Increase in Collateral Arterial Endothelial Cell Proliferation Induced by Captopril After Renal Artery Stenosis in the Rat

TERUO ODORI, M.D., ANDREA PASKINS-HURLBURT, PH.D., AND NORMAN K. HOLLENBERG, M.D., PH.D.

SUMMARY Studies to assess the role of blood pressure rise in the growth of the collateral arterial supply following renal artery stenosis were performed in 70 rats. Assessment of the proliferative response was made by coded reading of endothelial cell turnover following tritiated thymidine administration, 5 days after renal artery stenosis. Stenosis induced the anticipated brisk increase in endothelial cell turnover in arterial collaterals and in the ipsilateral renal vein, and ureteric epithelium. Blood pressure elevation did not appear to play the dominant role, as the proliferative response did not parallel blood pressure changes; moreover, neither bilateral renal artery stenosis, designed to enhance the hypertension, nor hydralazine administration, to reduce the blood pressure, influenced endothelial cell turnover. A contribution of elevated blood pressure to the vasoproliferative response, however, was not ruled out definitively in this study. Captopril, also administered to assess the same question, resulted in an enhanced endothelial cell proliferative response, both in frequency and in degree, an observation that became the central thrust of our study. The mechanism by which converting enzyme inhibitor modified endothelial cell turnover is not clear, but may well provide insight into the responsible factors. (Hypertension 5: 307-311, 1983)

KEY WORDS • tritiated thymidine • hypertension • angiotensin converting enzyme inhibition

We have documented a hyperplastic response of the endothelium and smooth muscle elements of growing arterial collateral vessels in the rat1 and dog2 following renal artery stenosis. Because biophysical factors are thought to influence vascular proliferative responses,1 and hypertension (a common feature in the models in our earlier studies) is known to promote an increase in arterial cell turnover,2 we initiated a systematic assessment of the role of hypertension in the hyperplastic response. Maneuvers were designed either to promote increased hypertension, or to prevent the hypertension with antihypertensive drugs. A surprising finding that the converting enzyme inhibitor, captopril, increased the vascular proliferative response rather than reduced it represents the primary thrust of this report.

Methods

Studies were performed in 70 Sprague-Dawley white rats weighing about 200 to 250 g. Anesthesia was induced with intraperitoneal sodium pentobarbital (30 mg/kg). Renal artery stenosis was induced through a flank incision with sterile technique. The main renal artery was exposed at its origin, and a silver clip was employed to induce the stenosis. In preliminary experiments we documented that a 0.229 mm diameter, measured with a spark plug "gapper," routinely induced incomplete but severe stenosis in rats of this size. In "sham" procedures, an identical sequence was followed, including the placement of a clip to induce stenosis of the renal artery, but the clip was not left in place. The decision to leave the clip in place, or remove it, was made by means of a randomization procedure after the clip was placed. The incision was closed, and the rat was returned to its cage.

In the drug-treated groups, captopril (5 mg/kg) or hydralazine (5 mg/kg), was injected intraperitoneally once daily for 5 days, beginning on the day of the stenosis. Control rats received a vehicle injection.

Five days after the stenosis was induced, tritiated methyl thymidine (3HTdR) 1 μCi/g was injected intra-
peritoneally in two doses, 1 hour apart. Beginning 2 hours after the second \( ^3 \)HTdR injection, the abdominal aorta was catheterized by way of the femoral artery with a PE50 polyethylene catheter under pentobarbital anesthesia, and blood pressure was measured with a Statham transducer and Grass polygraph recorder. Two hours after the second \( ^3 \)HTdR injection, the rats were sacrificed with saturated KCL.

After sacrifice, both kidneys and ureters as a unit, renal arteries, and a small segment of small intestine were removed for radioautography.

The radioautographs were prepared as described earlier. At the preparation of ureteric specimens, four 5 \( \mu \) sections were examined, including one from the renal hilum and three from the periureteric including one from the renal hilum and three from the periureteric tissues. Transverse sections of renal arteries and veins were also examined for peripelvic collateral formation. Labelled nuclei were assessed on a coded basis from transverse serial sections of the ureter and periureteric tissues. As previously, the nucleus was considered to be endothelial when it was unequivocally at the surface of the lumen, with no evidence of cytoplasm medially. Because of the large number of rats and sections examined, and the convenience of the method, we employed an ordinal assessment system, which ranged from 0 to 3 +. Zero (0) indicated no positive endothelial cells and 1 + indicated an occasional tritiated thymidine-labelled endothelial cell, 2 + an unequivocal increase over the low, spontaneous normal turnover rate, and 3 + an unequivocal, striking increase in endothelial cell turnover. In general, 0 and 1 + indicate a cell turnover rate that is indistinguishable from 0.1%.

The small intestine served as an internal, technical control. When an unequivocal 3 + was not identified in that organ, the examination of other specimens in two rats were not included in the study.

All readings and data compilation were made on a coded basis, and the code was not broken for any experimental group until data analysis was complete.

Protocols

Two series of experiments were performed in this study. In the first series, an assessment was made of 18 rats with unilateral renal artery stenosis. An additional 10 rats with unilateral renal artery stenosis were treated with captopril. In 19 rats, bilateral renal artery stenosis was induced in an effort to promote more severe hypertension.

Because the first series of experiments indicated that hypertension had not played a dominant role in the increase in arterial endothelial cell turnover in collateral-forming segments, and more important, because captopril induced an unanticipated increase in cell turnover, a second series of experiments in 23 rats with unilateral renal artery stenosis was undertaken. In this series, an additional set of 11 rats were treated with captopril, as previously, and compared with 12 rats treated with hydralazine. Hydralazine was employed as a vasodilator-antihypertensive control.

Statistical Analysis

Where appropriate, mean values have been presented with the standard error of the mean as the index of dispersion, and statistical significance was assessed by Student's t test or analysis of variance. For the nonparametric data, primarily involving the ordinal assessment of microscopic evidence of collateral formation, statistical significance was assessed by a nonparametric test, including chi square Fisher Exact, and the Spearman rank correlation tests. The null hypothesis was rejected when \( p \) was less than 0.05.

Results

Renal artery stenosis induced the anticipated increase in endothelial cell tritiated thymidine incorporation in the involved periureteric arteries (fig. 1). None of the nine sham-operated rats showed a proliferative response, whereas 12 of 18 rats with renal artery stenosis did (\( p < 0.001 \)). There was an equivalent increase in endothelial cell turnover in the renal vein (\( p < 0.001 \)), and a smaller, increase in turnover in the ureteric epithelium (\( p < 0.05 \)).

Treatment with captopril did not reduce the proliferative response. Indeed, each of the 10 rats in the first series and of the 11 rats in the second series showed a proliferative response (figs. 1 and 2). The increase in response induced by captopril was evident not only when the readings were divided as "positive" or...

---

**Figure 1.** The number of rats showing a positive proliferative response following either a sham operation or renal artery stenosis. Note that renal artery stenosis induced a proliferative response in the endothelium of arteries and veins and the epithelium of ureters, that the response was not modified by hydralazine, but was enhanced by captopril.
Table 1. Influence of Bilateral Renal Artery Stenosis and Hypertension on the Endothelial Response to Renal Artery Stenosis

<table>
<thead>
<tr>
<th>Blood pressure (mm Hg)</th>
<th>Control (n = 9)</th>
<th>Unilateral (n = 18)</th>
<th>Bilateral (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>9 + 0 0 8 1</td>
<td>18</td>
<td>19 5 21 12 1</td>
</tr>
<tr>
<td>≥ 100 mm Hg*</td>
<td>0 0 0 7</td>
<td>1</td>
<td>6 3 6 3 0</td>
</tr>
<tr>
<td>&lt; 100 mm Hg</td>
<td>9 0 0 8 1</td>
<td>11</td>
<td>13 2 15 8 1</td>
</tr>
<tr>
<td></td>
<td>8.48 p &lt; 0.01</td>
<td></td>
<td>7.15 p &lt; 0.01</td>
</tr>
<tr>
<td>Veins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>9 0 0 9 0</td>
<td>18</td>
<td>19 8 20 8 2</td>
</tr>
<tr>
<td>≥ 100 mm Hg*</td>
<td>0 0 0 0</td>
<td>11</td>
<td>6 2 4 4 2</td>
</tr>
<tr>
<td>&lt; 100 mm Hg</td>
<td>9 0 0 9 0</td>
<td>7</td>
<td>13 6 16 4 0</td>
</tr>
</tbody>
</table>

*Rats have been subdivided into those in which arterial blood pressure at study was normal (< 100 mm Hg) or elevated (BP ≥ 100 mm Hg).
artery stenosis played an important role in the genesis of the endothelial mitosis. Several observations in this study suggest that hypertension did not make a quantitatively important contribution: first, bilateral renal artery stenosis, which has been known to induce a more severe and better sustained hypertension since the classic studies of Goldblatt, did not enhance the proliferative response. Second, no association could be identified between blood pressure elevation at the time of reevaluation and the proliferative response. Third, hydralazine and captopril, effective antihypertensive agents administered in dosage documented to be effective in this model, and in this study, did not reduce the proliferative response. Because no attempt was made to monitor blood pressure continuously or serially in this study, a contribution of hypertension cannot be ruled out. However, because only a minimal blood pressure rise might be required to induce endothelial cell turnover, and because blood pressure was not measured serially, the findings do not rule out an initial blood pressure effect.

The major thrust of our investigation became the exploration of an unanticipated finding, that captopril enhanced the proliferative response. Because two cycles of experiments produced a virtually identical result, and because the influence of captopril was evident not only in the frequency of the proliferative response, but also in its degree, the enhanced response is difficult to deny. Hydralazine, employed as a vasodilator antihypertensive control did not modify the proliferative response, despite a similar fall in blood pressure on the study day. This observation makes it less likely that the action of captopril responsible for the proliferative response was its vasodilator or blood pressure lowering effect, but rather some specific influence for the agent.

Endothelial cells are known to synthesize a host of potentially relevant agents, including angiotensin, prostaglandins, and kinins. Moreover, multiple experiments suggest that captopril is capable of modifying not only angiotensin II formation, through its impact on converting enzyme, but also to modify the metabolism of kinins and to induce prostaglandin release, at least under some circumstances. Whether captopril’s influence on vascular turnover reflected a local action on these systems, an unrelated local action, or a systemic effect cannot be determined from these experiments. The well-documented influence of prostaglandins on cell turnover and the cell cycle make that line of investigation especially attractive.

There has been continuing interest in the role of biophysical and humoral or chemical factors as mediators in collateral vessel formation. One of the lines of investigation that has favored a humoral mediation has been the unanticipated finding that the renal vein and the ureter participate in the proliferative response; the biophysical forces, pressure, and flow velocity, which could be enhanced in small, preformed arteries — and, thus, participate in their proliferative response — could not be responsible for enhanced turnover in the renal vein and the ureter. This study has confirmed the earlier observation; following captopril, an enhanced response was evident not only in the small arteries, but also in the renal vein and in the ureter. Any explanation for the ability of captopril to enhance the vascular proliferation must account for enhanced turnover in the renal vein and ureter as well. An explanation based on an enhanced response to a humoral agent is very attractive.

No attempt was made in these studies to ascertain whether the striking increase in the proliferative response induced by captopril resulted in a more extensive collateral formation. Indeed, as pointed out in our earlier studies, the quantitative assessment of total collateral vascular mass is difficult. It was evident in the histologic sections and on gross examination, however, that there was both an increase in the number and size of arterial vessels supplying the kidney through various arteries following renal artery stenosis. Thus the increase in mitoses reflects true growth of the collateral vessels. Until such a study is performed, and until the underlying mechanism for the proliferative response has been delineated, no therapeutic conclusion should be drawn from this investigation. Despite the similar fall in blood pressure induced by captopril and hydralazine on the fifth day, it remains possible that more effective blood pressure reduction by captopril over the 5 days enhanced the ischemia distal to the stenosis, and thus the stimulus to endothelial cell proliferation. If increased ischemia due to more effective blood pressure reduction is responsible for the enhanced collateral formation, this would represent another factor in selecting medical therapy for patients with renovascular hypertension: if collateral formation was inadequate to reverse the ischemia, progressive renal atrophy and a reduction in excretory function could be a consequence. The unequivocal influence of captopril on the vascular proliferative response, however, may provide a strong clue as to the responsible humoral mediators.

Acknowledgment

It is a pleasure to acknowledge the technical assistance provided in various aspects of this study by Deborah Williams, Paula Goodwin and Diane Johnson. We are grateful to Marie Bifolck and Carolyn James for their secretarial aid.

References

Increase in collateral arterial endothelial cell proliferation induced by captopril after renal artery stenosis in the rat.
T Odori, A Paskins-Hurlburt and N K Hollenberg

Hypertension. 1983;5:307-311
doi: 10.1161/01.HYP.5.3.307

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/5/3/307

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/