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• COLORECTAL CANCER •

Application of autologous tumor cell vaccine and NDV vaccine in treatment of tumors of digestive traet

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Abstract

AIM: To treat patients with stage I-IV malignant tumors of digestive tract using autologous tumor cell vaccine and NDV (Newcastle disease virus) vaccine, and observe the survival period and curative effect.

METHODS: 335 patients with malignant tumors of digestive tract were treated with autologous tumor cell vaccine and NDV vaccine. The autologous tumor cell vaccine received were assigned for long-term survival observation. While these failed to obtain the autologous tumor tissue were given with NDV vaccine for a received short-term observation on curative effect.

RESULTS: The colorectal cancer patients treated with autologous tumor cell vaccine were divided into two groups: the controlled group (subjected to resection alone) (n=257), the vaccine group (subjected to both resection and immunotherapy) (n=310). 25 patients treated with NDV immunotherapy were all at stage IV without having resection. In postoperation adjuvant therapy patients, the 5, 6 and 7-year survival rates were 66.51 %, 60.52 %, 56.50 % respectively; whereas in patients with resection alone, only 45.57 %, 44.76 % and 43.42 % respectively. The average survival period was 5.13 years (resection alone group 4.15 years), the median survival period was over 7 years (resection alone group 4.46 years). There were significant differences between the two groups. The patients treated with resection plus vaccine were measured delayed-type hypersensitivity (DTH) reactions after vaccination, (indurative scope >5 mm). The magnitude of DTH was related to the prognosis. The 5-year survival rate was 80 % for those with indurations greater than 5 mm, compared with 30 % for those with indurations less than 5 mm. The 1-year survival rate was 96 % for 25 patients treated with NDV immunotherapy. The total effective rate (CR+PR) was 24.00 % in NDV immunotherapy; complete remission (CR) in 1 case (4.00 %), partial remission (PR) in 5 cases (20.00 %), stabilizedin in 16 cases (64.00 %), progression (PD) in 1 case (4.00 %). After NDV vaccine immunotherapy, the number of NK cell increased and immune function imporved obviously.

CONCLUSION: The autologous tumor cell vaccine and NDV vaccine can prolong the patients' life. NDV vaccine is notably effective for short-term with promotion of quality of life and can be used whenever necessary with good prospects.

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INTRODUCTION

Malignant tumors of digestive tract is common. It is difficult to improve the survival rate only by resection plus radiotherapy and chemotherapy. This study reported 335 patients treated with autologous tumor cell vaccine and NDV vaccine. 310 of 335 patients with colorected cancer of stages I-IV received autologous tumor cell vaccine postoperatively and the controlled group (resection alone) constituted 257 patients. The duration of follow-up was 7 years. There were statistically significant differences in the survival rate, the median survival period and the mean survival period between the two groups and the controlled group. The 1-year survival rate was 96 % for 25 late cancer patients treated with NDV vaccine. This study provided a basis for the clinical application of autologous tumor cell vaccine and NDV vaccine[1,2,5,6,24,25,26,30].

MATERIALS AND METHODS

Clinical data

Those receiving autologous tumor cell vaccine is consisted of 567 patients with colorectal cancer in 1993-1995 with a further follow-up of 7-year. 25 patients with late cancer were given NDV vaccine from June, 2001 to June, 2002: 15 men, 10 women; 41 to 83-year-old, mean age 62.3 years,they were all at stage IV patients and were followed up for 1-year. 310 postoperative patients treated with autologous tumor cell vaccine consisted of 204 men and 106 women range from 19 to 79 years, mean age 55.8 years: 136 patients having Miles operation, 98 colonic operation, including 74 anterior resection and 2 partial resection. The resection alone group (controlled group) consisted of 257 patients: 155 men, 102 women; age ranged 18 to 77 years of age, mean age 55.1 years; among these,120 patients had Miles operation, 78 colonic operation, 59 anterior resection. The DTH reactions were observed in 20 patients treated with immunotherapy after resections. Among these 20 all were stage II, III colorectal cancer among them 10 were male and 10 female age ranged 35 to 65 years, mean age 52.7 years. The follow-up results were shown in Table 1, 2 and 4. The TNF stages were shown in Table 3.

Preparation of autologous tumor cell vaccine

Material and reagent Tumor tissue speciacus ($\phi \geqslant 1$ cm), elemene for injection (Da Lian Jin Gang Pharmaceutical Factory), mitomycin C, 25 % glutaraldehyde (E merk), RPMI 1640 complete culture media.

Method The tumor tissure obtained under sterile conditions was placed in a sterile container and sent to the lab in two

hours. Prepare the suspended tumor cell solution by routine methods. Then add mitomycin C and elemene 0.3 mg/ml. placed in 37 ℃ incubator (0.5 h). heated at 42 ℃ for 30 min. centrifuged, washed, and fixed in 0.0625 % glutaraldehyde (10 min). After washing out the fixation solution, the sterile suspended autologous tumor cell solution ($\geqslant 10^7$/ml) was then prepared for use.

Immune procedure First Vaccination: The Patients treated received first vaccination a week after surgical resection, once a week for four weeks.

Intensive immunotherapy: Began at 1 to 2 months intervals after the first vaccination.

Method of injection: The vaccine was injected in three adjacent sites each 3-5 an apact on the arterior arm or deltoid region. one 0.2 mlintradermically and two 0.4 ml hypodamically. DTH test: Prior to the vaccination and two weeks after vaccination, skin test was performed. For the controlls distilled water was used. DTH reaction was concided position when the indenation was at 72 hours >5 mm (+) , 6-10 mm (++).

Table 1 Follow-up results of survival status for colorectal cancer patients with resection plus autelogous tumor cell vaccine from 1993 to 2000

Year	*n*	Survival period							
		0	1	2	3	4	5	6	7
1993-2000	50	50	50	44	39	35	31	31	29
1994-2000	133	133	125	104	94	90	85	75	
1995-2000	127	127	126	116	106	98	93		
Total	310	310	301	264	239	223	209	106	

Table 2 Follow-up results of survival status for colorectal cancer patients with resection alone from 1993 to 2000

Year	*n*	Survival period							
		0	1	2	3	4	5	6	7
1993-2000	73	73	71	60	54	41	35	34	33
1994-2000	94	94	85	68	56	47	43	43	
1995-2000	90	90	84	69	57	50	47		
Total	257	257	240	197	167	138	125	77	33

Table 3 TNF staging in colorectal cancer patients with resection plus vaccine group and resection alone group

Treatment	*n*	TNF stages			
		I	II	III	IV
Resection plus vaccine	310	46(14.8%)	121(39%)	80(25.8%)	63(20.3%)
Resection alone	257	55(21.4%)	94(36.6%)	66(25.7%)	42(16.3%)
Total	567	101	215	146	105

Statistical analysis

Using SPSS computer program, analyses were performed by *t*-test for the mean value between the two groups by life table and logrank test for survival rate, by graphical method and linear inner insert method for the median survival period and by Kaplan-Meier method for the mean survival period.

Preparation of NDV vaccine

Primary fluid of NDV La Sota IV weak toxicant stem was vaccinated in chick embryo chorioallantoic cavity. SPF fertilized egg was placed in the 37 ℃ incubator. On day 10, injecting 0.2 ml NDV diluted with 0.5 % LH into the chorioallantoic cavity in sterile conditions, sealed it with wax, and obtained the virus after 72 hours in 37 ℃ incubator. Before getting the virus, placed the chick embryo overnight at 4 ℃ refrigerator. Then removed the eggshell in air champer, opened the egg membrance and aspirated the chorioallantoic fluid containing virus by sterile technique. The fluid was centrifuged for 30 minutes at low speed (2 800 r/min, 800 g) to get rid of the sediment. The supernatants of NDV was centrifuged 60 minutes at low temperature and high speed (4 ℃, 30 000 r/min, 90 000 g) and the viruses were precipitated. Resuspended the virus sediment by pH7.2, 0.1 mol/L PBS, assayed the hemagglutinin unit (Hu) of NDV by 0.5 % fresh chick erythrocyte suspended solution and then diluted to 1:1 280 Hu/ml. The solution was packed into ampoules separately and stored at -20 ℃. Unfreeze it before using.

Immune procedure

Injected 1-2 ml in three sites intradermally at deltoid muscle region, once every three days. Three times constituted one course, and the injections also could be given continually.

Immune function assay

Prior to and after the immunotherapy, assayed the NK, CD_3^+, CD_4^+, CD_8^+, CD_4^+/CD_8^+ by flow cytometry.

RESULTS

Table 4 Comparison of yearly survival rates of resected colorectal cancer patients in vaccination group and controll group

Treatment method	*n*	[a]Yearly survival rates (%±s$\bar{x}$)						
		1	2	3	4	5	6	7
Vaccination group	310	97.05 ±0.97	84.35 ±2.10	76.42 ±3.23	71.12 ±3.43	66.51 ±3.84	60.52 ±4.57	56.50 ±6.52
Controll group	257	93.16 ±1.60	74.83 ±2.51	62.50 ±2.06	50.58 ±1.99	45.57 ±1.32	44.76 ±0.81	43.42 ±1.32

[a]Life table χ^2 test $P<0.005$ of the two groups.

By life table method, the 7-year survival rate of the vaccination group was 56.5 % and the median survival period was approximately 50 %. Hence by graphical method the median survival period was over 7 years, whereas by linear inner insert method, the median survival period of the controll group was 4.46 years. The average survival period of vaccination group was 5.13±0.60 years where that of the controlled group 4.15±0.60 years (Table 5).

Table 5 Comparison of median survival period, mean survival period of resected colorectal cancer patients of vaccination group and controlled group

Treatment method	*n*	Median survival period (year)	Mean survival period (year)
Resection plus vaccination group	310	>7[a]	5.13±0.60[b]
Resection alone group	257	4.46	4.15±0.60

[b]$P<0.01$ *vs* resection alone group.

The positive rate of DTH reactions in resection plus vaccine group was over 90 % whereas in resection alone group all negative. Most active immunotherapy for patients succeeded (Table 6). The remission rate of late digestive tract carcinoma treated with NDV vaccine (Table 7,9). The charge of immune function pre- and post-NDV vaccine therapy (Table 8).

Table 6 Comparison of fve-year survival rate, survival period of resected colorectal cancer patients in vaccination group with positive and negative DTH reaction

DTH reactions	*n*	5-year survival cases	Survival rate[b]	[a]Survival period ($\bar{x}$±s)
Positive group	10	8	80 %	4.7±0.67
Negative group	10	3	30 %	3.5±1.80

χ^2 test [a]P<0.05, [b]P<0.0 1 *vs* negative group.

Table 7 The remission rate of late cancer of digestive tract carcinoma treated with NDV vaccine

Types of disease	*n*	CR(%)	PR(%)	SD(%)	PD(%)	CR+PR(%)
Colorectal cancer	13	1	3	8	0	4
Stomach cancer	6	0	0	5	0	0
Liver caner	4	0	2	1	0	2
Pancreatic head cancer	1	0	0	1	1	0
Gall bladder cancer	1	0	0	1	0	0
Total	25	1(4.00%)	5(20.00%)	16(64.00%)	1(4.00%)	6(24.00%)

Table 8 Immunobgice function prior to and after NDV vaccination therapy

Item	*n*	Before therapy($\bar{x}$±s)	After therapy ($\bar{x}$±s)	*P*
NK	10	24.65±7.21	36.58±11.87	<0.05
CD_3^+	10	67.15±2.43	71.12±2.86	<0.01
CD_4^+	10	38.23±3.01	41.62±2.71	<0.01
CD_8^+	10	30.55±1.21	29.84±2.43	>0.05
CD_4^+/CD_8^+	10	1.23±0.12	1.41±0.13	<0.05

Table 9 Comparison of treatment courses and therapeutic effect of colorectal cancer patients with NDV immunotherapy

Group	*n*	CR	PR	SD	PD	CR+PR
1 Course	6	0	0	5	1	0
>2 Courses	7	1	3	3	0	4

χ^2=10, P<0.01.

DISCUSSION

The major treatments of malignant tumors of digestive tract malignant tumor postoperatively are radiotherapy and chemotherapy. However, it is very difficult to improve the 5-year survival rate. We treated these patients with autologous tumor cell vaccine and NDV vaccine and made a 7-year follow-up survey of postoperative patients with autologous tumor cell vaccine. The 5, 6, 7-year survival rate are 66.51 %, 60.52 %. 56.50 %, respectively, whereas they were only 45.57 %, 44.76 % and 43.42 % respectively in resection plus radiotherapy or chemotherapy group. The survival rate increased by 20.94 %, 15.76 %, 13.08 %. The increase of amplitude arrived at 46.00 %, 35.20 % and 30.12 % respectively. This indicated autologous tumor cell vaccine could really increase the long-term survival rate of colorectal cancer patients[7,9,10].

The median survival period increased more obviousely: for over 7-year in vaccination group but only 4.46 years in resection alone group, it increased by over 2.5 years. The amplitude of increase was 57 %. The mean survival period was 5.13 years in vaccination group yet only 4.15 years in resection alone group, an increase of 1 year in average.

The DTH reaction in postoperative colorectal cancer patients showed positive reaction two weeks after the immunotherapy in over 90 %, but negative before therapy. The 5-year survival rate was 80 % in the positive response group, and the 30 % in negative response group, the difference was significant. It demonstrated that active specific immunotherapy by autologous tumor cell vaccine could yield positive DTH reaction in resected colorectal cancer patients. The 5-year survival rate increased obviously as a result of augmented antitumor immunity after immunotherapy[2,3,7]. We conclude that most of the patients who are treated with active specific immunization lead to a specific antitumor effect. Our vaccine is effective in prevention of tumor progression. The protection achieved can be augmented by serial vaccinations and can be maintained for a long period of time[4-20].

With booming of biotherapy lately, more attention have been paid to NDV vaccine. Many reports from abroad showed some effectiveness of NDV in malignant tumor of digestive tract and malignant melanoma[21-23, 27-29] with few adverse effects. After transfection NDV, immune response could be induced with production of cytokines and trigguing of active tumor vaccine reaction. In the 1950s, people tried to treat malignant tumor with virus and discovered that much virus had greater killing effect on tumor cells than on normal cells. For example, NDV-73-T could infect all kinds of human tumor cells, with intracellular replication, elicit cell fusion and multinuclear body, and the tumor cells die eventually. NDV had selective killing effect on tumor cells, but not the normal cells. Despite there were many reports about the antitumor effect of NDV[27-36], yet long-term observation on the use of NDV was scarce, especially about its side effects. The NDV vaccine given to 25 patients in our study were 2-6 courses, some of them 8 courses. The duration of continuous injection was over 3 months, no significant side effects were found. We compared the effectiveness of one course and two course in 13 colorectal cancer patients. See Table 9, the curative effect of the latter was superior to that of the former. A few patients had flu-like symptoms, such as fatigue, lowgrade-fever, soreness of joints. which disappeared in 1-2 days. Now we had treated 80 patients bearing all kinds of late malignant tumor with NDV vaccine. We found that the curative effects on urinary bladder cancer and colorecal cancer were notable. Take for instance, one bladder cancer patient developed a 2.0×2.0 cm tumor in the right wall of his bladder, having hematuria and 2.5×2.7 cm hypodermic metastatic nodule over his left thigh. After four courses of NDV immunotherapy, the mass in the bladder wall and the metastate nodule over his left thigh regressed, hematuria also ceased. One colon cancer patient had a 3.0×3.5 cm cauliflower-like tumor on the left wall of the rectum, about 4 cm away from the anus,having hematochezia. After four courses of NDV vaccine, the mass in the recturn regressed and hematochezia ceased. Another patient with primary liver cancer, measuring 5.5×7.2 cm before the NDV therapy, after three courses, the mass decreased somewhat to 5.5×5.3 cm and the backache ameliorated. NDV injection can remit patients in a late gastric cancer impending to death with extensive peritoneal and pelvic metastasis, anuria, comatous and mantained his life only by intravenous nutrients and renal dialysis. After 12 days of large doses of NDV vaccination, the patient became conscious and could urinate by himself, with stoppage of dialysis, and could eat a little, the survival period prolonged to 30 days. As from Table 8, we could see that NDV could improve the immune functions by activing the lymphocytes. The preliminary use of NDV showed a new thapeutic approach to the treatment of on late malignant tumors, especially for those who were inable to obtain the autologous tumor tissue. After NDV vaccination, the curative efficancy

occures rapidly and long-term observation is undertaking. From this study we can expect that the NDV vaccine has a good prospect.

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