Protease Inhibitor and Metabolic Alteration

Inhibidor de la Proteasa y Alteraciones Metabólicas

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SUMMARY: Protease inhibitors (PIs), part of HAART (Highly Active Antiretroviral Therap) are selective, competitive inhibitors of protease, a crucial enzyme to viral maturation, infection and replication. A lipodystrophic syndrome has been reported in individuals treated with HAART, and associated to hyperglycemia, hypercholesterolemia, hypertrigliceridemia, hyperlipidemia, hypertension and hypreinsulinemia. The HAART-associated metabolic abnormalities were first associated with protease inhibitors, Ritonavir mostly, but the mechamisns that underlie these metabolic alterations are to date, not completely understood. Since PIs are candidate to be the drug of choice for other diseases treatment, such as the Hepatitis C, malaria and some types of cancer, it seems to be important to clarify the metabolic alterations associated to PIs. Wistar rats were treated twice a week with 30mg/kg Ritonavir for 4 and 8 weeks. Total cholesterol, HDL, LDL, VLDL, triglycerides and glycemic levels were measured by the end of each period of time selected. To avoid confunding effects of food intake, the animals were fasted 16 hours before. Our results showed rapid increase in serum triglycerides, total cholesterol, LDL-C and glycemic levels. No significant differences were observed for HDL-C or VLDL serum levels. Our study addresses the importance to observe the possible family history of dyslipidemia or diabetes, and control any other cardiovascular and diabetes risk factors when using protease inhibitors

KEY WORDS: Protease inhibitors; Dyslipidemia; Metabolic disorders.

INTRODUCTION

The Highly Active Antiretroviral Therapy (HAART), also known as ART (antiretroviral therapy), is available since 1996 and uses three or four different antiretrovirals, in general two nucleoside analogue reverse transcriptase inhibitors (NRTIs) + one or two protese inhibitors (PIs), or the non-nucleoside reverse transcriptase inhibitors (NNRTIs) and cell membrane fusion inhibitors (Lenhard *et al.,* 2000; Wynn *et al.*, 2004).

HAART is the treatment of choice for established HIV-infection and for post-exposure prophylaxis. HAART had a dramatic impact on the morbidity and mortality of individuals living with human immunodeficiency virus (HIV). However, strict adherence to the HAART is imperative to limit the development of viral resistance, and noxious side effects, adverse drug reactions and drug interactions may lead to nonadherence to HAART, placing the patient at risk for treatment failure. Studies have shown up to 70% of HIV patiens are intentionally nonadherent to treatment (Wynn *et al.*).

Protease inhibitors (PIs) are selective, competitive inhibitors of protease, a crucial enzyme to viral maturation, infection and replication. They are the largest class of drugs against HIV, acting at a very late stage in the HIV replication process. HIV protease acts right before the viral proteins leave the cell. However, the ability of these agents to suppress virus replication is limited, and the selection of drug resistant HIV variants is not uncommon(Patick & Potts, 1998). Also, the HIV protease is a member of the aspartyl-protease enzyme family, which includes renin and pepsin, and side effects of PIs may include gastrointestinal disruptions, dysfunction of lipids and glucose metabolism, sexual dysfunction, hepatic toxicity, and increased risk of bleeding (Wynn *et al.*).

All PIs are inhibitors of metabolism of cytochrome P450 system, specifically at the 3A4 enzyme (CYP3A4). Cytochrome P4503A is involved in the metabolic clearance of approximately 50% of drugs in the market, and accounts for about 70% of the cytochrome P450 content in the small intestine. Patients with inhibition of CYP system may be at risk for drug interactions and metabolic complications (Kalitsky-Szirtes *et al.*, 2004).

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Ritonavir is "the pan-inhibitor" or "multiple P450 inhibitor". The concurrent use with other agents which are metabolized by CYP3A4, such as different analgesics, antiarrythmic agents, antibiotics, anticoagulants, anticonvulsivants, antiemetics, and antifungal agents, may result in increased levels of these drugs in plasma, and a potential risk for adversal effects (Wynn *et al.*). Also, it seems like PIs may inhibit GLUT-4, the predominant glucose transporter in muscle and fat, and that alterations in GLUT-4 expression may alter insulin sensitivity (Hruz *et al.*, 2011).

A lipodystrophic syndrome has been reported in individuals on HAART, and includes fat redistribution with peripheral lipoatrophy, central or visceral lipohypertrophy, associated to hyperglycemia, hypercholesterolemia, hypertrigliceridemia, hyperlipidemia, hypertension and hypreinsulinemia (Leow *et al.*, 2003). The prevalence of lipodistrophy in patients with HAART is approximately 40%, and levels are differentially affected by anti retrovirals. The HAART-associated metabolic abnormalities were first associated with PIs, Ritonavir mostly, but other antivirals have also been implicated (Kinlaw & Marsh, 2004).

Serum lipid abnormalities seem to occur shortly after HAART beguining therapy (weeks to months) depending on the drug choice and dose, but the pathogenic pathways of PI-associated dyslipidemia is not completely understood (Calza *et al.,* 2003).

Drug therapy for dyslipidemia in persons receiving HAART may be problematic, since many statins (3-Hydroxy-3-Methylglutaryl coenzyme A or HMG-Co A reductase inhibitors), are extensively metabolized by CYP3A4. Overexposure to statins may significantly increase risk for liver and skeletal muscle toxicity (Calza *et al.*).

MATERIAL AND METHOD

Ritonavir is a prototype of PI, and causes the most severe metabolic abnormalities in humans and animal models. Lypodistrophy syndrome may occur in up to 50% taking PI (Temple *et al.*, 2003). Ritonavir (Norvirx solution) has been used in experimental studies (10mg/kg/dose for Sprague-Dawley rats and Rhesus monkeys, and 45-60mg/kg/d, 50ml or 2mg/day for mice). It was well absorbed, 80-100% in both animal species. The use of ritonavir for two weeks caused a elevation of plasma triglycerides and alterations on glycemic levels was observed some time after (Lenhard *et al.*; Xu *et al.*, 2004).

Our animals (Wistar rats) were treated twice a week with 30mg/kg Ritonavir for 4 and 8 weeks. To determine the

effects of the medication on *in vivo* lipoproteins, triglycerides, total cholesterol, HDL, LDL and VLDL were measured by the end of each period of time selected. To avoid confounding effects of food intake, the animals were fasted 16 hours before the blood collection. Blood samples were collected in the morning, from rats under anesthesia. Glycemia were evaluated by the end of the period of the time selected for the study, by measuring serum glycemic levels.

RESULTS

Data about cholesterol, triglycerides and glycemia are presented in Tables I and II.

DISCUSSION

The complexity about the effects of HIV infections and HAART regimens, associated with the effects of other related comorbidities, has challenged the studies of adverse effects in HIV-infection treatment (Hruz *et al.*).

HAART, and mostly PIs, have been associated with lipid disorders, such as hypertriglyceridemia (Friis-Møller *et al.,* 2003; Noor *et al.,* 2004; Dubé *et al.*, 2008), lipodistrophy (Carr *et al.,* 1999), increased total cholesterol and LDL-C serum levels, and lower HDL-C serum levels (Friis-Møller *et al.*). Longitudinal study with HIV infected patients suggests that there are decreases in total cholesterol, HDL-C and LDL-C at the time of infection, before treatment, and with initiation of HAART, total and LDL cholesterol increases but HDL-C low levels persists (Grinspoon & Carr, 2005). Studies indicated that some protease inhibitor (PIs), mostly Ritonavir, may increase hepatic triglycerides synthesis and plasma triglyceride levels (Lenhard *et al.*). Some protease inhibitors (PIs) also tend to increase total cholesterol serum levels, but this effect varies among different individual PIs (Périard *et al.,* 1999).

Our results showed rapid increase in serum triglycerides (Table I), but tendency to come back to normal levels with prolonged use (8 weeks), increased LDL-C levels (Table I), and increased total cholesterol serum levels soon after the initial use of PI. No significant differences were observed for HDL-C or VLDL serum levels.

Impaired insulin uptake is associated with PIs use (Noor *et al.*, 2004) but there are controversies (Dubé *et al.*). It is believed that ritonavir and liponavir induce insulin resistance at therapeutic concentrations, mostly by direct inhibition of GLUT4 but also modulating insulin sensitivity

Table I. Serum triglycerides, total cholesterol and fractions in animals control and treated with protease inhibitor.

	Control		Trated	
	4 weeks	8 weeks	4 weeks	8 weeks
Triglycerides	47.58	63.77	76.98	67.58
	64.32	78.30	92.45	74.01
	36.12	52.83	101.32	65.20
	66.08	61.89	74.53	76.65
	86.34	71.13	98.11	89.87
	60.08±19.19	**65.58±9.64**	**88.67±12.24**	**74.66±9.68**
Mann Whitney U Test			**U canc. = 2 P[U] =0.016**	**U canc. = 6 P[U] =0.111**
			↑	
Total Cholesterol	60.12	80.25	78.89	100.43
	63.19	90.30	70.37	97.91
	61.53	92.91	75.93	92.64
	60.92	90.49	69.75	104.91
	67.73	85.19	69.88	94.48
	62.69±3.03	**89.82±7.17**	**72.96±4.19**	**96.07±6.67**
			U canc. = 0 P[U] =0.004	**U canc. = 1 P[U] =0.008**
			↑	↑
HDL-C	40.84	37.33	52.27	52.16
	43.34	43.20	36.27	41.95
	37.35	44.67	49.20	48.78
	38.47	38.27	35.73	48.22
	43.41	45.60	37.73	45.02
	40.68±2.76	**41.81±377**	**42.24±7.86**	**47.21±3.88**
			U canc. =11 P[U] =0.421	U canc. = 5 P[U] =0.075
LDL-C	9.79	30.17	11.23	38.76
	6.99	37.44	15.61	31.16
	16.96	41.68	16.47	37.50
	10.14	39.85	19.12	41.36
	7.06	23.37	12.53	31.49
	10.17±4.08	**34.50±3.77**	**14.99±4.75**	**36.05±4.53**
			U canc. = 4 P[U] =0.048	**U canc. = 4 P[U] =0.048**
			↑	↑
VLDL	9.51	12.75	15.39	9.51
	12.86	15.66	18.49	14.80
	7.22	10.56	10.26	13.04
	13.21	12.37	14.90	15.33
	17.26	16.22	19.62	17.97
	12.01±3.83	**13.51±2.92**	**15.73±3.65**	**14.13±3.12**
			U canc. = 7 P[U] =0.155	U canc. = 11 P[U] =0.421

via other insulin signaling pathways, including IRS-1 and AKT (Yan & Hruz, 2005; Hurz *et al.*). Our results showed increased glycemic levels in treated animals. The mechanisms involved deserves additional studies.

Table II. Glycaemia in animals control and treated with protease inhibitor.

	Control		Trated	
	4 weeks	8 weeks	4 weeks	8 weeks
Glycaemia	72.41	108.87	72.59	129.65
	66.45	113.09	78.96	144.37
	69.38	118.33	77.52	120.26
	62.87	98.39	80.46	105.79
	74.92	102.89	70.59	141.48
	69.28±4.76	108.31±7.92	76.02±4.23	128.31±15.05
Mann Whitney U Test			U canc. = 3 P[U] =0.0028	U canc. = 3 P[U] =0.028

Dyslipidemia and hyperglycemia may be observed in HIV-positive patients not on HAART, and dyslipidemia may be caused by the HIV infection, as well as any other infectious disease (Baza Caraciolo *et al.*, 2007; Estrada & Portilla, 2011). However, among HIV-infected adults with HAART metabolic alterations are more prevalent than in HIV-infected not on HARRT (Grinspoon & Carr). Our study with Ritonavir showed higher triglyceride, total cholesterol, LDL-C and glycose serum levels in animals not HIV-infected and without previous induction of any metabolic alterations.

Originally used as monotherapy, Ritonavir is now used as enhancer for other PIs (Hull & Montaner, 2011). Some PIs may also be used in the Hepatitis C treatment (Gordon *et al.*, 2011; Poordad *et al.*, 2011) as anti-protozoals, in malaria and other gastrointestinal infections (Dunn *et al.*, 2007). There are also studies investigating whether PIs could be used as a cancer therapy (Gills *et al.*, 2007).

In any case, our study addresses that it is important to observe the possible family history of dyslipidemia or diabetes, control any other cardiovascular and diabetes risk factors when using PIs. Ritonavir is a potent inhibitor of CYP3A4 and of CHP2D6, mostly when used in a dose of 600mg twice daily. However, studies have shown that even low-dose (100mg twice daily) may cause some collateral underisable effects (Aarnoutse *et al.*, 2005; Grinspon & Carr).

RESUMEN: Los inhibidores de la proteasa (IP), que forman parte de la terapia HAART (terapia antirretroviral altamente activa), son inhibidores selectivos y competitivos de la proteasa, enzima crucial para la maduración, infección y replicación viral. Un síndrome lipodistrófico, asociado a hiperglucemia, hipercolesterolemia, hipertrigliceridemia, hiperlipidemia, hipertensión e hiperinsulinemia, ha sido relatado en pacientes tratados con HAART. Las anomalías metabólicas asociadas a la HAART fueron relacionadas, inicialmente, a los inhibidores de la proteasa, principalmente el Ritonavir, pero los mecanismos que relacionados a estas alteraciones metabólicas son poco comprendidos. Dado que los IP son posibles candidatos a fármacos de elección para tratamiento de otras enfermedades, como hepatitis C, malaria y algunos tipos de cáncer, es importante esclarecer las alteraciones metabólicas asociadas a los inhibidores de la proteasa. Ratas Wistar fueron tratadas dos veces por semana con 30 mg/kg de Ritonavir por 4 y 8 semanas. Fueron determinados los niveles de colesterol total, HDL, LDL, VLDL, triglicéridos y glucemia, al final de cada período considerado. Para evitar la interferencia de la ingestión de alimentos en las determinaciones de laboratorio, los animales fueron sometidos a un ayuno previo de 16 horas. Nuestros resultados mostraron un rápido aumento sérico de los niveles de triglicéridos, colesterol total, LDL-C y glucemia. No se observaron diferencias significativas para los niveles séricos de HDL-C o VLDL. Nuestro estudio apunta a la importancia de considerar los posibles antecedentes familiares de dislipidemia o diabetes, y controlar cualquier otro factor de riesgo cardiovascular y de diabetes cuando se utilizan los inhibidores de la proteasa.

PALABRAS CLAVE: Inhibidores de la proteasa; Dislipidemia; Trastornos metabólicos.

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