Sildenafil and bosentan plasma concentrations in a human immunodeficiency virus- infected patient with pulmonary arterial hypertension treated with ritonavir-boosted protease inhibitor

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Abstract

Sildenafil and bosentan are increasingly used for the treatment of pulmonary arterial hypertension (PAH) in HIV-infected patients. However, concerns exist about pharmacokinetic interactions among sildenafil, bosentan and antiretroviral drugs, including protease inhibitors (PI). We describe here the case of an HIV-infected patient with PAH, who was co-administered bosentan 125 mg twice daily and sildenafil 40 mg three times per day, together with a ritonavir-boosted PI-based antiretroviral therapy; plasma levels of bosentan, sildenafil, N-desmethylsildenafil, and PI were measured. The patient had a sildenafil $C_{through}$ and C_{max} of 276.94 ng/mL and 1733.19 ng/mL, respectively. The $C_{through}$ and the C_{max} of bosentan were 1546.53 ng/mL and 3365.99 ng/mL, respectively. The patient was able to tolerate as high sildenafil blood concentrations as 10 times those usually requested and did not report any significant adverse reaction to sildenafil during the follow-up period. Therapeutic drug monitoring should be considered during sildenafil therapy in patients concomitantly treated with ritonavir-boosted PI.

Introduction

The approved sildenafil dosing regimen for the treatment of pulmonary arterial hypertension (PAH) is 20 mg three times per day (tid); in patients with human immunodeficiency virus (HIV)-associated PAH the same treatment algorithm as in patients with idiopathic PAH should be considered, taking into consideration comorbidities and drug-drug interactions.[1]

Maximum plasma concentrations of sildenafil at this dose are reached by approximately 1 hour after oral administration (t_{max}), and the $t_{1/2}$ is approximately 4 hours in healthy adults, with a C_{max} of about 145 ng/mL.[2] Sildenafil t_{max} and $t_{1/2}$ in PAH patients are similar to those of healthy volunteers.[3,4]

The main route of clearance is by hepatic metabolism, and the major metabolite is N-desmethylsildenafil; it has the same phosphodiesterase specificity as sildenafil, but about half the potency, and plasma levels are about 40-50% of the parent drug.[5] Sildenafil is primarily metabolized by the cytocrome P-450 (CYP) isoenzyme CYP3A4 and to a lesser extent CYP2C9. Hence, the inhibitors of CYP3A4 could interfere with sildenafil elimination.

The antiretroviral protease inhibitor ritonavir (500 mg twice daily), a strong inhibitor of CYP3A-mediated metabolism, has been demontrated to increase sildenafil AUC 11-fold and Cmax 3.9-fold, and multiple dosing with ritonavir delayed sildenafil t_{max} by 3.1 hours.[6] The ritonavir dose reported in the study by Muirhead *et al.*[6] is far above the dose currently administered to boost protease inhibitors in HIV-infected individuals. However, sildenafil exposure was also markedly increased when coadministered with darunavir plus low-dose ritonavir (400/100 mg twice daily).[7]

Concomitant use of sildenafil (Revatio®, Pfizer Labs, New York, NY, USA) with ritonavir and other potent CYP3A inhibitors is not recommended by the manufacturer.[8] However, successful coadministration of ritonavir and sildenafil in HIV-PAH patients has been recently reported.[9]

Bosentan, a non selective endothelin receptor antagonist, is a substrate and a known inducer of CYP2C9 and CYP3A4;[10] additional monitoring/dose adjustments are required when bosentan is coadministered with several drugs including lopinavir/ritonavir.[11]

Mutual pharmacokinetic interactions between steady-state bosentan and sildenafil have been described in healthy volunteers: bosentan decreased the C_{max} of sildenafil by 55%, while sildenafil increased bosentan C_{max} by 42%.[12] Herein, we describe the case of an HIV-infected patient with severe PAH, coadministered bosentan 125 mg twice daily (bid) and sildenafil at the increased dose of 40 mg tid together with a ritonavir-boosted protease inhibitor (PI)-based antiretroviral therapy (ART); the plasma levels of bosentan, sildenafil, N-desmethylsildenafil, and PI were measured.

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Case Report

A 62-year-old male was found to be HIV infected in 2006; therefore, he started ART including zidovudine 300 mg bid, lamivudine 150 mg bid, fosamprenavir 700 mg bid, and ritonavir 100 mg bid. His body mass index was 19. He wasn't coinfected with hepatitis B or C virus.

The patient complained of fatigue and exertional dyspnea, and HIV-PAH was ascertained on July, 2007 (NYHA stage III). The right-heart catheterization showed a mean PAP of 55 mmHg and bosentan therapy was prescribed. After an initial good response to bosentan, the patient presented a worsening of the PAH symptoms, and underwent a new invasive hemodynamic assessment showing a mean PAP of 63 mmHg.

Despite the possibility of drug interactions with PI, on March 2010 sildenafil 20 mg tid was added to the ongoing therapy, that included furosemide, canrenone, digoxin, and acenocumarol. After the introduction of sildenafil therapy, the patient experienced an improvement in his cardiopulmonary symptoms passing from NYHA stage III to NYHA stage II, with a reduction of mean PAP to 43 mmHg. However, on May 2012 because of a worsening exertional dyspnea with a mean PAP of 47 mmHg, sildenafil dose was increased

to 40 mg tid, while bosentan was continued at the standard 125 mg bid dose. Subsequently, the patient experienced a relief of the dyspnea with a mean PAP of 40 mmHg on October 2012 and a 6-minute walking test of 479 m. On September 2012 plasma levels of his drugs were ascertained.

Plasma concentrations of bosentan, sildenafil, N-desmethylsildenafil, and PI were evaluated before the intake of the morning dose (T_0) and every two hours thereafter (T_2, T_4, T_6, T_8). Heparinized venous blood samples were collected in 7.5-mL tubes and were stored after centrifugation at –80°C until analysis.

Plasma concentrations of bosentan, sildenafil, and N-desmethylsildenafil were measured by a liquid chromatography tandem mass spectrometry (LC-MS/MS) after a liquid-liquid extraction of analytes from the biological matrix. After the addition of 100 ng of deliverdine as internal standard (10 μL of 10 μg/mL methanolic solution) and 500 μL of phosphate buffer pH 10, plasma sample was extracted twice with 1.5 mL *tert*-butyl methyl ether. After centrifugation at 3500 rpm for 3 min, the organic phase was evaporated to dryness under a stream of nitrogen and re-dissolved in 100 μL of mobile phase. A 20 μL aliquot was injected into the liquid chromatograph.

The LC-MS/MS analyses were performed using an Alliance HPLC system (Waters, Etten-Leur, The Netherlands) interfaced to a Micromass Quattro micro API triple quadrupole mass spectrometer (Waters) equipped with electrospray ionization (ESI) probe. Analytes separation was achieved using a Zorbax Eclipse XDB C8 column (150×4.6 mm, 5 μm). The experiment was carried out at a flow rate of 0.5 mL/min with a mobile phase A (0.1% formic) and B (acetonitrile). The gradient elution started at 30% B maintained for 1 min, ramped linearly to 90% B in 6 min, and maintained at 90% for 2.5 min, then directly returned to initial percentage and maintained for 10 min.

The tandem mass spectrometer was operated in positive ionization mode with the following parameters: capillary voltage, 4 kV; source temperature, 120°C; desolvation temperature, 350°C; cone gas flow rate, 50 L/h; desolvation gas flow rate, 800 L/h. Cone voltage and collision energy were 40 V and 30 V, respectively. Acquisition was performed using multiple reaction monitoring with the following transition: sildenafil *m/z* 475→311, 475→283 and 475→100, N-desmethylsildenafil *m/z* 461→311, 461→283 and 461→85, bosentan *m/z* 552→311, 552→280 and 552→202, deliverdine *m/z* 457→221. The bold transitions were selected for quantification.

PI concentrations were measured in plasma by a validated high-performance liquid chromatography method.[13] The patient gave informed consent to the study.

The results of the study are summarized in Tables 1 and 2. The through concentration ($C_{through}$) of sildenafil was 276.94 ng/mL. Six hours after the morning assumption of sildenafil, the patient had a sildenafil maximum plasma concentration (C_{max}) of 1733.19 ng/mL. The $C_{through}$ and the C_{max} of bosentan were 1546.53 ng/mL and 3365.99 ng/mL, respectively. The $C_{through}$ and the C_{max} of amprenavir were 1495 ng/mL and 3962 ng/mL, respectively. Plasma levels of ritonavir were undetectable in all of the samples.

Discussion

According to the European Medicines Agency review of sildenafil 20 mg tablets (Revatio®, USA), a sildenafil plasma concentration between 10 and 100 ng/mL is associated with a significant effect on PAP and pulmonary vascular resistance (PVR); maximal reductions in PAP and PVR were obtained at plasma concentrations in the range of 100 ng/mL.[14] An area under the concentration-time curve above 2600 ng·h/mL and C_{max} values in excess of 500 ng/mL were associated with a 40% incidence of abnormal vision episodes, 15% incidence of gastrointestinal events, and 25% incidence of vascular events.[15] Our patient experienced as high sildenafil blood concentrations as 10 times those usually requested; however, he did not report any adverse reactions to sildenafil, such as headache, flushing, dyspepsia or priapism; there was no significant influence on systemic blood pressure, which was measured regularly during the investigation. This is of clinical relevance as severe hypotension and cardiogenic shock are major concerns in this type of patients.

The patient had eye fundus examination during follow-up and no significant changes were seen in respect of baseline conditions. On March 2013 the off-label prescription of sildenafil was stopped and tadalafil 40 mg once daily (Adcirca®, Eli Lilly Nederland B.V., Houten, the Netherlands) was started after cardiological consultation. At that point the patient was in satisfying general conditions, without significant sildenafil-related adverse events. On March 2014 the patient experienced an important hemoptysis successfully treated with right bronchial artery embolization. He is currently in relatively good health conditions, followed-up and treated with ART, bosentan, tadalafil and home oxygen therapy.

During the study, plasma concentrations of N-desmethylsildenafil were lower than the expected 40-50% of the parent drug; this was probably due to inhibition of CYP3A4 by ritonavir. Of note, ritonavir resulted undetectable in all of the blood samples drawn from the patient. Since bosentan is also an inducer of CYP3A and CYP2C9, plasma concentrations of drugs metabolized by these 2 isozymes (*e.g.* ritonavir, glyburide, statins) will be decreased when bosentan is coadministered;[11] this could explain the undetectability of ritonavir. However, the patient did not experience significant changes in his CD4+ cells count, CD4% or HIV viral load (persistently undetectable) under this drug regimen; his amprenavir levels were within therapeutic ranges.[16]

Bosentan C_{max} in healthy volunteers administered 125 mg bid of the drug was in the range

Table 1. Plasma concentrations of sildenafil, N-desmethylsildenafil, and bosentan.

Sample	Hour	SLD, ng/mL	DM-SLD, ng/mL	BST, ng/mL
t_0	8 a.m.	276.94	27.59	1546.53
t_2	10 a.m.	925.40	58.33	3365.99
t_4	noon	566.46	61.85	1750.15
t_6	2 p.m.	1733.19	140.95	1306.73
t_8	4 p.m.	900.49	121.35	1234.25

SLD, sildenafil; DM-SLD, N-desmethylsildenafil; BST, bosentan. t0: before the intake of sildenafil and bosentan; t_2, t_4, t_6, t_8: 2, 4, 6, 8 hours after the intake of sildenafil and bosentan, respectively.

Table 2. Plasma concentrations of amprenavir and ritonavir.

Sample	Hour	AMP, ng/mL	RTV, ng/mL
t_0	8 a.m.	1495	<50
t_2	10 a.m.	1682	<50
t_4	noon	3962	<50
t_6	2 p.m.	2167	<50
t_8	4 p.m.	1889	<50

t_0: before the intake of antiretroviral dose; t_2, t_4, t_6, t_8: 2, 4, 6, 8 hours after the intake of antiretroviral dose, respectively. AMP, amprenavir; RTV, rotnavir.

of 1000 ng/mL in the study by van Giersbergen and colleagues.[17] It is well known that sildenafil may increase the exposure to bosentan by inhibition of hepatic uptake via human organic anion-trasporting polypeptides OATP1B1 and OATP1B3.[18] Bosentan C_{max} in our patient was indeed increased to 3365.99 ng/mL. Moreover, Dingemanse and coll.[19] have described substantial increases in bosentan C_{max} (+512%) and AUC (+422%) at steady state when coadministered with lopinavir/ritonavir, probably due to a combination of OATP1B1 and CYP3A4 inhibition by ritonavir. As a consequence, the dose of bosentan should be adjusted when used in combination with ritonavir-containing HIV regimens.[11] In our patient, therapy could have been switched from a PI-based to an integrase inhibitor-based ART, or the endothelin receptor antagonist could have been changed to ambrisentan; at the time of the study these options were not considered because therapy was effective and well tolerated.

Conclusions

In conclusion, in our patient the coadministration of sildenafil and ritonavir-boosted PI resulted in an increase of sildenafil plasma concentration above the therapeutic range; this increase could have been diminished to some degree by the use of bosentan, which is known to decrease sildenafil levels. However, our patient did not experience significant adverse events related to raised sildenafil levels. Therapeutic drug monitoring should be taken into consideration during sildenafil therapy in patients concomitantly treated with ritonavir-boosted PI.

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