

Clinical Ritonavir and Lopinavir Hypersensitivity Confirmed by a Specific *In Vitro* Cellular Allergen Stimulation Test

Roberto Manfredi^{*,1}, Sergio Sabbatani¹ and Sergio Bergonzi²

¹Department of Clinical and Experimental Medicine, Division of Infectious Diseases, "Alma Mater Studiorum" University of Bologna, S. Orsola-Malpighi Hospital, Bologna, Italy; ²Laboratory of Clinical Pathology, "C.A. Pizzardi" Maggiore General Hospital, Bologna, Italy

Abstract: A HIV-infected patient treated since eight years with all antiretroviral classes save boosted protease inhibitors, at the time of changing therapy due to an emerging genotyping resistance to non-nucleoside reverse transcriptase inhibitors, experienced repeated episodes of hypersensitivity reactions to all available boosted protease inhibitors. After documenting a combined ritonavir and lopinavir hypersensitivity by means of a specific *in vitro* cellular antigen stimulation test (CAST), antiretroviral therapy was safely continued with unboosted atazanavir. According to our knowledge, we report the first case of application of the *in vitro* CAST assay to antiretroviral intolerance, and the subsequent, specific regimen selection in a HIV-infected subject who showed multiple allergy to all boosted protease inhibitors. Further, controlled investigation is strongly needed to implement *in vitro* allergometric testing in patients with HIV infection and related diseases, who are prone to show unpredictable drug intolerance reactions. In fact, HIV-infected patients may suffer from frequent allergic drug reactions which may be difficult to be systematically recognized (due to the frequent, multiple concurrent pharmacotherapy), while eventual drug rechallenges are expected to be potentially dangerous.

Keywords: Ritonavir, lopinavir, hypersensitivity, clinical issues, laboratory issues, cellular allergen stimulation test (CAST).

INTRODUCTION

The spectrum of dermatological and systemic findings directly or indirectly related to HIV disease includes a broad variety of cutaneous and/or mucous and/or systemic disorders. Untoward and allergic reactions to drugs including skin involvement are observed with increasing frequency in HIV-infected subjects compared with the general population [6]. In particular, hypersensitivity reactions are frequent adverse events among HIV-infected patients, and antiretroviral drugs represent the major challenge. However, the multiple, concomitant treatments established for HIV- and AIDS-related conditions (i.e. sulfonamides, cotrimoxazole, anti-tubercular drugs, antibiotics, cytotoxic agents, and many other compounds), may also play a significant and often confounding role, when multiple concomitant drug treatments are of concern [2,9]. Among antiretroviral drugs, the nucleoside analogue abacavir [10], and both non-nucleoside reverse transcriptase inhibitors nevirapine and efavirenz (especially nevirapine) [3,6,8,14] are the most frequently involved compounds [2], although case series plus numerous anecdotal reports also claim a possible role for almost all anti-HIV compounds, including all nucleoside/nucleotide reverse transcriptase inhibitors, protease inhibitors, and more recently fusion inhibitors [18]. Stevens-Johnson syndrome, Lyell syndrome, toxicodermia, and other severe hypersensitivity syndromes may be severe and become life-threatening, and strongly contraindicate both continuation and drug rechallenges [3]. Moreover, the recent introduction of fixed antiretroviral combinations increases diagnostic difficulties

and poses cumbersome problems at the time of re-treatment of patients who developed an hypersensitivity reaction when undergoing concurrent administration of at least three associated antiretroviral compounds [3], as recommended since one decade [10]. Genetic- and gender-based differences in both incidence and severity of hypersensitivity reactions to every single compound are increasingly known, and it is now possible to operate a genetic screening to identify some selected specific predispositions [13,18], in order to prevent their occurrence whenever possible. A case report of intolerance of lopinavir and all ritonavir-boosted protease inhibitors is presented, together with the potential role of *in vitro* allergometric assays in diagnosing complicated clinical pictures like ours, where only the individuation of ritonavir as one of the two potential allergens allowed us to re-start an effective and well tolerated antiretroviral therapy.

CASE REPORT

A 32-year-old homo-bisexual man followed since 12 years for a HIV-HCV co-infection, eight years ago initiated antiretroviral therapy with didanosine-stavudine-indinavir, after diagnosing an AIDS-related disease (esophageal candidiasis). After changing four different antiretroviral regimens, always containing nucleoside-nucleotide analogues associated with non-nucleoside reverse transcriptase inhibitors and non-boosted protease inhibitors (indinavir and nelfinavir), due to an increased viral load (HIV-RNA 42,000 copies/mL), a decreased CD4⁺ lymphocyte count (361 cells/ μ L), associated with an emerging cross-resistance to efavirenz and nevirapine, he attempted for the first time boosted protease inhibitors (saquinavir-ritonavir, followed three weeks later by fosamprenavir-ritonavir, two weeks later by atazanavir-ritonavir, and four weeks later by lopinavir-ritonavir). All the four last therapeutic regimens were

*Address correspondence to this author at the University of Bologna, c/o Infectious Diseases, S. Orsola Hospital, Via Massarenti 11 - I-40138 Bologna, Italy; Tel: +39-051-6363355; Fax: +39-051-343500; E-mail: roberto.manfredi@unibo.it

poorly tolerated and self-discontinued after some days, due to diffuse malaise, fatigue, sore throat, swallowing difficulties, anorexia, loss of body weight, and a worsening, pruriginous, skin rash which predominantly involved the head, neck, trunk, and upper arms; this clinical picture even worsened after the introduction of the another boosted protease inhibitor (lopinavir-ritonavir). Also a four-week antihistaminic treatment and later a two-week corticosteroidal one, failed in recovering the clinical picture during boosted protease inhibitor-based antiretroviral therapy, so that the entire antiretroviral regimen was temporarily changed towards a triple nucleoside/nucleotide combination (zidovudine, lamivudine, and tenofovir), which was well tolerated, but failed in achieving a suppression of viral load (11,000 HIV-RNA copies/mL), and was associated with a further decrease of CD4⁺ lymphocyte count (263 cells/ μ L). During the triple nucleoside/nucleotide association treatment, the diffuse erythematous lesions turned into a desquamative stage (accompanied by reduction of itching), and a significant improvement of enanthema and dysphagia also occurred. At that time, our patient underwent a specific *in vitro* cellular antigen stimulation test (CAST) performed on all available antiviral compounds, which disclosed a very elevated positivity for both ritonavir and lopinavir (>3,200 sLT pg/mL), while all other tested compounds (including all nucleoside-nucleotide inhibitors, non-nucleoside reverse transcriptase inhibitors, but also nelfinavir, amprenavir, fosamprenavir, indinavir, saquinavir, and atazanavir), tested well below normal limits (levels <0.1 sLT pg/mL, with normal values fixed within 70.0 sLT pg/mL). After obtaining this laboratory testing, and taking also into account the result of prior genotype resistance assay, non-boosted atazanavir (200 mg twice daily) was safely introduced and continued together with lamivudine-stavudine. Three months later, while the novel antiretroviral therapy was perfectly tolerated and all allergic manifestations disappeared, viral load dropped to undetectable levels (<40 copies/mL), while the CD4⁺ count rose up to 775 cells/ μ L.

DISCUSSION

Compared with other classes of antiretroviral agents, with regard to protease inhibitors we only have sparse and anecdotal reports of hypersensitivity reactions involving all the available compounds with a very low absolute frequency, leading to a poor clinical suspicion, and related, increased diagnostic difficulties. Only very recently also lopinavir and/or ritonavir were included as rare, but possible agents of hypersensitivity reactions [1,7,11]. To the best of our knowledge, a pruriginous maculo-papular rash has been described 7-10 days after administration of combined lopinavir-ritonavir in two patients, who showed a repeated reaction upon rechallenge [1]. Another case of acute generalized exanthematous pustulosis occurred during lopinavir-ritonavir administration, as a part of a post-exposure prophylaxis [7]. Finally, the last described case regarded lopinavir alone in a patient who previously took ritonavir and was favorably rechallenged with atazanavir-ritonavir, although she manifested a prior, serious Stevens-Johnson-like syndrome, associated with myeloid, liver, and kidney toxicity, at her first administration of lopinavir-ritonavir [11].

During the last 3-4 years, the further development of laboratory flow cytometry allergology techniques available since the year 1993, enabled to test a series of compounds, including inhalants, food, insect venoms, occupational agents, and also medicines and their excipients, in order to contribute to specifically predict or prevent persistent allergies, and their related signs and symptoms. In particular, an assay for flow cytometric sulfidoleukotriene LCT4, LTD4, and LTE4 release by interleukin-3-primed basophils stimulated *in vitro* by the supposed allergens (the so-called cellular antigen stimulation test, or CAST), is the development of basophil activation test (BAT), and an *in vitro* allergen-induced specific activation of basophils (i.e. BASOTEST). The CAST assay seems to show elevated sensitivity and specificity rates (around 80-90%) for antigens for which a prick and/or intradermal test or other allergometric examinations are not feasible or reliable. All these assays also allow to detect allergic reactions due to mechanisms other than allergen-specific IgE antibody release [4,5,12,15-17], and also avoid some cumbersome and potentially dangerous provocation (re)challenges. To the best of our knowledge, *in vitro* immunoassays like BAT, CAST, and their combination, now have broad experiences in the field of aspirin and non-steroidal anti-inflammatory compounds [4,5,16,17], but they are not fully standardized for a broad spectrum of substances, and they have not ever been exploited to assess possible intolerance to antiretroviral compounds, although in our personal experience immediate or late allergic reactions were identified very reliably, and led to relevant clinical decisions in several examined patients, like the presented one. The role of CAST testing and its related immunometric assays seems very promising, since they allowed us to identify specific intolerances, not common to all agents belonging to the same pharmaceutical class (in our cases, ritonavir and lopinavir, but not other protease inhibitors), so that they may add significantly to the long-term management of HIV infection, when adverse or untoward events are very frequent, and sometimes difficult to distinguish from different reactions elicited by concomitant antiretroviral agents, concurrent diseases, and their pharmacological management. Since a broad spectrum of antiretroviral drugs may be responsible for immediate or delayed hypersensitivity reactions, and no experience is available until now with these proportionally innovative immunometric techniques, we believe that further, controlled studies will aid us to join a systematic appraisal of these assays in the field of antiretroviral treatment, which encompasses a very prolonged administration of different compounds and classes of associated antiretroviral agents. Moreover, the possibility to identify the compound(s) related to hypersensitivity reactions prevents further patient's exposure and dangerous rechallenge attempts, and allows to detect the specific drug to be withdrawn, and preserve all the remaining therapeutic options. In our emblematic case report, hypersensitivity to both ritonavir and lopinavir would be very difficult to be clinically identified and an effective antiretroviral treatment could be seriously delayed, since only regimens including protease inhibitors was recommended by *in vitro* resistance assays. As predicted by the CAST immunometric assay, an atazanavir-based treatment excluding ritonavir booster was perfectly tolerated and fully effective, after its clinical introduction.

REFERENCES

- [1] Calista D. (2005). Maculo-papular rash induced by lopinavir-ritonavir. *European Journal of Dermatology*. 15:97-98.
- [2] Caumes E, Bossi P, Katlama C, Bricaire F. (2003). Antiretroviral-induced toxiderma in HIV-infected patients. *Presse Medicale*. 32:1325-1333.
- [3] Daniel OJ, Krain AB, Ogun SA, Odusoga OL, Boyle BA. (2005). Nevirapine-related adverse events in a patient receiving a fixed-drug combination pill. *Journal of the National Medical Association*. 97:1719-1721.
- [4] De Weck AL, Sanz ML. (2004). Cellular allergen stimulation test (CAST) 2003, a review. *Journal of Investigative Allergology and Clinical Immunology*. 14:253-273.
- [5] De Weck AL, Gamboa PM, Esparza R, Sanz ML. (2006). Hypersensitivity to aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). *Current Pharmaceutical Design*. 12:3347-3358.
- [6] DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents – A Working Group of the Office of AIDS Research Advisory Council (OARAC). Guidelines for the use of antiretroviral agents in HIV-1 infected adults and adolescents. October 10, 2006. Available on the world wide web, at <http://AIDSinfo.nih.gov:1-113> (accessed January 25, 2007).
- [7] Ghosn J, Duvivier C, Tubiana R, Katlama C, Caumes E. (2005). Acute generalized exanthematous pustulosis induced by HIV postexposure prophylaxis with lopinavir-ritonavir. *Clinical Infectious Diseases*. 41:1360-1361.
- [8] Hartmann M, Brust J, Schuster D, Mosthaf F, Procaccianti M, Rump JA, Klinker H, Petzoldt D. (2005). Rashes in HIV-infected patients undergoing therapy with nevirapine or efavirenz. *Hautartz*. 56:847-853.
- [9] Kreuter A, Schugt I, Hartmann M, Rasokat H, Altmeyer P, Brockmeyer NH. (2002). Dermatological diseases and signs of HIV infection. *European Journal of Medical Research*. 7:57-62.
- [10] Lanzafame M, Trevenzoli M, Lattuada E, Faggian F, Vento S, Concia E. (2003). Enanthema as the first clinical manifestation of abacavir hypersensitivity reaction: a case report. *Le Infezioni in Medicina*. 11:40-41.
- [11] Manfredi R, Sabbatani S. (2006). Serious, multi-organ hypersensitivity to lopinavir alone, involving cutaneous-mucous rash, and myeloid, liver, and kidney function. *AIDS*. 20:2399-2400.
- [12] Moneret-Vautrin DA, Kanny G, Fremont S. (2003). Laboratory test for diagnosis of food allergy: advantages, disadvantages and future perspectives. *Allergie et Immunologie (Paris)*. 35:113-119.
- [13] Ofotokun I, Pomeroy C. (2003). Sex differences in adverse reactions to antiretroviral drugs. *Topics in HIV Medicine*. 11:55-59.
- [14] Pitche P, Drobacheff-Thiebaut C, Gavignet B, Mercier M, Laurent R. (2005). Cutaneous drug reactions to nevirapine: study of risk factors in 101 HIV-infected patients. *Annals of Dermatology and Venereology*. 132:970-974.
- [15] Sanz ML, Maselli JP, Gamboa PM, Oehling A, Dieguez I, De Weck AL. (2002). Flow cytometric basophil activation test: a review. *Journal of Investigative Allergology and Clinical Immunology*. 12:143-154.
- [16] Sanz ML, Gamboa P, De Weck AL. (2005). A new combined test with flowcytometric basophil activation and determination of sulfidoleukotrienes is useful for *in vitro* diagnosis of hypersensitivity to aspirin and other nonsteroidal anti-inflammatory drugs. *International Archives of Allergy and Immunology*. 136:58-72.
- [17] Sanz ML, Gamboa PM, Garcia-Aviles C, De Weck A. (2005). Drug hypersensitivities: which room for biological tests? *Allergie et Immunologie (Paris)*. 37:230-235.
- [18] Shibuyama S, Gevorkyan A, Yoo U, Tim S, Dzhangiryan K, Scott JD. (2006). Understanding and avoiding antiretroviral adverse events. *Current Pharmaceutical Design*. 12:1075-1090.

Received: January 25, 2007

Revised: February 26, 2007

Accepted: February 28, 2007

Copyright of Current HIV Research is the property of Bentham Science Publishers Ltd. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.