brief communication

Penetration of macromolecules into contracted blood clot

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ABSTRACT The effectiveness of thrombolytic therapy is determined by accessibility of thrombus compartments to plasminogen activators and, therefore, depends on permeability of thrombus to blood born macromolecules. Accumulation of ^{125}I labeled proteins with molecular masses ranging from 150 to 450 kD into partly contracted blood clot or plasma clot was consistent with diffusion coefficients 3.2×10^{-11} and 2.7×10^{-11} $m^2 s^{-1}$, respectively. So far as the model conditions imitated those for venous thrombi, these data indicate that such thrombi are porous enough for immunoconjugates of relatively big size.

INTRODUCTION

Lysis, growth, or stabilization of blood clots in an organism depend on exchange of macromolecules which are not incorporated into the fibrin gel between the thrombus and blood flowing nearby. This process is diffusion driven mostly when the inner part of the thrombus is concerned. Evaluation of mass transfer or, rather, rate of accumulation of plasminogen activators or immunoconjugates infused during thrombolytic therapy in the blood clot is of great importance for construction of more effective thrombolytic molecular complexes. Penetration of proteins into a thrombus may be restricted by the size of the protein itself or its complex with blood constituents (1). It was shown previously that the diffusion coefficient for albumin in the gel formed from purified fibrinogen (4×10^{-11} $m^2 s^{-1}$) (2) is quite similar to the same in solution. But, a real blood clot differs from pure fibrin gel. First, more than half of its volume comprises blood cells, mostly red blood cells (RBC); second, it is contracted by means of activated platelets (3). Therefore, restriction of macromolecules will vary for blood clots or plasma clots or fibrin gels. In this work, the rate of penetration of labeled proteins from the surrounding solution into partly contracted canine blood clots imitating venous thrombus was experimentally measured. The data were compared with the rate of accumulation of the proteins in plasma gels as well as fibrin gels formed from pure fibrinogen. Proteins of particular interest for targeting to thrombi were chosen, namely, fibrinogen (canine, Mr ~ 300 kD), fibrinogen-antibody immune complex (Mr ~ 450 kD) and nonimmune rabbit IgGs (Mr ~ 180 kD).

EXPERIMENTAL

Highly purified proteins were labeled to $\sim 10^8$ cpm per mg by incubation with Na ^{125}I in the presence of iodogen (Pearce Chemical Co., Rockford, IL). The unbound isotope was thoroughly removed by chromatography on a column with Sephadex G25 fine in the presence of 10 mM NaI. The content of free isotope did not exceed 0.5% of the total radioactivity as estimated by TCA precipitation of proteins. The control was critical because the diffusion coefficient for iodine is 100-fold higher than the diffusion coefficient measured for a protein. Contamination of free iodine higher than 2% would influence the results notably. The correspondence of the molecular weight of labeled proteins to estimated values of 180–450 kD was confirmed by analytical gel filtration on Sephacryl S 300 in buffer with albumin or in the presence of canine autocoagulated plasma. More than 70% of labeled rabbit antibody to canine fibrinogen bound specifically to the fibrinogen immobilized on CNBr-activated Sepharose 4 B.

Fresh citrated blood or plasma was used for the experiments. Clotting, incubations and washings of the gel were performed in 0.3 ml cylindrical wells of polystyrene 1 × 8 strips used for immunoassay (Titertek). Coagulation was initiated by raising the consentration of $CaCl_2$ to 15 mM, and 0.05 ml samples were immediately underlaid into the wells filled with 0.1 ml of PBS without mixing to produce sharp interface between liquids without meniscus. Gels with even smooth surface were formed within 2 h at 37°C. Blood clots were contracted, the height decreasing by ~30% while the gel was still tightly bound to the walls. The volume of plasma gels remained unchanged. After removal of liquid covering the gel it was washed by incubation with 0.2 ml PBS (phosphate buffer saline) containing 200 KIU aprotinin (trasylol) for 60 min at room temperature. This solution was replaced with 0.2 ml of solution containing ~1 μg (10^5 cpm) of labeled protein either in citrated canine plasma or in PBS with 1 mg/ml bovine serum albumin (BSA). Aprotinin was always present in this solution as mentioned before. After incubation with stirring in the thermostated microplate shaker (coefficient of external mass transfer $h_{ex} = 3.44 \times 10^{-6}$ m/s) the liquid phase was withdrawn and counted and the gel was rinsed with 0.2 ml PBS for ~30 s. Washed clots were dissolved by adding 0.2 mg of trypsin in 0.2 ml PBS and counted using the Minigamma counter (LKB). The percent of label accumulated in the gel related to total radioactivity was calculated for each well separately. Quadrate of this ratio was plotted against time to linearize the proportion $Q^2 \sim Dt$, where D is relative diffusivity and t is time.

RESULTS AND DISCUSSION

Experimental data fitted the linear approximations within inherent 20% error estimation. Diffusion coefficients were determined by relating slopes of experimental curves (Fig. 1) and the line calculated according to Fick's law applied to our conditions. Slope of the lines depends on diffusion coefficient D so that $tg = kD$, where K is the coefficient common for any molecule at the given conditions.

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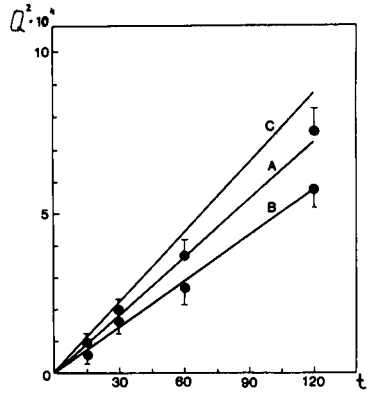

FIGURE 1 Accumulation of macromolecules into plasma gels and blood clots. AXIS: Q^2 refers to quadrate of ratio "counts in gel/total counts," t refers to time (min). (A) Experimental data for accumulation of immunocomplex ($^{[125\,I]}$antibody-fibrinogen) into canine plasma clot (average of four experiments); (B) same for canine whole blood clot (n = 4); (C) "theoretical" line, calculated according to Fick's law $Q/A = S \times (4Dt/\pi)^{1/2(*)}$ (4), where Q is the amount of accumulated molecules; A is the concentration of molecules in the stirred liquid; S is the contact surface (m^2); t is the time (s); D is the diffusion coefficient taken as 4×10^{-11} ($m^2\ s^{-1}$); and (*) for external mass transfer coefficient 3.44×10^{-6} m/s in our conditions this approximation is true from 1 s to 2 h of incubation.

The penetration of immunoglobulins, fibrinogen or antibody-fibrinogen complexes into gels in the presence of plasma was quite similar to plasma clots or fibrin gels ($D = 3.5 \times 10^{-11}\ m^2\ s^{-1}$), and was just slightly diminished for contracted blood clots ($D = 2.7 \times 10^{-11}\ m^2\ s^{-1}$). These data are close to diffusion coefficients of related molecules in liquid media ($D = 4 \times 10^{-11}\ m^2\ s^{-1}$) (5).

The structure of the blood clot formed in our conditions corresponds to the structure of venious thrombi where small pressure difference cannot compress the clot or tear it from the wall (6). Probably, these clots are partly contracted along the vessel axis. Our data demonstrate that diffusional exchange of macromolecules between such clots and the surrounding blood plasma is not restricted significantly. Some decrease of diffusion coefficient of macromolecules in blood clots in comparison with plasma clots may be attributed not to reduction of pores but to exclusion of effective contact surface by cells incorporated into blood clots. Otherwise, one could expect dependence on molecular dimensions which was not observed in our experiments.

Therefore, insufficient results with targeting to thrombi (7) are most probably caused not by sterical limitations but by low intensity of mass transfer into compartments with poor convection.

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REFERENCES

1. Okada, M., and B. Blomback. 1983. Factors influencing fibrin gel structure studied by flow measurement. *In* Molecular Biology of Fibrinigen and Fibrin. Annals of the New York Academy of Sciences, Vol. 408. M. W. Mosesson and R. S. Doolittle, editors. The New York Academy of Sciences, New York. 233–253.
2. Park, I. H., C. S. YR Johnson, M. R. Jones, and D. A. Gabriel. 1988. Probes of fibrin gel porosity. *In* Fibrinogen 3 Biochemistry, Biological Functions, Gene Regulation and Expression. Mosesson, M. W., D. L. Amrani, K. R. Siebenlist, and J. P. DiOrio, editors. Experta Medica, Amsterdam, 123–128.
3. Mustard, J. F. 1979. Platelets, vessel wall and thrombosis. *In* Haemostasis and Thrombosis: Proceedings of the Sereno Symposia, Vol. 15. G. G. N. Serneri and C. R. M. Prentice, editors. Academic Press, London. 77–90.
4. Sakharov, D. V., M. Yu. Matveyev, and S. P. Domogatsky. 1991. Binding of affinity ligand with a three-dimensional target: the role of diffusion limitations. *Biophysika.* 36:49–55.
5. Stenberg, M., M. Werthen, S. Theander, and H. Nygren. 1988. A diffusion limited reaction theory for a microtiter plate assay. *J. Immunol. Methods.* 112:23–29.
6. Marchesi, V. T. 1977. Disturbances of body water and circulation of blood. *In* Pathology. W. A. D. Anderson and J. M. Kissane, editors. The CV Mosby Company, Saint Louis, MO. 148.
7. Gargon, P. E., V. A. Ploplis, and J. D. Scheu. 1988. A fibrin specific monoclonal antibody which interferes with the fibrinolytic effect of tissue plasminogen activator. *Thromb. Haemostasis.* 59:426–431.