0.44
Met^-Hcy^+	0.90±0.14	0.08±0.11	0.03±0.04	1.75±0.45

Different media in combination with chemotherapeutic drugs

The surviving gastric cancer cells in the test group without chemotherapeutic drugs were fewer than those in the corresponding control group, with lower A value. The proliferation of gastric cancer cells in the Met^-Hcy^+ group was suppressed much more strongly than that in the Met^+Hcy^- group, no matter which drug was added. The former group had the manifestation of the decrease in surviving gastric cancer cells to various extents, with lower A value (Table 3).

Table 3 The influence of Met^+Hcy^- or Met^-Hcy^+ in combination with chemotherapeutic drugs to gastric cancer cells ($\bar{x}\pm s$, A)

Drug	Met^+Hcy^-	Met^-Hcy^+
ADM	0.3807±0.3114	0.3175±0.2003[a]
DDP	0.3878±0.3050	0.3189±0.1848[b]
5-FU	0.3657±0.2597	0.3182±0.2049[a]
MMC	0.3861±0.2734	0.3105±0.2103[b]
MTX	0.3649±0.2811	0.3120±0.2003[b]
Control	0.4834±0.4337	0.3981±0.3056[b]

[a]P<0.05, [b]P<0.01, *vs* Met^+Hcy^-.

DISCUSSION

Met-dependence and Met starvation

Methionine is an essential amino acid containing an S-methyl, which is used to synthesize through methylation quite a few important physiological active substances. Homocysteine is the direct precursor of Met. Hcy and Met may be converted into each other *in vitro*, but it is not as such *in vivo*. Hcy is continuously consumed with the need of metabolism, and hence is transformed and supplied by Met. Met depletion *in vivo* is easily produced once Met supply is stopped, as there is no pathway *in vivo* of Hcy transforming to Met, which is an essential amino acid. Normal cells can grow well in the environment containing Hcy instead of Met. The proliferation of tumor cells, however, may be suppressed under such condition. This phenomenon is called Met-dependence. It is one of research focuses in the past decades that Met starvation is made artificially in tumor-bearing body to inhibit the tumor growth based on the therapy of Met-dependence. There are several methods to produce Met starvation[32-34]: (1)degradation of Met by methioninase; (2)fast plus Met-depleting total parenteral nutrition (Met^--TPN).Due to its unsatisfactory specificity methioninase can degrade Met irreversibly,but it can also degrade Hcy,homocystine, cysteine and cystine during a short period of time so that the normal cells are hard to survive.Only through fast and Met^--TPN may Met starvation be produced and have little impact on normal tissue cells.

Met-dependence of tumor cells

Studies *in vivo* and *in vitro* can be used to judge whether tumor cells have the property of Met-dependence and to explore the impact of Met starvation on the proliferation of tumor cells. The research *in vivo* is not easy to conduct because of many inevitable factors. The prominent characteristic of the research *in vitro* is that the cells cultured are not influenced by complex internal environment and the rule of cell life activity can be studied. The change of cell physiological functions under the influence of single or multi-factors may be further observed when culture conditions such as physical, chemical and biological factors are altered artificially. Goseki *et al* explored the relationship of tumor growth and Met-dependence by animal experiment. We have also done such work in the past years[35,36]. The studies suggested that Met starvation in tumor-bearing animals could inhibit the proliferation of tumor cells. In the experiment *in vitro* of gastric cell line SGC-7901, we found that the percentages of SGC-7901 cells in S and G_2M phases in the Met^-Hcy^+ medium were both obviously elevated and the percentage of G_0G_1 cells significantly declined in comparison with those in the Met^+Hcy^- medium. The results meant that the cell cycle of SGC-7901 was inhibited in the G_2M phase. The present study showed again that *in vitro* the proliferation of human primary gastric cancer cells in the environment of Met^-Hcy^+ had the similar results to that of human gastric cancer cell line SGC-7901. The cell cycle of tumor cells was disturbed and the amount of cancer cells decreased significantly; however, no same phenomenon was observed in the gastric mucosa epithelial cells. The results suggested that human gastric cancer cells were Met-dependent. The cause of Met-dependence of tumor cells remains unclear. As far as we know now, the causes include: (1)decreased amount and activity of Met synthetase; (2)deficiency of 5-methyltetrahydrofolate reductase; (3)inability to make use of endogenous Met; (4)rise in the rate of basal transmethylation; (5)activated EJ/T24HRAS1 oncogene leading to the expression of Met-dependence.

Significance of Met starvation in combination with chemotherapeutic drugs

Chemotherapy can not be replaced by surgery and radiotherapy in the treatment of malignancies. At present most chemotherapeutic drugs are phase-specific, despite some without phase-specificity, all of which are hard to kill tumor cells completely. So the aim of tumor therapy is to take measures to improve the effect of chemotherapy. It is a new trial to drive the G_0 cells to enter into the proliferating phase. Our previous clinical study and tumor-bearing rat experiment[37-39] showed that after the treatment of parenteral nutrition, the percentages of S and $S+G_2+M$ gastric cancer cells increased obviously while that of G_0G_1 cells decreased dramatically. Met deprivation can produce Met starvation in Met-dependent tumor cells, then metabolism of cancer cells is suppressed and more cells remain in the phases of S and G_2. As a result, the effect of phase-specific chemotherapeutic drugs is enhanced significantly. This provides a new way to improve the therapeutical effect on tumors by using Met^--TPN combined with chemotherapeutic drugs. Many authors[40-44] have made the researches, both experimentally and clinically, on Met^--TPN in

combination with chemotherapeutic drugs to improve the effect of chemotherapy. They confirmed that Met-TPN in combination with 5-FU, DDP and ADM respectively can enhance the sensitivity of chemotherapy of gastric cancer, breast cancer and Yashida sarcoma. Our present study supported the above results. It is hypothesized that Met starvation could make primary gastric cancer cells blocked in the phases of S/G_2M. Theoretically, the more numerous are the cells in the proliferating phase, the stronger effect of phase-specific chemotherapeutic drugs have been produced.

REFERENCES

1 Cai L, Yu SZ. A molecular epidemiologic study on gastric cancer in Changle, Fujian Province. *Shijie Huaren Xiaohua Zazhi* 1999; 7: 652-655
2 Wang Q, Jin PH, Lin GW, Xu SR. Cost effectiveness of population based Helicobacter pylori screening to prevent gastric cancer. *Shijie Huaren Xiaohua Zazhi* 2000; 8:262-265
3 Wong BC, Lam SK, Ching CK, Hu WH, Kwok E, Ho J, Yuen ST, Gao Z, Chen JS, Lai KC, Ong LY, Chen BW, Wang WH, Jiang XW, Hou XH, Lu JY. The China gastric cancer study group. Differential Helicobacter pylori infection rates in two contrasting gastric cancer risk regions of South China. *J Gastroenterol Hepatol* 1999; 14:120-125
4 Gao GL, Yang Y, Yang S, Ren CW. Relationship between proliferation of vascular endothelial cells and gastric cancer. *Shijie Huaren Xiaohua Zazhi* 2000; 8:282-284
5 Zou SC, Qiu HS, ZhangCW, Tao HQ. A clinical and long term follow up study of peri operative sequential triple therapy for gastric cancer. *World J Gastroenterol* 2000; 6:284-286
6 Zhang XQ, Lin SR. The study advance of Helicobacter pylori and gastric cancer. *Shijie Huaren Xiaohua Zazhi* 2000; 8:206-207
7 Ma JL, Liu WD, Zhang ZZ, Zhang L, You WC, Chang YS. Relationship between gastric cancer and precancerous lesions. *World J Gastroenterol* 1998; 4: 180-182
8 Xue XC, Fang GE, Hua JD. Gastric cancer and apoptosis. *Shijie Huaren Xiaohua Zazhi* 1999; 7: 359-361
9 Xia HX. Association between Helicobacter pylori and gastric cancer:current knowledge and future research. *World J Gastroenterol* 1998;4:93-96
10 Wang WX, Yuan Y, Gao H, Wang L, Wu YQ, Dong M. Screening of Helicobacter pylori infection in 16 villages of high risk population of gastric cancer. *World J Gastroenterol* 1998; 4: 112
11 Cai L, Yu SZ, Zhang ZF. Helicobacter pylori infection and risk of gastric cancer in Changle Country, Fujian Province, China. *World J Gastroenterol* 2000; 6: 374-376
12 Yuan Y, Cong W, Xu RT, Wang XJ, Gao H. Gastric cancer screening in 16 villages of Zhuanghe region: a high risk area of stomach cancer in China. *World J Gatroenterol* 1998; 4: 111
13 Wu YA, Lu B, Liu J, Li J, Chen JR, Hu SX. Consequence alimentary reconstruction in nutritional status after total gastrectomy for gastric cancer. *World J Gastroenterol* 1999; 5: 34-37
14 Hu PJ. *Hp* and gastric cancer: challenge in the research. *Shijie Huaren Xiaohua Zazhi* 1999; 7: 1-2
15 Liu XB, Li L, Zhuang BZ, Jiang YD, Wang JH. Cycline E expression in gastric carcinoma and its clinicopathological significance. *Shijie Huaren Xiaohua Zazhi* 1999; 7:656-658
16 Folli S, Morgagni P, Roviello F, De Manzoni G, Marrelli D, Saragoni L, Di Leo A, Gaudio M, Nanni O, Carli A, Cordiano C, Dell'Amore D, Vio A. Risk factors for lymph node metastases and their prognostic significance in early gastric cancer (EGC) for the Italian Research Group for Gastric Cancer (IRGGC). *JpnJ Clin Oncol* 2001; 31: 495-499
17 Yokota T, Kunii Y, Teshima S, Yamada Y, Saito T, Takahashi M, Kikuchi S, Yamauchi H. Significant prognostic factors in patients with early gastric cancer. *Int Surg* 2000; 85:286-290
18 Kocher HM, Linklater K, Patel S, Ellul JP. Epidemiological study of oesophageal and gastric cancer in south-east Englang. *Br J Surg* 2001; 88: 1249-1257
19 Barchielli A, Amorosi A, Balzi D, Crocetti E, Nesi G. Long-term prognosis of gastric cancer in a European Country: a population-based study in Florence (Italy). 10-year survival of cases diagnosed in 1985-1987. *Eur J Cancer* 2001; 37: 1674-1680
20 Stein HJ, Feith M, Siewert JR. Cancer of the esophagogastric junction. *Surg Oncol* 2001; 9: 35-41
21 Wang XS, Wang RB, Zhang ZL, Cao TJ, Jin WD. Effect of short time heating with MMC and 5-FU on cancer cells. *Shijie Huaren Xiaohua Zazhi* 1999; 7: 576-578
22 Tu SP, Jiang SH, Qiao MM, Cheng SD, Wang LF, Wu YL, Yuan YZ, Wu YX. Effect of trichosanthin on cytotoxicity and induction of apoptosis of multiple drugs resistence cells in gastric cancer. *Shijie Huaren Xiaohua Zazhi* 2000; 8: 150-152
23 Zhang XY. Some recent works on diagnosis and treatment of gastric cancer. *World J Gastroenterol* 1999; 5: 1-3
24 Li GF, Xie SB, Sun H, Yang XH, Liu WJ, Zhai Q, Zhou YX, Li ZH, Zhang GM. An investigation of intra arterial chemotherapy infusion and embolization combined with abdominal chemotherapy for advanced gastric cancer. *World J Gastroenterol* 1998; 4: 71
25 Yu WL, Huang ZH. Porgress in studies on gene therapy for gastric cancer. *Shijie Huaren Xiaohua Zazhi* 1999; 7:887-889
26 Murashima N, Gochi A, Kenmotsu M, Hamazaki K, Funaki M, Ohtsuka S, Tanaka N. Schedule-dependent combined sensitivity testing of anti-cancer agents in human gastric carcinoma cell lines. *J Int Med Res* 2001; 29: 189-197
27 Itoh Y, Fuwa N, Shinoda M. A case of esophageal carcinoma surgically treated after discontinuance of the simultaneous application of radiotherapy and chemotherapy with low doses of CDDP and 5-FU. *Radiat Med* 2000; 18:55-58
28 Takahashi M, Yanoma S, Yamamoto Y, Rino Y, Amano T, Imada T. Combined effect of CDDP and caffeine against human gastric cancer cell line *in vivo*. *Anticancer Res* 1998; 18: 4399-4401
29 Harstrick A,Gonzales A,Schleucher N,Vanhoefer U,Lu K,Formento JL, Milano G,Wilke H,Seeber S,Rustum Y.Comparison between short or long exposure to 5-fluorouracil in human gastric and colon cancer cell lines: biochemical mechanism of resistance. *Anticancer Drugs* 1998;9:625-634
30 Raida M, Kath R, Arnrich M, Kahler G, Scheele J, Hoffken K. A phase II study of weekly high-dose 5-fluorouracil and leucovorin plus biweekly alternating doxorubicin and cisplatin for advanced gastric carcinoma. *Cancer Res Clin Oncol* 1998; 124: 335-340
31 Guo HY, Herrera H, Groce A, Hoffman RM. Expression of the biochemical defect of methionine dependence in fresh patient tumors in primary histoculture. *Cancer Res* 1993; 53: 2479-2483
32 Kreis W, Baker A, Ryan A, Bertasso A. Effect of nutritional and enzymatic methionine deprivation upon human normal and malignant cells in tissue culture. *Cancer Res* 1980; 40: 634-641
33 Yoshida S, Yomasaki K, Kaibara A, Takagi K, Noake T, Ishibashi N, Kakegawa T. Effect of methionine-deprived total parenteral nutrition on tumor protein turnover in rats. *Cancer* 1995;76:1275-1282
34 Poirson-Bichat F, Gonfalone G, Bras-Goncalves RA, Dutrillaux B, Poupon MF. Growth of methionine dependent human prostate cancer (PC-3) is inhibited by ethionine combined with methionine starvation. *Br J Cancer* 1997; 75: 1605-1612
35 Xiao HB, Cao WX, Yin HR, Lin YZ. The study of methionine-deprived total parenteral nutrition on gastric carcinoma bearing rat. *Changwai Yu Changnei Yingyang* 1997; 4: 16-18
36 Cao WX, Xu N, Yin HR, Chen XH. The implication of tumor cells' methionine dependence in chemotherapy. *Zhonghua Shiyan Waike Zazhi* 1999; 16: 319-320
37 Cao WX, Yan M, Lin YZ, Yin HR, Zhu ZG, Li SF, Lu YP. The influence of intravenous nutrition on gastric cancer cell kinetics. *Zhonghua Zhongliu Zazhi* 1992; 14:418-420
38 Cao WX, Xiao HB, Yin HR, Yan M, Zhu SZ, Lin YZ. The influence of intravenous nutrition on the effect of chemotherapy in gastric cancer. *Zhonghua Zhongliu Zazhi* 1994; 16:137-140
39 Yan M, Cao WX, Zhu ZG, Yin HR, Zhu SZ, Lin YZ. The study of the parenteral nutrition on the gastric cancer cell kinetics in Wistar rats. *Zhongguo Linchuang Yingyang Zazhi* 1993; 1: 31-34
40 Hoshiya Y, Kubota T, Matsuzaki SW, Kitajima M, Hoffman RM. Methionine starvation modulates the efficacy of cisplatin on human breast cancer in nude nice. *Anticancer Res* 1996; 16: 3515-3517
41 Goseki N, Maruyama M, Nagai K, Kando F, Endo M, Shimoju K, Wada Y. Clinical evaluation of anticancer effect of methionine-depleting total parenteral nutrition with 5-fluorouracil and / or mitomycin C. *Gan To Kagaku Ryoho* 1995; 22: 1028-1035
42 Kitamura S, Ohtani T, Kurihara M, Kosaki G, Akazawa S, Sasaki T, Takahashi H, Nakano S, Tokunaga K. A controlled study of AO-90, a methionine-free intravenous amino acid solution, in combination with 5-fluorouracil and mitomycin C in advanced gastric cancer patients (internal medicine group evaluation). *Gan To Kagaku Ryoho* 1995; 22: 765-775
43 Taguchi T, Kosaki G, Onodera T, Endo M, Nakagawara G, Sano K, Kaibara N, Kakegawa T, Nakano S, Kurihara M. A controlled study of AO-90, a methionine-free intravenous amino acid solution, in combination with 5-fluorouracil and mitomycin C in advanced gastric cancer patients (surgical group evaluation). *Gan To Kagaku Rhoyo* 1995; 22: 753-764
44 Cao WX, Cheng QM, Fei XF, Li SF, Yin HR, Lin YZ. A study of preoperative methionine-depleting parenteral nutrition plus chemotherapy in gastric cancer patients. *World J Gastroenterol* 2000; 6: 255-258

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