Fetal Plasma Contains Coagulation Factor XIIIa Inhibitor Absent in Normal Human Plasma

O. V. Mit'kevich, I. S. Kolotushkina, Yu. A. Blidchenko, and G. T. Sukhikh

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A protein inhibitor of fibrin-stabilizing coagulation factor XIII was isolated from fetal human plasma. The inhibitor is absent in newborns and adults. The purified protein is a 67-kD single-chain immunoglobulin; factor XIIIa activity in inhibited by 80% with 100 μl fetal plasma (16-22 weeks gestation) and by 100% with pure inhibitor (0.2 mg/ml). The persistence of this inhibitor and low concentration of factor XIIIa are probably responsible for umbilical and intracranial hemorrhage in preterm newborns.

Key Words: *factor XIII; inhibitor; fetal plasma*

Coagulation factor XIIIa belongs to the transglutaminase family catalyzing the formation of peptide bond between glutamine and lysine. Factor XIIIa is formed from inactive proenzyme after thrombin-catalyzed removal of a 37-amino acid N-terminal peptide [12]. Factor XIIIa is a polyfunctional enzyme: it stabilizes the clot by cross-linking of fibrin molecules, catalyzes binding of α_2-plasmin inhibitor to fibrin, which protects the clot from plasminolysis, and promotes wound healing by cross-linking of extracellular matrix proteins, in particular, fibronectin, collagen, and thrombospondin [7]. Factor XIIIa deficiency is associated with serious disorders such as sustained posttraumatic and postoperative hemorrhages, poor wound healing, abortions, umbilical and intracranial hemorrhage in newborns [9,11]. Current methods for assaying factor XIIIa concentration in fetal human plasma are not precise, since they are based on evaluation of lysis of a clot formed in the presence of test plasma [1,3]. In our study factor XIIIa concentration was determined by the direct quantitative method of enzyme-linked immunosorbent assay [8] and its activity was measured by dansyl cadaverine incorporation into casein (fluorescence method) [5]. These methods allowed as to study the effects of human fetal plasma inhibitor on factor XIIIa activity. About 15 cases of multiple hemorrhages associated with the presence of factor XIIIa inhibitors in patients aged from 9 to 78 (some cases with lethal outcome) have been described [2], but these inhibitors were never detected in children under 9. All factor XIIIa inhibitors were proteins, most of them were immunoglobulins. These antibodies interfere factor XIIIa-fibrin/fibrinogen interaction or inhibit its transglutaminase activity [2,4,9]. Human fetal inhibitor identified by us also belongs to immunoglobulins and directly inhibits factor XIIIa enzyme activity. Our findings are of practical importance, since some authors reported beneficial effect of factor XIIIa therapy in preterm newborns with intracranial hemorrhages [10,11].

MATERIALS AND METHODS

Fetal plasma (0.5 ml) was obtained from 16-22-week fetuses of healthy women via heart puncture (19-G plastic syringe). Pregnancy was interrupted (prostaglandin F_{2a}, enzaprost) for social reasons in accordance with Helsinki Declaration, Special Resolution of Ministry of Health of the Russian Federation and Russian Ethical Committee. Newborn plasma was obtained from the umbilical cord immediately after delivery.

Factor XIIIa concentration was measured by sandwich enzyme-linked immunosorbent assay in 96-well

Russian Cardiology Research-and-Production Complex, Ministry Of Health, Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, Moscow

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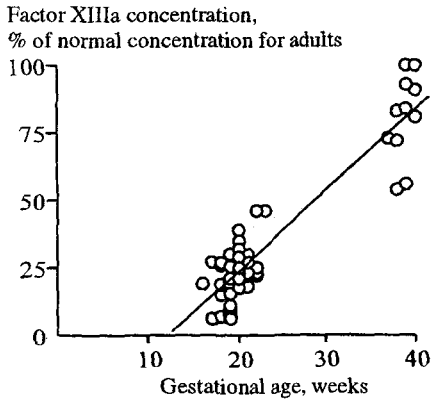

Fig.1. Correlation between concentration of factor XIIIa in fetal plasma (% of adult) and gestational age (16-22 weeks). Factor XIIIa concentration was measured by sandwich enzyme-linked immunosorbent assay. Here and in Fig. 2: gestational age 38-40 weeks corresponds to full-term newborns.

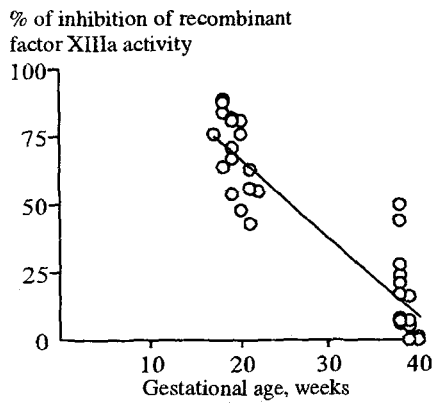

Fig. 2. Correlation between activity of fetal factor XIIIa inhibitor (% of inhibition of recombinant factor XIIIa activity) and gestational age.

plates [8]. To this end, polyclonal goat antibodies against human factor XIII (0.1 mg antibodies in 50 μl 50 mM Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl) were incubated in multiwell plates at 37°C for 1 h or at 4°C overnight. Nonspecific binding sites were saturated with 0.2% casein with 0.1% Tween-20 in the same buffer (200 μl/well, 1 h at room temperature). Fetal plasma (50 μl) was added to each well for 1 h (room temperature). Rabbit polyclonal antibodies against human factor XIII were used as second antibodies (0.75 mg in 50 μl per well, 1 h at room temperature). Peroxidase substrate o-phenylenediamine (0.1 mg/ml, 100 μl per well) with 0.03% hydrogen peroxide in 0.02 M citrate buffer (pH 4.7) was added to wells. The reaction was stopped with 50 μl 50% sulfuric acid and the optical density was measured at 492 nm (Labsystem multiscan).

Factor XIIIa activity was measured by a fluorescent method based on the enhancement of dansyl cadaverine fluorescence upon binding with dimethylcasein [5,6]. Reaction between 0.02 M dansyl cadaverine (Sigma) and 5% bovine dimethylcasein (Sigma) was carried out in 1 ml borate buffer (0.1 M, pH 9) in the presence of 5 mM dithiothreitol and 10 mM $CaCl_2$. The concentration of recombinant factor XIIIa (Zymo-Genetics) was 0.016 mg/ml. Inhibitor activity was measured by adding 50-150 μl fetal plasma or purified inhibitor to the incubation medium. Fluorescence was measured at λ_{ex}=360 nm and λ_{em}=510 nm on a Hitachi P-3000 spectrofluorimeter.

Preactivation of recombinant factor XIIIa was carried our in 50 mM Tris-HCl (pH 7.4) containing 0.15 M NaCl; 2 mg/ml recombinant factor XIIIa was incubated with human thrombin (10 U/0.1 mg factor XIIIa, Sigma) in the presence of trace amounts of fibrinogen (0.005 mg/ml, Sigma) and 10 mM $CaCl_2$ at 37°C for 40 min. The reaction was stopped with recombinant hirudin (provided by M. D. Ter-Avanesyan) in a concentration of 10 U/0.1 mg factor XIIIa.

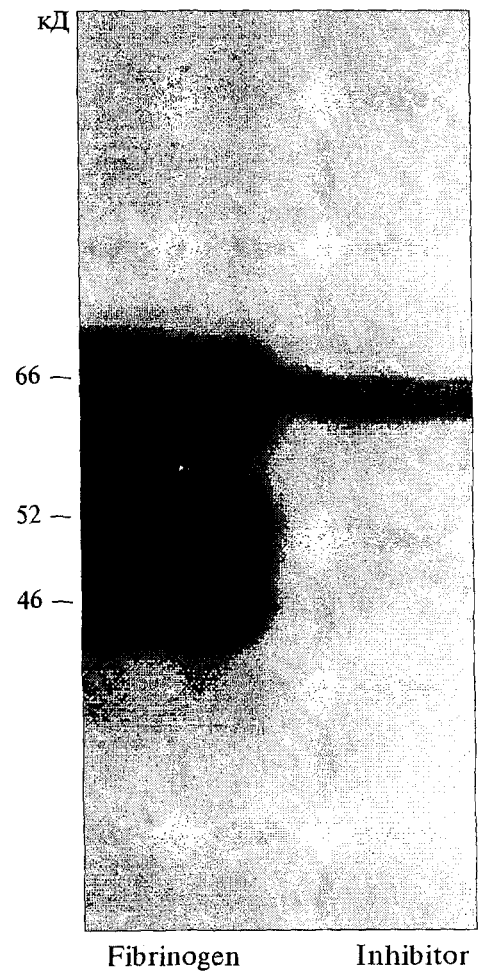

Fig. 3. Electrophoregram of purified inhibitor from fetal human plasma (9% polyacrylamide gel-electrophoresis according to Laemmli under reducing conditions, Coomassie staining).

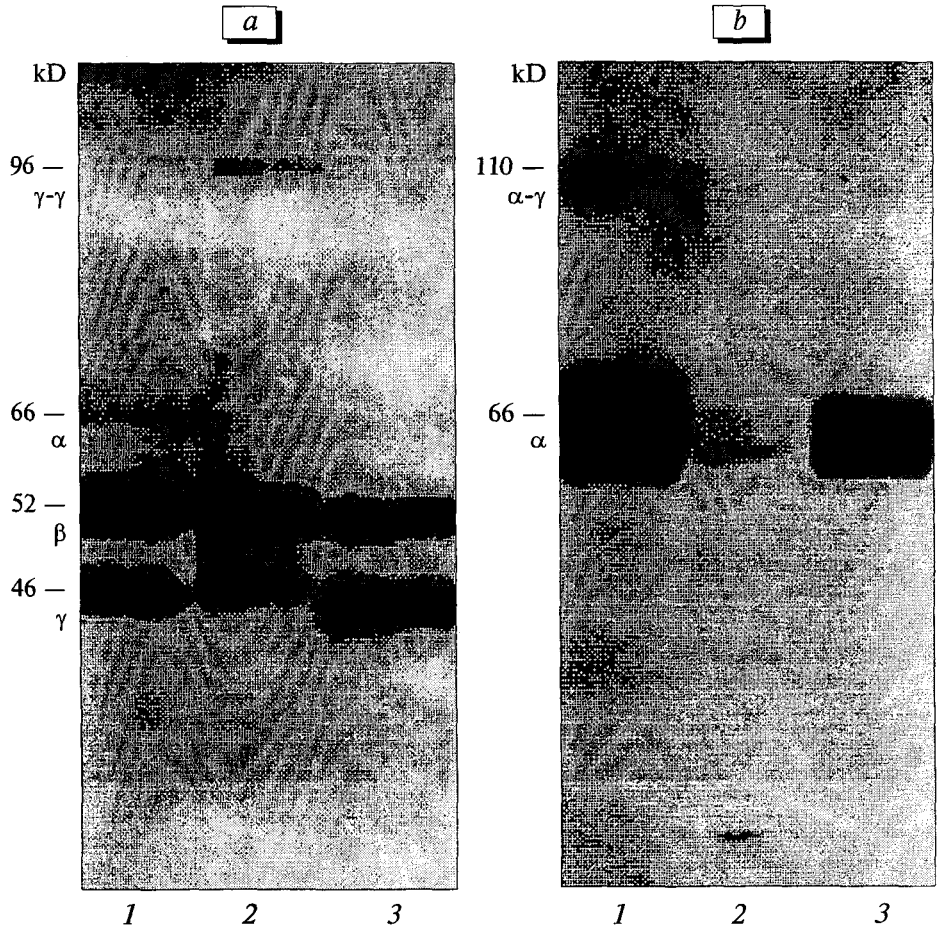

Fig. 4. Inhibition of fibrinogen cross-linking by recombinant factor XIIIa in the presence of purified fetal inhibitor. *a*) electrophoregram, *b*) Western-blot. Intact fibrinogen (*1*), fibrinogen in the absence(*2*) and presence (*3*) of fetal factor XIIIa inhibitor. *a*) 9% SDS-polyacrylamide gel electrophoresis according to Laemmli under reducing conditions, Coomassie staining; *b*) interaction with peroxidase-conjugated monoclonal antibodies to fibrinogen α-chain (5A2). Reaction mixture (75 μl) contained 1.7 mg/ml fibrinogen, 0.9 mg/ml inhibitor, 0.05 mg/ml factor XIIIa, 20 mM $CaCl_2$, 50 mM Tris-HCl, and 0.01 NaCl (pH 7.4); overnight incubation at 37°C.

RESULTS

The concentration of factor XIII was measured in 37 plasma samples from human fetuses (gestation weeks 16-22) and 11 newborns (38-40 weeks) by enzyme-linked immunosorbent assay. The content of factor XIII in 16-17-week fetuses constituted 8-10% of its normal concentration in adults (10 μg/ml), but this parameter markedly rose during prenatal ontogeny and attained 80% at birth (Fig. 1).

In the next experimental series we compared cross-linking activity of fetal and normal factor XIII. To this end, serial dilutions of defibrinated plasma were incubated for 1 h at 37°C with 2 mg/ml fibrinogen (Sigma) in 0.25 ml 50 mM Tris-HCl (pH 7.4) containing 0.15 M NaCl, 2 mM $CaCl_2$, and 1 U/ml thrombin (Sigma) and clots lysis with 5% monochloroacetic acid was assessed. It was found that activity (per mg protein) of fetal factor XIIIa (from 18-week fetus) was 4-fold lower than activity of normal factor XIIIa, which probably indicates structural differences between fetal and normal factor XIIIa (electrophoretic mobility of these two factors was identical), or the presence of a factor XIIIa inhibitor in fetal plasma. To verify this assumption we studied the effect of fetal plasma on activity of recombinant factor XIIIa (GomoGenetics). Fetal plasma inhibited recombinant factor XIII and this effect inversely correlated with the gestational age (Fig. 2). Newborn plasma inhibited recombinant factor XIIIa by about 20%, while plasma from adults possessed no inhibiting activity.

When analyzing chemical nature of factor XIIIa inhibitor, we first attempted to find out whether or not this inhibitor is a protein. To this end, fetal plasma was fractionated by precipitation with ammonium sulfate (Sigma). The inhibitor precipitated with 2.53 M ammonium sulfate, and hence is a protein. Then immunoglobulins were isolated from the protein fraction by adsorption on Protein-A-Sepharose (Sigma) [9]. The inhibitor bound to Protein-A-Sepharose can be

eluted with 0.1 M citrate buffer (pH 5.0, fraction 1) and then with 0.1 citrate buffer (pH 3.0, fraction 2). Fraction 1 protein was 2-fold more active (data not shown). The purified inhibitor was a protein with molecular weight about 67 kD (determined by electrophoresis according to Laemmli, Fig. 3). The corresponding band on the blot interacts with antihuman immunoglobulin antibodies (data not shown). Hence, factor XIIIa inhibitor identified in human fetal plasma is a protein similar to an immunoglobulin heavy chain.

Special experiments demonstrated that this fetal inhibitor suppressed cross-linking of fibrin/fibrinogen α-chains catalyzed by recombinant factor XIIIa (Fig. 4).

Thus, a protein (immunoglobulin) inhibitor of factor XIIIa was identified in human fetal plasma, which is absent in normal plasma from adults. This finding is of fundamental and practical importance, since the observed phenomenon suggests different regulation of factor XIIIa activity in human fetuses and adults. Preterm newborns often had sustained umbilical and intracranial hemorrhages associated with low activity of factor XIIIa. Sometimes intracranial hemorrhages in newborns were stopped by recombinant factor XIIIa therapy [9,10]. On the other hand, intracranial hemorrhage can be stopped by an non-invasive method, for instance, by removal of factor XIIIa inhibitor (plasmapheresis on a column with immobilized factor XIIIa). Further investigations of the phenomenon of decreased plasma factor XIIIa activity (or high concentrations of factor XIIIa inhibitor) in newborns with intracranial hemorrhages are required.

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