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THE EFFECT OF HYPO - OSMOLAR SOLUTIONS WITH HIBITANE ON SHED CANCER CELLS IN PERITONEAL CAVITY OF PATIENTS WITH GASTRIC CANCER

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For 100 patients with gastric cancer, intraoperative intraperitoneal perfusion (IOIPP) using double - distilled water(D. D. W) with hibitane 37℃ for 5 minutes was performed. The anticancer effects were assessed cytologically in pre - IOIPP and post-IOIPP specimens of the abdominal effusion and/or lavaged peritoneal fluid. The radical gastrectomy was performed in 65 of 93 cases with advanced gastric cancer, shed cancer cells in peritoneal cavity were detected in 16 of 65 cases with pre - IOIPP (24.62%) and in 3 of post - IOIPP cases (4.62%). There was significant difference between the two groups ($P < 0.01$). Shed cancer cells were inactivated *in vivo* estimated by the trypan - blue staining technique in 7 cases with radical surgery. But the effect of the IOIPP on shed cancer cells was not significant in cases with peritoneal metastasis. Also there were shed cancer cells in peritoneal cavity of patients with SS cancer histologically before the IOIPP (15.56%) and iatrogenic diffusion of cancer cells during operation (7/24) in thisarticle. The above results indicated that the IOIPP with hypo - osmolar solution containing hibitane, 37℃ for 5 min was indispensable and useful for the killing of peritoneal shed cancer cells in order to prevent postoperative peritoneal recurrence in patients with radical surgery of advanced gastric cancer regardless of conditions of serosal invasion.

Key words: Stomach neoplasm/drugs therapy, Shed cancer cells, Hibitane, Hypo - osmolar solutions, Intraperitoneal perfusion.

In most patients, peritoneal seeding of shed cancer cells and local recurrence from gastric cancer is an irreversible fatal process and peritoneal carcinomatosis is the most common postoperative recurrence of gastric cancer with radical resection, which is one of the key factors affecting treatment effects of advanced gastric cancer.[1-3] The prevention of peritoneal seeding and the treatment of subclinical foci in peritoneal cavity have been known as the key subject of gastric cancer surgery in recent years, to which many authors have paid more attention.[4,5] For that, we had already reported the killing effect of hypo-osmolar solution with surfactant and hyperthemia on a gastric cancer cell line MGC - 803 cultrued *in vitro*.[6,7] In this paper, it was reported that lethal effect of IOIPP with double distilled water containing hibitane on shed

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cancer cells in peritoneal cavity of patients with gastric cancer and preliminary evaluation of IOIPP with double distilled water containing hibitane has been done based on cytology results.

MATERIALS AND METHODS

Patients

One hundred patients with gastric cancer were randomly selected in our instituute from 1990 to 1993, including 7 cases of early gastric cancer, 5 cases of middle stage cancer (mp) and 88 cases of advanced cancer. Gastrectomy was performed in 93 cases, including 72 cases of radical surgery (early cancer 7 cases, advanced cancer 65 cases) and 21 cases of palliative resection. Three cases underwent gastroenterostomy and four cases underwent laparotomy.

Methods of IOIPP and Sample Collection

After the abdominal cavity was opened, 60 ml normal saline was perfused into Douglas pouch, lavage was aspirated as much as possible after stirring slightly. Sometime abdominal effusion was withdrawn directly, the speciments of lavage or effusion were separated by centrifugation at 2500 rpm for 10 minutes, then smears of sediments were prepared. Impression slides of serosa overlying the gastric cancer had been carried out for all the cases (3—4 slides per case).

4000 ml double-distilled water containing hibitane (0.01%) at 37℃ had been perfused into the peritoneal cavity for 5 min before closure of the abdomen, then amears and centrifugation of lavage form Douglas pouch were done as speciments of pre - IOIPP cases did.

Cellular layer of speciments in pre - IOIPP and post-IOIPP cases were collected after centrifugation in order to estimate the viability of shed cancer cells in 65 cases with radical surgery of gastric cancer.

Staining and Pathology Procedures

The above smears and impression slides were stained by the Papanicolaou and H. E methods for all the cases. Shed cancer cells were identificated according to Papanicolaou's criteria on cytological grades. The viability of shed cancer cells was estimated by the trypan-blue staining technique. Lastly, systematic pathological examination was made by professional pathologists at our institute after operation.

Observation of Side - effects

The examinations of peripheral blood routine, routine urine, liver and kidney function were carried out from the first postoperative day to the day when the above examination were normal.

All data were statistically compared between pre - IOIPP and post - IOIPP groups by the chi - square test.

RESULTS

The Changes of Shed Cancer Cells in Peritoneal Cavity Before and After the IOIPP

The positive rate of peritoneal shed cancer cells in this series was 39% in pre - IOIPP group, 24% in post - IOIPP group. There was significant difference between the two groups ($P < 0.05$). In cases with radical resection, the positive rate of shed cancer cells was 22.22% in pre - IOIPP group, 4.17% in post - IOIPP group. Also there was statistically significant difference ($P < 0.01$). The killing effect of the IOIPP on peritoneal shed cancer cells was more significant in radical resection group than in non - radical surgery group ($P < 0.05$). In post - IOIPP group, there were shed cancer cells in 7 cases with negative shed cancer cells during operation (Table 1 and Table 2).

In patients with radical resection of advanced cancer, the shed cancer cells were inactivated that was estimated by trypan - blue staining technique in cases with shed cancer cells after the IOIPP. The cytologic changes included nuclear pyknosis and

karyolysis in addition to breakage of cells as reported previously by us.[6,7]

Table 1. The changes of shed cancer cells before and after IOIPP

Operation	No. of cases	No. of positive cases of shed cancer cells		
		Pre-IOIPP	Post-IOIPP	Pre- and Post-IOIPP
Radical	72	16	7	3
Palliative	21	16	10	7
Gastroenterostomy	3	3	3	3
Laparotomy	4	4	4	4
Total	100	39	24	17

Table 2. The changes of shed cancer cells in different operation groups with the IOIPP

Operation	No. of cases	No. of positive shed cancer cells (cases)			
		Pre-IOIPP (%)		Post-IOIPP (%)	
Radical	72	16	(22.22)	3	(4.17)*
Non-radical	28	23	(82.14)	14	(50.00)**

Note: *$P < 0.01$ **$P < 0.05$

The Changes of Shed Cancer Cells in Peritoneal Cavity in Different Depth of Gastric Cancer Before and After the IOIPP

Of 39 cases with positive shed cancer cells in advanced gastric cancer before the IOIPP, 22 cases were negative with shed cancer cells after the IOIPP. There was significant difference between the two groups ($P < 0.01$).

In this paper, there were 45 cases without serosal invasion histologically (S_0). Shed cancer cells in abdominal cavity were positive in 7 of 45 cases before the IOIPP (15.56%). The invasion depth of gastric cancer cells was subserosa (SS) in all 7 cases. Cancer cells of serosal impressions were positive in one of 7 cases and growth pattern of cancer cells was infiltrative in 4 of 7 cases. The rate and degree of lymph node metastasis were 100% and 73.02% (46/63). Coat of lymph nodes was infiltrated and penetrated in 3 of 7 cases. There was significant difference on the changes of shed cancer cells between S_0 and S_2 groups before and after the IOIPP ($P < 0.01$) (Table 3).

Shed Cancer Cells, Peritoneal Metastasis and the Effects of the IOIPP

Of 100 cases, peritoneal metastasis was positive in 17 cases, negative in other 83 cases. The killing effect of the IOIPP on shed cancer cells was more obvious in negative group than in positive group ($P < 0.01$) (Table 4).

Side Effects

The main side effects of the IOIPP using D.D.W with Hibitane observed in this paper as follows: (1) Microscopic hematuria for about 3 days (12 cases). (2) Hepatic dysfunction, i.e. the value of ALT was slightly higher than normal value for weeks (2 cases). There was no other side effects with the IOIPP in this paper

Table 3. The effect of the IOIPP on peritoneal shed cancer cells in different serosal invasion groups

Serosal Invasion	The IOIPP	No. of cases	Serosal impressions n*	Serosal impressions %	Shed cancer cells n*	Shed cancer cells %	P
Negative (S_0)	Before	45	1	2.22	7	15.56	<0.01
	After	45			2	4.65	
Positive (S_2)	Before	48	36	75.00	25	52.08	<0.01
	After	48			8	16.67	

Note: * Number of cases

Table 4. The effect of the IOIPP on shed cancer cells in positive and negative peritoneal metastasis groups

Peritoneal metastasis	No. of cases	Positive peritoneal shed cancer cells: No. of pre-IOIPP	(%)	No. of post-IOIPP	(%)
Positive	17	13	(76.47)	11	(64.71)*
Negative	83	26	(31.33)	6	(7.23)**

Note: * $P > 0.05$ ** $P < 0.01$

DISCUSSION

There Are the Killing Effect of the IOIPP Using Hypo-osmolar Solution with Hibitane on Peritoneal Shed Cancer Cells *in vivo*.

In our previous studies, we had already reported that Hibitane could enhance and accelerate immediate lethal effect of D.D.W on gastric cancer cell line MGC-803 cultured *in vitro* at 37℃, the mechanism of lethal effect of surfactant on cancer cells in our research was the samd as that reported by Aránzazu.[7,8] In patients with radical surgery of advanced gastric cancer, peritoneal shed cancer cells were positive in 16 cases before the IOIPP, 3 cases after the IOIPP. The lethal effect of the IOIPP using D. D. W with Hibitane on peritoneal shed cancer cells was more obvious in radical surgery group than in non-radical surgery group ($P < 0.05$). The changes of morphology and viability of shed cancer cells in cases with radical resection suggested that there were the lethal effect of the IOIPP using hypo-osmolar solution with Hibitane on peritoneal shed cancer cells *in vivo* similar to the results reported *in vitro*.[6,7] So the authors proposed that the synergistic effect of killing and washing of IOIPP using D. D. W with Hibitane on peritoneal cancer cells, 37℃ for 5 min was indispensable and useful for the prophylaxis of postoperative peritoneal recurrence in patients with advanced gastric cancer after radical resection.

Some Problems on Application of the IOIPP Using D.D.W with Hibitane

Firstly, what is the indication of the IOIPP? In our research, shed cancer cells were positive in 7 cases without serosal invasion (SS) before the IOIPP, the probable reasons as follows: (1) The area of the penetrated serosa was not taken, i.e. misdiagnosis of pathology. Because cancer cells of scrosa impressions were positive in one of 7 cases and there was infiltrative growth pattern in 4 of 6 cases with SS. Also in cases without serosal invasion

(SS), there was the probability of "spotty penetration of serosa" due to the effect of hydrolase from cancer cells, for example β-glucuronidase, so the cancer cells exfoliated.[9] (2) "Second diffusion" of cancer cells, i.e. the cancer cells exfoliated from the lymph nodes after the cancer cells metastasis.[10] Both the rate and degree of lymph node metastasis were high and coat of lymph node was penetrated in 3 of 6 cases with SS. The above results indicated that there were shed cancer cells in abdominal cavity of patients with SS cancer, also iatrogenic diffusion of cancer cells during operation was found in 7 of 24 cases with advanced cancer in this article. The positive rate of shed cancer cells was 31.33% in patients without peritoneal metastasis, this means that there was a great probability for peritoneal recurrence to occur after surgery. So the authors put forward that the indication of the IOIPP was the cases with advanced gastric cancer after radical resection regardless of conditions of serosal invasion. The results in this article also suggested that the effect of the IOIPP using D.D.W with Hibitane on shed cancer cells was not significant in cases with peritoneal metastasis of cancer and intraperitoneal chemotherapy with carboplatin (400—500 mg) was necessary for the cases.[4] Secondly, observation of the IOIPP show that the main side effects were slight and harmless to patients as reported by us.[4,7] In a word, the IOIPP using hypo-osmolar solution with Hibitane was active and safe in clinical application of gastric cancer surgery.

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