ESTRAMUSTINE PHOSPHATE IN THE TREATMENT OF ENDOMETRIUM CARCINOMA

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ABSTRACT

Objective: To study the clinical effects and side effects of Estramustine phosphate (EMP) on the treatment of endometrial carcinoma. Methods: Fifty-eight patients with endometrial carcinoma diagnosed in our hospital from Oct. 1996 to Feb. 1998 were randomly divided into 3 groups and clinically observed. EMP group (n=21): after oral EMP 280 mg, bid, for 21 days, surgical operation followed in one week. Radiotherapy (RT) group (n=19): surgical operation was preformed after intra-cavity irradiation with half of the standard dosage. Control group (n=18): surgical operation alone. Histopathological changes in the samples from the removed uterus were observed. Estrogen receptor/progestin receptor (ER/PR) and nuclear proliferate antigen (ki-67) index of the endometrial carcinoma tissues of the EMP group were tested by immunohistochemical methods. Results: The microscopic changes induced by irradiation were much heavier than those induced by chemotherapy. In EMP group, 5/21 cases were found with no tumor lesion in the postoperation samples, all of those 5 cases being with ER strong positive (++) and 4/5 cases well differentiated tumor before chemotherapy. In RT group, the tumor lesion was disappeared in 6/19 cases, and 5 cases of which being with the moderate differentiation. No significant difference was shown between those two groups. No any histopathological changes were seen in control group. Immunohistochemical tests revealed a significant decrease in ER staining after EMP treatment and a decrease in ki-67 index, especially for the ER positive tumors, ki-67 index reduced significantly from 49.5% before medication to 35.1% after medication ($P<0.05$). Only 5 cases in EMP group reported slight nausea and vomit at the beginning of taking medicine. No changes in body weight, blood pressure, WBC count, or liver and kidney functions were seen at all. However, some patients experienced symptoms and signs such as darkening of areola and perineum, an increase in vaginal discharge, breast discomfort, significant increase of serum E_2 level, reduction of GnH level, and rise of TG and HDL, all of which disappeared after stop the medication. Conclusion: EMP has the effect for treating endometrial carcinoma, especially for ER-positive carcinoma. Increase of estrogen level dominates the side effects with slight and tolerable degree.

Key words: Estramustine phosphate, Endometrial carcinoma, Chemotherapy

Estramustine phosphate (EMP) is a combination of estrogen and nitrogen mustard, used in the treatment of prostatic carcinoma in the past, with its anti-androgen effects of the estrogen and cytotoxic action of nitrogen mustard.[1] Recent studies show it not only has cytotoxic effect on prostatic cancer cells, but also can lead the drug to target cells, i.e., cancer cells, especially the ER positive cancer cells, by the affinity of the drug and ER, and finally produce a specific cytotoxic effect. It is suggested this drug can be used in the treatment of ER positive endometrial and mammary cancer.[2-4] Till now, clinical study on this drug is still lacking. This study is to observe the clinical effects and side effects of EMP in treating endometial carcinoma, and its relation with ER, and to determine the possibility of EMP being a new oral chemotherapeutic drug in the treatment of endometrial carcinoma by specifically destroying cancer cells.

MATERIALS AND METHODS

Patients

Fifty patients with endometrial carcinoma admitted into our hospital from Oct. 1996 to Feb. 1998 were

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randomly divided into three groups: 21 cases in EMP group, 19 cases in radiotherapy (RT) group and 18 cases in control group. All cases in EMP group had no contradictions to use estrogen such as recent myocardial infarction, cerebral vascular accident, transient cerebral ischemic attack, acute hepatitis, or thrombotic diseases. The clinical staging made by Federation of International Gynecology and Obstetrics (FIGO) was applied pre-operationally. There was no statistical difference in clinical characteristics among the three groups regarding age, obesity, blood pressure, diabetes, clinical staging and cell differentiation ($P>0.05$).

Treatment

EMP Group

Oral EMP 280 mg, twice a day for 21 days. No other anti-cancer therapy was used before or during the medication. One week after stopping the medication, a hysterectomy plus removal of bilateral appendices were performed, and the lymph notes in para-aorta or in the pelvic cavity were send for biopsy, or were cleaned. If any risk factor of the following presents after the operation, such as: (1) Stage Ib or above; (2) Moderate or poor differentiation of cancer cells; (3) cytological positive ascites; (4) Positive biopsy of the lymph notes in pelvic cavity; and (5) Positive uterine edge, ^{60}Co pelvic extraradiation on DT4000 cGY and ^{192}Ir brachytherapy with dosage of 1000 cGY at A point would be given. Progesterone was be given to all patients after the operation for 3–6 months.

RT Group:

^{192}Ir brachytherapy with dosage of 2000 cGY at A point, 500 cGY each time and twice a week, was given for four times. After an interval of 2 weeks, a hysterectomy plus removal of bilateral appendices was performed. Indications for postoperational external radiotherapy were the same as those in EMP group.

Control Group

For patients with endometrial carcinoma diagnosed after curettage, a hysterectomy plus removal of bilateral appendices was performed, and lymph nodes of the para-aorta or pelvic cavity were send for biopsy, or were cleaned.

Histopathological Changes after Treatment in EMP Group and RT Group

To define the different reactions of cancer tissues to chemotherapy, staging criteria was adopted from the book Chemotherapy for malignant tumor written by Zhiyi Zhang:[5]

Mild reaction: Characterized by retrograde changes of cancer cells;

Moderate reaction: Characterized by formation of granuloma;

Severe reaction: characterized by proliferation of fiberous tissue and scarring.

The short-term reaction after radiotherapy resembles the responses to chemotherapy, so the same staging criteria was used.

Immunohistochemical Test of ER and PR in EMP Group before and after Medication

Positive staining: ER and PR could be seem in the nuclei of cancer cells, in the form of brown, or dark brown granules or mass.

Assessment criteria: on the basis of the degree of staining:

Strongly positive (++): ≥50% of cancer cell nuclei was stained.

Weakly positive (+): <50% of cancer cell nuclei was stained

Negative (-): Failure of staining in cancer cell nuclei

Immunohistochemical Test of Ki-67 in EMP Group before and after Medication

Criteria: Ki-67 was in the form of brown, or dark brown granules or mass in the cancer cell nuclei. At least 4 high power field were observed, staining index was calculated by the number of stained nuclei per 1000 tumor cells.

Observation of Side Effects in EMP Group

Response in digestive tract: changes in the amount of food intake and body weight, nausea, and vomiting.

Routine test of peripheral blood: once a week till two weeks after the medication. Biochemical test of hepatorenal function: twice, before and after the medication, respectively.

Blood pressure: measurement once a week.

Blood-lipid spectrum: measurement for three times, i.e.: before the medication, 0, and 3–6 months after the medication, respectively.

Gonadal hormone: Measurement for three times, before the medication, 0, and 3–6 months after the medication.

The last three tests were performed to determine the possible effects of estrogen in EMP when it dissociated in the body.

Drugs and the Main Preparation

EMP: produced by LEO Pharmacy Company, Sweden, with 140 mg estramustine phosphate in each capsule. Antibody: ER, PR, Ki-67 were all ready preparations made by American ZYMED Company. Antibody titer used was 1:50. Immunohistochemical reagent boxes were provided by Zhongshan Company of China. Positive control slide were provided by Zhongshan Company. Negative control: using PBS instead of first antibody in immunohistochemical test.

Statistical Method

Statistical software in Microsoft office from Microsoft Company was used. *t* test was used for quantitative data, and chi-square for counting data.

RESULTS

Histopathological Changes

EMP Group

Histopathological changes by chemotherapy reactions in different degrees were seen in all the 21 cases taking the medication. Changes in interstitial tissue, such as infiltration of lymphocytes, proliferation of capillary vessels, formation of granulation tissues prevailed, while nuclear changes existed. Among them, 13 cases had mild changes, 7 moderate, and 1 severe. Five cases failed to find focus of carcinoma in the specimen sections postoperatively, only to see moderate to severe atypical proliferative glands were seen.

RT Group

Large area of necrosis of a strange shape, with nuclear pyknosis, and vacuoles inside cytoplasm was seen. Nuclear change was the main reaction, while interstitial change being additional. Mild change occurred in no case, while moderate in 14 cases, and severe in 5 cases. In addition, foci of cancer disappeared in 6 cases. There was large area of necrosis, but no tissue structure could be recognized under the microscope. A significant difference was seen in these two groups in terms of chemotherapeutic change grading. Change in RT group was heavier than that in EMP group ($P<0.01$). No significant difference existed in terms of the number of cases in which the tumor disappeared. However, no foci of cancer in control group disappeared, which was significantly different from the previous two groups (Table 1).

No reversion of cancer cells was seen in either EMP group or RT group.

Table 2 shows the condition of cancer disappearing cases in EMP group and RT group. No difference in age and stage were discovered. But in EMP group, most were highly differentiated cancer cases (4/5), all ER were (++) and all chemotherapeutic reactions, except 1 case being moderate, were mild. Whereas in RT group, most cases were moderately differentiated (5/6), and all chemotherapeutic reaction, except 1 case being moderate, were severe.

Relationship of Drug Mechanism with ER, PR and Ki-67

In the 21 pre-medication endometrium samples, ER positive rate was 18/21 (85.7%), PR positive rate 15/21 (71.4%), and both ER and PR positive rate 13/21 (63.3%). Mean Ki-.67 index of endometrium sample for the 21 cases before the medication was: 48.3 % for highly differentiated cases, 35.7% for moderately differentiated cases, and 70.0% for low differentiated with only one case. Seemed that Ki-67 index increased with cell grading, but no significant difference was seen among grading groups, which may due to small sample.

By comparing the endometrial ER, PR and Ki-67 results in EMP group before and after the medication, significant decrease in ER positive rate was shown after the medication ($P=0.00019$) (Table 3). It means Ki-67 index decreased from 42.3±21.5% to 38.0±27.2% with no statistical significance. However, when we grouped the EMP group by endometrial ER staining before the medication, and made a statistical analysis, different results were obtained. In ER positive cases, Ki-67 index significantly dropped from 49.5±18.1% to 35.1±26.8 % ($P<0.05$). It indicated the number of proliferative cancer cells was decreased significantly, which was related to ER (Table 4).

Side Effects

Five patients in EMP group had nausea and vomit in the early stage of taking medicine, which then disappeared without treatment. One case had abdominal pain. No abnormality in liver and kidney function, and no marrow inhibition were seen. Compared with other chemotherapy drugs, EMP had milder cytotoxic action with lower incidence, which can be tolerated by most patients, and required no special treatment.

No severe cardiovascular sign reported in literature was shown as an estrogen-related side effects. All the patients had areola and vulva pigmentation. Twenty patients (95%) had increased vaginal discharge, and 12 (57%) had mammary swelling and thalalgia. Two cases had headaches. All the disorders disappeared after the stopping of medication without any special treatment. After medication, pituitary gonadotropin decreased

significantly, while estrogen level increased very significantly, total blood-lipid increased with very significant difference, high density lipoprotain (HDL) increased significantly, while total cholesterol and low density lipoprotain (LPL) decreased with no significance after medication (Table 6).

Table 1. Histopathological changes after treatment

	EMT group	RT group	Control group
Disappearance of cancer lesion	5/21	6/19	0/18*
Radiotherapy and chemotherapy reaction*			
Mild	13	0	
Moderate	7	14	
Severe	1	5	

* $P<0.01$

Table 2. The condition of tumor disappeared cases

	Age	Stage	Grade	Chemotherapeutic reaction	ER
EMP group					
1	58	I	1	Moderate	++
2	47	I	1	Mild	++
3	51	I	2	Mild	++
4	63	I	1	Mild	++
5	47	I	1	Mild	++
RT group					
1	56	I	2	Severe	
2	66	I	2	Severe	
3	41	I	1	Severe	
4	46	I	2	Severe	
5	47	I	2	Severe	
6	60	I	2	Moderate	

Table 3. Changes in ER before and after medication

ER	Pre-medication	Post-medication
++	10	3
+	8	8
-	3	5

$P=0.00019$

DISCUSSION

The Effects of EMP in the Treatment of Endometrial Carcinoma

EMP is a combination of estrogen and nitrogen mustard, an oral chemotherapeutic drug, previously used in the treatment of prostatic cancer with good results. Experimental study showed the strong killing action on endometrial cancer cells, especially ER positive endometrial cancer cells.[3, 4] But there is still no report of any clinical study on this subject. This report is the first clinical study on EMP in the treatment of endometrial carcinoma.

In this study, 21 subjects clearly diagnosed as endometrial carcinoma received chemotherapy of EMP with a dosage of 280 mg, twice a day for 21 days. Histopathological observations were made on the removed endometrium after the medication stopped, graded according to the changes of cancer cells and interstitial reaction, and compared to the samples from RT group. Changes in different degrees could be seen in all cases. In the EMP group, interstitial reaction prevailed, shown by proliferation of capillary vessels and lymph cells infiltration, whereas in RT group, nuclear reaction was the main with large necrotic area of tumor cells. Reaction was heavier in RT group than that in EMP group with significant differences ($P<0.01$). Foci of cancer disappeared in 5 cases in EMP group. After repeated sampling from these cases by pathologist, still no focus was found. These patients were not special in such aspects as age and staging, but were strongly positive in ER and were better differentiated. Foci of cancer disappeared in 6 cases in RT group, all of whom were poorly differentiated. This indicates that the effects of

EMP are related with ER; the higher ER, the better results. And the effects of radiation are related with the differentiation of cells. The poorer the differentiation, the better the results. In our previous clinical practice, a few cases found with endometrial carcinoma after diagnostic curettage, failed to see any tumor after hysterectomy. It is thought so because curettage removed all the foci. In this study, no cancer focus in the 18 cases of control group disappeared, which was significantly different from treatment groups. It indicated that disappearance of foci in EMP Group was not an accident.

Immunohistochemical test of nuclear proliferative antibody (Ki-67) was adopted to observe the effects of the drug on cancer cells. Ki-67 mono-clone antibody exists only in the human proliferative nucleus with highly correlation to cell proliferation.[6] To understand the relation between EMP and ER, PR, immunohistochemical method was again adopted to measure ER and PR content in endometrium before and after the medication. Seen from content changes in all cases, positive ER decreased after medication with a high significance, while the Ki-67 index decreased without a statistical significance. When we subdivided EMP group by the measurement results of endometrial ER before medication, and then did statistical calculation, a different result was obtained. Ki-67 index of ER positive cancer decreased with statistical significance ($P<0.05$). Therefore, it is estimated that EMP, upon entering the body, bonds to ER in cancer cells using estrogen as a vehicle, and kills cancer cells specifically, especially proliferative cancer cells, with cytotoxic action of nitrogen mustard, which reduces proliferative ER positive cancer cells. As a result, immunohistochemical staining of ER was weakened, and the Ki-67 index decreased. So this study showed EMP are effective in the treatment of endometrial carcinoma, especially in ER positive tumor.

Table 4. Changes in Ki-67 before and after the medication (%)($\bar{x}\pm s$)

	Pre-medication	Post-medication	*P*
All cases	42.3±21.3	38.0±27.2	0.46
ER positive cases	49.5±18.1	35.1±26.8	0.046

Table 5. Changes in gonadal hormone

	Pre-medication	Post-medication
FSH (μ/L)	36.74	2.87**
LH (μ/L)	48.09	3.27*
E_2 (ng/L)	45.2	500.0**
P (μg/L)	0.66	0.87

*$P<0.05$ **$P<0.01$

Table 6. Changes in TG before and after the medication ($\bar{x}\pm s$)

	Pre-medication	Post-medication
TG	1887±1276	2565±1396**
TCH	1917±373	1667±363
HDL	303±126	412±161*
LDL	1567±584	1396±557

*$P<0.05$ **$P<0.01$

Side Effects of EMP on the Treatment of Female Patients

EMP is a combination of estrogen and nitrogen mustard. The side effects caused by cytotoxic action of nitrogen mustard and by estrogen on females were studied.

Nitrogen mustard, as an alkyl agent, has the common side effects such as marrow inhibition, gastrointestinal reaction, headache, dizziness, and fatigue. Convulsion and motor nerve paralysis may occur with a large dosage.[7] In this study, 5/21 cases had slight nausea and vomiting in the early stage after medication, but had no changes in biochemical measurements of hepatorenal function and peripheral blood routine test.

The physiological and pharmacological action of estrogen are manifested as follows: 1. It enhances the nutrition and growth of internal and external reproductive organs, stimulates mucus secretion, vulva staining, and the proliferation of endometrium, cervical glandular epithelium, and mammary gland duct; 2. By feedback, it inhibits the hypothalamus and pituitary glands from secreting gonadotropin; 3. It reduces blood cholesterol and changes blood-lipid components, increasing HDL and decreasing LDL; and 4. It results in retention of water and sodium.

In the previous treatment of prostatic cancer, the most serious side effect of EMP was cardiovascular complications, such as cardiac infarction and cerebral embolism, which were all absent in our study. However, after medication, some symptoms particular to estrogen appeared. For example, all the patients had areola and vulva pigmentation, two cases had improved symptoms of chronic vulva dystrophy, 95% of patients had their vaginal discharge increased, and 57% of patients had

breast swelling and thalalgia. It was shown that estrogen level was significantly elevated, with significantly decreased FSH after taking EMP. The blood-lipid components changed accordingly, with significantly increased HDL and TG, and decreasing trend in LDL and TCH but with no statistical significance, this may be caused by short duration of taking medicine. According to the introduction of EMP, after it entering the body, approximately 10% of estrogen will be separated from the nitrogen mustard. In this study, symptoms from the increased estrogen caused by EMP were obvious, but breast swelling, vulva and areola pigmentation would disappear 3-6 months after the stop of medication blood-lipid content could go back to the pre-medication level and gonadotropin equaled or was lower than pre-medication level.

Observation results indicate side effects of EMP in female patients were slight, and tolerable with the symptoms caused by estrogen. Previous studies showed incidence of endometrial carcinoma was related to high estrogen level, and long-term estrogen stimulation. Besides, mammary cancer also seemed to be related to high level of estrogen.[8] In addition, a large dosage of progestogen can push tumor towards benign reversion and has direct killing effects on both endometrial carcinoma and mammary cancer. Therefore, progesterone with a large dosage was given after operation and radiotherapy in EMP group, and breast examination was done in the follow-up as a routine on our patients.

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