

## 白蛋白的存在對革蘭氏陽性菌光動力殺菌的影響

### Effect of Albumin on Photodynamic Inactivation of Gram-Positive Bacteria

#### 中文摘要

光動力殺菌 (Antimicrobial photodynamic inactivation, PDI) 利用光感物質，在照射特定波長的可見光之下，因單態氧或自由基的產生而對微生物造成毒性。近年來為了解決日漸嚴重的抗藥性問題，使得光動力殺菌得以發展成爲另一項治療微生物感染的方式。當菌體存在於生物體感染部位時，週遭的生物性因子的影響決定了光動力殺菌的效果。傷口部位週遭的白蛋白爲影響光動力效果的主要原因之一。在本研究當中，將探討白蛋白的存在，對於革蘭氏陽性菌光動力殺菌效果的影響。金黃色葡萄球菌 (*Staphylococcus aureus*) 與 MRSA 懸浮在 PBS 或牛血清白蛋白 (Bovine Serum Albumin, BSA) 中，加入光感物質 chlorin e6 (Ce6) 或 Toluidine Blue O (TBO) 避光培養後，以紅色發光二極體矩陣進行照光。Ce6 爲游離型式或 PF127 微胞載體型式。照光之後並加入具有提升光動力殺菌效果之生醫材料 CX 再與菌體培養。最後將菌液取出，以 PBS 序列稀釋 (10<sup>1</sup>~10<sup>6</sup>)，再將稀釋菌液滴於 TSB agar plate 上推盤，於 37°C 下隔夜培養後進行菌落數計數。實驗結果顯示，相較於在 PBS 的環境中，金黃色葡萄球菌及 MRSA 懸浮在有蛋白質存在的環境時，光動力殺菌的效果皆受到抑制。然而將 CX 合併光動力處理過後，無論以 Ce6 或 TBO，以及對金黃色葡萄球菌及 MRSA，均有提升光動力殺菌效果的作用。微胞載體具有避免 Ce6 在水溶液產生聚集而可增進殺菌效果的功能；PF127-Ce6 及 free Ce6 在白蛋白的存在下，以光動力處理的部分殺菌效果差異較小，然而在加入 CX 培養後，可進一步提升殺菌的效果。因此，以微胞載體以及合併 CX 的使用，有助於白蛋白的存在，對於革蘭氏陽性菌光動力殺菌效果的影響。

#### 英文摘要

Antimicrobial photodynamic inactivation (PDI) employs a photosensitizer and visible light to produce singlet oxygen and reactive oxygen species that can cause cytotoxic effect on microorganism. In recent years, the growing resistance to antibiotics among pathogenic bacteria rendered PDI as an alternative antimicrobial therapeutics of infection disease. Under the wound environment, several biological factors may affect the effectiveness of PDI. The presence of albumin around the wound is one of the factors that influence the efficiency of PDI. The purpose of this study is to evaluate the effect of albumin on the photodynamic inactivation of gram-positive bacteria. Suspensions of *Staphylococcus aureus* and MRSA in PBS or bovine serum albumin (BSA) were treated in dark and red LED light conditions in the presence of

photosensitizers using chlorin e6 (Ce6) and toluidine blue O (TBO). Ce6 was prepared in free form or micellar form. After illuminated, a biomaterial that can evaluate the antimicrobial effect of PDI called CX was added to bacteria suspensions. The suspensions were serially 10-fold diluted with PBS to give dilution of  $10^{-1}$ ~ $10^{-6}$  times then drop the aliquots on agar plate. Plates were incubated at 37°C overnight. The number of colony forming units (CFU) was counted. The results in this study show that the PDI sensitivity was inhibited by the presence of albumin compared with suspensions of *S. aureus* and MRSA in PBS. However, the combination of CX and PDI markedly reduced cell growth of *S. aureus* and MRSA by using photosensitizers as Ce6 and TBO. A micellar carrier system prevents the likelihood of aggregation of Ce6 provides a better PDI efficiency. There were little differences of bacteria killing between free-Ce6 and PF127-Ce6 under an albumin suspending medium. After the CX was added, the antimicrobial efficacy was also increased. In conclusion, the present findings have demonstrated that using an micelle as the carrier of Ce6, and the combination of PDI and CX, may promote the PDI efficiency when albumin was present.