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Effects of antiretroviral treatment on paraoxonase 1 (PON1) activity in rats

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ABSTRACT

Highly active antiretroviral therapy (HAART), especially protease inhibitors (PIs), commonly used in HIV-infected patients, effectively suppresses a viral replication. However, it is frequently associated with significant side effects, including fat redistribution, lipodystrophy, hyperlipidemia, insulin resistance and diabetes mellitus. Currently, metabolic complications and atherosclerosis resulting from them become the major cause of mortality in HIV-infected patients receiving HAART. Paraoxonase 1 (PON1) is the HDL-bound esterase, which inhibits development of atherosclerosis by decomposing lipid peroxidation products and hydrolyzing homocysteine thiolactone.

The aim of this study was to characterize the effects of HIV protease inhibitors on PON1 activity, total plasma homocysteine and protein-bound homocysteine thiolactone as well as lipid profile in rats.

The study was performed on seven groups of male Wistar rats: (1) control; (2) and (3) receiving ritonavir (RTV) at doses of 10 and 50 mg/kg, respectively; (4) and (5) receiving atazanavir (ATV) at 10 and 100 mg/kg, respectively; (6) and (7) receiving saquinavir (SQV) at 10 and 50 mg/kg, respectively. All drugs were administered orally for 4 weeks.

Compared to control animals, rats receiving PIs had significantly higher concentration of triglycerides and total cholesterol, but the levels of HDL-cholesterol were not different between groups. PON1 activity toward paraoxon was decreased in groups receiving PIs (control: 149 ± 5 U/ml; PIs-treated: RTV at doses 10 mg/kg 133 ± 9.5 U/ml, RTV at doses 50 mg/kg 134 ± 10.8 U/ml, SQV at doses 10 mg/kg 131 ± 9.2 U/ml, ATV at doses 10 mg/kg 132 ± 11.8 U/ml, ATV at doses 100 mg/kg 108 ± 7.8 U/ml). ATV reduced total homocysteine level around 25–28%, whereas other PIs had no effect on its concentration. In contrast, 10–15% increase in protein-bound homocysteine thiolactone was observed in PIs-receiving groups, such as RTV10, RTV50, SQV50, ATV10.

In conclusion, dyslipidemia induced by PIs is associated with reduced PON1 activity as well as increased protein homocysteinylation. PON1 deficiency may contribute to increased risk of atherosclerosis in these patients.

1. Introduction

Highly active antiretroviral therapy (HAART) has changed human immunodeficiency virus (HIV) infection to a chronic condition. HAART effectively suppresses viral replication. Currently, with the introduction of antiretroviral therapy (ART), co-formulations and once daily taking drug regimens, HIV-infected patients with excellent adherence and immunological response may expect a life span similar to those in the general population [1].

HAART was first presented at the XI World Conference on AIDS in Vancouver [2]. It is based on the activity of combined medicines,

Abbreviation: ART, Active Antiretroviral Therapy; ATV, atazanavir; HAART, Highly Active Antiretroviral Therapy; Hcy, homocysteine; HDL, High-density lipoprotein; HIV, Human Immunodeficiency Virus; LDL, Low-density lipoprotein; NRTIs, nucleoside reverse transcriptase inhibitors; PI, protease inhibitor; PON, paraoxonase; RTV, ritonavir; SQV, saquinavir; tHcy, total homocysteine; VLDL, Very-low-density lipoprotein.

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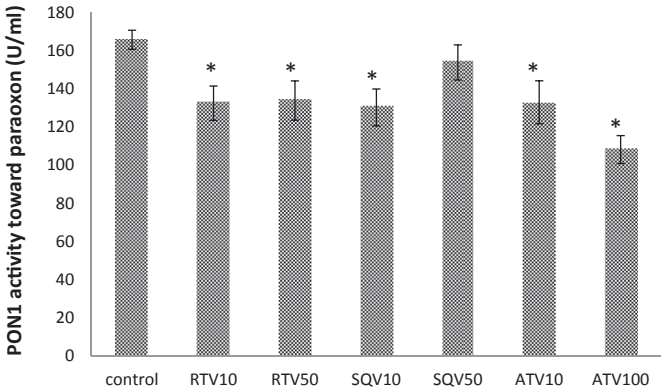

Fig. 1. Plasma paraoxonase (PON1) activity toward paraoxon in rats treated with PIs. RTV10–animals receiving ritonavir at a dose of 10 mg/kg/day, RTV50-animals receiving ritonavir at a dose of 50 mg/kg/day, SQV10-animals receiving saquinavir at a dose of 10 mg/kg/day, SQV50-animals receiving saquinavir at a dose of 50 mg/kg/day, ATV10-animals receiving atazanavir at a dose of 10 mg/kg/day, ATV100-animals receiving atazanavir at a dose of 100 mg/kg/day; ± SEM. $^{*}p < 0.05$ compared to the control.

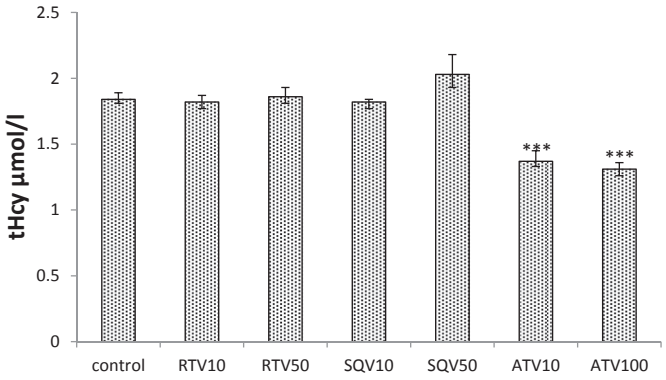

Fig. 2. The total homocysteine (tHcy) level in rats treated with PIs. RTV10–animals receiving ritonavir at a dose of 10 mg/kg/day, RTV50-animals receiving ritonavir at a dose of 50 mg/kg/day, SQV10-animals receiving saquinavir at a dose of 10 mg/kg/day, SQV50-animals receiving saquinavir at a dose of 50 mg/kg/day, ATV10-animals receiving atazanavir at a dose of 10 mg/kg/day, ATV100-animals receiving atazanavir at a dose of 100 mg/kg/day; ± SEM. $^{***}p < 0.001$ compared to the control.

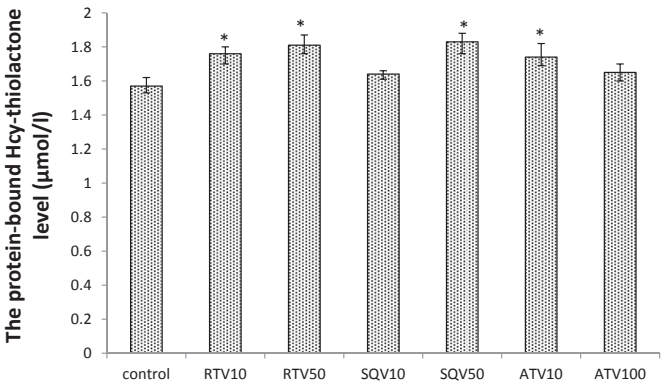

Fig. 3. The protein-bound Hcy-thiolactone in rats treated with PIs. RTV10–animals receiving ritonavir at a dose of 10 mg/kg/day, RTV50-animals receiving ritonavir at a dose of 50 mg/kg/day, SQV10-animals receiving saquinavir at a dose of 10 mg/kg/day, SQV50-animals receiving saquinavir at a dose of 50 mg/kg/day, ATV10-animals receiving atazanavir at a dose of 10 mg/kg/day, ATV100-animals receiving atazanavir at a dose of 100 mg/kg/day; ± SEM. $^{*}p < 0.05$ compared to the control.

which achieve significant and long term inhibition of HIV replication that viral RNA cannot be detected by highly sensitive molecular biology techniques like RT-PCR. Antiretroviral theraphy typically consist of three drugs, two nucleoside reverse transcriptase inhibitors (NRTI) and one of a protease inhibitor (PI), nonnucleoside reverse transcriptase inhibitor (NNRTI) or newer group of drugs-integrase inhibitors [3]. Although complete HIV eradication is not possible, the antiretroviral drugs (ARVs) restore, at least partially, and maintain immune system functions [4].

Nevertheless, current antiretroviral therapy causes numerous adverse side effects among which metabolic disorders, such as dyslipidemia, lipodystrophy, hypercholesterolemia, and insulin resistance are most frequent [5–8]. Patients suffer from undergoing antiretroviral treatment, develop dyslipidemia that is associated with significantly increased risk of atherosclerosis, ischemic heart disease, and pancreatitis. PIs seem to be associated with an increased cardiovascular diseases risk, the data are less clear for the NRTI abacavir [9]. HAART increases the risk of myocardial infarction by 26% per each year of treatment [4]. However, some studies indicate that HIV by itself increased a risk of coronary events in this population [10]. It has been demonstrated that HIV infection is associated with a number of compositional changes in HDL molecules [11]. Untreated HIV patients have defective HDL (High density lipoprotein) functions and low PON1 activity [11,12].

In 1997 the FDA (USA) published the first reports on the occurrence of diabetes mellitus in patients treated with PIs [6]. In addition, there were reports on hyperlipidemia associated with ritonavir administration [8]. Afterwards data on impaired distribution of adipose tissue (lipodystrophy) was published [13–15]. Lipohypertrophy and visceral fat deposits in HIV-positive patients receiving ART is frequently associated with the metabolic disturbances, including dyslipidemia, hypertriglyceridemia, low HDL-cholesterol level, insulin resistance and diabetes [10,16–19]. Those patient have increased Framingham risk scores and higher risk for cardiovascular diseases [10]. Mechanisms for lipohypertrophy are complex and not known in detail already, include high levels of circulating triglycerides, elevation of inflammatory cytokines, and free fatty acids stored in the liver and visceral fatty tissue [20,21].

At the time of HAART treatment, individual medicines induce different complications in adipose tissue. Nucleoside reverse transcriptase inhibitors, in particular thymidine analogues (stavudine and zidovudine) are responsible for lipoatrophy, while treatment with PIs is associated with lipohypertrophy, including visceral obesity, and systemic disturbances in the metabolism of lipids and glucose [22–24]. PIs affect the intracellular metabolism of fatty acids and glucose, and adipocyte differentiation [25–27].

During the RNA replication cycle of HIV infection, HIV proteins are translated to biochemically inert polyproteins, which are then transformed into different functional and structural proteins by cutting off the chain in the appropriate site by virus-specific proteases. HIV proteases play a key role in the formation of polyprotein components in the protein capsule of the virus [27].

Paraoxonase 1 (PON1) is well-known to inhibit the development of atherosclerosis. Most cardiovascular risk factors, including hyperlipidemia, insulin resistance and diabetes, are associated with reduced PON1 activity [28]. Taking into account adverse metabolic and cardiovascular effects of HAART, the effect of anti-HIV therapy on PON1 status is highly significant. In this study we investigated the effect of three HIV protease inhibitors, ritonavir (RTV), saquinavir (SQV) and atazanavir (ATV) on plasma PON1 activity. These drugs were chosen because they represent the main group of antiretroviral drugs affecting lipid disorders. In addition, because