Letters to the Editor

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Sir,

We are in agreement with Dr Lopardo¹ on various issues that affect antibiotic synergy testing, and in particular, on some of the intrinsic limitations of time–kill methodology. We also agree that the MIC is not a static parameter, and that one of the limitations of our study² (as mentioned in the Discussion section) was that we were unable to measure antibiotic concentrations within the testing medium. It is worth noting that MIC endpoints are read after 18 h of incubation, while the combination testing was performed over 24 h. It is also a point of interest that at least two other studies³,⁴ using similar time–killing assays against Acinetobacter baumannii have demonstrated bacterial regrowth at concentrations of 1 × MIC of colistin. It remains to be determined if this is a true phenomenon or a limitation of the testing methodology. With regard to the points raised regarding bacterial counts, we would like to point out that a turbidity equivalent to that of a 0.5 McFarland standard (barely detectable turbidity) equates to a bacterial count of 1.5 × 10⁸ cfu/mL, and Acinetobacter species will achieve substantially higher turbidity levels in Mueller–Hinton broth over a 24 h growth period. Finally, in our study, synergy was defined as a >2 log₁₀ decrease in cfu/mL for the antibiotic combination compared with its more active constituent. We were unable to ascribe the presence of synergy to one isolate in our study because the difference between the colony count in the combined antibiotic testing (≤20 cfu/mL) and the most active single antibiotic tested (colistin; 1200 cfu/mL) did not fulfill the study definition.

Transparency declarations

T. Y. T. has received funding from Wyeth Pharmaceuticals for unrelated research studies. L. S. Y. N.: none to declare.

References


Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkm473
Advance Access publication 11 December 2007

Comment on: Acinetobacter spp. and time–kill studies

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Sir,

We thank you for the opportunity to reply to the comments by Lopardo¹ on our recent article.²

First, as for the concentration of imipenem and colistin (each at 1 × MIC), there was no difference in the concentration between microdilution and time–kill studies; antibiotic dosage was adjusted according to the volumes of bacterial suspensions. It should be mentioned that substantial regrowth of Acinetobacter baumannii isolates at 24 h using 1 × MIC or even higher antibiotic concentrations has been also described by Owen et al.³

With regard to the titres in the time–kill assay (Figures 1 and 2 in Song et al.²), Lopardo points out that most Acinetobacter spp. isolates reach not more than 10⁷ cfu/mL in the stationary phase. In our opinion, however, there was no chance of carry-over while undertaking 10-fold dilutions. We undertook 10-fold dilutions cautiously to avoid carry-over and repeated experiments if the results did not show sequential, 10-fold decreases in colony counts with the serial dilutions. We are not sure why there is such a difference between previous reports and ours. We hypothesize that this might be due to conditions such as the small bacterial size, the negative post-antibiotic effect and structural changes related to the fitness cost of multidrug resistance.⁴ Previously, James et al.⁵ reported that Acinetobacter spp. were reverted to small coccoid forms (~1 μm in diameter) from bacillary morphology in the low-nutrient stationary phase, which were also aggregated. On the assumption that Acinetobacter isolates were in the small coccoid form (volume of one bacterial cell ≤0.2 μm³) of low-nutrient stationary phase, the concentrations of bacterial suspensions would reach 10¹⁵–10¹⁶ cfu/mL. However, further studies would be required to justify our high titres in time–kill assays.

Finally, we did not describe the MICs of rifampicin and sulbactam in the time–kill synergy tests, considering that they were in the non-susceptible ranges. The MICs of rifampicin were 4 mg/L in four cases (second, fourth, seventh and eighth isolates) and 8 mg/L in the others. In the case of sulbactam, the MICs in most cases were 32 mg/L except one (second isolate), which showed intermediate sulbactam resistance (MIC of 16 mg/L). On the assumption that imipenem and colistin were more active against multidrug-resistant A. baumannii isolates compared with sulbactam and rifampicin, respectively, individual time–kill curves of sulbactam and rifampicin were not taken, which might be the limitation of our study.

Transparency declarations

None to declare.

References

². Song JY, Kee SY, Hwang IS et al. In vitro activities of carbapenem/sulbactam combination, colistin, colistin/rifampicin combination
Letters to the Editor


Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkm478
Advance Access publication 11 December 2007

Comment on: Efficacy of liposomal amphotericin B for secondary prophylaxis of visceral leishmaniasis in HIV-infected patients

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Sir,

Molina et al.1 analyse the efficacy of liposomal amphotericin B (AmB) for secondary prophylaxis of visceral leishmaniasis in HIV-infected patients and conclude that AmB is useful for this purpose. However, I believe that there are some shortcomings in the study that limit the validity of the results reported.

A major drawback is the small sample size, as pointed out by the authors, owing to the low incidence of leishmaniasis as a consequence of highly active antiretroviral therapy (HAART). The authors analyse 21 episodes of leishmaniasis as individual cases, although there were only 15 patients studied. Therefore, some patients had two or more episodes of leishmaniasis. I believe that it would have been better to analyse only the patients (n = 15) instead of the episodes (n = 21) for two main reasons. Firstly, continued exposure to AmB in the patients who relapsed could contribute to a decrease susceptibility of the parasite to the drug.2,3 

and further relapses are not unexpected in these circumstances (parasite causes). Secondly, those patients who experienced a first relapse are probably more prone to develop further relapses than those who had never relapsed4 (host causes). If the two small groups to be compared (relapse, n = 9 and non-relapse, n = 12) were unbalanced for these important aspects, then the comparisons between them might be difficult.

Not unexpectedly, the main factor that differentiates between relapsers and non-relapsers was the increase in CD4 counts (Figure 2 of the authors’ article). As in other opportunistic infections, immunological recovery is the best preventive factor regardless of the drugs used for primary or secondary prophylaxis. Therefore, I believe that the main efficacy for the prevention of relapses in visceral leishmaniasis in this study corresponded to the host immune response rather than to the use of AmB.

The authors also compare the 1 year relapse rate using AmB (79.1%) with a historical control study from the same authors using pentavalent antimonials (93%)1 and depict the combined results in their Figure 1. This comparison is also difficult because that study was carried out many years ago, before the introduction of HAART, and the immunological recovery, the most important factor in the prevention of relapses of opportunistic infections including leishmaniasis, was expectedly lower with the earlier antiretroviral regimens. However, if any cautious conclusion is to be extracted from this difficult comparison it is that pentavalent antimonials seem to be somewhat superior to AmB, because the former patients had somewhat lower rates of relapse despite their presumably poorer immunological recovery.

Also, it is not clear to what comparison the P = 0.037 reported both in Figure 2 and in the text corresponds with. While in the figure it seems to represent the comparison of CD4 counts in relapers and non-relapers at a non-specified time point (which should have been indicated), in the text it seems to represent the comparison of CD4 at 12 and 24 months only in non-relapsing patients. In the latter case, a paired test should have been used instead of the Mann–Whitney test, because the variables are not independent but related.

On the other hand, the use of statistical comparisons in such a small study is of questionable utility because the statistical power is so limited that significant differences are very difficult to obtain. In fact, Table 1 shows that there were no significant differences between the two groups in the frequency of treatment with HAART despite differences so marked as 88.8% and 41.6%, indicating that clinical significance is different from statistical significance, and that absence of evidence is not evidence of absence.5 In addition, the Mann–Whitney test was used for the comparison of continuous variables, despite the fact that the P value obtained from this test is inaccurate for groups with ≤15 observations. Moreover, 3 of the 15 patients died or were lost to follow-up, all of them well before the median time to relapse. Therefore, the statistical power of the study was further reduced.

Likewise, as pointed out by the authors, parasitological cure by bone marrow aspiration was not evaluated in nearly half of the patients, opening the possibility that the patients had treatment failures rather than relapses. In fact, the median time to relapse in these patients (8 months) was considerably shorter than in patients who had evidence of a parasitological cure (15.5 months, not 14 months as reported), although I agree that these episodes may represent true relapses rather than acute treatment failures.

I believe that AmB, like other drugs, might be of some utility for the prevention of recurrences of visceral leishmaniasis while awaiting for immunological recovery. However, its efficacy does not seem to be very high1,5 and, according to the authors’ previous study,1 it could be lower than pentavalent antimonials.

The key question to elucidate is the efficacy of AmB compared with other drugs and, currently, it can only be answered by means of multicentre studies with considerably larger sample sizes.

Transparency declarations

None to declare.