0022-5347/89/1413-0629$02.00/0
THE JOURNAL OF UROLOGY
Vol. 141, March
Printed in U.S.A.

ISOLATION AND CHARACTERIZATION OF COLLAGEN IN PEYRONIE'S DISEASE

KENNETH D. SOMERS,* EDWARD N. SISMOUR, GEORGE L. WRIGHT, JR., CHARLES J. DEVINE, JR., DAVID A. GILBERT AND CHARLES E. HORTON

From the Departments of Microbiology and Immunology, Urology and Plastic Surgery, Eastern Virginia Medical School, Norfolk, Virginia

ABSTRACT

Peyronie's disease is characterized histologically by excessive collagen deposition in the lesion. We examined the collagen types in Peyronie's disease plaque tissues compared to unaffected tissues from the same patient, other control tissues, and Dupuytren's contracture. Gel electrophoresis of pepsin-solubilized collagen demonstrated the presence of type I collagen and an increased content of type III collagen in plaque tissue. Increased type III collagen was detected in apparently normal tissue adjacent to the plaque and in Dupuytren's lesion, confirming previous findings. Although the cause of excessive collagen accumulation of Peyronie's disease is unknown, the results suggest an imbalance in the regulation of extracellular matrix production leading to pathologic fibrosis. (*J. Urol., 141: 629–631, 1989*)

Peyronie's disease is a pathologic fibrosis in the tunica albuginea of the penis and a common cause of penile curvature. The disease is characterized by an initial perivascular inflammatory cell infiltrate[1,2] and culminates in the excessive deposition of collagen into a fibrous plaque. Plaque formation is frequently accompanied by dystrophic calcification and ectopic bone formation.[1,3,4] The histological features of plaque tissue and the excessive accumulation of collagen in the plaque, suggest some localized imbalance in the regulation of extracellular matrix production. Pathologic overproduction of collagen occurs in hypertrophic scars, keloids, and Dupuytren's contracture, but the pathogenesis of these fibrotic disorders is poorly understood.[5]

In the present study, we report the isolation and characterization of collagen types in Peyronie's disease plaque tissue. The data demonstrate the presence of type I collagen and an increased content of type III collagen in plaque tissue.

MATERIALS AND METHODS

Patient specimens. Tissue specimens from seven Peyronie's disease patients were obtained during surgical operation and stored at −70C until used. In most cases, plaque tissue and perilesional tunica albuginea from the penis as well as dermis were obtained from the same patient. Perilesional tissue was derived from lateral incisions 0.5 to 1.0 cm. from the plaque. Control tissues (penile scar and tunica) were obtained from three patients who underwent surgical repair of hypospadias and chordee. Additional control tissue was obtained from the palmar aponeurosis of three patients with Dupuytren's contracture.

Preparation of pepsin-solubilized collagen. Soluble collagens were extracted by limited pepsin digestion of tissue specimens. Tissue specimens (~100 mg. wet weight) were minced and placed in 0.5 M acetic acid (one part wet weight tissue:10 parts 0.5 M acetic acid) containing the protease inhibitors N-ethylmaleimide (eight mM), phenylmethylsulfonyl fluoride (one mM), and ethylenediaminetetraacetic acid (20 mM) and digested overnight at 4C with 100 μg./ml. pepsin (EM Science, Cherry Hill, NJ; one part pepsin digests 3000 to 3500 parts of egg albumin). Pepsin-solubilized extracts were centrifuged (60 min. at 16,500 *g* and 4C) and the pepsin-insoluble material subjected to repeated digestion under the same conditions. Supernatants from the digestions were combined and the pH adjusted to >8.0 with one M Tris to inactivate pepsin. Soluble collagen was precipitated by the addition of NaCl to a final concentration of 4.5 M for 16 hours at 4C and collected by centrifugation. The pellet was resuspended in 0.5 M acetic acid, centrifuged to remove insoluble material, and the soluble collagens reprecipitated with 4.5 M NaCl. The collagens were collected by centrifugation and the pellet dissolved in 0.5 M acetic acid. Concentration of soluble collagen was determined by the Sirius Red assay.[6]

Gel electrophoresis. Collagen samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 6 per cent polyacrylamide slab gels. Urea (0.5 M) was included in the solutions to improve the separation of the collagen chains. Interrupted electrophoresis was carried out by the method of Sykes et al.[7] using 60 mM dithiothreitol (DTT). Collagen polypeptides were visualized by staining with Coomassie brilliant blue R250 and quantitated with a scanning densitometer (Biomed Instruments, Fullerton, CA). Type I and type III collagens purified from human placenta were used as standards. To demonstrate the collagenous nature of specific protein bands, selected samples were digested with 30 units of bacterial collagenase (Advance Biofactures Co., Lynbrook, NY) for 20 hr. at 37C in 50 mM Tris-HCl, pH 7.6 and 5 mM $CaCl_2$ prior to electrophoresis.

RESULTS

Collagen isolated by limited pepsin proteolysis was analyzed by SDS-PAGE for genetically distinct collagen types. Fig. 1 *A* shows interrupted gel electrophoresis patterns of solubilized collagens from seven independent Peyronie's plaque (lanes 2, 4–6), perilesional samples (lanes 3, 8, 9), dermis (lane 7), and nodular tissue from Dupuytren's contracture (lane 10). All samples demonstrated the presence of α1(I) and α2(I) chains of type I collagen. Variable amounts of α1(III) chains of type III collagen were visually detectable in Peyronie's plaque and perilesional tissue as well as Dupuytren's lesion, but not detected in normal human dermis. The most dramatic departure from the expected 2:1 ratio of α1(I) to α2(I) chains was observed in lanes 4 and 9 with ratios of 7.2:1 and 6.3:1, respectively, determined by scanning densitometry. The mean α1(I) to α2(I) ratios for samples in Fig. 1 *A* are 3.7:1 for plaque (n = 4) and 3.9:1 for tunica (n = 3) compared to 2.8:1 for both dermis (n = 1) and human placenta collagen (n = 1). The results demon-

Accepted for publication August 22, 1988.
* Requests for reprints: Microbiology/Immunology Dept., Eastern Virginia Medical School, PO Box 1980, Norfolk, VA 23501.
Supported by NIH grant AM 30674.

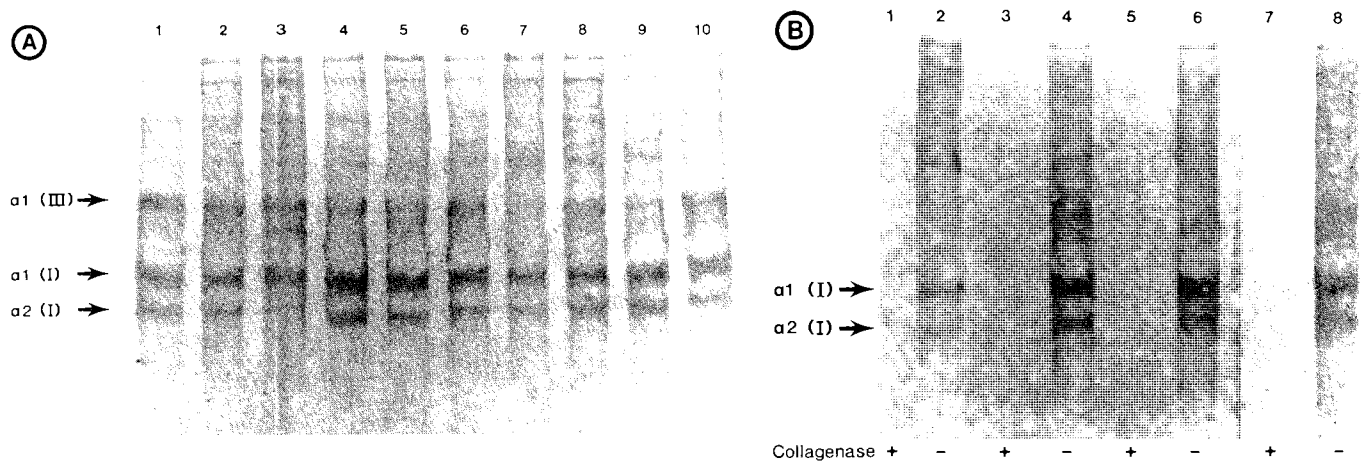

FIG. 1. SDS-polyacrylamide gel electrophoresis of collagen extracted from Peyronie's disease plaque, perilesional tissue, or normal dermis. Collagen was solubilized by limited pepsin digestion, precipitated in 4.5 M NaCl, resolved on 6% polyacrylamide gel and visualized by staining with Coomassie blue. *A*, interrupted gel electrophoresis patterns of collagens from human placenta (lane 1), independent Peyronie's plaques (lane 2, 4–6), perilesional tissues (lane 3, 8, 9), dermis (lane 7), and Dupuytren's nodule (lane 10). Migration positions of α1(I) and α2(I) chains of type I collagen and α1(III) of type III collagen are indicated by the arrows. *B*, susceptibility of collagens to bacterial collagenase. Collagen samples were divided into two equal portions, one of which was treated with 30 units of bacterial collagenase. Other was treated with buffer alone before gel electrophoresis under nonreducing conditions. Normal dermis (lane 1 and 2), plaque (lane 3 and 4) and normal tunica (lane 5 and 6) from Peyronie's disease patient; Dupuytren's cord (lane 7 and 8).

strate that the average amount of α1(I) chains is elevated suggesting the presence of type I trimer collagen chains in particular plaque or perilesional tissues of Peyronie's disease. Fig. 1 *B* demonstrates that type I and III collagens isolated from Peyronie's and Dupuytren's lesions were completely degraded by purified bacterial collagenase. These results establish the collagenous nature of the observed polypeptide chains and the absence of contaminating non-collagenous proteins.

To further characterize the collagen types in Peyronie's disease, collagen was isolated from the fibrous plaque, normal tunica albuginea, and dermis from the same patient. Solubilized collagen was analyzed by SDS-PAGE performed with or without delayed reduction of the disulfide bonds by DTT (fig. 2). Autologous samples demonstrated the presence of α1(I) and α2(I) chains of type I collagen in plaque, normal tunica, and dermis. However, only plaque tissue (lane 3) and, to a lesser extent, normal tunica (lane 2) contained α1(III) chains of type III collagen. Weakly stained bands migrating faster than α2(I) chains (lanes 8, 9) may represent collagen breakdown products. Quantitation of the different collagen types by densitometric scanning of fig. 2 indicated that the type III collagen content was 26 per cent, 10 per cent, and 2 per cent in plaque, tunica, and dermis, respectively. Ratios of type III to type I collagens and of α1(I) to α2(I) chains were, respectively, 0.70 and 1.34 for Peyronie's disease plaque, 0.19 and 1.12 for tunica, and 0.05 and 1.27 for normal dermis. Similar ratios were observed for autologous samples of dermis and penile scar tissue. Type III collagen was not detected in normal dermis, but the ratio of type III to type I collagens in the scar was 0.35. The ratio of α1(I) to α2(I) was 1.7 for normal dermis and 1.8 for scar.

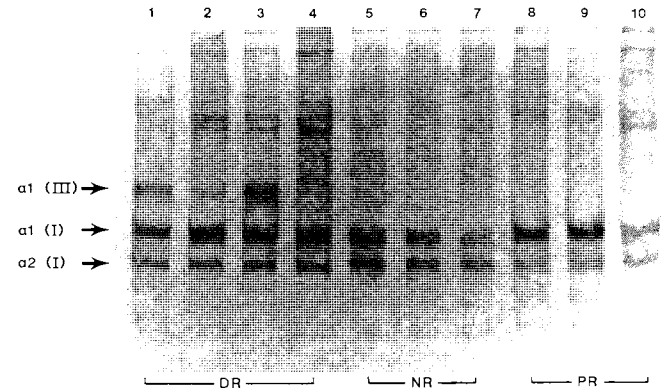

FIG. 2. Isolation of genetically distinct collagen types from plaque, normal tunica and dermis of Peyronie's disease patient. Gel electrophoresis was performed with delayed reduction (DR), no reduction (NR), or prior reduction (PR) with DTT. Human placenta (lane 1), normal tunica (lane 2, 5, and 8), plaque (lane 3, 6, and 9) and dermis (lane 4, 7, and 10).

DISCUSSION

The collagen composition of Peyronie's disease plaque has not been reported previously. The results described in this study demonstrate an altered composition of genetically distinct collagen types in plaque tissue, as compared to normal dermis. Increased amounts of type III collagen were consistently present in plaque tissue and penile scar tissue. The observed average ratio of α1:α2 chains was elevated above the expected ratio, suggesting the presence of other collagen chains, provisionally identified as type I trimer. Type V collagen chains were not detected in tissue specimens; however, the extraction procedure used may be suboptimal for recovery of type V collagen. Increased amounts of type III collagen have been associated with experimental granulation tissue, embryonic tissue and hypertrophic scar tissue.[8,9] Similarly, pathologic fibrosis associated with Dupuytren's contracture is characterized by the presence of type V and type I trimer collagens and a relative excess of type III collagen.[10,11] Type I trimer has also been reported in chronic inflammatory lesions[12] and embryonic tissue.[13] Of interest was the demonstration of type III collagen in apparently normal tissue adjacent to plaque tissue. This observation indicates that the disease is not localized solely to the plaque. Thus, perilesional tissue may represent sites of early active fibrosis that may coexist with or progress to mature relatively acellular fibrous plaque tissue.

The implications of changes in collagen composition and ratios in Peyronie's disease plaque and analogous pathologic fibroses remain speculative. The findings suggest an imbalance in the normal wound healing process with a shift toward a more embryonic or immature extracellular matrix. As a result, remodeling of granulation tissue is incomplete and the biological process of maturation is impaired. Excessive collagen deposition in vivo is a common feature of Peyronie's and Dupuytren's disease, yet nothing is known about the mechanisms responsible for collagen accumulation in these diseases. Theoretically, the excessive accumulation of collagen could result either from

locally increased production or decreased degradation of collagen in the lesions. Although the problem of relating these biochemical changes to the pathology of the disease remains, alteration in the quantity and location of collagen in many pathologic disorders, including Peyronie's and Dupuytren's disease, may result in the disruption of connective tissue structure and function.

Acknowledgments. We thank R. F. Diegelmann for helpful comments.

REFERENCES

1. Smith, B. H.: Peyronie's disease. Am J. Clin. Pathol., **45:** 670, 1966.
2. Vande Berg, J. S., Devine, C. J., Jr., Horton, C. E., Somers, K. D., Wright, G. L., Jr., Leffell, M. S., Dawson, D. M., Gleischman, S. H. and Rowe, M. J.: Peyronie's disease: an electron microscopic study. J. Urol., **126:** 333, 1981.
3. Vande Berg, J. S., Devine, C. J., Jr., Horton, C. E., Somers, K. D., Wright, G. L., Jr., Leffell, M. S., Dawson, D. M., Gleischman, S. H. and Rowe, M. J.: Mechanisms of calcification in Peyronie's disease. J. Urol., **127:** 52, 1982.
4. Rollandi, G. A., Tentarelli, T. and Vespier, M.: Computed tomographic findings in Peyronie's disease. Urol. Radiol., **7:** 153, 1985.
5. Smith, R.: Recovery and tissue repair. Br. Med. Bull., **41:** 295, 1985.
6. Marotta, M. and Martino, G.: Sensitive spectrophotometric method for the quantitative estimation of collagen. Anal. Biochem., **150:** 86, 1985.
7. Sykes, B., Puddle, B., Francis, M. and Smith, R.: The estimation of two collagens from human dermis by interrupted gel electrophoresis. Biochem. Biophys. Res. Comm., **72:** 1472, 1976.
8. Bailey, A. J., Sims, T. J., LeLous, M. and Bazin, S.: Collagen polymorphism in experimental granulation tissue. Biochem. Biophys. Res. Comm., **66:** 1160, 1975.
9. Bailey, A. J., Bazin, S., Sims, T. J., LeLous, M., Nicoletis, C. and Delaunay, A.: Characterization of the collagen of human hypertrophic and normal scars. Biochim. Biophys. Acta, **405:** 412, 1975.
10. Bazin, S., LeLous, M., Duance, V. C., Sims, T. J., Bailey, A. J., Gabbiani, G., D'Andiran, G., Pizzolato, G., Browski, A., Nicoletis, C. and Delaunay, A.: Biochemistry and histology of the connective tissue of Dupuytren's disease lesions. Eur. J. Clin. Invest., **10:** 9, 1980.
11. Ehrlich, H. P., Brown, H., and White, B. S.: Evidence for type V and I trimer collagen in Dupuytren's contracture palmar fascia. Biochem. Med., **28:** 273, 1982.
12. Narayanan, A. S., Page, R. C. and Kuzan, F.: Collagens synthesized in vitro by diploid fibroblasts obtained from chronically inflamed human connective tissue. Lab. Invest., **39:** 61, 1978.
13. Jimenez, S. A., Bashey, R. I., Benditt, M. and Yankowski, R.: Identification of collagen α_1(I) trimer in embryonic chick tendons and calvaria. Biochem. Biophys. Res. Comm., **78:** 1354, 1977.