Mucoid Dysplasia of Tricuspid and Congenital Bicuspid Aortic Valves in Syrian Hamsters (Mesocricetus auratus)

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Summary

A histological study was made of the aortic valves of 141 Syrian hamsters aged between 35 and 657 days, belonging to an inbred laboratory colony with a high incidence of congenital bicuspid aortic valves. A tricuspid aortic valve was found in 81 specimens, whereas the remaining 60 possessed a bicuspid aortic valve. In all bicuspid valves there were two aortic sinuses, a ventral and a dorsal, each supporting one cusp. Thirty-three (23.4%) of the 141 specimens showed mucoid dysplasia of the aortic valve. The defect was characterized by thickening of the valve cusps and disruption of the fibrosa layer accompanied by an increased amount of glycosaminoglycans. Ten (12.3%) of the 81 tricuspid aortic valves and 23 (38.3%) of the 60 bicuspid aortic valves were dysplastic. This difference was statistically significant (P < 0.001). The findings indicate that, in the Syrian hamster, the simultaneous occurrence of bicuspid aortic valve and aortic valve dysplasia is not a random event. However, the fact that these valve defects also occur independently suggests that there is no primary morphogenetic dependence between them, but that some other cause predisposes to their concurrence.

Introduction

Mucoid changes in the aortic valve, characterized by thickening of the valve cusps, disruption of the fibrosa layer, and an increased amount of glycosaminoglycans have been reported in man (Weaver et al., 1959; Davis et al., 1965; Hyams and Manion, 1968; Kern and Tucker, 1972; Cheitlin et al., 1978; Rippe et al., 1980; Allen et al., 1985; Bellitti et al., 1985; Cromme-Dijkhuys and Meuzelaar, 1991) and domestic animals (Rooney and Franks, 1964; Amberger et al., 1989; Watson et al., 1991). These tissue alterations in the aortic valve often lead to complications such as aortic incompetence because of weakening of the cusps (Allen et al., 1985; Bellitti et al., 1985) and aortic stenosis due to obstruction of the aortic orifice by thickening of the cusps (Davis et al., 1965; Cheitlin et al., 1978; Watson et al., 1991).

The aetiology of the mucoid changes in the aortic valve remains unclear. Several authors (Davis et al., 1965; Hyams and Manion, 1968; Cheitlin et al., 1978; Rippe et al., 1980; Allen et al., 1985; Bellitti et al., 1985; Cromme-Dijkhuys and Meuzelaar, 1991) and domestic animals (Rooney and Franks, 1964; Amberger et al., 1989; Watson et al., 1991). These tissue alterations in the aortic valve often lead to complications such as aortic incompetence because of weakening of the cusps (Allen et al., 1985; Bellitti et al., 1985) and aortic stenosis due to obstruction of the aortic orifice by thickening of the cusps (Davis et al., 1965; Cheitlin et al., 1978; Watson et al., 1991).
1978; Watson et al., 1991) believe that they are of congenital origin, as the result of an abnormal histogenesis, but others (Weaver et al., 1959; Kern and Tucker, 1972; Rippe et al., 1980; Allen et al., 1985; Bellitti et al., 1985) consider that they are of acquired origin, as the product of a degenerative process. An animal model might elucidate further the factor or factors producing this clinically important aortic valve disease.

In a histological study of the aortic valves of Syrian hamsters belonging to a laboratory inbred family with a high incidence of congenital bicuspid aortic valves, numerous specimens showed mucoid changes in the valve cusps, thought to be of congenital origin; these changes will be referred to as mucoid dysplasia. The aim is to report these findings, and to examine the possible relationship between mucoid dysplasia and the morphological condition of the aortic valves.

Materials and Methods

Animals

The animals used consisted of 141 Syrian hamsters (77 male, 64 female) aged 35 to 657 days, belonging to a single family subjected to high endogamous pressure by mating siblings, or, occasionally, the offspring of siblings. As reported by Sans-Coma et al. (1993), the incidence of bicuspid aortic valves is relatively high in this family, which originated from an unrelated pair with tricuspid aortic valves. The hamsters were housed in polypropylene cages in a temperature-controlled room. Commercial mouse food (UAR/Panlab s.l. A.04) and water were given ad libitum from the time of weaning. There was no known exposure of the animals to teratogenic agents. All the hamsters were handled in compliance with international policies of animal care and welfare. They were killed by overdosing with chloroform and the ventral aspect of the heart was exposed by means of a thoracotomy at the level of the fifth intercostal space.

Histopathological Examination

The hearts were perfused with heparinized 0.9% physiological saline, fixed in 10% neutral formalin buffered with magnesium carbonate, and embedded in paraffin wax. Transverse sections serially cut at 10 µm for light microscopy were stained with haematoxylin-eosin, toluidine blue, Mallory’s trichrome, Masson-Goldner’s trichrome, orcein-picrofuchsin or Weigert-Van Gieson stains. Other procedures applied were picrosirius for the specific detection of collagen by polarization microscopy (Junqueira et al., 1979), and differential staining for sulphated glycosaminoglycans by the critical electrolyte concentration method of Scott and Dorling (1965); in this method sections were stained in solutions of 0.1% alcian blue 8GX in 0.05 M acetate buffer (pH 5.8) plus magnesium chloride in concentrations of 0.3 M, 0.65 M, 0.9 M, and 1 M.

Statistical Analysis

The χ²-test was used. A probability of 0.05 or less was required as evidence for a significant difference.

Nomenclature

The nomenclature used for aortic valve components was that of Thubrikar (1990).
Results

In 81 (57.4%) of the 141 hamsters examined, the aortic valve was tricuspid (Fig. 1); it showed three aortic sinuses, right, left, and dorsal, and three cusps (or leaflets). The remaining 60 (42.6%) specimens possessed a bicuspid aortic valve (Fig. 2). In all of these anomalous valves there were two aortic sinuses, a ventral and a dorsal, each supporting one cusp. In 27 (45.0%) of the 60 bicuspid aortic valves a raphe was located in the ventral aortic sinus. In 22 cases the raphe reached the cusp (Fig. 2), whereas in the other five cases the raphe was more or less developed but did not contact the cusp. The remaining 33 (55.0%) bicuspid aortic valves were devoid of any raphe. Overall, 37 (48.0%) of the 77 males and 23 (35.9%) of the 64 females had a bicuspid aortic valve, thus giving a sex ratio of 1.3:1. This difference between sexes was not statistically significant ($\chi^2 = 2.064$; d.f. = 1; $P > 0.10$).

In 108 (76.6%) of the specimens (71 with a tricuspid aortic valve and 37 with a bicuspid aortic valve) the valve cusps were histologically normal, each consisting of a core of connective tissue covered by the endothelium. At the level of the coaptation region or redundant surface of the normal cusp, the connective tissue was stratified in two main layers, fibrosa and spongiosa (Fig. 3). The fibrosa, which occupied the arterial zone of the cusp, was composed principally of collagen fibres running parallel to the surface of the cusp. The spongiosa, located in the ventricular zone of the cusp, was a loose connective tissue layer, rich in glycosaminoglycans, and contained sparse connective tissue cells and collagen fibres. On the ventricular side of the spongiosa there was usually a thin layer of elastic and collagen fibres. At the basal portion of the cusp, the spongiosa showed a remarkable decrease in size or was even absent, except for the zones of attachment of the cusp to the wall of the aortic root. In several specimens, generally from older animals, the fibrosa was normal, whereas the spongiosa was somewhat thickened, mainly due to the deposit of glycosaminoglycans.

In the remaining 33 (23.4%) specimens, at least one valve cusp was dysplastic. The affected cusps were clearly thickened at the level of their coaptation region. At this site, the fibrosa was significantly disrupted, and the cusp mainly consisted of loose tissue containing large amounts of glycosaminoglycans as well as sparse connective tissue cells and randomly oriented collagen fibres (Fig. 4). In contrast, at the basal portion of the cusp, the fibrosa was intact. Foci of cartilage were detected in 23 (69.7%) of the dysplastic aortic valves (Fig. 2). However, no dysplastic valve showed inflammatory infiltrate, fibrosis or calcification.

In both normal and dysplastic cusps, alcian blue staining was strongly positive at concentrations of 0.3 M and 0.65 M of magnesium chloride, whereas the staining intensity decreased strikingly at concentrations of 0.9 M and 1 M, indicating that chondroitin sulphate was the most abundant glycosaminoglycan in both the normal spongiosa layer and dysplastic connective tissue.

Overall, 21 (27.3%) of the 77 males and 12 (18.7%) of the 64 females possessed a dysplastic aortic valve, thus giving a sex ratio of 1.5:1. This difference was not statistically significant ($\chi^2 = 1.436$; d.f. = 1; $P > 0.20$).
Fig. 1. Transverse section of a tricuspid aortic valve with histologically normal cusps, from a Syrian hamster aged 160 days. DS, dorsal aortic sinus; LS, left aortic sinus; LC, left coronary artery; RS, right aortic sinus. Weigert-Van Gieson stain. Scale bar = 250 μm.

Fig. 2. Transverse section of a dysplastic bicuspid aortic valve with a raphe (arrow) located in the ventral aortic sinus, from a Syrian hamster aged 160 days. The raphe widely encroaches toward the cusp. Both the cusps and raphe are thickened. A focus of cartilage (arrowhead) is present in front of the raphe, slightly penetrating this structure. DS, dorsal aortic sinus; VS, ventral aortic sinus. Toluidine blue. Scale bar = 250 μm.
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Fig. 3. Transverse section of the right and left cusps, at the level of their coaptation regions, of a tricuspid aortic valve with slight fusion of the ventral commissure (arrow), from a Syrian hamster aged 60 days. The cusps are histologically normal. The fibrosa (F) and spongiosa (S) can be clearly identified. Picrosirius. Scale bar = 50 μm.

Fig. 4. Transverse section of a cusp of a dysplastic tricuspid aortic valve, from a Syrian hamster aged 570 days. The cusp is thickened and the fibrosa is disrupted. The cusp consists of loose connective tissue containing large amounts of glycosaminoglycans, sparse connective tissue cells, and randomly oriented fibres of collagen. Weigert-Van Gieson stain. Scale bar = 50 μm.
The youngest and oldest hamsters with dysplastic aortic valves were 35 and 570 days old, respectively. The distribution of the affected animals according to age was as follows: age 35–180 days, 19 (22.1%) of 86 hamsters; age 181–365 days, seven (20.0%) of 35 hamsters; age > 365 days, seven (35.0%) of 20 hamsters. The differences were not statistically significant ($\chi^2 = 1.779$; d.f. = 2; $P > 0.30$).

Mucoid dysplasia occurred in 10 (12.3%) of the 81 tricuspid aortic valves and 23 (38.3%) of the 60 bicuspid aortic valves. This difference was statistically significant ($\chi^2 = 13.116$; d.f. = 1; $P < 0.001$). Seven of the 10 dysplastic tricuspid aortic valves showed an extensive fusion of the ventral commissure, between the right and left cusps. On the other hand, nine of the 23 dysplastic bicuspid aortic valves possessed a raphe that reached the cusp, a further three had a raphe confined to the aortic wall, and the remaining 11 were devoid of any raphe.

Discussion

Mucoid changes in the aortic valve have often been recorded in man, as mentioned above. In contrast, such changes seem to be uncommon in domestic animals (Watson et al., 1991); they have been described in the horse (Rooney and Franks, 1964), dog (Amberger et al., 1989) and cow (Watson et al., 1991). To our knowledge, this paper is the first to report mucoid changes in the aortic valve of the Syrian hamster.

In several specimens, generally from older animals, the spongiosa of the valve cusps, unlike the fibrosa, was somewhat increased in size because of the deposit of glycosaminoglycans. It is well known, however, that increased amounts of glycosaminoglycans are sometimes present in aortic valves as a result of ageing (Kern and Tucker, 1972; Rippe et al., 1980; Allen et al., 1985).

By contrast, in the present study a considerable proportion (23.4%) of specimens showed strikingly thickened aortic valve cusps, due to the accumulation of glycosaminoglycans, with significant disruption of the fibrosa layer. The thickened tissue mainly consisted of a glycosaminoglycan matrix containing sparse fibrocytes and randomly oriented collagen fibres, in the absence of inflammatory infiltrates, fibrosis or calcification. These tissue changes, the key feature of which was the disruption of the fibrosa, occurred only at the level of the coaptation region of the cusps, where the spongiosa is always present. In contrast, at the basal portion of the cusps, where the spongiosa is greatly decreased in size or even absent, the fibrosa was always intact. This suggests that disruption of the fibrosa and excessive accumulation of glycosaminoglycans are strongly related from the aetiological viewpoint. However, it remains uncertain whether the increase in glycosaminoglycans is a primary or secondary event.

Foci of cartilage occurred in a high proportion of dysplastic aortic valves. It is well known, however, that cartilage normally develops in the aortic valve of Syrian hamsters (Kelsall and Visci, 1970).

The results gave no statistically significant evidence that the occurrence of mucoid alterations in the valve cusps was age-related. A considerable propor-
tion of the specimens presenting such changes were from animals aged ≤180 days, the youngest being 35 days old. These findings, together with the fact that, in the Syrian hamster, the histogenesis of the cardiac semilunar valves continues until at least one month after birth (unpublished data), suggest that the abnormalities of the connective tissue reported herein were not the result of a degenerative process but were of congenital origin, hence the term mucoid dysplasia, which implies abnormal tissue development. However, a study in embryos and neonatal animals needs to be undertaken to verify this hypothesis.

Mucoid changes in the aortic valve tissue may lead to complications such as aortic incompetence and aortic stenosis. The tissue changes found in the hamsters examined probably caused disturbances in the aortic valve performance. However, the animals were in good general condition. In man mucoid changes may affect any of the cardiac valves, and patients often have multiple valve involvement (Bharati and Lev, 1973). Since our study was exclusively focused on the aortic valve, it remains unknown whether mucoid dysplasia also occurred in other cardiac valves.

Our findings showed that, in the Syrian hamster, mucoid dysplasia was significantly more common in bicuspid than in tricuspid aortic valves. In man, the bicuspid condition of the aortic valve is the most frequent congenital cardiac anomaly seen in adults (Roberts, 1987), with an incidence of 0.5–2% in autopsy surveys (Giusti et al., 1991). This valve defect may be inapparent clinically and compatible with normal functions (Roberts, 1989). However, it clearly predisposes to complications such as aortic stenosis by dystrophic calcification, incompetence, and infective endocarditis (Fenoglio et al., 1977; Cheitlin et al., 1978; Roberts, 1987; Giusti et al., 1991). Mucoid changes in the aortic valve, in association with the bicuspid condition, have been repeatedly reported in man (Kern and Tucker, 1972; Cheitlin et al., 1978; Allen et al., 1985), but whether there is a statistically significant link is not known.

The aetiological aspects of the association between bicuspid aortic valve and aortic valve dysplasia are still unclear. The present findings demonstrate that the simultaneous occurrence of these valve defects is not a random event. However, the fact that they also occur independently indicates that there is no primary morphogenetic dependence between them, but that some other cause predisposes to their concurrence. In the Syrian hamster, the development of bicuspid aortic valves is influenced by genetic factors (Sans-Coma et al., 1993); and it is likely that the mucoid dysplasia of the aortic valve is subordinated to genetic influence, as with similar mucoid changes affecting the human mitral valve (Devereux and Kramer-Fox, 1988). Therefore, genetic interaction as a possible cause of the association between bicuspid aortic valve and valvular dysplasia should not be overlooked. The Syrian hamster would seem to provide an appropriate animal model for further investigation of this question.

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