Photodynamic therapy: a new antimicrobial approach to infectious disease

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Joint Graduate Seminar Dec 2015

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Introduction-Photodynamic therapy (PDT)

- photosensitizers (PS)
  - absorb energy from light and transfer it to adjacent molecules
  - produces a chemical change
- Type I and Type II photoprocesses
- cytotoxicity

- accumulate preferentially in malignant cells (Cancer)
- kill microbial cells (Infectious diseases)

Type I pathway

- Type I pathway:
  - -PS + light -> activated PS
  - -activated PS + substrate -> radical ions
  - - radical ions + oxygen -> oxygenated cytotoxic species
  - -e.g. superoxide, hydroxyl and lipid-derived radicals

Type II pathway

- Type II pathway:
  - -PS + light -> activated PS
  - -activated PS + oxygen -> singlet oxygen ($^1O_2$)
  - -$^1O_2$ oxidize many biological molecules
  - -e.g. proteins, nucleic acids and lipids -> cytotoxicity

PDT for infectious disease

Emergence of antibiotic resistance :

->to find alternative antibacterial therapeutics

- PDT

  -Effectiveness?

  -Selectivity?

  (avoiding damage to host tissue)

Mechanisms of damage

bacteria, viruses and fungi

- (i) nucleic acid damage
- (ii) cytoplasmic membrane damage

Photoinactivation of Gram+ and Gram- bacteria *in vitro*

Species (Gram−)	Photosensitizer	References
Escherichia coli	Thiazines +, xanthenes +, acridines +, phenazines +, Cationic, neutral and anionic porphyrins 5-aminolevulinic acid, Zinc phthalocyanine tetrasulfonate +,	Martin & Logsdon 1987, Nitzan et al. 1995, Szocs et al. 1999, Gabor et al. 2001, Benov et al. 2002,
Acinetobacter baumannii	Cationic porphyrin	Nitzan & Ashkenazi 2001
Species (Gram+)	**Photosensitizer**	**References**
Staphylococcus aureus [MRSA too], *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Enterococcus faecalis*,	Methylene Blue+	Wainwright et al. 1998, Zeina et al. 2001, Usacheva et al. 2001
S. aureus	Tetraphenylporphyrins, Hematoporphyrin	Nitzan et al. 1995, Bertoloni et al. 2000

PDT difference between Gram+ and Gram– bacteria

neutral or anionic PS:

- effective for Gram+ bacteria

- bound to the outer membrane of Gram– bacteria
- but do not inactivate Gram– bacteria after illumination
- outer membrane: physical barrier

Act against Gram− bacteria

1) use a PS molecule with an intrinsic positive charge
   - e.g. Toluidine Blue O

2) use positively charged liposomes
3) add penetration enhancer e.g. EDTA/ polymyxin
   - increase the permeability of the Gram− outer membrane
   - allow PS to penetrate

Photoinactivation of viruses *in vitro*

Species[a]	Photosensitizer	References
Human immunodeficiency virus-1	Rose Bengal – Hypericin, Methylene Blue +	Lenard et al. 1993 Bachman et al. 1995
Herpes simplex virus	Hematoporphyrin derivative, Sapphyrins	Matthews et al. 1988, Judy et al. 1991
Influenza A virus	Hypericin, Rose Bengal -	Lenard et al. 1993

Photoinactivation of fungi and yeasts *in vitro*

Species	Photosensitizer	References
Aspergillus fumigatus	Green 2W	Friedberg et al. 2001
Saccharomyces cerevisiae	Glucosyl porphyrins, Hematoporphyrin, Eosin Y -	Cohn & Tseng 1977, Sharma & Jain 1994, Carre et al. 1999,
Candida albicans	Rose Bengal -, Zinc phthalocyanines	Bertoloni et al. 1992, Lazarova 1993

Selectivity

- human fibroblasts and keratinocytes were unharmed
- 1×10^5 cells /0.1–2.5 µM phthalocyanine solutions /600–700 nm light /1–5 min (Soncin et al. 2002)

- tissue distribution study

Specific killing of PDT

- covalently bound PS to a monoclonal antibody
- e.g. *P. aeruginosa* (cell surface antigens)
  (Friedberg et al. 1991)

Clinical applications (1)

Causative agent	Site of infection	Photosensitizer used	References
Herpes simplex virus	Cornea	Proflavine	Moore et al. 1972
Herpes simplex virus	Genitals	Methylene blue, neutral red	Chang et al. 1975
Bacteria	Brain abscess	Hematoporphyrin	Lombardet al. 1985
Human papilloma virus	Respiratory tract (in larynx)	Hematoporphyrin derivative— dihematoporphyrin ether	Abramson et al. 1992
Human papilloma virus	Genital warts	aminolevulinic acid	Fehr et al. 1996
Human papilloma virus	Hand and feet Skin	aminolevulinic acid	Karrer et al. 1999
Propionibacterium acnes	Skin/ sebaceous glands	aminolevulinic acid	Itoh et al. 2001

Clinical applications (2)

Causative agent	Site of infection	Photosensitizer used	References
Helicobacter pylori	Stomach	aminolevulinic acid	Wilder-Smith et al. 2002
Protozoa	Skin	aminolevulinic acid	Gardloet al. 2003
Candida or Trichophyton	Between toes	aminolevulinic acid	Calzavara-Pinton et al. 2004
Corynebacterium minutissimum	Skin	Endogenous porphyrins	Darras-Vercambreet al. 2006
Mycobacterium marinum	Hands	Endogenous porphyrins	Wiegell et al. 2006
Porphyromonas gingivalis *Fusobacterium nucleatum*	Dental pockets	Toluidine blue	de Oliveira et al. 2007
Enterococcus faecalis	Teeth/ root canal	Toluidine blue	Garcez et al. 2008

PDT: “anti-virulence factor therapy”?

- alter biological function of LPS from *E. coli*
- inactivate proteases of *P. aeruginosa*

  (Komerik et al. 2000)

- protease activity quantified by casein hydrolysis
- LPS ability to induce cytokine release reduced

Conclusion

Photodynamic therapy:
alternative antimicrobial approach to infectious disease

especially for

- multi-antibiotic resistant pathogens
- infection site: antibiotics not well perfused

End!

Thank you!

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