Cytokine 110 (2018) 204–212

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Plasma metabolic changes in Chinese HIV-infected patients receiving lopinavir/ritonavir based treatment: Implications for HIV precision therapy

Xiaolin Li, Tong Wu, Yongjun Jiang, Zining Zhang, Xiaoxu Han, Wenqing Geng, Haibo Ding, Jing Kang, Qi Wang, Hong Shang*

Key Laboratory of AIDS Immunology of National Health and Family Planning Commission, Department of Laboratory Medicine, The First Affiliated Hospital, China Medical University, Shenyang 110001, PR China
Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, Hangzhou, PR China

ARTICLE INFO

Keywords:
HIV
Antiretroviral therapy
Metabolomics
Prognosis
Dyslipidemia

ABSTRACT

Objectives: The goal of this study is to profile the metabolic changes in the plasma of HIV patients receiving lopinavir/ritonavir (LPV/r)-based highly active antiretroviral therapy (HAART) relative to their treatment-naïve phase, aimed to identify precision therapy for HIV for improving prognosis and predicting dyslipidemia caused by LPV/r.

Methods: 38 longitudinal plasma samples were collected from 19 HIV-infected patients both before and after antiretroviral therapy, and 18 samples from healthy individuals were used as controls. Untargeted metabolomics profiling of these plasma samples was performed using liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC–MS).

Results: A total of 331 compounds of known identity were detected among these metabolites, a 67-metabolite signature mainly mapping to tryptophan, histidine, acyl carnitine, ketone bodies and fatty acid metabolism distinguished HIV patients from healthy controls. The levels of 19 out of the 67 altered metabolites including histidine, kynurenine, and 3-hydroxybutyrate (BHBA), recovered after LPV/r-based antiretroviral therapy, and histidine was positively correlated with the presence of CD4 + T lymphocytes. Furthermore, using receiver operating characteristic (ROC) analyses, we discovered that butyrylcarnitine in combination with myristic acid from plasma in treatment-naïve patients could predict dyslipidemia caused by LPV/r with 87% accuracy.

Conclusions: Metabolites alterations in treatment-naïve HIV patients may indicate an inflammatory, oxidative state and mitochondrial dysfunction that is permissive for disease progression. Histidine may provide a specific protective function for HIV patients. Besides, elevated fatty acids levels including butyrylcarnitine and myristic acid after infection may indicate patients at risk of suffering from dyslipidemia after LPV/r-based HAART.

1. Introduction

Combinations of anti-HIV drugs are typically applied as highly active antiretroviral therapy (HAART), preventing acquired immunodeficiency syndrome (AIDS), and leading to a great reduction in mortality and prolonged course of chronic HIV infection. However, they cannot achieve complete health recovery, as treatment-mediated immune restorations are often incomplete even under long-term viral suppression [1]. Treatment with lopinavir/ritonavir (LPV/r), which is the most commonly used HIV protease inhibitors (PIs) in China, has been proven efficacious for HIV infection antiretroviral therapy (ART) in both naïve as well as experienced patients. However, LPV/r has also been correlated with the development of dyslipidemia, especially in cases presented with hypercholesterolaemia and hypertriglyceridaemia [2,3], which are independent risk factors for developing cardiovascular disease complications. Studies have reported that the prevalence of abnormal blood lipid metabolism and other risk factors for cardiovascular disease in HIV-infected patients ranged from 20% to 80% [4] both in developed and developing countries [5,6]. Thus, identifying the metabolic changes associated with clinical parameters during different phases of HIV infection is critical for designing precision strategies to help improving prognosis of HIV and identifying biomarkers for predicating dyslipidemia arising after LPV/r-based therapy.

Metabolomics is an unbiased method of identifying and quantifying small molecules in biological fluids. It has shed new insights into identification of novel clinical biomarkers since metabolites produced

* Corresponding author at: Key Laboratory of AIDS Immunology of National Health and Family Planning Commission, Department of Laboratory Medicine, The First Affiliated Hospital, China Medical University, Shenyang 110001, PR China.
E-mail address: hongshang100@hotmail.com (H. Shang).

https://doi.org/10.1016/j.cyto.2018.05.001
Received 15 November 2017; Received in revised form 30 March 2018; Accepted 2 May 2018

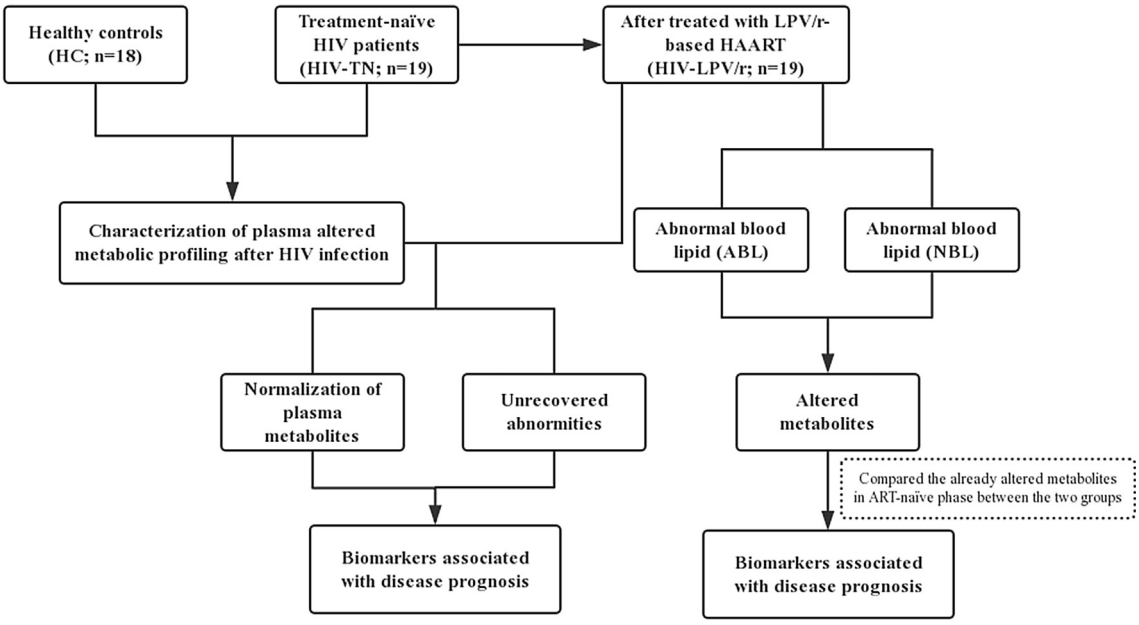

Fig. 1. Diagrammatic representation of the study's work flow design.

by organisms (cells, tissues, or organs) are indirect products of the genome transcriptome and proteome, and the number of metabolites is far smaller than proteins and genes. Metabolomics has been used in the diagnosis of many cancers, such as bladder [7] and ovarian cancer [8]. However, there is limited information on metabolomics approaches in the context of HIV infected patients. A recent study has characterized the metabolic profile of oral wash samples from 12 healthy HIV-infected patients from America to gain insight into how changes in the oral metabolome correlated with HIV disease, since occurrence of specific lesions in the oral cavity plays an important role in the status/progression of HIV disease [9]. Small studies on the differentially regulated metabolites in cerebrospinal fluid have also been performed in SIV-infected monkeys [10] and humans, indicating association between increased aerobic glycolysis and neurocognitive impairment in HIV-infected patients [11], which provided a new method of intervention to protect from HIV induced neurocognitive impairment.

At present, there is limited information on the characterization of longitudinal dynamic changes of plasma metabolites before and after LPV/r-based antiretroviral therapy in the background of HIV infection, especially in Chinese HIV-infected patients. In the current study, we compared longitudinal changes of metabolomic profiles in plasma from the same HIV-infected patients before and after HAART. The aim was to identify specific biomarkers that correlated with HIV disease prognosis and detect specific predictors associated with dyslipidemia induced by LPV/r-based HAART. Our approach would enable us to uncover implications for precision therapy to HIV which can improve disease prognosis and avoid side effects of antiretroviral therapy in Chinese HIV-infected patients.

2. Methods

2.1. Study subjects and study design

Plasma samples were collected from 19 adult HIV-infected patients both before and after HAART, and these samples were divided into two independent groups, the treatment-naïve group (HIV-TN; n = 19), and the LPV/r-treated group (HIV-LPV/r; n = 19) (400 mg and 100 mg of LPV/r, respectively, twice daily). Patients recruited in our study met the following criteria: subjects were without dyslipidemia before HAART, and HIV plasma viral load were < 400 copies/ml after antiretroviral therapy for the duration of at least 3 months.

Exclusion criteria were recent heavy alcohol consumption (> 3times/week, > 4drinks/d; 1 drink was considered to contain 12.5 g of ethanol), severe hepatotoxicity (alamine aminotransferase [ALT] > 50U/L, aspartate aminotransferase [AST] > 40U/L, gamma-glutamyl transpeptidase [GGT] > 60U/L), hyperglycemia, complicated with tuberculosis or other opportunistic infections including pneumocystis carinii pneumonia (PCP), Kaposi's sarcoma, oesophageal candidiasis, cytomegalovirus, wasting syndrome, lymphoma, atypical mycobacteria or various presumptive infections. Patients who were conforming to any of the above criteria were excluded. 18 healthy controls (HC) samples were HIV-negative, matched by age, gender, and body mass index (BMI). Written informed consents were obtained from all the participants before our study.

Blood samples were drawn in the morning, after 12 h of fasting, for routine examination and immunological assays, including cytometry, fasting blood lipids, CD4 + T lymphocyte counts, HIV-RNA viral load, total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglyceride (TG) levels were measured enzymatically in the serum. At the same time, 0.5 ml of plasma was additionally stored at −80 °C for metabolomics experiments. Hypercholesterolaemia and hypertriglyceridaemia were defined on the basis of cut-off values recommended by the US National Cholesterol Education Program guidelines [12]. Additional information on gender, age, BMI, transmission route (homosexual transmission, heterosexual transmission, injection drug use [IDU], transfusion/other), co-infection (hepatitis B/C virus), and current antiretroviral medications were also collected from these participants.

The study design is diagrammatically presented in the flow-chart of Fig. 1 (Fig. 1). We compared the metabolic changes in the 19 HIV treatment-naïve patients with those in the healthy controls, and then determined whether these alterations were recovered after HAART. Furthermore, we classified the HIV-LPV/r patients into two groups based on their blood lipid levels after antiretroviral therapy (abnormal blood lipid group, n = 10; normal blood lipid group, n = 9) and compared the difference between the two groups. Following, identified differences were compared to corresponding metabolites content alteration sat the ART-naïve phase for evaluating their effect on predicting dyslipidemia induced by HAART.