Correspondence

Modeling Metabolic Effects of the HIV Protease Inhibitor Ritonavir *In Vitro*

To the Editor-in-Chief:

Wang et al,¹ in an article entitled "Human Immunodeficiency Virus Protease Inhibitor Ritonavir Inhibits Cholesterol Efflux from Human Macrophage-Derived Foam Cells," attempts to relate the dyslipidemia, lipodystrophy, and metabolic syndromes associated with ritonavir to the ability of this drug to interfere with cholesterol efflux *in vitro*. However, the concentrations of ritonavir used are suprapharmacologic and thus of uncertain clinical significance.

The authors state in their abstract that "a clinically relevant concentration of ritonavir (15 $\mu\text{mol/L}$)" was used. They modify this statement in the Discussion by correctly noting that the recommended dose of ritonavir, as a sole protease inhibitor, is "600 mg every 12 hours resulting in a maximum plasma concentration of 8 to 15 $\mu\text{mol/L}$," equivalent to 5.6 to 10.5 $\mu\text{g/ml}$. Yet ritonavir is now rarely used at this dose, being most effective and most widely incorporated into combination antiretroviral regimens as part of a protease inhibitor-boosted strategy in which the maximum concentration is 0.677 $\mu\text{g/ml}$ for men and 0.918 $\mu\text{g/ml}$ for women, with an area under the concentration-time curve of 4 to 5 $\mu\text{g ml}^{-1} \text{ h}^{-1}$.² The clinical syndromes complicating ritonavir administration have been seen with both high and low (protease inhibitor-boosted) dosage regimens, demanding that pathophysiologic explanations derive from studies with the lower concentrations. Levels of ritonavir used by the authors have been reported as cytotoxic to cells of the monocyte/macrophage lineage.³

The use of similar suprapharmacologic levels of ritonavir has also led to other potentially spurious reports in the literature. For example, osteoclast formation is suppressed *in vitro* by levels of ritonavir exceeding 10 $\mu\text{g/ml}$,⁴ but doses in the appropriate pharmacological range of 0.7 to 3.6 $\mu\text{g/ml}$ (1 to 5 $\mu\text{mol/L}$) enhance osteoclast formation *in vitro*,⁵ and clinically, ritonavir seems to accelerate bone mineral density loss, not suppress it.⁶ Returning to lipid metabolism, Liang et al⁷ showed that ritonavir inhibits apolipoprotein B degradation at what they stated were "therapeutically relevant concentrations (5 to 100 $\mu\text{mol/L}$)." However, others have commented on the probable irrelevance *in vivo* of the very high concentrations required to inhibit this proteasome-based reaction, 25 to

40 $\mu\text{mol/L}$.⁸ To reveal clinically pertinent insights into the myriad metabolic disturbances that have been attributed to antiretroviral therapies over the years, care must be taken to use drug concentrations *in vitro* equivalent to pharmacologically appropriate levels, with attention paid to more than just the maximum concentration of the highest level of drug possibly achievable.

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Authors' Reply:

This is a response to the Correspondence written by Drs. Laurence and Modarresi regarding our recent publication¹ in *The American Journal of Pathology*. The authors have concerns that the concentrations of ritonavir used in our study are suprapharmacologic and thus of uncertain clinical significance. We believe the concentration of ritonavir around 15 $\mu\text{mol/L}$ used in our study is clinically relevant. The recommended clinical dose of ritonavir is 400 to 600 mg every 12 hours, which may result in maximum serum concentration (C_{max}) values of 7.1 to 11.2 $\mu\text{g/ml}$,^{2,3} equivalent to 10 to 16 $\mu\text{mol/L}$. Even though it is now rarely used as monotherapy, ritonavir at this dose range has been used in combination antiretroviral regimens in both adults and children.^{3–17}

Our current study may be of clinical significance. Ritonavir was exposed to human macrophages for 24 hours and showed significant inhibition in cholesterol efflux. This finding may be used to explain, at least in part, the clinical association of cardiovascular disease with ritonavir treatment in some HIV-infected patients. In addition, ritonavir induced oxidative stress in macrophages. These data may imply that clinical usage of antioxidant therapy may be effective in minimizing the adverse effects of ritonavir. For now, it is not possible to overinterpret our findings based solely on *in vitro* experiments. Different protease inhibitors may have different effects on cholesterol efflux or produce different degrees of oxidative stress. Because many different types of antioxidants with a variety of actions are available, it is likely that certain antioxidants may be more effective than others to minimize the adverse effects of ritonavir. Therefore, it is strongly warranted to investigate further these issues of cholesterol efflux and antioxidant therapy in animal models and clinical trials. It is also possible that future HIV drugs might be able to minimize effects on cholesterol efflux.

As part of highly active antiretroviral therapy, ritonavir or other anti-HIV drugs are usually used continually in patients for months or years. Even low-dose ritonavir used in humans may still have significant adverse effects on the cardiovascular systems due to long-term therapy. Because HIV infection became a chronic but controllable disease, we must pay great attention to the adverse effects of these anti-HIV drugs.

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