Changes in the Aortic Wall Oxygen Tensions of Hypertensive Rabbits

Hypertension and Aortic Wall Oxygen

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Hypertension is a known risk factor for atherosclerosis. We hypothesize that hypertension causes artery wall hypoxia that contributes to the formation of atherosclerotic lesions. Therefore, we examined the effect of hypertension on the transarterial wall oxygen gradient of the rabbit aorta. Hypertensive rabbits were created by unilateral nephrectomy and contralateral renal artery narrowing. Transarterial wall oxygen gradients of the infrarenal aorta were measured using an oxygen microelectrode 14–16 weeks (short-term hypertension) and 56–58 weeks (long-term hypertension) after the rabbits were made hypertensive. The transarterial wall oxygen gradients showed significant differences among the groups. Short-term hypertension caused significantly higher oxygen tensions in the outer 30% of the artery wall and significant thinning of the artery wall when compared with long-term hypertension and control groups. Long-term hypertension caused significantly lower oxygen tensions in the inner 40% of the artery wall and significant thickening of the artery wall when compared with short-term hypertension and control groups. These changes were noted despite no difference in the partial pressure of oxygen in arterial blood or visual evidence of atherosclerotic lesion formation in the three groups. These findings suggest that hypertension alters the transarterial wall oxygen gradient. This altered transarterial wall oxygen gradient may contribute to the formation of atherosclerotic lesions. (Hypertension 1992;19:33–39)

Hypertension has been shown to be a risk factor for atherosclerosis through numerous epidemiological studies. Despite considerable research, the etiology of atherosclerosis in hypertension remains unclear.

Evidence is accumulating that suggests hypoxia of the artery wall is important in the initiation and progression of atherosclerotic lesions. The oxygen economy of the artery wall is unique in that oxygen is derived from two sources: the outer two thirds of the artery wall is supplied with oxygen from the vasa vasorum and the inner one third is supplied with oxygen from luminal diffusion. Any alteration in the delivery of oxygen to the artery wall or change in the consumption of oxygen by cells in the artery wall could create a relatively hypoxic artery wall cellular microenvironment.

We hypothesize that hypertension creates a relatively hypoxic transarterial wall oxygen gradient that could facilitate the cellular events leading to the formation of atherosclerotic lesions. Therefore, we measured the effect of short- and long-term hypertension on the transarterial wall oxygen gradient using an oxygen microelectrode.

Methods

Animal Models

Female New Zealand White rabbits (1–3 kg) were used for study. All animals were fed standard chow and water ad libitum and housed according to the institutional guidelines at the University of Minnesota. These studies were approved by the University of Minnesota Animal Rights Committee.

Operative Procedures

For the hypertension operation, the rabbits were anesthetized with acepromazine maleate (1 mg/kg i.m., Aveco Co., Fort Dodge, Iowa) and ketamine HCl (50 mg/kg i.m., Aveco), and their abdomens were shaved and prepped with 70% povodine in alcohol. A midline laparotomy was performed, and dissection was carried down to the posterior peritoneum, which was opened in the area of the renal arteries. The left renal artery and vein were clamped, ligated, and divided. The left kidney was then removed. The right renal artery was carefully isolated,
and a silver clip was applied to occlude 75% of the internal diameter of the renal artery. The right kidney was then observed for 15 minutes to assure viability, and the abdomen was closed using 2-0 vicryl for the fascial layer and 2-0 silk for the skin.

For the control operation, the rabbits were anesthetized, a midline laparotomy was performed as described above, and the posterior peritoneum was opened in the area of the renal arteries. Both renal arteries were carefully exposed and observed for 15 minutes. The abdomen was then closed as described above.

**Experimental Groups**

The short-term hypertension (STH) group had aortic wall oxygen tension measurements performed 14–16 weeks after hypertension operations. Blood pressures were measured 8 weeks (midpoint) after the hypertension operation and just before the animals were killed. Arterial blood gas measurements were obtained immediately after measurement of the transarterial wall oxygen gradient \( n=6 \).

The long-term hypertension (LTH) group had aortic wall oxygen tension measurements performed 56–58 weeks after hypertension operations. Blood pressures were measured 28 weeks (midpoint) after hypertension operation and just before the animals were killed. Arterial blood gas measurements were obtained immediately after measurement of the transarterial wall oxygen gradient \( n=6 \).

The control group had aortic wall oxygen tension measurements performed 14–16 and 30–34 weeks after control operations. Blood pressures were measured 8–10 weeks, 14–16 weeks, and 30–34 weeks after control operation. Arterial blood gas measurements were obtained immediately after measurement of the transarterial wall oxygen gradient \( n=6 \).

**Arterial Blood Pressure**

Arterial blood pressure was recorded at the midpoint and at the completion of the experiment.

At midpoint, rabbits were anesthetized and the neck was shaved and prepped as previously described. The right carotid artery was exposed, an 18-gauge Teflon catheter was inserted into the carotid artery, and the catheter was brought out through a separate stab wound and was secured to the skin. Arterial blood pressure was recorded with a standard pressure transducer system while the rabbit was anesthetized. The catheter was then flushed every 8 hours with 100 units of heparin to maintain patency. Arterial blood pressures were again recorded 24 hours after catheter placement in the awake rabbit with a standard pressure transducer system. After the awake blood pressure measurement, the rabbit was again anesthetized as previously described, and the catheter was removed with ligature of the right carotid artery. The wound was closed with 2-0 vicryl for the fascia and 2-0 silk for the skin.

At completion of the experiment, after transarterial wall oxygen tension measurements, an 18-gauge Teflon catheter was inserted into the abdominal aorta at the iliac bifurcation. Blood pressure was measured using a standard pressure transducer system. After this blood pressure measurement, the rabbit was killed with an intravenous injection of pentobarbital sodium (Abbott Laboratories, Chicago, Ill.).

**Construction of the Oxygen Microelectrode**

Transarterial wall oxygen tension measurements were performed using an oxygen microelectrode constructed following the technique of Silver. Briefly, a 30-gauge 80% platinum-20% iridium wire (California Fine Wire Co., Grover City, Calif.) was electropolished to a 1–5 \( \mu \)m tip in a saturated alkaline NaCN solution using an alternating current of 0.5–2 A at 5–10 V. The wire was evenly tapered from the 1–5 \( \mu \)m tip to a diameter of approximately 100 \( \mu \)m at a distance of 200–300 \( \mu \)m from the tip. The electropolished wire was then coated with leaded glass leaving the wire tip exposed. The glass near the tip was so thin that it did not add to the overall diameter of the electrode. The performance of the electrode was then assessed in an oxygenated saline bath, and the size of the exposed tip was determined by assessing the amount of current per millimeters of mercury oxygen. A current-voltage polarogram was constructed to look for a plateau between \(-0.6 \) and \(-0.8 \) volts. If the electrode performed satisfactorily, it was coated with polymethyl methacrylate (Polysciences, Inc., Warrington, Pa.) leaving the tip exposed. The electrode was then tested by the criteria of Silver before use in the experiment. The six criteria needed to be met by the electrode were: 1) the current reading in the fluid bath was not affected by fluid movement, 2) the current per millimeter of mercury O2 was less than \( 1 \times 10^{-10} \) A, 3) there was a linear relation between oxygen tension and current measurement, 4) there was a negligible current measurement at an oxygen tension of zero, 5) the response of the electrode to changes in oxygen tension was rapid, and 6) the electrode tip surface was noted to be smooth without contour irregularities when examined with an operating microscope. The electrode was soaked in fresh rabbit serum (prepared in our laboratory using standard techniques) for 30 minutes to allow for surface coating with plasma proteins and then calibrated in an oxygenated saline bath at oxygen tensions of 0 mm Hg and 89 mm Hg immediately before and after measurement of the transarterial wall oxygen gradient.

**Transarterial Wall Oxygen Gradient Measurements**

The rabbits were anesthetized with acepromazine maleate and ketamine HCl 14–16 and 30–34 weeks (control), 14–16 weeks (STH), or 56–58 (LTH) weeks after hypertension operations. The abdomen was opened through a midline laparotomy, and the infrarenal aorta was exposed taking care not to disrupt the vasa vasorum. The aorta was first covered topically with a solution of 2% lidocaine HCl (Xylocaine, Astra Pharmaceutical Products Inc., Westboro, Mass.) to prevent vasospasm. The area in which
Arterial Blood Pressure

Mean arterial blood pressures at the midpoint of the experiment in anesthetized rabbits were 53.4±6.6 mm Hg in the control group, 102.1±5.7 mm Hg in the STH group, and 106.4±11.9 mm Hg in the LTH group. Mean arterial pressures at the completion of the experiment in anesthetized rabbits were 53.4±6.6 mm Hg in the control group, 39.3±9 mm Hg immediately before entering the lumen with a luminal value of 81.2±6 mm Hg. There were no differences in the oxygen tensions obtained from control rabbits measured 14–16 weeks or 30–34 weeks after hypertension operations (n=6) (Figures 1 and 2).

In the STH group, the oxygen tension at the adventitia was 55.5±4 mm Hg. The oxygen tension slowly fell to reach a low value of 23.5±4 mm Hg 75% of the distance through the wall toward the lumen. Oxygen tensions then slowly rose; they were 39.3±9 mm Hg immediately before entering the lumen with a luminal value of 81.2±6 mm Hg. When comparing STH with the control group, the oxygen tensions at the adventitia (70.5±3 versus 55.5±4 mm Hg), 10% (63.5±6 versus 55.5±3), 20% (60.7±4 versus 50.2±2 mm Hg), and 30% (51±4 versus 42.7±2 mm Hg) of the distance through the artery wall toward the lumen were significantly increased (p<0.05). There was no significant difference in luminal oxygen tensions between the two groups (n=6) (Figure 2).

In the LTH group, the oxygen tension at the adventitia was 61.5±4 mm Hg. The oxygen tension slowly fell to reach a low value of 11.8±4 mm Hg 75% of the distance through the wall toward the lumen. Oxygen tensions then slowly rose; they were 28.5±5 mm Hg immediately before entering the lumen with a luminal value of 82.2±4 mm Hg. When comparing LTH with the control group, the oxygen tensions at 60% (18.2±2 versus 28±3 mm Hg), 65%
artery wall when compared with control and LTH groups ($p<0.05$). LTH revealed significantly decreased oxygen tensions in the inner 40% of the artery wall when compared with both control and STH groups ($p<0.05$) (Figure 2).

Note that after measurement of the transarterial wall oxygen gradient the aorta was opened longitudinally and visually inspected for the formation of atherosclerotic lesions. No atherosclerotic lesions were noted by visual inspection in any group.

**Discussion**

Hypertension has been firmly established as a primary risk factor for atherosclerosis through numerous population-based studies. The temporal relation of cellular interactions in the formation of an atherosclerotic lesion has been well documented. The first changes noted were localized collections of monocytes attached to the endothelium throughout the arterial tree. These monocytes appeared to pass between endothelial cells and localize subendothelially. They then appeared to become activated taking up large quantities of lipids evolving into lipid-laden foam cells. With time, the lesions were noted to expand not only from macrophages but also due to smooth muscle cells, which migrate from the media to the intima, proliferate, and take up large quantities of lipids also evolving into foam cells. These cellular events resulted in the formation of an atherosclerotic lesion. This cellular activity occurs within the inner sections of the artery wall.

Heuper in 1945 first suggested artery wall hypoxia may have a role in the pathogenesis of atherosclerosis. Niinikoski et al demonstrated that a transarterial wall oxygen gradient is present in normal arteries with oxygen tensions falling from the adventitia and reaching a low value at the junction of the inner one third and outer two thirds of the vessel wall. Oxygen tensions then slowly rise until the lumen is entered. It is theorized that the oxygen supply to the outer two thirds of the artery wall is supplied by the vasa vasorum, and the inner one third is supplied by luminal diffusion of oxygen. Heughan et al demonstrated that in the presence of an established atherosclerotic lesion, the normal rise in oxygen tension found as you proceed through the inner one third of the vessel wall toward the lumen is eliminated. Oxygen tensions progressively fall from the adventitia until the lumen is entered. In the presence of an atherosclerotic lesion, the luminal diffusion of oxygen to the inner one third of the artery wall is impaired. Transarterial wall oxygen tensions of the inner one third of the artery wall are lower than controls after the formation of an atherosclerotic lesion; however, the question remains: What happens before atherosclerotic lesion formation to initiate and maintain the formation of atherosclerotic lesions?
We hypothesize that artery wall hypoxia caused by hypertension leads to the stimulation of endothelial cells, macrophages, and smooth muscle cells thereby contributing to the formation of atherosclerotic lesions. We examined the effect of renal artery hypertension on the transarterial wall oxygen gradient of the unmanipulated abdominal aorta 14–16 and 56–58 weeks after inducing hypertension and before the development of atherosclerotic lesions by visual inspection.

The transarterial wall oxygen gradient of control rabbits recorded in this study is similar in profile but with different oxygen tensions than those previously recorded by Niinikoski et al,8 although the model and electrode are similar. Two changes in the current study may account for this difference. Mineral oil was applied to our electrode after it was brought into contact with the artery wall adventitia, whereas in the study by Niinikoski et al,8 it appears that mineral oil was applied to the electrode tip before contacting the artery wall. Also in the current study, the electrode was soaked in fresh rabbit serum (FRS) for 30 minutes before calibration and use in the measurement of the transarterial wall oxygen gradient to allow for surface coating with plasma proteins. Initially these changes were omitted and the transarterial wall oxygen tensions recorded were quite similar to those recorded by Niinikoski,8 however we noted a rapid decrease in the sensitivity of the electrode to oxygen and a significant drift in electrode calibration following most measurements. Both of these problems were corrected by changing the time of application of the mineral oil and soaking of the electrode tip in FRS as previously mentioned. The oxygen tensions recorded in the current study, although not the same as those previously reported, were reliable and reproducible.

Our data demonstrate that STH significantly thins the artery wall and increases the oxygen tensions in the outer 30% of the artery wall when compared with controls. There were no differences in oxygen tensions noted in the inner 70% of the artery wall when compared with controls. These changes were noted despite no change in the partial pressure of oxygen in arterial blood when compared with controls. Thinning of the artery wall in the STH group would be expected because a relaxed, elastic vessel subjected to a positive transmural pressure will dilate.10 The volume of the artery wall will initially remain constant, and it is predicted that the artery wall will thin as the vessel dilates. Artery wall thinning in the STH group might be predicted to elevate oxygen tensions throughout the artery wall by increasing the luminal area for diffusion of arterial blood oxygen due to an increase in the internal diameter of the vessel from STH-induced dilatation. Our data suggest that this event is somewhat offset because there is no significant change in the oxygen tensions of the inner 70% of the artery wall when compared with controls. This could be explained by changes in wall tension and stress. The Law of Laplace10 states:

\[ T = P \times R \]

where \( T \) is the wall tension, \( P \) is the transmural pressure, and \( R \) is the vessel internal radius. The Law of Laplace would predict that as blood pressure rises and the vessel wall dilates, the artery wall tension will also increase. The circumferential wall stress10 can be calculated from:

\[ S = \frac{T}{h} \]

where \( S \) is wall stress and \( h \) is the wall thickness. Therefore, as wall tension increases, so will the circumferential wall stress. It is known that the circumferential wall stress is greatest on the luminal surface of a blood vessel.11 Elevated blood pressure will increase wall stress, which may impair the luminal diffusion of oxygen to the inner artery wall, resulting in no significant change in the oxygen tensions of the inner artery wall when compared with controls despite the increase in surface area for oxygen diffusion caused by artery wall thinning from the elevation in blood pressure with STH. Increased oxygen tensions in the outer 30% of the artery wall initially appear to be in disagreement with a previous study that demonstrated a decrease in flow through the vasa vasorum with acute elevations in blood pressure,12 which should decrease the oxygen tensions in the outer artery wall. However, the transarterial wall oxygen gradient in the STH group was measured 14–16 weeks after hypertension operations, not immediately after blood pressure elevation. Elevated blood pressure is known to alter endothelial cell morphology13 and presumably function, which may lead to endothelial cell production of a smooth muscle cell relaxing factor.14,15 This may be occurring 14–16 weeks after an elevation in blood pressure in the endothelial cells lining the vasa vasorum, resulting in higher flow through the vasa vasorum with a corresponding rise in oxygen tensions in the outer artery wall.

This study also demonstrates that LTH significantly thickens the artery wall and decreases the oxygen tensions in the inner 40% of the artery wall. This decrease in oxygen tension occurs in the region of the artery wall in which the cellular events involved in the formation of an atherosclerotic lesion are known to occur.6 These changes occur despite no difference in the partial pressure of oxygen in arterial blood when compared with controls, suggesting that LTH alters the delivery of oxygen to the artery wall independent of the partial pressure of oxygen in arterial blood. These changes occur before the formation of atherosclerotic lesions by visual inspection.

LTH artery wall thickening could be predicted based on previous research that revealed hypertension resulted in increased artery wall quantities of collagen and elastin16 as well as increased medial smooth muscle cell mass.17 The finding of lower oxygen tensions in the inner 40% of the artery wall with LTH could be in part caused by the known increase in artery wall mass resulting in a greater
area for the diffusion of oxygen. Also, elevated blood pressure resulting in increased wall tension and inner wall stress may impair oxygen diffusion. Probably of more importance is the known increase in artery wall oxygen consumption with hypertension caused by the increased medial smooth muscle cell mass. Smooth muscle cell oxygen consumption may serve as an oxygen sink resulting in the lower oxygen tensions noted in the inner 40% of the artery wall with LTH when compared with controls and STH as well as lowering the oxygen tensions of the outer artery wall to normal from the elevated levels found with STH.

The lower oxygen tensions recorded in the LTH group extended to the artery wall immediately before what we presume to be lumen entry with its marked rise in oxygen tension. It is assumed by most that the endothelial cell is exposed to the oxygen tensions commonly recorded in arterial blood; however, the flow characteristics of blood reveal a streaming effect with a relatively stagnant layer of red blood cells at the blood/artery wall interface. Also, at branch points and bifurcations flow patterns reveal stagnant pools of blood in areas prone to the future development of atherosclerotic lesions. The unique flow characteristics of blood in the vascular tree have led researchers to postulate that the endothelial cell is not subject to the oxygen tensions usually recorded in arterial blood but to much lower oxygen tensions, particularly at branch points and bifurcations where atherosclerotic lesions are known to form.

This work has led us to hypothesize that hypertension-induced artery wall hypoxia may lead to the development of atherosclerotic lesions. LTH leads to an increase in the smooth muscle cell mass of the artery wall media with an increase in oxygen consumption. This serves as an oxygen sink and, when combined with the thicker artery wall as well as increased inner wall stress, limits the diffusion of oxygen and results in hypoxia of the inner artery wall. Endothelial cells at branch points and bifurcations are exposed to low luminal oxygen tensions due to altered flow patterns as well as low artery wall oxygen availability. These factors combine to cause a hypoxic insult to endothelial cells, which leads to their production of transforming growth factor β (TGF-β) and platelet-derived growth factor (PDGF). Transforming growth factor β is a monocyte chemoattractant and may result in the margination of monocytes followed by their migration to the subendothelial space there converting to tissue macrophages. These macrophages are stimulated by artery wall hypoxia to secrete growth factors including angiogenesis factor. Endothelial cell–secreted PDGF is a smooth muscle cell chemoattractant and mitogen that results in the movement of smooth muscle cells from the artery wall media to the subendothelial space. Artery wall hypoxia, in addition to endothelial cell–produced PDGF, leads to smooth muscle cell conversion to their synthetic state resulting in smooth muscle cell hyperplasia, connective tissue secretion, and growth factor secretion. This subendothelial space cellular activity further lowers artery wall oxygen tensions resulting in a positive feedback loop with the eventual formation of an atherosclerotic lesion.

A concern expressed was the appropriateness of this rabbit model for human hypertension and atherosclerosis. Structurally the rabbit aorta and the human abdominal aorta are quite similar. Both have less than 30 lamellar units and no medial vascular vasorum. The human abdominal aorta is a frequent site for the development of atherosclerosis with hypertension and hypertension has been shown to accelerate the development of atherosclerosis in the rabbit independent of plasma renin activity. We feel this is an appropriate model to study artery wall oxygen tensions in hypertension.

Also, there is the lack of an age-matched control group for the LTH group. The rabbits were matched for age at the start of the experiment, and control groups were measured at 14–16 and 30–34 weeks. There was no difference in the transarterial wall oxygen gradients between these two groups. This suggested relative stability in the transarterial wall oxygen gradients of control rabbits, and measurements were not performed on control rabbits at 56–58 weeks.

Artery wall hypoxia induced by hypertension could lead to the accelerated form of atherosclerosis seen with hypertension. Further research in this area could lead to a greater understanding of this complication of hypertension.

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References


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