Effects of the PPAR-δ agonist MBX-8025 on atherogenic dyslipidemia

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LDL
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Triglyceride
Small dense LDL

A B S T R A C T

Objective: Determine the effects of treatment with a selective PPAR-δ agonist ± statin on plasma lipoprotein subfractions in dyslipidemic individuals.

Methods: Ion mobility analysis was used to measure plasma concentrations of subfractions of the full spectrum of lipoprotein particles in 166 overweight or obese dyslipidemic individuals treated with the PPAR-δ agonist MBX-8025 (50 and 100 mg/d) ± atorvastatin (20 mg/d) in an 8-week randomized parallel arm double blind placebo controlled trial.

Results: MBX-8025 at both doses resulted in reductions of small plus very small LDL particles and increased levels of large LDL, with a concomitant reduction in large VLDL, and an increase in LDL peak diameter. This translated to reversal of the small dense LDL phenotype (LDL pattern B) in ~90% of the participants. Modest increases in HDL particles were confined to the smaller HDL fractions. Atorvastatin monotherapy resulted in reductions in particles across the VLDL–IDL–LDL spectrum, with a significantly smaller reduction in small and very small LDL vs. MBX-8025 100 mg/d (−24.5 ± 5.3% vs. −47.8 ± 4.9%), and, in combination with MBX-8025, a reversal of the increase in large LDL.

Conclusion: PPAR-δ and statin therapies have complementary effects in improving lipoprotein subfractions associated with atherogenic dyslipidemia.

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1. Introduction

Peroxisome proliferator-activated receptor delta (PPAR-δ) belongs to a family of lipid-activated nuclear receptor transcription factors including two other members, PPAR-α and PPAR-γ. Studies in rodents and non-human primates have shown favorable effects of PPAR-δ agonists on fatty acid oxidation and utilization, diet-induced obesity, insulin resistance, and plasma lipid and lipoprotein metabolism [1–8]. There has, however, been considerable variation in lipid and lipoprotein response among different experimental models. In healthy humans, the PPAR-δ selective agonist GW501516 was reported to improve triglyceride clearance after a high fat meal, but without a change in total cholesterol (C) and LDL-C and fasting triglycerides [9]. Treatment with GW501516 in a small group of moderately obese men for 2 weeks showed significant reductions in total and LDL-C, apoB, triglycerides and nonesterified fatty acids (NEFA), without a change in HDL-C [10]. GW501516 treatment increased fatty acid oxidation and resulted in a significant reduction in liver fat (−20%) [10].

In a recent randomized, placebo-controlled study of 181 moderately obese mixed dyslipidemic patients treated for 8 weeks with two doses (50 mg/d and 100 mg/d) of a potent and selective PPAR-δ agonist (MBX-8025, Supplementary Fig. 1), we observed significant reductions in plasma triglycerides, apoB, and LDL-C. Treatment with atorvastatin (20 mg/d) resulted in a greater lowering of LDL-C, which was not further reduced in combination with MBX-8025 [11].

LDL is comprised of multiple subclasses differing in particle size and density [12], with evidence from a number of studies that smaller LDL particles are more strongly indicative of CVD risk than large LDL [13]. This relationship has been assessed both by measurement of absolute plasma concentrations of LDL subfractions, and by analysis of LDL particle size distributions, with determination of LDL subclass phenotypes representing predominance of smaller LDL (phenotype B) or larger LDL (phenotype A) [12]. In the present report, we have extended the findings of the recent trial of MBX-8025 [11] by analyzing the effects of MBX-8025 on concentrations of lipoprotein subfractions using the recently described technique of ion mobility (IM) [14,15]. The results demonstrate that atorvastatin reduced concentrations of all LDL subfractions, whereas MBX-8025 treatment resulted in reductions in smaller LDL particles with partially offsetting increases in large LDL.
particles. With the 100 mg/d dose of MBX-8025, reductions in smaller LDL were significantly greater than with atorvastatin, suggesting added benefit in CVD risk reduction.

2. Methods

2.1. Study design

Detailed characteristics of the participants and study design have been published elsewhere [11]. In brief, this study was a randomized, double-blinded, placebo-controlled, multicenter, parallel group trial carried out in non-diabetic overweight or obese individuals with mixed dyslipidemia. The eligible participants included men and women aged 18–75 years who were taking prescription or over-the-counter lipid lowering therapy with waist circumference ≥33” (women) and ≥38” (men) and with triglycerides 150–340 mg/dL, LDL-C 130–200 mg/dL, and HDL-C 35–60 mg/dL. Individuals on statins or ezetimibe/statin combination therapies had to meet the above lipid criteria after completing a wash-out during a single-blind placebo only run-in period. Also eligible were individuals who were untreated or diet treated, with triglycerides 150–550 mg/dL, LDL-C 130–280 mg/dL, and HDL-C ≤60 mg/dL. The study consisted of a screening period, a 5-week single-blind placebo only run-in period and an 8-week double-blind treatment period, followed by a final safety evaluation visit two weeks after the last dose was administered. Participants (n = 181, intention-to-treat, ITT) were randomized to one of six treatment groups: placebo; MBX-8025 50 mg/d; MBX-8025 100 mg/d; atorvastatin (ATV) 20 mg/d; MBX-8025/ATV 50/20 mg/d; or MBX-8025/ATV 100/20 mg/d. Of the ITT participants, 15 were excluded from the present analysis: 8 failed to complete the study (1 withdrew consent, 2 lost to follow-up, 5 with adverse events), 5 others did not have data at the 8 week time point, and 2 had significant protocol violations by using non-study lipid lowering therapy. The distribution of the 15 excluded patients was similar across treatment groups, and the results for the principal study endpoints in the ITT population [11] were nearly identical to those for the 166 patients whose data were used for the present analyses (Supplementary Tables 1 and 2). All participants provided informed written consent, and the study was approved by a central IRB.

2.2. Laboratory analyses

Blood was collected in the morning after an overnight fast for the measurement of plasma concentration of lipids (including total cholesterol, HDL-C, LDL-C, non-esterified fatty acids (NEFA), apoB-100), glucose and insulin (Supplementary Tables 1 and 2). Measurements were performed at ICON Central Laboratories, Inc. (Farmingdale, NY). The homeostasis model assessment of insulin resistance (HOMA-IR) index at day 1 (baseline) and Week 8 (end of treatment) was calculated as the product of the fasting plasma glucose (mg/dL) and fasting plasma insulin (mU/L) values, divided by 405.

A recently developed ion mobility method was used to directly quantify lipoprotein particles at baseline, mid-treatment (Week 4) and end of treatment (Week 8), over a wide range of sizes ranging from small HDL particles to large VLDL particles. This IM method measures both the size and concentrations of lipoprotein particle subclasses on the basis of gas-phase differential electric mobility. A detailed description of the IM technique is published elsewhere [14]. In brief, particle concentrations are measured in 11 size intervals ranging from 7.65 to 54.70 nm. For the analyses reported here, these have been grouped into the following fractions, using individual intervals previously defined [14]: VLDL-L (VLDL-Large, 42.40–54.70 nm), VLDL-M (VLDL-Medium, 33.50–42.39 nm), VLDL-S (VLDL-Small, 29.60–33.49 nm), IDL (IDL-Large, 25.00–29.59 nm), LDL-L (IDL-Small + LDL-L + LDL-2a, 22.20–24.99 nm), LDL-M (LDL-2b, 21.41–22.19 nm), LDL-S/VS (LDL-3a + LDL-3b + LDL-4a + LDL-4b + LDL-4c, 18.00–21.40 nm), HDL-L (IDL-Large, 10.50–14.50 nm), and HDL-S (IDL-Small, 7.65–10.49 nm).

2.3. Statistical analysis

All statistical analyses were performed using JMP 9 statistical software (SAS Institute, Cary, NC). Graphs were created using PRISM software (GraphPad v 5.01, San Diego, CA). Statistical comparisons were performed by paired t-test, one-way ANOVA, and two-way ANOVA at p < 0.05. The frequency distribution of the categorical variables for LDL phenotype conversion was compared between the groups with the Chi-square test (χ²). Data are expressed as means ± SEM. Values not sharing the same letter are significantly different by post hoc Tukey's multiple comparison tests. A multivariate analysis was used to assess potential correlations.

3. Results

3.1. Baseline characteristics and lipid levels

The 166 study participants were generally well-balanced across treatment groups in terms of demographic characteristics and baseline lipids (Supplementary Table 1). These data, as well as the percent changes in lipids, apoB, NEFA, and HOMA-IR from baseline (Supplementary Table 2) are similar to those of the ITT study population (n = 181) described previously [11]. There were no significant differences in particle concentrations of any of the lipoprotein subfractions measured by IM at baseline

| Table 1 |
|-----------------|--------|--------|--------|--------|--------|--------|
| Variables       | Placebo | 8025-50 | 8025-100 | ATV    | 8025-50/ATV | 8025-100/ATV |
| VLDL Total      | 194 ± 10 | 198 ± 10 | 193 ± 9  | 198 ± 10 | 224 ± 10 | 186 ± 10 |
| VLDL-L          | 26 ± 2  | 24 ± 2  | 25 ± 1   | 23 ± 2  | 27 ± 2  | 22 ± 2  |
| VLDL-M          | 76 ± 4  | 77 ± 4  | 76 ± 4   | 78 ± 4  | 87 ± 4  | 71 ± 4  |
| VLDL-S          | 92 ± 5  | 97 ± 5  | 92 ± 5   | 97 ± 5  | 111 ± 5 | 92 ± 5  |
| IDL             | 211 ± 11 | 224 ± 10 | 210 ± 10 | 218 ± 10 | 244 ± 10 | 207 ± 11 |
| LDL Total       | 1705 ± 76 | 1703 ± 74 | 1672 ± 68 | 1707 ± 73 | 1855 ± 74 | 1587 ± 76 |
| LDL-L           | 690 ± 47 | 712 ± 46 | 617 ± 42 | 701 ± 45 | 749 ± 46 | 628 ± 47 |
| LDL-M           | 342 ± 26 | 370 ± 25 | 393 ± 23 | 384 ± 25 | 376 ± 25 | 342 ± 26 |
| LDL-S/VS        | 673 ± 64 | 622 ± 63 | 662 ± 58 | 622 ± 62 | 731 ± 63 | 618 ± 64 |
| HDL Total       | 5454 ± 297 | 5722 ± 292 | 5447 ± 268 | 5160 ± 286 | 5206 ± 292 | 5224 ± 297 |
| HDL-L           | 923 ± 64 | 903 ± 63 | 905 ± 58 | 824 ± 62 | 1057 ± 63 | 839 ± 64 |
| HDL-S           | 4831 ± 266 | 4819 ± 261 | 4542 ± 240 | 4336 ± 256 | 4159 ± 261 | 4385 ± 266 |

There were no significant differences observed in all the baseline lipoprotein subclasses among treatment groups by one-way ANOVA at p < 0.05.
among the treatment groups (Table 1). The baseline and post-treatment particle concentrations correlate well with standard lipid and lipoprotein measurements as shown in Supplementary Fig. 2.

3.2. Changes in lipoprotein subfractions by treatments

Mean percent changes from baseline to Week 8 for all lipoprotein subfractions among treatment groups are shown in Fig. 1, and the statistical analyses of these data are presented in Table 2. Atorvastatin significantly reduced concentrations of all major apoB-containing lipoproteins (VLDL-Total, IDL and LDL-Total) to a similar extent (33–37%). MBX-8025 alone resulted in modest decreases in VLDL-Total (13–17%) and IDL (5–11%) particle concentrations that did not differ significantly from placebo. However, among the VLDL subfractions, there were significant reductions in VLDL-L in all treatment groups, and significant reductions in VLDL-M and VLDL-S for the ATV and combined drug groups. Changes in triglyceride were significantly associated with changes in VLDL-L ($p < 0.0001$) and VLDL-M ($p = 0.0028$) but not VLDL-S in all groups, including the placebo group. For VLDL-L and VLDL-M, there was a trend for additivity of the reductions with ATV plus MBX-8025, although there was no evidence of a dose–response effect for MBX-8025.

Changes in LDL subclasses were markedly different between MBX-8025 and ATV. With MBX-8025, the most pronounced changes were reductions of LDL-S/VS (40–48%) compared with a 25% decrease with atorvastatin treatment. The combination of MBX-8025 (50 mg/d) and atorvastatin led to an additional reduction in LDL-S/VS. In the case of LDL-L, there were also contrasting effects of MBX-8025 and ATV monotherapy: a 34–44% increase with the two doses of MBX-8025 vs. a 30% decrease with ATV. Reductions in LDL-M were significant in all treatment groups.
The moderate 13–14% decrease in LDL-Total with MBX-8025 monotherapy was due to increases in LDL-L partially offsetting the substantial decreases in LDL-S/VS (Table 2). With addition of MBX-8025 to ATV, there was additional reduction in LDL-S/VS particles compared to ATV alone, and attenuation of the increase in LDL-L seen with MBX-8025 alone.

Changes of total HDL particle concentrations did not differ significantly among the treatment arms, except for a much greater increase with MBX-8025 100 mg/d vs. ATV (44.2 vs. 0.9%, Table 2). With respect to HDL subfractions, in all treatment arms that included MBX-8025 there were greater increases of HDL-S than HDL-L, as well as reductions of HDL-L, compared to atorvastatin or placebo, but none of these differences were statistically significant by ANOVA except for the reduction of HDL-L with the combination of MBX-8025 vs. placebo and ATV.

There were significant differences among groups in the relative changes in plasma triglyceride vs. changes in VLDL particle concentrations (Supplementary Fig. 3). There were reductions in the triglyceride/VLDL particle ratio in both MBX-8025 groups, compared to increases in the ATV monotherapy group, with intermediate changes in the combination treatment groups. These results are consistent with differential effects of MBX-8025 and ATV on VLDL composition, and/or with greater reductions by MBX-8025 in larger, triglyceride-rich VLDL (>54.70 nm) that are not measured by the IM technique.

As shown in Table 3, in all groups combined, changes in LDL-S/VS were strongly correlated with changes in LDL-L and LDL-M, and there was a weaker and inverse correlation of LDL-S/VS with LDL-L. In contrast, changes in LDL-L were correlated strongly with changes in LDL-M, LDL-S, IDL and HLD-L, but weakly with VLDL-L. These relationships were not significantly altered after adjusting for change in plasma triglyceride as well as HOMA-IR (data not shown). Similar trends were observed with monotherapy with MBX-8025 and ATV, but most did not reach statistical significance after adjustment for multiple testing.

3.3. Changes in LDL peak particle size and LDL subclass phenotype conversions

Fig. 2 presents measurements of LDL peak particle diameter at baseline and following each of the drug treatments. Baseline values did not differ significantly among the groups. MBX-8025 significantly increased LDL peak diameter both as monotherapy and combined with ATV, whereas there was no significant change with ATV monotherapy.

LDL subclass phenotypes were determined based on peak LDL diameter as phenotype A (predominance of larger LDL with LDL peak diameter > 21.88 nm), phenotype B with predominance of smaller LDL particles (LDL peak diameter < 21.55 nm), or intermediate phenotype (phenotype I, with LDL peak diameter between 21.55 and 21.88 nm). For the purpose of the analyses presented here, because of relatively small numbers of individuals with phenotype I, it was included within the phenotype B category. Changes in LDL phenotypes with treatment (B → A and A → B) are shown in Fig. 3. As assessed by Chi-square analysis, and consistent with the changes in LDL peak diameter, there were significantly more conversions from phenotype B to phenotype A in all treatment groups compared to the placebo group (p < 0.0001) except the ATV group (Fig. 3A), whereas there were very few conversions from phenotype A to phenotype B in any of the groups (Fig. 3B). Overall >90% of individuals treated with MBX-8025 were classified as phenotype A by the end of treatment (Supplementary Table 3).

3.4. Atherogenic to anti-atherogenic lipoprotein particle ratio

The correlation analyses in Supplementary Fig. 2A indicates that non-HDL particle concentrations (sum of VLDL-Total + IDL + LDL-Total) were significantly correlated with multiple standard measures of CVD risk, specifically levels of LDL-C, apoB, and non-HDL-C, and that HDL-Total was correlated with HDL-C. To generate an overall estimate of treatment effects on lipoprotein-related CVD risk, we calculated the changes in ratio of non-HDL to HDL particle concentrations for each of the drug regimens (Supplementary Fig. 4). Compared with placebo, there were significant reductions in the ratio for all treatment arms, but there was a trend for greater reductions with MBX-8025 monotherapy and MBX-8025 plus ATV than with atorvastatin monotherapy.

![Fig. 2. LDL peak diameter at baseline and at Week 8 (nm, means ± SEM). Statistical comparisons were performed by two-way ANOVA. Values not sharing the same letter are significantly different by post hoc Tukey’s tests.](image-url)
Table 3
Correlations between lipoprotein subfractions (β-coefficients).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LDL-Subclass</th>
<th>VLDL-L</th>
<th>VLDL-M</th>
<th>VLDL-S</th>
<th>IDL</th>
<th>LDL-L</th>
<th>LDL-M</th>
<th>HDL-L</th>
<th>HDL-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>LDL-L</td>
<td>7.83</td>
<td>5.20</td>
<td>5.22</td>
<td>2.69</td>
<td>0.02</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDL-M</td>
<td>8.55</td>
<td>3.50</td>
<td>2.43</td>
<td>1.51</td>
<td>0.21</td>
<td>0.02</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDL-S/VS</td>
<td>15.82</td>
<td>4.17</td>
<td>1.64</td>
<td>0.98</td>
<td>0.38</td>
<td>0.36</td>
<td>0.17</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>LDL-L</td>
<td>15.44</td>
<td>5.84</td>
<td>4.23</td>
<td>2.04</td>
<td>0.08</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LDL-M</td>
<td>7.48</td>
<td>3.34</td>
<td>2.42</td>
<td>1.49</td>
<td>0.40</td>
<td>0.07</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>8025-100</td>
<td>LDL-S/VS</td>
<td>14.13</td>
<td>3.39</td>
<td>2.21</td>
<td>0.74</td>
<td>0.17</td>
<td>0.09</td>
<td>0.13</td>
<td>0.04</td>
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<tr>
<td></td>
<td>LDL-L</td>
<td>6.78</td>
<td>4.22</td>
<td>5.24</td>
<td>2.52</td>
<td>0.14</td>
<td>0.03</td>
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<tr>
<td></td>
<td>ATV-L</td>
<td>7.93</td>
<td>3.04</td>
<td>2.94</td>
<td>1.80</td>
<td>0.37</td>
<td>0.05</td>
<td>0.04</td>
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<tr>
<td></td>
<td>ATV-S/VS</td>
<td>10.54</td>
<td>2.46</td>
<td>0.57</td>
<td>1.02</td>
<td>0.37</td>
<td>0.78</td>
<td>0.23</td>
<td>0.05</td>
</tr>
<tr>
<td>Placebo</td>
<td>LDL-L</td>
<td>−2.48</td>
<td>0.01</td>
<td>3.14</td>
<td>1.51</td>
<td>0.36</td>
<td>0.13</td>
<td>0.01</td>
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<tr>
<td></td>
<td>LDL-S/VS</td>
<td>12.04</td>
<td>7.83</td>
<td>6.74</td>
<td>3.52</td>
<td>−0.57</td>
<td>0.26</td>
<td>0.36</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Only correlations with significance $p \leq 0.001$ are shown.

- $p \leq 0.001$.
- $p \leq 0.0001$.
- $p \leq 0.00001$.

4. Discussion

We have shown that in patients with mixed dyslipidemia, PPAR-δ activation by MBX-8025 results in a substantial reduction in levels of small LDL particles, and at a dose of 100 mg/d this decrease is nearly twice as great as that achieved by atorvastatin 20 mg/d. The reduction in LDL-S/VS with MBX-8025 was associated with, and partially offset by, an increase in LDL-L. Accordingly, there was a significant increase in LDL peak particle diameter, with reversal of the LDL subclass phenotype B in a high proportion of patients. In obese rhesus monkeys treated with GW501516, similar effects of PPAR-δ on LDL subclass particle redistribution were observed [1]. Among other dyslipidemic drugs, fenofibrate, a PPAR-α activator, also has been reported to induce a shift from smaller to larger LDL particles in conjunction with reduced plasma triglyceride [16–18]. Fenofibrate also increases LDL particle size, resulting in reversal of the atherogenic LDL phenotype B [16,19]. The underlying mechanism of fenofibrate on changes in LDL subclasses is attributed to the TG-lowering effect of fenofibrate, which is mediated both by decreased expression of apoCIII, a known inhibitor of lipoprotein lipase (LPL) activity, and by induction of LPL [20,21].

The effects of MBX-8025 on the lipoprotein profile differed distinctly from those of atorvastatin monotherapy. As shown by others [22], we found that atorvastatin resulted in reductions in particle concentrations of comparable magnitude across the full spectrum of apoB-100 containing lipoproteins. Although it has been reported that there is a small increase in LDL particle size with atorvastatin [22], we found no significant change in LDL peak diameter, consistent with other studies [17,23]. Notably, compared with MBX-8025, atorvastatin resulted in a reduction, rather than increase, of LDL-L, and a smaller reduction in LDL-S/VS. Moreover, in combination therapy, atorvastatin reversed the increase in LDL-L induced by MBX-8025, but did not result in attenuation of the MBX-8025-induced reductions in LDL-S/VS.

It has previously been proposed that the major LDL subclasses arise from lipolysis and intravascular remodeling by lipid exchanges and transfers among differing liver-derived lipoprotein precursors, with smaller LDL derived primarily from large VLDL precursors through intermediate size particles, and large LDL directly secreted by the liver or formed by lipolysis of small VLDL and IDL [12,24]. Atorvastatin’s primary effects on levels of apoB-containing lipoproteins are due primarily to increased LDL-receptor mediated clearance, although there is also evidence for reduced hepatic secretion of VLDL particles [25]. Overall, these effects result in relatively greater reductions of large LDL than small LDL by ATV (Fig. 1 and Table 2). In contrast, MBX-8025 causes a specific reduction in the large VLDL/small LDL pathway, with a corresponding shift to the pathway giving rise to large LDL. Because the ion mobility procedure as currently implemented does not measure VLDL particles larger than 54.70 nm, the present analyses do not allow an analysis of drug effects on triglyceride-rich particles in this size range. It is notable, however, that the ratio of plasma TG to VLDL particle number was reduced substantially more by...
MBX-8025 than by atorvastatin (Supplementary Fig. 3), consistent with a preferential reduction of TG-rich VLDL by MBX-8025. The extent to which this resulted from reduced VLDL TG secretion vs. increased peripheral TG clearance remains to be determined.

The mechanisms underlying PPAR-δ effects on lipid and lipoprotein metabolism remain poorly understood. In rodent models, PPAR-δ activation has been shown to decrease lipid accumulation in liver, muscle and adipose tissue and to ameliorate diet-induced weight gain primarily by promoting fatty acid oxidation and energy expenditure [3–5]. In PPAR-δ null mice on a Western diet, there was an increase in plasma triglyceride compared to PPAR-δ +/- mice that was associated with a greater rate of hepatic VLDL production, together with reduced post-hepatic lipoprotein lipase activity. Interestingly, hepatic fat content was reduced in this model, perhaps reflecting increased VLDL secretion [26]. A recent extensive metabolic profiling study in ob/ob mice treated with a PPAR-δ agonist further supports a systemic role of PPAR-δ activation in increasing hepatic and peripheral fatty acid oxidation [27]. The molecular basis of these effects is not understood. It is likely that a number of PPAR-δ targets that affect lipid metabolism, such as angiopoietin-like 4 [28] are involved. In humans, treatment of moderately obese men with the PPAR-δ activator GW501516 resulted in a significant increase in expression of the gene for carnitine palmityl-transferase 1b in skeletal muscle, in conjunction with increased fatty acid oxidation, as well as a reduction in liver fat [10]. Given the relation of hepatic triglyceride content to VLDL secretion [29,30], it is possible that our finding of reduced levels of larger VLDL with MBX-8025 reflects, in part, a smaller pool of hepatic triglyceride available for secretion.

Despite consistent increases of HDL cholesterol in monkeys and rodents treated with PPAR-δ agonists [1,2,6,7], only small changes in HDL cholesterol have been observed in humans [9,10], including those in the present study [11]. Although we observed a trend for increased particle concentration of total HDL with MBX-8025 that was confined to smaller HDL, none of the changes differed significantly from placebo. Nevertheless, estimated changes in CVD risk as assessed by ratios of non-HDL to HDL particle concentration indicated comparable reductions with MBX-8025 and ATV treatment, with the suggestion that MBX-8025 may add to the benefit of ATV.

In summary, PPAR-δ activation with MBX-8025 in patients with mixed dyslipidemia produced substantial reductions in small and very small LDL particles that were associated with lower levels of larger VLDL, and resulted in reversal of LDL phenotype B in a high proportion of patients. The reciprocal increase in large LDL was reversed by co-administration of atorvastatin 20 mg/d, which had a lesser effect on smaller LDL. The mechanisms of these effects, and their clinical significance, merit further study.

Disclosure
Metabolex funded this clinical trial (Clinical trial registration number: NCT00701883).

Ronald M. Krauss is a member of the Metabolex Scientific Advisory Board and the Merck Global Atherosclerosis Advisory Board, and is co-inventor on a patent for use of ion mobility methodology for lipoprotein particle analysis. Yun-Jung Choi, Brian K. Roberts, Xueyan Wang, and Sue Naim are employees of Metabolex Inc., the sponsor of this clinical trial. David B. Karpf was an employee of Metabolex, but he is not employed by Metabolex at the time of manuscript submission. J. Casey Geaney, Kathleen Wojnoonski and Ronald M. Krauss are employees of Children’s Hospital Oakland Research Institute.

Appendix A. Supplementary data

References


Supplement

Supplementary Table 1. Baseline characteristics of plasma lipids and lipoprotein in 166 study participants (means ± SEM).

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>Placebo</th>
<th>8025-50</th>
<th>8025-100</th>
<th>ATV</th>
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<td>32</td>
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<td>27</td>
<td>26</td>
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<td>Age (years)</td>
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<td>55.8 ± 2.0</td>
<td>54.3 ± 1.9</td>
<td>55.1 ± 1.8</td>
<td>53.8 ± 1.9</td>
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<td>53.2 ± 2.0</td>
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<td>Male (n, %)</td>
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<td>13 (50)</td>
<td>16 (59)</td>
<td>15 (47)</td>
<td>13 (46)</td>
<td>12 (44)</td>
<td>11 (42)</td>
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<td>BMI (kg/m²)</td>
<td></td>
<td>33.5 ± 1.1</td>
<td>33.3 ± 1.1</td>
<td>31.8 ± 1.0</td>
<td>34.0 ± 1.1</td>
<td>33.8 ± 1.1</td>
<td>35.3 ± 1.1</td>
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<tr>
<td>Total cholesterol (mg/dL)</td>
<td></td>
<td>247.6 ± 6.3</td>
<td>253.6 ± 6.3</td>
<td>250.6 ± 5.7</td>
<td>243.3 ± 6.0</td>
<td>255.6 ± 6.2</td>
<td>240.7 ± 6.3</td>
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<td>LDL cholesterol (mg/dL)</td>
<td></td>
<td>160.0 ± 5.4</td>
<td>169.0 ± 5.3</td>
<td>163.6 ± 4.9</td>
<td>164.8 ± 5.2</td>
<td>172.7 ± 5.3</td>
<td>158.3 ± 5.4</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td></td>
<td>44.0 ± 1.5</td>
<td>44.0 ± 1.5</td>
<td>44.3 ± 1.4</td>
<td>42.2 ± 1.5</td>
<td>43.6 ± 1.5</td>
<td>42.7 ± 1.5</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td></td>
<td>235.1 ± 15.5</td>
<td>213.2 ± 15.5</td>
<td>236.3 ± 14.0</td>
<td>192.9 ± 15.0</td>
<td>200.4 ± 15.2</td>
<td>207.7 ± 15.5</td>
</tr>
<tr>
<td>Apo B-100 (mg/dL)</td>
<td></td>
<td>116.7 ± 3.5</td>
<td>122.8 ± 3.4</td>
<td>121.1 ± 3.2</td>
<td>120.0 ± 3.3</td>
<td>125.1 ± 3.4</td>
<td>115.6 ± 3.5</td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td></td>
<td>0.57 ± 0.05</td>
<td>0.58 ± 0.05</td>
<td>0.58 ± 0.04</td>
<td>0.58 ± 0.04</td>
<td>0.48 ± 0.04</td>
<td>0.58 ± 0.05</td>
</tr>
<tr>
<td>HOMA-IR*</td>
<td></td>
<td>3.1 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>2.7 ± 0.3</td>
<td>2.9 ± 0.3</td>
<td>3.0 ± 0.4</td>
<td>2.7 ± 0.5</td>
</tr>
</tbody>
</table>

There were no significant differences observed in any of the baseline variables among treatment groups by one-way ANOVA at p < 0.05.

* Data are shown as median ± SE
Supplementary Table 2. Percent change from baseline to Week 8 in plasma lipids and lipoproteins in 166 study participants (means ± SEM).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo</th>
<th>8025-50</th>
<th>8025-100</th>
<th>ATV</th>
<th>8025-50/ATV</th>
<th>8025-100/ATV</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.1 ± 0.4</td>
<td>0.1 ± 0.4</td>
<td>0.2 ± 0.3</td>
<td>-0.1 ± 0.4</td>
<td>0.3 ± 0.4</td>
<td>0.1 ± 0.4</td>
<td>0.9746</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>-1.6 ± 2.1*</td>
<td>-15.6 ± 2.1 b</td>
<td>-16.9 ± 1.9 b</td>
<td>-32.4 ± 2.0 c</td>
<td>-32.6 ± 2.1 c</td>
<td>-33.1 ± 2.1 c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>-1.8 ± 2.8*</td>
<td>-18.5 ± 2.7 b</td>
<td>-21.8 ± 2.5 b</td>
<td>-42.5 ± 2.7 c</td>
<td>-42.8 ± 2.7 c</td>
<td>-42.1 ± 2.8 c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>1.0 ± 3.2 ab</td>
<td>10.6 ± 3.2 ab</td>
<td>13.2 ± 2.9 a</td>
<td>0.9 ± 3.1 b</td>
<td>14.0 ± 3.2 a</td>
<td>2.1 ± 3.2 ab</td>
<td>0.0018</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-3.1 ± 6.2 a</td>
<td>-34.5 ± 6.2 b</td>
<td>-32.7 ± 5.6 b</td>
<td>-17.1 ± 5.9 ab</td>
<td>-35.8 ± 6.1 b</td>
<td>-33.7 ± 6.2 b</td>
<td>0.0005</td>
</tr>
<tr>
<td>Apo B-100</td>
<td>0.2 ± 2.9 a</td>
<td>-20.4 ± 2.9 b</td>
<td>-19.9 ± 2.7 b</td>
<td>-37.3 ± 2.8 c</td>
<td>-38.0 ± 2.9 c</td>
<td>-34.2 ± 2.9 c</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NEFA</td>
<td>11.5 ± 8.4</td>
<td>-16.9 ± 8.4</td>
<td>-14.2 ± 7.7</td>
<td>1.1 ± 8.2</td>
<td>-19.4 ± 8.2</td>
<td>-8.0 ± 8.4</td>
<td>0.0719</td>
</tr>
<tr>
<td>HOMA-IR*</td>
<td>-5.2 ± 7.5 a</td>
<td>-8.0 ± 8.0 a</td>
<td>-27.3 ± 7.7 b</td>
<td>-11.0 ± 7.6 a</td>
<td>-13.2 ± 22.0 a</td>
<td>-1.6 ± 16.8 a</td>
<td>0.0141</td>
</tr>
</tbody>
</table>

Statistical comparisons were performed by one-way ANOVA at p < 0.05. Values not sharing the same letter are significantly different by *post hoc* Tukey’s tests. * Data are shown as median percent change ± SE and statistical comparisons were performed by Kruskal-Wallis test.
Supplementary Table 3. LDL subclass pattern conversion for individual study participants from baseline to Week 8.

<table>
<thead>
<tr>
<th>Baseline</th>
<th>8 week</th>
<th>Placebo</th>
<th>8025-50</th>
<th>8025-100</th>
<th>ATV</th>
<th>8025-50/ATV</th>
<th>8025-100/ATV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6 (60%)</td>
<td>9 (100%)</td>
<td>6 (100%)</td>
<td>9 (75%)</td>
<td>8 (89%)</td>
<td>7 (100%)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4 (40%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>3 (25%)</td>
<td>1 (11%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0 (0%)</td>
<td>16 (89%)</td>
<td>24 (92%)</td>
<td>3 (19%)</td>
<td>16 (89%)</td>
<td>15 (79%)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>16 (100%)</td>
<td>2 (11%)</td>
<td>2 (8%)</td>
<td>13 (81%)</td>
<td>2 (11%)</td>
<td>4 (21%)</td>
<td></td>
</tr>
</tbody>
</table>
Supplementary Figure Legends

Supplementary Fig. 1. MBX-8025 is a potent and selective PPAR-δ Agonist.

Human PPAR-α, PPAR-δ and PPAR-γ activity was monitored in transiently transfected cells treated with increasing concentrations of MBX-8025 (●) in comparison with reference compounds (■) for individual subtypes. The PPAR subtype selectivity of MBX-8025 was evaluated in a cell-based GAL4 reporter assay system. MBX-8025 selectively activated the human PPAR-δ with an EC₅₀ of 2 nM when compared to micromolar range potency for the PPAR-α and PPAR-γ subtypes. Data are expressed as fold inductions compared with vehicle-treated cells and represent the mean of assays performed in triplicate ± SEM.

Supplementary Fig. 2. Relationships between biochemical and IM measurements in approximately 490 plasma samples combined from all treatment groups at baseline, Week 4 and Week 8.

Correlation coefficient (r) and linear regression line are shown for each comparison. X-axes represent IM measured particle concentrations of LDL, IDL plus LDL, Non-HDL and HDL subclasses. Y-axes represent biochemical measurements of LDL cholesterol, Non-HDL cholesterol, ApoB-100 and HDL cholesterol. As expected, ApoB-100 and Non-HDL particle concentrations show the strongest correlation.

2A. LDL cholesterol, Non-HDL cholesterol and ApoB-100 vs. IM-measured LDL, IDL plus LDL and Non-HDL.

2B. HDL cholesterol vs. IM-measured HDL, HDL small and HDL large.
Supplementary Fig. 3. Mean percent changes in TG/VLDL ratio from baseline to Week 8 with placebo, MBX-8025, atorvastatin and their combination (means ± SEM). Statistical comparisons were performed by one-way ANOVA. Values not sharing the same letter are significantly different by post hoc Tukey’s tests.

Supplementary Fig. 4. Changes in non-HDL/HDL particle ratio (means ± SEM) Statistical comparisons were performed by one-way ANOVA at p < 0.05. Values not sharing the same letter are significantly different by post hoc Tukey’s tests.
Supplementary Fig. 1

Human PPAR-δ

Human PPAR-α

Human PPAR-γ
Supplementary Fig. 2

2A

2B
Supplementary Fig. 3

TG/VLDL

% Change from Baseline

Placebo 8025-50 8025-100 ATV 8025-50 /ATV 8025-100 /ATV

abc bc c a ab abc
Supplementary Fig. 4

Non-HDL/HDL Particle Ratio

% Change from Baseline