Hypothesis Paper

Uric Acid, Hominoid Evolution, and the Pathogenesis of Salt-Sensitivity

Susumu Watanabe, Duk-Hee Kang, Lili Feng, Takahiko Nakagawa, John Kanellis, Hui Lan, Marilda Mazzali, Richard J. Johnson

Abstract—Humans have elevated serum uric acid as a result of a mutation in the urate oxidase (uricase) gene that occurred during the Miocene. We hypothesize that the mutation provided a survival advantage because of the ability of hyperuricemia to maintain blood pressure under low-salt dietary conditions, such as prevailed during that period. Mild hyperuricemia in rats acutely increases blood pressure by a renin-dependent mechanism that is most manifest under low-salt dietary conditions. Chronic hyperuricemia also causes salt sensitivity, in part by inducing pregglomerular vascular disease. The vascular disease is mediated in part by uric acid–induced smooth muscle cell proliferation with activation of mitogen-activated protein kinases and stimulation of cyclooxygenase-2 and platelet-derived growth factor. Although it provided a survival advantage to early hominoids, hyperuricemia may have a major role in the current cardiovascular disease epidemic. (Hypertension. 2002;40:355-360.)

Key Words: uric acid ■ hypertension, sodium-dependent ■ renal disease ■ mutation

Cardiovascular disease and hypertension are epidemic in modern society. Cardiovascular disease is the number one cause of death in the United States, claiming nearly 1 million lives yearly and accounting for 40% of all-cause mortality and more deaths than the next 7 leading causes combined.1 The most common form of cardiovascular disease is hypertension, which is present in approximately 20% of the population over the age of 60.2,3 Hypertension frequency increases dramatically with age, affecting the majority of the population (50 million people) in the United States and whose hypertension in industrialized societies is the dietary sodium factor that may account for the increased prevalence of hypertension among the nonindustrialized.8 Hypertension markedly increases the risk for myocardial infarction, stroke, congestive heart failure, peripheral vascular disease, and end-stage renal disease.4,5 Treatment of hypertension reduces show to induce salt sensitivity.

There is evidence that the increased prevalence of hypertension is a recent event in human history and that it correlates with changes in the diet with industrialization.8 Dahl9 and Tobian10 have suggested that a key nutritional factor that may account for the increased prevalence of hypertension in industrialized societies is the dietary sodium intake. Primitive societies, such as the Yanomamo, whose populations ingest a very small amount of sodium, have an absence of hypertension.11 The sodium content of early hunter-gatherers of the Paleolithic Period was also extremely low and has been estimated to be only 690 mg/d (equivalent to 30 mEq Na+ or 1.9 g NaCl).12 In contrast, the average sodium intake in the current American diet averages 4000 mg/d (170 mEq Na+ or approximately 10 g NaCl). Although individuals with normal kidneys might be able to excrete the increased sodium content without altering systemic blood pressure, there is evidence that individuals who develop essential hypertension have a relative defect in their ability to excrete sodium.13 It has thus been speculated that the sudden increase in sodium content in the diet of industrialized nations will “unmask” those individuals with this physiological renal defect and thereby precipitate the development of hypertension.10 We now present a hypothesis that the development of salt sensitivity in humans may be related to environmentally driven mutations of the urate oxidase (uricase) gene, which occurred during the Miocene. The mechanism relates to an increase in serum uric acid (urate), which we have previously shown to regulate blood pressure14,15 and, in this paper, will show to induce salt sensitivity.

Parallel Mutations in the Uricase Gene in Early Hominoids: Evolutionary Implications

Uric acid is generated during the metabolism of purines and in most mammals is further degraded to allantoin by the hepatic enzyme, uricase (urate oxidase), resulting in serum uric acid levels in the range of 0.5 to 1.5 mg/dL. In contrast, serum uric acid is higher in hominoids (apes and humans) as well as in certain New World monkeys. The increase in uric acid is due to distinct mutations in the uricase gene that made it nonfunctional. In humans, the chimpanzee (Pan), and the gorilla (Gorilla), 3 mutations have been identified, including a nonsense mutation of codon 33, a nonsense mutation of codon 187, and a splice mutation in exon 3.16 The mutation at

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The levels of uric acid correlated with changes in hepatic urate oxidase activity in these species. Although studies determining the site of the mutations responsible for the loss of uricase in the New World monkeys have not been performed, the mutations were likely independent from those observed during hominoid evolution, giving evidence that the divergence of the Platyrhini infraorder (the New World monkeys) from the Catarrhini (Old World monkeys and hominoids) occurred approximately 40 million years ago. Interestingly, although urate oxidase was retained in the Old World monkeys (Cercopithecoidae), the enzyme activity is relatively unstable compared with other mammalian (nonprimate) species, suggesting that evolutionary mechanisms that impair urate oxidase activity may also have been operative in this superfamily.

The observation that several independent mutations involving uricase occurred during hominoid evolution and in parallel during the evolution of the Old World and New World monkeys has been interpreted as evidence that there must have been an evolutionary advantage for early primates in having an elevated uric acid level. The time frame of the mutations for the uricase gene indicates that they occurred during the Miocene (24 to 8 million years ago). It is thus important to understand the environmental pressures that were occurring during this period of hominoid history.

Fossil evidence suggests that the earliest hominoids, such as Proconsul, originated in East Africa approximately 22 to 17 million years ago, where they lived in lush tropical forests and wetlands. These early hominoids were arboreal quadrupeds with a diet that was frugivorous (ingesting primarily soft fruits). By the early Miocene, there were numerous hominoid species, particularly in Eurasia. However, by the middle to late Miocene (14 to 8 million years ago), there was an extinction of many Miocene apes, especially in Europe. This appears to be associated with an environmental change to a drier and more seasonal climate and with a change in the habitat to more open areas of savannas interspersed with tropical and subtropical forests.

In certain areas of East Africa, wet and wooded habitats may have been maintained. Molecular DNA studies suggest that there was a rapid period of positive selection for various genes during this period, particularly for a gene family (morphus) in the short arm of chromosome 16, and there were significant changes in dentition and axial skeleton that may have facilitated adaptation to a more arid environment.

As discussed above, the sodium intake of the early hunter-gatherers in the middle to late Pleistocene was in the range of 690 mg (1.9 g NaCl) per day. The sodium intake of early hominoids during the Miocene epoch (from 24 to 5 million years ago) was likely even lower, because the diets consisted primarily of fruits (frugivorous) or leaves (folivorous). Eaton and Konner estimated that the sodium content of a strictly vegetarian Paleolithic diet may have amounted to only 225 mg sodium (10 mEq Na+ or 0.6 g NaCl). Thus, the climatic shift to more arid conditions in the middle to late Miocene may have placed selection pressure on the early primates toward a genotype that would maximally conserve sodium with the maintenance of blood pressure.

Uric Acid Maintains Blood Pressure Under Low-Sodium Conditions

Most authorities have proposed that the mutations in the uricase gene provided an evolutionary advantage because uric acid may function as an antioxidant. Although an antioxidant role for uric acid may be one mechanism to explain the beneficial effects of the uricase mutation, we examined the hypothesis that uric acid might regulate blood pressure. This hypothesis was based on previous studies demonstrating that serum uric acid correlates with blood pressure and predicts the development of hypertension in population-based studies.

To test this hypothesis, mild hyperuricemia was induced in rats by administering the uricase inhibitor, oxonic acid, and then, to recreate the prehistoric conditions, we placed the rats on a low-salt (0.125% NaCl) diet. Normal rats on a low-salt diet have either no change or a gradual fall in blood pressure; in contrast, hyperuricemic rats increase their blood pressure (Figure 2). The increase in blood pressure corre-
Angiotensin system. This hormonal system has a key role in tubular injury. These renal lesions, as well as the renin activation, could be prevented by lowering the uric acid levels with allopurinol (a xanthine oxidase inhibitor) or with benzbiodarone (a uricosuric agent). The increase in blood pressure in hyperuricemic rats was shown to be mediated in part by stimulation of the renin angiotensin system. This hormonal system has a key role in sodium balance under low-salt dietary conditions. We also found that hyperuricemic rats develop vascular disease, particularly of the afferent arteriole of the renal microvasculature, and tubular injury. These renal lesions, as well as the renin activation, could be prevented by lowering the uric acid with either allopurinol or benzbiodarone. The arteriolar lesion resembled the arteriolosclerosis observed in patients with essential hypertension, but the lesion occurred independently of blood pressure as a consequence of a direct stimulation of the vascular smooth muscle cells by uric acid. Goldblatt originally postulated that primary renal microvascular disease might be the major pathogenic mechanism for essential hypertension. Recent studies support this hypothesis. The induction of preglomerular arteriolar disease in experimental models leads to tubular ischemia, the interstitial infiltration of lymphocytes and macrophages, local oxidant generation, and alterations in the expression of vasoconstrictors and vasodilators that favor local vasoconstriction. These changes result in both a decrease in sodium filtration (caused by a decrease in the ultrafiltration coefficient, $K_f$) and increased sodium reabsorption (via direct tubular effects) and result in an enhanced blood pressure response to sodium (salt sensitivity).

### Hyperuricemia Induces Salt Sensitivity in Rats

Given that hyperuricemia induces primary renal arteriolar lesions, we hypothesized that chronic hyperuricemia might also induce salt sensitivity. The uricase inhibitor, oxonic acid (2%), was administered with a low-salt diet to rats for 7 weeks. The diet resulted in mild hyperuricemia (uric acid level at 1 week of 2.4±0.7 mg/dL versus 1.2±0.1 mg/dL in low-salt diet controls, $P<0.005$, 6 rats tested per group). The oxonic acid diet was then stopped and rats continued on a low-salt diet for 2 weeks, allowing the serum uric acid levels to decrease to levels no different from those observed in low-salt diet controls. Renal tissue obtained from a random subset of rats sacrificed at 9 weeks showed that the previously hyperuricemic rats had a persistent afferent arteriolopathy, with decreased arteriolar lumen diameter and an increased media:lumen ratio compared with low-salt diet controls (Table 1). The rats also showed mild interstitial inflammation and low-grade interstitial collagen deposition (Table 1). Rats were then randomized to a low- or high-salt (2% NaCl) diet. An increase in blood pressure from the high-salt diet (ie, salt sensitivity) was observed only in the rats that had been previously hyperuricemic (Figure 3). This experiment therefore demonstrates that hyperuricemia in the rat can induce salt sensitivity and is consistent with other models in which salt sensitivity can be induced as a consequence of the development of preglomerular arteriolar disease.

### Hyperuricemia Induces Preglomerular Microvascular Disease and Interstitial Inflammation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Previously Hyperuricemic</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afferent arterioles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial wall area, $\mu$m²</td>
<td>171.0±14.5</td>
<td>150.2±22.7</td>
</tr>
<tr>
<td>Media:lumen ratio</td>
<td>2.7±0.1*</td>
<td>2.4±0.3</td>
</tr>
<tr>
<td>Lumen area, $\mu$m²</td>
<td>96.1±9.8*</td>
<td>116.0±15.3</td>
</tr>
<tr>
<td>Intersitium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrophages, ED-1+ Cells/mm²</td>
<td>222.4±30.4*</td>
<td>163.7±18.5</td>
</tr>
<tr>
<td>Collagen III, % area</td>
<td>5.5±0.7*</td>
<td>4.3±1.2</td>
</tr>
</tbody>
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Analysis was performed at 9 weeks (2 weeks after stopping the oxonic acid diet) using perfusion-fixed tissue. Measurements were blinded and were performed by computer image analysis as described elsewhere. $P<0.05; n=6$ per group.

Uric Acid Stimulates Smooth Muscle Cell Proliferation

We further explored the mechanism by which uric acid causes vascular disease. Soluble, endotoxin-free uric acid (3 to 5 mg/dL) stimulates vascular smooth muscle cell proliferation with activation of the mitogen-activated protein (MAP) kinase extracellular signal–regulated kinase (Erk1/2) and stimulation of platelet-derived growth factor (PDGF) and its receptor (Figure 4). Rao et al have also showed that uric acid induces PDGFA-chain expression and that the smooth muscle cell proliferation can be prevented with neutralizing antibodies to PDGF. We also found that the smooth muscle cell proliferation is mediated by uric acid–induced cyclooxygenase-2 (COX-2) expression with the generation of thromboxane (Duk-Hee Kang, Takahiko Nakagawa, Lili Feng, Marilda Mazzali, Susumu Watanabe, Lin Han, Luan Truong, Raymond Harris, Richard J. Johnson. Unpublished manuscript, 2002), and partially by activation of the renin angiotensin system.
The increase in uric acid that occurred with the mutation of uricase during the Miocene may have therefore provided a survival advantage (Figure 5). The stimulation of the renin-angiotensin system would be expected to acutely increase blood pressure and sodium reabsorption, whereas the preglomerular vascular disease induced by activation of MAP kinase, PDGF, and COX-2 systems would lead to chronic salt sensitivity. The net effect would be to maintain blood pressure and sodium balance.

Uric Acid: Potential Role in Hypertension and Cardiovascular Disease in Industrialized Societies

Whereas an elevated serum uric acid might have been advantageous for maintaining blood pressure under low-salt dietary conditions, the induction of chronic salt sensitivity would be expected to result in hypertension in modern society with its high-salt diet. It is of interest that hyperuricemia predicts the development of hypertension and is strongly linked to cardiovascular disease. Serum uric acid is also higher in almost all high-risk groups, including males and postmenopausal women (because estrogen is uricosuric), in the obesity insulin-resistance syndrome (because insulin may stimulate uric acid reabsorption), in blacks, and in patients with renal disease (secondary to decreased excretion). One might speculate that the higher uric acid levels in blacks may reflect a longer period of environmentally induced selection pressure after the deletion of the uricase gene.

Controversy has existed over the role of uric acid in cardiovascular disease for several reasons. First, some authorities have viewed hyperuricemia as a “marker” for patients at increased cardiovascular risk, as opposed to being truly pathogenic. This conclusion is based on several epidemiological studies that could not show uric acid to be indepen-
dent of other factors such as hypertension for predicting cardiovascular events.\textsuperscript{40} However, a factor does not need to be independent to have a causal role in a disease process. For example, if hyperuricemia is a cause of hypertension, then it would not be expected to be independent of it as a risk factor for cardiovascular events. Evidence that uric acid may have a causal role in hypertension is suggested by the experimental studies of mild hyperuricemia in rats.\textsuperscript{14,15} Furthermore, although hyperuricemia is not always an independent risk factor for cardiovascular events, it has always been found to be an independent risk factor for the development of hypertension.\textsuperscript{30,31} This suggests that a causal relationship between uric acid and hypertension may in part explain the variance in epidemiological studies about the role of uric acid in cardiovascular disease.

Second, some authorities have suggested that hyperuricemia may be a secondary response to the reduced renal blood flow that is a characteristic hemodynamic finding in hypertension.\textsuperscript{41} There is strong evidence that renal vasoconstriction results in increased proximal urate reabsorption and an increase in serum uric acid.\textsuperscript{42} Hyperuricemia may also occur in patients with congestive heart failure or peripheral vascular disease because of tissue ischemia that increases uric acid generation (from ATP breakdown) and reduces urate excretion (caused by the effects of lactate on the organic anion exchanger). Although there is no doubt that these conditions do result in an increase in serum uric acid levels, this should not necessarily imply that the increase in serum uric acid is without biological effect. Indeed, our studies would suggest that the increase in uric acid in these conditions may well represent a feedback mechanism to augment the renin angiotensin system to maximally stimulate sodium reabsorption and maintain blood pressure, because a reduced renal blood flow and/or tissue ischemia may be signaling the organism that its overall blood volume is low.

Finally, if uric acid truly causes hypertension, one might expect evidence showing that allopurinol treatment can lower blood pressure. We have not been able to document any controlled studies that have examined this possibility. The studies to examine whether allopurinol can lower blood pressure will need to be performed carefully. Thus, as we recently reported,\textsuperscript{44} once animals have significant preglomerular disease, such as in newly transplanted patients placed on cyclosporine or patients with preeclampsia. Second, it may be possible to show a hypertensive effect with allopurinol in patients with established hypertension who are first sodium depleted, because this will remove the salt-sensitivity mechanism mediated by the vascular disease. The sodium restriction will be necessary to remove the role of the preglomerular vascular disease in mediating the blood pressure response.

**Perspectives**

We present the hypothesis that the uricase mutation that occurred during early hominoid evolution was originally advantageous because it helped to maintain blood pressure both acutely (via stimulation of the renin angiotensin system) and chronically (by inducing salt-sensitivity via the development of renal microvascular and interstitial disease). In modern society the switch to a high salt diet, coupled with this mutation, may have an important role in the current epidemic of hypertension and cardiovascular disease. Other dietary factors (such as dietary changes in potassium and magnesium) and the development of obesity are also likely to be contributory factors for the development of hypertension. Although one must be cautious in the interpretation of experimental models, we suggest that studies be performed to determine the effect of lowering uric acid in man on the development of hypertension, particularly before the development of significant microvascular and renal injury.

**Acknowledgment**

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**References**


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