AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 29, Number 3, 2013

DOI: 10.1089/aid.2012.0232

Short Communication
Fasting Increases Serum Concentrations of Bilirubin in Patients Receiving Atazanavir: Results from a Pilot Study

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Abstract

Unconjugated hyperbilirubinemia resulting from therapy with atazanavir is physiologically related to hyperbilirubinemia in Gilbert's syndrome (GS). In patients with GS, changes in diet have a significant impact on bilirubinemia. Our aim was to investigate whether changes in diet affect the level of serum bilirubin in patients receiving atazanavir. Thirty patients on stable therapy with ritonavir-boosted atazanavir without evidence of GS were enrolled. Hemolysis and chronic hepatitis were excluded. After a baseline period of normal intake of calories, the patients were randomized to follow a 24-h 400-calorie diet (fasting), then a 48-h period of normal calorie intake and, afterward, a 24-h period of a high-calorie diet, or the same interventions in inverse order. Serum bilirubin concentrations were measured before and after each intervention. A high adherence to the recommended diet was observed. The mean unconjugated bilirubin concentration before the high-calorie diet was 2.79 ± 1.53 mg/dl and after such intervention it was 2.70 ± 1.40 mg/dl. The mean difference between preintervention and postintervention was -0.08 ± 0.69 mg/dl (p = NS). The mean unconjugated bilirubin concentration before the fasting diet was 2.31 ± 1.23 mg/dl and it was 3.84 ± 1.90 mg/dl after. The mean difference between prefasting and postfasting was 1.53 ± 1.17 mg/dl ($p = 0.001$). According to these results, short periods of fasting seem to increase the unconjugated bilirubin concentration in patients on atazanavir. A high-calorie diet did not have any impact in bilirubin probably because most patients follow similar diets in their everyday life.

Introduction

Indirect bilirubin is conjugated with glucuronic acid to be excreted in the bile. This process is mediated by the enzyme uridinediphosphate-glucuronyltransferase (UGT). Reduced activity of this enzyme leads to predominantly indirect hyperbilirubinemia. In Gilbert's syndrome (GS), there is a reduced expression of the UGT enzyme due to a mutation (UGT1A1) in the promoter region of this enzyme's gene[1,2] generating unconjugated hyperbilirubinemia and consequent jaundice.

According to the most recent United States Department of Health and Human Services (DHHS) and International AIDS Society (IAS) guidelines for the use of antiretroviral agents in HIV-1-infected people, atazanavir is one of the preferred antiretroviral drugs for the initiation of therapy among antiretroviral treatment-naive HIV-1-infected adults and adolescents.[3,4] This drug reduces the activity of UGT1A1 in a competitive manner producing unconjugated hyperbilirubinemia of a variable magnitude with an incidence of 6–52%.[5–13] This situation may lead to a discontinuation or change in antiretroviral therapy, or to social stigmatization of the patients due to the appearance of jaundice. The mechanism of hyperbilirubinemia in patients taking atazanavir would be similar to that observed in GS.[14]

In addition to the activity of the UGT enzyme, there are other factors that may influence, in a not very well-known manner, the development of unconjugated hyperbilirubinemia. Among people with GS, it has been reported that fasting reciprocally relates to the degree of hyperbilirubinemia, and that a fractioned diet decreases the frequency of the occurrence of hyperbilirubinemia in this disorder.[15]

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Fever, stress, and menses have also been associated with the increase in unconjugated bilirubin levels.[1]

Several mechanisms have been proposed to explain the increase of unconjugated hyperbilirubinemia during fasting such as downregulation of the polypeptide transporter of organic anions in the hepatocyte cell membrane,[16] competitive inhibition of bilirubin captation by free fatty acids in serum (which increase during fasting),[17] and a decrease of intestinal motility with a subsequent accumulation of biliary pigments, increase in bilirubin enterohepatic circulation, and increase in reflux to the plasma.[18] Ishihara *et al.*[19] observed an increase in the total bilirubin values to 1.2 ± 0.42 mg/dl (20.5 ± 7.2 μmol/liter) with respect to baseline values in patients with GS homozygotes for the sequence A(TA)7TAA exposed to a 24-h hypocaloric diet.

Based on these data, we hypothesized that factors influencing hyperbilirubinemia in GS may be similar to those causing hyperbilirubinemia in patients receiving a ritonavir-boosted atazanavir. Consequently, fasting may increase bilirubin levels while a fractioned diet would be partially protective.

The aim of this study was to evaluate the impact of a hypocaloric (fasting) vs. a hypercaloric fractioned diet on bilirubin levels in HIV-1-infected individuals treated with ritonavir-boosted atazanavir-containing combination antiretroviral therapy.

Materials and Methods

This was a randomized, unblinded, crossover pilot study. Inclusion criteria were as follows: (1) treatment with ritonavir-boosted atazanavir for a period not less than 6 months previous to study enrollment, (2) $CD4^+$ lymphocyte count $>200/mm^3$ in the 3 months previous to the baseline visit, (3) undetectable HIV-1 viral load (less than 50 copies/ml) within the 3 months previous to baseline, and (4) agreeing to sign the Informed Consent Form.

Exclusion criteria were (1) GS diagnosed before starting treatment with ritonavir-boosted atazanavir, (2) diabetes mellitus, (3) evidence of significant hemolysis (normal hemoglobin concentration, reticulocyte count, serum lactate dehydrogenase and plasma hemoglobin, and a negative Coombs' test), (4) concomitant hepatic conditions, positive serology for hepatitis C virus and/or hepatitis B surface antigen, and (5) the presence of concurrent opportunistic infection. All the patients were recruited in a private Infectious Diseases Center in Buenos Aires, Argentina.

Although atazanavir may produce hyperbilirubinemia either when administered with boosted ritonavir or unboosted, we chose to enroll in our study exclusively patients using ritonavir-boosted atazanavir-containing combination antiretroviral therapy since the majority of our patients take ritonavir-boosted atazanavir.

After the patients signed the Informed Consent Form, the following data were obtained from the medical records: age; sex; current $CD4^+$ lymphocyte count; time on treatment with ritonavir-boosted atazanavir; concurrent use of other antiretroviral drugs; bilirubin values during the 12 months prior to initiation of antiretroviral treatment to rule out GS; and concomitant medication at the time of enrollment; laboratory data (ALT, AST, alkaline phosphatase, hematocrit, hemoglobin, LDH, total and direct bilirubin) previous to the initiation of antiretroviral therapy and previous to the initiation of ritonavir-boosted atazanavir to rule out the presence of liver disorders and/or hemolysis. In addition, hepatobiliary ultrasound imaging was performed at baseline within 1 month of randomization.

An interview with patients was performed before the baseline visit in order to obtain information about their routine diet. Diets prescribed for the patients enrolled in this study were elaborated by a dietitian. These consisted of a hypocaloric diet, fractionated in four meals, containing 400 kcal in 24 h equivalent to fasting (intervention A) and a hypercaloric diet, fractionated in seven meals, containing 2,400–2,900 kcal in 24 h, according to the participant's sex (intervention B).

The randomly assigned diet instructions were provided to the participants, specifying the servings through measurements or food dish photos, with a diary to register all meals consumed for the duration of the study to assess adherence to the diets. A preliminary alimentary survey was designed by the dietitian to evaluate each patient's usual diet to design the study diet based on the patient's preferences since meals were not provided by the study.

Each intervention lasted 24 h, and serum bilirubin values were determined before and after each one (Table 1). Patients were not allowed to exercise during the intervention period.

All patients were enrolled by only one physician and after signing an Informed Consent, they were interviewed on a different day by the dietitian or another physician. The appointments for these interviews were given according to the patients' preferences and the order was not related to the enrollment sequence. This randomly determined the group, so patients were randomized in a 1:1 fashion to start with

Table 1. Intervention Timetable

	Day 1	*Day 2*	*Day 3*	*Day 4*	*Day 5*
Group	*08:00 h blood sample no. 1*	*08:00 h blood sample no. 2*		*08:00 h blood sample no. 3*	*08:00 h blood sample no. 4*
1	***Intervention A*** 400 kcal/day diet	Washout period		***Intervention B*** 2,400 (women) or 2,900 (men) kcal/day diet fractioned in 6 meals	
2	***Intervention B*** 2,400 (women) or 2,900 (men) kcal/day diet fractioned in 6 meals	Washout period		***Intervention A*** 400 kcal/day diet	

intervention A on day 1 followed by intervention B on day 4 after a 2-day washout period between interventions or to start with intervention B on day 1 followed by intervention A on day 4 after a 2-day washout period between interventions. The crossover design was intended to compensate for bias between interventions.

The main outcome measure was the change in the values of total bilirubin after each intervention. Secondary outcome measures were the changes in indirect bilirubin after each intervention.

Before collecting blood samples, a registered dietitian or a trained nurse conducted a 24-h dietary recall to ensure that the patients followed the prescribed diet. In the 24-h dietary recall, the individual attempted to remember all of the foods and beverages consumed in the preceding day.

The following methods were used for blood test determinations: conjugated bilirubin by the Jendrassik–Grof procedure (colorimetric test) with reagents manufactured by Roche, automatic equipment by Roche (MODULAR P 800); analytic variability: conjugated bilirubin: Cv%: 1.83% (0.7 mg/dl) and Cv%: 3.24% (2.6 mg/dl); total bilirubin by the colorimetric test with 2,5-dichlorophenyldiazonium (DPD), reagents by Roche and automatic equipment by Roche (MODULAR P 800); and analytic variability: Cv%: 3.55% (1.33 mg/dl) and Cv%: 3% (5.9 mg/dl). Indirect bilirubin was calculated by determining the difference between total bilirubin and direct bilirubin.

The sample size was calculated according to data from previous studies about fasting and changes in bilirubin concentrations in Gilbert's syndrome.[1,15] Using the prevalence of patients with significant changes in bilirubin values after fasting, it was calculated that 30 patients would be necessary to show a significant difference in bilirubin concentration after each intervention, with a power of 80% and a level of significance of 5%.

Descriptive results of continuous variables were expressed as mean and standard deviation (SD) values. Continuous variables were compared with parametric (Student's *t*) or nonparametric (Mann–Whitney *U*) tests, as required. Proportions were compared using the chi-square test, with Yates or Fisher corrections if needed. Univariate and multivariate logistic regression analyses were performed for identification of possible factors that might have an impact on changes in bilirubin after fasting.

Results

Thirty patients were included in the study. The mean age was 44.0 (SD ± 9.86) years; 93.3% were male. The median $CD4^+$ cell count was 462 (IQR 366–563) cells/mm^3. All the baseline characteristics were similar between the two groups (Table 2).

A high adherence to the recommended diets was observed according to the 24-h dietary recall performed before each intervention. All included patients were randomized and completed the study. The analyses were performed on an intention to treat basis; every patient followed the sequence as assigned. Since every patient completed the study, all the analyses were performed for the total of individuals included in each group.

The mean total bilirubin concentrations (TBC; mg/dl) before and after intervention B (hypercaloric and fractioned diet) were 2.79 ± 1.53 mg/dl and 2.70 ± 1.40 mg/dl, respectively. The mean difference before and after the intervention was − 0.08 ± 0.69 mg/dl of TBC ($p = 0.8$). The mean total bilirubin concentrations (mg/dl) before and after intervention A (hypocaloric diet) were 2.31 ± 1.23 mg/dl and 3.84 ± 1.90 mg/dl, respectively. The mean difference in TBC (mg/dl) before and after the intervention was + 1.53 ± 1.17 of TBC ($p = 0.001$).

To identify possible factors that have an impact on changes in bilirubin after fasting (hypocaloric and unfractionated diet), we performed a multivariate analysis. Neither $CD4^+$ lymphocyte count nor duration of time on treatment with ritonavir-boosted atazanavir had an impact on the values of bilirubin after fasting.

The bivariate analysis between total bilirubin values before fasting diet and the difference in total bilirubin before and after fasting diet showed that there was not a statistically significant relationship between the two variables: the increase in total bilirubin after fasting 24 h was not influenced by the value of baseline total bilirubin, r^2: 0.06.

Discussion

As far as we know, this is the first study to evaluate the impact of diet on bilirubin levels in patients on chronic treatment with ritonavir-boosted atazanavir-containing combination antiretroviral therapy. Although isolated hyperbilirubinemia does not result in histological liver damage, the consequent jaundice may produce aesthetic alterations, distress, and stigmatization impacting the adherence to antiretroviral therapy.

Two dietary interventions were designed in order to assess their impact on the hyperbilirubinemia related to atazanavir. A fractioned diet with high intake of calories did not demonstrate an impact on bilirubin levels. This may be explained by the fact that a hypercaloric and fractionated diet was reported by most of the patients participating in this study, according to the observations from the alimentary survey performed at the baseline visit. Nevertheless, short periods of fasting increased serum bilirubin levels at the expense of unconjugated bilirubin in patients on antiretroviral therapy with ritonavir-boosted

Table 2. Baseline Characteristics of the Included Patients

	Total	*Group 1*	*Group 2*	p *value*
Randomized patients	30	15	15	
Age [years, mean (SD)]	44.00 (9.86)	47.33 (11.09)	40.67 (7.36)	0.12
Male sex [*n*, (%)]	28 (93.3%)	14 (93.3%)	14 (93.3%)	1
Basal CD4 [cells/mm^3, median (IQR)]	462 (366–573)	448 (347–668)	490 (367–542)	0.8
Time on atazanavir [months, median (IQR)]	32 (14–51)	34 (20–51)	30 (14–40)	0.63
Basal total bilirubin [mg/dl, mean (SD)]	2.52 (1.19)	2.40 (0.93)	2.64 (1.43)	0.77

SD, standard deviation; IQR, interquartile range.

atazanavir, as has been described in patients with GS.[5] The explanation of this phenomenon is not clear.

As stated above, the effect of fasting and the mechanism responsible for the increase in TBC in patients with Gilbert syndrome, and even in the general population, are also unclear. Possible mechanisms, such as increased bilirubin turnover and a diminished hepatic bilirubin clearance, have been proposed. A plausible hypothesis suggests that in the fasting state an increased hepatic uptake of nonesterified fatty acids interferes with the hepatic clearance of bilirubin and thus contributes to the unconjugated hyperbilirubinemia of fasting. The same physiopathogenic mechanisms might occur with atazanavir-induced hyperbilirubinemia.

Among the variables that might modify the impact of fasting on bilirubin levels analyzed, none of them had a statistically significant relationship. Neither $CD4^+$ lymphocyte count nor duration of time on antiretroviral treatment with ritonavir-boosted atazanavir seems to attenuate the impact of fasting on indirect bilirubin levels. Baseline bilirubin values did not predict a response to fasting in patients on ritonavir-boosted atazanavir.

We did not, however, analyze plasma levels of atazanavir or genetic polymorphisms in our patients. Polymorphisms at MDR1-3435 were shown to significantly influence atazanavir plasma concentrations, as does being a white patient with CT/TT genotypes, with lower atazanavir levels, even when administering ritonavir for boosting. In addition, it was also shown that although atazanavir plasma concentrations directly correlate with bilirubin levels, the risk of severe hyperbilirubinemia is further increased in the presence of the UGT1A1-TA7 allele.[20–23] These interventions might have had a different impact in patients with different atazanavir plasma levels or different genetic polymorphisms.

Conclusions

Avoiding fasting and very low-calorie diets might prevent the increase of unconjugated bilirubin levels in patients on ritonavir-boosted atazanavir-containing combination antiretroviral therapy. Larger studies are needed to confirm this observation.

Acknowledgments

The authors would like to thank Dr. Roberto Arduino for his critical review of the manuscript. Funding is by the Fundación del Centro de Estudios Infectológicos, FUNCEI, Buenos Aires, Argentina. Dr. Emiliano Bissio receives funding as a trainee from the following NIH grant: Fogarty AIDS International Training and Research Program (AITRP), number D43 TW001037

Author Disclosure Statement

No competing financial interests exist.

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