Effect of metformin and rosiglitazone on lipid metabolism in HIV infected patients receiving protease inhibitor containing HAART

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ABSTRACT

Aims. Insulin resistance may be the primary event in the protease inhibitor-associated metabolic syndrome. Treatment with insulin sensitizers (metformin, rosiglitazone) can ameliorate insulin resistance. So far, the effects of these agents on blood lipids have not been well determined. The aim of the present study was to evaluate the effects of metformin and rosiglitazone treatment on lipid metabolism in HIV infected patients receiving protease inhibitors containing HAART.

Design and Methods. HIV infected male patients (>18 years) were eligible for the study if they had impaired glucose tolerance with insulin resistance, characterized by fasting insulin concentration greater then 20 mIU/L and if they were on stable antiretroviral therapy regimen including a protease inhibitor for at least 12 months prior to the study enrolment. The patients were randomly assigned to receive either 1g/day metformin (metformin group, n=30) or 4 mg/day rosiglitazone maleate (rosiglitazone group, n=30) or no treatment (control group, n=30). The primary efficacy parameters were fasting plasma lipids, glucose levels and fasting insulin levels compared between baseline and week 48, by treatment groups.

Results. The total cholesterol concentration in rosiglitazone group increased from 5.76 ±1.2 to 7.1±1.6 mmol/l (23% increase, $p<0.05$), HDL levels increased from 0.91± 0.44 to 1.3± 0.2 (38% increase, $p<0.01$) and LDL levels increased from 3.5± 0.98 to 4.5± 1.0 (28% increase, $p<0.05$). Treatment with metformin had no significant effect on total, HDL and LDL cholesterol. After 48 weeks of treatment, the fasting triglycerides concentration in the metformin group declined from 4.1±1.6 to 3.2±1.3 mmol/l (22% decrease, $p<0.05$) but in the rosiglitazone group no statistically significant effect on plasma triglycerides was noted. Furthermore, after 48 weeks of treatment the fasting insulin concentration in the rosiglitazone group declined by 49% and in the metformin group by 28%. This improvement in insulin secretion could be clearly demonstrated when the sums of insulin concentrations after oral glucose tolerance test were compared: 548±13 to 345±11.8 mIU/l in the rosiglitazone group (37% decrease, $p<0.01$) and from 552±15 to 420±12 mIU/l in the metformin group (24% decrease, $p<0.01$).

Conclusions. The study demonstrates that both therapies improve insulin resistance. However, treatment with metformin has no effect on total, HDL and LDL cholesterol, but significantly reduces triglycerides, which has beneficial effect on the lipid status in these patients. Rosiglitazone causes significant increases in total cholesterol, HDL and LDL, but has no effect on triglycerides concentrations.

KEY WORDS

HIV, protease inhibitors, serum lipids, metformin, rosiglitazone

Table 1. Baseline clinical characteristics in patients receiving rosiglitazone, metformin and no treatment.

characteristics	rosiglitazone (n=30)	metformin (n=30)	controls (n=30)
age (years)	41.5±11.4	42.1±9.8	43.4±10.9
weight (kg)			
baseline	71.4±8.6	72.8±6.4	73.1±8.4
week 48	75.2±7.3	70.6±4.9	72.8±7.6
BMI (kg/m^2)			
baseline	24.2±3.6	23.8±2.8	24.7±3.1
week 48	25.4±3.1	22.9±2.4	24.4±2.9
duration of HIV infection (years)	8.4±1.4	7.8±2.7	6.0±1.9
duration of treatment (years)	3.4±1.2	3.1±2.1	2.8±1.6
CD4 cell count (cells/mm^3)			
baseline	380±100.5	364±211	315±119
week 48	395±140	370±221	388±105
HIV RNA (copies/mL)			
baseline	7450±4291	9686±3265	7850±3851
week 48	8190±3765	9725±4157	7430±2791

*Data are means ± SEM (standard error of mean); *p <0.05* ***week 48 versus baseline****; **p <0.01* ***week 48 versus baseline;***

Introduction

The use of the highly active antiretroviral therapy (HAART) has been associated with a dramatic improvement of morbidity and mortality in HIV-infected patients (1, 2). In recent years, however, concern has been raised by the long-term toxicity of HAART (3). Since the introduction of protease inhibitors (PI) for treatment of HIV infection, metabolic abnormalities (dysregulation of glucose metabolism, dyslipidemia) and morphologic changes (fat accumulation and lipoatrophy) have been extensively reported in treated patients. We are still not able to fully define and understand the exact mechanism and pathogenesis of these metabolic disorders, but association with increased risk of cardiovascular disease is likely to become a significant problem (4, 5).

It is apparent that the effects of PI on glucose and lipid metabolism need to be examined independently of syndromes of fat redistribution (lipodystrophy), which occur in patients who have never been treated with PI (6). Insulin resistance may be considered the first pathogenetic step towards impaired glucose tolerance and, eventually, diabetes mellitus in HIV infected patients, treated with PI containing HAART (7-9). It is well known that insulin resistance and dyslipidemia are intimately linked (10). PI can directly impair insulin signaling in insulin-responsive tissues at pharmacological doses which provides a plausible basis for the hypothesis that insulin resistance is the primary event in the PI-associated metabolic syndrome (11-13). Insulin resistance results in reduced uptake of glucose in muscle and increased release of non-esterified fatty acids. Raised serum concentration of non-esterified fatty acids stimulates hepatic glucose production, reduces glucose utilization in muscle, and enhances insulin secretion, thus exacerbating the state of hyperinsulinaemia. This complex interactive system between insulin, glucose, and non-esterified fatty acids seems to be regulated by liver, muscle and adipose tissue (7). Concerning insulin resistance, dietary interventions and moderate aerobic exercise are accepted as general health measures (14). If a pharmacological intervention is needed, the best choices are probably insulin sensitizing agents like metformin and glitazones (15, 16).

The aim of the present study was to evaluate the effects of insulin sensitizers on lipid metabolism in HIV infected patients receiving PI containing HAART with respect to the improved insulin sensitivity. The effects of two insulin sensitizers, metformin and rosiglitazone, were studied and compared.

Materials and Methods

Study design

In this prospective randomized study, 90 male patients were recruited at the beginning of 2001. The study group consists of 60 Slovenian patients who were treated at the Department of Infectious Diseases in Ljubljana, Slovenia, and 30 American patients who were treated at UCSD in San Diego, USA. Patients were considered eligible on the basis of the following inclusion

criteria: documented HIV infection, age >18 years, stable PI-containing HAART regimen for at least 12 months prior to the study enrolment, impaired glucose tolerance (IGT) with insulin resistance characterized by fasting insulin concentration greater then 20 mIU/L. Eligible patients were randomly assigned to receive either metformin (Aglurab® – Medis) 1g/day (metformin group, n=30) or rosiglitazone maleate (Avandia® – Glaxo Smith-Klein) 4 mg/day (rosiglitazone group, n=30) or no treatment (control group, n=30). All patients were taking one protease inhibitor (indinavir) and two nucleoside analogues (lamivudine, stavudine, zidovudine or didanosine). Clinical examinations and laboratory tests were performed at baseline and after 48 weeks.

Laboratory measurements

Plasma HIV RNA concentrations (HIV viral load) were determined by the Cobas Amplicor HIV-1 Monitor Assay version 1.5 (Roche Molecular Systems, Brancburg, NJ, USA); results below the limit of detection were assigned a value of <20 copies/mL plasma. CD4-lymphocytes counts (CD4 cell count) were measured by three-color flow cytometry and hematology analyzers.

Normal and impaired glucose tolerance were defined according to the American Diabetes Association guidelines-fasting blood glucose of less than 6.1 mmol/l, 6.1-6.9 mmol/l, respectively, or blood glucose 2 h after glucose-tolerance test (75g) of less than 7.8 mmol/l, 7.8-11.1 mmol/l, respectively. For purposes of clarity, patients with impaired fasting glucose 6.1-6.9 mmol/l were defined as having impaired glucose tolerance. Total cholesterol, LDL, HDL and triglycerides were measured spectrometrically by an Olympus Corp. Analyzer (New Hyde Park, NY, USA). Fasting plasma glucose was measured by the hexokinase method with an Olympus Corp. Analyzer (New Hyde Park, NY, USA). Basal insulin levels were determined using a commercial radioimmunoassay method- IRMA Biosource Europe S.A. (Nivelles, Belgium).

Fasting total cholesterol, HDL and LDL cholesterol and triglycerides were determined. Patients were considered to have hypercholesterolaemia, low HDL-cholesterol concentration, high LDL-cholesterol concentration or hypertriglyceridaemia, if their plasma measurements were >5.0 mmol/L, <0.9 mmol/L, >3.0 mmol/L or >2.0 mmol/L, respectively.

Statistical analysis

Data is given as means and standard error of means (SEM). Continuous variables were compared with the t-test. Statistical analysis was performed using the SPSS 10.0 statistical software package. All given p-values are two-tailed and a p-value of < 0.05 was considered to indicate statistical significance.

Results

Patient population

90 male HIV infected patients on PI containing HAART were included in the study and randomly assigned to receive rosiglitazone (n=30), metformin (n=30) or no treatment (n=30). All patients completed 48 weeks protocol. Compliance with the study medication based on pill count was not different between all groups (was similar among the groups). Baseline clinical characteristics of the patients in each treatment group are presented in Table 1. All groups matched for age, BMI and duration of HIV infection. The mean age of study population was 42.3 ± 10.7 years and BMI 24.2 ± 3.1 kg/m^2. The mean duration of HIV infection was 7.8 ± 2.7 years, while the mean duration of HAART therapy was 3.1 ± 2.1 years.

There were no significant changes in blood counts, viral load or CD4 counts with rosiglitazone and metformin treatment. There were no statistically significant changes in the control group.

Effects on fasting blood lipid levels

Baseline metabolic data and data after 48 weeks of treatment with metformin, rosiglitazone or without treatment are presented in Table 2. Baseline levels of serum triglycerides, total, HDL, and LDL cholesterol did not differ between the two treatment groups, but patients in the control group had higher baseline HDL cholesterol levels compared to the rosiglitazone and metformin groups ($p=0.05$). After 48 weeks of treatment no significant changes in total, HDL, and LDL cholesterol were found in the metformin group. However, statistically significant increases from baseline were observed in total, HDL and LDL cholesterol levels in the rosiglitazone group. The total cholesterol concentration in the rosiglitazone group increased from 5.76 ±1.2 to 7.1±1.6 mmol/l (23% increase, $p<0.05$) HDL levels increased from 0.91± 0.44 to 1.3± 0.2 (38% increase, $p<0.01$) and LDL levels increased from 3.5± 0.98 to 4.5± 1.0 (28% increase, $p<0.05$). After 48 weeks of treatment, the fasting triglycerides concentration in the metformin group declined from 4.1±1.6 to 3.2±1.3 mmol/l (22% decrease, $p<0.05$), while in the rosiglitazone group no statistically significant effect on plasma triglycerides was noted.

Total cholesterol/HDL cholesterol ratio and LDL cholesterol/HDL cholesterol ratio did not change significantly in any treatment group.

The analysis also assessed the proportion of patients in each treatment group at the conclusion of the study who had total cholesterol levels greater than 5.0 mmol/

Table 2. Metabolic data in patients receiving rosiglitazone, metformin and in the control group.

characteristics	rosiglitazone (n=30)	metformin (n=30)	controls (n=30)
total cholesterol (mmol/L)			
baseline	5.76±1.2	6.0±1.3	5.8±1.7
week 48	7.1±1.6*	5.9±1.58	5.6±1.25
HDL cholesterol (mmol/L)			
baseline	0.91± 0.44	1.0±0.4	1.3±0.5
week 48	1.3±0.2**	0.97±0.6	1.2±0.3
LDL cholesterol (mmol/L)			
baseline	3.5±0.98	3.36± 1.1	3.21±0.91
week 48	4.5±1.0*	3.4±1.5	3.4±0.8
triglycerides (mmol/L)			
baseline	3.9±1.8	4.1±1.6	3.7±1.3
week 48	4.2±1.5	3.2 ±1.3*	3.85±1.2
fasting glucose (mmol/L)			
baseline	6.5±0.22	6.7±0.12	6.6±0.1
week 48	4.5±0.36*	4.3±0.46*	6.4±0.4
fasting insulin (mIU/L)			
baseline	39.5±7.4	40.3±5.6	39.7±4.5
week 48	20.3±5.4**	29.2±6.4**	40.2±3.6
sum insulin (mIU/L)			
baseline	548±13	552±15	550±10
week 48	345±11.8**	420±12**	547±11

*Data are means ± SEM; *p <0.05 week 48 versus baseline; **p <0.01 week 48 versus baseline; HDL, high density lipoprotein; LDL, low density lipoprotein*

l – the threshold for increased incidence of coronary heart disease in epidemiologic studies. These proportions were 63% in the metformin group, 90% in the rosiglitazone group and 30% among HAART naïve patients. Finally, 12% of patients had a highly atherogenic lipid profile (total cholesterol >6.5 mmol/L, LDL:HDL >4.1, triglycerides >4 mmol/L) at baseline; this profile was found in 20% patients at study end.

Glycemic control

Although the mean fasting plasma glucose (FPG) concentrations did not change significantly between the rosiglitazone and metformin groups, they significantly decreased compared with the control group. The mean FPG concentrations in the rosiglitazone group decreased from 6.5±0.22 to 4.5±0.36 mmol/L (P<0.05) and in the metformin group from 6.7 ±0.12 to 4.3±0.46 mmol/L (P<0.05) compared to the control group (6.6±0.1 mmo/L) (Table 2).

Effect on fasting insulin levels

All the study groups had high basal fasting insulin levels exceeding the normal range for the assay (5-20mIU/L). After 48 weeks of treatment, the fasting insulin concentration in the rosiglitazone group declined from 39.5±7.4 to 20.3±5.4 mIU/L (49% decrease) (p<0.01) and in the metformin group 40.3±5.6 to 29.2±6.4 mIU/L (28% decrease) (p<0.01) (Table 2). This improvement of insulin secretion could be clearly demonstrated when the sums of insulin concentrations after oral glucose tolerance test were compared: 548±13 to 345±11.8 mIU/L (37% decrease) (p<0.01) in the rosiglitazone group and from 552±15 to 420±12 mIU/L in the metformin group (24% decrease) (p<0.01) (Table 2).

Discussion

An investigation of 60 male patients in our study who received metformin or rosiglitazone established different effects on serum lipid concentrations associated with each drug; specifically, the changes observed with rosiglitazone therapy produced more pronounced disturbances of lipid levels.

The implications of dyslipidemia in HIV infected individuals are not completely known, but the type, incidence and degree of the abnormalities that are observed with this disease and its treatment may be ex-

pected to result in increased cardiovascular morbidity (17, 18). The alterations in blood lipid profiles associated with HAART may be secondary to insulin resistance (7,10,15,16). Insulin resistance is often described only in terms of an impairment of the effects of insulin on the uptake of glucose in peripheral tissues. However, insulin influences several metabolic pathways. Insulin increases peripheral uptake of glucose, but decreases endogenous glucose production, the rate of lipolysis and of proteolysis (19). It is clear that insulin resistance and post-prandial lipid/fatty acid metabolism are intimately linked. It is possible that insulin resistance is the primary event in the PI-associated metabolic syndrome but the question what comes first and what triggers this disease remains unanswered (20, 21). It is also possible that the primary defect induced by PI may be different (22). Careful evaluation of pre-existing individual risk factors and monitoring of lipid and glucose parameters during HAART are recommended. If altered glucose homeostasis is diagnosed, it should be treated. Treatment with insulin sensitizers can ameliorate HAART-associated insulin resistance (23-25). In adipose tissue metformin promotes re-esterification of free fatty acids and inhibits lipolysis, which may indirectly improve insulin sensitivity through reduced lipotoxicity. According to randomized placebo-controlled studies, metformin improves insulin sensitivity, decreases triglyceride concentration, and induces modest reductions of body weight, predominantly occurring in the intra-abdominal fat, but carries a risk of lactic acidosis (23, 24). So, it may not be an ideal medication for everyone. Limited data is available on the safety and efficacy of glitazone agents in HIV infection. Especially for newer agents like rosiglitazone there are not enough data on their efficacy and tolerability in HIV infected patients. Sutinen et al. conducted a randomized double-blind placebo-controlled study in which 30 patients with HAART-associated lipodystrophy received rosiglitazone (8 mg daily) or placebo for 24 weeks. Rosiglitazone had no effect on morphologic parameters, however, it decreased serum insulin concentrations and percentage of liver fat detected by spectroscopy, but it unexpectedly caused significant increases in serum triglyceride and cholesterol concentrations (26). However, patients were not preselected on the basis of a specific fat distribution criterion, and peripheral subcutaneous fat was not assessed. In contrast, Gelato et al. demonstrated increased peripheral subcutaneous fat levels in response to rosiglitazone in a small pilot study of HIV-infected patients (27).

Our data suggest that the effects of metformin therapy used in insulin resistant HIV-infected patients are encouraging not only because of the improvement of glucose metabolism but also because of a reduction of fasting triglycerides by 22%. There were no changes in total, HDL and LDL cholesterol in the metformin group. Rosiglitazone has a role in managing insulin resitance and glucose abnormalities, but it disappointed in regard to the lipid metabolism. During the 48 weeks of rosiglitazone treatment, serum total cholesterol, HDL and LDL fractions significantly increased. Results of our study are consistent with prior studies that demonstrated effects of metformin and rosiglitazone on serum lipid levels. Both drugs vary in their ability to affect lipids. Greater reduction of triglycerides in the metformin group than reported in preceding studies may be a result of longer duration of therapy (48 weeks in our study vs. 8 and 12 weeks, respectively) (23, 24). Metformin reduced triglycerides while rosiglitazone did not affect them. Details of the pathophysiological mechanisms of hypertriglyceridemia remain to be clarified. In addition to increasing triglycerides, rosiglitazone also elevated total cholesterol, therefore careful monitoring of serum lipids is mandatory when glitazones are used, especially in patients with pre-existing dyslipidemia. For patients with insulin resistance and lipid abnormalities metformin is a better choice, though it has less of an effect on managing insulin resistance in these patients.

Because of prolonged survival of HIV infected patients, PI-induced development of diabetes mellitus and dyslipidemia is of potential concern, as both disorders are closely associated with an increased risk of cardiovascular disease.

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