ELSEVIER

Atherosclerosis 193 (2007) 343–351

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

The different effect of pioglitazone as compared to insulin on expression of hepatic and intestinal genes regulating post-prandial lipoproteins in diabetes

S. Lally, D. Owens, G.H. Tomkin *

Department of Diabetes and Endocrinology, Trinity College, Dublin 2, Ireland

Received 16 May 2006; received in revised form 30 August 2006; accepted 27 September 2006
Available online 15 November 2006

Abstract

This study investigates lipoprotein composition in diabetes before and after treatment with insulin or pioglitazone and its relationship to gene expression of five genes found in liver and intestine which are involved in cholesterol homeostasis. Thirty zucker diabetic fatty fa/fa and 10 lean rats were examined. mRNA for 3-hydroxy3-methylglutaryl coenzyme A reductase (HMGCoA), microsomal triglyceride transfer protein (MTTP), Niemann Pick C1-like 1 (NPC1L1) and ATP binding cassette transporters (ABC) G5 and G8 was determined using real-time, reverse transcriptase (RT-PCR). Cholesterol, triglyceride, apo B48 and apo B100 were elevated in chylomicrons and very low density lipoproteins (VLDL) of untreated diabetic animals ($p < 0.02$). For similar blood glucose pioglitazone was more effective than insulin in normalising the lipoproteins. In diabetic animals, HMGCoA reductase, MTTP and NPC1L1 mRNA were significantly elevated ($p < 0.02$) and ABCG5 and ABCG8 were significantly reduced ($p < 0.02$) in the liver. Pioglitazone significantly reduced hepatic MTTP and NPC1L1 mRNA ($p < 0.0001$) and significantly increased ABCG5 and G8 mRNA ($p < 0.0001$) as compared to insulin. In conclusion diabetes was associated with major changes in mRNA levels of proteins involved in the regulation of post-prandial lipoproteins. Pioglitazone and insulin have different effects on post-prandial lipoprotein metabolism in part due their effect on genes regulating cholesterol synthesis and lipoprotein assembly.

Keywords: ABCG5; ABCG8; apo B48; apo B100; Chylomicron composition; Microsomal triglyceride transfer protein (MTTP); Neimann Pick C1-like1 (NPC1L1); 3-Hydroxy 3-methylglutaryl co-enzyme A (HMGCoA) reductase; Type 2 diabetes

Diabetes is associated with an increase in atherosclerosis. The major risk factors include dyslipidaemia and, in diabetes, triglyceride is often raised and HDL is low. The post-prandial phase in diabetes is thought to be particularly atherogenic [1,2]. We have previously suggested that the early increase in apo B48 in diabetic patients in the post-prandial phase may be due to increase in synthesis of chylomicron particles [3] and we have confirmed this to some extent by demonstrating an increase in microsomal triglyceride transfer protein (MTTP) mRNA in diabetic patients [4,5]. Synthesis of the chylomicron particle depends on the availability of both cholesterol and triglyceride in the intestine. Intestinal cholesterol synthesis has been shown to be increased in type 2 diabetes whereas cholesterol absorption is usually normal or may be reduced [6,7]. The recent discovery of Niemann Pick C1-like1 (NPC1L1) protein which regulates sterol absorption [8] and the ATP binding cassette proteins (ABC) G5 and G8 which work in tandem to excrete enterocyte cholesterol back into the lumen of the intestine [9] has given us new tools to explore chylomicron and VLDL synthesis in diabetes. In human studies we have been able to examine the intestine but not liver and have demonstrated a significant increase in NPC1L1 and a decrease in ABCG5 and G8 in type 2 diabetic patients which was related to abnormal chylomicron composition suggesting an important role for these

Abbreviations: ABC, ATP binding cassette protein; MTTP, microsomal triglyceride transfer protein; NPC1L1, Niemann Pick C1-like 1; HMGCoA, 3-hydroxy 3-methylglutaryl co-enzyme A reductase

* Corresponding author at: Diabetes Research Foundation, Clontra, Quinns Road, Shankill, Co Dublin, Ireland. Tel.: +353 1 2390658; fax: +353 1 2721395.
E-mail address: gerald.tomkin@tcd.ie (G.H. Tomkin).

0021-9150/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2006.09.031

genes in the the dysregulation of chylomicron metabolism in diabetes [5]. The peroxisome proliferator activated receptor (PPAR) agonists, the thiazolidinediones, improve insulin sensitivity and dyslipidaemia by altering fat storage and lipid metabolism. PPARα has a specific effect on triglycerides through reduction in synthesis and increase in turnover [10]. PPARγ agonists improve insulin sensitivity, perhaps mostly by their effect on the adipocyte [11]. The thiazolidinediones however have many other effects and alter more than 100 genes. It has been shown in a large prospective study that they may improve cardiovascular risk [12] though this has been disputed [13]. They have been shown to increase lipolysis of VLDL by increasing lipoprotein lipase mass and decreasing plasma levels of apo C111 [10]. Insulin has been shown in diabetic patients to reduce apo B48 and apo B100 post-prandial lipoproteins [14]. The purpose of the present study was to investigate both hepatic and intestinal expression of genes regulating chylomicron and VLDL synthesis in zucker diabetic fatty rats compared to lean littermates and to examine the effect of pioglitazone as compared to insulin treatment on mRNA expression and lipoprotein composition.

1. Methods

Thirty male zucker diabetic fatty rats and 10 lean littermates (non-diabetic) were investigated. Animals were housed under licence from the Department of Health, Dublin Ireland and experiments were carried out according to Irish law as administered by the Department of Health. Diabetes was confirmed when the rats were 9 weeks old by a blood sugar of >7.5 mmol/l. Ten of the diabetic rats were then treated with pioglitazone (0.1 mg/day administered by gavage) for 2 weeks and 10 with insulin (0.25 IU/day by injection) (Novorapid, NovoNordisk Denmark). Rats were fed a normal rat chow diet during this period. They were weighed at the beginning of the study and again before sacrifice and food intake was monitored on a daily basis. At the end of the study all of the rats were exsanguinated in the post-prandial phase and plasma was stored for chylomicron and VLDL isolation. The liver was removed and samples stored in RNAlater and the intestine was washed, mucosa scraped and samples stored in RNAlater for MTTP, ABCG5 and G8 HMGCoA reductase and NPC1L1 mRNA determination.

1.1. Lipoprotein isolation and apo B48 and B100 determination

Chylomicrons and VLDL were isolated from the plasma by sequential ultracentrifugation as previously described [14]. Lipoprotein fractions were stored at 4 °C and lipoproteins measured within 1 week. Chylomicron and VLDL apo B48, and apo B100 were separated by SDS-polyacrylamide gel electrophoresis using 4–15% gradient gels (Biorad, Hercules, CA, USA) as previously described [14].

1.2. Biochemical analyses

Venous blood glucose levels were determined according to an enzymatic colorimetric method using a commercially available diagnostic kit (Boehringer Mannheim GmBH, Mannheim, Germany). Total cholesterol and triglyceride content of lipoprotein fractions were measured by an enzymatic colorimetric method using kits from Boehringer Mannheim GmBH (Mannheim, Germany).

1.3. Total RNA extraction

Ten mg of liver or intestine which had been stored in RNAlater was homogenised in RLT buffer (Qiagen, Crawley, UK) using a Mixer-Mill 300 (Qiagen Crawley, UK) and 5 mm stainless steel beads. The RNA content from this homogenised sample was then extracted using the RNeasy® Mini Isolation Kit (Qiagen, Crawley, UK). The sample was treated with an RNase-free DNase set to eliminate any contaminating DNA. The RNA was then eluted into 50 μl RNase-free H_2O, aliquoted and stored.

1.4. RNA quantification

RNA was quantified using the Quant-iT Ribogreen RNA Assay Kit (Molecular Probes, Eugene, Origon, USA). An RNA standard curve was constructed using dilutions of a supplied, known amount of RNA, and read on a dual band plate reader with an excitation wavelength of 500 nm and an emission wavelength of 525 nm. Sample RNA concentrations were calculated using linear regression from this standard curve.

1.5. Reverse transcription

RNA was reverse transcribed as part of the two-step real-time RT-PCR to cDNA using TaqMan Reverse Transcription Reagents (Applied Biosystems, Dublin, Ireland). A GeneAmp 2400 PCR System (Applied Biosystems, Dublin Ireland) was used and the conditions were: 10 min at 25 °C, 30 min at 48 °C and 5 min at 95 °C. Eight hundred ng of each sample in 100 μl was reverse transcribed to cDNA, with 80 ng of the cDNA to be used in the next step.

1.6. Real-time PCR

Ten μl of the cDNA containing 80 ng was used in the real-time PCR analysis. The rat-specific primers and probes for the genes of interest were purchased from Applied Biosystems using the assay on demand system. Thermal cycling conditions and volumes of ingredients are already optimised using this system. The thermal cycling conditions used were: stage 1, 50 °C for 2 min; stage 2, 95 °C for 10 min; stage 3, 40 repetitions of 95 °C for 15 s alternating with 60 °C for 1 min. A sample volume of 25 μl was used in each well. Primers and probes were used from a 20× stock solution and the final