Nitazoxanide as a Broad-Spectrum Antiparasitic Agent

To the Editor—In a randomized, double-blind, placebo-controlled study of nitazoxanide, Rossignol et al. [1] reported excellent therapeutic response in patients with diarrhea caused by *Giardia intestinalis* and *Entamoeba histolytica* and/or *Entamoeba dispar* infection. Earlier open-label clinical investigations of therapeutic response to nitazoxanide in 125 patients with *Fasciola hepatica* infection, with parasitologic examination of stool samples 30 days after initiation of treatment, pointed toward its safety and efficacy [2]. Furthermore, nitazoxanide has been effective during experimental investigations of treatment of antibiotic-induced *Clostridium difficile* intestinal disease in hamsters. Intragastric treatment with nitazoxanide for 6 days, when followed by an inoculation of toxigenic *C. difficile*, resulted in survival of the animals during a 15-day observation period. Necropsy disclosed no signs of toxicity or of *C. difficile* intestinal disease [3].

The encouraging antibacterial and antiparasitic efficacy of nitazoxanide in experimental open-label or placebo-controlled, double-blind investigations [1–3] would be sustained through constant maintenance of its potency and bioavailability. Nitazoxanide formulations would require constant storage under controlled temperatures. Inadvertent exposure to extremes of humidity or temperature would affect drug potency. That was evident recently during field monitoring of the quality of the antiparasitic drug mebendazole. In Nigeria, 5 of the 37 samples being offered to patients did not contain the active drug ingredient, as specified by the British pharmacopeia [4]. An identical scenario with nitazoxanide in the field would almost certainly be associated with frequent therapeutic failures in bacterial and/or parasitic infections. Such cryptic nitazoxanide failures in the field would be best addressed by an on-the-spot evaluation of nitazoxanide quality.

Simple assay formats that could accomplish both qualitative and quantitative analyses of nitazoxanide formulations in clinical settings should be standardized. Recently, a quick and simple test was used to identify counterfeit artesunate in the field without the use of many chemicals or sophisticated equipment [5]. A similar test for nitazoxanide would speedily guide infectious disease practitioners about any linkage of the poor bioavailability with the recorded therapeutic failures of nitazoxanide.

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Human Immunodeficiency Virus Protease Inhibitors and *Pneumocystis carinii*

To the Editor—We read with great attention and interest the article by Walzer et al. [1] stating that anti–human immunodeficiency virus (HIV) drugs (mainly HIV protease inhibitors) are ineffective against *Pneumocystis carinii* in vitro and in vivo. The paper presented data that, according to the authors, conflict with our previously published results [2].

In an attempt to better understand these differences, we would like to underline some apparently missed or misunderstood facts:

1. The in vitro system that we used for testing drugs was not the spinner flask (as erroneously mentioned by Walzer et al. in the Discussion section of their article [1, p. 1357]) but, instead, a well-characterized, quantitative, reproducible, and standardized protocol using multiwell plates that was confirmed by several tests of experimental chemotherapy [3–5] followed by clinical use, as was done with clindamycin-primaquine. The spinner flasks...
[6] were used only to obtain clean trophozoites for the enzymatic study (aspartyl protease detection).

2. The monolayer of human embryonic lung feeder cells (HEL 299) was unaffected by the drugs and solvent that were used (as was clearly stated in our article [2]), after the absence of detachment, vacuolization, and other signs of cell toxicity were assessed.

3. The P. carinii isolate that we used was an original strain (provided by the Indiana University Medical School, Indianapolis) that has been successfully propagated since 1990 in our laboratory; the biomolecular properties of this strain have been very well characterized.

4. In vivo results from the study by Walzer et al. [1] show that a 3–6-fold reduction in cyst load was observed in the lungs of animals treated with indinavir, saquinavir, and nelfinavir. Cysts were enumerated by cresyl violet, which may have over-scored the degree of infection, since empty (dead) cysts also were counted. For these reasons, “ineffective” appears to be a strong word to describe HIV protease inhibitors, as applied by Walzer et al. and as used in the title of their article.

The data presented by Walzer et al. [1] agree, rather than conflict, with the partial inhibitory effects observed in our experiments: As a comparison, it would have been interesting to know the plasma levels in mice of the drugs used.

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References


Reply

To the Editor—We thank Drs. Atzori and Cargnel for their comments [1]. Our study [2] was part of a broader effort to present our experience investigating the effect against Pneumocystis carinii of drugs that are marketed for other clinical purposes [2–4]. The purpose was to urge caution in extrapolating in vitro P. carinii drug susceptibility data to human studies until the results could be confirmed in animal models and by other investigators. In vitro testing of drugs against P. carinii has the same problems as in vitro culture of P. carinii: rodent Pneumocystis serves as the source of organisms, modest replication (usually ≤ 10-fold increase) is achieved, and there are no standard criteria to measure P. carinii growth and the effects of drugs. Thus, the results from 1 group cannot be directly compared with those from another.

We began our in vitro studies of anti–P. carinii drugs with a cell-culture system that was similar to the system used by Atzori and Cargnel and their colleagues [5, 6], and, thus, we are very familiar with the methods used by this group. We switched to the bioluminescent luciferin-luciferase ATP assay because it is a cell-free system; measures Pneumocystis viability, which cannot be done with the Giemsa stain; is quantitative, more sensitive, and more objective than microscopic analysis; and can be used to study drug interactions or the cytotoxic effects of drugs on monolayers that cannot be visualized [3, 7, 8]. The ATP assay also evaluates the viability of the total population of Pneumocystis at a given time point, not just those organisms that happen to be in the supernatant of a monolayer-based system. We presented our data and the reasons why our results differed from those of Atzori and Cargnel and their colleagues within the space limitations of the Concise Communication format of the Journal [2]. In addition, we noticed an error in the Discussion section of our article [2, p. 1357]: the phrase “the Spinner flask cell culture system counts” should have read “the Spinner flask and cell culture systems count.” Perhaps these factors contributed to the concerns raised by Atzori and Cargnel [1] about our interpretation of their results.

Immunosuppressed rodents with P. carinii pneumonia (PCP) have served as the reference standard for drug testing for > 2 decades because they are reliable predictors of activity in humans [9]. Our mouse data, which correlated well with our in vitro results, were obtained with doses used in previously established experimental models [9–11]. Our statistical techniques in this study were more sensitive than the nonparametric techniques that we used in other studies [3, 4]; yet, the small differences that we detected were neither statistically nor biologically significant. We welcome studies by Atzori and Cargnel of protease inhibitors in an established animal model of PCP.

We used a different in vitro technique than that used by Atzori and Cargnel and obtained different results, and we confirmed our findings in an animal model of PCP; thus, we stand by our con-