Lipid and apolipoprotein levels during therapy with pinacidil combined with hydrochlorothiazide

C. N. Corder 1, M. R. Goldberg 2, P. A. Alaupovic 1, M. D. Price 1, and S. S. Furste 2

1 Oklahoma Medical Research Foundation, Oklahoma City, and 2 Eli Lilly Co., Indianapolis, USA

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Summary. This study determined the effect of pinacidil on the concentration of plasma lipids and apolipoproteins in male patients previously equilibrated with 25 mg hydrochlorothiazide twice daily.

Pinacidil therapy given to 52 hypertensives at 25 to 100 mg daily for 8 weeks resulted in a reduction of systolic and diastolic blood pressure concurrently to reductions in plasma cholesterol and triglycerides with no change in low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C). There was an associated decrease in apolipoproteins (Apo)B, C-III and E and an elevation in ApoA-I. A parallel placebo group of 44 patients experienced reduction in diastolic blood pressure and an elevation in ApoA-I.

These changes indicate that pinacidil will be a useful antihypertensive agent having properties on lipoprotein metabolism which would favor decreased risks of atherosclerosis.

Key words: Lipids, Pinacidil, Hydrochlorothiazide; hypertension, triglyceride, cholesterol, apolipoproteins

Pinacidil is one of a series of hypotensive cyano guanidines to be evaluated extensively [1]. It is a direct vasodilator with a good absorption, half-life of 2 to 4 h, and side effects similar to those of other vasodilators [2-5]. Pinacidil exerts its vasodilator effect by activating the potassium channels in vascular smooth muscle, resulting in a decrease in vascular tone [6-7]. When given alone to untreated patients, pinacidil is effective in reducing blood pressure but is associated with a dose-related incidence of edema that may be treated with concurrent administration of a diuretic. Accordingly, the combination of pinacidil and hydrochlorothiazide is appropriate to prevent edema and to enhance efficacy either as initial therapy or as a second step for patients who are unresponsive to low doses of either drug as monotherapy.

Some antihypertensive drugs such as thiazide diuretics and β-adrenergic blockers have adverse effects on blood lipid and apolipoprotein levels, while others have none or potentially positive effects [9, 10]. Pinacidil administered as monotherapy has been shown to reduce plasma cholesterol, low density lipoprotein-cholesterol (LDL-C), and triglycerides, with an increase in high density lipoprotein-cholesterol (HDL-C) [11]. The present report describes the effect of pinacidil on lipids and apolipoproteins in hypertensive patients stabilized on the thiazide diuretic, hydrochlorothiazide.

Materials and methods

Treatment protocol

This multicenter study (B6C-MC-EFBH, Eli Lilly Corp., Indianapolis, IN) enrolled male patients with a diagnosis of moderately severe essential hypertension. A baseline evaluation of 4-8 weeks was initiated with signed informed consent, then withdrawal from prohibited antihypertensive medication and initiation or continuation of treatment with hydrochlorothiazide, 25 mg twice daily. Patients were required to have a supine diastolic blood pressure (DBP) of 91-115 mm Hg for continuation into the randomization phase of the study (Table 1). Each patient completing this 4 week baseline period had a serum sample assayed for lipids and apolipoproteins.

Patients were subsequently randomized double-blind to one of three treatment groups: pinacidil, placebo, or alphamethyldopa. An internal monitor at SCICOR Laboratories (Indianapolis, IN) was authorized, by a review committee of Lilly Research Laboratories, to identify the samples subsequently collected on patients randomized to pinacidil and placebo, to allow shipment of only pinacidil and placebo specimens (in a blinded manner) to Oklahoma City for lipid and apolipoprotein analyses.

After randomization, the doses were titrated using four levels of pinacidil (12.5 mg, 25 mg, 37.5 mg, and 50 mg) taken twice daily (i.e. total dose 25, 50, 75, or 100 mg/day) and four matched doses of placebo, until arterial pressures were controlled, or the maximum dose was reached. This dose titration period varied from 2 to 8 weeks post-randomization (Table 1). Patients not obtaining at least a partial arterial pressure response were excluded from the study. A complete blood pressure (BP) response was defined as supine DBP less than 90 mm Hg and a decrease of 10 mm Hg or more from baseline; a partial response was defined as supine DBP less than 90 mm Hg or a decrease of 10 mm Hg or more to a level between 90 and 100 mm Hg from baseline. Systolic blood pressure (SBP) and DBP were measured 2 to 5 h after dosing with study medication, after the patients were supine for 10 min, using the mercury sphygmomanometer and the fifth Korotkoff sound for DBP.
Normolipidemic, age-matched male subjects were used as a second-vals.

Blood chemistries had serum samples properly collected and assayed for lipid and subsequently completed the 8 week stable dosing phase and who also were randomized to pinacidil, 87 to placebo and 82 to alphamethylhydrochlorothiazide treatment phase. Subsequently, 82 patients

Table 1. Protocol treatment assignments

<table>
<thead>
<tr>
<th>Period</th>
<th>Duration (weeks)</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4-8</td>
<td>Unassigned, n = 289</td>
</tr>
<tr>
<td>Titrationb</td>
<td>2-8</td>
<td>Randomly assigned to treatment with placebo, pinacidil or alphamethyl-dopa</td>
</tr>
<tr>
<td>Maintenancec</td>
<td>8</td>
<td>Completed maintenance and lipid analyses</td>
</tr>
</tbody>
</table>

- Placebo 25 mg (n = 8)
- Pinacidil 25 mg (n = 13)
- Placebo 50 mg (n = 10)
- Pinacidil 50 mg (n = 19)
- Placebo 75 mg (n = 8)
- Pinacidil 75 mg (n = 14)
- Placebo 100 mg (n = 18)
- Pinacidil 100 mg (n = 6)

Alphamethyl-dopa, not analyzed

a All patients on 25 mg hydrochlorothiazide BID at baseline.

b Titrated supine DBP to <90 mm Hg, or to 90–100 mm Hg with concurrent ≥ 10 mm Hg decrease DBP from baseline. Supine DBP was 91–115 mm Hg on titration entry.

c Those patients successfully completing titration and in which there were complete lipid analyses.

Table 2. Lipid and apolipoprotein levels prior to randomization

<table>
<thead>
<tr>
<th>Apo</th>
<th>Hypertensive</th>
<th>Normotensive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 289</td>
<td>n = 15</td>
</tr>
<tr>
<td>Age (y)</td>
<td>49.0 (9.9)</td>
<td>49.8 (5.2)</td>
</tr>
<tr>
<td>Cholesterol mg·dl⁻¹</td>
<td>230 (46.5)</td>
<td>217 (30.9)</td>
</tr>
<tr>
<td>HDL cholesterol mg·dl⁻¹</td>
<td>48.9 (13.2)</td>
<td>47.4 (7.1)</td>
</tr>
<tr>
<td>LDL cholesterol mg·dl⁻¹</td>
<td>140 (40.5)*</td>
<td>154 (28)</td>
</tr>
<tr>
<td>Triglyceride mg·dl⁻¹</td>
<td>211 (208)*</td>
<td>96.1 (29.6)</td>
</tr>
<tr>
<td>ApoA-I mg·dl⁻¹</td>
<td>138 (30.5)*</td>
<td>126 (18.5)</td>
</tr>
<tr>
<td>ApoB mg·dl⁻¹</td>
<td>120 (29.0)</td>
<td>118 (23.3)</td>
</tr>
<tr>
<td>ApoC-III mg·dl⁻¹</td>
<td>14.7 (7.8)*</td>
<td>8.7 (1.7)</td>
</tr>
<tr>
<td>ApoE mg·dl⁻¹</td>
<td>14.2 (7.4)</td>
<td>12.5 (5.4)</td>
</tr>
</tbody>
</table>

* P < 0.05 Grouped Student t. Values are mean with (SD). All hypertensive subjects on 25 mg hydrochlorothiazide BID

Patients that obtained a partial arterial pressure response at the end of dose titration (maximum dose) or obtained a complete response on a smaller dose were subsequently continued on that maintenance dose for 8 weeks. Patients were seen approximately weekly for measurement of vital signs.

There were 289 patients who completed the four week baseline hydrochlorothiazide treatment phase. Subsequently, 82 patients were randomized to pinacidil, 87 to placebo and 82 to alphamethyl-dopa; 38 patients failed to meet DBP criteria for randomization. There were 52 patients on pinacidil and 44 on placebo who subsequently completed the 8 week stable dosing phase and who also had serum samples properly collected and assayed for lipid and apolipoprotein levels at both baseline and endpoint.

Lipids and apolipoproteins were measured in serum specimens obtained after baseline qualification (2 weeks prior to randomization) and after the 8 week maintenance period. Blood chemistries and electrocardiograms were monitored for safety at frequent intervals.

Normolipidemic subjects

Normolipidemic, age-matched male subjects were used as a secondary reference population for measurement of lipid and apolipoprotein levels.

Processing of blood

Blood samples were collected into 10 ml vacutainer tubes by venipuncture after the patient fasted at least 12 h, except for water and study medication given 2-5 h before blood sampling. The tubes were allowed to stand for 30 min to allow clotting and then centrifuged at low speed. The serum was separated and then shipped at ambient temperature on the same day as collection, by overnight mail to a central laboratory (SCICOR, Indianapolis, IN) and stored in 3 ml aliquots at −20°C. Accumulated samples from the multicenter sites were appropriately identified, then periodically shipped on dry ice to Oklahoma City laboratories for analyses of lipids and apolipoproteins.

Assays

Serum triglycerides were determined enzymatically as modified by the A-GENT Triglycerides Test purchased from Abbott Laboratories (North Chicago, IL 60064) [12]. Reference sera (Q11 and Q16) from the Centers for Disease Control (CDC) (Atlanta, GA 30333) of known triglyceride content and glycerol standard were used for quality control and calibration, respectively.

Serum cholesterol was determined enzymatically as modified by the Cholesterol High Performance-K (992005) test purchased from Boehringer Mannheim Diagnostics (Indianapolis, IN 46250) [13]. CDC reference sera (Q11 and Q16) of known cholesterol content were used for quality control.

HDL cholesterol was determined by first precipitating serum with manganese chloride and sodium heparin, and then measuring cholesterol in the supernate [13,14].

LDL-C was calculated using the Friedewald formula [15]. No calculations were made when triglycerides were greater than 400 mg/dl.

Serum apolipoproteins (A-I, A-II, C-III, and E) were measured by rocket electroimmunoassays. The isolation of antibodies, preparation of their corresponding monospecific antisera, and individual assays have previously been described [16–19]. Normal pooled human plasma of known apolipoprotein content served as reference, and a normal individual serum of known apolipoprotein content was used as a quality control serum.

Serum samples with triglyceride concentrations above 600 mg·dl⁻¹ were partially delipidized prior to assays of apolipoproteins C-III and E [20]. Concentrations are expressed as mg·dl⁻¹.

Data analyses

These data sets do not contain data from all randomized patients because some patients were discontinued due to lack of target DBP efficacy during dose titration, protocol violation, physician or patient decision before they attained their final dose level of pinacidil, or inadequate blood specimens from the designated time points preventing the complete lipid analyses. These limitations were necessary because the objective of the study was to determine whether pinacidil altered lipid and apolipoprotein levels in hypertensive patients on steady dosing with hydrochlorothiazide. The statistical analysis was conducted using paired t test SAS programs [21]. Because this analysis is not “intention to treat,” statistical comparisons between placebo and pinacidil dose groups are limited. In fact, the process of selection, based upon DBP responses, to attend the maintenance phase of study drug dosing ensured much greater similarity of pressure response between the groups than would have been apparent had nonresponding patients and patients with severe adverse events been forced to complete all study visits.

Results

The purpose of this study was to evaluate the lipid and apolipoprotein levels in patients receiving hydrochlorothiazide and concurrently treated for approximately