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The Substance Aggregating Platelets Derived from Advanced Malignant Tumor of Head and Neck*

晚期头颈恶性肿瘤组织含促血小板聚集物

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Abstract To explore whether the substance aggregating platelets are contained in the extraction of malignant tumor tissue of head and neck, the tumor tissue from 15 cases of malignant tumor and 14 cases of nonmalignant tumor in head and neck are homogenized as crude extraction, which are found to aggregate platelets in 21 cases of malignant tumor, 33 cases of non-tumor and nonmalignant tumor of head and neck, and 16 controls respectively. The positive rate of aggregation induced by extraction from malignant tumor is higher than that from nonmalignant tumor; the aggregating function induced by extraction from the malignant increases with disease developing; the platelets of the cases in the malignant tumor group are apt to be aggregated by the crude extraction of tumor tissue, in which the maximum aggregation rate of platelets increases with disease developing. It is suggested that the aggregation substance is contained in the tissue of advanced malignant tumor of head and neck, and the aggregating substance from malignant tumor might directly affect the function of platelets in circulation of blood.

Key words tumor of head and neck, platelet aggregation, tumor extraction

摘要 为探讨头颈恶性肿瘤组织提取液是否含有聚集血小板的物质，将15例头颈恶性肿瘤和14例非恶性肿瘤组织匀浆取组织提取液，分别致聚于头颈恶性肿瘤21例、非肿瘤、非恶性肿瘤33例及健康人16例的血小板。发现，头颈恶性肿瘤组织提取液致聚的阳性率比非恶性头颈肿瘤组织提取液致聚的高；头颈恶性肿瘤组织提取液对血小板的聚集作用随病期进展而增强；头颈部恶性肿瘤病人的血小板易受组织提取液激聚，其血小板最大聚集率随病期进展而增强。表明，晚期头颈恶性肿瘤组织含促血小板聚集物，这可能是直接引起恶性肿瘤机体血小板聚集增强的原因之一。

关键词 头颈肿瘤 血小板聚集 肿瘤提取液
中图法分类号 R739.91

In many studies, it was demonstrated that platelet function played an important role in the development of metastasis and infiltration of cancer. We reported previously that enhancement of platelet aggregation, enhancement of fibrinolytic activity and a coagulable state occurred in the circulating blood of patients with nasopharyngeal carcinoma, and cancer of the head and

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neck[1,2]. Although some researches revealed that enhanced platelet aggregation associated with proteinase, prostaglandin, plasminogen activator, thrombin and metabolizing substances which might be produced by the destroyed tumor tissue and tissue degrading, the mechanisms were still obscured[3,4]. Based on the above mentioned reports, there might be a substance, which could aggregate platelets, contained in malignant tumor tissue. To explore the mechanism of platelet hyper-aggregation, we first examined platelet aggregation using extraction derived from advanced malignant tumor of the head and neck.

1 Materials and method

Specimens of tumor tissue were obtained from biopsy and operation without pretreatment. All samples were taken in the Department of Otorhinolaryngology, First Affiliated Hospital, Guangxi Medical University, China. The specimen was divided into two parts, and one was used for histopathological examination, another was used in the present study. In order to keep specimens in fresh, the samples used in the experiment were stored under 4 C during operation, and were washed many times using cold physiological saline solution until removal of pus and blood.

All samples were stored at-70 C deepfreeze before experiment. The samples from tumor of head and neck consisted of malignant and benign tumors. Of the 15 malignant tumors, eleven were men and 4 were women, aged from 27 to 74 years, averaged at 53 years. Histologically, squamous cell carcinoma was found in 8 cases and additional malignant tumor in 7 cases consisting of adenocarcinoma, adeno cystic carcinoma, malignant melanoma and olfactory neuroblastoma. The distribution of the primary sites of tumors is summarized in Table 1. The clinical classification was performed based on the Union International Control Cancer (UICC) Classification[5]. Fifteen cases were staged as follows: 1 case was in stage Ⅱ; 6 in stage Ⅲ and 8 in stage Ⅳ.

There were 14 cases with benign tumors of the head and neck (11 men, 3 women), aged from 6 to 43 years, averaged 30 years. The benign tumors consisted of fibroangioma, meningioma, nasal polyp, tonsil, adenoid, chronic inflammatory membran, infective lymphaden of neck, tuberculotic neck node and neck normal tissue (Table 1).

Table 1 Distribution of tumors samples

Primary sites	Malignant*	Benign*
Larynx	4(SCC)	—
Neck lymphaden	2(metastatic SCC)	2(TB & IF)
Nasal cavity	2(ACC & SCC)	2(polyp)
	1(ON)	2(MO)
Trachea	1(SCC)	—
Nasopharynx	1(SCC)	2(FA)
Hypopharynx	2(AC & SCC)	1(Adenoid)
Ethmoid sinus	1(AC)	—
Hard palate	1(MM)	
Chronic tonsillitis	—	2
Normal tissue	—	1(paralarynx)
Maxillary sinus	—	2
Total	15	14

* SCC: Squamous cell carcinoma; ACC: Adeno cystic carcinoma; MM: Malignant melanoma; TB: Tuberculosis; IF: Inflammation; MO: Meningioma); FA: Fibroangioma; ON: Olfactory neuroblastoma; AC: Adenocarcinoma

1.1 Extraction

The tumor tissue was minced with scissors and homogenized in the suspended solution. This procedure was carried out at 4 C in a cold room. Physiological saline was added to tumor tissue at wet weight of tissue ratio 0.1 g/0.5 ml. The homogenate was centrifuged at 5 000 r/min for 15 min. The supernatant was used as tissue extract of the tumor tissue, and stored in 0.1 ml/bottle at -20 C until subsequent experiment. The protein content of each kind of tissue extract was measured by Lowry's method[6,7]. For an inducer of platelet aggregation, protein concentration of tumor extract was employed at 4.0±0.6 mg/ml.

1.2 Platelet aggregation

We measured platelet aggregation of 70 patients which were separated into three groups. The group of malignant tumor of head and neck (Malignant group) consisted of 21 patients, 12 males and 9 females, aged from 37 to 65 years and averaged 50 years old. Twelve patients were diagnosed as nasopharyngeal carcinoma, five as cancer of larynx, two as hypopharyngeal carcinoma, one as malignant granuloma anl and onc as maxillary carcinoma. Based on the UICC classification, one patient was classified in stage Ⅱ, 8 in stage Ⅲ, 9 in stage Ⅳ, 3 in nonstage after treatment.

The groups of nontumor, nonmalignant tumor of

head and neck (benign group) consisted of 33 patients, 18 males and 15 females, aged 48 years in average, ranging from 22 to 77 years. The diseases included chronic sinusitis, chronic tonsillitis, Meniere disease, angiofibroma of nasopharynx, hemangioma of nasal cavity, branchial cyst, tuberculous lymphadenitis of the neck, vocal cord polyps, circumscribed labyrinthitis, idiopathic sudden sensorineural hearing loss, meningioma of nasal cavity, secretory otitis media, cerebral hemorrhage, arteriosclerosis, myocarditis, hyperlipemia, coronary heart disease, allergic thrombocytopenia purpura, rheumatic heart disease, rheumarthritis.

The control group consisted of 16 healthy adult men and women, who are 36 years old in average, ranging from 18 to 67 years.

1.3 Preparation of platelets

Blood was withdrawn using 3.8% sodium citrate as an anticoagulant (the ratio of blood to sodium citrate was 9:1 in volume). After sampling, the blood was immediately centrifuged at 160×g for 10 min at room temperature, and the supernatant was utilized as the platelet-rich plasma (PRP). The platelet count in the PRP was adjusted to 3×10^5 cells/mm^3 by dilution with platelet-poor plasma (PPP), which had been prepared by the centrifugation at 2000×g for 10 min at room temperature. The count of platelets in the PRP was measured by Brecher-Cronkite's method[8]. In these procedures, only plastic or siliconized tubes and pipettes were used. Aggregation of PRP was estimated with a Hematracer NKK 1 aggregometer (Niko Bioscience Co., Tokyo, Japan) by the method of Born and Cross[8,9]. An inducer of platelet aggregation which contains adenosine 5'-diphosphate (ADP) (Sigma Chemical Co., St. Louis, MO) and tumor extract derived from tumor tissue was employed in the experiment, and physiological saline was used as control. After 250 μl of PRP in the cuvette was incubated at 37°C for 2 min, 25 μl of inducer was added to PRP to make ADP be a final concentration of 4.5 μM, and the change of the absorbance was recorded. The maximum platelet aggregation rate (MAR) was calculated from the curve recorded on an X-Y recorder connected to the aggregometer. PRPs from malignant group, benign group and control group were induced by malignant tumor extract, benign tumor extract, ADP and physiological saline respectively.

1.4 Data Analysis

Statistical analysis was performed using the student's t-test for between-group comparison according to the procedure of Snedecor and Cockran[10]. Data are expressed as the means ± standard deviation.

2 Results

2.1 Positive rate of aggregation induced by tumor extract

The positive rates of platelet aggregation induced by malignant tumor extract in benign group, malignant group and control group were 54.5% (18/33); 76% (16/21) and 56% (9/16) respectively. The positive rate of platelet aggregation induced by extract from malignant tumor in malignant group was significantly higher than that in benign group and control group ($P < 0.05, t = 3.687$), but the difference between benign group and control group was not significant (Fig. 1). The positive rates of platelet aggregation induced by benign tumor extract in benign group, malignant group and control group were 42% (14/33), 86% (18/21) and 44% (7/16) respectively. The positive rate of platelet aggregation induced by benign tumor extract in malignant group was significantly higher than that in benign group and control group ($P < 0.001, t = 8.263$), but the difference of positive rates induced by malignant tumor extract and benign tumor extract was not significant ($P > 0.05$) (Fig. 1).

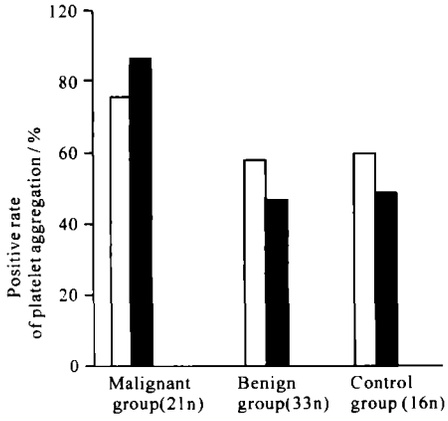

Fig. 1 Positive rate of platelet aggregation in different groups
□Malignant tumor extract; ■Benign tumor extract

2.2 MARs induced by malignant tumor extract

The mean MARs induced by malignant tumor extract in benign group, malignant group and control group were 28.3%± 31.1%, 49.8%± 29.9% and 26.5%± 21.5% respectively. The mean MAR induced by malignant tumor extract in malignant group was significantly higher than that in benign group and control group ($P < 0.02, t = 2.537$). MAR induced by stage Ⅳ malignant tumor extract was significantly higher than that induced by stage Ⅲ malignant tumor extract, but the difference between benign group and control group was not significant ($P > 0.05$) (Table 2).

2.3 MARs induced by benign tumor extract

The mean MARs induced by benign tumor extract in benign group, malignant group and control group were 24.3%± 30.4%, 57.1%± 32.2% and 25.2%± 26.8% respectively. The mean MAR induced by benign tumor extract in malignant group was significantly higher than that in benign group and control group ($P < 0.05, t = 3.653$), but the difference between benign group and control was not significant ($P > 0.05$) (Table 3). In malignant group in which there were 21 patients with malignant tumor of head and neck, the mean MARs were 59.5%± 34.8% in stage Ⅲ, 74.0%± 7.2% in stage Ⅳ; MARs in stage Ⅳ were higher than that in stage Ⅲ, but neither was significant difference between stage Ⅳ and stage Ⅲ, nor was different in the mean MARs between after treatment and control (Table 3).

3 Discussion

Many studies demonstrate that as the malignant tumor develops to some extent, coagutalion, hyperfibrinolysis and the enhanced platelet aggregation would occur. Above mentioned phenomenon might play an important role in the process of tumor development, metastasis and DIC. In a preceding study, we reported the enhancement of platelet aggregation appeared in patients with nasopharyngeal carcioma and advanced malignant tumor of head and neck[11] the mechanism is still not clear. The phenomenon of occurance of coagulation, hyperfibrinolysis and enhancement of platelet aggregation in circulating blood of patients with malignant tumor associated with proteinase and metabolic substances that are released from tumor cells in the process of tumor development[10,12,13]. Kosugi[14] found that some substances promote platelet aggregation existed in tumor extract derived from carcinoma linguae and hard palate carcinoma. According to Kosugi's method, we took the tumor extract from malignant and benign tumors as crude inducers to aggregate platelets from malignant patients, non-tumor patients and healthy persons. The aims are to explore whether platelet aggregating substances are contained in the extraction of malignant tumor tissue of head and neck. In the present study, the substance enhancing platelet aggregation was found in malignant tumor extraction, and demonstrated that there was a trend that the more substances promote platelet aggregation, the more advanced malignant tumors were contained in the extraction. The extract derived from malignant tumor of head and neck induced platelets either from patients with malignant tumor or without tumor. MARs

Table 2 MARs(%) induced by malignant tumor extract

Stage extract derived malignant tumor	Control group	Benign group	Malignant group				
			Stage Ⅱ	Stage Ⅲ	Stage Ⅳ	After treatment	Amount
Ⅰ(1)	20.4±16.3(5)	0(2)					
Ⅱ(6)	26.6±22.2(7)	20.7±25.6(15)	61(1)	35.5±35.0(4)	9(4)	0(1)	34.5±35 (10)△
Ⅳ(8)	28.5±25.5(4)	40.1±32.9(16)	52(1)	66.8±9.8(4)	63±15(4)	69.5±0.5(2)	64.5±11.8(11)△
Total(15)	26.5±25.1(16)	28.9±31.1(33)#					49.8±29.9(21)#

The value in brackets are number of cases; MAR $\bar{X} \pm s\%$. #$P < 0.02$ △$P < 0.05$

Table 3 MARs(%) induced by benign tumor extract

	Control group	Benign group	Malignant group			
			Stage Ⅱ	Stage Ⅲ	Stage Ⅳ	After treatment
	25.2±26.8(16)	24.3±30.4(33)	0(1)	59.5±34.8(8)	74±7.2(9)	23.5±21.3(3)
Total	25.2±26.8(16)	24.3±30.4(33)△			57.1±32.2(21)△	

The values in brackets are number of cases; MAR $\bar{X} \pm s\%$; △$P < 0.05$

increase in parallel with the development of the stage, and these results are corresponded with the findings in previous reports.

The substances promoting platelet aggregation were also contained in small amount in the extraction derived from benign tumor tissue. The extract from benign tumor induced platelet aggregation which increases with the development of malignant tumor, and the results were corresponded with that induced by ADP, but MARs were not significantly different between groups of non-tumor and control. Our study suggests that the platelets from patients with malignant tumor of head and neck are qualitatively change, so are that induced by tumor extract either from malignant or benign, and MARs are not different between inductions of malignant tumor extract and benign tumor extract and are as same as that induced by ADP in agrregation. Platelet aggregation induced by tissue extraction is complex[15], the mechanism is obscure. The platelet aggregation induced by tumor extract associated with content of extract containing collagen, thrombin, ADP, thromboxane A, adrenalin, prostaglandin, proteases and metabolic substance releasing from tumor cell. There would be different in content of extract as differences of tumor malignant extent and stage progress. Once these substances enter circulation, dynamic system of coagulation, fibrinolysis and function of platelet aggregation would be influenced. Our investigation demonstrates that there is difference of platelet aggregation between malignant tumor extract and benign tumor extract. Therefore the further investigations are necessary according to different extracts derived from malignant tumor of head and neck at various stages.

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