Application of phthalocyanines in blood sterilization

Luís Teles 1, Leandro Lourenço 2, ÂngelaCunha1, M. Amparo F. Faustino2, M. Graça P. M. S. Neves2, José A. S. Cavaleiro2, João P. C. Tomé2,3, Adelaide Almeida1*

[1]Department of Biology and CESAM; [2]Department of Chemistry and QOPNA, University of Aveiro, 3810-193, Aveiro, Portugal.

[3]Department of Organic and Macromolecular Chemistry, Ghent University, B-9000 Gent, Belgium.

Introduction

The therapeutic properties of light have been known for thousands of years, but it was only in the last century that antimicrobial photodynamic therapy (aPDT) was developed. aPDT involves interaction between light and a photosensitizer in the presence of molecular oxygen. This interaction produces reactive oxygen species (ROS) which cause oxidative damage of microbial vital molecules.

The medical importance of blood combined to the advent of the AIDS epidemic and other infectious diseases led to an improved pathogen screening techniques for donated blood. However, due to the "window" periods where infective agents cannot be detected, the ability to disinfect blood and its derivatives has assumed great importance. Whereas conventional disinfection techniques (solvent-detergent treatment or ultra-violet irradiation) may be employed in plasma or protein concentrates, the collateral damage associated with such treatments disallows their use with cellular fractions.

Objective

The aim of this study was to evaluate the suitability of the phthalocyanines to disinfect blood products.

Materials and Methods

The blood products (plasma and complete blood) and phosphate buffered saline (PBS) were challenged with 10^8 colony forming units (CFU)/mL of *Escherichia coli* and exposed to red light at 150 W m^{-2} for 270 min. The *E. coli* reduction was determinate at 0, 30, 60, 90, 180 and 270 min and compared with that of light (irradiated in the absence of phthalocyanine) and dark (incubated with phthalocyanine but non-irradiated) controls.

Microorganism and growth conditions

E. coli cells were grown on 10 ml of trypticase soy agar (TSB , Merck) for 12 h at 37 °C . The optical density of culture was measured at 590 nm and used only the cultures with optical density between 0.7-0.8.

Irradiation conditions

The effect of ZnH8Pc(NPyO)8Me8 was evaluated by exposing the samples and controls to red light (PAR radiation,13 OSRAM 21 lamps of 18 W each) with a fluence rate of 150 W m^{-2} , for 270 min, under 100 rpm mechanical stirring.

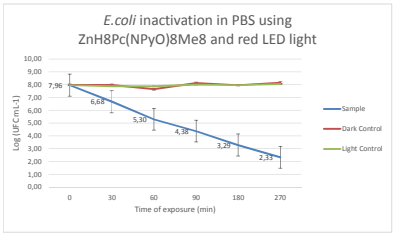
***E. coli* inactivation**

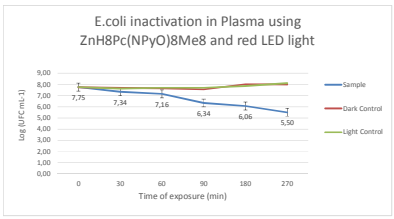
The efficiency of the ZnH8Pc(NPyO) 8Me8 at a concentration of 20.0µM was evaluated through quantification of the number of colony forming units (CFU). All the experiments were performed using the same experimental conditions.

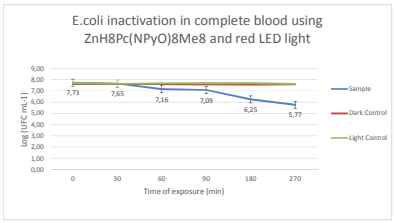
A volume of 1.0 mL of test and control samples was collected at time 0 and after predefined times (30, 60, 90, 180 and 270) of light exposure, then serially diluted and plated in duplicate in TSA medium.

The Petri plates were kept on the dark immediately after plating and during the incubation period. After 24 h of incubation at 37 °C the number of colonies was counted in the most convenient series of dilution.

Results







Red blood cells count in Neubauer chamber before and after the photoinactivation test

Time of exposure (min)		$\bar{X}$ (Cells/L)	σ (Cells/L)
0	Dark Control	1,00E+12	0,00E+00
	Sample	5,00E+11	2,50E+11
	Light Control	8,13E+12	4,88E+12
270	Dark Control	6,25E+11	1,25E+11
	Sample	2,00E+12	1,25E+12
	Light Control	6,00E+12	1,75E+12

Leukocyte cells count in Neubauer chamber before and after the photoinactivation test

Time of exposure (min)		$\bar{X}$ (Cells/L)	σ (Cells/L)
0	Dark Control	5,72E+10	2,00E+08
	Sample	6,33E+10	3,81E+10
	Light Control	1,04E+11	5,45E+10
270	Dark Control	1,08E+11	3,83E+10
	Sample	4,23E+10	7,50E+09
	Light Control	8,33E+10	1,05E+10

Conclusions

E. coli was efficiently photoinactivated using a 20.0 µM concentration of phthalocyanine in PBS (decrease of 5.6 log). The *E. coli* inactivation in plasma and complete blood was also effective, reductions of 2.0 and 2.5 log , respectively.

The blood cells were not significantly affected by the aPDT treatment, the differences in cells counts before and after aPDT in the treated samples were similar to those observed in the light control .

In conclusion, aPDT is effective to inactivate *E. coli* in blood and blood products, being a promising alternative approach to traditional methods of disinfection.

Bibliography

1. Wainwright M. The emerging chemistry of blood product disinfection. Chem Soc Rev. 2002;31(2):128-36.
2. Wainwright M. Pathogen inactivation in blood products. Curr Med Chem. 2002;9(1):127-43. 3. Solheim BG, Seghatchian J. Update on pathogen reduction technology for therapeutic plasma: an overview. Transfus Apher Sci. 2006;35(1):83-90.