Amiloride, a Specific Drug for Hypertension in Black People With T594M Variant?


Abstract—The T594M polymorphism of the epithelial sodium channel is found in ≈5% of people of African origin and is significantly associated with high blood pressure. Although the T594M polymorphism could increase renal sodium absorption through affected channels, it is not known whether this polymorphism causes hypertension. Amiloride specifically inhibits overactive sodium channels and effectively controls blood pressure in Liddle’s syndrome, in which hypertension is caused by separate epithelial sodium channel mutations. The aim of this study was to determine whether amiloride was effective in lowering blood pressure in individuals with the T594M polymorphism. In an open, controlled study, 14 black hypertensive individuals with the T594M polymorphism were withdrawn from their usual medication and treated with amiloride. On entry to the study, individuals taking a mean of 2 drugs had blood pressure of 142/89±3/3 mm Hg. Amiloride alone (10 mg BID) controlled blood pressure effectively to the same level (140/91±4/2 mm Hg). When amiloride was withdrawn for 2 weeks, there was a large increase in blood pressure of 17/8±4/2 mm Hg (systolic, P<0.05; diastolic, P<0.01). On restarting amiloride, blood pressure was again controlled to 140/88±6/2 mm Hg. These results demonstrate that 10 mg BID amiloride is effective in controlling blood pressure in hypertensive individuals of African origin who have the T594M polymorphism. Our study supports the concept that the T594M polymorphism contributes to the elevation of blood pressure and suggests that consideration should be given to the use of amiloride in affected individuals. (Hypertension. 2002;40:13-17.)

Key Words: hypertension, essential ■ blacks ■ sodium channel ■ polymorphism ■ epithelium

In London, nearly half of middle-aged adults of African origin have high blood pressure requiring treatment.1 In those in whom high blood pressure is found, adequate blood pressure control is only achieved in a minority.1 This poor blood pressure control is likely to account for the high incidence of hypertensive complications seen in people of African origin, particularly at a younger age, such as stroke and end-stage kidney failure.2 Many of the drugs most commonly prescribed in the United Kingdom for the treatment of hypertension are less effective in black people than white people. For instance, ACE inhibitors and β-blockers, which work by inhibiting the renin-angiotensin system, are unlikely to control blood pressure when used as single agents in black individuals who have lower levels of renin and angiotensin II.3,4 Improved understanding of the mechanisms causing hypertension in people of African origin is important to ensure that drugs that control blood pressure adequately can more rationally be prescribed.

Abnormal activity of the epithelial sodium channel may be an important cause of high blood pressure in black people. The epithelial sodium channel is present in the apical membrane of cells in distal renal tubule and collecting duct. It permits reabsorption of sodium from distal tubular fluid. Increased activity of the epithelial sodium channel caused by genetic mutations as seen in Liddle’s syndrome results in inappropriate reabsorption of sodium and the development of high blood pressure.5 Mutations of the sodium channel separate from those causing Liddle’s syndrome have also been identified in black people.6 We have previously shown that one polymorphism, the T594M polymorphism, is found in ≈5% of the black population7 and is significantly associated with high blood pressure.8 The T594M polymorphism affects the regulatory C-terminal region of the sodium channel β-subunit and alters a putative binding site for protein kinase C.9 This binding site is thought to mediate inhibition of sodium channel activity. The T594M polymorphism could therefore contribute to the development of high blood pressure by disrupting negative regulation and increasing sodium channel activity in a similar but less florid way to that seen in Liddle’s syndrome.

In Liddle’s syndrome, the high blood pressure is controlled by amiloride, which blocks the overactive epithelial sodium channels.10 The aim of our study was to determine whether amiloride would also control blood pressure in hypertensive black people with the T594M polymorphism.
Methods

Recruits
Hypertensive black people attending the Blood Pressure Unit at St George’s Hospital were screened for presence or absence of the T594M polymorphism of the epithelial sodium channel. Individuals who were heterozygous for the polymorphism were invited to take part in the study. These individuals either had blood pressure ≥140/90 mm Hg on no treatment after becoming accustomed to the measurement or were taking drug treatment for high blood pressure. Ethnicity was defined by skin color, place of birth or parents’ birth, and cultural identity.

Subjects were excluded from entry into the study if they had renal impairment, plasma creatinine >150 μmol/L, serum potassium >5.0 mmol/L, or they had evidence of target organ damage, for example, previous stroke, heart failure, myocardial infarction, heart failure, or other evidence of ischemic heart disease. Women of childbearing potential were only admitted to the study if they were taking oral contraceptives. Subjects were to be withdrawn from the study if they had creatinine >200 μmol/L, serum K+ >5.5 mmol/L, or blood pressure consistently >180/110 mm Hg.

Informed consent was obtained from all individuals before entry into the study, which was approved by the Local Research Ethics Committee of Merton, Sutton, and Wandsworth. Procedures followed were in accordance with institutional guidelines.

Study Design
This was a longitudinal, open-label study. Subjects were first observed on their usual treatment, then underwent 4 treatment phases: change from usual treatment to amiloride, treatment with amiloride alone, withdrawal of amiloride, and reinstitution of amiloride. At entry to the study, all individuals were advised, if not already doing so, to reduce salt intake modestly; this modest reduction in salt intake was continued throughout the study with dietary advice being reinforced at each visit. All individuals were then observed for 1 month on their usual blood pressure therapy (Table 1). After this 1-month observation period, 5 mg BID amiloride was added to their usual medication, and over the ensuing month, patients underwent gradual reduction of their usual medication with an increase in dose of amiloride to a maximum of 10 mg BID under careful supervision. Amiloride alone was then continued for 1 month. Next, the amiloride was stopped and subjects remained on no treatment under close supervision for a period of 2 weeks. Amiloride was then reintroduced at an initial dose of 5 mg BID for 2 weeks and increased to 10 mg BID for 4 weeks. Appropriate measurements of blood pressure, weight, urea, electrolytes, creatinine, plasma renin activity, plasma aldosterone, and atrial natriuretic peptide, as well as 24-hour collections of urine for sodium, potassium, and creatinine excretion, were made throughout the study.

Clinical Measurements
Blood pressure was measured with a semiautomatic Omron HEM-705CP oscillometric blood pressure recorder. Individuals rested for 5 minutes, after which blood pressure recordings were done in triplicate with the use of the appropriate cuff size, based on the upper mid-arm circumference. Blood pressure was measured with the patient sitting, and the values given are the mean of 3 recordings. Readings were therefore free of observer bias. Blood was taken patient sitting, and the values given are the mean of 3 recordings. Careful verbal and written advice was given to ensure that 24-hour urine collections were accurate. Weight was measured at each visit on the same set of digital electronic scales (Secca, Marsden).

Screening for the T594M Polymorphism
DNA was extracted from whole blood from each patient, and a 245-bp fragment of the sodium channel β-subunit containing the region affected by the T594M polymorphism was amplified from DNA by polymerase chain reaction as previously described. Subjects were genotyped to look for the T594M polymorphism by restriction digestion of amplified DNA.

Polymerase chain reaction products were verified by electrophoresis and digested at 37°C overnight with 0.25 U of Nla III. The restriction digestion was predicted to produce fragments of the following sizes: T594 homozygotes; one fragment of 245 bp, T594M heterozygotes; three fragments of 53, 192, and 245 bp; M594 homozygotes; two fragments of 53 and 192 bp. Digest products were verified by electrophoresis and ethidium bromide staining. The 192- and 245-bp fragments could be seen by this method, but the 53-bp fragment was too small to be

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Treatment at Entry</th>
<th>Blood Pressure on Initial Treatment, mm Hg</th>
<th>Blood Pressure on Amiloride (10 mg OD), mm Hg</th>
<th>Blood Pressure on No Treatment, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Enalapril, 10 mg BD</td>
<td>154/93</td>
<td>164/101</td>
<td>168/107</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>141/86</td>
<td>142/86</td>
<td>153/91</td>
</tr>
<tr>
<td>3</td>
<td>Enalapril, 5 mg OD</td>
<td>128/87</td>
<td>114/80</td>
<td>142/101</td>
</tr>
<tr>
<td>4</td>
<td>Bendrofluazide, 2.5 mg OD</td>
<td>141/101</td>
<td>141/95</td>
<td>143/96</td>
</tr>
<tr>
<td>5</td>
<td>Enalapril, 5 mg BD</td>
<td>145/88</td>
<td>132/84</td>
<td>138/90</td>
</tr>
<tr>
<td>6</td>
<td>Lisinopril, 2.5 mg OD</td>
<td>119/75</td>
<td>148/97</td>
<td>138/94</td>
</tr>
<tr>
<td>7</td>
<td>Amiloride, 5 mg OD</td>
<td>146/93</td>
<td>134/87</td>
<td>145/97</td>
</tr>
<tr>
<td>8</td>
<td>Atenolol, 50 mg OD</td>
<td>160/106</td>
<td>148/96</td>
<td>171/110</td>
</tr>
<tr>
<td>9</td>
<td>Amiloride, 5 mg OD</td>
<td>127/76</td>
<td>116/82</td>
<td>165/89</td>
</tr>
<tr>
<td>10</td>
<td>Valsartan, 80 mg OD</td>
<td>133/91</td>
<td>130/88</td>
<td>141/94</td>
</tr>
<tr>
<td>11</td>
<td>Verapamil, 120 mg BD</td>
<td>160/84</td>
<td>147/96</td>
<td>181/107</td>
</tr>
<tr>
<td>12</td>
<td>Nifedipine LA, 60 mg OD</td>
<td>160/107</td>
<td>150/103</td>
<td>185/116</td>
</tr>
<tr>
<td>13</td>
<td>Amiloride, 10 mg OD</td>
<td>136/91</td>
<td>134/96</td>
<td>141/98</td>
</tr>
<tr>
<td>14</td>
<td>Lisinopril, 20 mg OD</td>
<td>143/79</td>
<td>158/84</td>
<td>184/94</td>
</tr>
<tr>
<td>15</td>
<td>Verapamil, 120 mg BD</td>
<td>127/73</td>
<td>122/82*</td>
<td>141/92</td>
</tr>
</tbody>
</table>

OD indicates once daily; BD, twice daily.

*Patient taking 2.5 mg BD amiloride under careful supervision.
resolved on the gel. Genotype was confirmed in a proportion of samples by direct sequence analysis with the use of a dye terminator kit on an ABI 377 automated sequencer.

Statistical Analysis
Group values are given as mean±SEM for normally distributed data and as median and interquartile range for plasma renin activity, aldosterone, and atrial natriuretic peptide concentrations, which are not normally distributed. Paired Student \( t \) tests were used to test for differences in variables that were normally distributed. Wilcoxon signed ranks tests were used to test for differences in plasma renin activity and plasma aldosterone and atrial natriuretic peptide concentrations. Two-tailed probability values of \(<0.05\) were considered significant.

**Results**

**Subject Recruitment**
Fifteen black hypertensive individuals were recruited into the study (Table 1). One was withdrawn in accordance with the protocol after entry into the study after the development of hyperkalemia during treatment with 10 mg BID amiloride. This subject was noted to have mild kidney impairment at entry (urea, 10.4 mmol/L; creatinine, 128 \( \mu \)mol/L) and previously had undergone unilateral adrenalectomy for hyperaldosteronism. Subsequent treatment of this individual with 2.5 mg BID amiloride alone under careful supervision resulted in blood pressure control to 122/82 mm Hg compared with entry blood pressure of 123/73 mm Hg on two-drugs, enalapril (5 mg BID) and verapamil (120 mg BID), and with blood pressure of 141/92 mm Hg on no treatment.

**Blood Pressure Was Adequately Controlled on Amiloride Alone**
In the remaining 14 subjects at entry into the study taking usual medication, blood pressure was 142/89 mm Hg (Table 1). After 4 weeks of a moderate reduction in salt intake in addition to usual medication, blood pressure was unchanged at 142/90±3/3 mm Hg. After 1 month during which amiloride was substituted for usual medication and a further month of treatment with 10 mg BID amiloride alone, blood pressure was controlled to the same level as that achieved by the 1.93±0.3 drugs taken at entry, to 140/91±4/2 mm Hg (Figure 1). Cessation of amiloride treatment for 2 weeks caused a significant increase in blood pressure of 17±4/8 mm Hg (systolic, \( P=0.048\); diastolic, \( P=0.005\)). Twenty-four–hour sodium excretion was 102±15 mmol/24 hours on low salt diet and usual medication and did not change after 4 weeks of treatment with amiloride alone (103±12 mmol/24 hours) or after 2 weeks off all treatment (114±12 mmol/24 hours), indicating that sodium intake remained constant during the study.

Changes in body weight and serum sodium and potassium occurred when commencing and stopping amiloride treatment. Body weight fell by 1.1±0.5 kg with amiloride treatment and increased significantly by 0.8±0.3 kg on cessation of amiloride (\( P=0.033\)). Serum sodium concentration fell by 1.3±0.6 mmol/L with amiloride treatment and increased significantly by 1.4±0.5 mmol/L on cessation of amiloride (\( P=0.024\)). Serum potassium concentration rose by 0.74±0.14 mmol/L with amiloride treatment and fell significantly by 0.77±0.12 mmol/L on cessation of amiloride (\( P<0.0001\)).

**Plasma Hormone Changes Indicate Retention of Sodium After Stopping Amiloride**
Plasma hormone activity was measured after 4 weeks of treatment with amiloride alone and repeated after 2 weeks on no treatment (Table 2). On cessation of amiloride, plasma renin activity fell by 0.73±0.27 ng/mL per hour (\( P=0.008\)), plasma aldosterone concentration fell by 720±222 pmol/L (\( P=0.001\)), and plasma atrial natriuretic peptide rose by 12.4±2.0 pmol/L (\( P=0.001\)).

**Table 2. Plasma Hormone Levels in Participants After 4 Weeks of Amiloride Treatment and 2 Weeks After Stopping Amiloride Treatment**

<table>
<thead>
<tr>
<th>Hormone Measured</th>
<th>4 Wk of Treatment With Amiloride</th>
<th>2 Wk No Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA, ng/mL per h (n=13)</td>
<td>0.65 (0.42–1.65)</td>
<td>0.27 (0.06–0.58)</td>
</tr>
<tr>
<td>Aldosterone, pmol/L (n=14)</td>
<td>1013 (587.3–1395.5)</td>
<td>492 (220.3–606.8)</td>
</tr>
<tr>
<td>Atrial natriuretic peptide, pmol/L (n=13)</td>
<td>9.5 (7.9–15.2)</td>
<td>21.6 (15.2–32.1)</td>
</tr>
</tbody>
</table>

Reference ranges for laboratory: PRA, 0.5 to 2.5 ng/mL per hour; aldosterone, 100 to 600 pmol/L; ANP, 1 to 16 pmol/L.
Confirmation of Antihypertensive Effect of Amiloride on Restarting Treatment

Paired measurements were available in 9 individuals who restarted amiloride after 2 weeks on no medication (Figure 2). Amiloride alone (10 mg BID) again controlled blood pressure to 140/88 ± 6/2 mm Hg. Decrease in body weight and serum sodium and an increase in serum potassium again indicated that participants were taking amiloride tablets (Figure 2).

Effect of Amiloride in Patients Previously Taking Thiazide Diuretics

Seven individuals were taking thiazide diuretics as part of combination antihypertensive therapy at entry into the study. Of these, 2 were taking a thiazide in combination with 1 other drug and 5 were taking a thiazide in combination with 2 other drugs (Table 2). Blood pressure in these 7 patients at entry was 144/89 ± 5/4 mm Hg. Blood pressure was slightly but not significantly lower in these 7 patients when they had been changed to amiloride alone (10 mg BID) (blood pressure on amiloride) 137/89 ± 6/3 mm Hg.

Discussion

We have shown in this open study with observer-blind measurement of blood pressure that amiloride alone effect-
pressure, and a second period of amiloride treatment was as effective as the first. The study, therefore, in our view does show that amiloride is effective in black hypertensive patients with the T594M polymorphism. A further important consideration is that we have no control group to be certain that amiloride would not be equally effective in black hypertensive patients without the T594M polymorphism.

In view of the above considerations, we are now conducting a double-blind study comparing the effect of amiloride in black hypertensive people both with and without the T594M polymorphism. In the meantime, we feel that our study provides strong evidence that amiloride at a dose of 10 mg BID is very effective as a single agent combined with modest salt restriction in black hypertensive individuals with the T594M polymorphism. These results provide further support for the concept that the T594M polymorphism may, in part, be responsible for the rise in blood pressure in these individuals that would indicate that this is the most common cause of secondary hypertension in the black population. Currently, we feel that all black patients with high blood pressure should be genotyped for the T594M polymorphism, and, if positive, consideration should be given to the use of amiloride to control their blood pressure.

Perspectives

Genetic investigations have successfully identified rare disorders in which single gene mutations cause hypertension. These discoveries not only have allowed insight into the structure and function of genes that regulate blood pressure but also have provided clinical tools for diagnosis and targeted therapy of these conditions. The challenge for genetic research in hypertension is now to see if these studies can be extended to identify diagnostic and therapeutic targets for essential (primary) hypertension. In pursuit of this, many investigators have found allelic variants in genes that regulate blood pressure, and some of these are associated with hypertension or blood pressure variation in the general population. Our study is the first to suggest that possession of one of these genetic variants is associated with response to specific therapy targeted to the affected gene product. Our study is an early example of the use of pharmacogenomics, the association between genetics and drug response, in essential hypertension. New technologies including the rapid identification of single nucleotide polymorphisms and simultaneous analysis of multiple genes could lead to rapid expansion of this field and further advances in the treatment of essential hypertension.

Acknowledgments

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References

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