Efficacy and Safety of Oral Methazolamide in Patients With Type 2 Diabetes: A 24-Week, Placebo-Controlled, Double-Blind Study

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OBJECTIVE
To evaluate the safety and efficacy of methazolamide as a potential therapy for type 2 diabetes.

RESEARCH DESIGN AND METHODS
This double-blind, placebo-controlled study randomized 76 patients to oral methazolamide (40 mg b.i.d.) or placebo for 24 weeks. The primary efficacy end point for methazolamide treatment was a placebo-corrected reduction in HbA1c from baseline after 24 weeks (ΔHbA1c).

RESULTS
Mean ± SD baseline HbA1c was 7.1 ± 0.7% (54 ± 5 mmol/mol; n = 37) and 7.4 ± 0.6% (57 ± 5 mmol/mol; n = 39) in the methazolamide and placebo groups, respectively. Methazolamide treatment was associated with a ΔHbA1c of −0.39% (95% CI −0.82, 0.04; P < 0.05) (−4.3 mmol/mol [−9.0, 0.4]), an increase in the proportion of patients achieving HbA1c ≤6.5% (48 mmol/mol) from 8 to 33%, a rapid reduction in alanine aminotransferase (≈10 units/L), and weight loss (2%) in metformin-cotreated patients.

CONCLUSIONS
Methazolamide is the archetype for a new intervention in type 2 diabetes with clinical benefits beyond glucose control.

Methazolamide is a carbonic anhydrase (CA) inhibitor that was approved by the U.S. Food and Drug Administration in 1959 as a treatment for glaucoma. The safety profile of methazolamide has been well characterized through its long history of clinical use at doses from 50 to 100 mg b.i.d. or t.i.d. The most common side effect reported for methazolamide is reversible, dose-dependent, metabolic acidosis, which is a consequence of CA inhibition (1).

The potential antidiabetes activity of methazolamide was identified using a Gene Expression Signature screening technology (2). The glucose-lowering efficacy of methazolamide was established in studies using db/db and DIO mice, where methazolamide was also found to have more-than-additive efficacy in combination with metformin. Methazolamide was ineffective in insulin-deficient streptozotocin-treated rats but significantly enhanced the glucose-lowering effect of exogenous...
insulin administered to these animals (3). These data suggest that methazolamide is a novel insulin sensitizer that is complementary to metformin. Ongoing mechanistic studies have shown that methazolamide exerts its metabolic effects by a mitochondrial mechanism distinct from CA inhibition.

**RESEARCH DESIGN AND METHODS**

This was a randomized, double-blind, placebo-controlled, proof-of-concept study conducted at three sites in Australia between 2010 and 2012. The protocol and amendments were approved by independent ethics committees and institutional review boards. The study was implemented according to Good Clinical Practice and the Declaration of Helsinki. All study patients gave written informed consent prior to screening.

Eligible patients included men and women between the age of 18 and 75 years with type 2 diabetes. Key inclusion criteria were a hemoglobin A1c (HbA1c) at screening of 6.5–8.5% (48–69 mmol/mol), body weight >50 kg, and a BMI ≤40 kg/m². The study included participants who were not treated with any diabetes medication (Non-MET) and patients who had been treated with metformin for at least 3 months and had a stable metformin dose for at least 8 weeks prior to study entry (MET). Metformin doses were not altered throughout the study (Supplementary Fig. 1).

Patients were randomized in a 1:1 ratio to receive either methazolamide (40 mg b.i.d.) or matching placebo for 24 weeks. Blood samples for biochemistry, venous blood gases, fasting blood glucose, and insulin were at screening, day 0, and weeks 1, 2, 4, 8, 12, 18, and 24. HbA1c measurements, hematology, lipid profile, and urinalysis were conducted at screening, day 0, and weeks 12 and 24. Patients were consulted by telephone at weeks 3, 10, 15, and 21, and a telephone follow-up was conducted 30 days after study completion.

The primary efficacy end point was the change in HbA1c from baseline to 24 weeks (ΔHbA1c) in the pooled (Non-MET + MET) methazolamide group compared with the pooled placebo group (Non-MET + MET). The primary safety end point was the incidence of metabolic acidosis (based on venous blood gas parameters), defined as one of the following: pH ≤7.25 in repeated venous blood gas analysis and confirmed by arterial blood gas analysis, bicarbonate <20 mmol/L, or base excess below –5.

The study was powered to demonstrate superiority of the pooled (Non-MET + MET) methazolamide group over the pooled placebo group for the primary efficacy end point. There were insufficient patient numbers for comprehensive statistical comparisons between the Non-MET and MET groups. Statistical analyses were performed in accordance with ICH E9 guidelines using SAS, version 9.2 (SAS Institute, Cary, NC). Changes in HbA1c, fasting blood glucose, serum alanine aminotransferase (ALT), microalbumin, and blood pressure were evaluated using repeated-measures ANCOVA with missing data imputed using a last observation carried forward strategy. Treatment group differences were estimated using least squares means and 95% CIs based on the mean square error from ANCOVA using a one-sided significance level (P < 0.05).

**RESULTS**

A total of 132 patients were screened, and 76 were enrolled in the study. Baseline demographic parameters and HbA1c were well matched between groups (Table 1 and Supplementary Table 1). Ten patients (13%) discontinued the study: 5 placebo-treated and 5 methazolamide-treated.

The clinical study achieved its protocol-specified primary efficacy end point, demonstrating a statistically significant reduction in HbA1c from baseline to week 24 in methazolamide-treated patients relative to placebo (Table 1). Greater reductions in HbA1c were observed in patients with higher baseline HbA1c, and a statistically significant regression was noted between ΔHbA1c and baseline HbA1c values in methazolamide-treated patients (Supplementary Fig. 2). The proportion of patients with HbA1c ≤6.5% (48 mmol/mol) increased from day 0 to week 24 in the methazolamide group but was unchanged in the placebo group (Table 1).

Methazolamide treatment caused a significant reduction in serum ALT from baseline to week 24 compared with placebo (Table 1). The ALT reduction occurred as early as week 1 and continued for the duration of the study (Supplementary Fig. 2). There were no significant alterations to γ-glutamyltransferase (mean ± SD for placebo 1.6 ± 13.4 units/L, methazolamide –1.8 ± 10.4 units/L) or other liver markers.

Methazolamide-treated patients also had reduced urinary microalbumin compared with placebo at week 24 owing exclusively to microalbumin reductions in MET patients. There were no significant changes in creatinine or urea, and the incidence of abnormal renal function (defined as >10% reduction in eGFR) was similar for methazolamide (30%) and placebo (36%).

Changes in body weight were not different between the pooled methazolamide and pooled placebo groups; however, in the MET group, methazolamide-treated patients lost more body weight (–2.2 ± 3.6 kg, n = 21) than placebo-treated patients (–0.3 ± 1.7 kg, n = 18) at week 24 (P = 0.04, ANOVA with two-sided t test). In the Non-MET group, methazolamide- and placebo-treated patients lost similar amounts of body weight (Supplementary Table 3).

There were no incidences of hypoglycemia or hypotension and no significant effects of methazolamide on fasting blood glucose; homeostasis model assessment of insulin resistance; fasting insulin; HDL, LDL, or total cholesterol; triglycerides; blood pressure; venous pCO2 or pO2; electrocardiograms; safety laboratory measures; or electrolyte disturbances. Metabolic acidosis was diagnosed in seven methazolamide-treated patients (five MET, two Non-MET) and no placebo patients. None of the patients with acidosis showed any clinically significant symptoms, and all remained on study (Supplementary Table 2). A greater proportion of patients in the methazolamide treatment arm (26 of 37 [70%]) reported adverse events (primarily respiratory tract infections and nausea) compared with placebo (23 of 39 [59%]). None of the adverse events were considered to be definitely related to study medication (Supplementary Table 3).

**CONCLUSIONS**

Methazolamide is the archetype for a potential new class of type 2 diabetes therapy. In addition to reducing HbA1c, methazolamide provided unexpected additional clinical outcomes, including a rapid and persistent reduction in ALT and weight loss in metformin-cotreated patients. Type 2 diabetes is a risk factor for the development of liver diseases (4,5),
Table 1—Baseline demographics and changes in key parameters upon methazolamide treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Methazolamide</th>
<th>Treatment effect.§</th>
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<tbody>
<tr>
<td>HbA_1c (%)</td>
<td>7.4 ± 0.6</td>
<td>7.1 ± 0.7</td>
<td>0.0371</td>
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<tr>
<td>Microalbumin (mg/mL)</td>
<td>68.2 ± 211.1</td>
<td>29.7 ± 100.7</td>
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<tr>
<td>ALT (units/L)</td>
<td>33.9 ± 16.1</td>
<td>31.5 ± 15.3</td>
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<tr>
<td>GGT (units/L)</td>
<td>42.6 ± 37.0</td>
<td>38.4 ± 29.1</td>
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<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>83.7 ± 9.1</td>
<td>81.6 ± 11.1</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>136.2 ± 15.2</td>
<td>137.3 ± 14.4</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD or least squares means (95% CI) unless otherwise indicated. GGT, γ-glutamyltransferase. *Safety population included all patients who received at least one dose of study medication. †Percentage of all patients with at least one of the following: hypertension, hypercholesterolemia, and/or hyperlipidemia; patients often had more than one. ‡The intent-to-treat population included all randomized patients who received at least one dose of study medication and who had at least one valid observation for an efficacy variable while on study medication. §Methazolamide change – placebo change. ¶P < 0.05 by post hoc analysis using a two-sided t test.

References


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