Effect of Poststroke Captopril Treatment on Mortality Associated with Hemorrhagic Stroke in Stroke-Prone Rats†

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ABSTRACT

We tested the ability of captopril treatment (50 mg/kg/day p.o.), initiated 2 weeks before stroke or up to 5 days after stroke, to alter the onset of stroke and death after stroke in Kyoto Wistar stroke-prone spontaneously hypertensive rats (SHRsp). The benefits of blood pressure and aldosterone suppression during captopril treatment were assessed. SHRsp developed a 100% mortality rate with intracerebral hemorrhage within 16 weeks of age. Captopril treatment, started 2 weeks before or at the initiation of stroke, suppressed plasma aldosterone and equally prevented mortality to a mean age of >27 weeks. Treatment started 5 days after stroke extended the mean lifespan to >23 weeks. The re-elevation of plasma aldosterone (via osmotic pumps to levels in untreated SHRsp) during captopril treatment, before stroke, allowed stroke to develop. The initiation of the latter manipulation in pre- or poststroke captopril-treated SHRsp at a latter age (23 weeks) didn’t alter the lifespan of SHRsp (death occurred at about 28 weeks). The antistroke effects of captopril treatment occurred without an antihypertensive effect, weren’t altered by enhancing hypertension during treatment (with dexamethasone), and couldn’t be duplicated by antihypertensive treatment with hydralazine. Spironolactone treatment didn’t duplicate the effects of captopril. The suppression of plasma aldosterone may retard the onset of stroke in SHRsp during captopril treatment but likely other factors prolong life in pre- and poststroke SHRsp receiving long-term captopril treatment. The observation that spironolactone treatment couldn’t duplicate the effects of captopril suggests that aldosterone may facilitate stroke through non-genomic receptor mechanisms.

KYOTO WISTAR STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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ABBREVIATIONS: SHRsp, Kyoto Wistar stroke-prone spontaneously hypertensive rats; SHR, Kyoto Wistar spontaneously hypertensive rats; ACEI, angiotensin-converting enzyme inhibitor; MANOVA, general linear model of multivariant analysis of variance; AII, angiotensin II; MCA, middle cerebral artery.
management of intracerebral hemorrhage occurs after stroke has developed, often in previously undiagnosed hypertensive patients. The potential ability of ACEIs to prevent stroke during established hypertension or to prolong life after stroke has not been tested.

In the present study we tested the ability of captopril to alter mortality associated with stroke in SHRsp under conditions where: 1) treatment was started after hypertension was established in SHRsp at 10 weeks of age, 2 weeks before stroke; 2) at the first behavioral signs of stroke, indicated by the abrupt onset of seizures, and 3) 5 days after the onset of seizures. In addition, the effects of captopril treatment started from 5 days after the initial onset of stroke were compared with the effects of hydralazine treatment initiated at the same time. To determine whether elevated levels of aldosterone played a role in altering the onset of stroke development within SHRsp or mortality after stroke during captopril treatment, experiments were performed to test the effects of captopril treatment on SHRsp during established hypertension under conditions where the levels of aldosterone suppressed by captopril treatment were re-established by the coinfusion of aldosterone into the animals via osmotic pumps. In addition, SHRsp were treated with spironolactone during established hypertension to determine whether this type 1 mineralocorticoid receptor antagonist altered stroke development within SHRsp.

Materials and Methods

Only male SHRsp were used in the study. These were taken from a colony maintained at Memorial University of Newfoundland (St. John’s, Newfoundland, Canada). The studies were performed with institutional approval in a manner consistent with the Canadian Council on Animal Care. The rats were fed a Japanese style diet containing 4% NaCl (Zeigler Bros., Gardners, PA) from weaning. The systolic blood pressure of the animals was measured using a tail cuff compression method (IITC model 29, pulse/pressure amplifier; Woodlands Hills, CA). The SHRsp used in the study were: 1) untreated SHRsp; 2) SHRsp treated with captopril (50 mg/kg/day p.o.) before stroke from 10 weeks of age (the captopril levels within the drinking water were adjusted 2 times/week so that the rats ingested the appropriate level of captopril based on their drinking rates); 3) SHRsp treated with captopril (50 mg/kg/day p.o.) from the first onset of seizure associated with stroke and SHRsp treated with captopril or hydralazine (80 mg/liter drinking water) 5 days after the onset of seizure (the latter events typically occurred between the 12th and 14th week of age); 4) SHRsp treated before stroke from 10 weeks of age with captopril followed after 3 days by the continuous infusion of aldosterone (0.66 μg/h s.c.) via osmotic pumps (Alzet, Palo Alto, CA) within polyethylene glycol (300 MW) vehicle; 5) SHRsp treated with captopril from 10 weeks of age before stroke or from the first onset of seizure up to 23.3 weeks of age, followed by the infusion of aldosterone or vehicle for the balance of their lifespan; and 6) SHRsp treated before stroke from 10 weeks of age with daily injections of spironolactone (150 mg/kg/day s.c.) or spironolactone vehicle (polyethylene glycol; 0.1 ml/200 g s.c.).

The rats were monitored on a daily basis for behavioral signs of stroke. The behavioral alterations associated with stroke development are described in detail elsewhere (Smeda, 1989). A common initial sign of stroke was the occurrence of seizures. This symptom had an abrupt onset within previously well-groomed normal looking animals and consisted of repetitive involuntary flexion of the right or left forepaw over the head of the animal. Animals sampled at this stage had very minor cerebral lesions typically consisting of small pinpoint-sized hemorrhages and the presence of small fluid-filled cerebral blisters with traces of blood. One to two days after the occurrence of a seizure, the rats often behave in a near normal manner but exhibit poor grooming. Three to ten days after the initial signs of seizure, the rats undergo a dramatic behavioral change and exhibit stupor and immobility. This was often associated with the presence of a huddled sitting posture with the legs hyperextended beneath the body in what has been termed a “Kangaroo stance”. Death was usually imminent at this stage. SHRsp typically died 1.5 weeks after the first signs of stroke are observed (Smeda, 1989). Rats within the end stages of stroke often exhibit large multiple cerebral hematomas. Within our colony, the most common lesion observed is intracerebral hemorrhage within the cerebrum, primarily within the perfusion domain of the middle cerebral artery (MCA).

SHRsp that were at a stage of behavior where death was imminent were euthanized and the brains of these animals, as well as those that died spontaneously, were removed and fixed in 84 mM PO4 buffer containing 4% formaldehyde, 1% glutaraldehyde at pH 7.4 for preservation and histological analysis. In the majority of cases, the presence of intracerebral hemorrhage on, or slightly below, the surface of the cerebrum was evident. If any doubt existed, the entire brain from the point where the vertebral arteries join to form the basilar artery to the most anterior region of the brain was sectioned and examined by microscope to determine the presence or absence of hemorrhage.

Plasma aldosterone levels were measured in blood samples taken via cardiac puncture. The blood was centrifuged at 5000g for 15 min and the plasma was separated from the packed cells. Radioimmunoassays of aldosterone were performed using the Coat-A-Count radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA) by the Memorial University Health Science Center, Renal Diagnostic Service Lab. Personnel performing the assays were blinded to the identity of the samples. Aldosterone, spironolactone, and polyethylene glycol used in the study were purchased from Sigma-Aldrich Canada Ltd. (Oakville, Ontario, Canada).

Systolic blood pressure was measured on a weekly basis within the SHRsp. A general linear model of multivariate ANOVA (MANOVA) was used to determine whether the amplitude of blood pressure significantly differed with respect to age between the different treatment groups. Such comparisons between groups were performed over the maximum common age range of the groups. Differences in the age of death associated with stroke between groups were analyzed using the Mann-Whitney rank order test. Significant differences in plasma aldosterone present between groups were assessed using a one-way ANOVA followed by a Fisher post hoc test for multiple comparisons. Results were considered significantly different at P < .05. All of the data in the paper is expressed as the mean ± one S.E.

Results

Figure 1 outlines the effects of captopril treatment (50 mg/kg/day p.o.) on blood pressure and mortality associated with stroke. All of the untreated SHRsp within the study developed stroke and died by 16 weeks of age. Captopril treatment was started from 10 weeks of age about 2 weeks before stroke development. Because this group of rats served as a comparison group to SHRsp that were to receive captopril plus aldosterone infusion by osmotic pump, they were implanted with pumps containing vehicle (polyethylene glycol, 2.5 μl/h s.c.) for a 6-week period that started 3 days after the initiation of captopril treatment.

Captopril treatment produced a small but significant decrease in blood pressure within the animals and had a profound effect on stroke development in that no captopril-treated SHRsp developed stroke or died before the
Alterations in systolic blood pressure and mortality with age in untreated, captopril-treated, captopril + aldosterone-treated, and captopril + dexamethasone-treated SHRsp. Captopril treatment (50 mg/kg/day p.o. + polyethylene glycol vehicle used to dissolve aldosterone, s.c. 2.5 µl/h s.c.) was started at 10 weeks of age and completely retarded mortality associated with stroke for the duration of the experiment (up to 24 weeks of age). Aldosterone was infused into captopril-treated SHRsp at a rate (0.66 µg/h s.c.) that replaced the levels of aldosterone suppressed by captopril treatment (see Fig. 2). This prevented captopril from exerting a strong antistroke effect. The treatment of SHRsp with captopril plus dexamethasone (0.1 mg/kg/day s.c.) increased the blood pressure of SHRsp to levels comparable to those observed during captopril plus aldosterone treatment when compared with vehicle-treated control SHRsp (Fig. 1). Under the latter conditions, none of the SHRsp died from stroke before the termination point of the experiment (24 weeks). These results suggest that the ability of captopril to retard the onset of stroke development could be through a suppression in plasma aldosterone, and that the protective effects of captopril treatment against mortality associated with stroke occur via a mechanism independent of the small suppression in blood pressure produced by the treatment in these experiments.

To further test the mechanisms by which a suppression of aldosterone might retard the onset of stroke development, we studied another group of SHRsp that were treated with dexamethasone (0.1 mg/kg/day s.c.) + captopril from 6 weeks of age. Dexamethasone is a glucocorticoid that is capable of producing hypertension. Captopril-treated SHRsp supplemented with dexamethasone had blood pressures equal to those observed in captopril plus aldosterone-supplemented SHRsp and higher than those of SHRsp treated with captopril alone (Fig. 1). Under the latter conditions, none of the SHRsp died from stroke before the termination point of the experiment (24 weeks). These results suggest that the ability of captopril to retard the onset of stroke development could be through a suppression in plasma aldosterone, and that the protective effects of captopril treatment against mortality associated with stroke occur via a mechanism independent of the small suppression in blood pressure produced by the treatment in these experiments.

To assure ourselves that the small elevations in blood pressure observed under conditions where aldosterone was infused into captopril-treated SHRsp did not potentiate stroke development, we studied another group of SHRsp that were treated with dexamethasone (0.1 mg/kg/day s.c.) + captopril from 6 weeks of age. Dexamethasone is a glucocorticoid that is capable of producing hypertension. Captopril-treated SHRsp supplemented with dexamethasone had blood pressures equal to those observed in captopril plus aldosterone-supplemented SHRsp and higher than those of SHRsp treated with captopril alone (Fig. 1). Under the latter conditions, none of the SHRsp died from stroke before the termination point of the experiment (24 weeks). These results suggest that the ability of captopril to retard the onset of stroke development could be through a suppression in plasma aldosterone, and that the protective effects of captopril treatment against mortality associated with stroke occur via a mechanism independent of the small suppression in blood pressure produced by the treatment in these experiments.

To further test the mechanisms by which a suppression of aldosterone might retard the onset of stroke development, we tested the ability of spironolactone to duplicate the effects of captopril. These results are shown in Fig. 3. Spironolactone and its metabolic by-product canrenone are potent type 1 mineralocorticoid aldosterone receptor antagonists. High doses of spironolactone treatment (150 mg/kg/day s.c.) initiated at 10 weeks of age did not alter the blood pressure of the animals when compared with vehicle-treated control SHRsp and retarded 50% mortality associated with stroke by about 2 weeks. One rat did respond to treatment favorably and lived to near 24 weeks of age, nearly twice the lifespan of the longest living vehicle control rat. However, it was clear that spironolactone treatment was less effective than captopril...
Captopril treatment started before stroke and aldosterone (0.66 mg/kg/day p.o.) treatment. Statistical analysis: Top, MANOVA: A versus B, p < .05; A versus C, p < .01; B versus C, NS. Bottom, Mann-Whitney: A versus B and A versus C, NS; B versus C, p < .01. (n values: A = 8, B = 5, C = 6, SHRsp).

In other studies, SHRsp were treated 5 days after seizure (13.7 ± 0.4 weeks of age). The results of this experiment are shown in Fig. 5. When treatment was started 5 days after the onset of seizure, the SHRsp survived on average about 9.6 weeks longer than untreated SHRsp. No significant differences in blood pressure were observed when compared with untreated SHRsp. To assess if poststroke captopril treatment was unique in prolonging life after stroke in SHRsp, we tested the effects of hydralazine treatment (80 mg/l, within the drinking water). When hydralazine treatment was initiated 5 days after the onset of seizure in SHRsp (13.6 ± 0.2 weeks of age), it produced a significant depression in blood pressure when compared with control and captopril-treated SHRsp (Fig. 5). However, despite being a more effective antihypertensive agent, hydralazine treatment only extended the lifespan of SHRsp on average by about 2.5 weeks (Fig. 5).

**Discussion**

Captopril treatment started at the first signs of stroke more than doubled the average lifespan of SHRsp and pro-

**TABLE 1**

The effect of aldosterone infusion on mortality in SHRsp receiving long-term captopril treatment prior to or at the first signs of stroke

Captopril treatment (50 mg/kg/day p.o.) was started prior to stroke (10 weeks of age) or at the first signs of seizure associated with stroke (12 to 13 weeks of age). At 22.3 weeks of age aldosterone (0.66 µg/h s.c.) or vehicle (polyethylene glycol; 2.5 µl/h) was infused into the rats by osmotic pumps (s.c.).

<table>
<thead>
<tr>
<th>SHRsp Treatment Groups</th>
<th>Mean Systolic Blood Pressure (mm Hg) from 23.3 Weeks of Age to Death</th>
<th>Mean Age (Weeks) at Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Captopril treatment started before stroke and aldosterone (n = 3)</td>
<td>273 ± 12</td>
<td>26.0 ± 1.7</td>
</tr>
<tr>
<td>Captopril treatment started before stroke + vehicle (n = 3)</td>
<td>264 ± 9</td>
<td>28.0 ± 0.8</td>
</tr>
<tr>
<td>Captopril treatment started at 1st seizure + aldosterone (n = 4)</td>
<td>267 ± 8</td>
<td>27.4 ± 0.8</td>
</tr>
<tr>
<td>Captopril treatment started at 1st seizure + vehicle (n = 3)</td>
<td>255 ± 12</td>
<td>28.4 ± 1.0</td>
</tr>
</tbody>
</table>

No significant (p > .05) difference in blood pressure or mean age at death between groups.
longed life as effectively as treatment started during established hypertension 2 weeks before stroke. Captopril treatment started 5 days after stroke expanded the lifespan of SHRsp by about 73%. Within our colony, SHRsp live, on average, for a period of 1.5 weeks after the first signs of stroke (Smeda, 1989). At 5 days after stroke, SHRsp are at a critical stage where death in the near future is imminent. This is emphasized by the fact that when the study was initiated, 3 of 16 SHRsp did not survive the 5-day interval between the onset of stroke and the initiation of captopril or hydralazine treatment. The effectiveness of captopril treatment in prolonging life at this stage after stroke was astonishing.

Plasma aldosterone levels tend to increase in our SHRsp between 9.5 to 12.5 weeks of age, before stroke (MacLeod et al., 1997). The present study shows that an acute quantitatively accurate elevation in plasma aldosterone during captopril treatment at an age (10 weeks) when plasma aldosterone levels elevate in untreated SHRsp initiates stroke in the animals. This suggests that elevations in plasma aldosterone before stroke may play an important role in initiation of stroke in SHRsp. Although the elevation of plasma aldosterone before stroke permitted stroke to develop in captopril-treated SHRsp, a similar manipulation after the long-term treatment of pre- and poststroke SHRsp (up to 22 weeks) did not shorten the lifespan of SHRsp. There may be an age-related window of opportunity during which elevations in plasma aldosterone can initiate stroke leading to death. The long-term treatment with captopril may promote structural and or functional changes in the vasculature or the physiology of the animal that subsequently makes the animals resistant to stroke during captopril treatment even if aldosterone levels are elevated to levels present in untreated SHRsp.

The mechanism(s) by which captopril treatment prolongs life within SHRsp after stroke development remains unclear.
In studies involving magnetic resonance imaging of SHRsp that had developed stroke, treatment with the ACEI imidapril prolonged the lifespan of the SHRsp and appeared to arrest edema formation (Takahashi et al., 1994). It was suggested that this beneficial effect against edema formation may have prolonged the lifespan of the animals. The mechanisms promoting this effect were not discovered. In previous studies we observed that before the onset of hemorrhagic stroke, the MCAs of SHRsp lost the ability to elicit constriction in response to pressure (Smeda, 1992). The ability of the vasculature to constrict in response to elevated pressure during hypertension could play an important role in maintaining constant cerebral blood flow, and a loss of such function could produce an overperfusion of the vasculature, endothelial shear and promote elevations in cerebrovascular pressures. These events may contribute to the onset of cerebral edema and hemorrhage. In preliminary studies of SHRsp, captopril treatment initiated before stroke development permitted the MCA to maintain an ability to constrict in response to elevated pressure (Copeman et al., 1996). More recently we have observed that captopril treatment initiated after stroke development allows the cerebrovasculature to regain its ability to constrict in response to elevated pressure (J.S. and S.R.K., unpublished results). This could decrease downstream blood pressure and favorably arrest the additional progression of edema and cerebral hemorrhage formation and thus prolong life after stroke.

In the present study we observed that high doses of spironolactone could not duplicate the effects of captopril treatment. This is consistent with previous studies in which we observed that long-term treatment (from 6 weeks of age) with a lower dose of spironolactone (20 mg/kg/day s.c.) suppressed blood pressure in SHRsp but only retarded the onset of stroke by 2 weeks (MacLeod et al., 1997). Canrenone, the metabolic by product of spironolactone, has a half-life of 10 to 35 h (Irish and Stitzel, 1986). At the high doses of spironolactone used, it is unlikely that inadequate blockade of the type 1 mineralocorticoid receptor occurred. Other studies we have performed demonstrated that treatment of SHRsp with a wide variety of potassium-sparing and nonpotassium-sparing diuretics also does not alter the onset of stroke development in SHRsp (Smeda and Trachenko, 1991). Recent studies involving SHRsp (Rocha et al., 1998) have shown that single daily injections of spironolactone at 10 mg/kg/day from 7.5 weeks of age offset the initiation of mortality in SHRsp from 13.5 to >19 weeks of age. The effect was unique in that costudies indicated that this dose of spironolactone did not induce diuresis or natriuresis and actually reduced sodium and water loss at certain ages in SHRsp. In the study, salt (1%) was included in the drinking water (as opposed to the diet in the present study) to induce stroke. These differences or perhaps a difference in strains of SHRsp may have accounted for a difference in the responsiveness of the rats to spironolactone observed in the present versus the latter study.

There are mechanisms that could promote an aldosterone-mediated effect that is insensitive to spironolactone. Cell surface aldosterone receptors may be present on vascular endothelium (Wehling et al., 1994) and smooth muscle (Wehling et al., 1994; Christ et al., 1995a,b). This receptor type has a high specificity for aldosterone (in the nM range) and can not be inhibited by canrenone (Wehling, 1993, 1995). Aldosterone activation of the receptor produces an increase in intracellular Ca$^{2+}$ (Wehling et al., 1994), a phospholipase C-mediated increase in diacylglycerol production and protein kinase C activation (Christ et al., 1995b), and an increase in Na$^+$ efflux, thought to be mediated by an enhanced Na$^+$/H$^+$ porter activity (Christ et al., 1995a). It is possible that the above actions or some other pathological action of aldosterone, mediated by this receptor, could alter animal physiology or cerebral blood flow autoregulation in a manner conducive to the initiation of hemorrhagic stroke during hypertension.

The treatment dose of captopril in our study was chosen on the basis of the therapeutic doses (30–200 mg/kg/day p.o.) commonly used to treat hypertension in Kyoto Wistar spontaneously hypertensive rats (SHR) (Antonacci et al., 1979; Giudicelli et al., 1980; Forslund et al., 1981), a breed of rats closely related to SHRsp. These are higher than the maximal doses used to treat hypertension in humans (<6.5 mg/kg/day p.o.). We have shown that even chronic treatment with captopril at 50 mg/kg/day p.o. is ineffective in lowering the blood pressure of SHRsp. Although lower doses of captopril may prove effective in preventing stroke or mortality after stroke in SHRsp, we feel that it is unreasonable to expect that the doses of captopril used to treat hypertension in humans should be equivalent to the alternative use of the drug to treat stroke in SHRsp. This is further emphasized by the fact that even when captopril is used as an antihypertensive agent, there is no dose equivalency between humans versus SHR or SHR versus our SHRsp. Our experimental results are consistent with the hypothesis that captopril is promoting the antistroke actions observed in SHRsp by the suppression of the renin-angiotensin system. Captopril treatment suppressed the elevated plasma aldosterone levels in SHRsp. Because AII is a potent stimulator of aldosterone release from the adrenal gland, it would be logical to assume that the captopril also lowered plasma AII. Any nonspecific effect of captopril would exist in the presence or absence of aldosterone reconstitution in 10-week-old SHRsp and would not explain why the manipulation in plasma aldosterone alone in the presence of captopril treatment modified stroke development in SHRsp.

Consideration was given to carrying out dose response relationships with captopril in SHRsp. However, we felt that these experiments alone would be limited in clarifying the mechanisms of captopril’s action. Low doses of ACEIs often inhibit plasma angiotensin-converting enzyme activity and produce a therapeutic decrease in blood pressure. However, this can be achieved in the absence of a significant depression in plasma AII or aldosterone (Biollaz et al., 1982; Kawamura et al., 1982; Giudicelli et al., 1980; Mento and Wilkes, 1987; Schaison et al., 1996). This suggests that pools of angiotensin-converting enzyme outside the plasma contribute to the production of AII and that ACEIs can produce an antihypertensive effect in a manner independent of plasma AII suppression. In view of this, even if we observed that captopril was effective as an antistroke agent at a low dose it would be incorrect to interpret this information alone as indicating that captopril was acting specifically to inhibit the renin angiotensin system. Measurement of the components angiotensin-aldosterone system would be required and reconstitution experiments such as those we have performed would be needed to clarify the issue. In our view, duplicating such experiments at a lower dose of
Captopril would not provide additional mechanistic information than that provided by the same experiments we have performed at a 50 mg/kg/day p.o. dose of captopril treatment.

References


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