Pathogen inactivation in cellular blood products by photodynamic treatment

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CHAPTER I

INTRODUCTION

1. BLOOD TRANSFUSION SAFETY

Blood product safety is a major priority of blood suppliers. Transmission of viruses or other infectious agents can have deleterious effects for the transfused patients. To maximize the safety of blood products, various procedures are initiated starting as from the moment a person is willing to donate blood.[1]

RECRUITMENT AND SELECTION OF VOLUNTEERS

Before each blood donation, donors must fill a questionnaire as a control of good health and faithfulness. The questionnaire is meant to exclude high-risk behavior individuals, potential sick donors, and travelers to endemic areas for infections.
Donors must be willing to give blood without any kind of remuneration. Almost all studies have shown that unpaid donors are safer than paid donors.[2,3] Indeed, the latter failed more frequently than non-remunerated donors to mention illness or lifestyle involving risks.[4]

SCREENING FOR INFECTION

The presence of antigens or antibodies against Human Immunodeficiency virus (HIV) 1 and 2, Human T-lymphoma virus (HTLV) 1 and 2, Hepatitis C virus (HCV), Hepatitis B virus (HBV) and the presence of *Treponema pallidum* (Syphilis) is determined in donor blood samples. A major concern in the determination of antibodies is the window period. This is the period in which the presence of

infection is not detectable yet because the amount of either antibodies and/or antigens is below the detection limit. To shorten the window phase, more advanced techniques such as nucleic acid test (NAT) are used to detect the presence of viral genetic material from HIV, HCV[5,6], West-Nile Virus (WNV; In North America)[7,8] and HBV (Japan)[9].

Furthermore, besides these serological and molecular tests, additional precautions are taken.

Plasma is kept in quarantine for at least 6 months until the donor, coming back for a new donation, is tested again for infection.[10] If the donor is healthy and testing for infectious disease markers is negative, the plasma donation is released for transfusion. Platelet concentrates (PC) are cultured to detect bacterial contamination.[11]

For cellular blood products that are destined to high-risk patients like severely immunocompromised patient, pregnant women, or neonates, detection of not routinely tested viruses such as Cytomegalovirus (CMV) and human B19 Parvo-virus is performed to avoid, sometimes even fatal, transfusion complications.

2. THE PERMANENT THREAT OF PATHOGEN CONTAMINATION

A wide list of pathogens is permanently threatening the blood supply, whereas no screening test is available or readily available.

New viruses or pathogens are emerging. Since the bird flu virus (H5N1) was discovered to be able to infect humans[12], with fatal outcome in children and elders, increased attention was necessary to ensure the safety of the blood supply. Although it is not yet known if this virus can be transmitted through blood transfusion, travelers in regions where this virus spread out, were subjected to a temporarily deferral for at least 3 weeks as recommended by WHO.[13,14]

Another example of an emerging virus is WNV, which emerged in 1999 for the first time in North America where it became endemic.[15] In 2002, the virus was discovered to be transmitted through blood transfusion.[16,17] Since 2003, routine detection for this virus was implemented for all blood donations in the USA.[18,19] In

UK, the National Blood Service performs a test for this virus in donors who donates within 28 days of a visit to the USA or Canada. In the Netherlands, such donors are temporarily suspended for donation during 60 days.

Another potential danger for blood transfusion is prions. Prions are pathogenic proteins which can lead to Creutzfeldt-Jakob disease and its variant (CJD or vCJD).[20]. It is still unclear if prions are transmissible through blood transfusion.[21,22] Direct evidences were only obtained in animal experiments.[23] In addition, no test is available to detect contamination of an individual with prions. To counterpart a potential epidemic, leukodepletion of blood components was implemented, as prions may be associated to leukocytes[24]; although this declaration was denied by other researchers.[25] A deferral strategy was introduced to exclude blood donors who had received blood transfusion before 1980 or who resided in UK for longer than 6 months.

New viruses are still discovered, thanks to advanced molecular techniques. SenV[26], TTV[27], and Hepatitis G virus[28] were found to be transmissible through blood transfusion; however, no disease is associated with these infections.[29] HTLV-3 and HTLV-4 were recently discovered in rural Cameroon but it is not known yet if they can cause diseases.[30]

People travel more, especially from western countries to developing countries where some diseases are endemic such as malaria-like diseases caused by infection with parasites *Plasmodium* or *Babasia* and Chagas disease caused by infection with parasite *Trypanosoma cruzi.* Infections with these pathogens are mostly asymptomatic, and blood donors which have journeyed in these regions are temporarily deferred for donations.[31] However, a case of a very long incubation period before the appearance of detectable anti-*Plasmodium* antibodies was reported.[32] Moreover, parasites carriers such as mosquitoes can be transported to other, non endemic-countries by plane, exposing airport workers to infection risks.[33]

Besides the efforts made to minimize risks for virus and tropical parasite transmission, bacterial contamination of blood products remains a challenge.

About 17-22% of all reported transfusion-related fatalities in USA were caused by transfusion of contaminated blood products with bacteria.[34] The frequency of clinical apparent sepsis through transfusion amounts 1 in 15.000 platelet units[34] and 1 in 10.4 million red blood cell (RBC) units[35]. Only PCs are cultured for bacteria but the standard techniques available to detect the presence of bacteria last several days. If the post-donation cultured sample is negative, PC can be released with the mention "negative to date". However, if the cultured sample appeared to be positive after that time, hospital employees are informed of the bacterial contamination and asked to inform about transfusion reactions. In addition, the red blood cell concentrate (RBCC) originated from the same donor is recalled and discarded.

However, this strategy cannot completely prevent the transfusion of a contaminated product and served rather to determine the frequency of bacterial contamination in related blood products. Other attempts to reduce contamination by diversion of the first 20 ml of blood withdrawal and improvement of skin disinfection at time of donation have been introduced. As the majority of contaminating bacteria were shown to originate from skin flora[36], this new regulation has been shown to reduce the frequency of bacterial contaminated blood products.[37]

In summary, stringent selection of donors, deferral strategies and the use of highly sensitive tests make that blood transfusion has never been so safe. In industrialized countries, residual risks of infection through blood transfusion amount lower than 1 in 205.000 for HBV, 1 in 270.000 units for HCV and 1 in 3.1 million units for HIV, and almost inexistent for HTLV and *Treponema pallidum*.[38-40] However, the safety of blood products is not only challenged by the well known viral infections mentioned above, but also by increased donor travel, patients health conditions, and the time of infection of the blood donor. Emerging pathogens raise the concern as tests are either not available or supplementary tests must be required to ensure a pathogen-free product for high-risk patients. Infection can develop in an unexpected way (long incubation period). Bacterial contamination tests last too long. Considering the wide range of pathogens, it may be unrealistic to implement test for each of them. Increased costs and time

consuming leading to longer storage of labile blood cells prior to release may have enormous consequences on the blood supply. Finally, it is clear that, even though blood transfusions are presently considered to be very safe, continuous efforts are necessary to keep it safe.

3. UMBILICAL CORD BLOOD: A PARTICULAR BLOOD PRODUCT

Umbilical cord blood cells can be used for hematopoietic stem cell transplantation and tissue engineering e.g. megakaryocyte and RBC expansion.

Normally, during the gestation, placenta acts as a filter that protects the fetus from opportunistic infections. That is why cord blood is in principle considered as free from infections.[41] However, this filter does not always work perfectly and contamination from mother to child occurs.[42-47]

To ensure the safety of cord blood transplants, routine viral detection is performed in maternal blood. Tests for the detection of HIV1/2, HCV, HBV, HTLV1/2, *Treponema pallidum* are standard. A recall after 6 months helps to determine if the umbilical cord blood donor (the baby) has not developed any congenital diseases [source Eurocord]. A major problem with umbilical cord blood donation is the high rate of bacterial contamination. Contamination takes principally place at delivery and during the cord blood collection. Depending on the sample size and the method of testing, up to 13% of cord blood stem cell (CBSC) products have been found to be positive in bacterial cultures.[48,49] No case of sepsis or bacterial contamination related complications were reported after stem cell transplantation. This is probably due to the low bacteria titers (1-10 CFU/ml) contaminating cord blood product. As cord blood products are stored at very low temperature (-190°) until transplantation purposes, bacteria cannot grow to clinically significant levels. However, bacterial contamination of cord blood products may limit expansion protocols. Cord blood hematopoietic progenitor cells culture and expansion may create conditions in which low numbers of contaminating bacteria may reach levels of clinical significance, compromising cell growth and exposing immuno-compromised patients to high

risks of infection. Therefore for such purposes, pathogen-free cord blood products are required.

Efforts to minimize the risks of bacterial contamination, for example by stringent disinfection and intensive monitoring of clean areas, did not reduce the rate of microbial contamination.[50] In addition, the addition of antibiotics *in vitro* appeared to be unsuccessful and gave a new opportunity for contamination.[51] In general, the use of antibiotics is preferably avoided, considering the development of bacteria resistance to this kind of antimicrobial agents.[52] Recently, the FDA guidelines and Netcord FACT Standards requested the release of pathogen-free CBSC products for unrelated transplantation.[53] In case of contamination, the product should be discarded. This may be a problem in case the cord blood is from a related donor, intended for transplantation e.g. to a diseased sibling and for patients from ethnic minorities. Indeed, the cord blood bank has an advantage on bone marrow donations because it may help to reduce the shortage in stem cell products for non-Caucasian patients. Mothers originating from immigrant populations are easily reachable through regular maternity controls whereas those persons are largely less represented among blood and bone marrow donation institutions. Some of the cord blood donations from ethnic minorities may have a particular HLA typing. The discard strategy because of contamination may lead to the loose of these valuable products. As for common blood products, pathogen inactivation may represent an approach to circumvent the problem of microbial contamination.

4. PATHOGEN INACTIVATION

Since pathogens involved in transfusion-transmitted infection are very heterogeneous, a non-specific approach of inactivation of a broad range of pathogens would be desired. The ideal method to ensure the production of pathogen-free blood products should be a method that inactivates at once a broad range of pathogens in blood products. Such method of decontamination is commonly but often wrongly referred to as sterilisation. Sterilisation is a process that destroys or removes all microorganisms (pathogenic or not) from an object or

a solution. A sterilized item cannot support life in any form. Despite platelets and RBCs do not contain a nucleus and thus are not able to reproduce themselves, they anyway have an active respiratory metabolism thereby they are living cells, forming the essential therapeutic components of the blood products. Treatment of cellular blood products to inactivate pathogens must be applied in such a way that the therapeutic effect of the blood cells such as platelets and RBCs remain intact. Therefore, the common sterilization procedures are not suitable for PC and RBCC. However, pathogen inactivation procedures that will have a broad antimicrobial activity while preserving all blood components will represent a proactive approach to blood safety. The challenge of pathogen inactivation is to reduce maximally the greatest number of potential pathogens in blood without significantly compromising the cellular or protein constituents or introducing some new toxicity, carcinogenicity, or teratogenicity.[54]

PRINCIPLES OF PATHOGEN INACTIVATION TECHNIQUES

I. METHODS OF EVALUATION

The key requirement in the evaluation of the efficacy of pathogen inactivation strategies is to show that the procedure is effective in reducing the risk of infection by pathogens.

Potential infectivity is determined by several measures. Genomic equivalents refer to the number of viral particles detected by molecular techniques. The tissue culture infectious dose (50%) $TCID_{50}$ refers to the dose of viral particles in a tissue culture assay capable of infecting 50% of susceptible cells or animals. The term $TCID_{50}$ log_{10} reduction is usually used to define the degree of pathogen reduction. Although the “acceptable” log_{10} reduction number for a given organism is not standardized, experience suggests the fewer than 4 log reductions may be insufficient and greater than 6 log_{10} reduction is ordinarily sufficient.[54] However, in practice a 6 log_{10} reduction order is not always possible to demonstrate because of the limitations in inocula for some specimens; thereby a maximal of 5 log_{10} reduction can be demonstrated. As no all conceivable organisms can be tested, different classes of organisms are selected to cover the range of potential

pathogens. Model viruses are used in place of pathogens that cannot be cultured in vitro (table 1).

Virus	**Family**	**Virus Group RNA/DNA**	**Size (nm)**	**Model for**
Pseudorabies virus (PRV)	Herpesviridae	Lipid-env. DNA	100-200	HBV, CMV
Duck Hepatitis B virus (DHBV)	Hepadnaviridae	Lipid-env. RNA	40-45	HBV
Bovine viral diarrhorea virus (BVDV)	Flaviviridae	Lipid-env. RNA	37-50	HCV, WNV
Porcine Parvovirus (PPV) Canine Parvovirus (CPV)	Parvoviridiae	Non lipid-env. DNA	18-26	Human Parvo-B19
Vesicular Stomatitis virus (VSV)	Rhabdoviridae	Lipid-env. RNA	100	HIV

Table 1: Viruses, their properties and their use as model

II. CHEMICAL TREATMENTS

The combination of organic solvent and detergent (SD) is widely used for pathogen inactivation. The combination most often used is TNBP (tri-(n-butyl)-phosphate) with Triton-X100.[55] TNBP acts as an organic solvent which removes lipids from the membranes, while Triton X-100 is a non-ionic detergent which stabilizes TNBP and disrupts lipid bilayers for easier extraction of lipids. Solvent and detergent must be removed because the chemical reaction is non-selective. The detergent keeps the lipids from the membranes in suspension in order to be removed before the final product is transfused.

Lipid-envelop viruses as HIV, HBV and HCV or bacteria are sensitive to this treatment and are inactivated in an irreversible way. Non-lipid enveloped viruses

like Hepatitis A virus (HAV) and human B19 parvo-virus are insensitive to the SD-treatment. This chemical treatment is only suitable for pathogen inactivation in plasma products. As the SD treatment disintegrates plasma membranes, it is not suitable for pathogen inactivation of cellular blood components. Therefore, alternative methods to inactivate pathogens without affecting the therapeutic blood cells were developed. For the treatment of RBC, Pen 110 and Frangible Anchor Linker Effector compounds S-303 are under investigation.

Pen 110 is an alkylating ethyleneamine oligomer of low-molecular weight, highly water-soluble, and positively charged, diffusing readily through cell membranes and having a high selectivity for nucleic acid guanine bases. The disruption of nucleic acid replication forms the biochemical basis for pathogen reduction by the Inactine Pen 110 process.[56,57] Pen 110 is capable of inactivating a broad spectrum of viruses, bacteria, protozoa, and mycoplasma in RBCC[56,58-62] RBC membrane integrity seems to be maintained in Pen 110-treated RBCs. Hemolysis was less than 1% after 42 days of storage at 4°C. In vivo RBC recovery after autologous transfusion of treated RBCs in healthy subjects was comparable with the untreated control group.[62] Alteration of RBC surface antigens was not detected and neoantigens formation was not identified during subsequent testing. However, phase III clinical trials were halted when antibody formation was reported in repeatedly transfused patients. All other clinical studies have been suspended.[54]

S-303 (N,N-bis(2-chloroethyl)-2-aminoehtyl-3-[(acridin-9-yl)amino] propionate dihydrochloride) is an alkylating agent derived from a quinacrine mustard and belongs to a class of compounds called frangible anchor-linked-effectors (FRALE). The molecule consists of three components: an acridine ring, a planar compound that reversibly intercalates with double-stranded and single-stranded DNA and RNA; a bi-functional alkylating moiety that crosslinks DNA and RNA strands; and an alkyl ester linker that hydrolyses when exposed to neutral pH.[63] The compound is rapidly taken up by cells, bacteria and viruses. S-303 molecule becomes active as a result of pH change attendant upon admixture with the blood component and induces DNA and RNA cross-linkage inhibiting pathogen replication and transcription. Extracellular reactions are minimized by the inclusion of a

glutathione quenching agent. The alkyl ester linker spontaneously degrades releasing S-303 that diffuses outside of cell and is absorbed by an appropriate compound.[64] S-303 binds to other proteins and cell membranes as well as to nucleic acids, and up to 20% can potentially remain bound to the surface or contained within the RBCs.[54] S-303 has demonstrated pathogen inactivation of a wide range of viruses, bacteria, and protozoa.[65] No unexpected toxicities have been described. Assays for RBC storage lesions (extracellular potassium (K^+) leakage, plasma free hemoglobin (Hb), adenosine triphosphate (ATP), 2,3-diphosphoglycerate (2-3 DPG), glucose, and lactate) are comparable to control RBCs. The RBC function appears to be normal, and in-vivo survival studies with radioactive chromate labeling comply with the required standard of at least 75% recovery at 24 hours. However, as seen with Inactine Pen110 treatment, phase III clinical trials were halted when antibodies to residual RBC bound S-303 were discovered in 2 subjects.[66] Additional studies have revealed that 1% of patients and healthy donors that had never been exposed to S-303 had naturally occurring antibodies that reacted with S-303 treated RBC. Modifications have been made to the S-303 treatment process to reduce the amount of RBC bound S-303 in attempts to eliminate immunoreactivity and immunogenicity. Preliminary finding indicate that RBCs from the modified S-303 treatment process were crossmatch-compatible with the anti-S-303 antibodies formed after exposure to the original S-303 formulation as well as the anti– S-303 antibodies found in the patients and donors never exposed to S-303. The antibodies do not appear to impair RBC survival or to pose any clinical problem.[67]

PHOTOCHEMICAL TREATMENT

In photochemical treatment, light is used as a source of energy for intermolecular reactions, which consists of a reaction between the light-excited molecule and a different non-excited molecule. A good example of intermolecular reaction is the photoaddition of furocoumarins to pyrimidines.[68] By the use of psoralens (family of furocoumarins), photochemical treatment can be applied in cellular blood components. Psoralens are molecules that can intercalate into DNA base pairs. Upon activation by UVA-light (320 – 400 nm), formation either of monofunctional

adducts (single strand) or bifunctional adducts (interstrand cross-links) with DNA takes place. These adducts block the replication and transcription machinery and cause cell death.[69]

INTERCEPT, the S-59 amotosalen system, can currently be applied in PC.[70] It has been evaluated in clinical trials which, albeit not unequivocally, demonstrated comparable platelet transfusion requirements and posttransfusion platelet increments between photochemically treated platelets and control platelets.[71] Application in plasma is currently under investigation.[70] Functional quality of plasma proteins (factor VII, factor VIII, von Willebrand factors, metalloprotease, fibrinogen, protein C, protein S, and antithrombin) decreased slightly but were considered still sufficient for therapeutic use.[72] Antibodies against neo-antigens have not been detected thus far. Clinical trials are ongoing.[54]

Platelet and plasma are substantially transparent above 300 nm, but hemoglobin present in RBC has several absorption bands in the range 300-630 nm. Therefore photoactivated compounds for use in RBCC should exhibit significant absorption outside this spectrum (i.e. at longer wavelengths) and for this reason psoralens like S-59 cannot be used for pathogen inactivation in RBCC treatment.

PHOTODYNAMIC TREATMENT

A special form of photochemical reactions is photosensitization. Photosensitized reactions can occur when a mixture of at least two compounds is irradiated: one compound absorbs the light but the other is chemically altered. The term photodynamic refers to oxygen-requiring photosensitized reactions in biological systems.[73]

In photodynamic reactions, oxygen (O_2) acts as an intermediate transporting electrons or energy between the light-absorbing molecule and the altered molecule.[73] Figure 1 shows a schematic overview of the successive reactions involved in a photodynamic process. The light absorbing molecule is referred to as the photosensitizer (P). The altered molecule is termed a substrate (S). After absorption of light, the photosensitizer is transformed from its singlet electronic ground state ($^1P_0$) to an electronically excited state ($^1P^*$) (equation 1). Via

intersystem crossing, the short-lived singlet excited stated is converted to the triplet excited state ($^3P^*$) (equation 2) which has lower energy than the singlet excited state, but the lifetime of the triplet excited state is much longer (typically > 500 ns).[74]

There are two mechanisms by which the triplet excited state photosensitizer can react with biomolecules: type I and type II reactions.
In a type I reaction, $^3P^*$ can either abstract an electron from, or donate an electron to a substrate molecule (S). This reaction produces radical forms of both the photosensitizer and the substrate (equations 3). The substrate radicals ($S_1^{-\bullet}$) can react with other oxygen, to give oxidized forms of the substrate (equation 4). The photosensitizer radicals ($P^{+\bullet}$) can react with oxygen to give superoxide radical ($O_2^{-\bullet}$; equation 5) which can rapidly oxidize cellular targets (S_2) (equation 6).

Type II reaction can occur when the energy difference between the triplet excited state and the ground state of the photosensitizer exceeds 94.5 kJ/mol. $^3P^*$ can then directly transfer its energy to ground state oxygen ($^3O_2$), thereby generating the highly reactive singlet oxygen ($^1O_2$) (equation 7).
The photosensitizer returns to its ground state without being chemically altered and is able to repeat the process of energy transfer to oxygen many times. Singlet oxygen can react with a variety of cellular components such as amino acids (cysteine, histidine, tryptophan, tyrosine and methionine), nucleoside (mainly guanine) and unsaturated lipids.[75] Because of the short lifetime of $^1O_2$, damages are induced at, or close to, the sites where the photosensitizer is activated.

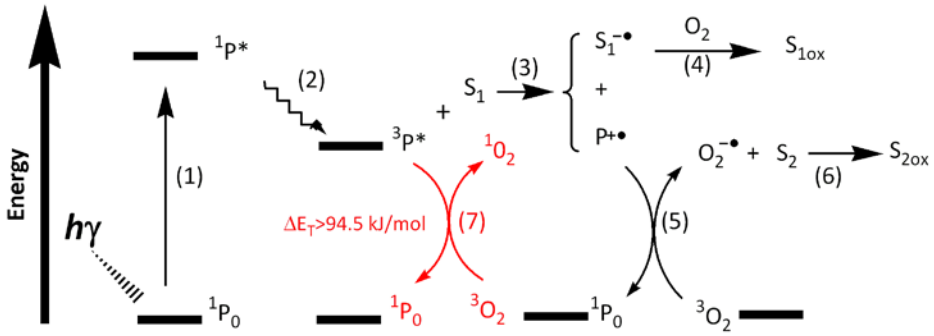


Figure 1: Schema of type I and type II photodynamic reactions

5. PHOTOSENSITIZERS USED FOR PATHOGEN INACTIVATION OF RBC

OPTIMAL PHOTOSENSITIZER PROPERTIES

Photosensitizers used for pathogen inactivation should have a high selectivity for the pathogens so that the pathogens are inactivated without altering the quality of cells. For RBC containing blood products, the sensitizer should absorb light of a long wavelength (> 600 nm) to avoid absorption by hemoglobin. An extra advantage of the use of long wavelength light is the greater penetration of the light in tissues and cell containing suspensions. During the past 15 years, a number of photosensitizers have been studied for their ability to inactivate pathogens in cellular blood products.

PHENOTHIAZINES

Phenothiazinium photosensitizers are efficient producers of singlet oxygen. The Phenothiazinium dye Methylene Blue (MB) is positively charged and planar molecule with affinity for nucleic acids and the surfaces of viruses.[76,77] Upon UV light exposure, most enveloped viruses are inactivated; however, non-enveloped viruses are more resistant to treatment. As the photosensitizer is easily reduced by biological systems to a neutral species MB has a low antibacterial and antiprotozoa activity.[78,79] Because of its virucidal activities, MB has been used in Europe for approximately 15 years for pathogen inactivation of single unit plasma. Millions of MB single units of plasma have been transfused without unexpected adverse outcome.[80]

Methylation of this dye results in Di-Methyl-Methylene Blue (DMMB) which is considerably more bactericidal in-vitro than MB and less susceptible to reduction.[77] DMMB (Fig.3) is a more hydrophobic dye with a permanent positive charge and can pass through the cellular plasma membrane.[81] The photosensitizer intercalates into DNA[82] and is a highly efficient photobactericide against a wide range of bacteria and against both extra- and intracellular viruses and non-enveloped species upon light activation at wavelength >600 nm.[83,84] However, up to one-half of an added DMMB is associated with RBCs. This binding is responsible

for the photoinduced hemolysis.[76]. The use of quinacrine which inhibits photosensitizer binding to RBC membranes was shown to enhance VSV inactivation and to diminish hemolysis of photo-treated RBCs.[82] However, DMMB exhibited (dark) toxicity[85] and post-treatment removal of the molecules e.g. by filtration is required before transfusion.

RIBOFLAVIN

Riboflavin is a natural compound, a proven and accepted non toxic agent known as vitamin B2 an essential dietary requirement in humans. The Riboflavin molecule is a polycyclic planar molecule with a sugar side chain that confers water solubility. The planar portion of the molecule is capable of intercalating into the bases of DNA and RNA of pathogens, and absorbing visible light (max absorption at 475 nm and 444 nm) or UV light. Light-activated, intercalated riboflavin oxidizes guanine in nucleic acids, a process preventing replication of the pathogen's genome.[86] Especially type I reaction (formation of radicals through electron transfer) are involved in the photoreaction of Riboflavin.[87]

Upon light activation, the photosensitizer breaks down to secondary photosensitizers Lumichrome and Lumiflavin.[88,89] The advantages of these photoproducts are that they are also naturally generated in situ by light transmitted through the skin and eyes and are naturally excreted by the human body.[90]

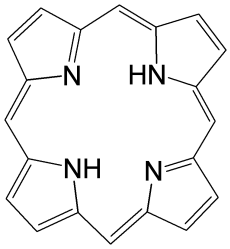
The particularity of Riboflavin is that this photosensitizer is sensitive to UV light and blue light (250 - 500 nm) and not to red light (> 600 nm), therefore Riboflavin seems not very, suitable for pathogen inactivation of RBCC. In contrast, Riboflavin was shown to be effective in inactivating pathogens in this blood product. A reduction of 4 to 7 $\log_{10}$ of extra- and intracellular HIV, BVDV, and PRV was obtained after treatment with Riboflavin. The non-enveloped PPV which has a tight-capsid, was more resistant. The antibacterial efficacy of Riboflavin was demonstrated with the inactivation of *S. aureus*, *E. coli*, *K. pneumoniae*, and *Y. enterocolitica*.[91] However, for this, it was necessary to reduce the RBCC

hematocrit to 30-37% instead of normally used 60% before the suspension was subjected to visible light (λ = 450 nm).[64,86,91,92,93]

PORPHYRINS

Porphyrins are a class of deeply colored red or purple pigments, of natural or synthetic origin, which vary in their substituent around the same basic skeleton. The latter is an aromatic macrocyclic ring consisting of four pyrrole rings, linked together by four methane bridges (Fig.2). Porphyrins are probably the most important class of compounds in biological systems because of their central role in photosynthesis, biological oxidation and reduction, and oxygen transport.[74,94]

The interest for porphyrins in clinical use rose when a mixture of porphyrin, called Hematoporphyrin derivatives (HpD), and later a purified form of HpD known as Photofrin was found to localize in tumour cells, making it useful in detecting and treating cancers.[95-97]To improve therapies and diminish side effects, in particularly long lasting skin photosensitivity, the development of new photosensitizers was necessary. In photodynamic treatment of cancer, red light (>600 nm) is usually used because long wavelength enter deeper in tissue. Since porphyrins have only a small absorption band in this region, research was focused on the synthesis of new photosensitizers with a strong absorption in the red.[98] This resulted to a second generation of photosensitizers, among which phthalocyanines and meso-substituted porphyrins appeared to be promising for RBCC decontamination.



Figre 2. Basic skeleton of porphyrin

I. Phthalocyanines

Phthalocyanines are porphyrin derivatives with an increased aromatic structure. They absorb intensively in the visible region with a maximum absorption around 670-680 nm and to a lesser extend in the near-ultraviolet region (see spectrum, Fig.4).[74] Therefore phthalocyanines are suitable for application in RBCC. The most effective molecules in inactivating pathogens were silicon phthalocyanine (Pc4) and chloro-aluminium sulfonated phthalocyanine (AlPcS4) (Fig.3).

Pc4 is a hydrophobic molecule which targets the lipid envelope of pathogens. Formulated into liposomes to maximize delivery into viruses with minimum distribution to RBCs, Pc4 was able to inactivate HIV-1 is three forms: cell-free virus, cell-associated virus and virus in latency infected cells, and to inactivate both enveloped and non-enveloped viruses.[99,100] AlPcS4 is a water-soluble derivative which is efficient in cell-free lipid-enveloped virus inactivation, but not capable of inactivating intracellular virus.[101,102] However, with both phthalocyanines RBC damage was apparent which required the addition of antioxidant such as vitamin E or a mixture of antioxidants containing either tocopherol succinate and carnitine or Trolox, Mannitol and glutathione to diminish the extensive hemolysis, K^+ efflux and binding of IgG to phototreated RBCs.[100,103-105]

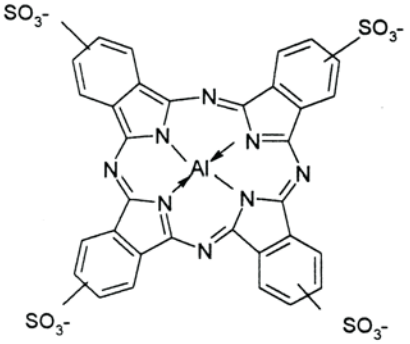
II. Meso-substituted porphyrins

The growing interest in the meso-substituted porphyrins began when besides their photosensitizing properties[106], their capacity to intercalate into DNA was discovered.[107] Their potential antimicrobial effect was shown by Merchat et al.[108,109]; especially cationic meso-substituted porphyrin were able to inactivate both Gram-positive and Gram-negative bacteria.

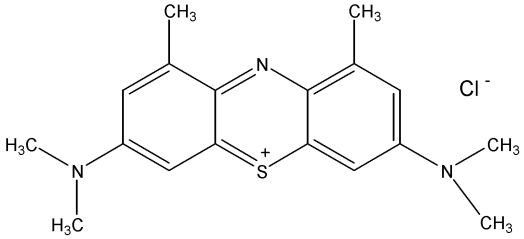
Meso-substituted porphyrins absorb radiant energy intensively in the UV region, at their Soret band (400-410 nm) and to a lesser extent in the long visible bands called the Q bands (580 – 650 nm) (see spectrum, Fig.4).[74] Again their capacity to absorb red light make these porphyrins suitable for application in RBCC.

In this thesis, 5 different meso-substituted porphyrins, from which the most powerful was the mono-phenyl-tri-(N-methyl-4-pyridyl)-porphyrin chloride (Tri-P(4)) (Fig.3) were studied for their pathogenical potential in RBCs (chapter 3).

AlPcS4



DMMB



Tri-P(4)

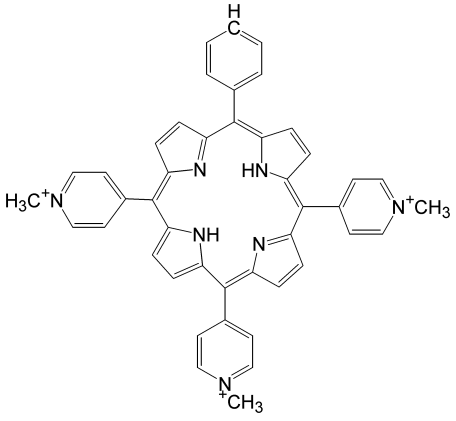


Figure 3. Main photosensitizers used in this thesis

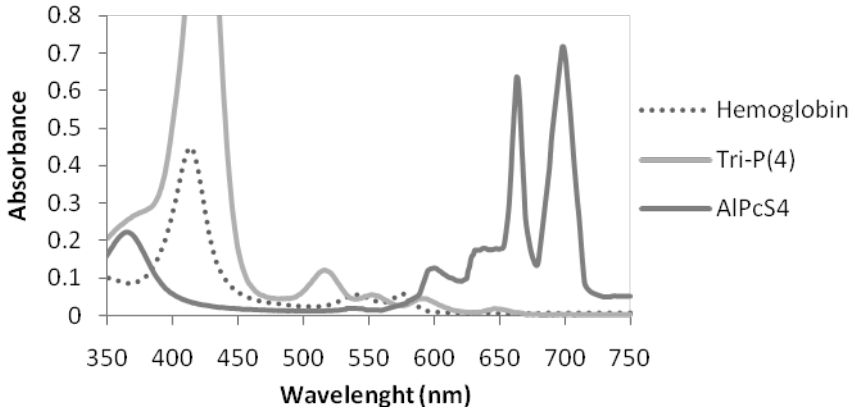


Figure 4. Absorption spectrum in Methanol of Hemoglobin and photosensitizers Tri-P(4) and AlPcS4.

6. CURRENT STATUS OF PATHOGEN INACTIVATION OF RBC

Many different techniques were studied to inactivate microorganisms in RBCC. Most of the treatments are able to induce a considerable reduction of viable pathogens. However, too much damage to the RBCs, such as hemolysis, RBC aggregation[105], K^+ leakage, enhanced transbilayer mobility of lipids[110] and IgG binding[111] are induced by pathogen inactivation techniques, especially the one based on the use of light. To reduce the damage to the RBCs, various antioxidants had to be added to the RBC suspension before treatment.[100,104,105] Such additives may have side-effects and may have to be removed before transfusion.

Besides the direct damage to RBCs, several treatments have shown to induce antibodies against either the agent added to the suspension or to the treated RBCs. Despite encouraging in-vitro results concerning the efficacy of S-303 and P110 treatments to inactivate a broad spectrum of pathogens while preserving in-vitro RBC qualities (Hb, ATP, 2-3 DPG level and storage time) and in-vivo survival, unexpected results in clinical trials showed up which resulted to their suspension.

The future of both S-303 and PEN110 as pathogen inactivation methods for RBCC is uncertain until the immunogenicity is understood and resolved.[54] For Riboflavin, extended animal studies are required to ensure the absence of neoantigenicity to Riboflavin and its breakdown product before proceeding clinical studies.[64]

7. OUTLINE OF THIS THESIS

This thesis describes different studies regarding the photo-decontamination of RBC and Cord-blood-derived stem cell products. Besides the inactivation of pathogens, major efforts were taken to preserve the therapeutic quality of the treated blood products. By using the photosensitizer DMMB, we tried to identify the target for RBC damage induced by photodynamic treatment (PDT). Based on this, we evaluated the possibility to protect the RBCs against photo-damage without reducing the virucidal capacity of the photo-treatment (**Chapters 2 and 3**). A series of meso-substituted porphyrins were tested for their potential to inactivate viruses in RBCC. From the series, one photosensitizer Tri-P(4) stood out as a very promising compound to be applied in pathogen inactivation in RBCC (**Chapters 4 and 5**). Photosensitization with Tri-P(4) was further tested in Cord-blood derived stem cell products (**Chapter 6**). Since the treatment did not seem to affect the viability of leukocytes, the potential immunomodulatory effect on leukocytes was investigated (**Chapter 7**).

8. REFERENCES

1. Meulenbroek A.J. Van bloed tot geneesmiddel, gezond vertrouwen. Sanquin ed. Amsterdam: Artoos Nederland; 2002.

2. Kalibatas V. Payment for whole blood donations in Lithuania: the risk for infectious disease markers. Vox Sang. 2008 Apr;94(3):209-15.

3. van der Poel CL. Remuneration of blood donors: new proof of the pudding? Vox Sang. 2008 Apr;94(3):169-70.

4. Boltho Massarelli V., Van Aken W, Genetet B. Ethics of transfusion: the Council of Europe's view. Transfusion Medicine 2004; An European Course on Blood transfusion:11-39.

5. Koppelman MHGM, Sjerps MC, Reesink HW, Cuypers HTM. Evaluation of COBAS AmpliPrep nucleic acid extraction in conjunction with COBAS AmpliScreen HBV DNA, HCV RNA and HIV-1 RNA amplification and detection. Vox Sang. 2005;89(4):193-200.

6. Council of Europe Health.Deptmt. K-FB. Pathogen inactivation of labile blood products. Strasbourg: Council of Europe; 2001. Report No.: ISBN 92-871-4560-1.

7. Laino C. ASH: Screening of blood supply for West Nile Virus justified By past Prevention Success 2004. Available from www.docguide.com/news/content.nsf.

8. Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL, Stobierski MG, Signs K, Newman B, Kapoor H, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. N.Engl.J.Med. 2003 Sep 25;349(13):1236-45.

9. Yoshikawa A, Gotanda Y, Itabashi M, Minegishi K, Kanemitsu K, Nishioka K. Hepatitis B NAT virus-positive blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. Vox Sang. 2005;88(2):77-86.

10. Roback JD, Caliendo AM, Newman JL, Sgan SL, Saakadze N, Gillespie TW, Lane TA, Kurtzberg J, Hillyer CD. Comparison of cytomegalovirus polymerase chain reaction and serology for screening umbilical cord blood components. Transfusion 2005;45(11):1722-8.

11. Hillyer CD, Josephson CD, Blajchman MA, Vostal JG, Epstein JS, Goodman JL. Bacterial contamination of blood components: risks, strategies, and regulation: joint ASH and AABB educational session in transfusion medicine. Hematology 2003;575-89.

12. Huai Y, Xiang N, Zhou L, Feng L, Peng Z, Chapman RS, Uyeki TM, Yu H. Incubation period for human cases of avian influenza A (H5N1) infection, China. Emerg.Infect.Dis. 2008 Nov;14(11):1819-21.

13. Likos AM, Kelvin DJ, Cameron CM, Rowe T, Kuehnert MJ, Norris PJ. Influenza viremia and the potential for blood-borne transmission. Transfusion 2007 Jun;47(6):1080-8.

14. Epidemic and Pandemic alert and response - Recommendation concerning SARS and blood transfusion safety 2003. Available from www.WHO.int.

15. Wüllenweber J, Cassens U, Sica S, Sibrowski W, Herrmann M. West Nile Virus - an Emerging Transfusion-Transmissible Pathogen. Transf. Med. Hemoth. 2004;31(6):373-7.

16. Bianco C. West Nile Virus transmission by blood transfusion and transplantation. Blood Therapies in Medicine 2003;3(3):78-83.

17. Harrington T, Kuehnert MJ, Kamel H, Lanciotti RS, Hand S, Currier M, Chamberland ME, Petersen LR, Marfin AA. West Nile virus infection transmitted by blood transfusion. Transfusion 2003;43(8):1018-22.

18. Biggerstaff BJ, Petersen LR. Estimated risk of transmission of the West Nile virus through blood transfusion in the US, 2002. Transfusion 2003;43(8):1007-17.

19. Hollinger FB, Kleinman S. Transfusion transmission of West Nile virus: a merging of historical and contemporary perspectives. Transfusion 2003;43(8):992-7.

20. Haltia M. Human prion diseases. Ann. Med. 2000 Oct;32(7):493-500.

21. Goodnough LT, Hewitt PE, Silliman CC. Transfusion Medicine: Joint ASH and AABB Educational Session. Hematology 2004 Jan 1;2004(1):457-72.

22. Dorsey K, Zou S, Schonberger LB, Sullivan M, Kessler D, Notari E, Fang CT, Dodd RY. Lack of evidence of transfusion transmission of Creutzfeldt-Jakob disease in a US surveillance study. Transfusion 2009 May;49(5):977-84.

23. Houston F, Foster JD, Chong A, Hunter N, Bostock CJ. Transmission of BSE by blood transfusion in sheep. Lancet 2000;356(9234):999-1000.

24. Seghatchian J. nvCJD and leucodepletion: an overview. Transfus Sci 2000;22(1-2):47-8.

25. Turner ML. Variant Creutzfeldt-Jakob disease and blood transfusion. Curr. Opin. Hematol. 2001 Nov;8(6):372-9.

26. Akiba J, Umemura T, Alter HJ, Kojiro M, Tabor E. SEN virus: epidemiology and characteristics of a transfusion-transmitted virus. Transfusion 2005;45(7):1084-8.

27. Okamura A, Yoshioka M, Kubota M, Kikuta H, Ishiko H, Kobayashi K. Detection of a novel DNA virus (TTV) sequence in peripheral blood mononuclear cells. J. Med. Virol. 1999;58(2):174-7.

28. Hadlock KG, Foung SK. GBV-C/HGV: a new virus within the Flaviviridae and its clinical implications. Transfus. Med. Rev. 1998;12(2):94-108.

29. Viazov S, Ross RS, Varenholz C, Lange R, Holtmann M, Niel C, Roggendorf M. Lack of evidence for an association between TTV infection and severe liver disease. J. Clin. Virol. 1998 Dec;11(3):183-7.

30. Mahieux R, Gessain A. [New human retroviruses: HTLV-3 and HTLV-4]. Med. Trop. 2005 Nov;65(6):525-8.

31. Kitchen AD, Chiodini PL. Malaria and blood transfusion. Vox Sang. 2006 Feb;90(2):77-84.

32. Kitchen A, Mijovic A, Hewitt P. Transfusion-transmitted malaria: current donor selection guidelines are not sufficient. Vox Sang. 2005 Apr;88(3):200-1.

33. Thellier M, Lusina D, Guiguen C, Delamaire M, Legros F, Ciceron L, Klerlein M, Danis M, Mazier D. Is airport malaria a transfusion-transmitted malaria risk? Transfusion 2001;41(2):301-2.

34. Wagner SJ. Transfusion-transmitted bacterial infection: risks, sources and interventions. Vox Sanguinis 2004 Apr;86(3):157-63.

35. Andreu G, Morel P, Forestier F, Debeir J, Rebibo D, Janvier G, Herve P. Hemovigilance network in France: organization and analysis of immediate transfusion incident reports from 1994 to 1998. Transfusion 2002 Oct;42(10):1356-64.

36. de Korte D, Marcelis JH, Soeterboek AM. Determination of the degree of bacterial contamination of whole-blood collections using an automated microbe-detection system. Transfusion 2001 Jun;41(6):815-8.

37. de Korte D, Curvers J, de Kort WL, Hoekstra T, van der Poel CL, Beckers EA, Marcelis JH. Effects of skin disinfection method, deviation bag, and bacterial screening on clinical safety of platelet transfusions in the Netherlands. Transfusion 2006 Mar;46(3):476-85.

38. Kluter H. The Struggle for Safer Blood: Pathogen Inactivation of Cellular Blood Preparations. Blood Therapies in Medicine 2002;2(2):42-7.

39. Dodd RY, Notari EP, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. Transfusion 2002 Aug;42(8):975-9.

40. Stramer SL. Viral diagnostics in the arena of blood donor screening. Vox Sang. 2004 Jul;87 Suppl 2:180-3.

41. Koi H, Zhang J, Parry S. The mechanisms of placental viral infection. Ann. N.Y. Acad. Sci. 2001 Sep;943:148-56.

42. Van Geertruyden JP, Thomas F, Erhart A, D'Alessandro U. The contribution of malaria in pregnancy to perinatal mortality. Am. J. Trop. Med. Hyg. 2004 Aug 1;71(suppl):35-40.

43. Saback FL, Gomes SA, de Paula VS, da Silva RR, Lewis-Ximenez LL, Niel C. Age-specific prevalence and transmission of TT virus. J. Med. Virol. 1999;59(3):318-22.

44. Pahwa S. Human immunodeficiency virus infection in children: nature of immunodeficiency, clinical spectrum and management. Pediatr. Infect. Dis. J. 1988;7(Suppl):61-71.

45. Linder N, Sirota L, Aboudy Y, German B, Lifshits T, Barnea BS, Lieberman B, Mendelson E, Barzilai A. Placental transfer of maternal rubella antibodies to full-term and preterm infants. Infection 1999;27(3):203-7.

46. Zufferey J, Hohlfeld P, Bille J, Fawer CL, Blanc D, Pinon JM, Vaudaux B. Value of the comparative enzyme-linked immunofiltration assay for early neonatal diagnosis of congenital Toxoplasma infection. Pediatr. Infect. Dis. J. 1999;18(11):971-5.

47. Alpert SG, Fergerson J, Noel LP. Intrauterine West Nile virus: ocular and systemic findings. Am. .J. Ophthalmol. 2003 Oct;136(4):733-5.

48. Honohan A, Olthuis H, Bernards AT, van Beckhoven JM, Brand A. Microbial contamination of cord blood stem cells. Vox Sang. 2002;82(1):32-8.

49. Kogler G, Callejas J, Hakenberg P, Enczmann J, Adams O, Daubener W, Krempe C, Gobel U, Somville T, Wernet P. Hematopoietic transplant potential of unrelated cord blood: critical issues. J. Hematother. 1996;5(2):105-16.

50. Cassens U, Ahlke C, Garritsen H, Krakowitzky P, Wullenweber J, Fischer RJ, Peters G, Sibrowski W. Processing of peripheral blood progenitor cell components in improved clean areas does not reduce the rate of microbial contamination. Transfusion 2002;42(1):10-7.

51. Stroncek DF, Fautsch SK, Lasky LC, Hurd DD, Ramsay NK, McCullough J. Adverse reactions in patients transfused with cryopreserved marrow. Transfusion 1991;31(6):521-6.

52. Engelhard D. Bacterial and fungal infections in children undergoing bone marrow transplantation. Bone Marrow Transplant. 1998 Apr;21 Suppl 2:S78-80.:S78-S80.

53. Netcord, Foundation for the Accreditation of Cellular Therapy. International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection, and Release. 2006 Dec. Report No.: 3rd edition.

54. Bryant BJ, KLEIN HG. Pathogen inactivation: the definitive safeguard for the blood supply. Arch. Pathol. Lab Med. 2007 May;131(5):719-33.

55. Horowitz B, Lazo A, Grossberg H, Page G, Lippin A, Swan G. Virus inactivation by solvent/detergent treatment and the manufacture of SD-plasma. Vox Sang. 1998;74 Suppl 1:203-6.

56. Lazo A, Tassello J, Jayarama V, Ohagen A, Gibaja V, Kramer E, Marmorato A, Billia-Shaveet D, Purmal A, Brown F, et al. Broad-spectrum virus reduction in red cell concentrates using INACTINEtm PEN110 chemistry. Vox Sang. 2002;83(4):313-23.

57. Purmal A, Valeri CR, Dzik W, Pivacek L, Ragno G, Lazo A, Chapman J. Process for the preparation of pathogen-inactivated RBC concentrates by using PEN110 chemistry: preclinical studies. Transfusion 2002;42(2):139-45.

58. Zavizion B, Serebryanik D, Chapman J, Alford B, Purmal A. Inactivation of Gram-negative and Gram-positive bacteria in red cell concentrates using INACTINE PEN110 chemistry. Vox Sang. 2004 Oct;87(3):143-9.

59. Zavizion B, Pereira M, de Melo JM, Serebryanik D, Mather TN, Chapman J, Miller NJ, Alford B, Bzik DJ, Purmal A. Inactivation of protozoan parasites in red blood cells using INACTINE PEN110 chemistry. Transfusion 2004 May;44(5):731-8.

60. Zavizion B, Purmal A, Chapman J, Alford B. Inactivation of mycoplasma species in blood by INACTINE PEN110 process. Transfusion 2004 Feb;44(2):286-93.

61. Mather T, Takeda T, Tassello J, Ohagen A, Serebryanik D, Kramer E, Brown F, Tesh R, Alford B, Chapman J, et al. West Nile virus in blood: stability, distribution, and susceptibility to PEN110 inactivation. Transfusion 2003;43(8):1029-37.

62. AuBuchon JP, Pickard CA, Herschel LH, Roger JC, Tracy JE, Purmal A, Chapman J, Ackerman S, Beach KJ. Production of pathogen-inactivated RBC concentrates using PEN110 chemistry: a Phase I clinical study. Transfusion 2002;42(2):146-52.

63. Allain JP, Bianco C, Blajchman MA, Brecher ME, Busch M, Leiby D, Lin L, Stramer S. Protecting the Blood Supply From Emerging Pathogens: The Role of Pathogen Inactivation. Transf. Med. Rev. 2005 Apr;19(2):110-26.

64. Benjamin RJ. Red blood cell pathogen reduction: in search of serological agnosticism. Transfusion 2006 Sep;1(1):222-6.

65. Corash L. Helinx technology for inactivation of infectious pathogens and leukocytes in labile blood components: from theory to clinical application. Transfus. Apher. Sci. 2001;25(3):179-81.

66. Benjamin RJ, McCullough J, Mintz PD, Snyder E, Spotnitz WD, Rizzo RJ, Wages D, Lin JS, Wood L, Corash L, et al. Therapeutic efficacy and safety of red blood cells treated with a chemical process (S-303) for pathogen inactivation: a Phase III clinical trial in cardiac surgery patients. Transfusion 2005;45(11):1739-49.

67. Conlan MG, Garratty G, Castro G, Erickson A, Schott MA, Arndt P, Cook D, Corash L, Stassinopoulos A. Antibodies to S-303 Treated RBC Prepared with the Original Treatment Process (OSRBC) for Pathogen Inactivation Do Not React with RBC Prepared with a Modified S-303 Treatment Process (MSRBC). ASH Annual Meeting Abstracts 2005 Nov 16;106(11):430.

68. Coyle J.D., Hill R.R., oberts D.R. Light, chemical change and life: a source book in photochemistry. The Open University Press ed. Milton Keynes: 1988.

69. Gasparro FP, Dall'Amico R, Goldminz D, Simmons E, Weingold D. Molecular aspects of extracorporeal photochemotherapy. Yale J. Biol. Med. 1989 Nov;62(6):579-93.

70. Wollowitz S. Fundamentals of the psoralen-based Helinx technology for inactivation of infectious pathogens and leukocytes in platelets and plasma. Semin. Hematol. 2001;38(4 Suppl 11):4-11.

71. Janetzko K, Lin L, Eichler H, Mayaudon V, Flament J, Kluter H. Implementation of the INTERCEPT Blood System for Platelets into routine blood bank manufacturing procedures: evaluation of apheresis platelets. Vox Sang. 2004 May 1;86(4):239-45.

72. Ciaravino V, McCullough T, Cimino G, Sullivan T. Preclinical safety profile of plasma prepared using the INTERCEPT Blood System. Vox Sang. 2003 Oct;85(3):171-82.

73. Tappeiner H.V., Jesionek A. Therapeutische versuche mit fluoreszierenden stoffen. Muench.Med.Wochenschr. 1903;7:2042-4.

74. Milgrom L.R. The colours of life: an introduction to the chemistry of porphyrins and related compounds. New York: 1997.

75. van Steveninck J., Dubbelman T.M.A.R., Verweij H. Photodynamic membrane damage. In: Kessel D & Dougherty TV, editor. Porphyrin Photosensitization. New York: Plenum Press; 1983. p. 227-40.

76. Wagner SJ. Virus inactivation in blood components by photoactive phenothiazine dyes. Transfus. Med. Rev. 2002;16(1):61-6.

77. Wainwright M. Pathogen inactivation in blood products. Curr. Med. Chem. 2002 Jan;9(1):127-43.

78. Wainwright M, Phoenix DA, Laycock SL, Wareing DR, Wright PA. Photobactericidal activity of phenothiazinium dyes against methicillin-resistant strains of Staphylococcus aureus. FEMS Microbiol.Lett. 1998 Mar 15;160(2):177-81.

79. Wainwright M, Phoenix DA, Marland J, Wareing DR, Bolton FJ. A study of photobactericidal activity in the phenothiazinium series. FEMS Immunol Med.Microbiol. 1997 Sep;19(1):75-80.

80. Mohr H. Methylene blue and thionine in pathogen inactivation of plasma and platelet concentrates. Transfus. Apher. Sci. 2001 Dec;25(3):183-4.

81. Paardekooper M, Van den Broek PJ, de Bruijne AW, Elferink JG, Dubbelman TM, van Steveninck J. Photodynamic treatment of yeast cells with the dye toluidine blue: all-or-none loss of plasma membrane barrier properties. Biochim. Biophys. Acta 1992 Jul 8;1108(1):86-90.

82. Jori G, Brown SB. Photosensitized inactivation of microorganisms. Photochem. Photobiol. Sci. 2004 May;3(5):403-5.

83. Wagner SJ, Skripchenko A. Comparison of the sensitivity of Duck Hepatitis B and Vesicular Stomatitis VIrus to photoinactivation by DMMB. Transfusion 2000;40(SP230):99S.

84. Wagner SJ, Skripchenko AA, Robinette D, Mallory DA, Hirayama J, Cincotta L, Foley J. The use of dimethylmethylene blue for virus photoinactivation of red cell suspensions. In: Vyas BF, editor. Advances in Transfusion Safety: Dev Biol. 1999 ed. Basel, Karger; 1999. p. 125-9.

85. Wainwright M, Phoenix DA, Rice L, Burrow SM, Waring J. Increased cytotoxicity and phototoxicity in the methylene blue series via chromophore methylation. J. Photochem. Photobiol. B: Biology 1997 Oct;40(3):233-9.

86. Corbin F. Pathogen inactivation of blood components: current status and introduction of an approach using riboflavin as a photosensitizer. Int. J. Hematol. 2002 Aug;76 Suppl 2:253-7.

87. Douki T, Cadet J. Modification of DNA bases by photosensitized one-electron oxidation. Int. .J. Radiat. Biol. 1999 May;75(5):571-81.

88. Wainwright M. The emerging chemistry of blood product disinfection. Chemical Society Reviews 2002;**31**(**2**):128-36.

89. Dodd RY. Pathogen inactivation: mechanisms of action and in vitro efficacy of various agents. Vox Sang. 2002 Aug;83 Suppl 1:267-70.

90. Schuyler R. Use of riboflavin for photoinactivation of pathogens in blood components. Transfus. Apher. Sci. 2001 Dec;25(3):189-90.

91. Reddy HL, Doane S, McLean R., McDaniel S., Dawson L. Reduction of Virus in Red Blood Cell Suspensions with Riboflavin and Light. Transfusion 2002;42(S9):16S.

92. Goodrich RP, Murthy KK, Doane SK, Fitzpatrick CN, Morrow LS, Arndt PA, Reddy HL, Buytaert-Hoefen KA, Garratty G. Evaluation of potential immune response and in vivo survival of riboflavin-ultraviolet light-treated red blood cells in baboons. Transfusion 2008 Oct 14.

93. McAteer MJ, Tay-Goodrich BH, Doane S, Hansen E, McBurney LL, Stewart L. Photoinactivation of virus in packed red blood cell units using Riboflavin and visible light. Transfusion 2000;40(SP231):99S.

94. Rest A. Porphyrins and phthalocyanines. In: Coyle J.D., Roberts D.R., Hill R.R., editors. Light, chemical change and life: a source book in photochemistry. Milton Keynes: The Open University Press; 1988. p. 43-51.

95. Policard A. Etude sur les aspects offerts par des tumeurs expérimentales examinées à la lumière de Wood. C. R. Soc. Biol. (Paris) 1924;91(1423):1424.

96. Inaguma M, Hashimoto K. Porphyrin-like fluorescence in oral cancer: In vivo fluorescence spectral characterization of lesions by use of a near-ultraviolet excited autofluorescence diagnosis system and separation of fluorescent extracts by capillary electrophoresis. Cancer 1999 Dec 1;86(11):2201-11.

97. Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, Moan J, Peng Q. Photodynamic therapy. J. Natl. Cancer Inst. 1998 Jun 17;90(12):889-905.

98. Dougherty TJ. A brief history of clinical photodynamic therapy development at Roswell Park Cancer Institute. J. Clin. Laser Med. Surg. 1996 Oct;14(5):219-21.

99. Ben Hur E, Oetjen J, Horowitz B. Silicon phthalocyanine Pc 4 and red light causes apoptosis in HIV-infected cells. Photochem. Photobiol. 1997 Mar;65(3):456-60.

100. Ben-Hur E, Chan WS, Yim Z, Zuk MM, Dayal V, Roth N, Heldman E, Lazo A, Valeri CR, Horowitz B. Photochemical decontamination of red blood cell concentrates with the silicon phthalocyanine PC 4 and red light. Developments in Biological Standardization 2000;102:149-55.

101. Margolis-Nunno H, Ben-Hur E, Gottlieb P, Robinson R, Oetjen J, Horowitz B. Inactivation by phthalocyanine photosensitization of multiple forms of human immunodeficiency virus in red cell concentrates. Transfusion 1996;36(8):743-50.

102. Horowitz B, Rywkin S, Margolis-Nunno H, Williams B, Geacintov N, Prince AM, Pascual D, Ragno G, Valeri CR, Huima-Byron T. Inactivation of viruses in red cell and platelet concentrates with aluminum phthalocyanine (AlPc) sulfonates. Blood Cells 1992;18(1):141-9.

103. Ben-Hur E, Rywkin S, Rosenthal I, Geacintov NE, Horowitz B. Virus inactivation in red cell concentrates by photosensitization with phthalocyanines: protection of

red cells but not of vesicular stomatitis virus with a water-soluble analogue of vitamin E. Transfusion 1995;35(5):401-6.

104. Rywkin S, Lenny L, Goldstein J, Geacintov NE, Margolis-Nunno H, Horowitz B. Importance of type I and type II mechanisms in the photodynamic inactivation of viruses in blood with aluminum phthalocyanine derivatives. Photochem. Photobiol. 1992;56(4):463-9.

105. Ben-Hur E, Barshtein G, Chen S, Yedgar S. Photodynamic treatment of red blood cell concentrates for virus inactivation enhances red blood cell aggregation: protection with antioxidants. Photochem. Photobiol. 1997;66(4):509-12.

106. Diamond I, Granelli SG, McDonagh AF. Photochemotherapy and photodynamic toxicity: Simple methods for identifying potentially active agents. Biochem. Med. 1977 Apr;17(2):121-7.

107. Fiel RJ, Howard JC, Mark EH, Datta Gupta N. Interaction of DNA with a porphyrin ligand: evidence for intercalation. Nucleic Acids Res 1979 Jul 11;6(9):3093-118.

108. Merchat M, Bertolini G, Giacomini P, Villanueva A, Jori G. Meso-substituted cationic porphyrins as efficient photosensitizers of gram-positive and gram-negative bacteria. J. Photochem. Photobiol. B, Biology 1996;32(3):153-7.

109. Merchat M, Spikes JD, Bertoloni G, Jori G. Studies on the mechanism of bacteria photosensitization by meso-substituted cationic porphyrins. J. Photochem. Photobiol. B, Biology 1996;35(3):149-57.

110. Deuticke B, Henseleit U, Haest CW, Heller KB, Dubbelman TM. Enhancement of transbilayer mobility of a membrane lipid probe accompanies formation of membrane leaks during photodynamic treatment of erythrocytes. Biochim. Biophys. acta 1989;982(1):53-61.

111. Sato Y, Kamo S, Takahashi T, Suzuki Y. Mechanism of free radical-induced hemolysis of human erythrocytes: hemolysis by water-soluble radical initiator. Biochemistry 1995;34(28):8940-9.