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Rapid Determination of Fibronectin by Laser Nephelometry[1])

Fibronectin concentrations in plasma in human diseases, I.

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Summary: Fibronectin (Synonyms: cold-insoluble globulin, antigelatin factor) is a cell surface glycoprotein. It has been shown that remarkably large amounts of fibronectin are present in human plasma. Owing to the difficult and expensive radioimmunoassays, only a few reports on the relation of fibronectin and human diseases have so far been published.

In this communication a rapid and easily handled immunoassay, using the light scattering effect, is described. The first results on the measurement of fibronectin concentrations in the plasma of patients with chronic active liver diseases are reported.

Schnelle Bestimmung von Fibronectin durch Lasernephelometrie; Fibronectinkonzentrationen im Plasma bei Erkrankungen des Menschen, 1. Mitteilung

Zusammenfassung: Fibronectin (Synonyma: Kälteunlösliches Globulin, β-Kryoglobulin, Antigelatine-Faktor) ist ein Glykoprotein von Zelloberflächen. Im menschlichen Plasma sind hohe Konzentrationen an Fibronectin nachweisbar. Wegen des schwierigen und kostenintensiven Nachweises durch Radioimmunoassay sind bislang nur wenige Berichte über Fibronectinkonzentrationen im Plasma von Patienten bekannt geworden. In dieser Mitteilung wird ein schneller und einfacher Immunoassay durch Lasernephelometrie beschrieben. Über erste Ergebnisse von Patienten mit chronisch aktiven Leberkrankheiten wird berichtet.

Introduction

Glycoproteins are characteristic constituents of membranes in mammalian cells. Some structural glycoproteins are associated with the cell membrane. Their physiological function and their role in the pathological status of cells are not completely elucidated.

One of the cell surface glycoproteins is fibronectin (Synonym: cold-insoluble globulin) (for reviews see l.c. (1, 2)), which is identical or closely related to the antigelatin factor (3). In recent years interest has been focussed on fibronectin, because cultured proliferating or virus-transformed fibroblasts as well as smooth muscle cells and endothelial cells secrete fibronectin into the culture medium (4).

There have been recent reports on fibronectin as a protein of connective tissue matrix, and increased levels of fibronectin have been reported in fibrotic processes (5, 6). Some authors suggest that increased fibronectin concentrations in the plasma of patients with accelerated connective tissue metabolism would reflect the activity of fibroplasia (3), but so far there are no reports concerning fibronectin and connective tissue diseases.

Up to now only time-consuming gel diffusion assays (7) or difficult and expensive immunoassays (8) have been used to measure fibronectin concentrations in bio-

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logical fluids. This may be why no intensive study has been made of plasma concentrations of fibronectin in patients with various diseases. In this communication we describe a rapid and easily handled determination of fibronectin by laser nephelometry, using a monospecific antibody.

Material and Methods

Preparation of fibronectin

Fibronectin was purified from human plasma, using adsorption chromatography with coupled denaturated collagen type I, as described previously (3, 9).

Antibodies were raised against fibronectin in rabbits (3). The resulting anti-human fibronectin rabbit serum was compared with an anti-human fibronectin rabbit serum from Behring-Werke Marburg, FRG. Fibronectin and monospecific antibodies were produced in the same way. Purity was shown by formation of a single band in various gel electrophoretic systems. Both antisera were tested against fibrinogen, and no reaction was observed.

Laser nephelometric immunoassay

Basic principles of laser nephelometry are described elsewhere (10). The nephelometer of Behring-Werke-Messer-Griesheim, FRG, was used in the present studies.

A plasma pool was made of 20 healthy male and female persons (20–50 years of age). Blood was collected in sodium citrate and plasma was separated by centrifugation at room temperature. Plasma was cleared by Frigen® (Hoechst AG, Frankfurt, FRG). A standard plasma (Behring-Werke) with a known concentration of fibronectin was also used. Antisera were cleared by filtration through a Millipore filter (Millipore S. A., Molsheim, France). Both methods for plasma clearing were without significant effects on the fibronectin concentration.

Dilution series both for antisera and plasma were prepared as described in Figure 1 and 2. The output of the nephelometer did not exceed 0.5 volt for either the antisera (dilution 1:2), or plasma (dilution 1:5).

The final test consists of:

100 µl plasma, dilution 1:10 in 0.15 mol/l NaCl

200 µl antiserum, dilution 1:5 (Behring-Werke); 1:10 (own preparation) in 0.15 mol/l NaCl. Using this dilution no output of the nephelometer was observed with plasma or antiserum blanks. Reaction time was 60 min.

Determination of serum activity of N-acetyl-β-glucosaminidase and histological estimation of fibrosis in liver biopsy specimens of patients with chronic active liver diseases were performed as previously described (11).

Results

Plasma fibronectin reacts with corresponding antibodies, visible by the measurable turbidity of the immunoprecipitate in the reaction mixture. The immune reaction is concentration-dependent in a nearly linear range from 0.6 to 3.0 mg/dl fibronectin (antiserum dilution 1:5, antiserum Behring-Werke, and 1:10 for the self-prepared anti-human fibronectin serum). Dilution experiments with the antisera are demonstrated in figure 1. Reaction capacity is sufficient after a dilution up to 1:16 (1:32). For clinical use, a dilution of the plasma specimens was made 1:10 in 0.15 mol/l NaCl (figure 2).

Figure 3 shows mean values and standard deviations of fibronectin in the plasma specimens of normal persons

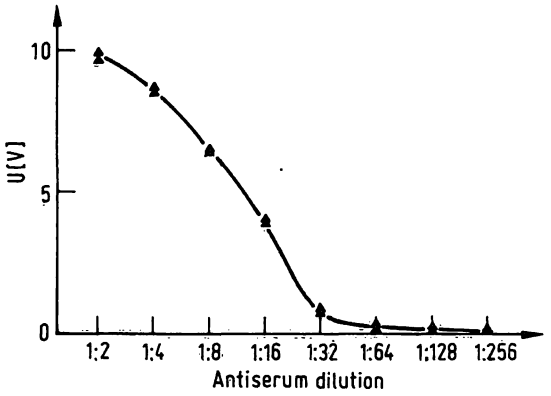

Fig. 1. Dilution curve of a monospecific antiserum (from a self-prepared antigen) against a human plasma pool containing fibronectin (21 mg/dl). Plasma pool dilution was 1:10.
U[V] = intensity of the light scattering signal.

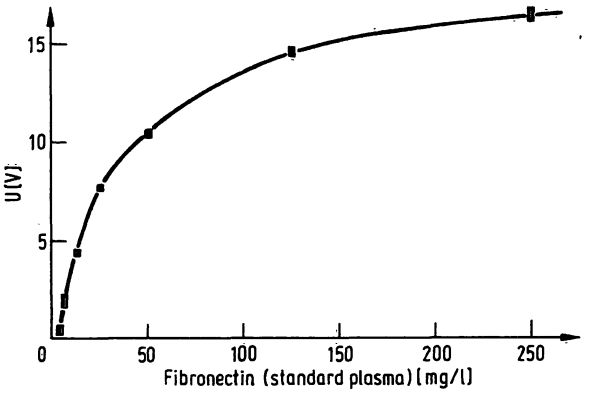

Fig. 2. Dilution curve of a standard plasma (Behring-Werke), containing 25 mg/dl fibronectin. The dilution of the monospecific antiserum was 1:5.
U[V] = intensity of the light scattering signal.

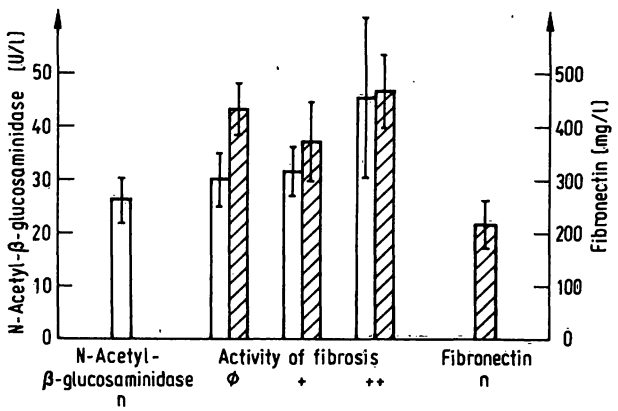

Fig. 3. Preliminary results on fibronectin concentrations (hatched bars) in the plasma of patients with chronic active liver diseases (Mean values and standard deviations: ⊢⊣). For comparison the histologically estimated fibrosis activity (ϕ; +; ++) in the liver as well as the serum activity of N-acetyl-β-glucosaminidase (open bars) from the same patients are given. n = normal healthy controls.

(n = 23) and patients with chronic active liver diseases (n = 79). Our own plasma pool and the available standard plasma contain nearly the same amounts of fibronectin. Preliminary results indicate that fibronectin concentrations in plasma specimens of patients with chronic active liver diseases could be elevated.

Discussion

Determination of fibronectin concentrations in various biological fluids and supernatants of homogenized tissues has been reported by only a few authors (1, 5, 12). The fact that immunoassays in particular are not used by other authors for clinical purposes reflects the relative difficulty in purifying fibronectin and mainly the poor performance of labeled fibronectin (6). In general a radioimmunoassay procedure is not necessary to determine fibronectin in plasma specimens because of its high concentration. Using the light scattering of immunoprecipitates, we have established a laser nephelometer assay that circumvents the problem encountered with previous methods. Our dilution series and reference curves show that at the concentrations of antisera and plasma specimens chosen no significant surplus of either antiserum or plasma is detectable. The dilution experiments with the antisera show that the concentration elected for the assay is found in the middle of the right side of the *Heidelberger* curve (8).

Our preliminary results show that the fibronectin concentrations in the plasma of patients with chronic liver diseases (fig. 3) may be increased twofold over the mean value for normal persons or standard plasma. An interpretation of the increased fibronectin concentrations with respect to the pathogenesis of the mentioned diseases and the possible clinical implications is difficult on the basis of these preliminary results.

As demonstrated in figure 3, no relation between fibronectin concentrations in the plasma and the histologically estimated fibrous activity in the liver tissue could be detected, whereas the serum activity of N-acetyl-β-glucosaminidase – as previously reported (9) – is increased in relation to the activity of fibrosis. Further investigations will be necessary to elucidate the diagnostic value of determining the fibronectin concentration in the plasma of patients with liver fibrosis.

References

1. Mosesson, M. W. (1977), Thrombos. Haemostas. *38*, 742–750.
2. Vaheri, A., Mosher, D., Wartiovaara, J., Keski-Oja, J., Kurkinen, M. & Stenman, S. (1976), Interactions of fibronectin, a cell-type specific surface-associated glycoprotein. Academic Press, New York and London.
3. Dessau, W., Jilek, F., Adelmann, B. C. & Hörmann, H. (1978), Biochim. Biophys. Acta *533*, 227–237.
4. Vaheri, A. & Rouslahti, E. (1975), J. Exp. Med. *142*, 530–535.
5. Hahn, E., Wick, G., Pencer, D. & Timpl, R. (1980), Gut, in press.
6. Pott, G., Rauterberg, J., Voss, B., Allam, S., Brehmer, U., Liehr, H. & Gerlach, U. (1980), Connective tissue metabolism and lysosomal glycosidases in nonparenchymal cells from normal and fibrotic human livers and from livers of galactosamine-treated rats. In: RES and related liver diseases (Liehr, H. & Grühn, M. eds.), Elsevier, Amsterdam.
7. Bruhn, H. D. & Heimburger, N. (1976), Haemostasis *5*, 189–192.
8. Ruoslahti, E., Vuento, M. & Engvall, E. (1978), Biochim. Biophys. Acta *534*, 210–218.
9. Engvall, E. & Ruoslahti, E. (1977), Int. J. Cancer *20*, 1–5.
10. Conrad, A., Schürmann, J., Kreutz, F. H., Sieber, A. (1978), J. Clin. Chem. Clin. Biochem. *16*, 299–305.
11. Pott, G., Eberhardt, G. & Gerlach, U. (1978), Z. Gastroenterologie *16*, 32–37.
12. Mosher, D. & Williams, E. M. (1978), J. Lab. Clin. Med. *91*, 729–735.

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