Selection Criteria for Drug-Treated Animals in Two-Kidney, One Clip Renal Hypertension

SHIRLEY H. SMITH AND SANFORD P. BISHOP

SUMMARY The two-kidney, one clip (2K1C) model of hypertension in the rat does not uniformly result in increased blood pressure. That is, the placement of a clip around one renal artery in a two-kidney rat will usually, but not always, produce hypertension. This is an important problem in studies designed to evaluate the ability of antihypertensive therapy to prevent hypertension. Therefore, an additional objective means other than blood pressure is needed to assess animals that are treated from the outset with antihypertensive therapy. The purpose of this study was to correlate the relative fresh weights of left (clipped)/right (nonclipped) kidneys (LK/RK) with tail-cuff systolic blood pressure in the 2K1C model of renal hypertension and to identify an LK/RK range that would exclude the animals least likely to become hypertensive (failures of the clipping procedure). On a scale of 0.0 to 1.0, an LK/RK ratio of 0.0 was present when the clipped kidney was completely infarcted or atrophied and a ratio of 1.0 was present when the clip did not cause sufficient renal artery stenosis to alter kidney weight. In a series of 72 untreated 2K1C male Sprague-Dawley rats examined 6 to 8 weeks after clipping, 100% of the animals with an LK/RK ratio of 0.5 to 0.8 (n = 19) and 75% with an LK/RK ratio of 0.4 to 0.9 (n = 38) had a blood pressure greater than 150 mm Hg. Less than 50% with an LK/RK ratio below 0.4 or above 0.9 (n = 34) were hypertensive. Two 2K1C groups treated with hydralazine (n = 32) or spironolactone (n = 24) were compared with the untreated 2K1C model, and the mean blood pressure of each group was found to be underestimated if the elimination criteria (LK/RK ratio, 0.4–0.9) were not applied. These results indicate that LK/RK ratios provide an objective means to compare treatment groups and to evaluate whether a given 2K1C animal would have become hypertensive, had it not been treated with antihypertensive therapy.

(Hypertension 8: 700–705, 1986)

KEY WORDS • kidney weight • Goldblatt hypertension • hydralazine • spironolactone

THE two-kidney, one clip (2K1C) model of hypertension has been used widely to study cardiovascular and renal physiology. Unfortunately, the clipping procedure does not always result in the development of hypertension. Approximately two thirds of the animals that undergo renal artery clipping attain a systemic blood pressure of 150 mm Hg or greater. The success of the clipping procedure in producing hypertension generally is evaluated by the presence or absence of high systemic blood pressure. In a study designed to use only those animals with documented hypertension, the selection of animals on the basis of blood pressure alone is satisfactory. In studies designed to evaluate the capacity of antihypertensive therapy to hypertension, however, it is necessary to predict precisely which animals would have become hypertensive, had there been no antihypertensive therapy. Given an objective means of evaluating the success of operation other than blood pressure, it would be possible to identify those animals in which the clipping procedure was unsuccessful. During the course of studies with the 2K1C model, we observed that the relative weights of clipped versus nonclipped kidneys reflected the fact that the clipped kidney varied in its degree of atrophy and that the nonclipped kidney varied in its degree of hypertrophy. Certainly, this observation has been made by several early investigators who described atrophy of the clipped kidney accompanied by hypertrophy of the unclipped kidney. Although Wilson and Byrom described two patterns of blood pressure elevation in the 2K1C model in the rat that distinguish sustained from malignant hypertension, blood pressure was not correlated specifically with a particular degree of atrophy or hypertrophy. Therefore, we examined the cor-

From the Department of Pathology, University of Alabama at Birmingham, and the VA Medical Center, Birmingham, Alabama.

Supported in part by grants from the National Institutes of Health (1-K11-HL01596, 5-T32-HL07457, 5-P50-HL25451) and Veterans Administration Medical Research Service.

Address for reprints: Shirley H. Smith, M.D., Department of Pathology, University of Alabama at Birmingham, Birmingham, AL 35294.

Received June 17, 1985; accepted February 5, 1986.
relation between blood pressure and the ratio of clipped/nonclipped kidney weight in an effort to find an easily obtainable and objective criterion other than blood pressure to judge the success of the renal clipping operation. To determine its usefulness in evaluating the results of antihypertensive therapy, we applied this criterion in a study of the effects of two drugs that stimulate renin production, hydralazine and spironolactone, on 2K1C hypertension.12,13

Materials and Methods

Male Sprague-Dawley rats were purchased from Charles River Kingston (MA, USA) Laboratories at 4 weeks of age (weight, 50–75 g) and were placed in one of three experimental groups: 2K1C (n = 72), 2K1C plus hydralazine therapy (n = 32), and 2K1C plus spironolactone therapy (n = 24). An additional 32 Sprague-Dawley rats served as controls: 14 nonoperated, 5 nonoperated treated with hydralazine, 7 sham-operated, and 6 sham-operated treated with spironolactone. Rats were anesthetized with sodium pentobarbital (30 mg/kg i.p.). A U-shaped silver clip standardized to 0.20 mm internal diameter was placed on the proximal portion of the left renal artery. In 4-week-old rats, this size clip is snug but does not constrict the renal artery. The sham operation consisted of left renal artery dissection without clipping. The right renal artery was untouched.

The rats were allowed to grow for 6 to 8 weeks and were fed Agway Rodent Lab Chow 5001 (Syracuse, NY, USA) and drinking water ad libitum. Hydralazine was administered in the drinking water at a dose of 160 mg/L. Spironolactone (SC 9420, donated by G.D. Searle, Skokie, IL, USA) was administered by continuous subcutaneous infusion at a rate of 2 mg/kg/day using Alzet 2002 osmotic minipumps (Palo Alto, CA, USA). Each minipump was checked at the time of removal to ensure that its contents had been delivered. Weekly tail-cuff pressures were measured using a Narco Bio-Systems PE-300 electrosphygmomanometer (Houston, TX, USA). A systolic blood pressure of 150 mm Hg or greater was accepted as hypertensive. Rats were killed by decapitation. The left (clipped) to right (nonclipped) kidney weight (LK/RK) ratios were calculated from fresh whole kidney weights, measured by a Mettler H80 balance (Hightstown, NJ, USA), after careful dissection from surrounding fat. Renal vessels and ureter were removed at the hilum of each kidney.

Statistically significant differences between groups were calculated by one-way analysis of variance and the unpaired Student’s t test. Differences were considered significant when the p value was less than 0.05.

Results

The LK/RK ratio ranged from zero (infarction or complete atrophy of the clipped kidney) to 1.0 (no effect on clipped kidney), as shown in Figure 1. In the untreated 2K1C group, 43 of 72, or 60%, were hypertensive (Figure 2). In the hydralazine-treated 2K1C
In the spironolactone-treated group, 16 of 24, or 67%, were hypertensive (Figure 4). The population distribution of each of these three groups was similar in terms of the LK/RK ratios, although the spironolactone group contained few infarcted kidneys. Overall (without regard to LK/RK ratios), mean systolic blood pressure was 168 ± 4 (SEM), 139 ± 4, and 162 ± 7 mm Hg in the untreated 2K1C, hydralazine-treated 2K1C, and spironolactone-treated 2K1C groups, respectively. There was a significant difference in blood pressure between hydralazine-treated and untreated 2K1C groups, while there was no difference between spironolactone-treated and untreated 2K1C groups. Nonoperated control rats had a mean systolic blood pressure of 128 ± 2 mm Hg, and there was no significant difference in blood pressure between this control group and sham-operated (128 ± 2 mm Hg), hydralazine-treated (122 ± 4 mm Hg), or spironolactone-treated (130 ± 2 mm Hg) control groups. However, there was a significant difference in blood pressure between nonoperated control and untreated 2K1C rats, hydralazine-treated 2K1C rats, and spironolactone-treated 2K1C rats.

Examination of the LK/RK ratio in the untreated 2K1C group revealed that 100% of the rats with an LK/RK ratio of 0.5 to 0.8 (n = 19) were hypertensive (Figure 5). Flanking this range, 75% of the rats with an LK/RK ratio of 0.4 to 0.49 (n = 4) or 0.8 to 0.89 (n = 15) were hypertensive. Less than 50% of the rats having an LK/RK ratio below 0.4 or above 0.9 (n = 34) were hypertensive. Similarly, in the hydralazine-treated 2K1C group, the rats that remained hypertensive fell primarily into the midrange of LK/RK ratios; only a few hypertensive rats had an LK/RK ratio greater than 0.8 (Figure 6). Again, most LK/RK values in the hypertensive, spironolactone-treated 2K1C group were in the midrange (Figure 7).

To compare these results with those obtained without regard to LK/RK values, two LK/RK ranges were chosen for the exclusion of animals for study. A wide range of 0.4 to 0.9 included 74 (58%) of the original 128 clipped animals. A narrower range of 0.5 to 0.8 included 36 (28%) of the original 128 clipped animals. This distribution of LK/RK ratios was similar in the untreated 2K1C group alone, in which 19 of 72 (26%) had an LK/RK ratio of 0.5 to 0.8 and 38 of 72 (53%) had an LK/RK ratio of 0.4 to 0.9. The LK/RK ratio in nonoperated controls was 1.00 ± 0.01 and was not altered in sham-operated or drug-treated control groups.

When only those 2K1C rats with an LK/RK ratio between 0.4 and 0.9 were accepted for study, the mean systolic blood pressure was 190 ± 5 (n = 37) in the untreated 2K1C group, 147 ± 4 (n = 21) in the hy-
FIGURE 5. Percentage of hypertensive two-kidney, one clip rats in each of 10 groups equally divided according to clipped/nonclipped kidney weight (LK/RK) ranging from 0.0 to 1.0. Note that 100% of rats between 0.5 and 0.8 were hypertensive. Also, 75% of rats with an LK/RK ratio of 0.4 to 0.49 and 0.8 to 0.89 were hypertensive.

FIGURE 6. Percentage of hypertensive two-kidney, one clip rats treated with hydralazine in each of 10 groups equally divided according to ratio of left to right kidney weight (LKIRK) ranging from 0.0 to 1.0.

FIGURE 7. Percentage of hypertensive two-kidney, one clip rats treated with spironolactone in each of 10 groups equally divided according to ratio of left to right kidney weight (LK/RK) ranging from 0.0 to 1.0.

FIGURE 8. Effect of clipped/nonclipped kidney weight (LKIRK) ratio on mean systolic blood pressure. Untreated two-kidney, one clip (2K1C) rats, hydralazine-treated 2K1C rats (2K1C + HYD), and spironolactone-treated 2K1C rats (2K1C + SPI) all showed higher blood pressure when the data were confined to an LK/RK range of 0.4 to 0.9. Also, a slight decrease in the blood pressure of spironolactone-treated rats as compared with 2K1C rats was shown when the data were confined to an LK/RK range of 0.4 to 0.9.

The mean absolute kidney weight in nonoperated control rats was 1.2489 ± 0.043 (SEM) g for the left and 1.2446 ± 0.040 g for the right kidney (Table 1). In comparison, the untreated 2K1C rats with an LK/RK ratio between 0.4 and 0.9 had a left kidney weight of 1.1449 ± 0.044 g and a right kidney weight of 1.6751 ± 0.064 g. Note that the body weight was 367 ± 22 g in the control (n = 14) and 366 ± 12 g in the 2K1C rats with an LK/RK ratio between 0.4 and 0.9 (n = 37). Thus, the absolute kidney weights indicated that hypertrophy did occur in the nonclipped kidney and that partial atrophy of the clipped kidney also occurred. In further examining the absolute kidney weights of untreated 2K1C rats, the severely stenosed group, which had an LK/RK ratio of less than 0.4 (body weight, 376 ± 17 g; n = 19), had kidney weights of 0.3756 ± 0.076 g on the left and 2.0244 ± 0.146 g on the right, indicating that the enlargement of the nonclipped kidney was truly compensatory. The hydralazine-treated group, which had an LK/RK ratio between 0.4 and 0.9, had absolute kid-
Renal Hypertension

TABLE 1. Absolute Kidney Weights and Body Weight Normalization of Kidney Weights for Two-Kidney, One Clip Renal Hypertension

<table>
<thead>
<tr>
<th>Variable</th>
<th>2K1C hypertension</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 19)</td>
<td>(n = 37)</td>
</tr>
<tr>
<td>LK/RK</td>
<td>&lt;0.4</td>
<td>0.4-0.9</td>
</tr>
<tr>
<td>BW (g)</td>
<td>376 ±17</td>
<td>366 ±12</td>
</tr>
<tr>
<td>BP (mm Hg)</td>
<td>145 ±4</td>
<td>190 ±5</td>
</tr>
<tr>
<td>LK wt (g)</td>
<td>0.3756 ±0.076</td>
<td>1.1499 ±0.044</td>
</tr>
<tr>
<td>RK wt (g)</td>
<td>2.0244 ±0.146</td>
<td>1.6751 ±0.064</td>
</tr>
<tr>
<td>LK/BW (g/g x 1000)</td>
<td>0.9853 ±0.185</td>
<td>3.1665 ±0.099</td>
</tr>
<tr>
<td>RK/BW (g/g x 1000)</td>
<td>5.360 ±0.263</td>
<td>4.6676 ±0.175</td>
</tr>
</tbody>
</table>

Values are means ± SEM. 2K1C = two-kidney, one clip; LK = clipped kidney; RK = nonclipped kidney; BW = body weight; BP = systolic pressure; Control = nonoperated rats.

Discussion

Use of the LK/RK ratio is an effective means of determining which of the animals that underwent the 2K1C procedure would have become hypertensive in a study with antihypertensive agents. Partial atrophy of the clipped kidney and compensatory hypertrophy of the nonclipped kidney in 2K1C renal hypertension have been observed by several investigators. It has been postulated that an angiotensin II–mediated increase in vascular resistance in the nonclipped kidney is important in the maintenance of hypertension in this model. However, this study, as well as most physiological studies, used elevated arterial blood pressure as the criterion for inclusion in the study. There is evidence that the lesions associated with hypertension, such as cardiac hypertrophy, may be mediated by factors other than high blood pressure alone. Investigation of the effects of such nonhemodynamic factors on these other lesions would require the use of a model in which the success of the preparation itself could be evaluated by an objective criterion other than blood pressure. The data presented here demonstrate that in untreated 2K1C renal hypertension, an LK/RK ratio between 0.4 and 0.9 is indicative of successful operation. Elimination of those animals outside this range objectively excludes those animals that may never have achieved a renal hypertensive state. In most rats, the clipped kidney was either severely atrophied or partially to completely infarcted at an LK/RK ratio less than 0.4 and the clip did not produce sufficient renal artery stenosis to induce hypertension at an LK/RK ratio greater than 0.9.

Of the animals with untreated 2K1C hypertension, all those with an LK/RK ratio between 0.5 and 0.8 were hypertensive: systolic blood pressure was 150 mm Hg or greater (see Figures 1 and 4). Among the animals with an LK/RK ratio of 0.4 to 0.49 and 0.8 to 0.89, mean systolic blood pressure was 150 mm Hg or more in 75%. Outside the LK/RK range of 0.4 to 0.9, most animals were normotensive. In choosing an LK/RK range as a criterion for elimination of animals for study, it would seem appropriate to allow the elimi-
nation of animals that might have been hypertensive, rather than to risk the inclusion of large numbers of animals that probably would not have been hypertensive. Also, the mean systolic blood pressure was underestimated by the inclusion of all animals without regard to relative kidney weight ratio (190 vs 168 mm Hg).

Failure to exclude animals that would not have become hypertensive can give erroneous results. Including all of the rats in the hydralazine-treated 2K1C group without regard to LK/RK values yielded blood pressure within normal limits (139 mm Hg); however, the continued presence of a modest hypertensive state (147 mm Hg) despite hydralazine treatment became apparent when the data were confined to the 0.4 to 0.9 LK/RK range.

Spironolactone therapy had only a minimal effect in reducing blood pressure in the 2K1C model. However, the effect was apparent only when exclusion criteria were applied. According to these data, spironolactone (2 mg/kg/day) would not be an effective antihypertensive agent in this model. However, the use of the LK/RK ratio for the evaluation of this drug provided an objective means to detect a minimal antihypertensive effect. Perhaps a higher dose of spironolactone would be more effective in reducing blood pressure.

According to absolute kidney weights and kidney weights normalized to body weight, our data support the concept that the 2K1C renal model of hypertension involves a partial atrophy of the clipped kidney and a compensatory hypertrophy of the nonclipped kidney that is not altered by drug therapy or blood pressure. The compensatory nature of the nonclipped kidney enlargement is demonstrated by the finding that lower LK/RK ratios were associated with higher RK/BW ratios. Blood pressure did not seem to affect the degree of hypertrophy, since the blood pressure was lower in the rats that had an LK/RK ratio below 0.4 than in those with an LK/RK ratio between 0.4 and 0.9, while the absolute right kidney weight and the RK/BW ratio were higher in the rats with a low LK/RK ratio. Conversely, the lower blood pressure seen in rats with an LK/RK ratio above 0.9 was associated with a lower absolute right kidney weight and RK/BW ratio than that seen in the hypertensive rats with an LK/RK ratio of 0.4 to 0.9.

In our experience, the inclusion of animals with an LK/RK ratio between 0.4 and 0.9 would allow acceptance of 58% of all clipped animals for study, while the narrower LK/RK range of 0.5 to 0.8 would allow only 28% acceptance. As the blood pressure was 190 ± 6 (SEM) mm Hg in the group with an LK/RK ratio of 0.4 to 0.9 and 200 ± 6 mm Hg in the group with an LK/RK ratio of 0.5 to 0.8, we have found the use of the wider range of acceptance to be more practical for our experimental use. Of course, all investigators should run appropriate controls to ensure that hypertension was achieved and to analyze their own group of animals with regard to LK/RK ratio and blood pressure.

In summary, the prevention of high blood pressure in the 2K1C model through the use of antihypertensive agents may be useful in the evaluation of other nonhemodynamic aspects of hypertension that result from the clipping procedure. To eliminate the animals in which the clipping procedure itself failed, our results indicate the use of the ratio of clipped/nonclipped kidney weight is a simple and objective criterion for the selection of animals for study.

Acknowledgments

The authors thank Searle Pharmaceutical, Dr. Richard Katholi, and Dr. David Ploth for their valuable assistance.

References

17. Frohlich ED, Tarazi RC. Is arterial pressure the sole factor responsible for hypertensive cardiac hypertrophy? Am J Cardiol 1979;44:959-963
Selection criteria for drug-treated animals in two-kidney, one clip renal hypertension.
S H Smith and S P Bishop

Hypertension. 1986;8:700-705
doi: 10.1161/01.HYP.8.8.700

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 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/8/8/700

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/