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COAGULATION ACTIVITY IN VARIOUS STRAINS OF YOSHIDA ASCITES HEPATOMA TISSUES AND CELLS OF RAT

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To investigate the mechanism of chemotherapy related DIC, a quantitative and comparative study was carried out on the coagulation activities of tumor cells and tumor tissues in three strains of rat ascites hepatoma AH109A, AH272 and LY80. The content of thromboplastin was expressed as the relative concentration against that in the brain tissue. Ascites hepatoma cell and tissue of each strain obviously have different quantities of coagulants, such as tissue thromboplastin. Coagulation activities of tumor tissues are higher than that of tumor cells. The coagulation activities of tumor tissues and cells of AH272 were higher than that of AH109A and LY80. Our findings suggest that the initiation of DIC not only depend on the tissue thromboplastin, the other factors or the other mechanisms should be considered.

Key words: Coagulation activity, Thromboplastin, DIC, Tumor tissue, Tumor cell.

Abnormalities of the coagulation system in cancer patients have been repeatedly noted in clinical studies.[1,2] Thromboplastic and fibrinolytic activities of tumor cell themselves are considered an important factor in the thrombosis and disseminated intravascular coagulation syndrome (DIC).[3,4] Recently, the authors have reported that DIC had occurred following a successful chemotherapy in rats which bearing Yoshida ascites hepatoma cells.[5] To clarify the mechanism of the DIC, investigation on the coagulation activity of tumor cells and tumor tissues is basically important. For this purpose, thromboplastic activities of three strains of transplantable rat ascites hepatoma cells and tissues were estimated by modified Astrup's method.[6] The relationships between chemotherapy followed DIC and coagulation activities of tumor tissues and cells are discussed.

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MATERIALS AND METHODS

Male inbred Fub: KS rats (a substrain of Donryu rats), weighing 170-190 g, and tumor cells of Yoshida ascites hepatomas AH109A, AH272 and LY80, maintained in our laboratory were used in the study. AH109A cells (3×10^6), AH272 cells (10×10^6) and LY80 cells (2×10^6) in 0.1 ml phosphate buffered saline each was inoculated either intra peritoneally or subcutaneously. Within 4 to 8 days after intraperitoneal inoculation, when tumor cells reached a state of nearly pure culture, they were collected and washed three times with cooled saline. Suspension was diluted to 10% by saline and stored at -20 ℃. Fresh tumor tissues were obtained from the subcutaneous solid tumor. The brain, liver and subcutaneous tissues were also collected. All of the solid tissues (100 mg of tissue in 0.9 ml of saline), were homogenized in a Teflon homogenizer, then stored.

At the time of assay, the cells and tissues were thawed and then sonicated by an ultra sonicator

(Model UR-150P., Tominaga Works Ltd., Tokyo) for 1 min. at 0 ℃. The homogenate was substituted for the commercial thromboplastin in the system of Quick prothrombin test,[7] i.e., the principle of the assay was illustrated in Figure 1. 0.1 ml of plasma was added to 0.1 ml of 0.025M $CaCl_2$ solution and 0.1 ml of homogenate in serial twofold dilution in saline. The test was started at 37 ℃ in a water bath after the solutions in the tubes (8 mm diam) were prewarmed for 2 min. Coagulation was examined while the tube was shaking, and the coagulation time was determined when a small and solid clot was formed. An entire course of the test was completed within 3 h after thawing of the plasma. An empirical equation was employed for a systemic survey of concentrations of thromboplastin in various organs from different animal species, reported by Astrup,[6] as follows: lot t = $-a$ log C $-b$, where t is the clotting time, C is the concentration of thromboplastin in arbitrary units, and a and b is constant. This equation shows that when the clotting time and tissue dilution are plotted in a double logarithmic scale, there has to be a linear relationship. If two lines obtained in the same assay system are in parallel, the concentration of thromboplastin in one can be expressed by the relative concentration of that in the other, because the difference reflects only the difference of concentration.

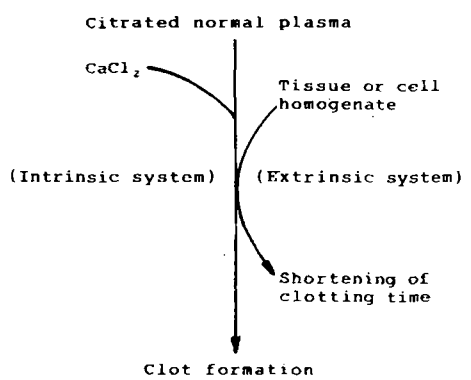

Fig. 1. Assay system

RESULTS

Figure 2 shows the coagulation activities in the three normal tissues of rats. With serial dilution of test homogenate, clot-promoting effect appeared markedly in the brain and liver, and all the three curves proved to form straight lines. The types of the brain and liver suggested the presence of anticoagulant compounds, the effect of which decreased with dilution as mentioned by Astrup. The linear part of the curve was extrapolated toward the direction of 100% concentration of homogenate to obtain the expected clotting time without anticoagulant compounds. The equation of the brain used as standard curve in the present study was as follows: log t = -0.24 log C+ 1.77.

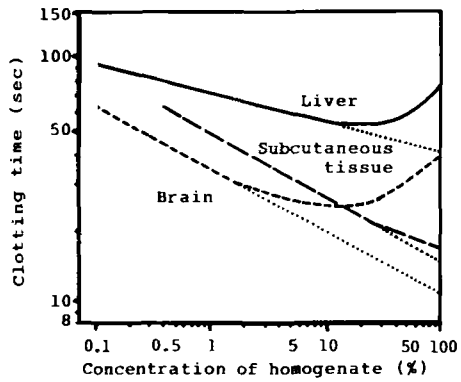

Fig. 2. Coagulation activities in liver, brain, and subcutaneous tissue of rat.

The slopes of the linear part of the curves in the brain and subcutaneous tissues were parallel (a=0.24). Therefore, the relative concentration of thromboplastin in the subcutaneous tissue was 32.7% of that in the brain. The coagulation activities in tumor cells and tissues and the normal brain tissue are showing in Figure 3. We obtained clotting time of test homogenate in serial dilution. The content of thromboplastin was expressed as the relative concentration against that in the brain tissues. It appeared clearly that cells and tissues of AH272, AH109A and LY80 have different quantities of coagulants considered as tissue thromboplastin and anticoagulant compounds. All of the 7 curves proved to form straight lines. The slopes of the linear part in cells and tissues of AH272, AH109A and LY80 were parallel with that of the brain. So that the relative concentrations of thromboplastin were calculated as described above. These results are summarized in

Figure 3 and compared in Figure 4. There was a marked difference in the concentration of thromboplastin according to each homogenate examined. Tissues of AH272 exhibited 13.3 times than that of brain on the thromboplastin activity. Nevertheless, in AH109A and LY80 were 5.5 times and 1.58 times than that of brain. As for tumor cells, the activity of AH272 was strong and exhibited 4.6 times than that of brain, but AH109A and LY80 showed very weak activities and was only 3.2% and 3.8% of that of brain.

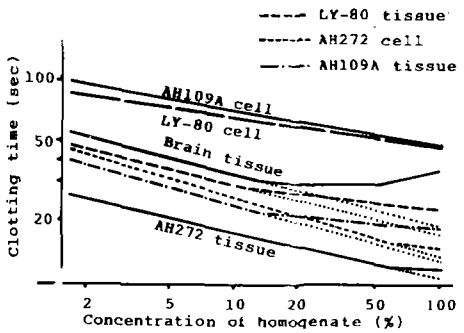

Fig. 3. Comparison of coagulation activities in tumor cells and tissues.

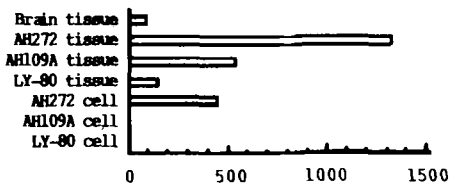

Fig. 4. Spectrum of the concentration of thromboplastin contained in tumor cells and tissues.

DISCUSSION

Disseminated intravascular coagulation (DIC) is a well-recognized phenomenon in some malignant diseases,[8,9] especially after the onset of cytotoxic treatment. This has been attributed to the release of the procoagulant activity from the malignant cells.[10] Although there are some reports about the coagulation activities of tumor tissues, literatures on the activities of tumor cells without stroma tissues are scanty. In the present study, we have investigated thromboplastic activity of three strains of tumor tissues and cells *in vitro*, because thromboplastic activity of the cancer cells might be very important in terms of pathophysiology of the patients with malignancy, such as thrombosis and DIC. The thromboplastic activities seem to be due to tissue thromboplastin of the tumor cells themselves. Tissue thromboplastin is the most potent cofactor for initiation of the extrinsic pathway of coagulation. Together with factor VII/VIIa its leads to a direct activation of factor IX and factor X. It is a membrane glycoprotein surrounded by phospholipid, which is essential to its procoagulant activity.[11] The results showed that tumor cells and tissues have higher coagulation activity than that of brain, thromboplastic activity of tumor tissues is higher than that of tumor cells. According to these findings, the biological characteristics of tumor tissues and cells on the coagulation activity are detected. The method that we improved is valuable to use for basic or clinical study on the investigation of coagulation activity of tumor cells and tissues.

Recently, a newly-developed cancer chemotherapy FCC was achieved by Suzuki, et al..[12] FCC could selectively enhance the drug concentration and its retention time in tumor tissues. It was done on the rats that bearing many strains of Yoshida ascites hepatoma cells. The therapeutic effect on the tumors was more marked than conventional chemotherapy. Following the rapid tumor size reduction, many rats, which bearing AH109A and LY80 cells are dead by DIC syndrome. Nevertheless, it has not occurred in AH272 rats, although the therapeutic effect in AH272 rats are as the same as that in AH109A and LY80 rats.[5] The mechanism of DIC should be elucidated. Although most explanations for the formations of thrombosis have been offered, there is no consensus agreement on the causative factors. Edgington[13] has speculated that there are three general mechanisms by which tumor cells may activate the coagulation system. The first mechanism is related to the production and secretion of molecules capable of activating the coagulation system. The second, necrosis of tumors could activate the coagulation system by release of intracellular thromboplastin. For the third mechanism, activation of the coagulation system is result from an entirely indirect mechanism. By the result of present study, the thromboplastic activities of tumor tissues and cells of AH272 were showing higher than that of AH109A and LY80, but DIC has not occurred. On the contrary, DIC occurred in AH109A and LY80 rats through their thromboplastic activities are lower than that of AH272. It is hard to define that intracellular

tissue thromboplastin that released from damaged tumor cells should be a major mechanism for the occurrence of DIC. However, in rats with a solid tumor it is also unlikely that a large quantity of the tissue factor is released into the blood. The trigger of DIC maybe considerably obscure by most unknown factors, the coagulation system is a complex interaction of many different components. Activation of the coagulation system in malignant disease not only produces life-threatening clinical problems but also may enhance the ability of tumor to avoid destruction by the host's immune system or help the tumor thrive in other way. Coagulation abnor-malities in cancer and their causes and consequences have been a great challenging biochemical and clinical problems requiring further investigation.

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