The Brazilian Journal of
INFECTIOUS DISEASES
www.elsevier.com/locate/bjid

Letter to the editor

Efficacy of tigecycline, polymyxin, gentamicin, meropenem and associations in experimental *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* non-lethal sepsis

Dear Editor,

Retrospective clinical data suggest that antibiotic combinations, including tigecycline (TIG) and polymyxin (POL), result in better outcomes than monotherapy against *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* (KPC-KP).[1] An *in vitro* study with time-kill assay has shown that TIG, POL and meropenem (MPN) as single agents do not exhibit efficient bactericidal activity against most of the KPC-producing strains, and TIG alone might be a therapeutic option for infections caused by KPC-producers when bacteriostatic activity is adequate, or combined with POL when bactericidal activity is required. The TIG and MPN association was neither synergistic nor bactericidal against KPC-KP strains, suggesting an antagonist effect, as demonstrated by Pournasara et al. in a previous publication in the IJAA.[2] Additional *in vivo* tests are warranted to better assess killing kinetics of TIG in combination with other antibiotics against KPC-producers.

After approval by the local Ethics Committee for Animal Experimentation, a non-lethal experimental murine model of KPC-KP sepsis was conducted, aiming to observe the effect of different monotherapies and antimicrobial combinations on blood cultures and quantitative peritoneal cultures. Thirty-six rats were inoculated with a low dose inoculum (9×10^8 CFU/mL) of a KPC-KP. MPN minimal inhibitory concentration (MIC) was above 16 μg/mL, but the strain was susceptible to TIG (MIC = 1 μg/mL), POL B (MIC < 0.5 μg/mL) and gentamicin (MIC = 4 μg/mL). Antibiotic dosages and combinations are described in Table 1. Dosages were the same in combined and monotherapy groups. A control group without antibiotic was included.

No differences were observed in untreated controls and animals treated with MPN, since both groups had positive blood cultures and peritoneal fluid cultures with 10^3–10^4 colony-forming units/mL (CFU/mL). All animals treated with TIG, GEN and POL as monotherapy had negative blood cultures, as did the animals treated with POL plus GEN, POL plus TIG, TIG plus GEN and triple therapy. Those groups also presented negative peritoneal fluid cultures, except for two animals treated with TIG monotherapy, who had 10^3 CFU/mL in peritoneal fluid cultures. When POL and TIG were combined with MPN, three out of six blood cultures turned out positive after 12 h of incubation, a worse microbiological result than monotherapies with TIG, POL and GEN and other combinations.

All monotherapies with TIG, POL and GEN (total $n = 9$) showed higher efficacy in sterilizing peritoneal and blood cultures than the group of untreated controls and animals treated with MPN ($n = 6$) ($p = 0.011$). Triple therapy (MPN + TIG + POL) and double therapies with no MPN (TIG + POL, TIG + GEN, POL + GEN) (total $n = 12$) were significantly more effective than controls (untreated controls and animals treated with MPN alone) in sterilizing cultures ($p = 0.011$). On the other hand, when the TIG + MPN and POL + MPN combinations ($n = 6$) were compared to untreated controls and animals receiving MPN ($n = 6$), there were no significant differences in culture positivity ($p = 0.275$).

This finding must be confirmed with larger samples, but these data suggest that combinations of MPN with TIG or POL might have an antagonist effect *in vivo*.

As previously reported by Pournarasa, 2011, MPN plus TIG was non-synergistic in this *in vivo* experiment. Additionally, POL plus MPN or TIG plus MPN associations may be less effective than monotherapies or other combinations to treat KPC-KP sepsis.

Antibiotic associations should be cautiously used, even for carbapenemase-producing Enterobacteriaceae strains. *In vitro* studies showing this paradox or antagonist effects used empirical combination therapies in humans with infections by KPC-KP. An experimental study with concentrated inoculum aiming to evaluate the effect of antimicrobial combination in lethal sepsis is ongoing and may elucidate this subject. Clinical trials could answer those questions, but, until then, the combined therapy for synergistic effect of carbapenems and

Table 1 – Experimental study with non-lethal sepsis in rats by KPC-producing *Klebsiella pneumoniae*. Monotherapy was compared with antibiotic combinations. Meropenem combined with other antibiotics besides having no benefit, positive cultures suggest an antagonistic effect.

Rats	Positive blood culture	$>10^3$ CFU/mL in peritoneal fluid culture
No antibiotics	100%	100%
Meropenem	100%	100%
Gentamicin	0	0
Tigecycline	0	67%
Polymyxin B	0	0
Polymyxin B + tigecycline	0	0
Gentamicin + tigecycline	0	0
Polymyxin B + gentamicin	0	0
Gentamicin + meropenem	0	0
Polymyxin B + meropenem	67%	0
Tigecycline + meropenem	33%	0
Polymyxin B + tigecycline + meropenem	0	0

CFU/mL, colony-forming units per milliliter.

other classes of antimicrobials is not recommended, at least for strains with higher MPN MIC.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgment

Authors acknowledge the staff of Animal Laboratory of Universidade Estadual de Ponta Grossa.

REFERENCES

1. Lee GC, Burgess DS. Treatment of *Klebsiella pneumoniae* carbapenemase (KPC) infections: a review of published case series and case reports. Ann Clin Microbiol Antimicrob. 2012;11:32.
2. Pournarasa S, Vrioni G, Neou E, et al. Activity of tigecycline alone and in combination with colistin and meropenem against *Klebsiella pneumoniae* carbapenemase (KPC)-producing Enterobacteriaceae strains by time-kill assay. Int J Antimicrob Agents. 2011;37:244–7.

Paula Virginia Michelon Toledo [a,b,*]

[a] *Medicine Department, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil*
[b] *Internal Medicine Postgraduate Programme, Universidade Federal do Paraná, Curitiba, PR, Brazil*

Felipe Francisco Tuon [a,b,c]

[a] *Medicine Department, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil*
[b] *Division of Infectious Diseases, Universidade Federal do Paraná, Curitiba, PR, Brazil*
[c] *Internal Medicine Postgraduate Programme, Universidade Federal do Paraná, Curitiba, PR, Brazil*

Lavinia Arend

Bacteriology Section, Laboratório Central de Saúde Pública do Estado LACEN, PR, Brazil

Ayrton Alves Aranha Junior [a,b]

[a] *Medicine Department, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil*
[b] *Surgery Postgraduate Programme, Universidade Federal do Paraná, Curitiba, PR, Brazil*

[*] *Corresponding author at*: Rua Mamoré, 981 L1, 80810-080, Brazil.
E-mail address: paulavmtoledo@yahoo.com.br (P.V.M. Toledo).

Received 15 April 2014
Accepted 12 May 2014
Available online 5 June 2014

http://dx.doi.org/10.1016/j.bjid.2014.05.003