Long-Term Effects of Hypertension on the Rat Aortic Wall and Their Relation to Concurrent Aging Changes

MORPHOLOGICAL AND CHEMICAL STUDIES

By Harvey Wolinsky

ABSTRACT

The effects of long-term (16 months) hypertension on the thoracic aorta of male rats were compared to previously reported short-term (2.5 months) changes and to concurrent aging changes. Hypertension was produced by clipping a renal artery. Although short-term hypertension was characterized by a disproportionate increase in noncollagenous alkali-soluble proteins, which have been attributed primarily to vascular smooth muscle, with long-term hypertension there was no further increase in these proteins but instead there were striking increases in mural accumulations of elastin and collagen. Chronically elevated wall tension in hypertensive vessels was associated with a progressive increase in wall thickness which resulted in a value for wall stress no different from that of control vessels. Concurrent aging changes were qualitatively similar to, but much less pronounced than, those seen with hypertension and were attributed to an increase in wall tension in controls resulting from a combination of significant increases in diameter and systolic blood pressure with age. This study of the interaction of vessel structure and function has revealed common features of what appears to be a diverse group of vascular alterations.

KEY WORDS

- vessel structure and function
- elastin
- collagen
- aortic wall tension
- aortic wall stress
- vascular disease

Hypertension results in a strikingly increased thickness and stiffness of the entire vascular tree (1, 2); similar changes are seen to a lesser degree in the vessel wall during growth and aging (3, 4). Morphological and chemical studies of hypertensive vessels have shown that the changes in vessel dimensions and properties are due to smooth muscle hypertrophy (1) and hyperplasia (5) and increased amounts of mural mucopolysaccharides (6), elastin, and collagen (7).

Elastin and collagen also accumulate in vessels during aging (4), though the relative degree of increase in each of the fibrous proteins is controversial (4, 8-10). These similarities and others have led some to suggest that hypertension is an accelerated form of aging (3).

Although the changes in vascular morphology associated with hypertension have been well described (1) and no qualitative morphological differences between vessels exposed to acute hypertension and chronic hypertension were found (11), a detailed comparison of short-term and long-term effects of hypertension on the dimensions and chemical composition of the vessel wall has not been done. This would be of considerable interest for several reasons. First, the lack of significant effects of treatment on mortality from coronary artery disease in hypertensive patients has been postulated to be due to...
irreversible changes in the vessel wall resulting from the long duration of hypertension prior to treatment; it was predicted that shorter duration of hypertension prior to treatment would favorably influence the prognosis (12). Second, recent work has suggested that structural adaptive changes in vessel walls of hypertensive animals may be the cause of sustained hypertension (13, 14). Finally, experimental evidence suggests that it is much easier to restore blood pressure to normal after correction of renal artery stenosis in animals with short-term hypertension than it is in animals with long-term renal hypertension (11, 15-17); structural changes in vessels could be responsible.

As part of another study (18) we previously reported the detailed effects of hypertension of 10 weeks' duration on the male rat thoracic aorta; these will be referred to as the short-term (2.5 months) results. The present study was undertaken to study the effects of long-term (16 months) hypertension on rat aortic wall dimensions and chemical composition and to compare these to short-term effects and to concurrent aging changes in the vessel wall.

Methods

Male Carworth (CFN) rats were 7 weeks old at the start of the experiment and weighed 135-145 g. All animals were housed two per cage and were given Purina laboratory chow and ordinary drinking water ad libitum.

Hypertension was produced by clipping the renal artery as previously described (7). The criterion for hypertension was systolic blood pressure of 150 mm Hg or more. Blood pressures were taken biweekly for the first 3 months, then monthly for the duration of the experiment. A tail cuff connected to a mercury manometer and a pressure transducer connected to a signal amplifier and recorder were used to measure blood pressure.

Animals were killed after 2.5 months and after 16 months of documented hypertension; corresponding groups of animals with documented normotension were studied after the same periods of time. The animals killed after 2.5 months were approximately 5.5 months old; those killed after 16 months were approximately 19 months old. Each group consisted of ten animals (Table 1); six were used for chemical studies and four for morphological studies.

MORPHOLOGICAL STUDIES

Description of the methods used to distend and extend the thoracic aorta prior to fixation and subsequent handling for histological evaluation are given elsewhere (7). Diameters of the segments filled with barium-containing perfusion mixture were obtained from x-rays of the segments; micrometric techniques were used to measure wall thickness and medial lamellae (7). Cross-sectional area of the media was calculated according to the formula: area of a ring between two circles of radius \( r_1 \) and \( r_2 \) is equal to \( \pi (r_1^2 - r_2^2) \) (7). Tangential tension was calculated according to the law of Laplace: tension (dyne/cm) = blood pressure (dyne/cm\(^2\)) \( \times \) radius (cm); wall stress (dyne/cm\(^2\)) was calculated by the additional step of dividing tangential tension by medial thickness. All measurements were corrected for tissue shrinkage due to fixation and further processing (7, 19). Student's t-test for differences between means was used to test for significance of results.

CHEMICAL STUDIES

The methods used to measure total absolute amounts of elastin, collagen, and noncollagenous alkali-soluble proteins in intima-media preparations from identical anatomically defined segments of thoracic aorta were the same as those used in the previous study of short-term hypertension (18) with one exception. Because of the greater amounts of tissue present, constant dry aortic weight was achieved in the 16-month groups only after 72 hours of vacuum drying at 50-55°C instead of the 48 hours which were adequate for the 2.5 month groups.

Results

CHARACTERISTICS OF GROUPS

Characteristics of the groups studied are shown in Table 1. No difference in body weight was seen between control and

<table>
<thead>
<tr>
<th>Characteristics of Animal Groups Studied</th>
<th>C</th>
<th>H</th>
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<tbody>
<tr>
<td>Final body weight (g)</td>
<td>488 ± 16</td>
<td>448 ± 19</td>
</tr>
<tr>
<td>Final systolic blood pressure (mm Hg)</td>
<td>116 ± 3</td>
<td>186 ± 5</td>
</tr>
<tr>
<td>Heart weight/body weight (%)</td>
<td>0.27 ± 0.01</td>
<td>0.35 ± 0.02</td>
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</table>

Values are means ± se; C = control; H = hypertensive.

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hypertensive rats at 16 months ($t = 1.59; 0.2 > P > 0.1$), whereas body weight of hypertensive rats was significantly less than that of controls at 2.5 months ($t = 1.59; 0.2 > P > 0.1$). Weight gains in both control and hypertensive rats between 2.5 and 16 months were significant ($P < 0.001$ for both). Systolic blood pressures were significantly greater in hypertensive rats than in controls at both 2.5 and 16 months ($P < 0.001$ for both). Although no change in blood pressure was seen with aging in hypertensive rats between 2.5 and 16 months ($t = 0.63; 0.6 > P > 0.5$), a significant increase in systolic blood pressure occurred in controls over this period ($t = 2.5; P < 0.001$). The ratio of postmortem heart weight to body weight corresponded well to blood pressure levels with the exception that no change was seen in this ratio in controls between 2.5 and 16 months.

**MORPHOLOGICAL FINDINGS**

Aortic diameters are shown in Table 2; vessel diameters of hypertensive aortas were significantly greater than those of controls ($P < 0.05$) as they had been at 2.5 months (18). Interestingly, the diameters of hypertensive vessels at 2.5 and 16 months were not significantly different ($t = 1.11; 0.4 > P > 0.3$), whereas the diameter of normotensive vessels increased significantly during aging ($t = 4.18; P < 0.01$).

Medial thicknesses of aortic segments are shown in Table 2. Again, the significant difference between control and hypertensive aortas seen at 2.5 months (18) was maintained at 16 months ($t = 11.55; P < 0.001$). Wall thickness increased significantly with age in both hypertensive and normotensive rats ($t = 5.52$ and $P < 0.01$; $t = 4.00$ and $P < 0.01$, respectively). In addition to the increase in wall thickness of control and hypertensive aortas associated with age, an apparent increase in connective tissue staining in hypertensive rats at 16 months was seen compared to those at 2.5 months. As found in our previous studies of hypertension in rats (7, 18), no difference in number of medial lamellar layers between control and hypertensive animals was found ($P > 0.7$) (Table 2). In addition, no change was seen with aging over the period covered in this study ($P > 0.3$).

Calculated tension per lamellar unit (Table 2) was significantly greater in hypertensive rats than in controls ($P < 0.001$) as it was at 2.5 months (18). No change was seen with increased duration of hypertension from 2.5 to 16 months, since all the factors used in calculation, that is, diameter, blood pressure, and lamellar units did not change in hypertensive vessels, as noted above. However, in controls, the significant increase in blood pressure (Table 1 and ref. 18) and diameter (Table 2) which occurred with aging resulted in a significant increase in the calculated value for the tension per lamellar unit during aging ($t = 4.03; P < 0.01$).

Calculated wall stress relates wall tension to wall thickness; these values are shown in Table 2. Although calculated wall stress was strikingly increased in hypertensive aortas at 2.5 months (18), after prolonged hypertension, the sharply continued increase in wall thickness (Table 2) resulted in a calculated wall stress no different from that of control vessels ($t = 2.05; 0.01 > P > 0.05$) and strikingly below the value for hypertensive aortas at 2.5 months ($t = 4.00; P < 0.01$). Wall stress did not change significantly with aging in controls ($t = 1.26; 0.3 > P > 0.2$), presumably because the increase in wall thickness in those vessels paralleled the increases in blood pressure and diameter over the same period (Table 2 and ref. 18).

### Table 2

Dimensions of and Calculated Stresses on the Aortic Wall

<table>
<thead>
<tr>
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<th>C</th>
<th>H</th>
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<tbody>
<tr>
<td>Diameter (mm)</td>
<td>2.74 ± 0.05</td>
<td>3.08 ± 0.11</td>
</tr>
<tr>
<td>Wall thickness (mm)</td>
<td>1.178 ± 0.027</td>
<td>1.743 ± 0.041</td>
</tr>
<tr>
<td>Lamellar units</td>
<td>8.0 ± 0.1</td>
<td>8.1 ± 0.1</td>
</tr>
<tr>
<td>Tension/lamellar unit (dynes/cm × 10⁶)</td>
<td>2.65 ± 0.12</td>
<td>4.50 ± 0.19</td>
</tr>
<tr>
<td>Wall stress (dynes/cm² × 10⁶)</td>
<td>1.80 ± 0.08</td>
<td>2.09 ± 0.12</td>
</tr>
<tr>
<td>Medial area (mm²)</td>
<td>1.058 ± 0.030</td>
<td>1.779 ± 0.072</td>
</tr>
</tbody>
</table>

Values are means ± se. C = control; H = hypertensive.
Medial area represents a synthesis of diameter and wall thickness (Table 2). Differences between normotensive and hypertensive aortas were significant at both 2.5 months (18) and 16 months (P < 0.001). Significant increases were also seen with aging in both control and hypertensive rats (t = 8.5; P < 0.001 and t = 5.50; P < 0.01, respectively). The increase in aortic medial area after long-term hypertension could be attributed solely to changes in wall thickness since diameter did not increase with increased duration; the increased medial area in long-term controls reflected increases in both wall thickness and diameter with time.

CHEMICAL FINDINGS

Values for total aortic dry weight at 16 months are given in Table 3; the difference between control and hypertensive aortas was significant (P < 0.001). The increases seen between 2.5 and 16 months in values for control (t = 4.20; P < 0.01) and hypertensive rats (t = 4.73; P < 0.001) were also significant. Absolute amounts of elastin, collagen, and noncollagenous alkali-soluble proteins are shown in Table 3. Comparison between control and hypertensive aortas at 16 months showed significant increases in absolute amounts of both elastin and collagen (P < 0.01 for both proteins), a finding already present at 2.5 months (18). Striking increases of elastin and collagen occurred in hypertensive aortas between 2.5 and 16 months (P < 0.001 for both fibrous proteins). Interestingly, sharp increases in absolute amount of both fibrous proteins were seen in aging controls as well (P < 0.01 for elastin; P < 0.001 for collagen). Although the rate of accumulation of collagen between 2.5 and 16 months was similar in control and hypertensive vessels, it appears that the rate of elastin accumulation in hypertensive vessels was faster than that in controls over the same period.

Absolute amounts of noncollagenous alkali-soluble proteins were also significantly increased in hypertensive aortas compared to controls at 16 months (P < 0.001), a finding seen also at 2.5 months (18). Whereas an increase in this component occurred in controls with aging (t = 3.74; P < 0.01), no change in absolute amount was seen in hypertensive rats as the duration of hypertension increased from 2.5 to 16 months (t = 0.55; 0.6 > P > 0.5).

The percents of elastin, collagen, and noncollagenous alkali-soluble protein in the aortic wall are shown in Table 4. Although the percent of elastin in the hypertensive vessel was significantly less than that in the control vessel at 2.5 months (18), this difference was not maintained at 16 months. By then, the fixed amount of noncollagenous alkali-soluble protein and the continued increase in elastin combined to give a percent of elastin no different from that of controls (P > 0.9). No difference in elastin content was seen in controls with aging (t = 0.25; 0.9 > P > 0.8).

The percent of collagen increased sharply in aortas of both control and hypertensive rats over the period from 2.5 to 16 months (Table 4 and ref. 18) (P < 0.001). The collagen percent in hypertensive vessels was less than that of controls at 16 months (t = 2.55; P < 0.05).

### TABLE 3

<table>
<thead>
<tr>
<th>Total Aortic and Wall Component Weights</th>
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<tbody>
<tr>
<td>C</td>
</tr>
<tr>
<td>Total aortic dry weight</td>
</tr>
<tr>
<td>Elastin</td>
</tr>
<tr>
<td>Collagen</td>
</tr>
<tr>
<td>Alkali-soluble protein</td>
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</tbody>
</table>

Values are means ± se. C = control; H = hypertensive.

### TABLE 4

<table>
<thead>
<tr>
<th>Percents of Aortic Wall Components</th>
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<tbody>
<tr>
<td>C</td>
</tr>
<tr>
<td>Elastin</td>
</tr>
<tr>
<td>Collagen</td>
</tr>
<tr>
<td>Alkali-soluble protein</td>
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<tr>
<td>Total</td>
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</table>

Values are means ± se. C = control; H = hypertensive.
HYPERTENSION AND AGING

Whereas the percent of noncollagenous alkali-soluble proteins decreased slightly (and significantly) with aging in controls ($t = 4.99; P < 0.05$), a large decrease in percent of this component occurred with duration of hypertension ($t = 4.99; P < 0.001$). This latter decrease reflected the fixed absolute amount of this component combined with the continued accumulation of fibrous proteins. Although the percent of noncollagenous alkali-soluble protein in vessels of rats with long-term hypertension remained significantly higher than that of controls ($t = 3.14; P < 0.02$), it was much more similar to controls at 16 months than it was at 2.5 months (18). In sum, then, the composition of the aortas of rats with long-term hypertension much more closely resembled those of controls than did the aortas of rats with short-term hypertension. This difference could be attributed to a biphasic response of the vessel wall to hypertension. The early phase consisted of a disproportionate increase in the noncollagenous alkali-soluble component and relatively smaller increases in fibrous proteins; the later phase was characterized by a "catching-up" of the fibrous elements and no further increase in the absolute amount of the noncollagenous alkali-soluble fraction with chronic hypertension. Corresponding to the tendency for the composition of the vessel wall exposed to chronic hypertension to approach that of controls, the morphology of the hypertensive vessel at 16 months much more closely resembled that of controls than it did at 2.5 months.

It was previously shown that additional evidence for new elastin synthesis in hypertensive vessels could be adduced from the higher lysine values in elastin from these vessels than from those of controls (18). This difference between control and hypertensive vessels was maintained to the sixteenth month with the value for control aortas being $8.88 \pm 0.45$ residues/1000 residues and that for hypertensive aortas $11.38 \pm 0.93$ residues/1000 residues at that time. Whereas no difference was found between 2.5 and 16 months in hypertensive aortas ($t = 0.28; 0.6 > P > 0.7$), of note is the finding that the value in older controls ($8.88 \pm 0.45$) was significantly greater than that in younger controls ($7.1 \pm 0.66$ residues/1000) ($t = 2.22; P = 0.05$). This suggests that there was a new "burst" in synthesis of elastin in the normal vessel during aging. The possible relation of this finding to calculated stresses on the vessel wall during aging is discussed below.

Discussion

The two major findings of the present study are (1) that the response of the aortic wall to hypertension is biphasic, the phases being related to the duration of the elevated tension and (2) that the aortic wall response to increases in tension resulting from increases in blood pressure and diameter associated with aging is similar to that seen with overt hypertension; the difference is one of magnitude only.

No systematic comparison of the vessel wall exposed to short-term and long-term hypertension has been previously reported. Still (20) described the changes in the intima in early experimental hypertension in rats to consist of focal accumulations of cells and granular material, which increased slightly in diffuseness but not in severity with increased duration of hypertension. Thus, most of the changes in aortic dimensions and morphology found in this study and the one previously reported (18) were presumably due to medial alterations. Whereas many of the striking changes in aortic morphology and dimensions associated with hypertension appeared to increase in relation to the duration of hypertension, closer evaluation showed many differences in details of the response. Thus the early (2.5 month) response consisted of a disproportionate increase in the noncollagenous alkali-soluble proteins, which we have previously attributed to the smooth muscle component of the vessel wall, with a minor contribution from the protein moiety of mucopolysaccharides (18). Somewhat surprisingly, however, with chronic hypertension (16 months) no further increase in the noncollagenous alkali-soluble component was seen; instead, the continued increase in wall

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thickness and medial area reflected considerable accumulation of the fibrous proteins, elastin and collagen. The end-result was an aortic wall with nearly twice the medial area of the control vessel at that age but with a calculated wall stress no different from that of controls and a composition much closer to that of the control vessel than to that seen early in hypertension.

In hypertension, hypertrophy of the media of the entire arterial side of the vascular tree occurs (1). The cellular changes in the aorta and renal and mesenteric vessels have been described in detail (5, 21-23) usually after relatively short-term hypertension. In addition to evidence for cellular hypertrophy and hyperplasia, degenerative changes are seen in the medial smooth muscle cells (23). Photographs of mesenteric vessels exposed to hypertension of 5 months' duration in the study by Aikawa and Koletsky show the medial cells as veritable islands in a sea of medial connective tissue (23). I submit that the findings described here for the aorta probably pertain to the entire arterial tree. It would therefore appear that the response of the vascular tree is biphasic, that is, an initial nearly maximal early response of smooth muscle to elevated wall stress, followed by continued synthesis of fibrous proteins by these cells which serves to restore the composition of the stressed wall to near-normal values.

I have previously shown that after reversal of hypertension of 2.5 months' duration in rats of both sexes, the noncollagenous, alkali-soluble protein (smooth muscle) component reverts completely to normal; only the fibrous proteins which accumulated to the point of reversal remain (18). Especially in the female, this is accompanied by a complete restoration of wall dimensions to normal. Clearly, after long-term hypertension, with the later phase consisting solely of fibrous protein synthesis, even if the same amount of smooth muscle could revert to normal, it is unlikely that much change in aortic dimensions could occur since so much more of the wall consists of fibrous protein. There is evidence to suggest that fibrous protein accumulations in the vessel wall predispose that vessel to lipid accumulation (24). It should therefore not be surprising to observe little if any favorable effect of treatment of human hypertension on subsequent morbidity and mortality from coronary artery disease (12), since hypertension usually develops insidiously and is undoubtedly present for many months if not years prior to detection (1). The suggestion by Freis that treatment of hypertension earlier in its course would favorably influence the toll from coronary disease (25) carries the implication that screening of the general population for the presence of hypertension would be necessary to accomplish this; the findings here would support that contention.

The relative reversibility of the early phase and apparent relative irreversibility of the late phase of vessel wall exposure to hypertension would also seem to be pertinent to other features noted in hypertensive animals. Folkow et al. (13) and Sivertsson (14) found increased resistance to blood flow in the maximally dilated vascular bed of the limbs of hypertensive humans and postulated that a structural adaptation of the vessels occurs in hypertension which increases wall thickness and encroaches on the lumen at maximal dilation. In addition, others have found that experimental renal constriction of a single kidney in the rabbit (11, 16) and dog (15, 17) results in hypertension which consists of two phases, an early phase of several months' duration in which removal of the clip restores blood pressure promptly to normal and a later, chronic phase in which after removal of the clip blood pressure comes down slowly, incompletely, or not at all. It has been postulated that an irreversible structural vascular change is responsible for the later phase and that the early phase is due to smooth muscle constriction (1), though apparently activation of the renin-angiotensin system is much too transient to account for the latter (26), and experimentally induced antibodies to these hormones do not influence the subsequent development of hypertension (27). Perhaps the chronic structural and compositional alterations described here are
the basis for these postulated irreversible changes. Since the degree of fibrous protein accumulation is related to the duration and severity of hypertension (7), differences in rate of fibrous tissue accumulation could account for individual and species variations. For example, renal hypertension in the rat appears to be completely reversible even after a duration of 8 months (28); perhaps a still longer period of hypertension is necessary in this species.

The many changes found previously in vessels during aging include: a two- to three-fold increase in wall thickness (3), an increase of vessel diameter (4), and decrease in wall distensibility (29). Although it is generally agreed that connective tissue accumulates in the vessel wall during aging (4, 30), sampling variations, the question of chemical reliability, and the use of relative terms such as percent have clouded the precise degree to which each wall component changes in absolute terms (4). There is a general tendency, even when the findings appear otherwise (31), to ascribe aging changes to increased amounts of collagen and a net loss of elastin; the frayed appearance of elastin with time and a fall in its percent have been offered as evidence for its degeneration (4, 31).

From the findings here and elsewhere (18) I submit that aging of the rat thoracic aorta is accompanied by considerable accumulation of both fibrous proteins and that the frayed appearance of elastin is not due to degeneration but probably represents newly synthesized fibrils (3). Of considerable interest is that these compositional changes seem to reflect increased levels of calculated tension on the vessel wall arising from an increasing diameter and slowly rising blood pressure. The tension per lamellar unit found in the aged rat (19 months old) was well above that found for this species and others with avascular aortas in a previous study (32). The increases in wall thickness and fibrous proteins associated with aging are not unexplained findings, therefore, but represent a response to tension no different from that seen in hypertension, albeit to a much smaller degree.

In essence then, these findings suggest that "hypertension" can be present even when blood pressures are within the accepted range of normal. This concept is closely aligned with Pickering's view that hypertension is a quantitative entity, not a qualitative one (1); it is therefore just as appropriate, if not more so, to consider aging to be a muted form of hypertension as it is to consider hypertension to be an accelerated form of aging.

With the introduction of this concept, new ways of evaluating vessels are possible. For example, increased vessel diameter due to any cause, whether increased body weight and size in a given species (unpublished observations) or increased caliber of vessels supplying a hypertrophied organ (33), should yield an elevated value for calculated tension even in the presence of normal blood pressure. Since, even in the presence of significant hypertension, the vessel wall thickens to such a degree as to reduce wall stress to normal but does not increase the number of lamellar units, an important and easily overlooked clue to the presence of increased tension resides in the combination of an increased tension per lamellar unit and a normal wall stress; fewer lamellar units than expected for that wall thickness must be found. This combination of findings has been seen in aging and hypertension (Table 2) and in one other previously described (32) situation. The human abdominal aorta has a diameter appropriate for the adult body weight of man. Calculated wall stress is the same as that found in other mammals, but tension per lamellar unit is clearly excessive and becomes so early in life (34). This segment is normally avascular so that the increase in wall thickness which parallels the increased tension results in an avascular wall of a thickness far exceeding that of any other species. We have speculated on the consequences of this in terms of medial nutrition and susceptibility of this segment to vascular disease (32); other regions of predilection for disease involvement may represent similar interactions between caliber, tension, and architectural and nutritional limitations. What seems clear is that closer
study of the interaction of structure and function under carefully reproduced conditions can yield new insights into and common features of what appears to be a diverse group of vascular alterations.

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References


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