Anomalous Origin of the Left Coronary Artery from the Dorsal Aortic Sinus and its Relationship with Aortic Valve Morphology in Syrian Hamsters

M. Cardo, B. Fernández, A. C. Durán, M. C. Fernández, J. M. Arqué* and V. Sans-Coma

Department of Animal Biology (Zoology), Faculty of Science, University of Málaga, E-29071 Málaga and *Regional Hospital “Carlos Haya”, E-29011 Málaga, Spain

Summary
The condition of the aortic valve and the origin of the coronary arteries were examined in 2413 Syrian hamsters aged between 1 and 823 days, belonging to nine inbred laboratory families. The specimens were studied with either a stereomicroscope, a corrosion-cast technique, or histological techniques (light microscopy). The aortic valve was tricuspid in 1823 (75.5%) cases, and bicuspid in the remaining 590 (24.5%). In all bicuspid aortic valves there were two aortic sinuses, a ventral and a dorsal, each supporting one cusp. The left coronary artery arose from the dorsal aortic sinus in 71 (2.9%) hamsters, eight of which died unexpectedly between ages 1 and 622 days. In 29 (40.8%) of the 71 cases, the aortic valve was tricuspid and the right coronary artery originated from the right aortic sinus. In the remaining 42 (59.2%) cases, the aortic valve was bicuspid and the right coronary artery arose from the right side of the ventral aortic sinus. Results of a $\chi^2$ contingency test demonstrated that the frequency of left coronary artery arising from the dorsal aortic sinus significantly increased when the aortic valve was bicuspid. This fact, together with previously reported data on coronary artery anomalies in the Syrian hamster, suggests that the left coronary artery arising from the dorsal aortic sinus may be an expression of a single morphogenetic defect which is expressed as bicuspid aortic valve in some cases, anomalous origin of the left coronary artery in others, or in the simultaneous occurrence of these two cardiac abnormalities.

Introduction
In mammals there are generally two coronary arteries, right and left, which originate from the right and left aortic sinuses, respectively (Robb, 1965). A coronary artery that arises from the dorsal (posterior or non-facing in man) aortic sinus is considered as having an anomalous origin (Angelini, 1989).

Occurrence of a left coronary artery arising from the dorsal aortic sinus in coexistence with a right coronary artery originating (normally) from the right aortic sinus is an extremely uncommon event. In man, only nine cases have been reported (Ogden, 1970; Roberts, 1987; Click et al., 1989; Virmani et al., 1989; Ishikawa et al., 1990; Cohen et al., 1991), and in two of them, the patient died a sudden “cardiac death” (Virmani et al., 1989; Ishikawa et al., 1990). On the other hand, this anomalous arrangement of the coronary arteries has
been described in Syrian hamsters (Sans-Coma et al., 1989, 1991; Arqué et al., 1993) and in a rat (Durán et al., 1991).

In the Syrian hamster, a species which provides an animal model for the study of congenital anomalies of the coronary arteries and cardiac semilunar valves in man (Sans-Coma et al., 1991, 1992, 1993b; Cardo et al., 1994), the anomalous origin of the left coronary artery from the dorsal aortic sinus often occurs in association with a bicuspid aortic valve (Sans-Coma et al., 1991; Arqué et al., 1993), but whether there is a statistically significant link remains unknown. The present study was designed to elucidate this question in hamsters in which the left coronary artery arose from the dorsal aortic sinus.

Materials and Methods

Animals

Syrian hamsters (2413; 1214 male, 1199 female) belonging to nine inbred laboratory families were used. Each family was subjected to high endogamous pressure by mating siblings or, occasionally, the offspring of siblings. As previously reported (Sans-Coma et al., 1989), the incidence of coronary artery anomalies is relatively high in this complex of families. In addition, the frequency of bicuspid aortic valves is particularly high in one of the families, the characteristics of which have been described elsewhere (Sans-Coma et al., 1993b; Franco et al., 1994). Overall, 1346 of the hamsters (683 male, 663 female) belonged to this family.

The hamsters were housed in polypropylene cages in a temperature-controlled room. Commercial mouse food (UAR/Panlab s.i. A04) 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 the international policies of animal care and welfare as stated in EC Directive 86/609, 1986. Most of them (n = 2237) were killed at ages 1 to 816 days by overdosing with chloroform, according to the protocol established for cardiovascular studies in our laboratory. The remaining 176 died unexpectedly between ages 1 and 823 days. In all cases, the heart was exposed by means of a thoracotomy at the level of the fifth intercostal space.

Techniques

In 549 cases, the heart was removed after perfusion with heparinized 0.9% physiological saline, and transferred to a similar solution for dissection. The condition of the aortic valve and the origin of the coronary arteries were assessed by means of a stereomicroscope.

A further 1648 specimens were examined by a corrosion-cast technique. Vinyl resin (Rhodopas® AX 85/15) in a 20% ketone solution was injected via a cannula placed in the ventral aorta through the apex of the left ventricle. Whenever an anomalous arrangement of the coronary arteries was suspected, the right ventricle and the pulmonary trunk were also injected. Internal casts of the ventricles and arterial vessels were obtained by macerating the specimens in a 20% hydrochloric acid bath. In 24 cases, the cast was gold sputter-coated for observation on a scanning electron microscope (Jeol JSM-840), operated at 15 kV.

The remaining 216 specimens were studied by histological techniques. The hearts were fixed in 10% neutral formalin buffered with magnesium carbonate, and embedded in paraffin wax. Transverse sections serially cut at 10 μm thickness for light microscopy were stained with haematoxylin and eosin or Mallory’s trichrome stains.

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 71 (2.9%) of the 2413 specimens examined, the left coronary artery originated from the dorsal aortic sinus. The affected animals (39 male, 32 female) were aged between 1 and 622 days. The difference between sexes was not statistically significant.

In 1823 (75.5%) of 2413 hamsters, the aortic valve was tricuspid; three aortic sinuses were present, right, left and dorsal, and three cusps (or leaflets). The remaining 590 specimens (24.5%) possessed a bicuspid aortic valve: 314 of 1214 males (25.9%) and 276 of 1199 females (23.0%). In all of these anomalous valves there were two aortic sinuses, a ventral and a dorsal, each supporting one cusp. The slight difference between sexes was not statistically significant.

In 29 (40.8%) of the 71 cases of left coronary artery originating from the dorsal aortic sinus, the aortic valve was tricuspid and the right coronary artery arose from the right aortic sinus (Fig. 1). In the remaining 42 (59.2%) cases,
the aortic valve was bicuspid (Fig. 2) and the right coronary artery arose from the right side of the ventral aortic sinus. In all specimens, the proximal segment of the left coronary artery formed an acute angle with the aortic wall. The left coronary ostium was located either in the centre of the dorsal aortic sinus or near the left commissure of the valve.

Eight (11.3%) of the 71 hamsters with the left coronary artery arising from the dorsal aortic sinus died unexpectedly. They were in good general condition before death. Three of these eight hamsters (two male, one female) were neonatal animals; another was a young female aged 26 days; the remaining four (female) were adult animals aged 218 to 622 days. The aortic valve was tricuspid in one case, and bicuspid in the remaining seven. None of these valves showed signs of disease, and no other cardiac defects were detected. However, extracardiac causes of death could not be ruled out since the post-mortem examination was confined to the heart.

To test for any association between the occurrence of a left coronary artery originating from the dorsal aortic sinus and the morphology of the aortic valve, a $\chi^2$ contingency test was performed under the null hypothesis that they are independent events. Male and female data were pooled for the test since no statistical difference between sexes was found in the occurrence of the coronary artery anomaly or the bicuspid condition of the aortic valve. First, the frequency of either tricuspid or bicuspid aortic valve in the whole sample ($n=2413$) was calculated. The values obtained were 0.755 for tricuspid aortic valves and 0.245 for bicuspid aortic valves. In the group with the left coronary artery arising from the dorsal aortic sinus ($n=71$), the expected value of each aortic valve condition was obtained by multiplication of its frequency and the total number of specimens with the coronary artery anomaly. The calculated
expected values, the values obtained from the sample studied, and the results of the test are given in Table 1. The computed value of the $\chi^2$ statistic was 46.069, with one degree of freedom. Therefore, the null hypothesis was rejected at $P<0.001$.

**Discussion**

In the Syrian hamster, the normal aortic valve has three aortic sinuses, each supporting one cusp (Sans-Coma et al., 1992). Two well-developed coronary arteries, the right and left, arise from the right and left aortic sinuses, respectively, and become intramyocardial shortly after their origin from the aorta (Sans-Coma et al., 1993a). When the aortic valve is bicuspid, with the sinuses arranged in ventrodorsal orientation, the right and left coronary arteries originate from the right and left sides of the ventral aortic sinus, respectively (Sans-Coma et al., 1991; Cardo et al., 1994). These are the sites which topographically correspond to the right and left aortic sinuses of a tricuspid aortic valve. A coronary artery that arises from the dorsal aortic sinus of a bicuspid aortic valve can be regarded as having an anomalous origin.

In all of the present specimens with the left coronary artery arising from the dorsal aortic sinus, the proximal segment of this artery was orientated at an acute angle and not perpendicular to the aortic wall. According to Virmani et al. (1984), the acute "take-off" at the origin of a coronary artery can be worsened when dilation of the aorta occurs, producing a fall in blood flow.

Recent studies in quail (Bogers et al., 1989) and chick embryos (Waldo et al., 1990) have contradicted the classical hypothesis that the proximal coronary arteries develop as coronary artery buds that hollow out from the aorta to connect with the peritruncal plexus of capillaries, located in the subepicardial layer of the developing heart. These studies have shown that in birds, at least, the proximal coronary arteries do not grow outward from the aorta, but into the aorta. They develop with the penetration of the endothelia from the peritruncal ring of coronary artery vasculature into the media of the aorta. Therefore, a coronary artery that arises anatomically from the dorsal aortic sinus is connected to this sinus from the embryological viewpoint.

The statistical analysis in the present study demonstrated that the anomalous origin of the left coronary artery from the dorsal aortic sinus was significantly
associated with a bicuspid aortic valve. This indicates that the simultaneous occurrence of these two cardiac defects is not a random event. However, the fact that they also occur independently denotes that there is no primary morphogenetic dependence between them, but that some other cause predisposes to their concurrence.

In two previous studies (Sans-Coma et al., 1991; Cardo et al., 1994), it was demonstrated that, in our Syrian hamster colony, the frequency of left coronary artery originating from the right coronary artery and left coronary artery arising from the pulmonary trunk, significantly increased when the aortic valve was bicuspid. Interestingly, all of the congenital coronary artery defects that appear to be significantly associated with the bicuspid condition of the aortic valve in our hamsters are characterized anatomically by the anomalous origin of the left coronary artery. This suggests that bicuspid aortic valve and abnormal connection of the embryonic left coronary arterial tree to the great arterial vessels result from a single developmental diathesis. In the Syrian hamster, these cardiac anomalies seem to result from a common underlying morphogenetic defect, which is expressed as bicuspid aortic valve in some cases, anomalous origin of the left coronary artery in others, or in the simultaneous occurrence of both abnormalities.

Several reports have appeared in recent years suggesting that the anomalous behaviour of the cells from the cardiac neural crest gives rise to the bicuspid condition of the aortic valve (Kappetein et al., 1991; Miyabara et al., 1993a, b) and anomalies in the origin of the coronary arteries (Hood and Rosenquist, 1992). This gives rise to speculation that defects of the neural crest cells may be the common cause of the aortic valve and coronary artery anomalies found in our hamsters. However, further investigation is needed to verify this hypothesis.

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