Arnold W, Häusler R (eds): Otosclerosis and Stapes Surgery.
Adv Otorhinolaryngol. Basel, Karger, 2007, vol 65, pp 45–49

Expression of Collagens in the Otosclerotic Bone

H.P. Niedermeyer, E.T. Becker, W. Arnold

Department of Otorhinolaryngology, Head and Neck Surgery,
Klinikum rechts der Isar, Technical University Munich, Munich, Germany

Abstract

The etiopathogenesis of otosclerosis is still controversially discussed. The major hypotheses discussed are a viral infection on a genetic background and an (autoimmune) collagen disease. The aim of our study was to investigate by immunohistochemistry the expression pattern of collagens within the otosclerotic focus. Stapes footplates from 30 patients with clinical otosclerosis undergoing stapedectomy were formalin fixed, decalcified and paraffin embedded. As controls, 30 autoptic temporal bone specimens were employed. We investigated the expression of collagens I–V with immunohistochemistry. The expression of collagen I showed a diffuse homogeneous distribution with increased staining of the otosclerotic focus. Collagen II was exclusively expressed in chondrocytes including the globuli interossei. The pattern of collagen III in the otosclerotic bone was web-like in contrast to a lamellar pattern in the control bone. The mucoperiosteal layer and connective tissue such as the vessels of the resorption lacunae expressed collagen IV. An increased expression of collagen V around osteocytes was observed in the otosclerotic focus. In conclusion, in the otosclerotic tissue, in comparison with the control bone, a high expression of collagen IV occurred. The immunohistochemical analysis of collagen II, which has been suggested to be implicated in the etiopathogenesis of otosclerosis, revealed no differences between control and otosclerotic bones. The intense staining of the otosclerotic focus with collagen I is in good agreement with an inflammatory process but in contrast with lesions like those in osteogenesis imperfecta.

Otosclerosis is the major cause of hearing loss in western countries, the southern part of India and Japan, but its etiopathogenesis is not clear [1, 2]. The current etiopathogenetic hypothesis includes a measles virus infection as the triggering event on a genetic background although neither measles virus nor the genetic changes could be ascertained as a cause of otosclerosis. Histologically,

the otosclerotic foci exhibit all signs of a chronic inflammatory process which develops in three phases with the final formation of a scar. Otosclerosis shows some similarities with Paget's disease and osteogenesis imperfecta [3]. In the past, some authors reported blue sclera in patients with otosclerosis, suggesting a common pathogenetic base. A collagen (auto-)immune etiopathogenesis was supported by induction of lesions very similar to otosclerosis in rats which were immunized against collagen II [4, 5]. Chondrocytes are known to express collagen II. Antibodies against collagens II and IX in the serum of patients with otosclerosis supported a collagen involvement [6, 7]. To support a collagen pathogenesis in otosclerosis, McKenna et al. [8, 9] found mutations in COL1A1 which occur in the mild form of osteogenesis imperfecta. There is no doubt that otosclerosis develops in the border region of the enchondral and endosteal layer of the cochlea characterized by the presence of the unique globuli interossei. Thus, a collagen etiopathogenesis is possible.

The aim of this study was to investigate by immunohistochemistry the expression pattern of collagens I–V within the otosclerotic focus in comparison with normal temporal bone specimens.

Materials and Methods

Footplates from 30 consecutive patients with clinical otosclerosis subjected to stapedectomy were collected. The ratio of males:females was 1:1.3. The age ranged from 27 to 52 with an average of 41 years. As controls, 30 autoptic gender- and age-matched temporal bone specimens were employed. The specimens were formalin fixed (4% paraformaldehyde), decalcified (15% EDTA) and paraffin embedded. Two-micrometer sections were prepared.

Specific antibodies against collagens I–V (Quartett, Berlin, Germany; Eurodiagnostics, Apeldoorn, the Netherlands) were used for immunohistochemistry. For detection, the ABC peroxidase method (Vektor, Burlingame, USA) and the nonconjugated APAAP technique (Dianova, Hamburg, Germany) were employed.

Results

In 15 footplates, we observed areas of bone resorption and reorganization and the stage of scar formation in the other 15 samples. The control tissue did not present otosclerotic foci. The expression of collagen I showed a diffuse homogeneous distribution with enhanced staining of the otosclerotic focus (fig. 1). Collagen II was exclusively expressed in the globuli interossei and chondrocytes of the stapes footplate (fig. 2). The pattern of collagen III in the otosclerotic bone was web-like in contrast with a lamellar pattern in the control bone.

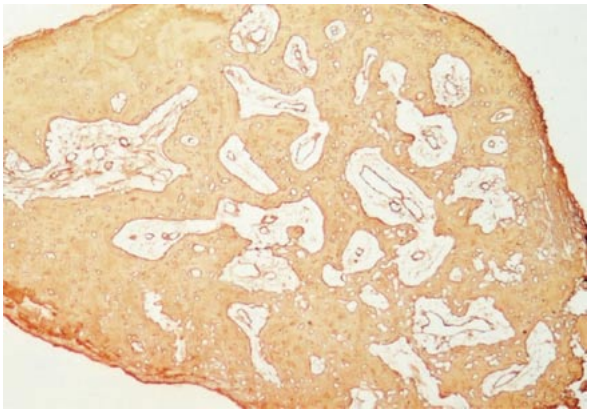

Fig. 1. Expression of collagen I in the otosclerotic focus (×10, ABC).

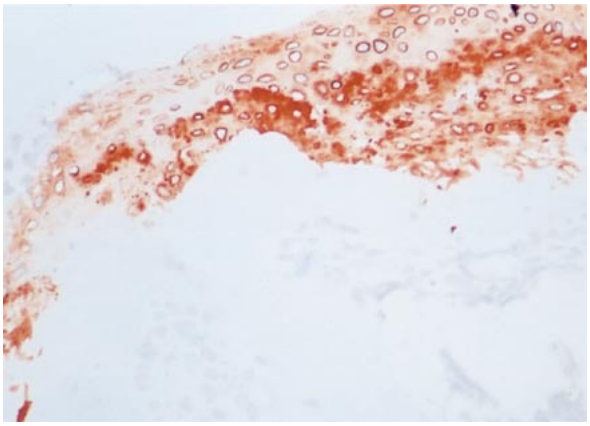

Fig. 2. Chondrocytes are stained with anticollagen II (×20, APAAP).

The mucoperiosteal layer and connective tissue such as the vessels of the resorption lacunae expressed collagen IV. A slightly increased expression of collagen V around osteocytes was observed in the otosclerotic focus.

Discussion

Otosclerosis develops in the ontogenetically 'weak' border region of the otic capsule. In the past, results of animal studies and serologic analysis of

patients with otosclerosis suggested a collagen etiopathogenesis of otosclerosis. Mutations of COL1A1 in familial cases of otosclerosis support this view [8]. In our immunohistochemical analysis, we found a slightly increased expression of collagen I in the otosclerotic focus. Collagen I is part of the extracellular matrix of the normal bone and an increased expression can be expected in regions of new bone formation. This observation is in contrast with the reduced expression of collagen I in osteogenesis imperfecta [10]. However, we cannot exclude mutations of COL1A1.

Experimental data from rats immunized against collagen II suggested an autoimmune process in otosclerosis [4, 5]. At the protein level, we could not find any difference in the expression of collagen II between otosclerotic and control tissue. This finding is supported by a case control study where the mutations in patients with otosclerosis could not be confirmed [11] (see also Solvsten-Sorensen et al. [12]). Finally, if a collagen II autoimmune etiopathogenesis was true, we would expect other inflammations of cartilage tissue in patients with otosclerosis.

We suppose that the increased expression of collagens IV and V is a consequence of the well-known inflammatory process that occurs in otosclerosis. Further studies on the RNA level could help to elucidate the role of collagens in otosclerosis.

Acknowledgement

This study was supported by KKF H-14/2000.

References

1 Vrabec JT, Coker NJ: Stapes surgery in the United States. Otol Neurotol 2004;25:465–469.
2 Ohtani I, Baba Y, Suzuki T, Suzuki C, Kano M, Deka RC: Why is otosclerosis of low prevalence in Japanese? Otol Neurotol 2003;24:377–381.
3 Pedersen U, Sogaard H, Elbrond O: Histological investigation of skin biopsies in otosclerosis and osteogenesis imperfecta. Arch Otorhinolaryngol 1984;240:1–6.
4 Yoo TJ, Stuart JM, Kang AH, Townes AS, Tomoda K, Dixit S: Type II collagen autoimmunity in otosclerosis and Ménière's disease. Science 1982;217:1153–1155.
5 Yoo TJ, Shea JJ Jr, Floyd RA: Enchondral cartilage rests collagen-induced autoimmunity: a possible pathogenetic mechanism of otosclerosis. Am J Otolaryngol 1987;8:317–324.
6 Bujia J, Alsalameh S, Jerez R, Sittinger M, Wilmes E, Burmester G: Antibodies to the minor cartilage collagen type IX in otosclerosis. Am J Otol 1994;15:222–224.
7 Bujia J, Burmester G: The presence of antibodies directed against specific cartilagenous collagens in patients with otosclerosis. Acta Otorrinolaringol Esp 1993;44:277–280.
8 McKenna MJ, Kristiansen AG, Bartley ML, Rogus JJ, Haines JL: Association of COL1A1 and otosclerosis: evidence for a shared genetic etiology with mild osteogenesis imperfecta. Am J Otol 1998;19:604–610.

9 McKenna MJ, Kristiansen AG, Tropitzsch AS: Similar COL1A1 expression in fibroblasts from some patients with clinical otosclerosis and those with type I osteogenesis imperfecta. Ann Otol Rhinol Laryngol 2002;111:184–189.
10 Verstreken M, Claes J, Van de Heyning PH: Osteogenesis imperfecta and hearing loss. Acta Otorhinolaryngol Belg 1996;50:91–98.
11 Rodriguez L, Rodriguez S, Hermida J, Frade C, Sande E, Visedo G, Martin C, Zapata C: Proposed association between the COL1A1 and COL1A2 genes and otosclerosis is not supported by a case-control study in Spain. Am J Med Genet A 2004;128:19–22.
12 Solvsten-Sorensen M, Nielsen LP, Bretlau P, Jorgensen MB: The role of type II collagen autoimmunity in otosclerosis revisited. Acta Otolaryngol 1988;105:242–247.

PD Dr. H.P. Niedermeyer
HNO Klinik und Poliklinik, Klinikum r.d. Isar, Technische Universität München
Ismaningerstrassse 22
DE–81675 Munich (Germany)
Tel. +49 89 4140 2371, Fax +49 89 4140 4853, E-Mail h.p.niedermeyer@lrz.tum.de