Liquid cell TEM (L-TEM) for observation of Polymyxin effect on E. coli.

HING, H.L.[1], Sahalan, A.Z.[1], Zhuang, C.[2], Wang, L.[2], Ang, L.[2], Ma, L.[2], Han, X.[2] and Md Ambia, K.[3]

[1] Universiti Kebangsaan, Malaysia, [2] Beijing University of Technology, China, [3] UniKL, Malaysia

Liquid Cell Transmission Electron Microscope (L-TEM) has the ability to observe the lie interaction of antibiotic on bacteria and the activity can be closely monitored. Polymyxin B (PMB) is an outer membrane active agent that interact primarily against Gram negative outer membrane especially *E. coli* (Ahmad Zorin et al 2013). It has quick destructive effect against the outer membrane (Sahalan et al 2008) with nepharotoxic effect when used clinically. The activity are dose dependent. The aim of this study is to use L-TEM to observe the effect of sub inhibitory concentration or sub-MIC of PMB against Gram negative bacteria. The method also helps to monitor ultrastructural changes directly in 'real time". Results have shown, although at sub inhibitory concentration, PMB has disorganized the Gram negative bacteria outer membrane and released of membrane vesicles. Other results also showed the increased of permeability of bacteria cell marked by the increased of cell wall size. The sub inhibitory concentration of PMB could enhance antibacterial activity when other types of antibiotic are combined synergistically.

Liquid Cell Transmission Electron Microscopy (L-TEM)

10 ml of *E.coli* culture was carefully injected into the L-TEM chamber holder using a very fine fiber tube to minimize any unwanted vibration or movement. The *E.coli* solution was then allowed to stabilize at temperature of 25^{o}C for 4 hours before sub-MIC of PMB was injected into the L-chamber containing *E.coli*. After injection, the L-chamber was then inserted into the TEM holder for observation. The spot size was put at 6 to minimize the effect of beam on the wet sample. Images were then taken at 0 time and progressively till 93 minutes to observe the effect of PMB on *E.coli*.

The *E.coli* without PMB (Figure A) showed a bacteria cells with clear outline of the bacteria outer membrane. The inner surface of the bacteria has dark image with lighter spot in the center.

"Real time" imaging system have benefits and advantages where timely framed analysis are made directly and reduce interferences of mis-interpretations (Shogi et al. 2016). The L-TEM used in this work has the application of a real time system. The micrograph of the interaction of sub-inhibitory concentration of PMB against *E.coli* has been documented with L-TEM. The micrographs showed many bench mark activity of PMB such as the presence of membrane vesicles and disorganization of outer membrane. Ultrastructural changes have been shown with change of colour on the surface of the bacteria. Other research had showed similar changes, which indicated some disorganization of the Gram negative outer membrane components such as LPS (Sahalan et al, 2008). However total bacterial cell destruction was not seen at the end of the experiment due to the application of PMB sub inhibitory concentration where it helps to reduce PMB activity.

Thus, although at sub inhibitory level, PMB could enhance Gram negative outer membrane disorganization and increased permeability.

References

1. Sains Malaysiana, (2013) Vol 42, No3:301-306.
2. International Journal of Antimicrobial Agents 31 (2008) 224 - 227.
3. Nature Chemistry 8, 743 - 752 (2016) doi:10.1038/nchem.2526.