Fitzgerald Factor (High Molecular Weight Kininogen) Clotting Activity in Human Plasma in Health and Disease in Various Animal Plasmas

By Hidehiko Saito, George Goldsmith, and Robert Waldmann

Fitzgerald factor (high molecular weight kininogen) is an agent in normal human plasma that corrects the impaired in vitro surface-mediated plasma reactions of blood coagulation, fibrinolysis, and kinin generation observed in Fitzgerald trait plasma. To assess the possible pathophysiologic role of Fitzgerald factor, its titer was measured by a functional clot-promoting assay. Mean ± SD in 42 normal adults was 0.99 ± 0.25 units/ml, one unit being the activity in 1 ml of normal pooled plasma. No difference in titer was noted between normal men and women, during pregnancy, or after physical exercise. Fitzgerald factor activity was significantly reduced in the plasmas of eight patients with advanced hepatic cirrhosis (0.40 ± 0.09 units/ml) and of ten patients with disseminated intravascular coagulation (0.60 ± 0.30 units/ml), but was normal in plasmas of patients with other congenital clotting factor deficiencies, nephrotic syndrome, rheumatoid arthritis, systemic lupus erythematosus, or sarcoidosis, or under treatment with warfarin. The plasmas of 21 mammalian species tested appeared to contain Fitzgerald factor activity, but those of two avian, two reptilian, and one amphibian species did not correct the coagulant defect in Fitzgerald trait plasmas.

FITZGERALD TRAIT is an asymptomatic disorder of blood coagulation with a prolonged partial thromboplastin time related to the deficiency of Fitzgerald factor.[1] The plasma of the index patient displayed abnormalities in other surface-mediated plasma reactions, including kinin generation, fibrinolysis, and enhancement of vascular permeability by diluted plasma (PF/Dil).[2] Fitzgerald factor has been isolated from normal human plasma free of other known clotting factors.[3] Five additional unrelated individuals have recently been described who have the same clotting defect as Fitzgerald trait.[4-8] Wuepper et al.[5] first identified an agent deficient in their patient's plasmas as high molecular weight (HMW) kininogen. This finding has been confirmed in other individuals with the same clotting defect.[3,4,6,7] Thus Fitzgerald factor appears to be identical to HMW kininogen, a component of the plasma kinin-generating system.

The site of action of Fitzgerald factor in the blood clotting sequence is not

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fully elucidated, but, as tested in vitro, it appears to participate in the early steps of the intrinsic pathway of thrombin formation subsequent to activation of Hageman factor (factor XII) and Fletcher factor (a plasma prekallikrein).[3] To assess the possible pathophysiologic role of Fitzgerald factor (HMW kininogen), we have examined variations in its clotting activity among normal human subjects and animals, and in pathologic conditions.

MATERIALS AND METHODS

Pooled normal human plasma, plasma from normal individuals (21 males and 21 females, age 20–40), plasma from patients with congenital clotting factor deficiencies and animal plasmas were prepared or obtained as described earlier.[9,10] Fletcher trait[11] plasmas were kindly supplied by Dr. C. F. Abildgaard, University of California, Davis, and by Dr. Jeanette Soria and Dr. Claudine Soria, Paris. Plasmas from patients with Fitzgerald trait,[4,8] Flaujeac trait,[5] Williams trait,[6] and Reid trait[7] were obtained through the courtesy of Dr. V. H. Donaldson, University of Cincinnati College of Medicine, Cincinnati, Dr. R. Fenning, Charlotte Memorial Hospital, Charlotte, N.C., Dr. K. Wuepper, University of Oregon, Portland, Dr. R. W. Colman, University Hospital of Pennsylvania, Philadelphia, and Dr. C. L. Lutcher, Medical College of Georgia, Augusta, respectively. Citrated plasma from an iguana was kindly supplied by Dr. A. M. Leash, Case Western Reserve University, Cleveland, Ohio.

Seven normal male volunteers underwent exercise at 90% age-corrected maximal effort for 5 min on a Schwinn bicycle ergometer exerciser.[12] Blood was drawn before and immediately after exercise. Blood samples were obtained from volunteer healthy pregnant subjects (second and third trimesters) undergoing routine antenatal care. Plasmas from patients with hepatic cirrhosis, with chronic renal failure, nephrotic syndrome, rheumatoid arthritis, systemic lupus erythematosus (SLE), sarcoidosis, and disseminated intravascular coagulation (DIC), or who were under treatment with warfarin were obtained at University Hospitals of Cleveland and Henry Ford Hospital, Detroit. The diagnosis of DIC was inferred from the presence of a prolonged thrombin time, reduced plasma fibrinogen, decreased platelet count, and a positive test for fibrin-related antigen (fibrin degradation products), as detected by the Thrombo-Wellco test (Wellcome Reagents, Ltd., England). Venipuncture in patients and normal subjects was performed after informed consent for the procedure was obtained and in accordance with the principals of the Declaration of Helsinki. Citrated plasmas were used in all studies.

Kaolin (Fisher Scientific Co., Fair Lawn, N.J.), Centrolex "0" (Central Soya Co., Chicago, Ill.), a crude preparation of soybean phosphatides, and kaolin-Centrolex were prepared as described earlier.[13]

Barbital-saline buffer, pH 7.4, contained 2.76 g of barbital, 7.3 g of sodium chloride, and 2.06 g of sodium barbital per liter.

Assays of Fitzgerald factor clot-promoting activity were performed by the method reported earlier.[3] In essence, a volume of 0.1 ml each of the test sample, suitably diluted in barbital-saline buffer (usually 40-fold), kaolin-Centrolex, and plasma from the index individual with Fitzgerald trait were incubated together for 8 min at 37°C in a 10 × 75-mm glass tube. The mixture was then recalcified with 0.1 ml prewarmed 0.025 *M* $CaCl_2$, and the clotting time was measured

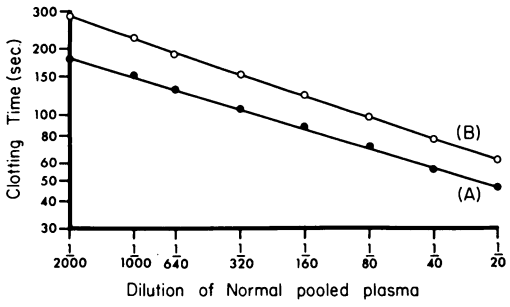

Fig. 1. Standard curves for Fitzgerald factor assay. Standard calibration curves were prepared by assaying serial dilutions of a standard pool of normal citrated plasma with (A) 0.1 ml and (B) 0.05 ml Fitzgerald trait plasma as a substrate.

Table 1. Fitzgerald Factor Activity in Some Physiologic Conditions

Conditions	No. of Subjects	Fitzgerald Factor Activity* (units/ml)
Normal men	21	0.99 ± 0.26
Normal women	21	0.99 ± 0.24
Pregnancy	13	0.99 ± 0.22
Physical exercise		
Preexercise	7	1.07 ± 0.41
Postexercise	7	1.05 ± 0.38

*Mean ± SD.

at 37°C. In some experiments, the assay was modified by using 0.05 ml each of the test sample and Fitzgerald trait plasma, while the volume of the other reagents was 0.1 ml each. Clotting times were converted to percentage activity by interpolation into a calibration curve prepared by assaying serial dilutions of a standard pool of normal plasma.[9] In both assay systems, a linear relationship existed between the logarithm of the clotting time and the logarithm of 1:20 to 1:2000 dilutions of the normal pooled plasma (Fig. 1). One unit of Fitzgerald factor activity was arbitrarily defined as that amount present in 1 ml of a standard pool of normal citrated plasma. Antihemophilic factor (AHF, factor VIII) activity was assayed as described previously.[9]

RESULTS

Fitzgerald factor (HMW kininogen) activity in 21 normal men was 0.99 ± 0.26 units/ml (mean ± SD), and that in 21 normal nonpregnant women was 0.99 ± 0.24 units/ml; thus there was no observable difference between sexes (Table 1). Fitzgerald factor activity was also normal in 13 pregnant women. There was no change in Fitzgerald factor activity after strenuous physical exercise in seven normal men, although the titer of AHF activity rose significantly in these subjects.

The level of Fitzgerald factor (HMW kininogen) was greatly reduced in the plasmas of eight patients with liver cirrhosis ($p < 0.001$) and ten patients thought to have undergone disseminated intravascular coagulation ($p < 0.005$) (Table 2). In nine patients with chronic renal failure, the titer of Fitzgerald factor was at a slightly decreased level ($p < 0.005$). In contrast, Fitzgerald factor activity was normal in plasmas of ten patients undergoing therapy with warfarin, six patients with nephrotic syndrome, seven patients with rheumatoid arthritis, eight patients with SLE, and five patients with sarcoidosis.

Table 2. Fitzgerald Factor Activity in Some Pathologic Conditions

Condition	No. of Subjects	Fitzgerald Factor Activity* (units/ml)	p Value
Normal	42	0.99 ± 0.25	
Liver cirrhosis	8	0.40 ± 0.09	< 0.001
Warfarin treatment	10	0.91 ± 0.10	
Chronic renal failure	9	0.75 ± 0.12	< 0.005
Nephrotic syndrome	6	1.09 ± 0.20	
Rheumatoid arthritis	7	0.92 ± 0.20	
SLE	8	0.86 ± 0.28	
Sarcoidosis	5	1.10 ± 0.19	
DIC	10	0.60 ± 0.30	< 0.005

*Mean ± SD.

Table 3. Fitzgerald Factor Activity in Some Congenitally Deficient Plasmas

Conditions	No. of Subjects	Fitzgerald Factor Activity* (units/ml)
Normal	42	0.99 ± 0.25
Fitzgerald trait	3	< 0.01
Flaujeac trait	1	< 0.01
Williams trait	1	< 0.01
Reid trait	1	< 0.01
Hageman trait	16	0.91 ± 0.20
Fletcher trait	2	0.96, 0.55†
PTA deficiency	10	1.05 ± 0.20
Christmas factor deficiency	5	0.83 ± 0.17
AHF deficiency	9	0.93 ± 0.20
von Willebrand's disease	6	1.00 ± 0.36
Stuart factor deficiency	1	0.64†
Factor VII deficiency	2	0.91, 0.88†
Factor V deficiency	1	0.95†
Combined deficiency of factor V and AHF	2	0.84, 0.51†

*Mean ± SD.
†Individual data given.

The patients reported by Donaldson et al.,[4] Wuepper et al.,[5] Colman et al.,[6] Lutcher et al.,[7] and Fenning et al.,[8] had no Fitzgerald factor activity (Table 3). In contrast, the plasmas of 54 patients with other congenital deficiencies of clotting factors contained normal titers of Fitzgerald factor activity. These included 16 individuals with homozygous Hageman trait, 10 with homozygous plasma thromboplastin antecedent (PTA, factor XI) deficiency, two with Fletcher trait, five with Christmas factor (factor IX) deficiency, nine with classic hemophilia (functional deficiency of AHF), six with von Willebrand's disease, one with Stuart factor (factor X) deficiency, two with factor VII deficiency, one with factor V (proaccelerin) deficiency, and two with combined deficiencies of factor V and AHF. Fitzgerald factor levels were slightly decreased, but still within normal range (above 0.49 units = mean − 2 SD) in individuals with Fletcher trait (0.55 units/ml), Stuart factor deficiency (0.64), and combined deficiency (0.51).

The capacity of 21 mammalian, 2 avian, 2 reptilian, and 1 amphibian plasmas to correct the clotting defect of Fitzgerald trait plasma was examined (Table 4). All mammalian plasmas, including those of Cetacea of both toothed and baleen varieties, contained detectable Fitzgerald factor activity; activities were relatively low in those obtained from cattle, cats, rabbits and one hamster. On the other hand, two fowl plasmas, two reptilian plasmas, and one amphibian plasma had little or no corrective effect.

DISCUSSION

Fitzgerald factor (HMW kininogen) is an agent in normal human plasma that shortens the prolonged partial thromboplastin time of plasma from individuals with Fitzgerald trait. HMW kininogen, a plasma protein with an approximate MW of 197,000, is a precursor of biologically active kinins.[14] It accounts for about 20% of the total plasma kininogen (80% belongs to low

Table 4. Fitzgerald Factor Activity of the Plasmas of Various Species

Species	Fitzgerald Factor Activity (units/ml)
Human*	1.0
Apes	
Chimpanzee†	0.54
Gibbons (2)‡	1.14, 1.19
Black ape†	0.75
Baboon (3)‡	0.45 (0.38–0.58)§
Rhesus†	1.05
Horse (Palomino)‖ (2)‡	0.34, 0.46
Bovine (Hereford)‖ (4)‡	0.07 (0.05–0.08)
Swine†	1.92
Goat (Motntot)‖ (4)‡	0.55 (0.43–0.66)§
Sheep (Merion)‖ (4)‡	0.31 (0.22–0.39)§
Dog (4)‡	0.97 (0.78–1.32)§
Cat (3)‡	0.20 (0.06–0.33)§
Rabbit (New Zealand albino)‖ (9)‡	0.11 (0.07–0.19)§
Guinea pig* (American short hair)‖	0.90
Hamster†	0.10
Mouse* (Swiss Webster)‖	0.26
Killer whale†	0.75
Beluga whale†	0.47
Baleen whale†	0.30
Texas dolphin†	0.36
Porpoise†	0.20
Chicken* (Inida River)‖	< 0.01
Duck-a (Peking)‖	< 0.01
Turtle†	0.03
Frog†	< 0.01
Iguana† (*Iguana iguana*)‖	< 0.01

*Pooled plasma specimens.
†Single animal studied.
‡Figure in parentheses indicates numbers studied.
§Mean (range).
‖Name in parentheses indicates the breed used.

molecular weight (LMW) kininogen) and is the preferred substrate of plasma kallikrein.[14] Kinin generation in Fitzgerald trait plasma is thereby impaired. Kinins are small peptides with very strong biologic activity; for example, they produce pain, dilate small blood vessels, and increase vascular permeability. Therefore, kinins have been regarded as one of the humoral mediators of inflammation.[15] Thus, these observations suggest that Fitzgerald factor (HMW kininogen) may be related not only to blood coagulation, but also to the process of inflammation. In this regard, it is interesting to note that Mr. Fitzgerald, the index patient with Fitzgerald trait, displays an abnormal cellular inflammatory response as observed by the skin-window technique.[16] Although five additional individuals[4-8] appear to have the same clotting defect as Mr. Fitzerald, these cases exhibit variable defects in the kinin-generating system. Mr. Fitzgerald's plasma is only deficient in HMW kininogen,[3] whereas plasmas of the patients reported by Donaldson et al.,[4] Wuepper et al.,[5] and Colman et al.[6] are deficient in both LMW and HMW kininogen. It appears that LMW kininogen has little

function in blood coagulation.[4,6] Studies on Fitzgerald trait, coupled with those on Fletcher trait,[11,17,18,19] demonstrate that prekallikrein and HMW kininogen, proteins of the plasma kinin-generating system, also participate in blood clotting, in which they were recognized first as Fletcher factor and Fitzgerald factor, respectively. These findings serve once again to emphasize the intimate relationships among apparently different mechanisms of host defense.[15]

Fitzgerald factor activity is greatly reduced in plasmas of patients with hepatic cirrhosis, whereas it is normal in patients under treatment with warfarin. These data suggest that Fitzgerald factor may be produced in the liver, but that its production does not require vitamin K. That the amount of total kininogen in plasma is low in liver cirrhosis has been reported earlier.[20]

The reduced plasma levels of Fitzgerald factor activity in patients with DIC, most of whom also demonstrated reduced titers of Hageman factor (factor XII) activity and concentrations of Hageman factor-like antigens,[21] is interesting in view of the observation that infusion of ellagic acid, an activator of Hageman factor,[22] depletes the plasma kallikrein-activatable kininogen (probably HMW-kininogen) in hamster plasma.[23] It is tempting to speculate that the decreased level of Fitzgerald factor (HMW-kininogen) in plasmas of patients with DIC may be mediated through activation of Hageman factor in these patients.

Normal titers of Fitzgerald factor activity were found in plasmas from patients with nephrotic syndrome, rheumatoid arthritis, SLE, sarcoidosis, and other congenital clotting factor deficiencies, whereas patients with chronic renal failure showed mildly reduced titers. The significance of the decrease in renal failure is not evident.

We also examined Fitzgerald factor (HMW kininogen) activity in 21 mammalian, 2 avian, 2 reptilian, and 1 amphibian plasmas. All mammalian plasmas, including those of both toothed whales such as dolphins and one baleen whale, contained Fitzgerald factor-like activity, but five nonmammalian plasmas studied were devoid of this property.

The data reported here provide only the beginning of our understanding of the pathophysiologic role of Fitzgerald factor (HMW kininogen). As more clinical data accumulate, we should obtain a clearer view of the function of this plasma protein.

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