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Fibronectin and Factor XIII in Spinal Cord Injured Patients with End-stage Renal Disease

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Summary

Fibronectin and factor XIII play a major role in blood coagulation cascade and contribute to wound healing and phagocytic function of macrophages. Spinal cord injured (SCI) patients with end-stage renal disease (ESRD) have been shown to exhibit a variety of coagulation abnormalities, a high incidence of infection and poor healing pressure ulcers. Earlier studies in SCI patients with no discernible renal disease revealed a marked rise in plasma fibronectin in patients with fast healing pressure ulcers. However, no significant rise is found in those with poor healing ulcers. We compared plasma concentrations of fibronectin and factor XIII in a group of 13 SCI-ESRD patients with those of a normal control group. Despite the presence of pressure ulcers, the SCI-ESRD patients as a group failed to show a significant rise in plasma fibronectin concentration. In addition, the mean plasma factor XIII value in the SCI-ESRD group was not significantly different from that of the normal control group. Accordingly, the combination of SCI, ESRD and associated conditions seems to impair the patient's ability to mount a rise in plasma fibronectin concentration in response to the presence of pressure ulcers. Failure of SCI-ESRD patients to produce a rise in plasma fibronectin concentration may, in part, account for the poor healing property of pressure ulcers in this population.

Key words: *Fibronectin; Factor XIII; Spinal cord injury; Chronic renal failure; Paraplegia.*

Fibronectin is a macromolecular glycoprotein which is present in soluble form in plasma and insoluble form in the connective tissue matrix, basement membrane and various cell surfaces (Mosher, 1980; Mosher, 1987). In conjunction with factor XIII, fibronectin plays a role in the final stage of blood coagulation, promotes, wound healing and serves as an opsonin to promote phagocytosis (Vaheri *et al.*, 1983; Nagleschmidt *et al.*, 1987; Falcone *et al.*, 1984: Saba *et al.*, 1984).

Previous studies in our laboratory have revealed a variety of coagulation and fibrinolytic abnormalities in spinal cord injured (SCI) patients with end-stage renal

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disease (ESRD) (Vaziri *et al.*, 1985-A, 1985-B, 1986, 1987). In addition, a great majority of SCI-ESRD patients exhibit poor healing pressure ulcers as well as a variety of acute and chronic infections involving the urinary tract and other systems (Vaziri *et al.*, 1982). In a recent study of SCI patients without renal failure harboring pressure ulcers, we have found that a rise in plasma fibronectin concentration signifies rapid healing while lack of fibronectin elevation denotes a poor healing process (Vaziri *et al.*, 1990). In this study we determined plasma levels of fibronectin and factor XIII in a group of SCI men with ESRD and pressure ulcers.

Patients, materials and methods

Thirteen men with longstanding SCI and ESRD, 36 to 64 years of age, were studied. The level and duration of spinal cord injury varied from C_2–L_5 and 6 to 63 years respectively. The aetiology of renal disease was multifactorial and consisted of chronic pyelonephritis, reflux nephropathy, secondary amyloidosis, urolithiasis, and hypertensive nephrosclerosis. Patients with significant liver disease, sepsis, acute intercurrent illnesses, disseminated intravascular coagulation and those consuming anticoagulants or other drugs known to affect the blood coagulation system were excluded from the study. All patients had chronic urinary tract infections and decubitus ulcers.

The duration of haemodialysis therapy ranged from 1 week to 8 years. Patients were dialysed for 3 to 4 hours three times weekly using a parallel-plate or hollow-fiber cellulosic dialyser, a conventional acetate-buffered dialysate, and a single pass dialysate delivery system. Access to the blood stream was provided by surgically created Cimino arteriovenous fistulas or most commonly polytetrafluoroethylene (PTFE) grafts. Dialysis was performed under systemic anticoagulation using heparin 4000 to 7000 units in 4 divided doses.

Dietary restrictions were limited to water, sodium and potassium intakes as needed. No protein or caloric restrictions were imposed. Aluminum carbonate was prescribed as a phosphate binder and was taken with meals to control hyperphosphatemia. Multi-vitamins and folic acid were given to prevent deficiency states. Oral or parenteral iron preparations were employed in patients with iron deficiency. Blood transfusions were used in patients with severe symptomatic anemia unresponsive to other modalities. Pressure ulcers were managed as described previously (Eltorai and Chung, 1977).

Control group

The control group consisted of 15 healthy men aged 25 to 45 years. They had normal prothrombin time (PT), partial thromboplastin time (PTT) and no evidence of coagulopathies, liver disease, renal, neurological or other disorders, and were not receiving any drugs including those known to affect the blood coagulation system.

Blood samples

Blood samples were drawn from the blood access site shortly before and after

dialysis in SCI-ESRD patients. Free-flowing venous blood samples were obtained by venipuncture from the normal control group. Plastic syringes were used in all instances and samples were immediately transferred to plastic tubes containing 0·1 molar sodium citrate solution (blood/citrate = 9/1, volume). The citrate anti-coagulated blood was promptly centrifuged at 2000 X g for 20 minutes and plasma separated and stored at −70°C in multiple small plastic tubes until assayed.

Internal standard

The internal standard used was assayed coagulation reference plasma (ARP) which was obtained from Helena Laboratories (Beaumont, Texas). ARP was prepared from a pool of fresh citrated plasma from healthy, non-smoking men aged 20 to 40 years. The pool was buffered and lyophilised to ensure stability of all plasma constituents.

Immunological assays

Monospecific antisera produced in rabbits against purified human fibronectin and factor XIII a-subunit were used in respective assays employing electroimmuno-diffusion. A standard curve was constructed with each assay session using several dilutions of the reference standard. The concentration of each protein was determined by extrapolation using the standard curve.

Data presentation and analysis

All data are given as mean ± SD and are expressed as per cent of the values found in the ARP standard. Student's t-test and regression analysis were employed as appropriate, and p-values equal to or less than 0·05 were considered statistically significant.

Results

The mean value for plasma fibronectin concentration obtained in the SCI-ESRD group prior to dialysis (106 ± 52%) was not significantly different from that observed in the normal control group (99 ± 14%). Plasma fibronectin concentration obtained after dialysis (108 ± 33%) did not significantly differ from that observed before dialysis. Likewise, mean plasma factor XIII concentration obtained pre-dialysis (128 ± 57%) was not significantly different from that of the control group (107 ± 19%). However, marked interindividual variations (48% to 308%) were noted in factor XIII levels in the SCI-ESRD patients. As with fibronectin, haemodialysis did not significantly change plasma factor XIII concentraiton (122 ± 30%, post-dialysis).

Discussion

Fibronectin is a nearly ubiquitous component of the body and plays a number of important functions related to haemostasis, tissue remodelling during embryo-genesis and wound healing (Mosher, 1980, 1987). In addition, fibronectin serves as

a non-specific opsonin, thereby facilitating the removal by macrophages of the circulating micoraggregates, especially those of self-associating proteins, e.g. fibrin, collagen and actin. It is a substrate for thrombin, plasmin and activated factor XIII (F-XIIIa). F-XIIIa catalyses the crosslinking of alpha and gamma chains of fibrin and as such contributes to the stability and strength of the thrombus. In addition, F-XIIIa promotes crosslinking of fibrin to fibronectin and fibronectin to collagen in the vessel wall and connective tissue. This results in firm anchoring of the clot to the site of injury. Moreover, fibronectin and factor XIII appear to enhance migration and proliferation of fibroblast which culminate in organisation of clot, wound repair and the healing process (Vaheri, 1983).

In a recent study of SCI patients without discernible renal disease, we found an association between a patient's ability to attain a significant rise in plasma fibronectin and rapid healing of pressure ulcers. In contrast, failure to raise plasma fibronectin was associated with the presence of poor healing ulcers. In addition, we found no significant difference in plasma fibronectin concentration between normal able-bodied controls and SCI controls who were otherwise healthy. Accordingly, the presence of SCI alone did not seem to affect plasma fibronectin concentration (Vaziri *et al.*, 1990). Our SCI patients with end-stage renal disease showed no significant rise in the plasma fibronectin concentration despite the presence of pressure ulcers. In fact, some patients exhibited markedly subnormal values. Accordingly, the SCI patients with ESRD included in this study seemed to have failed to mount a significant rise in their plasma fibronectin levels in response to pressure ulcers. The lack of elevation of plasma fibronectin concentration in these patients was associated with poor healing pressure ulcers. It thus appears that the combination of SCI and advanced renal disease with the associated nutritional disorders (Mirahmadi *et al.*, 1983), physical inactivity, chronic infections (Vaziri *et al.*, 1982), secondary amyloidosis and other factors impairs the affected individual's ability to attain an elevated fibronectin level and normal healing. Since pressure ulcer compounds the patient's disability and contributes to malnutrition, amyloidosis, local and systemic infections, its prevention and successful treatment can have far-reaching benefits. In view of the demonstrated role of fibronectin in wound healing, further research to identify the cause(s) of the abnormal fibronectin response to pressure ulcers and possible usefulness of fibronectin administration may prove to be highly rewarding.

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