Mechanical properties of the common carotid artery in Williams syndrome

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Abstract

Objective—To determine whether arterial wall hypertrophy in elastic arteries was associated with alteration in their mechanical properties in young patients with Williams syndrome.

Methods—Arterial pressure and intima–media thickness, cross sectional compliance, distensibility, circumferential wall stress, and incremental elastic modulus of the common carotid artery were measured non-invasively in 21 Williams patients (mean (SD) age 8.5 (4) years) and 21 children of similar age.

Results—Systolic and diastolic blood pressures were higher in Williams patients (125/66 *v* 113/60 mm Hg, p < 0.05). The mean (SD) intima–media thickness was increased in Williams patients, at 0.6 (0.07) *v* 0.5 (0.03) mm (p < 0.001). Normotensive Williams patients had a lower circumferential wall stress (2.1 (0.5) *v* 3.0 (0.7) mm Hg, p < 0.01), a higher distensibility (1.1 (0.3) *v* 0.8 (0.3) mm Hg^{-1}.10^{-2}, p < 0.01), similar cross sectional compliance (0.14 (0.04) *v* 0.15 (0.05) mm^2.mm Hg^{-1}, p > 0.05), and lower incremental elastic modulus (7.4 (2.0) *v* 14.0 (5.0) mm Hg.10^2; p < 0.001).

Conclusions—The compliance of the large elastic arteries is not modified in Williams syndrome, even though increased intima–media thickness and lower arterial stiffness are consistent features. Therefore systemic hypertension cannot be attributed to impaired compliance of the arterial tree in this condition.

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Microdeletion of chromosome 7q, encompassing the elastin locus, has been identified by fluorescent in situ hybridisation in patients with Williams syndrome.[1] The pathophysiological mechanism is not yet clear but elastin hemizygosity is certainly implicated in the pathogenesis of the arteriopathy of Williams syndrome.[2] Systemic hypertension is a common feature in this condition, and may be caused by renal artery stenosis.[3 4] However, hypertension often remains unexplained in these patients. It has been attributed to reduced compliance of the entire arterial tree associated with diffuse hypertrophy of the media.[5] In vitro and in vivo studies have shown that increased arterial wall thickness is a common phenotypic trait of the Williams syndrome.[6 7] However, arterial wall hypertrophy in hypertensive subjects is not necessarily associated with reduced distensibility.[8–10] In adult Williams syndrome patients, hypertension can cause remodelling of the conductance arteries, overriding primitive alterations of their mechanical properties related to elastin hemizygosity. In this study, we sought to determine whether arterial wall hypertrophy of the common carotid artery, a large proximal and conductance artery, was associated with alterations in its mechanical properties in young patients with Williams syndrome.

Methods

PATIENTS AND CONTROLS

Twenty one patients with Williams syndrome aged 3.5–19 years (mean (SD) age 8.5 (4) years) and 21 control children (9 (2) years) were included in the study. The diagnosis of Williams syndrome relied on typical facial appearance and hemizygosity at the elastin locus in all patients. Williams patients with either renal artery stenosis or receiving antihypertensive drugs were excluded from the study. Fifteen of the 21 Williams patients had a mild supravalvar aortic stenosis (range 12–20 mm Hg). The remaining six patients had no left heart obstruction. None of the Williams patients in this series had coarctation of the aorta and none underwent coarctation repair. Informed consent of the parents was obtained for all subjects.

The investigation was performed in a controlled environment kept at 22 (2)°C. Blood pressure was measured with a mercury sphygmomanometer with a cuff adapted to the arm circumference of the child, who was recumbent for at least 15 minutes before the measurement.

ARTERIAL MEASUREMENTS

Non-invasive arterial measurements were performed with a real time B mode ultrasound imager (Acuson XP128, Acuson, Mountain View, California, USA). The right common carotid artery was examined with a 7 MHZ vascular probe following a procedure described previously.[11 12] The intima–media thickness and lumen diameter measurements were performed in the same arterial segment in all subjects. This segment was located 1–2 cm above the bifurcation of the right common carotid artery. Echographic imaging of the common carotid artery was obtained in the anteroposterior projection with the patient lying supine and the head in the axis. The same physician

(YA) performed all measurements throughout the study.

During scanning, the operator adjusted the sound beam so that it was perpendicular to the far wall of the vessel, obtaining two parallel echogenic lines corresponding to the lumen–intima and media–adventitia interfaces. The gain setting was adjusted in order to visualise cross sectional images for proximal and distal common carotid artery walls with, for each wall, two parallel line echoes separated by a small echo-free space. The upper demarcation line of an echo is defined as the "leading edge" and the lower demarcation line as the "far edge". The location of the leading edge of an echo is at the same level as the level of the interface that creates the echo, and the location of this is not gain dependent. The far edge is partially dependent on the gain setting and the ultrasonographic properties of the vessel wall, and not only on the thickness of the common carotid artery wall. Thus the intima–media thickness was measured between the two leading edges corresponding to the far wall of the common carotid artery.

Once these two parallel echogenic lines were clearly visible along at least 1.5 cm of the segment measured, the frozen end diastolic vessel image (obtained by electrocardiographic R triggering) was transferred to a computer. Off-line image analysis was performed using the Iotec system program (Iô Data Processing Company, Paris, France), based on the analysis of grey level densities and on specific tissue recognition algorithms. The vessel image was transferred to the monitor of the computer and represented a 3.63-fold magnification of the anatomical structure examined. The observer chose a field of measurement that included the intima–media thickness and automatically drew a rectangle at least 1 cm long in the longitudinal axis of the vessel and at least 0.3 cm high in the direction perpendicular to the wall. The computer located the two interfaces (lumen–intima and media–adventitia) by discriminating changes in grey levels inside the sample area, and drew the two parallel lines, displaying these interfaces on the computer monitor. The average intima–media thickness obtained along the length of the sample area represented the mean value of at least 100 successive local measures. The intima–media thickness was measured every 10 μm along 1 cm of the length of the common carotid artery.

The internal lumen diameters of the common carotid artery were averaged along the same distance as the intima–media thickness, between the near and far lumen–intima interfaces. The diastolic diameter (Dd) was calculated as the mean of the minimum values of common carotid artery diameter for five consecutive cardiac cycles (R wave triggering). Systolic diameter (Ds) was calculated as the mean value of the maximum common carotid artery lumen during the same cardiac cycles (T wave triggering). The cross sectional area of the lumen (LCSA) was calculated as $LCSA = \pi Dd^2/4$, the cross sectional area of the arterial wall as $WCSA = \pi(Dd/2 + IMT)^2 - \pi(Dd/2)^2$, and wall/lumen ratio as (IMT/Dd), where IMT = intima–media thickness. Histological validation of computerised intima–media thickness measurements using this technique has been reported previously.[11] The cross sectional compliance (CSC) and distensibility of the common carotid artery were determined from changes in diameter during the systole and simultaneously measured pulse pressure (ΔP) according to following formulas: $CSC = [\pi(Ds^2 - Dd^2)]/4\Delta P$ (mm^2.mm Hg^{-1}); and distensibility = $(Ds^2 - Dd^2)/[Dd2.\Delta P]$ (mm Hg^{-1}.10^{-2}). Circumferential wall stress was calculated as [mean arterial pressure × Dd]/2 × IMT. In contrast to compliance, which provides information about elasticity of the artery as a hollow structure, the incremental elastic modulus (Einc) provides information on the properties of the wall material independent of the geometry. Einc was calculated as [3(1 + LCSA/WCSA)]/distensibility (mm Hg.10^2).

REPEATABILITY OF THE MEASUREMENTS

All measurements were performed by the same investigator. When two series of paired measurements were compared, the results were analysed in two steps according to Bland and Altman.[13] The correlation between the measured values (the linear relation equation, correlation coefficient, and p value) was calculated. The first step was used to gauge the degree of agreement between the two series of measures. Second, the relative differences within each pair of measures (Di) were plotted against the mean of the pair to make sure that no obvious relation appeared between estimated value (mean) and Di. The lack of agreement between the two measures was estimated by the mean difference Di and the standard deviation of the differences.

Repeatability of intima–media thickness and diastolic and systolic diameter measurements was investigated in 10 subjects by calculating the repeatability coefficient (RC), as defined by the British Standard Institution according to the formula $RC^2 = \sum Di^2/n$, where n is the sample size and Di the relative difference within each pair of measures. This coefficient is the standard deviation of the estimated difference between two repeated measurements. RC values for intraobserver repeatability (comparison of two determinations obtained two hours apart by the same observer) for intima–media thickness and diastolic and systolic diameter were 43 μm, 212 μm, and 153 μm, respectively. These values were small when compared with the actual values of common carotid intima–media thickness and the diastolic and systolic diameters—565 (21) μm, 5143 (68) μm, and 5847 (603) μm, respectively—and compared with the difference between Williams patients and controls.

STATISTICAL ANALYSIS

Summary statistics are presented as mean (SD) and range. Univariate comparisons of data in the two groups was made using the unpaired Student's t test or the Mann–Whitney test as appropriate for interval variables. Fisher's exact test was used for categorical variables.

Results

The characteristics of the Williams patients and of the control children are shown in table 1. Systolic and diastolic blood pressures were higher in Williams patients, while the pulse pressure was not different. Twelve Williams patients had a normal blood pressure for their age and body surface area, and nine Williams patients were hypertensive.[14]

Table 2 shows the arterial indices of normotensive Williams patients and control subjects. The carotid intima–media thickness was significantly increased in Williams subjects (0.61 (0.08) *v* 0.51 (0.02) mm; p < 0.0001), and the diastolic diameters were reduced. The ratio between intima–media thickness and diastolic lumen diameter (IMT/Dd) was higher in the Williams patients (0.15 (0.02) *v* 0.10 (0.01); p < 0.001). Consequently, the circumferential wall stress was reduced in the Williams patients. Cross sectional compliance was not modified in normotensive Williams patients. Distensibility was greater and the incremental elastic modulus lower in normotensive Williams patients.

The intima media thickness was slightly higher in hypertensive Williams patients when compared with the normotensive Williams group (0.68 (0.07) *v* 0.59 (0.04) mm, p = 0.04). The functional variables (distensibility, cross sectional compliance, and incremental elastic modulus) were not different in the hypertensive and the normotensive Williams patients (table 3), but a statistical comparison was not performed as the calculated variables depend on the pressure.

Discussion

The relation of the disorganisation of the medial elements in Williams syndrome with alterations of the elastin structure was only recently suspected in the light of the molecular findings in this syndrome.[1] While disruption or loss of elastin was believed to play a central role in arterial aneurysm formation, mutations or deletions in one allele encoding elastin are now known to cause obstructive arterial disease.[1 2 15 16] In our study, we showed—as have others[5–7]—that the intima–media thickness of the common carotid artery was increased in Williams patients, regardless of the mean arterial pressure. Increased intima–media thickness cannot be considered an "adaptative" process according to Laplace's law, as the ratio of intima–media thickness to diastolic diameter is also significantly increased in Williams patients; in fact we found a reduction in diastolic diameters in normotensive Williams patients. Further evidence for the role of disruption of elastin in producing thickening of the arterial wall has recently been produced in a mouse model lacking elastin.[15 16] Obstructive arterial disease in these mice is accompanied by a compensatory increase in the number of rings of elastic lamellae and by intimal smooth muscle proliferation and reorganisation. These morphological changes are independent of haemodynamic stress as they also occur in isolated arteries in organ culture.[15] Although elastin mRNA and protein were reduced by 50% in elastin +/− mice, arterial compliance at physiological pressures was nearly normal.[16] Consequently, elastin not only has a structural role in the extracellular matrix but also controls smooth muscle proliferation during arterial development.

In this study, we showed that the cross sectional compliance of the common carotid artery of Williams patients was not different from that of controls, despite increased wall thickness in the Williams patients. These findings are in contrast with hypertension induced arterial wall hypertrophy associated with decreased distensibility and increased elastic modulus of large elastic arteries observed in essential hypertension. In patients with Williams syndrome, sustained hypertension has an additional effect on arterial wall hypertrophy, as hypertensive Williams patients have significantly increased intima–media thickness compared with their normotensive counterparts. These observations suggest that increased intima–media thickness in Williams patients may have a dual origin—alteration of arterial wall development related to elastin haploinsufficiency, and the additional consequences of haemodynamic stress.

Another striking finding of our study was reduced stiffness of the common carotid artery in Williams patients, expressed by Young's elastic modulus.[10] This parameter gives direct information on the elastic properties of the arterial wall material, regardless of vessel geometry. The respective contribution of the

Table 1 Demographic data and pressure values of Williams patients and controls

Variable	Williams patients (n=21)	Controls (n=21)	p Value
Age (years)	8.5 (4.0)	9.0 (2.0)	NS
Weight (kg)	31 (20)	29 (9)	NS
BSA (m²)	1.05 (0.5)	0.97 (0.2)	NS
Systolic arterial pressure (mm Hg)	125 (18)	113 (10)	< 0.05
Diastolic arterial pressure (mm Hg)	66 (8)	60 (9)	< 0.05
Pulse pressure (mm Hg)	59 (14)	54 (7.3)	NS

Values are mean (SD).
BSA, body surface area.

Table 2 Morphometric and haemodynamic variables in normotensive Williams patients and controls

Variable	Normotensive Williams patients (n=12)	Controls (n=21)	p Value
Intima–media thickness (mm)	0.61 (0.08)	0.51 (0.02)	< 0.001
Diastolic diameter (mm)	4.1 (0.4)	5.1 (0.6)	< 0.001
IMT/diastolic diameter	0.15 (0.02)	0.10 (0.01)	< 0.001
Distensibility (mm Hg^{-1}.10^{-2})	1.1 (0.3)	0.8 (0.3)	< 0.01
Cross sectional compliance (mm².mm Hg^{-1})	0.14 (0.04)	0.15 (0.05)	NS
Circumferential wall stress (mm Hg.10²)	2.1 (0.5)	3.0 (0.7)	< 0.01
Young's elastic modulus (Einc) (mm Hg.10²)	7.4 (2.0)	14.0 (5.0)	< 0.001

Values are mean (SD).
IMT, intima–media thickness.

Table 3 Comparison of morphometric and haemodynamic variables in Williams patients with and without hypertension

Variable	Hypertensive Williams patients (n=9)	Normotensive Williams patients (n=12)
Intima–media thickness (mm)	0.68 (0.07)	0.61 (0.04)
Distensibility (mm Hg^{-1}.10^{-2})	1.0 (0.4)	1.1 (0.3)
Cross sectional compliance (mm².mm Hg^{-1})	0.18 (0.06)	0.14 (0.04)
Circumferential wall stress (mm Hg.10²)	2.5 (0.6)	2.1 (0.5)
Young's elastic modulus (Einc) (mm Hg.10²)	9.5 (7.0)	7.4 (2.0)

Values are mean (SD).

different components of the extracellular matrix to aortic tensile strength and stiffness are not well known.[17] Loss of medial elastin increases pressure dependent circumferential wall stress and it has been suggested that this promotes endothelial damage and aneurysm formation.[18] Anomalies of medial elastic function in Williams syndrome might not be directly related to quantitative loss of elastin in the arterial wall. Bruel and colleagues recently showed that inhibition of the formation of the cross links between collagens by the lysyloxidase resulted in destabilisation of the aortic wall, with increased diameter and reduced stiffness.[19] Such improvement in arterial wall stiffening has also been reported when the accumulation of advanced glycosylation end products on collagen is prevented by aminoguanidine treatment in diabetic rats as well as in normotensive aged WAG/Rij rats.[20 21] In these studies, no changes in elastin and collagen concentrations were found. Another recent study showed that removal of the microfibrils from elastin reduced the modulus of the pig aortic elastic tissue, even though no evidence of elastin hydrolysis could be observed.[22] A quantitative change in biosynthesis appears to alter the organisation of the various medial elements during development, and this is supported by knock out of the elastin gene in the mouse.[16 23] In addition, pathological observations in Williams patients show thick irregular elastic fibres, swirling collagen, and hypertrophied smooth muscle cells.[7] This abnormal deposition of elastin in the media could modify the distribution of the load throughout the arterial wall and shift load bearing to structures with a low elastic modulus. For these reasons, qualitative changes of elastin—or more specifically changes in the orientation of the elastic fibres—might explain the alterations in the mechanical performance of the common carotid artery in Williams syndrome.

CONCLUSIONS

We have shown that the functional compliance of the common carotid artery is not significantly modified in Williams syndrome, although increased intima–media thickness and lower arterial stiffness are consistent features. Thus systemic hypertension in Williams patients cannot be attributed to impaired compliance of the arterial tree in this condition. Other pathogenic mechanisms for systemic hypertension need to be evaluated, such as resetting of the baroreceptors in response to alterations in the sensing of wall stress.

1 Ewart AK, Morris CA, Atkinson D, *et al*. Hemizygosity at the elastin locus in a developmental disorder, Williams syndrome. *Nature Genet* 1993;**5**:11–16.
2 Tassabehji M, Metcalfe K, Donnai D, *et al*. Elastin: genomic structure and point mutations in patients with supravalvular stenosis. *Hum Mol Genet* 1997;**6**:1029–36.
3 Wessel A, Motz R, Pankau R, *et al*. Arterial hypertension and blood pressure profile in patients with Williams-Beuren syndrome. *Z Kardiol* 1997;**86**:251–7.
4 Daniels SR, Loogie JM, Schwartz DC, *et al*. Systemic hypertension secondary to peripheral vascular anomalies in patients with Williams syndrome. *J Pediatr* 1985;**106**:249–51.
5 Rein AJJT, Preminger TJ, Perry SB, *et al*. Generalized arteriopathy in Williams syndrome: an intravascular ultrasound study. *J Am Coll Cardiol* 1993;**21**:1727–30.
6 Sadler LS, Gingell R, Martin DJ. Carotid ultrasound examination in Williams syndrome. *J Pediatr* 1998;**132**:354–6.
7 O'Connor WN, Davis JB, Geissler R, *et al*. Supravalvular aortic stenosis. Clinical and pathologic observations in six patients. *Arch Pathol Lab Med* 1985;**109**:179–85.
8 Hayoz D, Rutschmann B, Perret F, *et al*. Conduit artery compliance and distensibility are not necessarily reduced in hypertension. *Hypertension* 1992;**20**:1–6.
9 Laurent S, Caviezel B, Beck L, *et al*. Carotid artery distensibility and distending pressure in hypertensive humans. *Hypertension* 1994;**23**:878–83.
10 Laurent S, Girerd X, Mourad JJ, *et al*. Elastic modulus of the radial artery wall material is not increased in patients with essential hypertension. *Arterioscler Thromb* 1994;**14**:1223–31.
11 Gariepy J, Simon A, Massonneau M, *et al*. Echographic assessment of carotid and femoral arterial structure in men with essential hypertension. Group PCVMETRA. *Am J Hypertens* 1996;**9**:126–36.
12 Gariepy J, Massonneau M, Levenson J, *et al*. Evidence for in vivo carotid and femoral wall thickening in human hypertension. Groupe de Prevention Cardio-vasculaire en Médecine du Travail. *Hypertension* 1993;**22**:111–18.
13 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;**i**:307–10.
14 Andre JL, Deschamps JP, Gueguen R. Pression artérielle chez 17,067 enfants et adolescents. Variation en fonction de l'âge et de la taille. *Arch Fr Pediatr* 1980;**37**:477–82.
15 Li DY, Brooke B, Davis EC, *et al*. Elastin is an essential determinant of arterial morphogenesis. *Nature* 1998;**393**:276–80.
16 Li DY, Faury G, Taylor DG, *et al*. Novel arterial pathology in mice and humans hemizygous for elastin. *J Clin Invest* 1998;**102**:1783–7.
17 Avolio A, Jones D, Tafazzoli-Shadpour M. Quantifications of alterations in structure and function of elastin in the arterial media. *Hypertension* 1998;**32**:170–5.
18 Boyle JR, McDermott E, Crowther M, *et al*. Doxycycline inhibits elastin degradation and reduces metalloproteinase activity in a model of aneurysmal formation. *J Vasc Surg* 1998;**27**:354–61.
19 Bruel A, Ortoft G, Oxlund H. Inhibition of cross-links in collagen is associated with reduced stiffness of the aorta in young rats. *Atherosclerosis* 1998;**140**:135–45.
20 Huijberts MS, Wolffenbuttel BH, Boudier HA, *et al*. Aminoguanidine treatment increases elasticity and decreases fluid filtration of large arteries from diabetic rats. *J Clin Invest* 1993;**92**:1407–11.
21 Corman B, Duriez M, Poitevin P, *et al*. Aminoguanidine prevents age-related arterial stiffening and cardiac hypertrophy. *Proc Natl Acad Sci USA* 1998;**95**:1301–6.
22 Lillie MA, David GJ, Gosline JM. Mechanical role of elastin-associated microfibrils in pig aortic elastic tissue. *Connect Tissue Res* 1998;**37**:121–41.
23 Martyn CN, Greenwald SE. Impaired synthesis of elastin in walls of aorta and large conduit arteries during early development as an initiating event in pathogenesis of systemic hypertension. *Lancet* 1997;**350**:953–5.