Effect of Hydralazine on Aortic Rupture Induced by B-Aminopropionitrile in Turkeys

CHARLES F. SIMPSON, D.V.M., PH.D., AND W. JAE TAYLOR, M.D.

SUMMARY. The effects of hydralazine on aortic rupture, hemodynamics and aortic ultrastructure were studied in turkeys fed B-aminopropionitrile (BAPN). A mortality rate of 24% due to hemopericardium and internal hemorrhage in turkeys fed only BAPN increased to 91% when turkeys were fed both BAPN and hydralazine, despite a significant reduction in blood pressure after both drugs. Death rates among turkeys fed BAPN and hydralazine were lowered by adding either dietary propranolol (53%), which lowered blood pressure and dP/dt max, or reserpine (67%), which reduced blood pressure and increased dP/dt max. Striking ultrastructural alterations of collagenous and elastic fibers of the aortic media, which were additive to the effects of BAPN alone, were induced by BAPN and hydralazine.

This study demonstrates that a 6-week feeding of high levels of BAPN and hydralazine, which accumulates in vessel walls, can produce vascular injury and increase mortality from hemorrhage in lathyritic turkeys.

THE LATHYRITIC TURKEY is used as a model for evaluating pharmacologic interventions in the therapy of life-threatening aortic dissections in humans. In both the human disease and the B-aminopropionitrile (BAPN)-induced disease of turkeys, death from aortic rupture into either the pericardial sac or an open space is common. Studies indicate that reduction of blood pressure or, perhaps more importantly, the rate of pressure increase (dP/dt max) retards or stops the progression of aortic dissection in in vitro models, the turkey, and man.1,8

The interactions between the tensile strength of the aortic wall and hemodynamic factors are not clearly delineated in aortic dissections. This study was designed to evaluate the effect of hydralazine, which interferes with collagen synthesis, accumulates in the arterial wall,4 and alters the elasticity of collagen,5,6 in the lathyritic turkey, singly and in combination with other hypotensive agents.

Materials and Methods

One-day-old male, broad-breasted white turkeys were obtained from a commercial hatchery. They were housed in a specially ventilated turkey house in which they were segregated into groups of six and shielded from undue noise or other stress. Control and experimental groups were in contiguous pens and were inspected daily to determine mortality and general health.

There were 150 turkeys each in the control group and in two of the four experimental groups; 90 turkeys were included in the other 2 experimental groups (table 1). All turkeys were fed turkey mash until the age of 4 weeks, at which time 0.07% BAPN was added to the experimental diets and continued for the remaining 6 weeks of the study. The turkeys that received BAPN are designated as lathyritic groups, One lathyritic group served as a control; 0.07% hydralazine was fed to three groups either alone or in combination with 0.04% propranolol or 0.0005% reserpine. The average daily consumption of feed was 0.14 kg/day, which calculates to daily doses of 95 mg of BAPN, 95 mg of hydralazine, 54 mg of propranolol, and 0.7 mg of reserpine in the respective groups.

After 2 weeks of the drug regimen, 20% of each treatment group was selected at random for hemodynamic measurements. A carotid artery was cannulated to measure heart rate, blood pressure and maximal rate of arterial pressure increase (dP/dt max) directly. Heart rate was calculated from the arterial pulse recording, blood pressure was measured with a linear-core transducer and dP/dt max was calculated using a differentiator coupler (Narco Biosystems). The carotid artery was ligated after these procedures, and no subsequent ill effects were noted.

Ten percent of the turkeys in each treatment group were sacrificed for histologic and electron microscopic studies of the aorta after 2 weeks of drug administration. The remaining turkeys continued to receive the experimental drug regimen for 4 more weeks. At the age of 10 weeks, 6 weeks after the initiation of drug intervention, the remaining turkeys were necropsied. Mortality was recorded daily, and the cause of death was determined by necropsy. Sections of the abdominal aortas of turkeys that died or were sacrificed were prepared for examination by light microscopy. The specimens were fixed in 10% neutral formalin, embedded in paraffin, cut at 5 µm, and stained with hematoxylin-eosin and also orcein Van Gieson stain for elastic fibers. Sections of the aortas for electron microscopy were fixed in 3% buffered glutaraldehyde, postfixied in 1% O2O4, and embedded in Araldite. Thin sections on grids were stained with uranyl acetate and lead citrate before examination by electron microscopy.

Data were tested by analysis of variance to determine if there was a significant difference between the measurements of each variable tested, followed by Duncan's multiple-range test to determine which specific variables were significantly different.

Results

Mortality was strikingly increased in turkeys fed hydralazine (table 1) compared with mortality in

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TABLE 1. Mortality Among Turkeys Fed B-aminopropionitrile and Hydralazine

<table>
<thead>
<tr>
<th>Medication</th>
<th>No. of turkeys*</th>
<th>Average day of death</th>
<th>Hemopericardium</th>
<th>Aortic rupture</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>150</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BAPN</td>
<td>150</td>
<td>24</td>
<td>5 (4%)</td>
<td>27 (20%)</td>
<td>32 (24%)</td>
</tr>
<tr>
<td>BAPN, hydralazine</td>
<td>150</td>
<td>20</td>
<td>48 (36%)</td>
<td>75 (55%)</td>
<td>123 (91%)</td>
</tr>
<tr>
<td>BAPN, hydralazine, reserpine</td>
<td>90</td>
<td>24</td>
<td>8 (10%)</td>
<td>46 (57%)</td>
<td>54 (67%)</td>
</tr>
<tr>
<td>BAPN, hydralazine, propranolol</td>
<td>90</td>
<td>29</td>
<td>3 (4%)</td>
<td>40 (49%)</td>
<td>43 (53%)</td>
</tr>
</tbody>
</table>

*Ten percent of the turkeys from each group were sacrificed at 2 weeks of age; the remainder were used to calculate mortality.

Abbreviation: BAPN = B-aminopropionitrile.

TABLE 2. Hemodynamics Among Turkeys Fed B-aminopropionitrile and Hydralazine

<table>
<thead>
<tr>
<th>Medication</th>
<th>Heart rate (beats/min)</th>
<th>Blood pressure (mm Hg)</th>
<th>dP/dt max (mm Hg/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td>Control</td>
<td>326a</td>
<td>171a</td>
<td>128a</td>
</tr>
<tr>
<td>BAPN</td>
<td>325a</td>
<td>175a</td>
<td>130a</td>
</tr>
<tr>
<td>BAPN, hydralazine</td>
<td>327a</td>
<td>154b</td>
<td>111b</td>
</tr>
<tr>
<td>BAPN, hydralazine, reserpine</td>
<td>286b</td>
<td>137c</td>
<td>82c</td>
</tr>
<tr>
<td>BAPN, hydralazine, propranolol</td>
<td>256b</td>
<td>133c</td>
<td>98c</td>
</tr>
</tbody>
</table>

Values with different superscripts are significantly different from each other (p< 0.05).

Turkeys fed BAPN alone. The 24% death rate from aortic hemorrhage in turkeys fed BAPN alone was comparable to that in other studies. The addition of hydralazine produced a 91% mortality rate, which was only moderately reduced by reserpine (67%) or propranolol (53%). This lethality of the BAPN and hydralazine regimen was caused in part by the early occurrence of hemopericardium, which was significantly reduced by the concomitant administration of reserpine or propranolol. Thirty-six percent of deaths among turkeys fed BAPN and hydralazine resulted from hemopericardium, but 4% of the turkeys fed BAPN alone or BAPN, hydralazine and propranolol died of hemopericardium. Ten percent of the turkeys fed BAPN, hydralazine and reserpine died from hemopericardium. Death from internal hemorrhage was not radically influenced by propranolol or reserpine in the presence of hydralazine and BAPN.

Hydralazine significantly lowered both systolic and diastolic blood pressures; addition of propranolol or reserpine caused further reductions (table 2). Reserpine had a more pronounced effect on diastolic pressure than did propranolol; however, propranolol lowered and reserpine increased dP/dt max. Reserpine and propranolol, but not hydralazine, lowered heart rate.

The ultrastructure of the abdominal aorta of the turkeys was profoundly altered by the drugs. In the BAPN-fed turkeys, the changes were similar to those described previously. Smooth muscle cells were often compressed and adjacent ones were usually separated by disorganized bundles of collagen fibers, pools of mucopolysaccharide, and fragmented and swollen elastic fibers with electron-dense surface elevations (fig. 1). These elevations consisted of disorganized, nonfragmented fibrils that overlay central portions of normal-appearing elastic fibers of various widths.

FIGURE 1. Aorta of a turkey fed B-aminopropionitrile showing electron-dense elevations (1-4) on the surface of elastic fibers. The interior of a fiber (arrow) has weak electron density and is apparently normal. Magnification X 5000.
Hydralazine and BAPN produced more severe alterations in the aortic media than did BAPN alone. Occasionally, a narrow central portion of an elastic fiber had a normal appearance, but in general, almost the entirety of the elastic fiber was swollen and fragmented and had irregular, opaque elevations on its surfaces (fig. 2). Fragmentation and disorganization of fibrils caused the larger elevations to have a granular appearance (fig. 2). Collagenous fibers in the intercellular spaces were swollen. The cytoplasm of most smooth muscle cells appeared to contain more glycogen than corresponding cells in the aortic media of turkeys that received BAPN alone.

When reserpine was added to the BAPN and hydralazine regimen, the elevations on the elastic fibers of the aortic media were not as extensive as with BAPN and hydralazine alone. The central portion of these fibers was normal in appearance, but they had knobby and electron-dense peripheral elevations (fig. 3). The elevations were less granular than those of the turkeys fed BAPN and hydralazine. Bundles of colla-

**Figure 2.** (A) Aorta of turkey fed B-aminopropionitrile and hydralazine. The elastic fibers (E) are swollen and fragmented. A small interior portion of a fiber (arrow) is weakly electron dense and apparently unaltered. Magnification ×4000. (B) Prominent swellings (arrows) on elastic fibers are granular because of fragmented fibrils. The interior of the fiber (E) is not electron dense, is apparently normal and contains an orderly array of fibrils. Magnification ×25,000.

**Figure 3.** Aorta of turkey fed B-aminopropionitrile, hydralazine and reserpine. Much of the interior of the elastic fiber (E) has a normal appearance. Elevations on a fiber are electron dense and some have a granular appearance (arrow). Bundles of collagenous fibers (C) are dense and matted. Magnification ×5000.
ol in the turkeys fed BAPN requires a consideration of both hemodynamic factors and direct chemical action on the aortic wall.

Previous studies showed that lowering arterial blood pressure and $dP/dt$ max with propranolol reduces BAPN-induced mortality in turkeys,

4) this principle is now widely used in the treatment of aortic dissections in man.

In the present experiment, the reduction in deaths from hemopericardium, and accordingly in overall mortality, with propranolol and reserpine therapy was probably due to hemodynamic factors, because the aortic root is particularly vulnerable owing to the slight torsion produced by cardiac contraction. Heart rate and blood pressure were lowered by both reserpine and propranolol. Propranolol, but not reserpine, also reduced $dP/dt$ max significantly, which probably explains its greater effectiveness in reducing hemopericardium and overall mortality. The higher mortality rate with hydralazine and BAPN cannot be explained by hemodynamic factors alone, because hydralazine lowered blood pressure without altering either $dP/dt$ max or heart rate. Accordingly, hydralazine had an injurious effect on the aortic wall, which was shown by ultrastructural studies to be additive to that of BAPN.

There are several mechanisms by which hydralazine can cause increased degeneration of the aortic wall, as compared with BAPN alone. Hydralazine accumulates in the media of the aorta and other blood vessels in mice.

By radioautography, radiocarbon from hydralazine-$\text{I}

\text{C}_{14}$ has been shown to be concentrated in the wall of muscular arteries of the kidney, liver, spleen, heart, lung, brain and muscle.

Hydralazine reacts with the aldehyde groups of collagen by means of hydralazine groups.

This might be the mechanism by which the elasticity module of collagen fibers from the tail tendon of the rat is reduced after incubation in hydralazine.

Hydralazine also inhibits both the synthesis and secretion of collagen in embryonic chicken cartilage.

Hydralazine accumulates in the aorta, but ultrastructural studies of this phenomenon have not been reported. Using electron microscopy, we found profound alterations of aortic collagenous and elastic fibers in all the hydralazine-treated groups that were in addition to the effects of BAPN. There were accumulations of dense bundles of extracellular collagen in the aortic media of reserpine-treated turkeys and, to a much lesser extent, in those that received propranolol. These accumulations might be attempts at repair. Destruction of elastic fiber was most severe in the aortas of turkeys fed hydralazine and BAPN. In vertebrate tissues, elastic fibers consist of two morphologically distinct components, amorphous elastin and fibrils, with the former being derived in maturation from the latter.

Controlled synthesis and balanced interactions between the two components are essential for normal fibrilogenesis. BAPN interferes with the cross-linking of elastin and with covalent incorporation of synthesized elastin into fibers.

Therefore, the granular elevations on elastic fibers in these experiments appear to represent an interference with

Discussion

Hydralazine dramatically increased the mortality rate from hemorrhage in turkeys also fed BAPN, compared with mortality in turkeys fed only BAPN. Either reserpine or propranolol partially protected against death in this situation, primarily by decreasing the incidence of hemopericardium, as the drugs did not lower the incidence of hemorrhage from rupture of the abdominal aorta. Evaluation of potential mechanisms of action of hydralazine, reserpine and propranolol in the aortic media of turkeys fed BAPN, hydralazine and propranolol was there bridging of elevations on elastic fibers with collagenous fibers (fig. 4). In addition, collagenous fibers in the intercellular spaces were swollen, but bundles of collagen were not dense or matted.

Collagenous fibers in the intercellular spaces were dense and matted, and extremely prominent throughout the sections.

Some elastic fibers in the aortic media of turkeys fed BAPN, hydralazine and propranolol were swollen and had nongranular elevations on their surfaces. The fibrils in such elevations were disorganized but, unlike elevations on elastic fibers in turkeys fed hydralazine, the fibrils were not fragmented. Unaltered elastic lamellae without elevations also were present in the aortic media. Only in the aortas of turkeys fed BAPN, hydralazine and propranolol was there bridging of elevations on elastic fibers with collagenous fibers (fig. 4). In addition, collagenous fibers in the intercellular spaces were swollen, but bundles of collagen were not dense or matted.

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formation of fibrils in the newly formed peripheral layer (elevations) of elastin on an elastic fiber. Destruction of elastic fiber and granularity of the elevations were less marked in turkeys fed propranolol or reserpine, possibly because of ameliorating hemodynamic stress.

The clinical implications of this study are not clear. Although the turkeys received quantities of hydralazine that exceeded the usual human dose, it did not produce an overwhelming reduction of blood pressure or the extreme toxicity reported in dogs. In combination with other data on the influence of hydralazine on collagen, our study provides evidence of potentially serious adverse effects in addition to the hydralazine-induced lupus syndrome. These undesirable effects should be considered, as hydralazine is being more widely advocated as an afterload-reducing agent in refractory heart failure and for long-term management of hypertension.

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References

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