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EXPERIMENTAL AND CLINICAL STUDY OF CONCOMITANT RADIATION THERAPY AND CHEMOTHERAPY FOR CERVICAL CARCINOMA

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The results of *in vitro* Hela cell experiment and human cervical cancer in nude mice therapeutic trial showed that combined anticancer drugs and radiation had more marked anticancer effect than radiation alone. On the bases of experiment, 25 patients with carcinoma of uterine cervix, FIGO stage Ib-IV, were chosen for our study. A weekly ^{60}Co intracavitary irradiation plus cisplatin (30 mg/M^2) and bleomycin (15 mg/M^2) were given to 11/25 cases, while the remaining 14, radiotherapy alone. The former group showed a superior response in tumor regression and histological improvement than the later one. The toxic side effect was acceptable. The results indicate that concomitant radiation therapy and chemotherapy may be benefit for treating cervical carcinoma.

Key words: Cervical cancer, Radiotherapy, Chemotherapy Hela cell, Nude mice.

Carcinoma of the cervix is one of the most common malignancies of women in China. The results for patients with advanced disease are still poor. According to FIGO reports (1988),[1] the 5-year survival rates of stage IIb, III and IV were 50–70%, 30–50% and 10–15%, respectively. The failure of radiotherapy to control the advanced cervical carcinoma may related to local-regional recurrences and distant metastasis. Attempts to improve the results of radiotherapy by increasing the radiation dose have been hampered by radiation sensitivity of the normal tissue. Hyperbaric oxygen and radiosensitising drugs have been used to increase the effectiveness of radiation. However, the results were unsatisfied and neither of these techniques showed sufficient advantage over conventional radiotherapy to warrant widespread use. So it would seem logical to combine radiotherapy with some antineoplastic modality to increase tumor control. The status of chemotherapy for cervical carcinoma is controversial.[2, 3] Although there is increasing clinical opinion that chemotherapy may have a role to play in the treatment of cervical carcinoma,[4] it still remained unsolved whether chemotherapy is really effective on cervical cancer, what is the optimal regimen of combined radiotherapy with chemotherapy, and which are the appropriate drugs to cervical cancer. Cisplatin is one of the most common chemotherapeutic agents used in squamous cell carcinoma and the response rate was reported as 50%. The present study was designed to investigate the initial response of combination therapy

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(radiotherapy immediately followed by chemotherapy) in cervical carcinoma, which based on *in vitro* Hela cell experiment and nude mice therapeutic trial. Further studies on long-term tumor control and survival are proceeding.

MATERIALS AND METHODS

Hela Cell Experiment

Cell Culture

Hela cells were provided by Shanghai Cancer Institute. The lines grew as monolayer cultures in RPMI 1640 Medium supplemented with 20% fetal calf serum and an appropriate amount of penicillin and streptomycin in a 5% CO_2 incubater at 37°C.

Drugs

Cisplatin (DDP) and Bleomycin (BLM), dissolved in 0.9% saline before use.

Irradiation

^{60}Co γ-ray irradiation, with dose rate of 86 cGY/min and FSD of 60 cm.

Assay for Cell Survival

According to preliminary experiment, we chose drug concentrations of 0.1 μg/ml and radiation dose of 400 cGy in this experiment. Drugs were given 1 h before or after radiation. Viable cells were counted by trypan blue dyeing method at 24 and 48 h after radiation.

Nude Mice Therapeutic Experiment

*Animal*BALB/C nude mice was from Shanghai Biologic Products Institute, at the age of 8–10 weeks, under SPF conditions (temperature 25°C, humidity 60%).

Tumor

The tumor tissue was from a 54-year-old female patient with squamous cell carcinoma of the cervix stage III, histological grade II. Two perpendicular diameters of the tumor were measured with graduated calipers at regular time intervals. The tumor volume was estimated according to this formula:

$$V = \frac{\text{length} \times \text{width}^2 \times \pi}{6}$$

Drugs

DDP 3 mg/kg and BLM 10 mg/kg, dissolved in 0.9% saline, injected intraparitoneally immediately and then 24 h after radiation.

Irradiation

^{60}Co irradiation, with dose rate of 100 cGY/min and FSD of 75 cm. A single dose of 800 cGY was given.

Clinical Trial

Patients and Methods

A total of 25 patients with cervical carcinoma stage Ib-IV were entered our study. Among them twenty-two were histologically confirmed squamous cell carcinoma and three were adenocarcinoma. The median age was 62 years (range, 35–77 years). High dose rate ^{60}Co intracavitary irradiation (dose rate:30 cGY/min) was given with the tumor dose of A point: 800 cGY each time. Drugs were given immediately after radiation with DDP 30 mg/M^2 and BLM 15 mg/M^2 infused intravenously. The treatment was applied once a week. The control group received intracavitary radiation only.

Pretreatment Evaluation

The disease was staged according to FIGO criteria. Investigations included a complete blood picture, renal

and liver function, chest X-ray, ECG, tumor size in two dimensions, tumor histological differentiation and flow cytometry measurement (FCM).

Response

The response to therapy was evaluated by tumor regression, histological improvement and FCM measurement at the 7th day after each treatment. Tumor volume was estimated by this formula: length × wide^2 × $\pi/6$. Histological alterations were evaluated by Shimosato's criteria[5] (shown in Table 1). FCM measurement was made by a routine procedure in the FCM section of our hospital.[6] DNA content was expressed by DNA Index (DI), which defined as the ratio of tumor G1 DNA content to that of human diploid cells.

Statistical Analysis

The medical statistic (t-test and/or x^2-test) software (SPSS/PC) was used to analyze the data.

Table 1. Histologic criteria for evaluation of therapeutic effects

Grade I	Characteristic changes are noted in tumor cells but tumor structures have not been destroyed (there is not defect in tumor nests resulted from lysis of individual tumor cells)
Grade II	In addition to characteristic cellular changes, tumor structures have been destroyed as a result of disappearance of tumor cells. However, variable number of "viable cells" still remain
	A Destruction of tumor structures is of mild degree, i.e., "viable tumor cells" are frequently observed
	B Destruction of tumor structures is of severe degree, that is, "viable tumor cells" are few in number
Grade III	Markedly altered, presumably no viable tumor cells are present singly or in small clusters and "viable cells" are hardly seen
Grade IV	No tumor cells remain in any of sections (local cure)

RESULTS

In cell experiment, the drugs given after rather than before radiation had a more marked inhibiting effect, and radiation with both cisplatin and bleomycin was more effective than with either one of the drugs and radiation alone, as shown in Table 2.

In animal experiment, the mean growth delay time of the xenografts was much longer in the group receiving combined radiation with cisplatin and bleomycin chemotherapy than in any other groups, as shown in Table 3. In clinical trial, patients receiving combined radiotherapy and cisplatin-bleomycin chemotherapy had a superior response in tumor regression (shown in Table2).

Table 2. Inhibiting effect of combined radiation with drugs on Hela cells

Group	Cell number (10^4/ml) 48 h after radiation
1. Radiation	73.92± 1.13
2. DDP+BLM radiation	33.68± 1.48*#
3. Radiation +DDP+BLM	16.88± 0.66*#
4. DDP after radiation	49.30± 1.20*
5. BLM after radiation	40.39± 0.54

*Group 1 vs group 2, 3, 4, 5, $P<0.05$.

#Group 2 vs group 3, $P< 0.05$.

Table 3. Mean growth delay time of xenografts after treatment

Group	Tumor	Growth delay Time (day)	Mean growth Delay time
1. Radiation	1	14	13.0
	2	12	
	3	13	
	4	13	
	5	13	
2. Radiation	6	15	15.8*
+DDP	7	15	
	8	16	
	9	17	
	10	16	
3. Radiation	11	12	12.3
+BLM	12	12	
	13	12	
	14	13	
4. Radiation	15	18	19.0**
	16	19	
+DDP+BLM	17	19	
	18	20	

*Group 1 vs group 2, $P<0.01$

**Group 1 vs group 4, $P<0.001$

Table 4), and the histopathological improvement was also more obvious in patients having combined radiotherapy and chemotherapy (shown in Table 5). By flow cytometry measurement, a decline of DNA content in aneuploid tumor was observed (shown in Table 6), but the difference of changes on cell progression of various phase in the cell cycle after the treatment was not found between the two groups, probably the biopsy was obtained too late in terms of changes on cell progression of various phases in the cell cycle.

Only one patient had a moderate nausea and vomiting, which was controlled by antiemetics. No patient developed severe or life threatening hematologic or renal toxicity.

DISCUSSION

As the survival of patients with advanced cervical carcinoma has remained unchanged over the past few decades, new and innovative approaches are needed. The concept of using systemic chemotherapy is appealing as it might be useful in reducing the tumor bulk with resultant improved tissue perfusion, controlling the metastatic foci and allowing greater radiosensitivity. A combination of chemotherapy and radiotherapy might thus lead to better survival rate of advanced cervical carcinoma. Cisplatin is fairly active as a single agent with response rate as high as 50%. Combination of cisplatin with bleomycin and vincristin (VBP) has achieved a better response in some patients with advanced cervical cancer.[2, 7] Kim[8] reported a overall response rate of 89% using the regimen to 35 cases with cervical cancer.

According to documents and our previous clinical practice we initiated this experimental and clinical study using cisplatin and bleomycin chemotherapy combined with radiation. The results revealed that the drugs given after rather than before radiation had a more marked inhibiting effect on Hela cells, radiation with both cisplatin and bleomycin seemed more effective than with either one of the drugs or radiation alone, and the growth delay time of transplanted tumor on nude mice was much longer again in the group receiving

Table 4. Tumor regression percentage after treatment

Group	No. of cases	R1	R2
Radiotherapy	14	0.48± 0.25	0.77± 0.19
Radio-chemotherapy	11	0.72± 0.20	0.93± 0.07
P		<0.05	<0.05

R1: Tumor regression after first course of treatment. R2: Tumor regression after second course of treatment.

Table 5. Histologic improvement after second course of treatment

Group	Grade I	Grade II	Grade III	Grade IV
Radiotherapy	0	9	5	0
Radio-chemotherapy	0	4	2	5
P		<0.05		

Table 6. Alteration of DNA index after treatment

	Radiotherapy	Radio-chemotherapy
Pre-treatment	1.12± 0.27	1.12± 0.23
After 1 course of treatment	1.07± 0.17	1.01± 0.07
After 2 course of treatment	1.03± 0.07	1.03± 0.06
P	>0.05	>0.05

combined radiation with cisplatin and bleomycin chemotherapy than in any other groups. These suggested that combined anti-cancer drugs with radiation had a more effective inhibition on tumor growth than radiation alone in laboratory, and drug used immediately after radiation is better than used before. Based on the above results, we compared radio-chemotherapy with radiotherapy alone on 25 patients of cervical cancer, the initial response showed that tumor size reduction and histopathological improvement was more obvious in radiochemotherapy group, the difference was significant. These findings suggested that the simultaneous administration of radiotherapy and cisplatin-bleomycin chemotherapy is an effective regimen in cervical carcinoma.

The mechanism for this is not well explained. We considered that chemotherapy given immediately after radiation may inhibit the repair of sublethal damage and potentially lethal damage following radia-tion;[9] moreover, radiation affects mostly on M phase cells in the cell cycle, while anticancer drugs, S phase cells. The combination of these two might be synergetic. More detailed mechanisms would be revealed by further *in vivo* and *in vitro* researches.

The other important effect of chemotherapy is to inhibit or kill the metastatic foci. Kim (1989) reported a series of 54 cases of cervical carcinoma receiving preoperative chemotherapy.[7] The incidence of lymph node metastasis remaining in the operative specimen was 20%, lower than those previous reported as high as 40%.[10] When lymph node metastasis was correlated with the response of primary tumor to preoperative chemotherapy, all nodal metastasis was found in the group of patients who had Grade I or II response and none was found in the group with Grade III or IV.[7] This suggested that chemotherapy may decrease the number of patients with positive nodes. In our department we also found that radiotherapy with cisplatin had a better effect than radiotherapy alone on cervical cancer patients with left supraclavicular lymph node metastasis. These findings suggested that chemotherapy might be able to eliminate effectively diseases in lymph node and possibly micrometastases.

Although the initial local response to treatment is favorable in these patients, the prediction of long-term advantages with this mode of treatment cannot be made until a follow-up of at least five years is completed.

There are many anticancer drugs used in cervical cancer patients. The effective ones are Cyclophosphamide, 5-Fluorouracil, Mitomycin, Doxorubin, Methotrexate, Bleomycin and Cisplatin, etc. Cisplatin is one of the most active agents with response rate of 50%. Combination chemotherapy are usually based on Cisplatin, Bleomycin and Doxorubin. VBP regimen (Vincristine, Bleomycin and Cisplatin) is reported as more effective one in cervical carcinoma, and the

cisplatin-bleomycin chemotherapy used in this experimental and clinical study also showed more effective than radiotherapy alone.

The mechanism of action of cisplatin and bleomycin is different, so is the toxicity. Thus the combination of these two drugs may strengthen the anticancer effect but not the toxic side effect. Cisplatin is known to bind directly to DNA to form DNA cross-links and to inhibit cell mitosis; while bleomycin, to inhibit DNA synthesis, to produce scission of single stranded DNA and to inhibit cell DNA repair by marked inhibition of DNA ligase. The toxic side effects of the former are nausea, vomiting, myelo-suppression, cytotoxicity and nephrotoxicity. Bleo-mycin has mild gastrointestinal side effect and almost no myelosuppression. Its main toxicities are pulmo-nary toxicity and allergic reaction. The use of cisplatin and bleomycin with radiotherapy in this study does not appear to have increased side effect. The cisplatin analogue, carboplatin, which offers the potential of equal efficacy with reduced toxicity, now has been used. If it replaces cisplatin in this regimen, the results might probably be more satisfied.

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