Spectrophotometrical Alterations in the Support Tissue of Large Bronchi in Humans

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Abstract: We investigated spectrophotometrically hyaline cartilage of large bronchi in 53 humans. The gradual increase of optical density (OD) both in cytoplasm of chondrocytes (CH) and extracellular matrix (EM) from the proliferative zone to the zone of hypertrophied CH was revealed in the first type of non-changed cartilage. The second type of non-changed and partly degenerative cartilage exhibited the same distribution of OD along all zones through insignificant changes of OD in the EM. The third type of degenerative and asbestotic cartilage showed either significant increase or decrease of OD in all zones. We suggest, that the different types of distribution of the OD corresponds to the degeneration of cartilage, but does not depend on age and duration of disease.

INTRODUCTION

In the course of differentiation a hyaline cartilage goes through several stages starting with initial hard core distribution of chondrocytes to soft core distribution and even diminishing of differentiation in the last decade of life [1]. These stages of differentiation may be disturbed due to several respiratory and non-respiratory diseases [2,3]. The above process is not rarely accompanied with cartilage ossification and development of asbestotic channels in cartilage [2,4].

In a recent paper [5] we described the spectrophotometrical differences of the non-changed, vacuolised and ossified bronchial cartilage of humans with chronic non-specific lung disease. The findings of different types of cartilage spectrophotometrically induced us further to characterise cartilage in normal and impaired bronchi. In the present study we compared normal and degenerative hyaline cartilage of large bronchi and gave criterias for the development of asbestotic channels spectrophotometrically.

MATERIALS AND METHODS

There were 53 humans of various age (9 to 95 years) under our investigations. Bronchial wall of 10 patients was obtained during lung operation due to cancer of bronchial soft tissue. Control tissue was obtained from 43 people died from non-respiratory disease. Tissue were fixed in 10% formalin and then embedded into paraffin. Further 7 μm thin sections were stained with hematoxilin and eosin, Bismarck Brown and PAS reactives. Three different zone of cartilage: proliferation zone, zone of mature and hypertrophied chondrocytes were analysed in the light microscope [6]. We employed the imaging technics to determine the topographical distribution of absorption of measuring optical density value [7] for cytoplasm and extracellular matrix. The automatic analyser of the microscopic images (Morphoquant - Karl Zeiss, Jena) was used for the measurements of optical transmission or density of the hyaline cartilage stained by PAS reaction or Bismarck Brown. Both baseline of light intensity (J_0) and intensity of the transmission light (J_1) passing through extracellular matrix, cytoplasm and granules of chondrocytes were measured utilising photo-multiplier in the photon-counting regimen. The optical density had been calculated from the transmission light intensity measurements according to the formula:

$$D = \sum_{i=1}^{N} \lg \frac{J_0}{J_i} = N \cdot \lg J_0 - \sum_{i=1}^{N} \lg J_i$$

D - optical density
J_0 - baseline of light intensity
J_1 - the transmission light intensity

Systematic error from the main sources (accumulation of light etc.) was taken into account by regular controls. The maximum error of the transmission light intensity measurements was estimated about 1%.

RESULTS

We could not detect any difference between cartilage of normal and disordered bronchial wall spectrophotometrically. 22 humans of various age possessed non-changed hyaline cartilage. Tissue showed large proliferative zone and zone of mature chondrocytes and thin zone of hypertrophied chondrocytes. The OD of extracellular matrix and cytoplasm of chondrocytes usually increased from the proliferative to the zone of hypertrophied chondrocytes in PAS stained-sections, although we observed insignificant variations of the OD in 6 cases. These variations included the decreasing of the OD in the extracellular matrix between mature chondrocytes with following increase of the OD in the extracellular matrix between hypertrophied cells. The OD in the cytoplasm of chondrocytes and extracellular matrix of non-changed cartilage also increased in the direction from the proliferative zone to the zone of hypertrophied chondrocytes with small decreasing of the OD in the extracellular matrix of the zone of hypertrophied chondrocytes in sections stained with Bismarck Brown.

Samples of 31 human of various age possessed degenerative alterations in the cartilage. Thin proliferative zone with only few chondrocytes were observed. Often the

lack of the zone of mature chondrocytes was seen. Hypertrophied and degenerative chondrocytes were detected in both - proliferative and zone of mature chondrocytes. The largest was the zone of hypertrophied chondrocytes practically in all cases. The extracellular matrix was stained with PAS and Bismarck Brown in all zones heterogenously.

The OD displayed 3 types of distribution in degenerative cartilage. The OD of extracellular matrix and cytoplasm generally has a tendency to increase from the proliferative zone to zone of mature chondrocytes with insignificant variations in the cytoplasm of cells in the mature chondrocytes and degenerative chondrocytes in 13 cases of sections stained with both - PAS and Bismarck Brown reactives. Degenerative changes of chondrocytes correlated with the either significant decrease or increase of OD in the extracellular matrix and cytoplasm of chondrocytes in all three zones. Especially high difference of the OD in the extracellular matrix and cytoplasm was seen in 3 degenerative cartilage with asbestotic channels. Extracellular matrix and cytoplasm of chondrocytes displayed an intensive staining with PAS, Bismarck Brown and even with hematoxilin and eosin.

PAS positive granules were measured spectrophotometrically in hyaline cartilage of disordered and normal bronchial wall. We have found PAS positive granules in cells of all zones only in 13 cases. Amount of granules has a tendency to increase in chondrocytes from the proliferative zone to zone of hypertrophied chondrocytes in 21 cases, mainly in cartilage of young people and in non-changed cartilage. The degenerative cartilage possessed PAS positive granules only in the zone of hypertrophied chondrocytes in 8 cases. Also decreasing in the amount of granules from the zone of mature chondrocytes to zone of hypertrophied cells was observed in degenerative cartilage of 13 humans of various age. We could not detect PAS positive granules in the zone of hypertrophied cartilage in 2 cases. Additionally, the OD of granules did not sowed significant differences in chondrocytes of all 3 zones.

DISCUSSION

OD of EM and C of CH increased normally from proliferative to hypertrophied cells. OD of EM seemed more unstable in process of degeneration than OD of C. The major components of cartilage are collagen II and the large tissue-specific proteoglycan [8]. It is known that ossification of cartilage may change type of collagen [9]. Proteoglycan constituent-glycosaminoglycans possessed changes before mineral deposition in cartilage due increasing of glycogen in PAS positive granules. Therefore we suggest that both of the above mentioned EM components are involved in process of degeneration of hyaline cartilage.

CONCLUSIONS

1. Graduall increase of OD in EM and C from proliferative to hypertrophied CH characterises non-changed bronchial hyaline cartilage.

2. Beginning of degeneration in cartilage correlates to changes of OD in EM via OD increasing or decreasing in all zones of cartilage. Significant changes of OD in EM characterises degenerative and asbestotic cartilage.

3. Degeneration of cartilage seems to depend on changes of EM components, but do not depend on character of airways disease and even on age.

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