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Clinical Observations

CLINICAL AND BIOLOGICAL BEHAVIOR OF NEUROGENIC TUMOR AFTER PREOPERATIVE CHEMOTHERAPY

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Objective: To study the significance of preoperative chemotherapy for the treatment of neurogenic tumor in children. Methods: VMA, MYCN gene and DNA content of 21 cases of neuroblastoma treated with preoperative chemotherapy were studied with a control group. Results: Resection rate was 95.5%. Mean survival time was 28.1±10.2 months, which was significantly higher than the control group (8.8±6.8 months, $P<0.01$). Post chemotherapeutic VMA was lower. DNA index was also reduced and the percentage of cells in G0+G1 phases was elevated. The MYCN expression was suppressed. Conclusion: Preoperative chemotherapy can induce the apoptosis of neurogenic tumor cells and inhibit its proliferative activity.

Key words: Neuroblastoma, Chemotherapy, Surgery, Gene, MYCN.

For advanced neurogenic tumor, the primary and metastatic lesions can be controlled through preoperative chemotherapy. After delayed or secondary operation, the resectability and the survival rate were elevated. 21 cases of neuroblastoma which were treated in our hospital during July 1990 to June 1996 were reported here. Using image analysis, *in situ* hybridization for MYCN and VMA assay, the mechanism of preoperative chemotherapy and its effect on the biological character of the tumor were discussed.

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MATERIALS AND METHODS

Clinical Data

There were 21 cases of neuroblastoma treated with preoperative chemotherapy and delayed or secondary operation, which is preoperative chemotherapy group, or test group. Among them 12 were male and 9 were females. The age span was between 6 months to 12 years, mean 5 years. There were 8 cases in stage 3 and another 13 in stage 4. Four cases underwent laparotomy for biopsy after admission. After 1 2/30 to 4 5/30 months of chemotherapy, they went through a secondary operation for radical removement of the tumors. For the other 17 cases the decisive diagnosis was made after CT, sonograph, urine VMA assay. 7 cases were treated with OPEC regime and another 4 with CCSG regime. 14 cases of neuroblastoma treated during 1989–1990 were used as the control group, which underwent primary operative group. They are in stage 3 or 4. The surgical achievement were classified according to Shemberge Criteria as (1) total resection: None remaining tumor can be seen with naked eyes. (2) subtotal resection: less than 20% remains. (3) partial resection: less than 50% remains. (4) Biopsy. The survival rate and surgical achievement were analysed statistically.

Active Observation of Urine VMA

Urine was collected routinely before and after chemotherapy. The result was statistically analysed.

DNA Content

Ten paraffin embedded samples respectively from primary operative and preoperative chemotherapy group were analysed. Statistically no difference can be detected between clinical stage, sex and age of the two groups. The samples were dyed with HE and Azure. DNA content and cell phase were assayed by image analysis system CAS200. According to Michie's criteria, DI of the DNA was classified into (1) Diploid: DI is between 0.9–1.1; (2) Quasi-diploid: DI<0.9 or 1.1–1.3; (3) Aploid: DI>1.3. The DI value and percentage of the cell phase were analyed for statistical difference.

In Situ Hybridization

Six cases from preoperative chemotherapy group and eleven cases from primary operative group were analysed. The single strand probe of MYCN cDNA (kindly provided by the Gene Base of Japanese Oncological Study Group) was ligated to the digoxin for hybridization. According to the Mannuals for Nonradiological *in situ* hybridization, the slides with a dark red color were regarded as positive ones. Using the system MIAS-300 the absorptance and the gray value were calculated. The results were statistically analysed.

RESULTS

Clinical Results

Table 1 shows the surgical achievement and survival time of the two groups. The differences between the sex, age, site of primary tumors have no statical significance. The total and subtotal resection rate was 90.5% in the test group which was higher than that in the primary operative group (21.4%, $P<0.01$). The difference between survival time had statical significance. For 14 cases with the complete removal of the tumors, 12 have survived for 8–62 months. In the control group, only one case of mediastinal neuroblastoma who has survived for 10 years after total resection of the tumor. Others died after 2–20 months after operation.

Urine VMA

The 24 h urine VMA at admission were 8.2 ng~36.5 ng/24 h, mean 19.35 ng±12.1 ng/24 h for 21 cases of the test group. Post chemotherapeutic VMA value were 3.2 ng~21.6 ng/24 h, mean 8.6 ng±11.2 ng/24 h. The VMA value were significantly lower after chemotherapy ($P<0.05$).

Table 1. Clinical data of preoperative chemotherapy group and primary operative group

		Preoperative chemotherapy	Primary operation
Sex	male	12	8
	female	9	6
Age (year)		5± 3	4± 2
Site	abdomen	17	11
	mediastine	3	3
	head	1	
Stage	3	9	9
	4	12	5
Operation	total	14	2
	subtotal	5	1
	partial		2
	biopsy	2	9
Survival time (month)		28.1± 10.2	8.8± 6.8

DNA Content and Cell Phase

DNA content and cell phase percentage were shown in the Table 2. There were more diploid in the preoperative group. The difference of DI value was of statical significance. The percentage of cells in G0 and G1 were higher in preoperative group while those in G2 and M were lower. It suggested that the proliferative activity were suppressed.

MYCN *In Situ* Hybridization

For 17 cases of neurogenic tumors, 11 were detected positive for MYCN by *in situ* hybridization (64.7%). The gene was inside the nuclear. There is no MYCN expression in the interstitial tissue. For primary operative group, 9 out 11 were positive (81.8%). The mean gray value was 7.51±4.56. While in preoperative group, 2 out 6 were positive. Their mean gray value was 3.77±3.13. The difference of this value was of statical significance. It suggested that the expression of MYCN was significantly inhibited after

chemotherapy.

Table 2. DNA content of preoperative chemotherapy group and primary operative group

	Preoperative chemotherapy	Primary operation
DNA content		
diploid	8	
quasi-diploid	2	2
aploid		8
DI	1.03± 0.12	1.84± 0.69
Cell phase		
G0+G1	76.23± 9.8	67.7± 17.6
S	20.89± 7.61	7.91± 12.5
G2+M	2.87± 3.16	4.39± 6.11

DISCUSSION

Neurogenic tumor is the second most common solid malignant tumor in children. It grows fast and metastasis occurs early. The patients often presented to a pediatrician with an advanced tumor in stage 3 or 4. The key to improve the clinical outcome is effective control of metastatic lesions and total removal of the primary tumor. Through delayed and secondary operation, we achieved a resection rate of 90.5% for total and subtotal removal. The mean survival time was 28.13±10.21 months, which was higher than that of primary group. It confirmed that preoperative chemotherapy can improve resectability, reduce the surgical complication, and elongate the survival time. Many researchers, believing in the role of preoperative chemotherapy for the treatment of advanced neurogenic tumors, studied its mechanism. The metabolite of catecolamine, proliferative activity and MYCN expression are the main criteria used to assess the malignancy and biological activity of neurogenic tumor. Through VMA assessment, DNA content analysis, and MYCN *in situ* hybridization, we can see that by preoperative chemotherapy the metabolite of catecolamine were reduced, the cell proliferation and MYCN expression suppressed. Through *in situ* hybridization, the MYCN expression can be located inside the nuclear and reduction of amplification can also be shown. It is a fast and convenient way for gene location, even for quantification, which is better than cell culture and PCR. It is an important and effective method for molecular biological study worthy of advocating.

Preoperative chemotherapy is helpful for the resectability of primary tumor and the control of metastatic lesions. So the survival time and living quality can be improved. The change of metabolism, gene expression, and DNA content suggested that preoperative chemotherapy alter the biological activity such as proliferative active, metabolic rate, and malignancy. Pathologically we can see apoptosis and thickening of the fibric layer. All of these will lead to the detection of the mechanism of preoperative chemotherapy.

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