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Short-term Acipimox decreases the ability of plasma from Type 2 diabetic patients and healthy subjects to stimulate cellular cholesterol efflux: a potentially adverse effect on reverse cholesterol transport

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Abstract

Aims To evaluate the effect of short-term administration of the anti-lipolytic agent, Acipimox, on the ability of plasma to stimulate cellular cholesterol removal, which represents one of the first steps in the anti-atherogenic process of reverse cholesterol transport.

Methods Eight male Type 2 diabetic patients and eight healthy subjects were studied after a 12-h fast at baseline, after 24 h of Acipimox administration, 250 mg every 4 h, and again after 1 week (recovery). Plasma lipids, apolipoprotein AI, phospholipid transfer protein (PLTP) activity, pre-β high-density lipoproteins (HDL) in incubated plasma and efflux of radiolabelled cholesterol from Fu5AH rat hepatoma cells to plasma were measured at each time point.

Results Acipimox lowered plasma triglycerides in diabetic patients ($P = 0.001$) and healthy subjects ($P = 0.002$), whereas plasma non-esterified fatty acids were decreased in diabetic patients ($P = 0.001$) compared with the averaged values at baseline and recovery. Acipimox decreased HDL cholesterol in healthy subjects ($P = 0.007$) and plasma apolipoprotein AI in both groups ($P = 0.001$ for diabetic patients; $P = 0.008$ for healthy subjects). Not only plasma PLTP activity ($P = 0.001$ for diabetic patients; $P = 0.01$ for healthy subjects), but also pre-β HDL in incubated plasma ($P = 0.001$ for diabetic patients; $P = 0.03$ for healthy subjects) and cellular cholesterol efflux to plasma ($P = 0.04$ for diabetic patients; $P = 0.005$ for healthy subjects) were lowered by Acipimox in both groups.

Conclusions Short-term Acipimox administration impairs the ability of plasma from Type 2 diabetic patients and healthy subjects to stimulate cellular cholesterol efflux, in conjunction with alterations in HDL parameters and in PLTP activity. If the impairment of cellular cholesterol efflux to plasma is sustained with long-term treatment, this potentially adverse effect should be considered when treating diabetic dyslipidaemia with Acipimox.

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Abbreviations apo, apolipoprotein; BMI, body mass index; HbA_{1c}, glycated haemoglobin; HDL, high density lipoproteins; NEFA, non-esterified fatty acids; PLTP, phospholipid transfer protein

Introduction

The cardioprotective effect of high density lipoproteins (HDL) [1] is commonly explained by the role of HDL in reverse cholesterol transport [2]. By this process cholesterol is removed from peripheral cells by HDL and then transported to the liver for metabolism and excretion. Efflux of free cholesterol from cell membranes to extracellular acceptors is one of the first steps in reverse cholesterol transport [3]. The mechanisms responsible for cellular cholesterol removal are complex and still incompletely understood [4,5]. Lipid-poor pre-β HDL particles are considered to represent the initial acceptors of cellular cholesterol [6,7]. Among other factors, plasma phospholipid transfer protein (PLTP) is involved in HDL remodelling. This lipid transfer protein converts HDL in smaller and larger particles [8], and during this process pre-β HDL particles are generated [7,9]. Over-expression of human PLTP in mice enhances pre-β HDL generation and inhibits cholesterol accumulation in macrophages [9]. PLTP may also promote cellular cholesterol removal via binding to the cell membrane [10]. Cholesterol efflux out of Fu5AH rat hepatoma cells, a well-validated cell culture system [4,11], is positively correlated with PLTP activity in human plasma [12]. This indeed suggests that a high PLTP activity enhances the potential of plasma to promote cellular cholesterol removal. It was recently shown that plasma PLTP activity is lowered after 24 h administration of the anti-lipolytic agent, Acipimox [13], a drug used to ameliorate diabetic dyslipidaemia. We hypothesized that an Acipimox-induced decrease in plasma PLTP activity would result in less pre-β HDL generation and in a diminished cellular cholesterol efflux-promoting capacity of plasma.

In the present study, we document the effect of 24 h Acipimox administration on the ability of plasma from Type 2 diabetic patients and healthy subjects to stimulate cellular cholesterol removal, as well as on pre-β HDL generation.

Patients and methods

The study was approved by the medical ethics committee and informed consent was obtained from all subjects. Eight male patients with Type 2 diabetes mellitus [14], and eight healthy men participated. Subjects with clinically manifest cardiovascular disease, renal disease (serum creatinine > 120 μmol/l or microalbuminuria > 20 μg/min), blood pressure ⩾ 160/95 mmHg, thyroid dysfunction, liver disease and smokers were excluded. Maximal alcohol allowance was three beverages per day. Four diabetic patients used sulphonylurea and four patients received sulphonylurea and metformin. No other drugs were used. The subjects were studied at baseline, after 24 h of Acipimox administration, 250 mg every 4 h, and again after 1 week (recovery). They refrained from alcohol and strenuous physical activity during 24 h before blood sampling. On each occasion venous blood was obtained after a 12-h fast. The participants consumed their habitual diet and the diabetic patients continued their blood glucose-lowering drugs during the study period. Body mass index (BMI, in kg/m^2) was calculated as weight divided by height squared.

Laboratory methods

Venous blood was collected into EDTA (1.5 mg/ml)-containing tubes. Plasma was separated immediately at 4°C and stored at –80°C. Plasma non-esterified fatty acids (NEFA), triglycerides and cholesterol were measured enzymatically [15]. Apo B-containing lipoproteins were precipitated with polyethylene glycol-6000 and HDL cholesterol was measured in the supernatant [15]. Plasma apolipoprotein (apo) A1 was assayed by immunoturbidimetry [15]. Plasma pre-β HDL generation was measured after incubation of plasma at 37°C for 16 h under conditions of lecithin:cholesterol acyltransferase inhibition with 1 mmol/l iodoacetate [9,16] and was expressed as its apo AI concentration in g/l. α HDL and pre-β HDL were separated using one-dimensional agarose electrophoresis with inclusion of a reference sample with a known amount of pre-β HDL (0.171 g of apo AI per ml in pre-β HDL) on each gel. This was done because different plasma dilutions may give rise to different proportions of pre-β HDL and α HDL [17]. Four-fold diluted plasma samples (3 μl) were loaded on the gels. The HDL subfractions were separated at 100 V during 1 h, followed by blotting on nitrocellulose membranes. After 1 h at room temperature in blocking buffer, the membranes were incubated with 100-fold diluted rabbit anti-human apo AI during 1 h, followed by overnight incubation at 4°C. Subsequently, the membranes were extensively washed, followed by 3 h incubation at room temperature with 1000-fold diluted donkey ^{125}I-labelled anti-rabbit $F(ab')_2$ fragments. Thereafter, the nitrocellulose membranes were extensively washed and dried in air, followed by quantification of radioactivity in pre-β HDL.

Plasma PLTP activity was measured by a radioisotopic method using a phospholipid vesicles-HDL system as described [15,18]. Plasma PLTP activity levels are linearly correlated with the amount of plasma added to the incubation system and were

expressed as percentage of the activity in a human reference plasma. Cellular cholesterol efflux to plasma was measured using Fu5AH rat hepatoma cells as described [11]. Fu5AH cells were grown to confluence in the presence of ^{3}H-cholesterol. After removal of the culture medium containing the radiolabelled cholesterol, the cells were allowed to equilibrate for 24 h. Thereafter, efflux of radiolabelled cholesterol from cells was determined during 4 h of incubation at 37°C with 20-fold diluted plasma samples, originally containing 50 U/mL of heparin. The radioactivity in the medium was then counted, and cholesterol efflux was expressed as percentage of the radioactivity initially present in the cells (fractional efflux). Data were corrected for the small amount of radiolabel in the medium in the absence of plasma. Similar efflux values were found with plasma and serum (data not shown). Fractional cholesterol efflux is a measure of the ability of a specific plasma to remove cholesterol from the cell membrane. Glycated haemoglobin (HbA_{1c}) was measured by high-performance liquid chromatography (BioRad, Veenendaal, The Netherlands; reference range 4.6–6.1%). Blood glucose was measured on an APEC glucose analyser (APEC Inc., Danvers, MA, USA).

Statistical analysis

Data are given in mean ± SD or in geometric mean (95% confidence interval (CI)). Changes in variables after Acipimox were compared with the averaged values at baseline and recovery by paired Student's *t*-tests and are given in mean (95% CI). Between group differences in baseline variables and between group differences in changes in variables after Acipimox were evaluated by unpaired Student's *t*-tests. $P < 0.05$ was considered significant. On the basis of a day-to-day coefficient of variation of cellular cholesterol efflux to plasma of 6.0%, it was estimated that with eight participants a relative change in cellular cholesterol efflux to plasma of 6.0% could be

Table 1 Blood glucose, plasma (apo)lipoprotein variables, non-esterified fatty acids (NEFA), phospholipid transfer protein (PLTP) activity and cholesterol efflux from Fu5AH cells to plasma after 24 h Acipimox administration in eight Type 2 diabetic patients and eight healthy subjects

	Baseline	Acipimox	Recovery	Change after Acipimox	*P*-value for between group difference in change
Blood glucose (mmol/l)					
Type 2 diabetic patients	7.8 ± 1.5*	6.4 ± 0.9	7.9 ± 1.9	–1.4 (–2.3 to –0.6) $P = 0.005$	$P = 0.007$
Healthy subjects	4.3 ± 0.4	4.2 ± 0.5	4.3 ± 0.7	–0.1 (–0.3–0.1) $P = 0.21$	
Plasma triglycerides (mmol/l)					
Type 2 diabetic patients	1.81** (1.46–2.25)	1.29 (1.02–1.64)	1.66 (1.19–2.31)	–0.48 (–0.71 to –0.25) $P = 0.001$	$P = 0.03$
Healthy subjects	0.97 (0.64–1.18)	0.68 (0.41–1.12)	0.87 (0.63–1.20)	–0.23 (–0.30 to –0.15) $P = 0.002$	
Plasma NEFA (mmol/l)					
Type 2 diabetic patients	1.29 ± 0.44***	0.31 ± 0.08	1.14 ± 0.23	–0.90 (–1.11 to –0.69) $P = 0.001$	$P = 0.001$
Healthy subjects	0.64 ± 0.30	0.52 ± 0.26	0.67 ± 0.27	–0.14 (–0.30–0.03) $P = 0.08$	
HDL cholesterol (mmol/l)					
Type 2 diabetic patients	0.98 ± 0.15	0.93 ± 0.13	1.01 ± 0.18	–0.06 (–0.14–0.01) $P = 0.08$	$P = 0.77$
Healthy subjects	1.12 ± 0.31	1.06 ± 0.34	1.15 ± 0.41	–0.07 (–0.12 to –0.03) $P = 0.007$	
Plasma apo AI (g/l)					
Type 2 diabetic patients	1.24 ± 0.11	1.15 ± 0.10	1.21 ± 0.09	–0.08 (–0.10 to –0.06) $P = 0.001$	$P = 0.90$
Healthy subjects	1.21 ± 0.20	1.16 ± 0.21	1.27 ± 0.26	–0.08 (–0.14 to –0.03) $P = 0.008$	
Pre-β HDL in incubated plasma (apo AI, g/l)					
Type 2 diabetic patients	0.18 ± 0.03	0.11 ± 0.03	0.16 ± 0.06	–0.06 (–0.07 to –0.04) $P = 0.001$	$P = 0.60$
Healthy subjects	0.16 ± 0.09	0.09 ± 0.05	0.11 ± 0.06	–0.05 (–0.09 to –0.01) $P = 0.03$	
Plasma PLTP activity (% of reference plasma)					
Type 2 diabetic patients	87.0 ± 5.9	81.3 ± 9.5	91.8 ± 10.1	–8.1 (–11.2 to –5.0) $P = 0.001$	$P = 0.58$
Healthy subjects	93.8 ± 19.1	84.0 ± 13.5	94.2 ± 15.0	–10.0 (–17.0 to –2.9) $P = 0.01$	
Cellular cholesterol efflux (%/4 h)					
Type 2 diabetic patients	31.4 ± 2.7	29.7 ± 2.5	30.8 ± 2.6	–1.4 (–2.8 to –0.1) $P = 0.04$	$P = 0.80$
Healthy subjects	29.9 ± 3.0	28.3 ± 2.7	29.1 ± 2.5	–1.3 (–2.0 to –0.5) $P = 0.005$	

Data are given in mean ± SD, except for plasma triglycerides which are given in geometric mean (95% confidence interval).
Changes in variables after Acipimox are compared with the averaged values at baseline and recovery and are given in mean (95% confidence interval).
*$P = 0.001$; **$P = 0.01$; ***$P = 0.002$ from healthy subjects at baseline.

demonstrated with a power of 0.80 and a two-sided *P*-value < 0.05.

Results

Age was not different between Type 2 diabetic patients and healthy subjects (58 ± 7 vs. 52 ± 9 years, $P = 0.11$), whereas BMI (27.7 ± 1.6 kg/m^2 vs. 24.7 ± 2.3 kg/m^2, $P = 0.003$) and HbA_{1c} ($7.1 \pm 0.8\%$ vs. $5.1 \pm 0.3\%$, $P = 0.001$) were higher in diabetic patients than in healthy subjects. Blood glucose decreased after Acipimox in diabetic patients, but not in healthy subjects (Table 1). Baseline plasma cholesterol was 5.4 ± 0.6 and 4.7 ± 0.8 mmol/l in diabetic and healthy subjects, respectively ($P = 0.06$). Baseline plasma triglycerides and NEFA levels were higher in diabetic compared with healthy subjects (Table 1). HDL cholesterol, plasma apo AI, pre-β HDL in incubated plasma, PLTP activity and cholesterol efflux from Fu5AH cells to plasma at baseline were not significantly different between the groups. Blood glucose decreased after Acipimox in diabetic patients, but not in healthy subjects (Table 1). Plasma triglycerides were lowered by Acipimox in both groups. The decrease in plasma NEFA was significant in diabetic patients. The changes in blood glucose, plasma triglycerides and NEFA in response to Acipimox were larger in diabetic patients than in healthy subjects. Acipimox administration resulted in a decrease in HDL cholesterol in healthy subjects, as well as in a decrease in plasma apo AI, plasma PLTP activity and pre-β HDL generation in both groups (Table 1). Of interest, cellular cholesterol efflux to plasma from both diabetic patients and healthy subjects was decreased by Acipimox (Table 1). The changes in these variables in response to Acipimox were similar in both groups.

Discussion

Using Fu5AH cells as model system, this study shows a similar impairment in the ability of plasma from Type 2 diabetic patients and healthy subjects to promote cellular cholesterol efflux after 24 h of Acipimox administration. As expected [13], plasma PLTP activity decreased in both groups in response to this treatment. A novel finding of our study is that the generation of pre-β HDL particles, as measured in incubated plasma, is also decreased after Acipimox in Type 2 diabetic patients and in healthy subjects. Previous studies have demonstrated that apart from the HDL cholesterol and HDL phospholipid concentration, plasma apo AI, pre-β HDL and plasma PLTP activity are determinants of cellular cholesterol efflux [4,6,7,9,11,12]. The diminished cellular cholesterol efflux to plasma after Acipimox could thus be due to the decrease in plasma PLTP activity which may result in less pre-β HDL generation [7,9], to the slight decrease in plasma apo AI and in HDL cholesterol, or to a combination of these changes. The presently studied Type 2 diabetic patients were not matched with healthy subjects for plasma lipid levels. Plasma triglyceride and NEFA concentrations at baseline were higher and the decreases in these variables in response to Acipimox were larger in Type 2 diabetic patients than in healthy subjects. Baseline levels of HDL cholesterol, plasma apo AI, pre-β HDL in incubated plasma and plasma PLTP activity were, however, not different between diabetic patients and healthy subjects. Likewise, cellular cholesterol efflux at baseline was similar in both groups. In comparison, cholesterol efflux out of Fu5AH cells has been reported to be impaired in Type 2 diabetic patients with a low HDL cholesterol [12].

The acute plasma triglyceride lowering effect of Acipimox in diabetic patients [19, present study] is sustained with long-term treatment [20–22]. HDL cholesterol remains unchanged [20–22] or tends to increase [23–25] with its prolonged use. Although hypertriglyceridaemia is an independent cardiovascular risk factor [26], the potential cardioprotective effect of Acipimox remains to be proven. An impairment in reverse cholesterol transport, as reflected by a low capacity of plasma to stimulate cellular cholesterol removal, probably represents an atherogenic phenomenon [2–4,27]. Further studies are therefore warranted to document whether the decrease in cellular cholesterol efflux to plasma persists with prolonged Acipimox treatment. If this potentially adverse effect on reverse cholesterol transport is sustained, it should be taken into account when treating diabetic dyslipidaemia with this anti-lipolytic agent.

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