Hemorrhagic Stroke Development in Spontaneously Hypertensive Rats Fed a North American, Japanese-Style Diet

John S. Smeda, PhD

The purpose of my study was to assess a North American, Japanese-style diet commercially available from Zeigler Brothers (Gardners, Pennsylvania) with respect to the initiation of stroke development in 34 stroke-prone spontaneously hypertensive rats (spSHR) and in 14 stroke-resistant spontaneously hypertensive rats (srSHR). Nineteen spSHR fed the diet containing 4% NaCl and 0.75% K+ (low-K+ diet) from weaning had an accelerated rate of stroke development (mean±SEM age at death 15.3±0.5 weeks). The same diet containing 2.11% K+ (high-K+ diet) increased the mean lifespan of 15 spSHR by 39% but did not prevent stroke. The locations of hemorrhagic lesions were similar in the groups of spSHR fed high- and low-K+ diets, being nearly equally divided between the territories of the anterior, posterior, and middle cerebral arteries. The 14 srSHR fed the low-K+ diet exhibited 50% mortality at 66 weeks of age. However, in the srSHR fed the low-K+ diet, death did not result from hemorrhagic stroke. The differing incidence of stroke between the spSHR fed high- and low-K+ diets and between spSHR and srSHR fed the low-K+ diet could not be explained on the basis of differing blood pressures. Compared with spSHR fed the low-K+ diet, both srSHR fed the low-K+ diet and spSHR fed the high-K+ diet exhibited higher drinking and urine excretion rates and elevated plasma K+ levels. My study indicates the availability of a commercial North American diet that produces a predictable high incidence of stroke within a compressed time period in spSHR but not in srSHR. This diet would be useful in studies attempting to determine the events preceding and leading to the development of stroke and in determining the genetic factors responsible for stroke resistance. (Stroke 1989;20:1212-1218)
There is currently no North American distributor for the Funahashi-SP diet, and importing the diet from Japan is prohibitively expensive. However, a Japanese-style diet is produced by Zeigler Brothers (Gardners, Pennsylvania). The objectives of my initial study were to determine if this Japanese-style diet containing 4% NaCl and 0.75% K+ accelerated stroke development in spSHR and to determine if srSHR fed the same diet retained their resistance to stroke. If spSHR fed the Japanese-style diet developed stroke, then the effects of 2.11% and 0.75% K+ would be studied to determine if elevated dietary K+ retarded or prevented stroke development. Various studies6,7,9 have indicated that elevated dietary K+ exerts an antihypertensive effect; therefore, particular attention was paid to the relation between blood pressure (BP) and stroke development.

Finally, I suspected that altering dietary Na+ and K+ levels also alters water uptake and urine excretion rates, and perhaps even plasma electrolyte levels in spontaneously hypertensive rats; such alterations may in turn play an important role in stroke development. Therefore, I measured the above parameters in spSHR and srSHR fed a low-K+ diet and spSHR fed a K+-supplemented diet.

Materials and Methods

The colony of spSHR originated from breeding pairs obtained from Dr. Hiroyuki Ito, Kinki University, Osaka, Japan; srSHR were obtained from a long-standing colony maintained at McMaster University for the past 10 years. I used 34 male spSHR (seven litters from seven breeding pairs) and 14 male srSHR (three litters from three breeding pairs). At weaning (5–6 weeks of age), the rats were placed on a Japanese-style stroke-inducing diet produced by Zeigler Brothers containing 4% NaCl. All 14 srSHR and approximately one half of each litter of spSHR (19 rats) were fed the Japanese-style diet containing 0.75% K+ derived from potassium citrate (low-K+ diet); the other spSHR littersmates (15 rats) were fed the same diet with 2.11% K+ (high-K+ diet). Twenty-one other male spSHR (19 rats) were fed the Japanese-style diet (low-K+ diet); the other spSHR littermates (15 rats) were fed a K+-supplemented diet. Seventeen srSHR (13 rats) were fed the same diet as spSHR (low-K+ diet) and seven age-matched srSHR fed the high-K+ diet (see Table 5 for n values). The samples were centrifuged at 5,000g and the plasma was then removed. Na+ and K+ levels in the plasma were subsequently determined using atomic absorption spectroscopy.

All data were analyzed using one-way analysis of variance combined with Student’s two-tailed unpaired t test; p < 0.05 was considered significant. The data are presented as mean±SEM.

Results

The first behavioral signs of stroke appeared just prior to 12 weeks of age in spSHR fed the low-K+ diet. In many rats, these signs consisted of mild lethargy. The affected rat would often be poorly groomed and would sit quietly in a corner of the cage. The rat’s immobility was often evident in that the bedding litter immediately surrounding it was saturated with urine. Touching the rat would cause it to move only a few steps, after which it would resume immobility. Within a few days, this often developed into more severe lethargy; if the rat were placed on its side, it required 5–8 seconds to right itself. At this time, the affected rat often sat in a peculiar posture, huddled, with its rear legs hyper-extended at the knee and maintained underneath the body in such a manner that its toes often extended past its head. Eventually, this led to a total inability of the rat to right itself if placed on its side.

A common sign was convulsive rhythmic movement of one fore limb. Often the rat’s right or left front foot and shoulder would move rhythmically every few seconds dorsally or anteroposteriorly. In some affected rats such movements were quite violent, resulting in the rat’s being knocked over on its side. A few rats exhibited repetitive head bobbing. In virtually all instances, the repetitive movements disappeared within 2–3 days. The affected rat then became quite immobile, exhibiting the lethargic signs described above.

The eye region was affected in many rats suffering stroke. Often one eyelid was maintained in a nearly closed position. In some instances, it appeared...
that partial facial paralysis had occurred and that the rat was incapable of opening its eyelid. This was often associated with a change in facial shape; the rat’s cheeks appeared puffy. In these instances, it was often difficult to determine whether the change in facial shape was due to paralysis or to edema. In other rats, the eyelid appeared to be voluntarily closed as a compensatory mechanism because the pupil was fully dilated. Many rats also exhibited microhemorrhages around the eyes, resulting in the eyelids being encrusted with blood. In some instances, the rat became totally blind and a cataract was visible. Most rats died ≤2.5 weeks after the first behavioral signs of stroke were observed.

As shown in Figure 2, the spSHR fed Purina 5015 exhibited 50% mortality at 29 weeks of age; when the experiment was terminated, five of these 21 spSHR aged >60 weeks remained alive. The commercial Japanese-style diet at either K+ level was effective in increasing mortality in spSHR. The high-K+ diet decreased the age at which half the spSHR had died compared with the low-K+ diet, by approximately 3.4 weeks. Although the low-K+ diet induced mortality in 100% of the spSHR by 20 weeks of age, the diet did not increase mortality in srSHR; when the experiment was terminated (after 72 weeks), only nine of 14 srSHR fed the Japanese-style diet had died (Figure 2), and the balance were alive and well. Postmortem examination of the brains of the srSHR that died failed to indicate any evidence of hemorrhagic stroke (Table 1). In view of this, the srSHR may have died of causes other than stroke.

Postmortem examination of the brains of spSHR indicated cerebral hemorrhage in all 19 fed the low-K+ diet and in 13 of 15 fed the high-K+ diet. The mean age at death was significantly less in the former, but the time between observation of the first behavioral signs of stroke and death did not differ significantly between K+ levels (Table 1), indicating that supplemental dietary K+ retards stroke development as opposed to affecting the ability of stroke to cause death. Hemorrhage occurred only in the cerebrum (never in the cerebellum or the brainstem) of spSHR fed the Japanese-style diet and was more prevalent in the left than the right cerebral hemisphere. Hemorrhagic areas in either hemisphere were nearly equally distributed between the territories of the anterior, middle, and posterior cerebral arteries. The incidences of hemorrhage in each territory were comparable between K+ levels. As shown in Table 1, neither the mean
Effect of Diet on Stroke in SHR

Figure 2. Mortality (% of total population) observed at various ages in stroke-prone (spSHR, ▲; n=19) and stroke resistant (srSHR, ◦; n=14) spontaneously hypertensive rats fed Zeigler Brothers Japanese-style stroke-inducing diet containing 4% NaCl and 0.75% K⁺, in spSHR fed the same diet containing 4% NaCl and 2.11% K⁺ (●, n=15), and in spSHR fed Purina 5015 diet (○, n=21).

Table 1. Mean Age at Death, Mean Time Between Appearance of Signs of Stroke and Death, and Severity of Cerebral Hemorrhage in Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>% K</th>
<th>n</th>
<th>Age at death (wk)</th>
<th>Time between stroke and death (wk)</th>
<th>% of rats</th>
<th>Lesions per brain</th>
<th>Severity grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>spSHR</td>
<td>0.75</td>
<td>19</td>
<td>15.3±0.5</td>
<td>1.7±0.2</td>
<td>100</td>
<td>2.8±0.5</td>
<td>2.4±0.3</td>
</tr>
<tr>
<td>spSHR</td>
<td>2.11</td>
<td>15</td>
<td>21.2±1.7*</td>
<td>1.6±1.0</td>
<td>87</td>
<td>2.5±0.4</td>
<td>2.3±0.4</td>
</tr>
<tr>
<td>srSHR</td>
<td>0.75</td>
<td>14</td>
<td>65.0†</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

spSHR, stroke-prone spontaneously hypertensive rats; srSHR, stroke-resistant spontaneously hypertensive rats. Data are mean±SEM.

*p<0.003 different from low-K⁺ spSHR by Student's two-tailed unpaired t test.
†Age at 50% mortality; nine rats died.
latter, thus raising the possibility that the depression of systolic BP in the high-K+ group may in fact be the mechanism whereby stroke development is retarded in these rats. In view of this, the data were further analyzed; mortality rates of spSHR fed the high- and low-K+ diets were compared in rats exhibiting similar BPs between 10 and 13 weeks of age (Table 3). Mean age at death still was significantly lower in spSHR fed the low-K+ diet than in spSHR fed the high-K+ diet, despite the fact that BPs of the two groups were comparable.

srSHR and spSHR fed the low-K+ stroke-inducing diet also exhibited comparable BPs during the phase of established hypertension (i.e., >13 weeks of age; Table 2). However, systolic BP increased more slowly in srSHR than in spSHR when both were fed the low-K+ diet, being approximately 37 mm Hg lower in the former than in the latter at 11 weeks of age (Table 2). Because none of the srSHR studied died of stroke, despite the fact their mean systolic BPs were >225 mm Hg for a good part of their lives, it would appear that factors other than a longer developing hypertensive phase play a role in stroke resistance in srSHR.

Table 4 summarizes the average daily water intake and urine excretion in spSHR and srSHR between 7 and 9 weeks of age. spSHR fed the high-K+ diet and srSHR fed the low-K+ diet exhibited greater daily water intake and urine excretion than spSHR fed the low-K+ diet. Urine excretion is considerably lower than water intake, more so than can be accounted for by perspiration. Urine is collected in a metabolic cage by funneling that excreted into a container below the cage. During 24 hours, significant evaporation takes place; hence the volume of urine excreted is underestimated. However, since the three groups of rats were studied simultaneously, comparisons of daily water intake and urine excretion indicate that spSHR fed the high-K+ diet and srSHR fed the low-K+ diet exhibited greater diuresis than spSHR fed the low-K+ diet.

Table 5 summarizes the plasma Na+ and K+ levels of 14-week-old spSHR and srSHR. There were no differences in the plasma Na+ levels. Plasma K+ levels were elevated in both spSHR fed the high-K+ diet and in srSHR fed the low-K+ diet compared with spSHR fed the low-K+ stroke-inducing diet.

Discussion

Feeding spSHR a Japanese-style stroke-inducing diet containing 4% NaCl and 0.75% K+ produced a very rapid and well-defined period of stroke onset. spSHR fed the diet started dying at 12 weeks of age, and 100% had died by approximately 20 weeks. The diet produced a greater incidence of stroke at a much younger age in spSHR than the Funahashi-SP diet (88% developed cerebrovascular lesions [cerebral hemorrhage/infarct] at 36 weeks of age3). The higher incidence of cerebrovascular lesions produced in spSHR by the Japanese-style stroke-inducing diet compared with the Funahashi-SP diet likely can be attributed to the elevated Na+ level in former diet, a modification that has been shown to accelerate stroke development in spSHR.4,10

srSHR fed the 4% NaCl and 0.75% K+ diet did not develop hemorrhagic stroke, despite the fact both spSHR and srSHR fed the diet had comparable systolic BPs during the phase of established hypertension. This suggests that some other, as yet unknown, mechanism or offsetting feature in srSHR protects these rats from stroke.

Elevating dietary K+ from 0.75% to 2.11% significantly retarded stroke development in spSHR. These findings confirm the observations of Tobian and colleagues.6,7 However, the effects appear to be more dramatic in that study (i.e., 83% mortality at 23 weeks of age in spSHR fed the low-K+ diet vs.

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**Table 2.** Systolic Blood Pressure Age Profiles in Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>% K</th>
<th>n</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11*</th>
<th>12</th>
<th>13</th>
<th>15</th>
<th>16</th>
<th>20</th>
<th>25</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>spSHR</td>
<td>0.75</td>
<td>19</td>
<td>141±4</td>
<td>146±7</td>
<td>164±3*</td>
<td>160±6</td>
<td>206±12</td>
<td>230±5</td>
<td>228±14*</td>
<td>223±9</td>
<td>—</td>
<td>233±3</td>
<td>220±11</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>spSHR</td>
<td>2.11</td>
<td>15</td>
<td>135±5</td>
<td>136±3</td>
<td>158±3</td>
<td>166±6</td>
<td>193±10</td>
<td>211±4</td>
<td>222±9</td>
<td>210±4</td>
<td>235±11</td>
<td>237±10</td>
<td>—</td>
<td>243±2*</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>srSHR</td>
<td>0.75</td>
<td>14</td>
<td>—</td>
<td>—</td>
<td>153±3</td>
<td>172±11</td>
<td>185±6</td>
<td>—</td>
<td>191±3</td>
<td>—</td>
<td>236±9</td>
<td>246±13</td>
<td>—</td>
<td>223±6</td>
<td>260±5</td>
<td>230±9</td>
</tr>
</tbody>
</table>

spSHR, stroke-prone spontaneously hypertensive rats; srSHR, stroke-resistant spontaneously hypertensive rats. Data are mean±SEM mm Hg.

*p<0.05 significant group effect by one-way analysis of variance.

tp<0.05 different from srSHR by Student's two-tailed unpaired t test.

‡p<0.05 different from low-K+ spSHR by Student's two-tailed unpaired t test.

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**Table 3.** Relation Between Mortality and Systolic Blood Pressure in Stroke-Prone Spontaneously Hypertensive Rats Fed High- and Low-K+ Diets

<table>
<thead>
<tr>
<th>Age at death (wk)</th>
<th>Blood pressure between 10 and 13 weeks of age (mean±SEM mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% K</td>
<td>n</td>
</tr>
<tr>
<td>0.75</td>
<td>8</td>
</tr>
<tr>
<td>2.11</td>
<td>6</td>
</tr>
</tbody>
</table>

*p<0.05 different from low-K+ by Student's two-tailed unpaired t test.
The mechanisms by which elevated dietary K⁺ protects the cerebral vasculature from stroke are presently unknown. Tobian et al.6 hypothesized that elevated dietary K⁺ may help to retard stroke development by protecting the endothelial layer of the cerebral arteries from damage associated with hypertension. In a recent study, Sugimoto et al.11 observed that the aorta of spSHR fed a diet containing 6% NaCl and 0.75% K⁺ exhibited an impaired endothelium-dependent ability to relax in response to acetylcholine compared with Wistar-Kyoto normotensive control rats fed the same diet or with spSHR fed the same diet containing a high (2.1%) K⁺ level. Compared with the latter two groups, spSHR fed the former diet also exhibited an increased incidence of intimal lesions in the aorta and superior mesenteric arteries. If the above types of morphologic changes in the intima were to occur in the cerebrovasculature of spSHR, they could lead to increased endothelial permeability and perhaps to a higher incidence of hemorrhage in spSHR fed a low-K⁺ diet compared with spSHR fed a high-K⁺ diet.

In other studies involving Dahl salt-sensitive and salt-resistant hypertensive rats, Tobian7 demonstrated that hypertension in the former fed a 4% NaCl, low-K⁺ diet was associated with progressive destruction of nephrons and reduction of plasma flow to the renal papilla. Elevating K⁺ in the diet protected the kidneys from nephron loss and promoted high plasma flows to the renal papilla. Such treatment also reduced stroke development without altering BP. Stroke development in spSHR could also be associated with renal failure. Nagaoka et al.12 demonstrated a direct relation between the time of stroke onset and proteinuria in spSHR. Proteinuria was shown to be produced by necrotic changes in the renal arterioles and cortex of the kidneys. In another study,13 renal necrotic changes that predisposed the kidneys to ischemia preceded the onset of cerebrovascular lesions in spSHR. In view of this, Nagaoka et al.13 hypothesized that, in spSHR, arteriolar changes in the kidney (particularly those producing renal ischemia) may be related to the pathogenesis of cerebrovascular lesions. In my study, spSHR fed the high-K⁺ diet exhibited increased diuresis compared with spSHR fed the low-K⁺ diet. Diet-induced diuresis in spSHR fed the high-K⁺ diet could help to prolong normal renal

<table>
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<th>Table 4. Daily Water Intake and Urine Collected From Rats</th>
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<tr>
<td><strong>Group</strong></td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>spSHR</td>
</tr>
<tr>
<td>spSHR</td>
</tr>
<tr>
<td>srSHR</td>
</tr>
</tbody>
</table>

spSHR, stroke-prone spontaneously hypertensive rats; srSHR, stroke-resistant spontaneously hypertensive rats. Data are mean±SEM. *, p<0.05 different from low-K⁺ spSHR by Student’s two-tailed unpaired t test. **p<0.05 significant group effect by one-way analysis of variance.

2% mortality in spSHR fed the high-K⁺ diet). Elevating dietary K⁺ did not prevent stroke in spSHR but retarded the mean age at death from stroke by approximately 6 weeks. The differences between the two studies can be resolved by examining the data. The studies of Tobian et al.6,7 were concluded when the rats were 23 weeks of age; if my study was stopped at approximately 15 weeks of age, the mortality of spSHR fed the low-K⁺ diet would be approximately 55% compared with 0% in spSHR fed the high-K⁺ diet. Unlike in my study, Tobian et al.6,7 demonstrated a much more significant difference in BP between spSHR fed low- and high-K⁺ diets (i.e., 233 vs. 187 mm Hg) during the phase of established hypertension. When BP was normalized between the two groups (212 mm Hg), mortality was still higher in spSHR fed a low-K⁺ diet than in those fed a high-K⁺ diet (64% vs. 9%); however, such normalization decreased the mortality in spSHR fed a low-K⁺ diet and slightly increased that in spSHR fed a high-K⁺ diet. Finally, stroke onset occurred at a much younger age in the spSHR I used (100% mortality by 20 weeks of age) than in the rats used by Tobian et al.6,7 (83% mortality at 23 weeks of age). Therefore, it might be expected that the differences in the mortality I observed between the high- and low-K⁺ diet groups of spSHR might be more compressed than those observed by Tobian et al.6,7. The latter differences between my study and those of Tobian et al.6,7 could be due to differences in the strains of spSHR used. In absolute terms, a 6-week increase in the mean age at death in spSHR fed a high-K⁺ diet compared with that in spSHR fed a low-K⁺ diet may not appear to be long; however, this represents a 39% increase in the average lifespan of the spSHR I used.

<table>
<thead>
<tr>
<th>Table 5. Plasma Na⁺ and K⁺ Levels in Rats</th>
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<tbody>
<tr>
<td><strong>Group</strong></td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>spSHR</td>
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<tr>
<td>spSHR</td>
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<tr>
<td>srSHR</td>
</tr>
</tbody>
</table>

spSHR, stroke-prone spontaneously hypertensive rats; srSHR, stroke-resistant spontaneously hypertensive rats. Data are mean±SEM mM.

†Numbers in parentheses, number of rats sampled.

††p<0.05 different from low-K⁺ spSHR by Student's two-tailed unpaired t test.
function, which in turn might retard stroke development in these rats.

srSHR fed the low-K⁺ diet exhibited intermediate diuresis, which was higher than that in spSHR fed the same diet but lower than that in spSHR fed the high-K⁺ diet. The resistance to stroke exhibited by srSHR cannot be explained completely on the basis of the moderately increased diuresis. It is worth noting, however, that renal function in srSHR and spSHR differs. Both srSHR and spSHR have comparable glomerular filtration rates (GFRs) under normal conditions; however, srSHR are capable of maintaining a constant (normal) GFR when renal perfusion pressures are decreased to 99 mm Hg via aortic constriction, whereas such pressure decreases produce a marked drop in GFR in spSHR. It would appear that, compared with spSHR, the kidneys of srSHR have an increased capacity to autoregulate. If the pathogenesis of cerebrovascular lesions in SHR is related to renal function, as suggested by Nagaoka et al., the increased autoregulatory capacity exhibited by the renovasculature of srSHR might contribute to their ability to resist stroke.

Hemorrhage was never noticed in the cerebellum or midbrain of spSHR, yet cerebral lesions do occur in this area in humans. This difference could be due to differences in vascular architecture between human and rat brains. In the human brain, vascular connections between the vertebrobasilar arterial system and the circle of Willis are much better established, the basilar artery dividing first into the superior cerebellar arteries, then terminating in the posterior cerebral arteries. In rats, the vertebrobasilar arterial system is separated to a greater degree from the arterial system fed by the internal carotid arteries; in rats, the basilar artery terminates in the superior cerebellar arteries and is then connected to the posterior cerebral arteries via very fine, small-diameter connections. This greater separation of the two arterial systems might make the vertebrobasilar arterial system of rats less vulnerable to stroke development.

In my study the incidence of cerebral infarction was low. Tissue softening and herniation, suggesting cerebral infarction, was observed; however, virtually all such areas were closely associated with hemorrhage (see Figure 1, Brain 3, black arrow). One possible explanation for this phenomena is that the rupture of a blood vessel as well as the vasospasm associated with the accumulation of blood could shut down blood flow to more distal regions, thus producing the appearance of an ischemic brain area.

References


Key Words • cerebral hemorrhage • diet • rats
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