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Urinary NO_3 excretion and renal failure in indinavir-treated patients

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Abstract

Treatment with indinavir (IDV), a protease inhibitor, is frequently associated with renal abnormalities. We determined the incidence of renal failure (creatinine clearance <80 mL min^{-1} 1.73 $(m^2)^{-1}$) in HIV patients treated with highly active antiretroviral therapy, including IDV, and investigated the possible mechanisms and risk factors of IDV nephrotoxicity. Thirty-six patients receiving IDV were followed for 3 years. All were assessed for age, body weight, duration of infection, duration of IDV treatment, sulfur-derivative use, total cholesterol, triglycerides, magnesium, sodium, potassium, creatinine, and urinalysis. We also determined renal function in terms of creatinine clearance, urine osmolality and fractional excretion of sodium, potassium, and water. Urinary nitrate (NO_3) excretion was measured in 18 IDV-treated patients and compared with that of 8 patients treated with efavirenz, a drug without renal side effects. Sterile leukocyturia occurred in 80.5% of the IDV-treated patients. Creatinine clearance <80 mL min^{-1} 1.73 $(m^2)^{-1}$ was observed in 22 patients (61%) and was associated with low body weight and the use of sulfur-derivatives. These patients also had lower osmolality, lower urine volume and a higher fractional excretion of water compared to the normal renal function group. Urinary NO_3 excretion was significantly lower in IDV-treated patients (809 ± 181 μM NO_3^-/mg creatinine) than in efavirenz-treated patients (2247 ± 648 μM NO_3^-/mg creatinine, $P < 0.01$). The lower NO_3 excretion suggests that IDV decreases nitric oxide production.

Key words
- HIV
- Indinavir
- Nephrotoxicity
- Nitric oxide

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Introduction

Since the introduction of HIV-1 protease inhibitors, the impact of antiretroviral therapy on the incidence of opportunistic infections has been thoroughly investigated and is now well defined. The availability of highly active antiretroviral therapy (HAART) has resulted in profound and sustained reduction of HIV replication, improving CD_4 cell counts, prolonging time to development of AIDS and improving AIDS survival rates (1-4).

The potential long-term consequences of HAART are studied continually (5). Indinavir sulfate (IDV), a protease inhibitor, was introduced as a combination therapy for HIV-infected patients in 1996. Renal and urologi-

cal complications are significant side effects of IDV use, and IDV has been implicated in the development of symptomatic crystalluria and nephrolithiasis in 3-50% of patients (6,7). These complications have been associated with elevated plasma IDV concentrations (8).

In addition, reports of IDV nephropathy secondary to multiple crystals in the collecting ducts of the kidney, have led to the inclusion of such nephropathy in the panoply of IDV-related renal disorders such as tubulointerstitial nephritis, hypertension and renal atrophy (9,10).

Boubaker et al. (11) demonstrated crystal nephropathy with progressive serum creatinine elevation in 18.6% of patients receiving standard IDV doses (800 mg tid), which was potentiated by a low-baseline body mass index and co-administration of trimethoprim-sulfamethoxazole (TMP/SMX).

Recent studies performed in our laboratory have demonstrated that IDV induces renal vasoconstriction and decreases glomerular filtration rate in rats. This effect was associated with a lower nitric oxide (NO) production, and that combining IDV with TMP/SMX elevates the extent of nephrotoxicity (12,13).

We conducted a prospective and observational clinical investigation of 36 HIV-seropositive individuals who had been treated with IDV for at least 12 months. We evaluated the incidence of renal failure, defined as starting at glomerular filtration rate or at creatinine clearance (CrCl) <80 mL min^{-1} 1.73 $(m^2)^{-1}$ body surface, and risk factors for impairment of renal function during the use of IDV. In addition, we measured urinary excretion of nitrate (NO_3) in these patients and compared the values obtained with those from another group of patients treated with efavirenz (EFV), a non-nucleoside reverse-transcriptase inhibitor that is in current use and is a drug without renal side-effects in humans (14).

Subjects and Methods

Patients

From March 2000 to October 2003, we recruited 36 patients infected with HIV who were being treated with IDV and two nucleoside analogs (AZT, 3TC, D_4T, or DDI) at the Instituto de Infectologia Emílio Ribas (São Paulo, SP, Brazil). The study group was composed of 28 (77.8%) men and 8 (22.2%) women ranging in age from 27 to 57 years (mean, 40.3). The inclusion criteria were: a) patient attended the clinic, b) age ≥18 years and seropositivity for HIV-1, and c) patient taking IDV for at least 12 months prior to entry into the study. Patients were excluded for proven non-compliance, if they had a recent diagnosis of an opportunistic infection requiring therapy, or if they were receiving concurrent systemic co-medication with nephrotoxic agents (amphotericin B, radiocontrast agents and aminoglycoside antibiotics) or diuretics. All participating patients provided written informed consent, and the Ethics Committee of the Instituto de Infectologia Emílio Ribas and Hospital das Clínicas da FMUSP approved the project.

According to the Brazilian national guidelines for HIV treatment, patients are seen at the clinic at approximately 2-month intervals for regular follow-up exams. A manual review of medical charts was performed for all patients recruited for information about demographic, clinical and laboratory parameters including age, sex, body weight, time of infection, beginning of IDV-treatment, and co-administration of TMP/SMX, as well as CD_4 cell counts and plasma HIV-1 RNA loads. There was no control regarding patient diet, including L-arginine ingestion. After an initial medical evaluation, laboratory data were collected for each patient during the study period (three years). The laboratory tests consisted of biochemical parameters (total cholesterol, triglycerides, magnesium, sodium, potassium, and creati-

nine). In addition, development of urological symptoms (such as flank pain or renal colic) and hypertension were evaluated periodically. We also obtained 2 or 3 urine samples for assessment of leukocyturia (i.e., >10,000 leukocytes/mL) and hematuria (i.e., >5,000 erythrocytes/mL), which were measured manually (microscopy). Urinary cultures were done for patients with persistent leukocyturia (i.e., on two or more occasions).

Individuals receiving EFV (N = 8) as part of HAART (with previously documented normal renal function) were used as the control group only for urinary excretion of NO_3.

Renal function studies

All patients had two accurate 24-h urinary collections (volume measured), as well as collection of blood samples. Urinary creatinine was determined by the Jaffe method. Renal function was reported as CrCl adjusted per 1.73 m^2 of body surface. Blood and urine samples were also used for determination of sodium and potassium with a flame spectrophotometer (model 143, Instrumentation Laboratories, Inc., Lexington, MA, USA). Urinary osmolality was measured with a freezing point depression osmometer (model 3D3, Advanced Instruments, Inc., Norwood, MA, USA). Standard formulas were used to calculate CrCl (UCr x V/PCr), as well as fractional excretion of sodium (FENa = UNa/PNa x PCr/UCr x 100), potassium (FEK = UK/PK x PCr/UCr x 100) and water (FEH_2O = V/CrCl x 100).

Patients were divided into two groups: those with CrCl >80 mL min^{-1} 1.73 $(m^2)^{-1}$, designated as the preserved function (PF) group, and those with CrCl <80 mL min^{-1} 1.73 $(m^2)^{-1}$, designated as the renal insufficiency (RI) group.

Nitrite and nitrate measurements

Eighteen patients from the IDV-treated group produced urine samples for nitrite (NO_2) + NO_3 (NO metabolites) measurement by chemiluminescence (15), which was performed separately using a model NOA 280 NO analyzer (Sievers Instruments Inc., Boulder, CO, USA) and corrected for urinary creatinine concentration (normal range = 1023-1818 μM NO_3^-/mg creatinine).

The same measurements (determination of urinary excretion of NO metabolites) were also performed on HIV-seropositive patients (N = 8) who were treated with EFV and nucleoside analogs, and the results were compared with those for the IDV-treated group.

Ultrasonography analyses

Renal ultrasound imaging was performed in 26 patients from the IDV-treated group without prior knowledge of creatinine clearance levels. Although requested for all patients, the exam was not performed in 10 of them who had various other problems.

Statistical analysis

Data were processed using means, medians and percentages. Results are reported as means ± SEM. Categorical variables were analyzed using the chi-square test (Fisher's exact test). Continuous variables were analyzed using the unpaired Student *t*-test or Mann-Whitney test, with the latter being often used because there were several variables that did not have a normal distribution. Statistical significance was set at $P < 0.05$.

Results

Study patients

There were 35 patients taking 800 mg IDV every 8 h and 1 receiving IDV (800 mg) and ritonavir (100 mg) twice a day. All patients were advised to maintain a 2-L daily intake of liquids. There were 7 patients (19.5%) receiving secondary prophylaxis against opportunistic infections, using TMP/

SMX and sulfadiazine for *Pneumocystis carinii* pneumonia and toxoplasmosis, respectively. Disease stage was determined according to the Centers for Disease Control and Prevention classification: 19 patients were in stage A (asymptomatic), 7 were in stage B (symptomatic) and 10 were in stage C (AIDS).

The 36 patients studied had all been receiving HAART (including IDV) for at least 12 months prior to enrollment in the study, and 19.5% had a history of opportunistic infections before initiation of HAART. Rates of CrCl were lower (54.8 ± 3.7 mL min^{-1} 1.73 $(m^2)^{-1}$) in 22 patients and normal (103.6 ± 6.5 mL min^{-1} 1.73 $(m^2)^{-1}$) in 14. Demographic and clinical characteristics of the study group, separated by level of renal function, are listed in Table 1. The groups were comparable in age, sex, duration of infection, time on IDV therapy, and disease stage. However, statistically significant differences in body weight were observed between PF patients and RI patients (79.4 ± 3.1 *vs* 62.0 ± 2.3 kg, $P < 0.001$). Only 7 patients were using TMP/SMX or sulfadiazine and all of them were in the RI group (31.8 *vs* 0%, $P < 0.05$). Urological symptoms such as flank pain occurred in 12 (33.3%) of the 36 subjects, and no difference was found between groups. Hypertension was present in 8 (36.4%) of the RI patients and in 6 (42.8%) of the PF patients ($P = 0.90$).

Table 1. Demographic and clinical characteristics of the 36 subjects studied.

	CrCl [>80 mL min^{-1} 1.73 $(m^2)^{-1}$] (N = 14)	CrCl [<80 mL min^{-1} 1.73 $(m^2)^{-1}$] (N = 22)	P value
Mean age (years)	41.3 ± 1.6	40.3 ± 1.7	0.72*
Sex			
Female, N (%)	3 (21.4%)	5 (22.7%)	1.0+
Male, N (%)	11 (78.6%)	17 (77.3%)	1.0+
Duration of IDV therapy (years)	4.7 ± 0.39	4.3 ± 0.36	0.51**
Duration of infection (years)	8.5 ± 0.82	8.3 ± 0.49	0.79**
Body weight (kg)	79.4 ± 3.1	62 ± 2.3	<0.05*
Sulfur-derivative use, N (%)	0	7 (31.8%)	<0.05+

Data are reported as means ± SEM, except where indicated. CrCl = creatinine clearance; IDV = indinavir; sulfur-derivative = trimethoprim-sulfamethoxazole or sulfadiazine.
*Student *t*-test; +Fisher's exact test; **Mann-Whitney test.

Table 2. Renal function of the 36 indinavir-treated patients according to creatinine clearance.

	CrCl [>80 mL min^{-1} 1.73 $(m^2)^{-1}$] (N = 14)	CrCl [<80 mL min^{-1} 1.73 $(m^2)^{-1}$] (N = 22)
FENa (%)	1.11 ± 0.09	1.41 ± 0.25
FEK (%)	8.94 ± 0.74	11.71 ± 1.27
FEH_2O (%)	1.29 ± 0.13	2.57 ± 0.38*
24-h urine volume (mL)	1934 ± 123	1607 ± 244*
Osmolality (mOsm/kg H_2O)	662 ± 63.6	499 ± 50.6*

Data are reported as means ± SEM. CrCl = creatinine clearance; FENa = fractional excretion of sodium; FEK = fractional excretion of potassium; FEH_2O = fractional excretion of water.
*$P < 0.05$ compared to CrCl >80 (Mann-Whitney test).

There were no differences in CD_4 lymphocyte counts between the RI group (199-1102 cells/µL; mean, 528 ± 54 cells/µL) and the PF group (287-1461 cells/µL; mean, 644 ± 82 cells/µL). We found no differences in viral load between RI patients (<400 to 120,000 copies/mL; median: <400) and PF patients (<400 to 17,977 copies/mL, median: <400). Leukocyturia was detected in 19 (86.3%) of the RI patients and in 10 (71.4%) of the PF patients ($P = 0.394$), and hematuria was found in 7 (32%) and 3 (21.4%) patients from the two groups, respectively ($P = 0.706$).

There were no significant differences when the biochemical parameters (total cholesterol, triglycerides, magnesium, sodium, potassium, and serum creatinine) of the RI group were compared with those of the PF group (data not shown). When evaluating the various nucleoside analog combinations (AZT + 3TC, AZT + DDI, D_4T + 3TC, and D_4T + DDI) between the groups we found no correlations with renal failure.

Renal function studies

Twenty-two of the IDV-treated patients (61%) had renal failure based on CrCl stud-

ies and 14 (39%) did not (Table 2). FENa and FEK were not significantly different in the RI and PF groups. Urine volume (24 h) was significantly lower in RI patients than in PF patients (1607 ± 244 and 1934 ± 123 mL, respectively; $P < 0.05$). In addition, as shown in Table 2, osmolality was significantly lower and FEH_2O was significantly higher in patients with renal failure than in those with preserved renal function (499 ± 50.6 *vs* 662 ± 63.6 and 2.57 ± 0.38 *vs* 1.29 ± 0.13 mOsm/kg H_2O, respectively; $P < 0.05$).

Nitrite and nitrate measurements

NO_2 was not detected in the urine of patients in either of the two treatment groups (IDV and EFV). However, Figure 1 illustrates that urinary excretion of NO_3 was significantly lower in the 18 patients treated with IDV than in the 8 patients treated with EFV (809 ± 181 *vs* 2247 ± 648 μM NO_3^-/mg creatinine, $P < 0.05$).

Ultrasound analysis

Ultrasound examinations were performed in 26 IDV-treated patients. Ten of the sonograms were interpreted as being abnormal and 6 of these 10 were from renal failure patients. The principal ultrasound abnormalities were nephrolithiasis (N = 4), followed by bilateral parenchymal nephropathy (N = 3) and renal cysts (N = 3).

Discussion

Indinavir is a potent protease inhibitor used for the treatment of individuals infected with HIV-1 and is a well-known cause of crystal-induced acute renal failure (16,17) and urinary complications. The risk factors associated with urological symptoms or renal injury during treatment have been already identified (7,9,11,18). Nevertheless, few clinical studies have been performed to investigate the mechanisms of reduction in renal function in patients receiving long-term treatment with IDV.

In the present study, we observed reduced creatinine clearance in patients treated with IDV, and this reduction was associated with low body weight and the use of sulfur-derivative drugs. Moreover, we investigated the possible role of NO in the renal failure associated with IDV.

Previous studies on rats have demonstrated that IDV reduces urinary excretion of NO_2 and that the vasodilator agents L-Arg, nifedipine and magnesium protect against IDV-induced nephrotoxicity (13). The present results indirectly suggest that a low NO production occurs and may contribute to a vasoconstriction mechanism (previously demonstrated in rats by decreased renal blood flow and increased renal vascular resistance), since patients treated with IDV presented lower urinary excretion of the NO metabolite NO_3 than did patients treated with EFV. Although suggestive of low NO production, one concern is that we did not employ dietary control for NO_3 intake. In addition, renal function studies in IDV-treated patients showed that FEH_2O was higher in RI patients and that the osmolality was lower than in PF patients. This difference in osmolality between groups may be caused by the lower osmolar clearance in patients with significant weight loss in the renal failure group. FENa and FEK did not differ between PF and RI patients.

Previous studies have shown a correlation between IDV nephrotoxicity and low body weight (11,18). This can be attributed

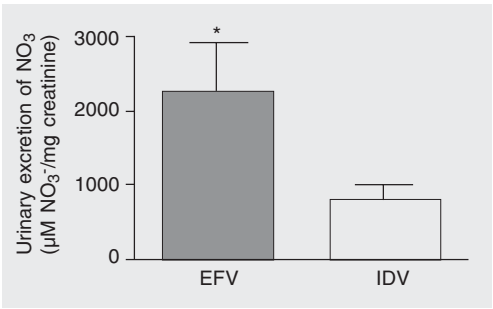

Figure 1. NO_3 excretion by IDV-treated (N = 18) and EFV-treated (N = 8) patients. IDV = indinavir; EFV = efavirenz. *$P < 0.05$ compared to the IDV group (Mann-Whitney test).

to a reduction in distribution volume, probably leading to higher IDV concentrations in such patients. In the present study, neither age nor sex was identified as a risk factor.

In conclusion, we have found that IDV-treated patients have reduced excretion of NO_3, a NO metabolite with vasodilator action. This lower NO_3 excretion suggests that IDV decreases NO production (directly or indirectly) and this lack of NO may contribute to a vasoconstriction mechanism, probably inducing to renal ischemia and renal failure.

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