Effect of troglitazone on plasma lipid metabolism and lipoprotein lipase

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Summary:To clarify how troglitazone, an insulin-sensitizing agent, affects lipid metabolism and post-heparin plasma lipoprotein lipase(LPL), fifteen patients (3 male, 12 female) [the average age 62 ± 7 y; the mean body mass index (BMI) 25 ± 3 kg/m²] were recruited and the serum lipids and postheparin plasma lipoprotein lipase(LPL) mass before and 4 weeks after oral administration of troglitazone (200 mg per day) were measured. Mouse preadipocyte cell line, 3T3-L1 cells were treated with this compound and LPL enzyme protein mass in the culture media was measured by an enzyme linked immunosorbent assay. A reverse transcription polymerase chain reaction (RT-PCR) and Northern blot analysis was conducted to investigate the effect of this compound on the expression of LPL. The average levels before treatment of fasting serum total cholesterol, triglycerides and high density lipoprotein-cholesterol, plasma glucose and glycohemoglobin A1c were 216± 34, 160±84, 57± 19, 145±30 mg/dl and 7.8± 1.6 %. Four weeks after treatment, those levels were 209±36, 105 ±27 (p=0.004 ), 63 ±19 (p=0.02 ) mmol/l, 139±41 mg/dl and 7.3±0.6 % (p=0.01), respectively. The postheparin plasma LPL mass increased from 226 ± 39 to 257 ± 68 ng/ml (p = 0.03 ) during that period. RT-PCR and Northern blot analysis revealed that in the cultured 3T3-L1 cells, the expression of LPL was enhanced in the presence of troglitazone. These results suggest that troglitazone improves plasma triglyceride-rich lipoproteins metabolism by enhancing the expression of LPL in adipocytes.

Key words. Troglitazone, Lipoprotein lipase, 3T3-L1 cells, Insulin

Introduction
Troglitazone, a thiazolidinedione derivative, is a novel oral anti diabetic agent that lowers plasma glucose levels and enhances insulin action in obese animals [1]. It is effective in lowering plasma glucose, reducing hyperinsulinemia, and correcting glucose metabolism in animal models of obesity and NIDDM as well as improving insulin sensitivity in cultured cells [2,3]. Lipoprotein lipase (LPL) is a lipolytic enzyme which catalyzes the hydrolysis of triglycerides in chylomicrons and very low density lipoproteins (VLDL). This enzyme is well known to be regulated by insulin both transcriptionally[4] and posttranscriptionally[5]. In this report, we investigated the effect of troglitazone, which enhances insulin action, on the plasma lipid metabolism in patients with hyperlipidemia and diabetes mellitus and its mechanisms for lowering plasma triglycerides from the aspect of LPL using mouse preadipocyte cell line, 3T3 - L1 cells.

EFFECT OF TROGLITAZONE ON PLASMA LIPOPROTEINS AND GLUCOSE LEVELS

The average levels before treatment of fasting serum total cholesterol, triglycerides and high density lipoprotein-cholesterol, plasma glucose and glycohemoglobin A1c were 216 ± 34, 160±84, 57± 19, 145±30 mg/dl and 7.8± 1.6 %. Four weeks after treatment, those
levels were 209 ± 36, 105 ± 27 (\( p=0.004 \)), 63 ± 19 (\( p=0.02 \)), 139 ± 41 mg/dl and 7.3 ± 0.6 % (\( p=0.01 \)), respectively. The postheparin plasma LPL mass increased from 226 ± 39 to 257 ± 68 ng/ml (\( p = 0.03 \)) during that period.

**EFFECT OF TROGLITAZONE ON MOUSE LPL DETERMINED BY RT-PCR (Fig 1)**

To determine how troglitazone affects expression of LPL mRNA from the 3T3-L1 cells, we carried out RT-PCR using primers specific for the carboxyl terminal mouse LPL cDNA in four separate PCR cycles. The area of the bands of the RT-PCR product for the carboxyl terminal mouse LPL in the cells extracts cultured in the presence of troglitazone was significantly larger than those from the cells cultured in the absence of troglitazone.

<table>
<thead>
<tr>
<th>cycles</th>
<th>Tro(-)</th>
<th>Tro(+)</th>
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<tbody>
<tr>
<td>20</td>
<td>![LPL band](405 bp)</td>
<td>![LPL band](405 bp)</td>
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<tr>
<td></td>
<td>![Cy B band](374 bp)</td>
<td>![Cy B band](374 bp)</td>
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<tr>
<td>25</td>
<td>![LPL band](405 bp)</td>
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<td>![Cy B band](374 bp)</td>
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Figure 1
A 405 base pair RT-PCR products for either the carboxyl terminal of mouse LPL or mouse cyclophilin B in 3T3-L1 cells cultured with or without 10 \( \mu M \) troglitazone. For identification of amplification products, 10 µl of each reaction mixture was electrophoresed on a 1.5 % agarose gel containing ethidium bromide.
- Tro(-), in the presence of 10 \( \mu M \) troglitazone
- Tro(+), in the presence of 10 \( \mu M \) troglitazone
- LPL, A cDNA fragment corresponding to carboxyl terminal mouse lipoprotein lipase
- Cy B, Mouse cyclophilin B cDNA

**NORTHERN BLOT ANALYSIS OF RNA IN THE 3T3-L1 CELLS FOR EXPRESSION OF LPL (Fig. 2)**

Northern blot analysis was carried out for the RNA from the 3T3-L1 cells cultured with or without 10 \( \mu M \) of troglitazone. The signal for LPL mRNA extracted in duplicate from 3T3-L1 cells in separate culture wells was found to be far stronger in 3T3-L1 cells cultured in the presence of 10 \( \mu M \) of troglitazone than without this compound, despite the fact that same amounts of total RNA (20 µg of RNA for each lane) were run.