Prolongation of the Saralasin Responsive State of Two-Kidney, One Clip Goldblatt Hypertension in the Rat by the Orally Administered Converting Enzyme Inhibitor Captopril (SQ14,225)

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SUMMARY Two-kidney, one clip Goldblatt rats were treated with oral converting enzyme inhibitor captopril (SQ14,225) 6 mg/kg/day for 3 weeks after they had developed hypertension. Before treatment, systolic blood pressure rose from 143 to 202 mm Hg \((p < 0.05)\). Tail vein infusions of saralasin 10 \(\mu\)g/kg/min in conscious rats reduced systolic blood pressure from 202 to 121 mm Hg \((p < 0.05)\) at 8 weeks after clipping the renal artery and before treatment with captopril. Chronic treatment with captopril for 3 additional weeks lowered blood pressure to 173 mm Hg \((p < 0.05)\). When saralasin infusion was repeated during treatment with captopril, blood pressure fell from 173 to 159 mm Hg \((p < 0.05)\). Blood pressure rose to 197 mm Hg within 4 days after captopril was discontinued and saralasin infusion 3 weeks after captopril (15 weeks after clipping the renal artery) again resulted in a dramatic fall in blood pressure from 197 to 142 mm Hg \((p < 0.05)\). Goldblatt rats who had not received captopril showed no blood pressure response to saralasin infusion at 12 weeks after renal artery clipping. The present study demonstrates that partial inhibition of the renin-angiotensin system with captopril results in a delay in the natural evolution of clip hypertension retarding the appearance of hypertension that is resistant to acute saralasin infusion. (Hypertension 1: 8-12, 1979)

KEY WORDS • renovascular hypertension • captopril • saralasin • blood pressure • renin-angiotensin-aldosterone system • converting enzyme inhibition • Goldblatt hypertension

THE experimental model of increased blood pressure produced by renal arterial narrowing was first described by Goldblatt et al.\(^1\) With this model, interest in the enzyme renin was renewed and the renin-angiotensin-aldosterone system eventually was defined.\(^2\) However, plasma renin activity has not been found to be elevated consistently in a variety of experimental models of hypertension that created a reduction in renal blood flow.\(^3\) These findings challenge the role for abnormal renin secretion as the mediator of the hypertension. The use of the angiotensin II competitive inhibitor (Sar\(^1\), Ala\(^9\))-angiotensin II (saralasin) and inhibitors of converting enzyme gave new insight into the pathogenesis of experimental renovascular hypertension. Miller et al.\(^7\) administered the converting enzyme inhibitor, teprotide (SQ20,881), to the uninephrectomized dog and prevented the characteristic rise in blood pressure that follows renal arterial narrowing. However, in rats with Goldblatt hypertension of 8 weeks duration, Brunner et al. found a reduction in blood pressure in response to saralasin infusion only when the contralateral kidney was intact (two-kidney, one clip model) and no reduction in blood pressure when the contralateral kidney was absent (one-kidney, one clip model).\(^8\) Accordingly, the authors concluded that there were two different mechanisms of hypertension of renal origin.

There is a growing body of evidence that the hypertension in these Goldblatt models is initially angiotensin-dependent and evolves to an “angiotensin-independent” phase. The duration of the initial phase is different in the one- and two-kidney models of...
Goldblatt hypertension. The two-kidney, one clip model develops hypertension that remains responsive to angiotensin blockers for several weeks, whereas in unilaterally nephrectomized Goldblatt animals, high blood pressure is responsive to angiotensin blockade for only a few days. Hence, the initial elevation of blood pressure is dependent on angiotensin II-induced vasoconstriction, but subsequently blood pressure is not affected by acute angiotensin blockade in either model. The natural history of clip hypertension is influenced by contralateral nephrectomy, sodium balance, the level of the blood pressure and the presence or absence of the adrenal glands.

Since many of these variables involve the renin-angiotensin-aldosterone system, we hypothesized that full expression of the system subsequent to renal artery clipping was a prerequisite for the sequence of events that characterize renovascular hypertension.

This study was designed to determine the effect of chronic blockade of converting enzyme by the oral administration of captopril (SQ14,225: 2-L-methyl-3-mercapto propanoyl-L-proline) on the natural history of hypertension in the two-kidney, one clip model.*

Materials and Methods

Male Wistar rats (150–200 g) were maintained on normal sodium (110 mEq/kg) chow and had free access to water. Under ether anesthesia, a 0.25-mm silver clip was placed on the left renal artery or a sham flank exploration was performed. The right kidneys were left intact.

Systolic blood pressure was monitored weekly by the tail cuff method. At 8 weeks, six hypertensive animals and six sham controls had intravenous infusion of saralasin 10 μg/kg/min in 5% dextrose (20 μl/min) for 20 minutes. Infusion was via percutaneous tail vein puncture with a 25-gauge scalp vein needle attached to a Harvard infusion pump. Blood pressure was monitored in these awake animals every 5 minutes during infusion and after a 10-minute recovery period.

The hypertensive animals were treated by gavage with captopril (3 mg/kg/12 hr) for 3 weeks. The dose of captopril was selected on the basis of acute studies in Goldblatt rats and inhibition of responses to angiotensin I. A second group of seven similarly prepared and maintained hypertensive animals was not treated. Animals that received captopril were rein- fused with saralasin at the end of the captopril treatment period (12 weeks) and 3 weeks following treatment with captopril (15 weeks). The untreated hypertensive group was challenged by saralasin infusion at 12 weeks after surgery. Control animals had sham flank operations and were studied in an identical manner but received 5% dextrose and water (D5W) by gavage instead of captopril.

Data were analyzed by the Student's *t* test. Each figure presents mean values ± the standard error of the mean.
**Results**

After clipping, the blood pressure rose from 143 ± 5.3 to 202 ± 12.8 mm Hg ($p < 0.05$) at 8 weeks when saralasin was infused. As shown in figure 1, saralasin induced a dramatic fall in blood pressure to 121 ± 6.9 mm Hg ($p < 0.05$) which rapidly returned to the pretreatment hypertensive level after termination of the infusion.

Initially the oral administration of captopril to the rats reduced blood pressure to normal. However, the chronic treatment regimen resulted in only a partial (but significant) depression of blood pressure to 173 ± 9.9 mm Hg ($p < 0.05$, fig. 2). Concomitant saralasin infusion after 3 weeks of captopril treatment resulted in a further fall in blood pressure to 159 ± 11.8 mm Hg ($p < 0.05$, fig. 3). Withdrawal of captopril was followed by a gradual return in blood pressure to the pretreatment level, 197 ± 11.5 mm Hg.

The blood pressure remained elevated and at 15 weeks (3 weeks following termination of captopril) the infusion of saralasin still dramatically lowered systolic blood pressure to 142 ± 12.8 mm Hg (fig. 4, broken line).

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**Figure 2.** Blood pressure of two-kidney, one clip Goldblatt rats before clipping the renal artery (pre-clip); 8 weeks post-clip; after SQ14,225 treatment for 3 additional weeks (total of 12 weeks post-clip); 2 weeks after SQ14,225 treatment had been discontinued. The number over each bar represents the number of animals studied.

**Figure 3.** Effect of saralasin on blood pressure in two-kidney, one clip Goldblatt rats during treatment with SQ14,225. The saralasin infusion (10 μg/kg/min) was at the end of a 3-week treatment period with SQ14,225 at 6 mg/kg/day and a total of 12 weeks after clipping the renal artery. $N =$ the number of animals studied.
In contrast, hypertensive animals treated with D5W by gavage for 3 weeks showed absolute resistance to saralasin infusion at 12 weeks with an actual rise in blood pressure from 179 ± 7.7 to 181 ± 10 mm Hg (fig. 4, solid line).

Sham operated control animals did not show a significant blood pressure response to either saralasin or captopril at any time during the 15-week study period.

Discussion

The well-documented increase in plasma renin activity that has been correlated with the initial vasoconstriction and hypertension following experimental renal artery clipping was not sustained. Hence, a role for the renin-angiotensin-aldosterone system in established (chronic) renal hypertension has been challenged. The development of hypertension independent of the renin-angiotensin system has been supported by observations that pharmacologic blockade of angiotensin II production or receptors does not totally prevent the development of renal hypertension. In addition, it is clear that the blood pressure in experimental two-kidney, one clip renal hypertension in the rat is unaffected by angiotensin II antagonists after it has become established for about 12 weeks.

The present study suggests that the renin-angiotensin-aldosterone system may contribute to the development and maintenance of established ("saralasin-resistant") Goldblatt hypertension. As expected, the untreated hypertensive animals were resistant to infusion of saralasin by 12 weeks. Previous studies have shown that mild sodium and water retention occurs in this model, plasma renin activity falls and blood pressure does not respond to acute angiotensin blockade unless the sodium retention is reversed either by diuretic or low sodium diet.

It is difficult to assess the importance of the reduction in blood pressure during treatment with captopril in prolonging the "saralasin-responsive" phase of Goldblatt hypertension. The partial control of blood pressure could represent a major factor in modifying the evolution of the hypertension to the "saralasin-resistant" phase. Studies were not attempted with vasodilators or diuretics to achieve a comparable reduction in blood pressure since these agents activate the renin-angiotensin system and are known to induce saralasin responsiveness.

**Figure 4.** Prolongation of angiotensin II dependency of blood pressure by captopril (SQ14,225) in two-kidney, one clip Goldblatt rats. The solid line represents the responses during a 20-minute infusion of saralasin in rats 12 weeks after clipping the renal artery. The broken line represents responses during saralasin infusion in hypertensive rats that had been treated for 3 weeks (Weeks 9 to 12 post-clip) with SQ14,225. The saralasin infusions were performed 3 weeks after SQ14,225 had been discontinued (or 15 weeks after clipping the renal artery). N = the number of experimental animals.
Blockade of angiotensin II formation with 6 mg/kg/day of captopril was not complete as evidenced by the additional drop in blood pressure when saralasin was infused during the treatment period with captopril. Initially, captopril did lower blood pressure to normal levels; however, by the fifth to seventh day of treatment, blood pressure had risen to about 170 mm Hg and remained at that level for the duration of the captopril treatment period. In spite of only partial blockade of converting enzyme, a marked prolongation of the “saralasin-responsive” phase of Goldblatt hypertension was achieved. This observation suggests that full expression of the renin-angiotensin-aldosterone system is involved in the development of saralasin-resistant renal hypertension. Furthermore, since sodium retention is characteristic of this resistant phase of hypertension, our findings suggest a major role for angiotensin II-induced aldosterone secretion as the mediator of the sodium retention. Converting enzyme inhibition has been shown to prevent angiotensin II production with a resultant fall in aldosterone levels. Hence, in the present study, sodium retention was blocked and upon termination of captopril, blood pressure rose and angiotensin II-induced vasoconstriction persisted as manifest by the depressor response to saralasin. Similarly, it has been reported that bilateral adrenalectomy did not prevent clip hypertension, but plasma renin activity remained high and saralasin responsiveness persisted in the established phase.

We suggest a common mechanism for all forms of experimental clip hypertension requiring an active renin-angiotensin-aldosterone system and subject to influence by the following five experimental manipulations:

1. Presence or absence of a normal contralateral kidney
2. Degree of renal artery occlusion (level of hypertension)
3. Dietary sodium intake
4. Presence or absence of adrenal glands
5. Chronic blockade of the renin-angiotensin-aldosterone system.

Renal artery clipping is followed by renin release and the hypertension is maintained initially by angiotensin II-induced vasoconstriction. Concurrent angiotensin II-induced aldosterone biosynthesis may act to mediate subtle sodium retention until blood pressure dependence upon angiotensin II vasoconstriction is lost. Contralateral nephrectomy seems to accelerate the transition to angiotensin II independence defined as failure of an acute blood pressure response to angiotensin blockers. In contrast, severe hypertension is associated with a greater contralateral natriuresis and retards the process. Sodium deprivation by stimulating the renin system can prevent or reverse this transition. Similarly, bilateral adrenalectomy prevents any aldosterone-induced sodium retention. Chronic partial inhibition of angiotensin II production and partial blood pressure control with captopril resulted in a similar delay in the natural evolution of clip hypertension further substantiating a continuous role of the renin-angiotensin-aldosterone system in the established phase of clip hypertension. It is reasonable to suggest that chronic treatment with captopril will reduce blood pressure at anytime in both one- and two-kidney Goldblatt hypertension.

References

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