Pathogen inactivation of blood components, impact on plasma and platelet function

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Pathogen inactivation technologies are photochemical treatments developed to decrease transfusion transmitted infections. However, the impact of pathogen inactivation technologies on the blood components themselves is not entirely clear. Therefore, we investigated the quality of blood components following pathogen inactivation. First, the impact of three different pathogen inactivation technologies on plasma was compared. The different methods all negatively affected ADAMTS13 activity and antigen level, but to different degrees. The pathogen inactivation technology using riboflavin as a photosensitizer had the largest effect. This effect was caused by reactive oxygen because removal of dissolved molecular oxygen prevented protein damage to occur. Next, we investigated the influence of three different pathogen inactivation technologies on platelet concentrates. For this, platelet function was assessed in microfluidic flow chamber experiments. These indicated a decreased platelet function compared to untreated controls for all pathogen inactivation technologies. Additional experiments showed that the underlying mechanisms of platelet damage were different for every pathogen inactivation technology, but all three resulted in similar thrombus formation deficiencies in flow chambers. We focused on one particular pathogen inactivation technology which combines the photosensitizer amotosalen (a psoralen) and UVA light (PUVA). The data showed a specific inhibition of the phosphatidylinositol 3-kinase signal transduction pathway caused by covalent binding of amotosalen to phospholipids during photoactivation. As the combination of a psoralen with UVA light is clinically used off-label for graft-versus-host disease treatment, phosphatidylinositol 3-kinase signal transduction in T lymphocytes of patient samples was studied and also here inhibition of phosphatidylinositol 3-kinase signal transduction was found. To conclude, research that initiated from the observation that platelet and plasma function is decreased following pathogen inactivation technologies has revealed an overall effect of PUVA on cellular phosphatidylinositol 3-kinase signal transduction by covalent modification of phospholipids.

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Introduction

In 2014, 372,759 successful donations were collected from 185,000 donors in Flanders. Before a candidate can donate blood, stringent donor selection criteria need to be fulfilled. This increases the safety of the donors, but also that of recipient patients and serves as a first-line tool to prevent transmission of disease.[1] Complementary to this, a vigilant screening of the

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blood in the laboratory needs to reduce the risk of transfusion transmitted infections (TTI) as much as possible. Despite these efforts, there is a small residual risk for viral and bacterial TTI. With the implementation of pathogen inactivation technologies (PIT) of blood components, blood banks want to reduce the residual risk further, in order to achieve current state-of-the-art product safety levels. In this regard, the most recent haemovigilance data of Belgium found neither bacterial nor viral transmission after transfusion with more than 150,000 pathogen inactivated platelet concentrates in a time period of five years. On the other hand, there were four bacterial TTI's after transfusion with approximately 186,000 untreated platelet concentrates during the same time period. Despite this improvement of product safety, off-target effects of PIT on blood components should be investigated. A recent meta-analysis found a significantly shorter transfusion interval and a 7% increase in platelet transfusions for PIT treated concentrates compared to untreated.[2] Therefore, our research group investigated the biochemical and functional impact of several PIT on plasma products and platelet concentrates.

Pathogen inactivation of plasma

Since 1991, the solvent/detergent treatment of pooled plasma products is licensed in Europe. It efficiently kills enveloped viruses.[3] More recently, several bench-top PIT have been developed to treat single plasma products using short wavelength light in combination with a photosensitizer. These show effectiveness against non-enveloped viruses as well. Three available methods are MIRASOL Riboflavin (vitamin B2) pathogen reduction technology with broad spectrum UV light, INTERCEPT amotosalen (S59) photochemical treatment with UV-A light and THERAFLEX Methylene Blue Technology with visible light.[4-6] Previous work of our team investigated the integrity of plasma proteins before and after PIT.[7] Although the requirements of the Council of Europe for PIT treated plasma were met, a significant decrease of several important plasma proteins was found following each PIT. Subsequently, we investigated the molecular mechanism causing this decrease. Our data showed that reactive oxygen species have a significant role in the modification of plasma proteins because protein carbonyls, which form as an end product of oxidative chemistry, were significantly increased after PIT. Moreover, our data showed that this oxidative damage could be prevented by lowering dissolved molecular oxygen and so prevent molecular damage to crucial labile factors FVIII, fibrinogen and ADAMTS13.[8] Whether these oxidative changes to plasma and coagulation factors are clinically significant for the treatment of factor deficiencies in thrombotic thrombocytopenic purpura or haemophilia's needs to be addressed by appropriate clinical studies.

Pathogen inactivation of platelets

The MIRASOL and INTERCEPT methods were originally developed for the treatment of platelet concentrates. A third method, THERAFLEX UV-Platelets applies short-wavelength UV-C light without exogenously added photosensitizer. The impact of all three PIT on platelet function in concentrates for transfusion was studied.

A microfluidic flow chamber experiment was used following the recommendations of the International Society on Thrombosis and Haemostasis Scientific and Standardisation subcommittee on Biorheology.[9,10] This method mimics platelet transfusion and models haemostasis in real-time using fluorescence video microscopy.[11] All three methods caused decreased platelet function in this model compared to untreated paired controls. This finding was confirmed by platelet aggregometry, metabolic analysis and flow cytometry. These assays indicated that the underlying biochemical mechanisms of platelet damage were different between PIT methods. For instance, the MIRASOL treated platelets had increased anaerobic metabolic respiration, continuous degranulation and increased phosphatidylserine exposure rates pointing to accelerated storage lesion.[12] The THERAFLEX UV-C treated platelets had an immediate but storage-independent conformational activation of integrin αIIbβ3 indistinguishable of physiologic receptor activation. Increased storage lesion like in MIRASOL treated platelets was however not found.[13] Finally, INTERCEPT treatment had a more targeted effect, specifically on platelet signal transduction. Integrin activation was affected in response to some, but not all, platelet agonists.[12] Targeted analysis of individual signal transduction pathway sections demonstrated attenuation of PI3K signalling by reduced phosphorylation of major effector proteins like Akt (formerly Protein Kinase B) and Bruton's tyrosine kinase (Btk). In addition, the INTERCEPT photosensitizer amotosalen photochemically formed covalent adducts with (phospho)lipids preventing efficient binding of membrane specific peptides. This suggests that lipid-lipid and lipid-protein interactions are compromised in amotosalen modified membranes. Therefore, we hypothesised that the binding of Akt and Btk to the

Key messages for clinical practice

1. **Pathogen inactivation of blood components is an important additional tool to tackle the problem of transfusion transmitted infections, but significantly affects the quality of these products.**
2. **Specifically for MIRASOL treatment of plasma, the generation of reactive oxygen species that adversely affect biomolecular integrity of relevant plasma constituents, can be bypassed by applying hypoxic conditions during the pathogen inactivation process.**
3. **Pathogen inactivation technologies of platelet concentrates significantly attenuates thrombus formation kinetics in a flow chamber model of haemostasis but by different underlying biochemical mechanisms.**
4. **Unsaturations in fatty acyl chains of phospholipids in the plasma membrane co-determine kinase-lipid binding and are selectively inhibited by a photochemical treatment combining a psoralen with UVA light in platelets, but also in other cells, like T lymphocytes.**

plasma membrane was affected. Indeed, following INTERCEPT treatment subcellular localisation of Akt and Btk to the plasma membrane was impaired, despite normal formation of PI(3,4,5)P_3 by PI3K. Our data show that acyl chains of vicinal phospholipids cooperate successful binding of Akt and Btk to PI(3,4,5)P_3 and that interference of amotosalen with this process underlies poor transduction of activation signals. This causes decreased integrin activation and we hypothesise that this is the reason for poor haemostasis in our flow chamber model. However, it is not clear if this observed platelet function decrease *in vitro* sustains *in vivo* or whether platelet function can recover once transfused to the patient. Clinical trials have shown that INTERCEPT treated platelets are cleared from circulation faster and this may reflect their decreased function. None of the trials was powered to assess clinically significant bleeding, and this remains an unresolved issue.

Similar to INTERCEPT, the combination of psoralen and UVA light (PUVA) is used to treat disorders like cutaneous T-cell lymphoma and graft-versus-host disease (GvHD). To extend our observations of PUVA on platelets, we investigated T lymphocyte signalling in GvHD patient samples. The data showed significant inhibition of Akt activation and thereby confirmed that PUVA treatment specifically but selectively inhibits the PI3K pathway. These results can help understand or even improve the mechanism of action of all PUVA treatments including this leukapheresis-based procedure.

Conclusions

Pathogen inactivation of blood components is a solid next step to eliminate the threat of transfusion transmittable disease in the blood supply. However, a well-balanced interplay of different aspects is needed to successfully implement these worldwide. First, a certain grade of effectiveness needs to be reached. This is the maximal log reductions of pathogens as well as the integrity of the whole production process and its materials. Second, off-target effects of PIT and the consequent loss of product quality needs to be taken seriously. For instance, the reduced component potency by dilution and loss of activity after PIT could limit their effectiveness in resuscitation of massive hemorrhage.[14] Additional clinical studies with well-defined research questions are needed. Finally, it is important that all the costs and their benefits for implementation and production are weighted. However, significant decreases in the quality of the blood components and the underlying molecular mechanisms show that there is room for improvement and second generation PI technologies with less side-effects on cells and proteins.

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