MECHANISM OF FIBRIN FORMATION IN GASTRIC ADENOCARCINOMA TISSUES

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Fibrin deposits in the tissues of malignant tumors have been found by many investigators [6, 7, 12, 13, 16] but the factors promoting the transition of fibrinogen into fibrin have been discovered by no means completely.

The aim of this investigation was to study the role of tissue hemocoagulating compounds of gastric adenocarcinoma in the mechanism of formation of fibrin deposits in the tumor.

EXPERIMENTAL METHOD

Intact organs and organs involved by tumor tissue were obtained from 10 patients (eight men and two women) aged from 50 to 69 years, undergoing operations for gastric carcinoma. The diagnosis of adenocarcinoma was confirmed histologically. The tumor in four patients was located in the middle third of the stomach, in five in the distal portion, and in one it had a double location: in the middle and upper third of the organ. Extracts in a dilution of 1:10 in buffered physiological saline (pH 7.4) were prepared from the mucosal and muscular layers of unaffected stomach tissues, taken as far away as possible from the tumor, in the line of the operative incision, and also from tissues of the tumor itself and of the muscular layer immediately below the tumor, and infiltrated by adenocarcinoma cells. The tissue homogenate was centrifuged for 4 min at 3000 rpm. The supernatant was used for testing. The effect of extract was determined on the following parameters of the coagulogram: recalcification time of platelet-free plasma [9], antiheparin activity [1], antithromboplastic activity [2], heparin time [8], antithrombin III activity [11], plasma factor XIII activity [3], and lytic activity also was discovered in a homogenate of the fibrinogen–heparin complex [5]. The hemocoagulating properties of the extracts were studied by the method described previously [4].

EXPERIMENTAL RESULTS

The study of intact stomach tissues revealed that they have high thromboplastic and antiheparin activity. This was manifested as the ability of extracts of the mucosal and muscular coats to shorten the recalcification time of platelet-free plasma by 11.1 and 7.1 times, respectively, and the thombin time of heparinized plasma by 6.2 and 3.5 times, respectively (Table 1). The gastric mucosa contains virtually no thromboplastin inhibitors, and it may actually depress activity of antithromboplastins with a progressive type of action in the blood serum. The muscular coat possessed average antithromboplastic activity. In extracts of the mucosal layer heparin was found, and it lengthened the heparin time of the plasma by 78.1%. No heparin was found in the muscle tissue of the stomach. The tissues studied also contained antithrombin III (AT III) and moderate fibrinase activity; activity of a complex compound of heparin with fibrinogen was discovered in them.

Compared with the intact mucosa, extracts of tumor tissues had a somewhat lower total blood clotting potential, but their antiheparin activity was high. Infiltration of the gastric muscles with adenocarcinoma cells led to an increase in its procoagulant and antiheparin properties. Considerable activity of antithromboplastins with both types of action was found in the tumor extracts, but was absent in homogenates of the intact spleen. Similar changes were observed in the tissues of the muscular layer during its invasion by the adenocarcinoma. The heparin concentration was considerably (by 2.3 times) reduced in the tumor compared with the mucosa, and antithrombin (AT) III activity was reduced a little. During malignant growth

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TABLE 1. Blood-Clotting Activity of Intact and Tumor Tissues of the Human Stomach ($\bar{X} \pm m$)

Parameter	Control	Mucous membrane	Muscular coat	Tumor tissue	Infiltrated muscular coat
Recalcification time of platelet-free plasma, sec	187,3±7,6	16,8±0,4	26,2±1,2	21,6±3	23,0±0,8
P		<0,001	<0,001	<0,001	<0,001
P_1				>0,1	<0,02
Antiheparin activity, sec	139,2±1,9	22,6±3,4	39,4±5,5	19,2±2,2	35,8±5,3
P		<0,001	<0,001	<0,001	<0,001
P_1				>0,05	>0,1
Antithromboplastins with instant type of action, sec	25,4±0,8	28,8±2,2	33,9±1,9	35,6±2	40,7±1,9
P		>0,1	<0,002	<0,001	<0,001
P_1				<0,02	<0,002
Antithromboplastins with progressive type of action, sec	5,9±0,6	0,5±1	17,8±4,8	14,2±3,4	19,8±3
P		<0,01	<0,05	<0,05	<0,001
P_1				<0,01	>0,5
Heparin time, sec	16,0±0,6	28,5±4,9	14,4±2	12,4±2,4	15,1±2,8
P		<0,05	>0,25	>0,1	>0,5
P_1				<0,01	>0,5
Antithrombin III, sec	30,3±1,1	48,5±3,6	39,8±1,6	41,2±2,4	46,2±2,8
P		<0,001	<0,001	<0,002	<0,001
P_1				<0,05	<0,05
Fibrin-stabilizing factor, sec	62,4±1,5	67,4±2,1	67,1±2,5	73,4±1,9	67,8±1,7
P		<0,01	<0,05	<0,001	<0,01
P_1				<0,01	>0,5
Fibrinolytic activity of fibrinogen–heparin complex, mm²	0	160,8±9,4	123,2±11,6	108,5±8	104,5±6,8
P		<0,001	<0,001	<0,001	<0,001
P_1				<0,001	>0,05

Legend. P) Significance of difference relative to control, P_1) relative to intact tissues.

a unique kind of inversion of the anticoagulant composition of the gastric tissue is thus observed: an increase in antithromboplastin activity and a decrease in concentrations of heparin and AT III. The reasons for the increase in the antithromboplastin concentration in the stomach wall when involved with carcinoma are not clear. This may perhaps be a compensatory response of the tissues to the fall in their heparin activity.

Tumor growth was also accompanied by strengthening of activity of plasma factor XIII analog in the tissues and by a considerable (by 32.5%) fall in the concentration of the fibrinogen–heparin complex (Table 1).

These results indicate a definite link between fibrin formation in the tumor and changes in the hemocoagulant structure of the tissues. A particularly important role is played here, evidently, by the increased antiheparin activity of the adenocarcinoma tissues. Antiheparin compounds bind the greater part of the heparin in the tissue fluid and displace the coagulable protein from the fibrinogen–heparin complex. When deprived of its natural stabilizer (heparin), and in the presence of a high concentration of thrombokinase in the tissue fluid of the tumor [6, 15], and also, possibly, under the influence of "cancer procoagulant A" [10, 14], fibrinogen is converted into fibrin. The increased concentration of tissue fibrinase in the tumor leads to stabilization of the fibrin polymer thus formed.

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ANTIBODIES INTERACTING WITH MOUSE MAMMARY TUMOR VIRUS PROTEINS IN SERA OF HEALTHY WOMEN

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It is stated in the literature that humoral antibodies reacting with structural proteins of mouse mammary tumor virus (MMTV) are found in man [1, 2, 4, 6, 8]. The prevalence of these antibodies in patients with breast carcinoma is 10-20 times higher than in healthy women, and accordingly the question of their importance for the diagnosis of breast cancer has been raised [2, 9]. According to data published by various workers the percentage of healthy women who produce antibodies capable of reacting with MMTV proteins varies from 3 to 5 [1, 2. 4, 6, 9]. These fluctuations are determined not only by differences in the populations studied, but also by the methods used to test the serum. However, virtually nowhere is there any exact definition of the term "healthy women," except that it implies the absence of breast cancer. Since data of different workers may be based on the investigation of groups that differ in age, or the presence of benign changes in the breast, and so on, it is important to know how all these factors may affect expression of antibodies reacting with MMTV proteins in man. With information of this kind it will be possible to make a more detailed analysis of the data for patients with breast cancer, and ultimately to judge the applicability of the MMTV antibody test in diagnosis.

In the investigation described below, in order to study expression of antibodies to MMTV in women without breast cancer, it was decided to study the age, inheritance, endocrine status, and benign changes in the breast in conjunction with analysis of the prevalence of antibodies to MMTV in different groups. The presence of antibodies was determined by enzyme-linked immunosorbent assay (ELISA).

EXPERIMENTAL METHOD

Sera were obtained from women during out-patient attendance at a Moscow factory medical clinic, kept at −20°C without overfreezing until required for the reaction. The presence of antibodies to MMTV in the sera was determined by ELISA on polystyrene plates [7] in the modification used by the writers previously [4]. A preparation of MMTV from C3H mice was used as the antigen: this consisted of virus precipitated from the culture medium of cells of mouse mammary tumor MM5/mt. Blood donors' sera were used in dilutions of 1:100-1:300, and incubation with the test sera was carried out overnight at 6-8°C. Rabbit antibodies against human immunoglobulins (produced by the N. F. Gamaleya Research Institute of Microbiology and Epidemiology, Academy of Medical Sciences of the USSR) were conjugated with peroxidase (1000 units/mg, from Serva, West Germany) by the method in [5]. Dilutions of sera and conjugate were made up in PBS buffer with 0.01% Triton X-100 and 5% normal rabbit serum. After each incubation the samples were washed with the same buffer without addition of the serum. As the chromogen, 5-aminosalicylic acid (0.8 mg/ml, pH 6.0) was used, with the addition of hydrogen peroxide. The marked difference between the positive and negative reactions made it possible to assess the reaction visibly.

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