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SUPPRESSIVE EFFECT OF HUMAN BLOOD COAGULATION FACTOR XIII ON THE VASCULAR PERMEABILITY INDUCED BY ANTI-GUINEA PIG ENDOTHELIAL CELL ANTISERUM IN GUINEA PIGS

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Abstract

We investigated the effect of blood coagulation factor XIII(FXIII) on enhanced permeability induced by anti-endothelial cell antiserum, that was produced by the immunization of guinea pig endothelial cells with adjuvant into rabbits repeatedly. We have found that this antiserum reacts to human and guinea pig endothelial cells but not guinea pig fibroblast cells. The permeability was enhanced by intradermal injection of 400-fold dilution of this antiserum into dorsal skin of guinea pigs. The mixture of equal volume of antiserum and FXIII was intradermally injected into dorsal skin of guinea pig after Evans blue injection, and 15 minutes later the quantity of Evans blue at the each injection site was determined. We recognized the suppressive effect of FXIII on the dye leakage. We also studied the suppressive effect on swelling induced by the antiserum. After the subcutaneous injection of the mixture of antiserum and FXIII into the back of guinea pigs, we measured the thickness of skins at the injection site after day 1, 2 and 3. As a result, FXIII significantly suppressed the swelling. We found that FXIII suppresses the acute and subacute permeability enhancement. These results suggest that FXIII plays an important role on an inflammatory site and that it may exert as an anti-inflammatory protein.

Blood coagulation factor XIII (FXIII), the last enzyme in the

Key words: Factor XIII, anti-endothelial cell antiserum, vascular permeability, anti-inflammatory protein, Schönlein Henoch purpura

blood coagulation cascade, is a transamidase that catalyzes the formation of γ - gultamyl - ε - lysyl peptide crosslinks between polypeptide chains in adjacent fibrin monomers and other plasma proteins(1,2,3). Crosslinks of each fibrin molecule caused a marked increase in the rigidity of the clot network(4). On the other hands, the crosslinks between fibrin and cellular matrix protein such as fibronectin may exert to connect fibrin molecules with the injury sites(5). It is well known that clots play an important role in the prevention of further tissue damage and in subsequent wound healing(6). Schönlein Henoch purpura (SHP) is characterized by hemorrhagic skin lesions, abdominal symptoms including gastro-intestinal bleeding, renal involvement with proteinuria and hematuria and swelling of joints(7). The symptoms are ascribed to generalized inflammation of arterioles and capillaries. That is, the local changes of the coagulation and fibrinolytic system due to immunoreaction were induced in the affected vessels. In 1977, Henriksson and colleagues described a lowering of FXIII activity during the acute phase of this disease(8). The mechanism of the decrease of FXIII activity in the acute phase of SHP has not yet been clarified. Destruction of FXIII molecules by protease derived from leukocytes which migrated into the inflammation sites has been proposed(9). In this connection, Kamitsuji and Fukui et al. reported that the administration of FXIII concentrate may contribute to the improvement of gastro-intestinal complications of SHP patients(10). Recently FXIII concentrate (Fibrogammin P) is used for the treatment of SHP patients(11). According to Matsuoka(12), Bowie et al.(13) and Ito et al.(14), this vasculitis of SHP is regarded as the immunovascular disease that antibody-antigen complexes on the vascular capillary endothelial cells enhances the vascular permeability. Consequently non-thrombocytopenic purpura caused by the injection of anti-endothelial cell antiserum(15). In the present study, we investigated whether or not human FXIII suppresses the enhancement of permeability and swelling induced by anti-endothelial cell antiserum in guinea pigs.

MATERIALS AND METHODS

Materials

Materials were purchased from the following suppliers: Dulbecco phosphate buffer, Dulbecco MEM, FCS(Gibco, USA), ECGS(Calbiochem, USA), Freund's adjuvant(Difco, USA), FITC conjugated anti-rabbit IgG(Cappel, USA), Evans blue, potassium hydroxide(KOH, Kanto Kagaku, Japan), phosphoric acid(Wako Pure Chemical, Japan), Guinea pig complement(Kyokuto, Japan), and Human FXIII(Fibrogammin P, Behringwerke, FRG).

Preparation of anti-guinea pig endothelial cell antiserum
Guinea pig endothelial cells were isolated from the main artery and vena cava(16), then cells were inoculated into tissue culture dishes and incubated for several days with Dulbecco MEM containing 15% FCS and 37.5 μg/ml ECGS till reaching confluency. Confluent monolayer was harvested by a cell scraper. The cells were rinsed twice with Dulbecco phosphate buffered saline(pH 7.2). These cells were used as an antigen for the production of anti-endothelial cell antiserum. The antiserum was obtained from rabbits immunized with emulsion of Freund's complete adjuvant with guinea pig endothelial cells, and boosted with emulsion of Freund's incomplete adjuvant. After several times of boosting, the antibody titer was measured with guinea pig endothelial cells by the methods of cytolysis and indirect immunofluorescence microscopy using FITC conjugated anti-rabbit IgG(17).

Measurement of antibody titer of anti-endothelial cell antiserum
Confluent monolayer of guinea pig endothelial cells in a 96-well plate was incubated with 50 μl of variously diluted antiserum in Dulbecco MEM-15% FCS for 30 min. The medium was then replaced to 50 μl of 5% guinea pig complement in Dulbecco MEM-15% FCS and the cells were further incubated for 30 min. After addition of 10 μl of trypan blue solution, the cell layers were photographed to evaluate the extent of cell lysis. Indirect immunofluorescence microscopy was done as follows. The antiserum was serially diluted two times. The diluted antiserum was then incubated with the main artery at room temperature for 1 hour and rinsed 3 times with Dulbecco phosphate buffer. After washing, 1000-fold dilution of FITC conjugated anti-rabbit IgG was added to the sections, incubated for 30 minutes at room temperature, and washed 3 times with Dulbecco phosphate buffer. All sections were observed by a Nikon microscope equipped with a mercury lamp. The titer was taken as a highest dilution which gave a fluorescent staining just above the background staining of normal serum controls.

Duration of activity of permeability enhancement
Measurement of permeability was studied according to Yamamoto et al.(18). A 100 μl portion of 50-fold diluted antiserum was intradermally injected into the back of a guinea pig before intravenous injection of 0.5 ml of 1 % Evans blue. After 15 minutes of the Evans blue injection, the back skins were harvested and the blue lesions were observed.

Suppressive effect of FXIII on the permeability enhancement
A 100 μl portion of either each diluted antiserum or the mixture of equal volume of FXIII and the diluted antiserum was

intradermally injected into the dorsal skin of guinea pigs after intravenous injection of Evans blue. After 15 minutes, skins were harvested and blue lesions in the skins were observed.

Extraction of Evans blue from guinea pig skins
Evans blue was extracted from skins, soaked with 1 ml of 1 M KOH solution, and incubated at 37°C overnight. After the incubation, 3 ml of 0.6 N phosphoric acid and 3 ml of FRIGEN(Behringwerke, FRG), a defatting agent, was added to each tube and mixed for 30 sec. with a Vortex mixer. Each tube was centrifuged at 3000 rpm for 15 minutes, and the absorbance of the supernatant was measured at 620 nm(19).

Suppressive effect of FXIII on the swelling
One milliliter of equal volume mixture of FXIII and the intact antiserum was subcutaneously injected into the dorsum of guinea pigs. After days 1, 2 and 3, the skins were harvested and the thickness was measured with a slide caliper at injection sites as a marker of swelling. The swelling was shown by the difference of the thicknesses between a injection and a non-injection site.

RESULTS

Characterization of polyclonal anti-guinea pig endothelial cell antiserum
The antibody titer was determined with guinea pig endothelial cells by the methods of cytolysis and indirect immunofluoresence microscopy using FITC conjugated anti-rabbit IgG. As a result, the 50% cytolysis was observed by the 60-fold dilution of antiserum, and the fluorescence was observed by 400-fold dilution. The antiserum exhibited the reactivity with not only guinea pig but also human endothelial cells. However it did not react with guinea pig fibroblasts. When the cryosection of the main artery of a guinea pig was used for the indirect immunofluorescence test, the fluorescence was observed on the inner membrane which was seemed to be endothelial cell. It was also found that the antiserum reacted with the extracellular matrix proteins produced by endothelial cells(data not shown).

Enhanced permeability
First, we studied whether this antiserum induced the permeability in guinea pigs. The variation of permeability after intradermal injection is shown in Fig. 1. The permeability reached the maximum within 5 minutes. This activity for enhancing the permeability almost disappeared within 30 minutes after the injection. This permeability enhancing phenomenon was classified as a short lasting reaction. We next investigated the dose response of this antiserum. As shown in Fig. 2, the activity of

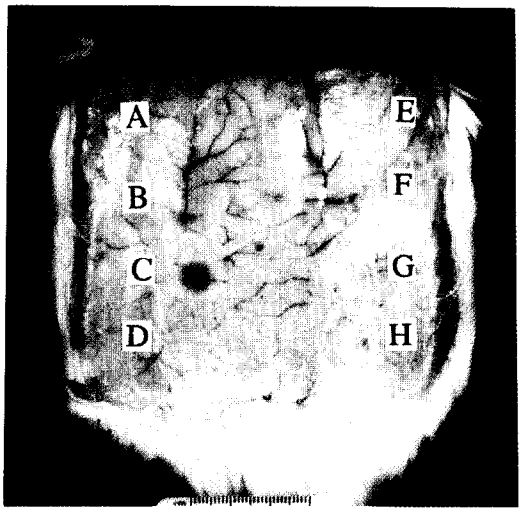

FIG. 1.

Time course of permeability enhancement induced by anti-endothelial cell antiserum. Antiserum was injected into a guinea pig at varying times before intravenous dye injection. Time 0 means an intradermal injection immediately after intravenous dye injection. (A): antiserum, 60 min, (B): antiserum, 30 min, (C): antiserum, 0 min, (D): saline, 30 min, (E): rabbit serum, 60 min, (F): rabbit serum, 30 min, (G): rabbit serum, 0 min, (H): saline, 0 min

enhancing the permeability is recognized by 400-fold dilution of antiserum. The effect of FXIII was examined on the vascular permeability induced by the antiserum. In this experiment, the mixture of antiserum was injected with various concentration of FXIII. As shown in Fig. 3, FXIII shows the suppressive effect on the dye leakage in a dose dependent fashion. We obtained a result that both 200-fold and 400-fold diluted antiserum exhibit the same tendency. Thus the effect of FXIII was examined in 10 guinea pigs and the dye leakage was measured in extravascular space. As shown in Fig. 4, FXIII exhibited the suppressive effect in a dose dependent manner.

Suppressive effect of FXIII on the swelling

When the antiserum was subcutaneously injected into a dorsal skin of guinea pig, edema, in addition to hemorrhage was observed at injection site(20). Thus we examined the suppressive effect of FXIII on the swelling. On injecting the mixture of FXIII and antiserum, the edema was significantly suppressed by FXIII on day 1 and 2(Fig. 5). This result indicates that FXIII suppresses the permeability in the acute and the subacute phase as well.

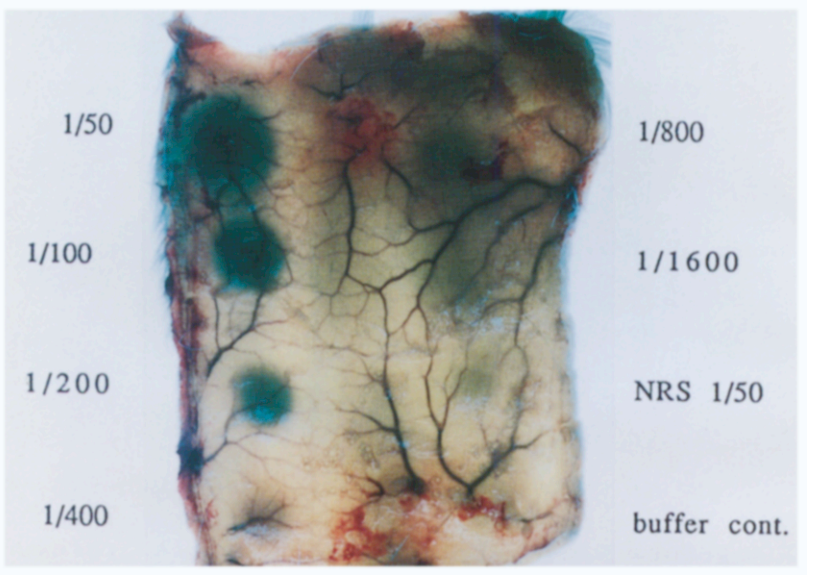

FIG. 2.

Dose response of anti-endothelial cell antiserum in a guinea pig. Each sample was injected immediately after a intravenous dye leakage. NRS: Normal rabbit serum

DISCUSSION

For more than 20 years after its detection of FXIII, many authors have reported that a clotting factor, FXIII, influenced a lot of other systems and thus it was often termed a connective tissue factors (21). The fibrin stabilizing effect is an example of general properties of this factor which crosslinks proteins with suitably configurated ε - lysyl- and γ - glutamyl - residues. Many kinds of proteins are listed as substrates for FXIII, e.g. fibrin(1), collagen(22), fibronectin(5), actin(23) and factor V(24). In this context, the binding of biogenic amines to proteins by FXIII may also participate in the elimination of toxic substances like histamine. FXIII concentrate has been recently used not only for the promotion of the wound healing but also for the treatment of Shönlein Henoch Purpura(SHP)(6,10). The clinical effects of FXIII on SHP are probably due to the stabilization of microvasculature leading to a reduction of the leakage at inflammatory sites. Pilger et al(25) has reported that FXIII shows the suppressive/sealing effect in a screlodermia patient. However none of these reports showed the sealing/suppressive effect on the permeability by FXIII in animal studies. This vasculitis of SHP is regarded as the immunovascular disease that

the vascular permeability is enhanced by the formation of the antigen-antibody complex not with standing ambiguity of trigger which may include drugs, foods, insect bites or bacterial infections(11,12,13,14). Thus we tried to demonstrate the suppressive effect of FXIII on permeability enhancement induced by anti-endothelial cell antiserum. As shown in Figs. 1 and 2, anti-endothelial cell antiserum induces the enhancement of permeability. This phenomenon can be caused by factors such as complement fragments and histamine etc. which are produced by the activation of complement system after complex formation of antiserum with endothelial cells(11,12,13,14). As this phenomenon shows the dose dependent manner by antiserum, condition of SHP patients may be influenced seriously depending on the extent of the antibody generation. SHP patients show the increase of plasma level of IgA and the imbalance of serum IgG subclass and IgM(13,14,26). As shown in Figs. 3, 4 and 5, FXIII suppresses the vascular permeability in acute phase and the edema in subacute phase. These results are supported by some clinical studies. Kamitsuji et al.(10) and Fukui et al.(11) have reported that FXIII shows the suppressive effect on the swelling of joints of SHP patients.

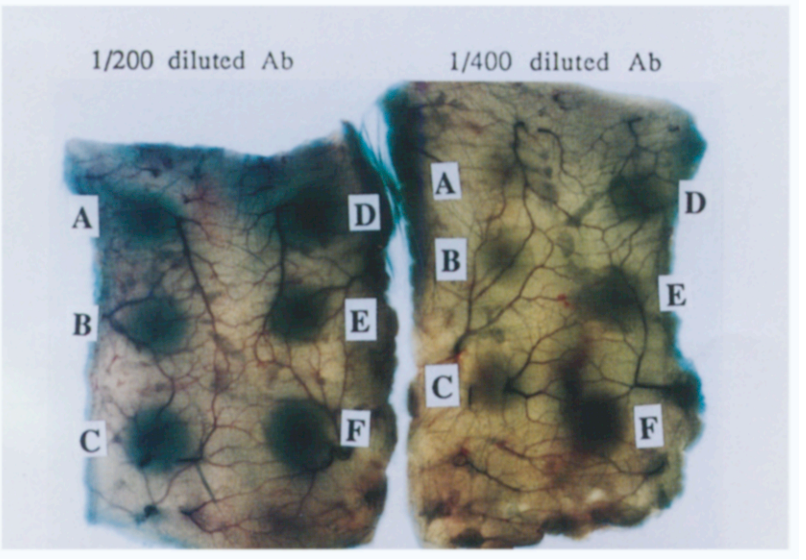

FIG. 3.

Suppressive effect of FXIII on the permeability enhancement induced by anti-endothelial cell antiserum. FXIII was used with the final concentration at a injection site of (A), 3.0 U; (B), 1.5 U; (C), 0.75 U; (D), 0.38 U; (E), medium control. The mixture of FXIII and either 200- or 400- fold diluted antiserum was injected immediately after the intravenous injection of dye.

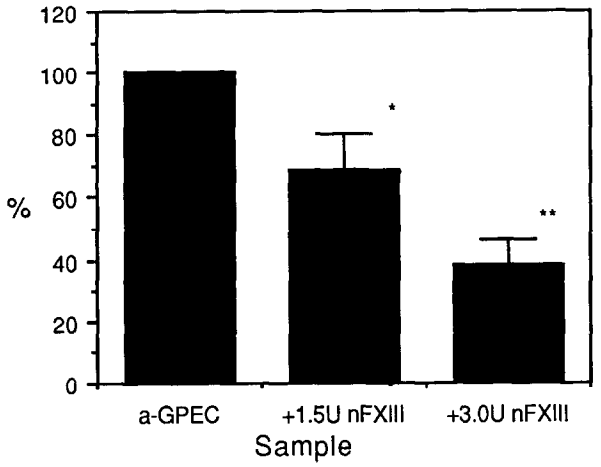

FIG. 4

Suppressive effect of FXIII on the permeability induced by anti-guinea pig endothelial cell antiserum. Extraction of Evans blue at the injection site was according to the materials and methods. n=10, *: $p<0.05$, **: $p<0.01$ In this experiment, we used the 300-fold diluted anti-endothelial cell antiserum as a permeability inducer. FXIII was mixed with antiserum, then the mixture was injected intradermally.

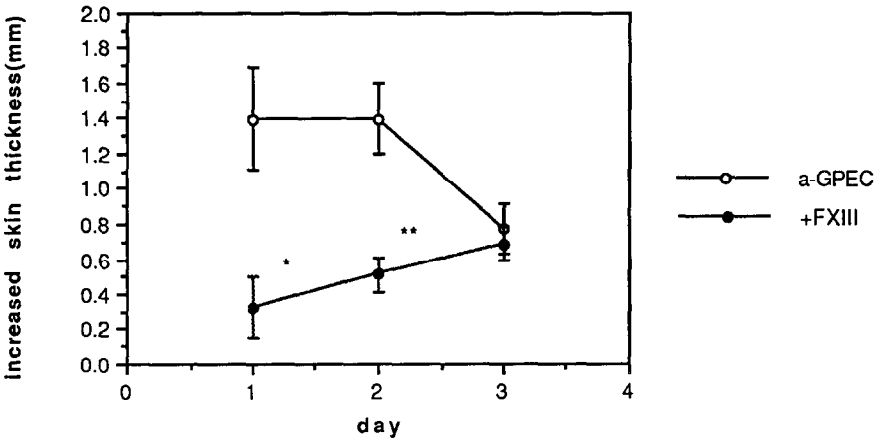

FIG. 5.

Effect of FXIII on the swelling induced by anti-guinea pig endothelial cell antiserum. Open circle (o) denotes the antiserum alone. Closed circle (●) denotes the FXIII plus antiserum. n=5, *: $p<0.05$, **: p,0.01

Pilger et al.(25) reported that FXIII also shows the suppressive effect on vascular permeability in sclerodermia patients. These results suggest that FXIII may crosslink cellular matrices to prevent the opening of the space between cells(27) and that it may crosslink the enhancing factors for the permeability(21). We have succeeded in demonstrating the suppressive effect of FXIII on vascular permeability in an animal study. This study indicates that FXIII may play a crucial role in an inflammatory site. Consequently it seems that FXIII therapies are necessary for the treatment of some inflammatory diseases (28,29).

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