EFFECTS OF PERIOPERATIVE CIMETIDINE ADMINISTRATION ON TUMOR CELL NUCLEAR MORPHOMETRY AND DNA CONTENT IN PATIENTS WITH GASTROINTESTINAL CANCER

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ABSTRACT

Objective: To explore the effects of perioperative cimetidine administration on tumor cell nuclear morphometric parameters and DNA content in patients with gastrointestinal adenocarcinoma. Methods: 49 patients with pathologically confirmed gastrointestinal adenocarcinoma were randomized into test group (n=25) and control group (n=24). The test group started oral cimetidine intake 400 mg, tid, 7-10d before operation, followed by standard curative operation. The control group did not receive cimetidine. Tumor specimens were paraffin embedded for microsection and stained with hematoxylin and eosin (HE) and Feulgen stain. Morphometric studies and DNA content of tumor nuclei were performed on IBAS Image Analyzer. Results: The tumor cell nuclear area (μm²), nuclear perimeter (μm), maximal nuclear diameter (μm) for test group/control group were 23.54 ±5.08/34.69±10.08 (P<0.001), 22.06±4.43/24.88±4.05 (P<0.05), 7.84±1.64/ 8.62±1.24 (P<0.05), 4.42±0.61/5.41±0.89 (P<0.001), Respectively. The percentages (%) of diploidy, triple-tetraploidy, quintuple ploidy, and >quintuple ploidy tumor cells for test group/control group were 16.64±2.58/5.33±2.14 (P<0.002), 39.84±2.28/35.70±3.58 (P>0.50), 12.42±5.00/14.48±0.74 (P>0.20), 31.11±6.86/ 45.97±3.82 (P<0.005), respectively. Conclusion: Perioperative administration of cimetidine in gasgtrointestinal cancer patients could decrease the nuclear size and raise the percentage of diploid tumor cells, and convert high aneuploid tumor cells into low-aneuploid tumor cells, which might help reduce the invasiveness of tumor cells.

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Cimetidine is a well-known histamine type 2 receptor antagonist widely used to treat peptic ulcer. Recent clinical trials and case reports also have confirmed its antitumor effect. Colorectal cancer patients treated with cimetidine had survival advantage over those who did not receive this drug.[1,2] *In vitro* studies have also demonstrated its inhibitory effect on colon cancer cells.[3] These effects generally have been attributed to immunomodulatory function of cimetidine.[1-3] And our previous study also supported this view.[4] Are there other mechanisms by which cimetidine deliver its tumor inhibitory effect? To explore this problem, we carried out the following clinical trial.

METERIALS AND METHODS

The study design

From September 1997 to May 1998, 125 consecutive patients with gastrointestinal (GI) cancers entered the Department of Oncology, the 2nd Affiliated Hospital of Hubei medical University. The criteria for entry into this study were: (1) primary GI cancers fit for surgery; (2) no preoperative evidence of distant metastasis; (3) no previous history of immune-impairing chronic diseases such as diabetes mellitus, systemic lupus erythematosus; (4) no history of preoperative chemotherapy, radiotherapy or immunotherapy. 49 eligible patients were recruited into this study. After giving informed consent, these patients were randomized into test (n=25) and control (n=24) groups. The former began oral cimetidine (also name Tagamet, produced by Tianjin Smith Kline & French laboratories Ltd.) intake at the dose of

400mg, tid, 7-10d before operation. Patients in the control group only received their routine treatment. All the patients in both groups underwent curative resection for cancer.

Tumor Cell Nuclear Morphometric Study and DNA Content analysis

Sections of 4-μm thick with paraffin embedded tumor specimens were made and stained by hematoxylin and eosin (HE) and Feulgen method.[5]

IBAS content studies. From each slide, stained by HE method, 100 tumor cells at the front of tumor invasion were selected randomly for the measurement of the following nuclear morphometric parameters: nuclear area (NA, μm^2), nuclear perimeter (NP, μm), maximal nuclear diameter (MAND, μm), and minimal unclear diameter (MNND, μm). On each slide stained by Feulgen method, the DNA content of 100 tumor cells at the front of tumor invasion were measured. 50 lymphocytes in the stroma on the same slide were measured at the same time and their mean value was used as normal diploid control (2C). DNA index (DI) was calculated based on the following equation: DI=(tumor nuclear optic density)/(lymphocyte nuclear optic density mean value). The cell was considered to be diploidy (2C) when DI• 1.25, triple-tetraploidy (3C-4C) when DI=1.25~2.5, quintuple ploidy (5C) when DI=2.25~2.75, and >quintuple ploidy (>5C) when DI>2.75. 3C-4C, 5C cells were regarded as low aneuploid cells and >5c cells were designated as high aneuploid cells. DNA content distribution was expressed as percentage of each ploidy. All results were expressed as mean±standard deviation ($\bar{\chi}\pm s$).

Statistical Analysis

Data from nuclear morphometric study were subjected to *t* test. Percentile data from DNA content study first underwent arcsine square root transformation and then were subjected to *t* test. The test was two-tailed with the level of significance P=0.05.

RESULTS

The Clinicopathological Features of the Patients

There was no statistically significant difference in the variables between the two groups (P>0.05) (Table 1).

Table 1. The clinicopathological features of 49 patients in the study

Item	Test group	Control group
Age (yr)		
Mean (range)	50(25-73)	53(27-78)
Sex (No. of cases)		
Male	13	16
Female	12	8
Tumor sites (No. of cases)		
Stomach	6	5
Colon	3	3
Rectum	16	16
Pathological types		
Tubular adenocarcinoma	14	12
Papillary adenocarcinoma	3	3
Villous adenocarcinoma	2	1
Signet-ring-cell carcinoma	2	3
Mucous adenocarcinoma	4	5
TNM stages (No. of cases)		
I	3	5
II	7	9
III	9	6
IV	6	4
Differentiation (No. of cases)		
Well differentiated	5	6
Moderately differentiated	8	7
Poorly differentiated	12	11

The Nuclear Morphometric Parameters

All the four parameters used to define the size of tumor nuclei were smaller in the test group than in the control group. The differences were of statistical significance in NA, NP and MNND(Table 2).

Table 2. Nuclear morphometric results of the two groups ($\bar{x}\pm s$)

Group	Number	NA(μm)	NP(μm)	MAND (μm)	MNND(μm)
Test	25	23.54±5.08	22.06±4.43	7.84±1.64	4.42±0.61
Control	24	34.69±10.08	24.88±4.05	8.62±1.24	5.41±0.89
P value		<0.001	<0.05	<0.05	<0.001

NA: nuclear area; NP: nuclear perimeter; MAND: maximal nuclear diameter, MNND: minimal nuclear diameter. *T* test

Nuclear DNA Content Distribution

From Table 3, we can see that the percentage of diploid cells was much higher in the test group than in the control group. In the test group, there was also a

tendency toward low aneuploidy from high aneuploidy. Whereas the percentage of high aneuploidy was much higher than that in the control group.

Table 3. Percentages (%) of different DNA ploidies in the two groups ($\chi \pm sd$)

Group	Number	2C	3C-4C	5C	>5C
Test	25	16.64± 2.58	39.84± 2.28	12.42± 5.00	31.11± 6.86
Control	24	5.33± 2.14	35.70± 3.58	14.48± 0.74	45.97± 3.82
P value		<0.50	>0.50	>0.20	<0.005

2C: dipleidy; 3C-4C: triplepleidy-tetrapleidy; 5C: quintuplepleidy; data first underwent are sine square root transformation and then subjected to *t* test.

DISCUSSION

Tumor Nuclear Size, DNA Content and Clinical Outcome

One of the most striking morphological characteristics of malignant tumor cell is the enlarged, deep-standed and varied nuclei. Large nuclei usually indicate active DNA, RNA and protein synthesis and rapid cell multiplication, a feature of cancer proliferation, invasion and metastasis. Study in colorectal cancer has confirmed that large nuclear size is an adverse prognostic indicator.[6] In our series, we found that large nuclei were accompanied by high DI and high percentage of aneuploid tumor cells. This finding is in agreement with that found in breast cancer by Uyterlinde.[7] Aneuploid tumor cells, especially high aneuploid ones, are usually poorly differentiated, highly proliferative, more aggressive, and less responsive to conventional chemotherapy. Microscopectrophotometric studies on gastric cancer have already confirmed that high aneuploid cancer cells had significantly higher potential for local invasion, lymphogenous and hematogenous metastasis, and shorter host survival.[8,9]

Possible New Mechanism of Cimetidene's Antitumor Effect

Cimetidine has long been observed during clinical study to possess certain adverse effect on tumor growth, leading to improved clinical outcome.[1-3] Up to now, immunomodulation has been considered as the only mechanism by which cimetidine exerts its action. The current study, however, has found that cimetidine's antitumor mechanism goes far beyond immunomodulation. Indeed, it has direct inhibitory effect on the morphology and pathophysiology of tumor cell nuclei in GI adenocarcinoma. The size of tumor cell nuclei and percentage of high aneuploid cells were reduced as a result of the treatment, implying possible reduction in invasive potential of these cells. This finding may add to the explanation of previous clinical boservations. By exactly what mechanism in terms of molecular biology does it impose such actions unclear at present. Further study in molecular biology may shed new light on this interesting topic.

In summary, the current study has gained new insight into the antitumor effect of cimetidine. It has direct inhibitory effects on tumor cell nuclei, in addition to immunomodulation actions. The finding has a very useful clinical significance. Since cimetidine is a low-cost, almost non-toxic drug, to use it as adjuvant therapy in GI cancer patients may help intervene some of the malignant biological behaviors of the tumor such as invasion and metastasis and improve clinical outcome.

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A CASE OF MAMMARY METASTASIS FROM CHORIOCARCINOMA

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A 25 years old female was hospitalized because a mass had been found in the right breast more than 5 months and progressively enlarged after delivery for 3 months. 5 months before, a bean-sized nodule found by the patient in her right breast asymptommatically. 3 months before the hospitalization, a little boy had been delivered in a natural labor. She had had intermittent postpartum vaginal bleeding, the mass enlarged gradually with a faint pain, coughing and occasional blood sputum. She had been diagnosed "mastitis" in the local county hospital but an anti-inflammation treatment produced little effect. Physical examination: General condition well. Lymph nodes of 1.5cm diameter were palpated in her right armpit. The mass in her right breast found purple blue, spherical, of 10cm diameter, firm, distended, movable and tender. A 2.5cm mass was palpated subcutaneous in her right back. Gynecological examination: no obvious nodules of metastasis in her vulva and vagina. Mild erosion found in cervix. Body of uterus was nearly the size of 50-day gestation, soft, movable, and no tenderness. A 7cm cyst was palpated in the right region of accessory, movable and no tenderness. In-patient chest X-ray film revealed shadow of multiple irregular 0.5- to 3.5-cm nodules scattering in the middle and lower fields of both lungs and mild right pleurasy. US detected an enlarged uterus with intra-uterine moderate echo in a 4.6cm×4.4cm area, and a 6.9cm×5.9cm dark area of liquid in the region of right accessory. Serum β-hCG>50ng/ml. Pathologic report of the right back mass after resection: malignant tumor in right back, probably metastasis of choriocarcinoma. Established diagnosis: Stage IV choriocarcinoma, with metastases in the lungs, right breast and right back. General chemotherapy with 5-Fu+VP-16+DDP administered for 4 cycles. Of which the first 3 cycles together with injection of 5-Fu into the right breast mass.

Result After the third cycle, the breast mass diminished completely, serum β-hCG decreased to normal. After the fourth, all supplementary examinations gave normal results except chest X-ray film revealing 1.5cm nodule of metastasis close to the right rib-diaphragm angle. Afterward the patient left hospital on her own accord with no further treatment. 1 year later, a letter follow-up found the patient alive.

Discussion One of the characteristics of choriocarcinoma is its early metastasis to anywhere of the body through the blood stream. The most common place of metastasis is the lungs, then the vagina, vulva, etc. The breasts are very rare sites of metastasis. So far, there are only 2 cases of such reports in literature. Mammary metastasis of choriocarcinoma is easy to be misdiagnosed. Especially, our case has clinical manifestations as postpartum mammary swelling and pain and local mass. It is liable to be misled to acute postpartum mastitis. However, there is no difficulty to establish a diagnosis by careful history-taking and physical examination together with measurement of serum β-hCG. Mammary metastasis of choriocarcinoma has got to its advanced stage and is frequently complicated with other metastases, even so it's very sensitive to chemotherapy. Therefore, the option of management is allied chemotherapy. The main approach is general, with local administering of drug supplemented. The chemotherapy should be started as soon as the diagnosis is established. After 3 cycles of therapy, our case has had the mammary mass of metastasis diminished completely, serum β-hCG decreased to normal, that shows that chemotherapy has definite effect on mammary metastasis of choriocarcinoma and its curing can be succeeded.

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