



Letter to the Editor

Mutant prevention concentration of pradofloxacin against *Porphyromonas gingivalis*

Keywords: Mutant prevention concentration; MPC; *Porphyromonas gingivalis*; Pradofloxacin

It has become clear, following careful consideration of fluoroquinolone resistance mechanisms, that prevention of clonal expansion of mutant populations is fundamental to reducing the impact of resistance development. Drlica and Malik (2003) made the important point that a successful strategy to restrict mutant selection is to ensure that drug concentrations are high enough to prevent the growth of mutants already present in a bacterial population. This comment was made in the context of the mutant prevention concentration (MPC) and the mutant selection window. The MPC has been defined as the drug concentration at which no mutant is recovered when more than 10^{10} cells are applied to an agar plate (Dong et al., 1999). Zhao and Drlica (2001) argued for a general strategy restricting the selection of antibiotic resistant fluoroquinolone mutants and presented the case for the “mutant selection window” (MSW). Baquero and Negri (1997) first forwarded the idea that there was a dangerous range of drug concentrations in which mutants were most frequently selected; this is now considered to be the range between the minimum inhibitory concentration (MIC), which is the concentration required to block the growth of wild-type bacteria and the concentration required to inhibit the growth of the least susceptible, single-step mutant (MPC). Thus the MSW is the drug concentration range between MIC and MPC. Placing antimicrobial concentrations inside the window will enrich resistant mutant subpopulations selectively, whereas placing

concentrations above the window is expected to restrict selective enrichment (Dong et al., 1999). To date all the published MPC data has related to aerobic bacteria. Pradofloxacin (PRA) is an 8-cyano-fluoroquinolone (FQ) being developed to treat bacterial infections in dogs and cats and is active against anaerobes as well as aerobes (Stephan et al., 2003). In clinical trials it has been shown to be effective in the treatment of periodontal disease and as such we would like to report the MPC for *Porphyromonas gingivalis*, isolated from a canine field case of periodontal disease. Wetzstein (2005) has already reported MPC data for PRA against *Escherichia coli* and *Staphylococcus aureus* and we utilised the same approach but modified for working with anaerobes (using Brucella Blood Agar supplemented with hemin, vitamin K plus 5% defibrinated horse blood as the plate medium, incubated under anaerobic conditions, 10% CO₂, 10% H₂, 80% N₂ for 10 days). A dense inoculum of the test strain was spread over two 245 mm × 245 mm agar plates at each PRA concentration, such that the total inoculum exceeded 10^{10} cfu. The large assay plates were used to facilitate adequate distribution of the inoculum over the agar surface and thus promote discrete colony formation. The intervals between PRA concentrations were 0.05 µg/ml so that the MPC could be accurately determined. A minimum of three replicates was carried out at each PRA concentration at or close to the MPC. All work was carried out inside an anaerobic work station. Using a total inoculum of 1.7×10^{10} cfu at each

PRA concentration, the MPC against *P. gingivalis* DWC 5625 was found to be 0.3 µg/ml; the MIC was determined as 0.031 µg/ml against pradofloxacin and 0.062 µg/ml against metronidazole, using standardised CLSI M11-A6 methodology. We believe this to be the first report of MPC data for an anaerobe isolated from periodontal disease. We would also like to comment on the difficulties in culturing these anaerobes to achieve the culture density requirements necessary for the determination of MPC. MPC data could only be generated for one of three chosen strains as it was not possible in all strains to achieve the required cell densities necessary for determining the MPC.

Whilst the MSW hypothesis has yet to be proven clinically there is increasing evidence from model studies that it does have relevance. It has been suggested that if the MSW can be closed mutants will not be selected (Zhao and Drlica, 2001). There are two means of closing the window: the first is to minimise the time at which the drug concentration lies inside the window, which is a function of the pharmacokinetics of the drug. The second mechanism is one in which the difference between MIC and MPC is greatly reduced. The optimal situation is for a drug to have a very low MIC and an equally low MPC. Indeed, PRA has a very low MIC and MPC against *P. gingivalis*. Considering the pharmacokinetics of PRA (C_{max} of 1.3 µg/ml; Fraatz et al., 2003), this data suggests that this new drug offers opportunities for treatment that also restrict the emergence of antimicrobial resistance. PRA should possess an exceptional potential in eliminating not only large populations of wild-type but also first-step resistant clones (Wetzstein, 2005).

Acknowledgement

Thanks to Bayer HealthCare AG for funding the reported studies.

References

- Baquero, F., Negri, M.C., 1997. Strategies to minimize the development of antibiotic resistance. *J. Chemother.* 9, 29–37.
- Dong, Y., Zhao, X., Domagala, J.M., Drlica, K., 1999. Effect of fluoroquinolone concentration on selection of resistant mutants of *Mycobacterium bovis* BCG and *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 43, 1756–1758.
- Drlica, K., Malik, M., 2003. Fluoroquinolones: action and resistance. *Curr. Topics Med. Chem.* 3, 249–282.
- Fraatz, K., Krebber, R., Edingloh, M., Heinen, E., 2003. Oral bioavailability of pradofloxacin tablets and renal drug excretion in dogs. *J. Vet. Pharmacol. Therap.* 26 (Suppl. 1), 88–89.
- Stephan, B., Pridmore, A., Silley, P., 2003. In vitro activity of pradofloxacin and metronidazole against anaerobic bacteria from dogs and cats. 43rd ICAAC, Chicago, USA, ASM, p. 223 (Abstract F-420).
- Wetzstein, H.-G., 2005. Comparative mutant prevention concentrations of pradofloxacin and other veterinary fluoroquinolones indicate differing potentials in preventing selection of resistance. *Antimicrob. Agents Chemother.* 49, 4166–4173.
- Zhao, X., Drlica, K., 2001. Restricting the selection of antibiotic-resistant mutants: A general strategy derived from fluoroquinolone studies. *Clin. Infect. Dis.* 33, 147–156.

Bernd Stephan
Heinrich A. Greife

Bayer HealthCare AG, Leverkusen, Germany

Andrew Pridmore
Don Whitley Scientific Limited, Shipley, UK

Peter Silley*
MB Consult Limited, Lymington, UK
Department of Biomedical Sciences,
University of Bradford, Bradford, UK

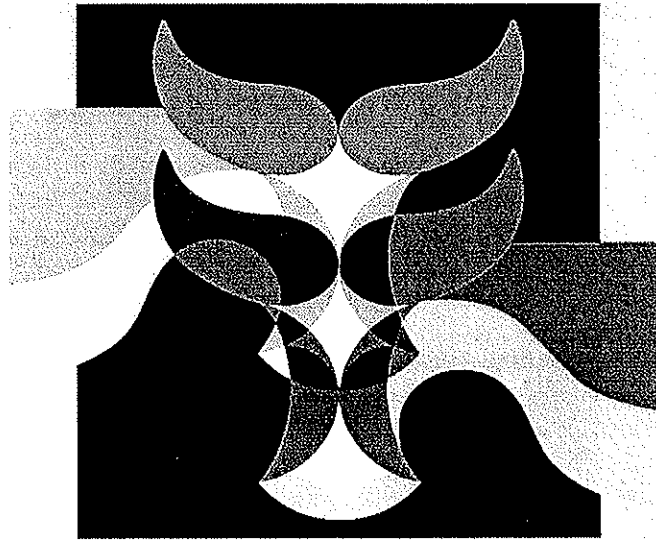
*Corresponding author. Tel.: +44 1590 678700;
fax: +44 1590 678751.
Email address: p-s@mbconsult.co.uk
(P. Silley)

Provided for non-commercial research and educational use only
Not for reproduction or distribution or commercial use



Volume 121, Issues 1-2, 31 March 2007

ISSN 0378-1135



This article was originally published in a journal published by Elsevier, and the attached copy is provided by Elsevier for the author's benefit and for the benefit of the author's institution, for non-commercial research and educational use including without limitation use in instruction at your institution, sending it to specific colleagues that you know, and providing a copy to your institution's administrator.

All other uses, reproduction and distribution, including without limitation commercial reprints, selling or licensing copies or access, or posting on open internet sites, your personal or institution's website or repository, are prohibited. For exceptions, permission may be sought for such use through Elsevier's permissions site at:

<http://www.elsevier.com/locate/permissionusematerial>