Indomethacin, Amiloride, or Eplerenone for Treating Hypokalemia in Gitelman Syndrome

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ABSTRACT

Patients with Gitelman syndrome (GS), an inherited salt-losing tubulopathy, are usually treated with potassium-sparing diuretics or nonsteroidal anti-inflammatory drugs and oral potassium and magnesium supplementations. However, evidence supporting these treatment options is limited to case series studies. We designed an open-label, randomized, crossover study with blind end point evaluation to compare the efficacy and safety of 6-week treatments with one time daily 75 mg slow-release indomethacin, 150 mg eplerenone, or 20 mg amiloride added to constant potassium and magnesium supplementation in 30 patients with GS (individual participation: 48 weeks). Baseline plasma potassium concentration was 2.8 ± 0.4 mmol/L and increased by 0.38 mmol/L (95% confidence interval [95% CI], 0.23 to 0.53; P < 0.001) with indomethacin, 0.15 mmol/L (95% CI, 0.02 to 0.29; P = 0.03) with eplerenone, and 0.19 mmol/L (95% CI, 0.05 to 0.33; P < 0.01) with amiloride. Fifteen patients became normokalemic: six with indomethacin, three with eplerenone, and six with amiloride. Indomethacin significantly reduced eGFR and plasma renin concentration. Eplerenone and amiloride each increased plasma aldosterone by 3-fold and renin concentration slightly but did not significantly change eGFR. BP did not significantly change. Eight patients discontinued treatment early because of gastrointestinal intolerance to indomethacin (six patients) and hypotension with eplerenone (two patients). In conclusion, each drug increases plasma potassium concentration in patients with GS. Indomethacin was the most effective but can cause gastrointestinal intolerance and decreased eGFR. Amiloride and eplerenone have similar but lower efficacies and increase sodium depletion. The benefit/risk ratio of each drug should be carefully evaluated for each patient.


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Gitelman syndrome (GS; Mendelian Inheritance in Man 263800) is a rare inherited autosomal recessive salt-losing tubulopathy characterized by hypokalemic metabolic alkalosis, hypomagnesemia, hypocalciuria, and secondary hyperaldosteronism.1 GS has a prevalence of heterozygotes of approximately 1% and an estimated prevalence of homozygotes of approximately 1 in 40,000 in Caucasians.2 It is mainly related to loss-of-function mutations in the SLC12A3 gene encoding for the apically expressed thiazide-sensitive NaCl cotransporter (NCC) of the distal convoluted tubule (DCT).2

The natural history of GS is highly variable in terms of age at clinical diagnosis, biologic phenotype, and severity of clinical manifestations. However, severe hypokalemic rhabdomyolysis or ventricular arrhythmias may occur when acute additional extrarenal potassium and magnesium losses occur (vomiting or diarrhea).3–7

Lifelong oral potassium and magnesium supplementation is the cornerstone of treatment aiming at relieving symptoms and preventing severe hypokalemia-related complications, including cardiac arrhythmias.8 However, oral supplementation may be insufficient to fully correct the electrolyte disorders or may have poor gastrointestinal (GI) tolerability, which can limit dosing.9,10 In the case of persistent symptomatic hypokalemia or intolerance to supplementation, potassium-sparing diuretics, such as amiloride, or mineralocorticoid receptor (MR) antagonists (spironolactone or eplerenone) usually constitute the first therapeutic option.11–14 Another option is low-dose indomethacin, a nonselective inhibitor of cyclooxygenase 1 (COX-1) and COX-2.11,15 However, evidence is limited for all these treatment options, and no randomized controlled trial has so far compared their efficacy, tolerability, or safety in patients with GS.

We designed an open-label, randomized, crossover study to compare the efficacy on plasma potassium concentration and tolerability of slow-release indomethacin, amiloride, and eplerenone in patients with type I GS treated with stable potassium and magnesium supplementation.

**RESULTS**

Baseline characteristics of 30 patients (17 women) randomized in the study are shown in Table 1; all had typical GS with low BP, hypokalemia, and hypomagnesemia with inappropriate urine potassium and magnesium excretion, low urine calcium excretion, high plasma renin, and normal plasma aldosterone concentrations. Supplemental Figure 1 shows the reasons for exclusion or study drug discontinuation, the number of patients randomized to each treatment sequence, and the number completing the study.

**Tolerability**

Indomethacin caused GI intolerance (heartburn, dyspepsia, or gastric irritation) in six patients, resulting in early discontinuation. Eplerenone (150 mg once a day [o.d.]) was discontinued in two other patients for palpitations and dizziness (Supplemental Figure 1). The 20-mg o.d. dose of amiloride was downtitrated to 10 mg o.d. in two patients and 15 mg o.d. in another two patients for palpitations and dizziness. Thus, 22 of 30 patients actually received all three study drugs. Their baseline characteristics were similar to those of the randomized population of 30 patients (Table 1).

**Effects of Indomethacin, Eplerenone, and Amiloride on Electrolytic and Hormonal Parameters and GFR**

All parameters returned to their respective baseline and pretreatment levels after the 6-week washout periods (Supplemental Table 1).
Table 1. Therefore, only treatment effects are reported for the on-treatment population who actually received all three study drugs (n=22 of 30). Treatment effects analyzed by an ANOVA for a crossover design were significant for the following parameters: plasma potassium, creatinine, aldosterone and renin concentrations, and urine magnesium excretion. Two-by-two comparisons are shown below.

Indomethacin significantly increased plasma potassium by 0.34 mmol/L (95% confidence interval [95% CI], 0.20 to 0.48 mmol/L) compared with the control period (n=22, P<0.001) (Table 2) but did not significantly change plasma magnesium or sodium concentration (Table 2). When using the whole dataset for the indomethacin period (n=24), the net potassium increase from baseline was 0.38 mmol/L (95% CI, 0.23 to 0.53; P<0.001 versus baseline), and 6 of 24 (25%) patients normalized their plasma potassium concentration (≥3.5 mmol/L) (Figure 1). As expected, indomethacin markedly reduced plasma renin concentration (n=22; P<0.001) (Table 2) but did not change plasma aldosterone concentration (n=22; P>0.99) (Table 2). Compared with the control period, eGFR significantly decreased by 10.0 ml/min per 1.73 m² (95% CI, 4.2 to 15.9 ml/min per 1.73 m²; n=22; P<0.01) (Table 2) after the 6-week treatment with indomethacin. Urine potassium, magnesium, and sodium excretion did not significantly change (Table 2).

Eplerenone increased plasma potassium by 0.13 mmol/L (95% CI, −0.01 to 0.27 mmol/L) compared with the control period (n=22; P=0.41) (Table 2). When using the whole dataset for the eplerenone period (n=28), the net potassium increase from baseline was 0.15 mmol/L (95% CI, 0.02 to 0.29; P=0.03 versus baseline), and 3 of 28 (11%) patients normalized their plasma potassium concentration (Figure 1). Eplerenone did not change plasma magnesium concentration and slightly decreased plasma sodium concentration (n=22) (Table 2). It markedly increased plasma aldosterone concentration compared with baseline (n=22; P<0.001) (Table 2), but the increase in plasma renin concentration was not significant (n=22) (Table 2).

eGFR and urine potassium, magnesium, and sodium excretion did not change significantly (Table 2).

Amiloride increased plasma potassium by 0.18 mmol/L (95% CI, 0.04 to 0.32 mmol/L) compared with the control period (n=22; P=0.09) (Table 2). When using the whole dataset for the amiloride period (n=30), the net potassium increase was 0.19 mmol/L (95% CI, 0.05 to 0.33; P<0.01 versus baseline), and 6 of 30 (20%) patients normalized their plasma potassium concentration (Figure 1). Amiloride increased plasma magnesium concentration and decreased plasma sodium concentration slightly (n=22) (Table 2). As expected, it markedly increased plasma aldosterone concentrations compared with the control period (n=22; P<0.001) (Table 2), but the increase in plasma renin concentration was not significant (Table 2). Compared with the control period, eGFR slightly decreased by 6.4 ml/min per 1.73 m² (95% CI, −0.54 to 12.20 ml/min per 1.73 m²; P=0.20) (Table 2) after the 6-week treatment with amiloride. Urine potassium and sodium excretion did not change significantly, but magnesium excretion significantly decreased (n=22) (Table 2).

The increase in plasma potassium concentration was significantly greater after the 6-week treatment with indomethacin than eplerenone (increase of 0.21 mmol/L; 95% CI,

Table 2. Effects of 6-week treatments with 75 mg o.d. slow-release indomethacin, 150 mg o.d. eplerenone, or 20 mg o.d. amiloride in 22 patients with genetically proven GS type I who received all three treatments

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Period (Week 6)</th>
<th>Indomethacin (Week 6)</th>
<th>Eplerenone (Week 6)</th>
<th>Amiloride (Week 6)</th>
<th>ANOVA (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>69.3±13.1</td>
<td>70.3±13.4*</td>
<td>68.5±12.6b</td>
<td>68.7±13.1b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>115±9</td>
<td>116±11</td>
<td>115±11</td>
<td>114±10</td>
<td>0.24</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>71.8±10.1</td>
<td>73.5±7.6</td>
<td>75.8±8.0</td>
<td>75.6±9.7</td>
<td>0.07</td>
</tr>
<tr>
<td>Plasma potassium, mmol/L</td>
<td>2.8±0.4</td>
<td>3.2±0.4c</td>
<td>3.0±0.5d</td>
<td>3.0±0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma magnesium, mmol/L</td>
<td>0.54±0.09</td>
<td>0.56±0.07</td>
<td>0.56±0.13</td>
<td>0.58±0.08</td>
<td>0.59</td>
</tr>
<tr>
<td>Plasma sodium, mmol/L</td>
<td>140.0±1.6</td>
<td>139.1±1.4</td>
<td>138.6±1.5</td>
<td>138.5±1.6</td>
<td>0.17</td>
</tr>
<tr>
<td>Plasma creatinine, µmol/L</td>
<td>61.7±15.1</td>
<td>66.6±17.6a</td>
<td>63.1±15.6</td>
<td>64.8±16.4</td>
<td>0.03</td>
</tr>
<tr>
<td>eGFR, ml/min per 1.73 m²</td>
<td>126±28</td>
<td>115±25*</td>
<td>122±24</td>
<td>119±28</td>
<td>0.05</td>
</tr>
<tr>
<td>Plasma renin concentration, mU/L</td>
<td>90 (61 to 133)</td>
<td>47 (32 to 69)b</td>
<td>113 (76 to 167)b</td>
<td>112 (76 to 166)b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma aldosterone, pg/ml</td>
<td>45 (33 to 62)</td>
<td>42 (30 to 59)</td>
<td>131 (94 to 183)c</td>
<td>155 (107 to 124)bc</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urine potassium excretion, mg/24 h</td>
<td>107 [93–141]</td>
<td>123 [89–134]</td>
<td>104 [90–129]</td>
<td>104 [94–137]</td>
<td>0.95</td>
</tr>
<tr>
<td>Urine magnesium excretion, mg/24 h</td>
<td>2.1 [1.0–3.3]</td>
<td>3.1 [1.4–7.3]</td>
<td>3.0 [1.5–4.6]</td>
<td>1.7 [0.8–3.3]d</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data are mean±SD, geometric mean (95% CI), or median [interquartile range]. ANOVA is P value for the treatment effect in ANOVA for a crossover study. Normal values for plasma renin concentration are 20.8 mU/L (95% CI, 17.7 to 24.4) in men and 16.2 mU/L (95% CI, 13.6 to 19.2) in women. Normal values for plasma aldosterone concentration are 45 pg/ml (95% CI, 39 to 52) in men and 34 pg/ml (95% CI, 28 to 40) in women. mU/L, milli-international units per liter.

*aP<0.05 versus control period.

*bP<0.001 versus indomethacin.

cP<0.001 versus control period.

dP<0.05 versus indomethacin.

*eP<0.01 versus control period.
The results of this crossover trial showed that the moderate-to-
severe hypokalemia of patients with genetically proven GS was
corrected in the short term but only partially by 75 mg/d slow-
release indomethacin, 20 mg/d amiloride, or 150 mg/d eplerenone
given for 6 weeks combined with a constant oral potassium and
magnesium supplementation. At the selected doses, indometh-
acin was more effective in increasing mean plasma potassium
concentration than amiloride or eplerenone, which were of
similar efficacy. The effects of the treatments on plasma mag-
nesium concentration were either negligible (amiloride) or ab-
sent (indomethacin and eplerenone). As expected, indomethacin
and the two diuretics had opposite effects on plasma renin and
aldosterone concentrations as well as the slight treatment-
induced BP changes. eGFR significantly decreased with in-
domethacin and less so with amiloride but did not change with
eplerenone. Clinical tolerability of the drugs was inversely re-
lated to their efficacy, with indomethacin being the least tol-
erated because of GI intolerance leading to early discontinuation
in 6 of 30 (20%) patients, despite coprescription of a proton
clearance pump inhibitor.

The crossover nature of the study design with rotation of the
three drugs showed that 40% of patients who were intermediate
responders, nonresponders, or patients intolerant to indometh-
acin became full responders by switching to amiloride or
eplerenone. Thus, 73% of patients who tolerated the three
treatments were responders to at least one of three treatments.

Our primary end point was treatment-induced increase in
plasma potassium concentration in patients with GS. Hypo-
kalemia is associated with the intensity of musculoskeletal
symptoms and the occurrence of more severe complications,
and it has previously been shown to respond to each of three
study drugs in preliminary reports. Hypokalemia in pa-
tients with GS is caused by secondary consequences of the
inherited inactivation of NCC in the proximal DCT, including
increased sodium delivery to late DCT and cortical connecting
duct and secondary renin-dependent hyperaldosteronism
triggered by sodium depletion. This biologic and hormonal
phenotype mimics the electrolytic consequences of chronic
administration of thiazide diuretics, which specifically block
NCC in the kidney. The rationale behind administering the
epithelial sodium channel inhibitor amiloride and the MR
antagonist eplerenone is, thus, to neutralize the compensatory
mechanisms in the distal nephron that are responsible for
hypokalemia in patients with GS.

The dose of 20 mg amiloride has previously been shown to
reverse the hydrochlorothiazide-induced hypokalemia and
increase plasma magnesium concentration more effectively
than 100 mg spironolactone in healthy volunteers. Because
eplerenone is approximately 75% less potent than spironola-
ctone, the selected dose was 150 mg on the basis of the healthy
volunteer study mentioned above. Eplerenone was uptitrat-
ed weekly from 50 to 100 mg o.d. and then, to the maximum
dose of 150 mg o.d. administered for 4 weeks. A 6-week

**DISCUSSION**

The results of this crossover trial showed that the moderate-to-
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corrected in the short term but only partially by 75 mg/d slow-
release indomethacin, 20 mg/d amiloride, or 150 mg/d eplerenone
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ed weekly from 50 to 100 mg o.d. and then, to the maximum
dose of 150 mg o.d. administered for 4 weeks. A 6-week
administration of either 20 mg o.d. amiloride or 150 mg o.d. eplerenone was equally effective in increasing plasma potassium concentration. However, amiloride and eplerenone normalized plasma potassium concentration in only 20% and 11% of the patients, respectively, when given combined with potassium and magnesium supplementation. Amiloride slightly increased plasma magnesium concentration and significantly reduced urine magnesium excretion, indicating the stimulation of tubular magnesium reabsorption. This observation is in line with a previous report in healthy volunteers.\textsuperscript{17} It is not known whether a higher dose of amiloride or eplerenone could be more effective in correcting hypokalemia in patients with GS, but this option would be limited by tolerability. Indeed, the use of amiloride or eplerenone is, in part, paradoxical, because both drugs may worsen sodium depletion and hypovolemia in these patients with a salt-losing nephropathy.\textsuperscript{6} In fact, both drugs induced a marked increase in plasma aldosterone concentrations secondary to (1) diuretic-induced sodium depletion associated with a mild decrease in systolic BP (approximately 2 mmHg) and eGFR (amiloride only), although the rise in plasma renin was nonsignificant, and (2) partial correction of hypokalemia. The progressive titration of amiloride and eplerenone to their highest respective doses may explain the satisfactory clinical and biologic tolerability of the two drugs. Amiloride was downtitrated in only two patients, and eplerenone was discontinued in only two patients for worsened hypovolemia. Whether additional MR activation by high aldosterone concentrations triggered by amiloride may contribute to long-term cardiovascular activation by high aldosterone concentrations triggered by amiloride and eplerenone.

In contrast to amiloride and eplerenone, indomethacin decreased plasma renin concentration. Indeed, indomethacin is known to inhibit renin secretion by inhibition of PGE\textsubscript{2} synthesis in the juxtaglomerular apparatus.\textsuperscript{20} Indomethacin blunted the expected physiologic increase in plasma aldosterone concentration in response to the increase in plasma potassium concentration. Moreover, indomethacin has been shown experimentally to inhibit the diuretic effect of thiazides and stimulate sodium reabsorption in the loop of Henle.\textsuperscript{22} Accordingly, systolic BP increased slightly by approximately 2 mmHg, suggesting that the potassium-sparing effect of indomethacin was associated with a slight correction of hypovolemia. In summary, the effect of indomethacin in patients with GS may be caused by (1) the increase in loop NaCl reabsorption, leading, in turn, to a decrease in NaCl delivery to DCT,\textsuperscript{23} and (2) the inhibition of renin release by the juxtaglomerular apparatus as observed in rats treated with hydrochlorothiazide.\textsuperscript{24}

Indomethacin normalized plasma potassium concentration in only 25% of the patients who tolerated the drug. A titration of indomethacin dosage by monitoring the effect on plasma renin concentration or urinary excretion rates of prostaglandins may have improved efficacy and/or tolerability of indomethacin. However, whether a higher dose of indomethacin would have been more effective in correcting hypokalemia in these patients is not known,\textsuperscript{15} but the use of slow-release indomethacin (even at the low dose of 75 mg o.d.) for only 6 weeks had limited tolerability. First, eGFR decreased by 10.0 ml/min per 1.73 m\textsuperscript{2}, despite the mild increase in systolic BP after 6 weeks of treatment. The indomethacin-induced decrease in eGFR was slightly greater than that observed with amiloride, and both decreases were reversible when the drugs were stopped. This result is probably related to the specific effects of COX-2 inhibition on renal hemodynamics, which are specifically enhanced in sodium- and volume-depleted patients with GS.\textsuperscript{25,26} The risk of a more marked decrease in GFR may be increased with longer-term indomethacin use in the setting of (1) additional extrarenal sodium loss or (2) drug-induced interstitial nephritis.\textsuperscript{27–30} Second, six patients suffered from GI intolerance related to the indomethacin-induced inhibition of COX-1 at the level of the GI mucosa, leading to early discontinuation, despite coprescription of a proton pump inhibitor. The risk of severe GI adverse events is increased with long-term use of nonsteroidal anti-inflammatory drugs, including indomethacin.\textsuperscript{31,32} Third, there may also be a theoretical increased risk of cardiovascular events with prolonged exposure to the drug.\textsuperscript{32} However, this risk is related to the degree of COX-2 inhibition\textsuperscript{33} and thus, remains probably low at a dose of 75 mg o.d. slow-release indomethacin. In addition, chronic use of a proton pump inhibitor could worsen magnesium depletion by decreasing net intestinal absorption of magnesium.\textsuperscript{34}

The principle limitations of this study were (1) the short duration of exposure to treatments, which may not have been sufficient to fully assess tolerability and sustained efficacy for a
Conclusions

In conclusion, this study is the first and largest randomized controlled study performed in patients with GS providing evidence of moderate efficacy of three treatments currently used on the basis of limited case series. Although the three drugs have different mechanisms of action, they all increase plasma potassium concentration on the short term, which however, is normalized in a minority (20%–25%) of patients with GS.

Because we did not specifically address the long-term benefits and risks with the three treatments used in our study, these treatments still remain the last resort in patients with GS who remain with severe or symptomatic hydroelectrolytic abnormalities, despite potassium, magnesium, and possibly, salt supplementation at optimally titrated and tolerated doses. The decision should also take into consideration the clinical setting (e.g., the need for nonsteroidal anti-inflammatory drugs for chondrocalcinosis), the specific contraindications of each drug, and the patient’s informed choice. BP and heart rate were measured at home with a validated electronic device (OMRON M6; Omron Co., Kyoto, Japan) as described previously. In addition, we assessed quality of life using the Short Form Health Survey self-administered questionnaire adapted for the French population at randomization and the end of each active treatment period.

Laboratory Methods

Biochemical and hormone measurements were performed blind to the randomization sequence in a centralized laboratory. Plasma and urinary electrolytes and creatinine were measured as described previously. eGFR was calculated by the Modification of Diet in Renal Disease formula. Plasma renin and aldosterone concentrations were measured as described previously.

Statistical Analyses

The primary objective of the study was to determine whether slow-release indomethacin would significantly increase the plasma potassium concentration compared with the control period, which was defined as the first 6-week period after the 2- to 6-week washout period. Using a within-patient SD of 0.3 mmol/L, we calculated that 30 patients were needed to detect a 0.3-mmol/L difference in plasma potassium concentration between the indomethacin and control periods with 80% power and 5% α-error for a two-tailed test.

Because eight patients had to stop one of the study drugs for intolerance, leading to a missing period for each of these patients (see Results), data were analyzed by an ANOVA for a crossover design on the on-treatment population who actually received all three study drugs (n=22 of 30). The model included treatment, sequence, and period as fixed effects and subject nested within sequence as the
random effect. When the F test was significant (P<0.05) and there was no period, sequence, or carryover effect, paired comparisons were made between treatments by the Holm method.41

We also performed an analysis restricted to main criteria, i.e., plasma potassium concentration, using paired t tests comparing 6-week on-treatment versus pretreatment values on all datasets of valid periods of treatments.

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DISCLOSURES

None.

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This article contains supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2014030293/-/DCSupplemental.
**Supplemental Table 1: Recovery after each treatment period.** The parameters were measured just before the treatment, at the end of the preceding washout treatment (WO-pre-tt) and after a 6-week washout post-treatment period (WO post-tt). Full recovery was obtained for the three treatments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Indomethacin</th>
<th>Eplerenone</th>
<th>Amiloride</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Indomethacin</th>
<th>Eplerenone</th>
<th>Amiloride</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Indomethacin</th>
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<th>Amiloride</th>
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<sup>a</sup>: pre-treatment vs. post-treatment washout periods.
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<td>p.Arg209Trp</td>
<td>5</td>
<td>c.625C&gt;T</td>
</tr>
<tr>
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<td>c.625C&gt;T</td>
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<td>F</td>
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<td>F</td>
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<td>10</td>
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<td>F</td>
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<td>c.2</td>
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<td>16</td>
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<td>p.? splice defect</td>
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<td>c.2581C&gt;T</td>
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M: Male; F: female. $Patients included, not randomized. § Numbering is according to the cDNA sequence (GenBank : NM_000339.2). The A of the ATG of the initiator methionine codon is denoted as nucleotide. Mutations are described using the HGVS recommendations CH: compound heterozygous, Ho : homozygous. ¹Both parents are heterozygous ²No deletions detected by MLPA ³Heterozygous deletion detected par MLPA, exon1 to exon 7 deletion without breakpoint characterization. New mutation: not previously published mutation. The
following mutations were previously expressed in vitro: p.Arg209Trp, p.Gly316Val and p.Pro349Leu and p.Thr1026Ile (2) ; p.Gly316Val and p.Ala588Val (13) ; p.Cys994Tyr (14) . The others were predicted to be pathogen in silico.

References to supplemental Table 2:

Supplemental Table 3: Results of the Short Form (Health Survey (SF36) quality of life self-questionnaire at randomization and at the end of each active treatment period.

<table>
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<td><strong>summary</strong></td>
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<td>Physical functioning</td>
<td>84 ± 21</td>
<td>75 ± 22</td>
<td>70 ± 24</td>
<td>68 ± 21</td>
<td>72 ± 23</td>
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<td>Role limitations (physical)</td>
<td>81 ± 32</td>
<td>48 ± 43</td>
<td>58 ± 39</td>
<td>50 ± 44</td>
<td>53 ± 36</td>
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<td>Bodily pain</td>
<td>73 ± 24</td>
<td>53 ± 24</td>
<td>62 ± 25</td>
<td>52 ± 25</td>
<td>54+/- 24</td>
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<td>General health perception</td>
<td>69 ± 19</td>
<td>45 ± 19</td>
<td>45 ± 20</td>
<td>44 ± 18</td>
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<td><strong>summary</strong></td>
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<td>Vitality</td>
<td>60 ± 18</td>
<td>37 ± 19</td>
<td>42 ± 22</td>
<td>34 ± 18</td>
<td>41 ± 19</td>
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<td>Social functioning</td>
<td>82 ± 21</td>
<td>54 ± 20</td>
<td>65 ± 24</td>
<td>55 ± 28</td>
<td>55 ± 24</td>
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<td>Role limitations (emotional)</td>
<td>82 ± 32</td>
<td>64 ± 44</td>
<td>63 ± 42</td>
<td>56 ± 42</td>
<td>59 ± 41</td>
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<td>General mental health</td>
<td>68 ± 18</td>
<td>57 ± 15</td>
<td>57 ± 18</td>
<td>54 ± 19</td>
<td>56 ± 17</td>
</tr>
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</table>

Physical functioning: Measurement of limitations in physical activities such as walking, climbing stairs, bending forward, lifting and significant and moderate physical effort.

Role limitations because of physical health problem: Measurement of discomfort, due to the physical condition, in daily activities; measurement of limitations in certain activities or difficult to achieve them.

Bodily pain: Measurement of the intensity of the pain and inconvenience.

Vitality: Self-assessment of vitality, energy, fatigue.

Social functioning: Measurement of limitations in social activities due to physical problems and mental health.

Role limitations because of emotional problems: evaluation of discomfort due to psychological problems in daily activities; time spent on less important work, sloppy work.

Mental health: Self-rated mental health: anxiety, depression, well-being (happiness?).

Supplemental Figure 1: Flow chart of the study

Patients enrolled (n=33)

Exclusion criteria (n=3)

Patients randomized (n=30)

Indomethacin (n=30)

Drug intolerance (n=6)

Completed (n=24)

All three treatments completed (n=22)

Eplerenone (n=30)

Drug intolerance (n=2)

Completed (n=28)

Amiloride (n=30)

Completed (n=30)
**Supplemental Figure 2:** Correlations between treatment-induced changes in plasma potassium concentration (ΔK mmol/L). The correlation between responses to indomethacin and amiloride was significant (panel A, $r^2=0.1713$, 0.0444). The correlation between responses to indomethacin and eplerenone (B panel) and between responses to eplerenone and amiloride (C panel) were not significant.
DETAILED METHODS

Participants

Eligible patients were men and women aged 18 to 60 years diagnosed with genetically proven GS at five tertiary French hospitals. Patients had either homozygous (n=8) or compound heterozygous for point mutations or large rearrangements in the SLC12A3 gene (n=27) (see supplemental Table 2). Exclusion criteria were: known intolerance to the study drugs, significant cardiac arrhythmia, no contraception in women of child bearing potential, pregnancy, or an estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m². All patients gave written informed consent before participating in the study. The protocol (ClinicalTrials.gov Identifier: NCT01146197) was approved by the “Comité de Protection des Personnes”, Paris-Île de France III, France.

Design of the study

This was a seven-period, three-treatment, open-label, randomized, crossover study with blind end-point evaluation. The initial baseline assessments were performed after a 2- to 6-week washout period to stop any COX inhibitor (2 weeks) or potassium-sparing diuretic (6 weeks) effects. Patients were then randomly assigned to sequentially receive slow release indomethacin, eplerenone or amiloride for 6 weeks each in addition to oral potassium and
magnesium supplementation. Each active period was intercalated with a 6-weeks washout period during which the potassium and magnesium supplementation was maintained.

Slow release indomethacin was given at a dose of 75 mg o.d. for 6 weeks in combination with a proton pump inhibitor (omeprazole 20 mg/day) for gastric protection. This dosage regimen was based on the low indomethacin doses ($\approx 1 \text{mg/kg}$) currently used in Bartter syndrome \(^{16}\) and preliminary reports in GS \(^{15}\).

Amiloride was uptitrated weekly from 10 mg o.d. to 15 mg o.d. and then to the maximum dose of 20 mg o.d. administered for 4 weeks. This dose of 20 mg amiloride has previously been shown to reverse the hydrochlorothiazide-induced hypokalemia and to increase plasma magnesium concentration more effectively than 100 mg spironolactone in healthy volunteers \(^{17}\).

Eplerenone was uptitrated weekly from 50 mg o.d. to 100 mg o.d. and then to the maximum dose of 150 mg o.d. administered for 4 weeks. Since eplerenone is $\approx$75\% less potent than spironolactone \(^{18}\), the selected dose was 150 mg based on the healthy volunteer study mentioned above \(^{17}\). The amiloride and eplerenone doses could be down-titrated if symptomatic hypotension occurred.

The oral potassium and magnesium supplementation doses were adjusted for each patient during the first 6-week washout period to maintain plasma K concentration at either $\geq 2.8 \text{mmol/L}$ or 0.2 to 0.4 mmol/L above their usual plasma K concentration. The mean dose
of slow release potassium chloride was $4.0\pm 1.7 \text{ g}$, and that of magnesium element was $226 \pm 77 \text{ mg/day}$. Thereafter, the dose was kept constant throughout the study.

**Randomisation**

The randomisation sequence was generated by computer without stratification, using randomised blocks of small size and permutation of treatments within each block. Investigators, patients, and research staff were blinded to the randomisation list.

**Study evaluations**

Biochemical, hormonal, hemodynamic, and safety assessments were performed after each 6-week washout and active treatment periods. Blood was sampled at ≈ 09:00 a.m. in fasting conditions after the patient rested for one hour in a semi-recumbent position. Seated blood pressure (BP) and heart rate (HR) was measured at home with a validated electronic device (OMRON M6®, Omron Co., Kyoto, Japan) as described previously. In addition, we assessed quality of life using the Short Form Health Survey (SF36) self-administered questionnaire adapted for the French population at randomization and the end of each active treatment period.

**Laboratory methods**

Biochemical and hormone measurements were performed blind to the randomization sequence in a centralized laboratory. Plasma and urinary electrolytes and creatinine were measured as described previously. Estimated GFR was calculated by the
Modification of Diet in Renal Disease (MDRD) formula.\textsuperscript{21} Plasma renin and aldosterone concentrations were measured as described previously.\textsuperscript{22}

\textit{Statistical analysis}

The primary objective of the study was to determine whether slow release indomethacin would significantly increase the plasma potassium concentration compared to the control period, defined as the first 6-week period following the 2- to 6-week washout period. Using a within-patient standard deviation (SD) of 0.3 mmol/L, we calculated that 30 patients were needed to detect a 0.3 mmol/L difference in plasma potassium concentration between the indomethacin and control period with 80\% power and 5\% alpha error for a two-tailed test. The secondary objectives were to compare the potassium- and magnesium-sparing effects as well as the hemodynamic and hormonal effects and tolerability of the three study drugs.

Patients with a clinically significant treatment-induced increase of at least 0.3 mmol/L in plasma potassium concentration were considered as full responders, those with 0.10 to 0.29 mmol/L increases were intermediate responders and those with less than 0.10 mmol/L increases were non responders.

Since eight patients had to stop one of the study drugs for intolerance leading to a missing period for each of these patients (see results), data were analyzed by an ANOVA for a crossover design on the on-treatment population who actually received all three study
drugs (n=22/30). The model included treatment, sequence, and period as fixed effects and subject nested within sequence as the random effect. When the F test was significant (P<0.05), and when there was no period, sequence or carryover effect, paired comparisons were made between treatments by Holm's method. The assumptions of ANOVA (homogeneity of variance and normality) were checked for each variable and natural logarithmic transformation was applied where appropriate. We also performed an analysis restricted to plasma potassium concentration using paired t-tests comparing 6-week on-treatment vs. pre-treatment values on all data sets of valid periods of treatments. Correlations were estimated using Pearson coefficients. SAS software version 9.3 (SAS Institute Inc., Cary, NC, US) was used for statistical analyses. Data are expressed as geometric means with 95% confidence intervals (CI) or medians (interquartile range, IQR) for non-normal parameters and as means ± one SD for normally distributed data. Two-sided P values of <0.05 were considered to be significant.