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Activity of boanmycin against colorectal cancer

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INTRODUCTION

Boanmycin (Bleomycin A6, BAM), a new antitumor antibiotic, was isolated from many components of bleomycin (BLM) produced by streptomyces pingyangensis which were obtained from a soil sample collected in Pingyang County, Zhejiang Province, China. Boanmycin has a similar chemical structure to that of BLM, but the terminal amine moiety is different [1]. Pingyangmycin (bleomycin A5), one of multicomponent bleomycin complex produced by the strain, was found to have a high activity against a wide spectrum of murine transplantable tumors, but have a relatively low pulmonary toxicity in mice[2-4]. Pingyangmycin, as a single agent, shows marked inhibition on the growth of human colon cancer, stomach cancer and nasopharyngeal cancer xenografts in nude mice[5-7]. It has been widely used clinically in the treatment of tumors since 1979 in China[8]. Because of a good antitumor efficacy of pingyangmycin as a single agent and low marrow toxicity, attempts have been made to develop new superior bleomycin derivatives[9]. BAM, a minor component of bleomycin complex, was also found to be highly active against murine tumors, human liver cancer and colorectal xenografts in nude mice, and markedly inhibit the spontaneous pulmonary metastasis of Lewis carcinoma in mice[10-14]. BAM-monoclonal antibody immunoconjugates were highly effective against related human tumor *in vivo* and *in vitro*[15-19]. BAM also reached a higher concentration and remained for a longer time in murine transplantable carcinomas as compared with other bleomycin components[3]. As observed under electron microscopy, the pulmonary toxic damage caused by BAM was less than that induced by bleomycin[20]. Phase I clinical study of BAM showed no myelosuppression and cardiac toxicity, and its major adverse reactions were fever, gastrointestinal reactions and hardening at the site of i.m. injection. All adverse effects disappeared after discontinuation of the therapy[21].

An ideal animal model for cancer is one that mimics human disease in every respect. Most tumor xenograft studies, including colorectal tumor, for the evaluation of antitumor activity of drugs used subcutaneous implantation system due to its convenience and access to direct detection and therapeutic effect. However, those models have limitations for the study of interaction of tumor cells with their relevant organ environment or organ distribution of drugs. Alteration of microenvironment surrounding tumor tissue will not only influence growth and spread of tumor but also is important for drug delivery[22-24]. Recently, use of orthotopic models for the growth of tumors in mice or rat has aroused more interest, including lung cancer, colorectal cancer, breast cancer, pancreatic cancer, etc[25-30]. However, whether such orthotopic colorectal tumor models apply to the evaluation of new anticancer agents remains unknown.

In the present study, human colorectal tumor xenograft model in nude mice and the orthotopic model of murine colon cancer were used to clarify the antitumor effect of BAM in comparison with that of mitomycin C and 5-fluorouracil, drugs commonly used in clinics against colorectal cancer. We attempted to determine the effect of BAM against colorectal cancer and whether the organ microenvironment could influence the response of a murine colon cancer to systemic therapy with BAM.

MATERIALS AND METHODS

Mice

Nude mice with a BALB/c genetic background were bred under specific pathogen-free conditions at the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences (CAMS). All experiments were carried out in the Institute of Laboratory Animal Sciences under specific pathogen-free conditions using laminar airflow racks. Six to seven week old male or female nude

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mice weighing 18 g-22 g were used for the experiment. All food, water and bedding were sterilized.

Tumors

Two human cell lines of colorectal cancer, HT-29 and Hce-8693, transplanted into nude mice, were used. HT-29 was an adenocarcinoma of the colon established in the Memorial Sloan-Kettering Cancer Center, New York. Hce-8693 was a poorly differentiated adenocarcinoma of the cecum established in the Zhejiang Tumor Hospital, Hangzhou.

Colon tumor NO. 26 (CT-26), a murine colon adenocarcinoma, was induced in a BALB/c mouse by chemical carcinogens. Initially, CT-26 cells were serially transplanted and then established as a cell line and used in a large number of chemotherapy studies.

Drugs

Commercially available mitomycin C (MMC) and 5-fluorouracil (5-FU) were used for the experiment. BAM, a single component of bleomycin A6, was purified from bleomycin complex at Institute of Medicinal Biotechnology, CAMS & PUMC, Beijing. A 1/9 LD50 equitoxic dose for each drug, MMC 1.0 mg/kg per injection, 5-FU 27 mg/kg per injection and BAM 10 mg/kg per injection, was used for *in vivo* comparative studies[12].

Chemotherapy of human colorectal cancer xenografted in nude mice

Two human tumor fragments of about 2 mm^3 were inoculated subcutaneously into the right axillary region of nude mice. When diameter of tumor reached 3 mm-4 mm, tumor-bearing mice were randomized into test groups of 5-6 each and drug treatment was initiated. Test mouse was weighed and size of tumor was measured with sliding calipers two times weekly, and tumor weight (W) was calculated by the formula W (mg) = a(mm) × b^2(mm)/2 where a was the width and b is the length. The drugs were administered i.p., *b.i.w.*, a total of 10 injections. Next day after last treatment, mice were killed and the tumors were removed and weighed. The antitumor effect of drug was evaluated in terms of inhibitory ratio [1-(mean tumor weight of treated group - mean tumor weight of control group)] × 100%[12].

Chemotherapy of murine CT-26

Orthotopic tumor implantation Six-week-old (18 g-20 g) female BALB/c mice were anesthetized with diethylether, and the abdomen was prepared for sterile surgery. A small incision was made, the cecum was exteriorized and CT-26 cells (1×10^6/0.02 mL) were injected with a 30-G needle between the submucosa and the subserosa. The lack of extracecal leakage was the criterion for a successful injection. The cecum was returned to the abdominal cavity, and the wound was closed in one layer[7].

Subcutaneous tumor implantation CT-26 cells (1×10^6/0.02 mL) were injected into *s.c* in right axilla region. Mice were randomized into treatment and control groups based on the body weight next day after tumor cell injection. The drugs were administered *i.m.* (hindlimb), *q.o.d.*, for a total of 10 injections. Mice with *s.c* tumors and cecal tumors were killed on day 21 after tumor cell injection, and tumors were removed and weighed. The antitumor effect of drug was also evaluated in terms of inhibitory ratio [1-(mean tumor weight of treated group - mean tumor weight of control group)] × 100%[7].

Pathologic examination

Samples from untreated and drug-treated tumors were collected from subcutis or cecum and fixed in Bouin's solution, and embedded in paraffin. Sections were made, stained with H & E and observed under microscopy with a rectangular net-like micrometer. Whole area and necrosis area in tumor section was measured, and the necrosis ratio of tumors was calculated. Ten optical fields were examined along the peripheral area of the tumor and the mitotic figures were recorded[12].

Examination of nucleated cells in bone marrow

Femurs were removed and the bone marrow cavity was washed out with 3 mL white-blood-cell diluting solution. The nucleated cells per femur were counted under microscopy[12].

RESULTS

Response of colon cancer to BAM in nude mice

The results shown in Table 1 reveals the same response of the two types of human colon cancer to intraperitoneal BAM in both 10 and 15 mg/kg dose groups. Both doses exerted marked inhibition on the growth of HT-29 and Hce-8693 xenografts in nude mice.

Table 1 Inhibitory effect of BAM on the growth of human colorectal xenografts in nude mice*

Drug	Tumor	No. of mice	Dose (mg/kg)	Tumor weight (g) $\bar{x} \pm s$	Tumor growth inhibition (%)
BAM	HT-29	6		1.300 ± 0.620	
		6	10	0.138 ± 0.064	89[b]
		6	15	0.098 ± 0.074	92[b]
	Hce-8693	5		1.501 ± 1.445	
		5	10	0.154 ± 0.125	90[b]
		5	15	0.122 ± 0.154	92[b]

*Treatment was started on day 3 and day 14 after HT-29 and Hce-8693 tumor transplantation, *ip.*, twice a week, with a total of 10 injections. [b]$P<0.01$, *vs* control group.

Comparative response of colon cancer to BAM, 5-FU and MMC in nude mice

On the basis of equitoxic doses (1/9 LD50), BAM and two clinically active drugs (5-FU and MMC) were evaluated against human colon cancer HT-29 xenografts in nude mice. Table 2 shows the response of the tumor to two weekly treatments with three drugs. BAM is the most active single agent with an inhibition rate of 82% and exerted much stronger growth inhibition against HT-29 xenografts than 5-FU(P<0.01) and MMC(P<0.05). MMC had moderate activity against HT-29 xenografts with an inhibition rate of 53%. 5-FU did not arrest tumor growth throughout the experiment, and tumor increased in size at the same rate as the untreated control with an inhibition rate of 12%.

Comparative response of CT-26 growing at subcutis and cecum to BAM 5-FU and MMC

In the experiment, BALB/c mice were given injections of CT-26 cells into subcutis and cecum, which produced s.c tumor and cecum tumor. In view of the drug distribution, BAM, 5-FU and MMC were administered *i.m.* rather than *i.p.* at an equitoxic dose. In the treatment, the equitoxic dose was administered, and the antitumor activity of the three agents was compared. Table 3 shows the antitumor activity of BAM on the intracecal and *s.c* CT-26 tumor in comparison with that of 5-FU and MMC. BAM displayed a striking activity against intracecal CT-26 tumor; the inhibition of tumor growth was higher at the cecum than at the s.c site (P<0.05). The mean tumor weight after BAM treatment was less than that in the control, 5-FU and MMC groups, and the difference was statistically significant (P<0.01). On the other hand, 5-FU did not effectively suppress the tumor growth, and MMC showed mild activity.

Changes in tumor necrosis and mitosis

In contrast to the control HT-29 tumors that maintained their feature of poorly differentiated adenocarcinoma, the BAM-treated HT-29 xenografts in athymic mice presented extensive tumor necrosis and fibrosis. In areas of residual tumor, tumor cells were frequently found to have giant, bizarre-shaped pyknotic nucleoli, or prominent inclusion-type nucleoli. Xenografts treated with 5-FU and MMC showed scattered giant, bizarre-shaped nuclei and nucleolar prominence but these changes were focal and less apparent than in BAM-treated tumors. More extensive necrosis was found in tumors treated with BAM than in those treated with 5-FU or MMC. The necrotic ratio of tumors (whole necrotic area/whole tumor area) in BAM-treated group (67%) was much higher than that in 5-FU-treated (35%), MMC-treated (43%) and control groups (35%). In contrast to the tumor of the control group, the pathologic mitosis figures in BAM-treated group were reduced by 69%, which were fewer than those in 5-FU-treated, MMC-treated and control group (Table 3).

Table 2 Inhibitory effect of BAM, MMC and 5-Fu on the growth and tumor cell mitosis of human colon cancer HT-29 xenografts in nude mice*

Drug	Dose** (mg/kg)	No. of mice	Tumor weight (g) $\bar{x} \pm s$	Inhibitory rate (%)	Necrotic ratio of tumors△ (%)	Mitotic figures△△	
						$\bar{x} \pm s$	(%)
Control		5	0.804±0.173		35[b]	104±12	100
BAM	10	5	0.148±0.059	82	67	41±4	39[b]
MMC	1	5	0.376±0.174	53[a]	43[b]	107±12	103
5-FU	27	5	0.707±0.168	12[b]	35[b]	130±17	130

*Treatment was started on day 9 after tumor transplantation, *ip.*, twice a week, with a total of 10 injections;
**Drugs were administered at equitoxic doses (1/9 LD50); △Ratio of necrotic areas in whole section of the tumor.
△△Number of mitotic figures in 10 optical fields of the section of tumor.
[a]P<0.05, *vs* BAM group; [b]P<0.01, *vs* BAM group.

Table 3 Inhibitory effect of BAM, MMC and 5-FU on the growth of CT-26 tumor at the cecum and *s.c* site in mice*

Exp.	Drug	Dose** (mg/kg)	No. of mice	*s.c.* tumor		Cecal tumor	
				Tumor weight (g) $\bar{x} \pm s$	Inhibitory rate (%)	Tumor weight (g) $\bar{x} \pm s$	Inhibitory rate (%)
I	Control		9	0.854±0.151		0.557±0.112	
	BAM	10	9	0.101±0.054	88[b]	0.005±0.010	99[b]
	MMC	1	8	0.311±0.105	64	0.159±0.043	71
	5-FU	27	8	0.459±0.118	46	0.310±0.148	44
II	Control		6	0.900±0.396		0.740±0.446	
	BAM	10	6	0.105±0.088	88[b]	0.011±0.019	99[b]
	MMC	1	5	0.674±0.270	25	0.159±0.510	78
	5-FU	27	6	0.657±0.322	27	0.434±0.255	41

*Treatment was started on next day after tumor cell injection, *im.*, *qod.*, with a total of 10 injections.
**Drugs were administered at equitoxic doses (1/9 LD50).
[b]P<0.01, *vs* any other group.

Toxicity

No death or body weight loss of more than 20% was seen in the control or treated mice during the experiment. No inhibition on bone marrow cellularity was found in HT-29-bearing nude mice and CT-26-bearing mice at therapeutic doses of BAM. There was no significant difference in nucleated cell counts of marrow between the control and BAM group. In the CT-26-bearing mice, 5-FU and MMC caused significant decrease in bone marrow cellularity (Table 4). At therapeutic doses, no pathologic changes were found in the heart, lung, liver, spleen, kidney and brain of BAM-treated mice.

Table 4 Bone marrow nucleated cells in mice bearing CT-26 treated by BAM, 5-FU and MMC

Exp.	Drug	Dose** (mg/kg)	No. of mice	Nucleated cells (10^6/femur) $\bar{x} \pm s$	%
I	Control		6	13±3	100
	BAM	10	6	13±3	100
	MMC	1	5	7±2	54[b]
	5-FU	27	6	7±2	54[b]
II	Control	9		12±3	100
	BAM	10	9	12±2	100
	MMC	1	8	3±1	25[b]
	5-FU	27	8	4±1	33[b]

*Drugs were administered at equitoxic doses (1/9 LD50).
[b]$P<0.01$, *vs* control group.

DISCUSSION

Colorectal carcinomas are generally not very sensitive to the established chemotherapeutic agents; only 5-FU and MMC have shown some activity against colon carcinomas; the effects achieved, however, are of only a little value with respect to patient survival[31-41]. In the present study, we investigated the antitumor effectiveness of BAM, a new antitumor antibiotic, against human colorectal adenocarcinoma heterotransplanted to nude mice and murine colon adenocarcinoma. On the other hand, BAM, an analog of BLM that is clinically characterized by marked antineoplastic activity against carcinoma of the head and neck[42,43], showed a pronounced antitumor effect against the colorectal carcinoma used in the present study. At a 1/9 LD50 equitoxic dose, BAM exerted much stronger growth inhibition against human colon cancer HT-29 xenograft in nude mice than MMC and 5-FU. However, the tumor that remained after treatment consisted of viable cells with no degenerative changes, which were the source of early recurrence[44]. These results indicated that the effect of cancer chemotherapy should be judged not only by tumor reduction rate but also by histological changes, such as the necrotic ratio of tumors. In the present study, more extensive necrosis and fewer mitosis figures were found in tumors treated with BAM than those with MMC and 5-FU, indicating that BAM was more active against colon cancer HT-29 xenografts among three agents.

Most patients with colorectal carcinoma will die from distant metastases that are not detectable at the initiation of treatment[45,46]. Two major factors that influence the outcome of systemic therapy of cancer are heterogeneity of malignant neoplasms and *in vivo* conditions. Organ environment effects on the response of tumor to systemic chemotherapy are multifactorial, including the nutritional status of cells, the presence of organ-specific growth factors and other single-transducing agents, the degree of oxygenation, pH, extent of the vascular network and its functionality, local immunity, extracellular matrix components and drug metabolism[22-24]. The current model of orthotopic implantation of a colon carcinoma provides a unique opportunity to study a human malignancy in a context that is as close as possible to the clinical condition. Since intracecal tumors were much closer to clinical tumors than *s.c* tumors from the view of the histology of tumor growth or metastasis, this system was applicable to the evaluation of the tumor growth inhibitory effect by BAM. The present study demonstrated that murine adenocarcinoma CT-26 can successfully, using the orthotopic implantation technique, produce an aggressive tumor which retained the morphological biological characteristics of the donor tumor and metastasized to the mesenteric glands. BAM inhibited tumor growth on CT-26 implanted into the cecum and *s.c* more than 5-FU and MMC at the equitoxic dose. Moreover, the inhibitory effect of BAM on the growth of CT-26 tumor was higher at the cecum than at the *s.c* site in mice, which implicates that BAM may have the organ-specific effect. Organ-specific differences in the chemosensitivity of tumor cells have been reported by a few authors. Staroselsky et al have reported that a murine fibrosarcoma growing s.c in syngeneic mice is more sensitive to DXR than the same tumor growing as lung metastases[24]. Pratesi *et al* investigated the antitumor efficacy of flavone acetic acid against human ovarian carcinoma cells xenografted into different organ sites in nude mice, while tumors in the liver and subcutis were sensitive to the flavone, and ascites and lung tumors were resistant[22].

Since current clinical chemotherapy of colorectal cancer generally gives poor results, the finding of the present study is of interest with respect to the growth inhibiting activity of BAM against human colon carcinoma xenograft and murine colon carcinoma. However, further and extensive studies are necessary to confirm this finding and to evaluate the actual antineoplastic effectiveness of BAM against colon carcinomas.

REFERENCES

1 Xu HZ, Dai LH, Zhang HY, Zhao GY, Zhang XR, Zhang GS. Zhengguangmycin A6 and its status in the complex of Zhengguangmycins. *Yaoxue Xuebao*, 1988;23:667-671
2 Xu HZ, Zhang HY. The isolation and identification of pingyangmycin. *Yaoxue Xuebao*, 1980;15:609-614
3 Li DD, Lin FT, Liu HT, Xue SB. Antimicrobial activity of various bleomycin components and their effects on transplantable esophageal cancer in mice. *Kangshengsu*, 1981;6:41-45
4 Zhen YS, Li DD, Lin FT, Li X, Tian PY, Xue YC, Yang XP. Study on antitumor effect and toxicity of bleomycin A5 and bleomycin A2. *Yaoxue Xuebao*,1979;14:83-89
5 Zhen YS, Zhang ZH, Wu SY, Huang J. Inhibitory effect of pingyangmycin on human nasopharyngeal cancer transplanted in nude mice. *Zhongguo Yixue Kexueyuan Xuebao*, 1986;8:51-53
6 Jiang M,Zhen YS.Inhibitory effect of pingyangmycin on human liver cancer and stomach cancer xenografts in nude mice. *Zhonghua Zhongliu Zazhi*, 1988;10:357-360
7 Deng YC, Zhen YS, Zheng S, Xue YC. Antitumor Activity of Pingyangmycin against Colorectal Cancer. *Zhongguo Zhongliu Linchuang*, 1999;26:759-771
8 Zhengguangmycin Coordinating Group. Therapeutic effect of pingyangmycin on Malignant tumors. *Zhonghua Zhongliu Zazhi*, 1979;1:172-175
9 Zhang H, Zhen YS. Pingyangmycin shows no inhibition on the restoration of depressed hemopoiesis and immune function in mice treated with cyclophosphamide. *Proc CAMS & PUMC*, 1987;2:202-206
10 Lin FT, Song KG, Xue YC, Zhen YS. Preclinical pharmacologic evaluation of bleomycin A6. *Zhonghua Zhongliu Zazhi*, 1989; 11:257-259
11 Liu JG, Jiang M, Xu LN, Zhen YS. Minocycline potentiates the antimetastatic effect of boanmycin. *Yaoxue Xuebao*, 1995;30: 668-673
12 Deng YC, Zhen YS, Zheng S, Xue YC. Inhibitory effect of bleomycin A6 on human colon cancer xenografts in nude mice. *Zhongguo Yixue Kexueyuan Xuebao*, 1990;12:335-340
13 Jiang M, Zhen YS. Antitumor activity of bleomycin A6 against human liver cancer in cell culture and in nude mice. *Yaoxue Xuebao*, 1987;22:881-885
14 Li DD, Hu JS, Yu B. Studies on antitumor effect of bleomycin A6. *Kangshengsu*, 1985;10:312-315
15 Peng Z, Zhen YS. Experimental studies on the antitumor activity of monoclonal antibody-bleomycin A6 conjugate against human liver cancer. *Yaoxue Xuebao*, 1991;26:331-335
16 Deng YC, Zhen YS, Zheng S, Jiang M. Experimental studies on the rapeutic effect of rat monoclonal antibody-bleomycin A6 conjugate against human colorectal cancer. *Yaoxue Xuebao*, 1993; 28:410-415
17 Zhen YS, Peng Z, Deng YC, Xu HZ, Chen YX, Tian PY, Li DD, Jiang M.Antitumor activity of immunoconjugates composed of boanmycin and monoclonal antibody. *Chin Med Sci J*, 1994;9: 75-80
18 Zhen YS, Chen YX, Yu B, Zhang H, Wu SY, Du P. Cytotoxicity of the monoclonal antibody anti-CCT2 and bleomycin A6 conjugate to leukemia cells. *Zhongguo Yixue Kexueyuan Xuebao*, 1986;8: 272-275
19 Tian PY, Zhang ML, Huang J, Yu B, Zhen YS. Specific binding and internalization of anti-CCT2 monoclonal antibody and bleomycin A6 conjugate in human leukemia cells. *Yaoxue Xuebao*, 1989;24:16-21
20 Tian PY, Huang J, Zhen YS. An electron-microscopic study on pulmonary toxicity of bleomycin A6 in mice. *Zhongguo Yaolixue Yu Dulixue Zazhi*, 1990;4:221-223
21 Feng FY, Zhou LQ, Man JL, Liu H, Sun Y, Tang ZQ. Phase I clinical study of a new anticancer drug boanmycin. *Zhongguo Yixue Kexueyuan Xuebao*, 1996;18:143-146
22 Pratesi G, Manzotti C, Tortoreto M, Audisio RA, Zunino F. Differential efficacy of flavone acetic against liver versus lung metastases in a human tumour xenograft. *Br J Cancer*, 1991;63:71-74
23 Fidler IJ, Wilmanns C, Staroselsky A, Radinsky R, Dong Z, Fan D. Modulation of tumor cell response to chemotherapy by the organ environment. *Cancer Met Rev*, 1994;13:209-222
24 Staroselsky AN, Fan D, O Brian CA, Bucana CD, Gupta KP, Fidler IJ. Site-dependent differences in response of the UV-2237 murine fibrosarcoma to systemic therapy with adriamycin. *Cancer Res*, 1990;15:7775-7780
25 Rho YS, Lee KT, Jung JC, Yoon C, An Z, Hoffman RM, Chang SG. Efficacy of new platinum analog DPPE in an orthotopic nude mouse model of human colon cancer. *Anticancer Res*, 1999;19: 157-161
26 Sun FX, Sasson AR, Jiang P, An Z, Gamagami R, Li L, Moossa AR, Hoffman RM. An ultra-metastatic model of human colon cancer in nude mice. *Clin Exp Metastasis*, 1999;17:41-48
27 Garcia-Olmo D, Garcia-Rivas M, Garcia-Olmo DC, Atienzar M. Orthotopic implantation of colon carcinoma cells provides an experimental model in the rat that replicates the regional spreading pattern of human colorectal cancer. *Cancer Lett*, 1998;132: 127-133
28 An Z, Jiang P, Wang X, Moossa AR, Hoffman RM. Development of a high metastatic orthotopic model of human renal cell carcinoma in nude mice: benefits of fragment implantation compared to cell-suspension injection. *Clin Exp Metastasis*, 1999; 17: 265-270
29 Pocard M, Muleris M, Hamelin R, Salmon RJ, Dutrillaux B, Poupon MF. Growth dependency of human colon cancer xenograft on organ environment is related with their original clinical stage. *Anticancer Res*, 1998;18:2743-2747
30 Liu QZ, Tuo CW, Wang B, Wu BQ, Zhang YH. Liver metastasis models of human colorectal carcinoma established in nude mice by orthotopic transplantation and their biologic characteristic. *World J Gastroenterol*, 1998;4:409-411
31 Thirion P, Wolmark N, Haddad E, Buyse M, Piedbois P. Survival impact of chemotherapy in patients with colorectal metastases confined to the liver: a re-analysis of 1458 non-operable patients randomised in 22 trials and 4 meta-analyses. Meta-Analysis Group in Cancer. *Ann Oncol*, 1999;10:1317-1320
32 Van Halteren HK, Roumen RM, Coebergh JW, Croiset van Uchelen FA, Keuning JJ, Vreugdenhil G. The impact of 5-FU-based bolus chemotherapy on survival in patients with advanced colorectal cancer. *Anticancer Res*, 1999;19:3447-3449
33 Kokudo N, Seki M, Ohta H, Azekura K, Ueno M, Sato T, Moroguchi A,Matsubara T, Takahashi T, Nakajima T, Aiba K. Effects of systemic and regional chemotherapy after hepatic resection for colorectal metastases. *Ann Surg Oncol*, 1998;5:706-712
34 Seitz JF, Perrier H, Giovannini M, Capodano G, Bernardini D, Bardou VJ. 5-Fluorouracil, high-dose folinic acid and mitomycin C combination chemotherapy in previously treated patients with advanced colorectal carcinoma. *J Chemother*, 1998;10:258-265
35 Li XF,Wang GX, Chen J, Hong BZ. Effect of regional antineoplastics in fusion on local recurrence and hepatic metastasis after colon carcinoma resection. *Shijie Huaren Xiaohua Zazhi*, 1999;7:958-959
36 Lu XP, Li BJ, Chen SL, Lu B, Jiang NY. Effect of chemotherapy or targeting chemotherapy on apoptosis of colorectal carcinoma. *Shijie Huaren Xiaohua Zazhi*, 1999;7:332-334
37 de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Le Bail N, Louvet C,Hendler D, de Braud F, Wilson C, Morvan F, Bonetti A. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol*, 2000;18:2938-2947
38 de Gramont A, Louvet C, Andre T, Tournigand C, Krulik M. A review of GERCOD trials of bimonthly leucovorin plus 5-fluorouracil 48-h continuous infusion in advanced colorectal cancer: evolution of a regimen. Groupe d Etude et de Recherche sur les Cancers de l Ovaire et Digestifs (GERCOD). *Eur J Cancer*, 1998;34: 619-626
39 Seitz JF, Perrier H, Giovannini M, Capodano G, Bernardini D, Bardou VJ. 5-Fluorouracil, high-dose folinic acid and mitomycin C combination chemotherapy in previously treated patients with advanced colorectal carcinoma. *J Chemother*, 1998;10:258-265
40 Kokudo N, Seki M, Ohta H, Azekura K, Ueno M, Sato T, Moroguchi A, Matsubara T,Takahashi T, Nakajima T, Aiba K. Effects of systemic and regional chemotherapy after hepatic resection for colorectal metastases. *Ann Surg Oncol*, 1998;5:706-712
41 Hartmann JT, Harstrick A, Daikeler T, Kollmannsberger C, Muller C,Seeber S, Kanz L, Bokemeyer C. Phase II study of continuous 120 h infusion of mitomycin C as salvage chemotherapy in patients with progressive or rapidly recurrent colorectal cancer. *Anticancer Drugs*, 1998;9:427-431
42 Amrein PC, Colecchi CH, Finkelstein DM, Fabian RL. Adjuvant chemotherapy in advanced head and neck cancer. *Oncologist*, 1997;2:135-141
43 de Vries N, van Zandwijk N, Pastorino U. Chemoprevention of head and neck and lung (pre) cancer. *Recent Results Cancer Res*, 1999;151:13-25
44 Yuan HY, Li Y, Yang GL, Bei DJ, Wang K. Study on the causes of local recurrence of rectal cancer after curative resection: analysis of 213 cases. *World J Gastroenterol*, 1998;4:527-529
45 Yang JH, Rao BQ, Wang Y, Tu XH, Zhang LY, Chen SH, Ou-Yang XN, Dai XH. Clinical significance of detecting the circulating cancer cells in peripheral blood from colorectal cancer. *Shijie Huaren Xiaohua Zazhi*, 2000;8:187-189
46 August DA, Ottow RT, Sugarbaker PH. Clinical perspective of human colorectal cancer metastasis. *Cancer Metastasis Rev*, 1984;3:303-324

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