P-071 **Hemocompatible polyelectrolyte microparticles loaded with synthetic glycoconjugates as novel immunosorbents**

Markvicheva E.[1#*], Selina O.,[1] Bartkowiak A.,[2] Bovin N.[1] and Grandfils Ch.[3]
[1] Shemyakin-Ovchinnikov Inst of Bioorganic Chem, R A S, Moscow, Russia, [2] West Pomeranian Univ of Technology, Szczecin, Poland, [3] CEIB, University of Liege, Belgium
email : lemarkv@hotmail.com

INTRODUCTION AND OBJECTIVES

The gap between the number of patients waiting for organ transplantation and the availability of suitable donor organs is very significant (US OPTN, 2006). Removal of blood group anti-A and anti-B antibodies can prevent hyperacute organ rejection in ABO-incompatible transplantation. Antibody-removing treatment, including plasma exchange and column immunoadsorption with antibody specific antigen covalently linked to solid microbeads are used in pre- and post-transplant periods to avoid hyperacute organ rejection. However, similar to other common techniques, complicated procedure of plasma separation is required before to eliminate the specific antibody by the adsorption column. The paper describes hemocompatible alginate-polycation (chitosan, DEAE-dextran etc) microparticles loaded with synthetic A and B antigens, namely Atri-PAA and Bdi-PAA glycoconjugates which could be used to directly remove ABO blood group antibodies from whole blood.

MATERIALS AND METHODS

Chemicals. Sodium alginate (Alg) (medium viscosity, was from Sigma, and polycations, namely DEAE-dextran (500 kDa, PKS, Germany), oligochitosan (Chit), 3.5 kDa, DD 89 %, modified oligchitosan in the form of quaternary ammonium base (Chit M), 6.5 kDA, DD 87%, substitution degree 0.65, were kindly provided by Prof. Bartkowiak. Synthetic antigens A-trisaccharide (Atri) and B-disaccharide (Bdi) were previously covalently attached to PAA to obtain glycoconjugates A_{tri}–PAA and B_{di}–PAA (MM ~ 2000 кДа), relatively, as described earlier (Shilova et al 2005).

Entrapment of glycoconjugates in Alg-polycation microparticles was carried out as previously described (Selina 2008). To increase microparticle mechanical stability in some cases Alg-polycation microparticles were coated with an additional Alg upper layer.

Study of antibody binding capacity of microparticles. Aliquots of microparticles or Sepharose (50 µl slurry) were incubated with antibody solution (1:10 v/v) for 1 h and 24 hs, and sorption was carried out at 24 и 37° C. Two types of antibodies were used : anti–Bdi (IgG + IgM, 254 µg/ml) isolated from human serum (Khraltsova L. et al. 2000), and anti-Atri (IgM) mouse antibody from ascitic liquid (Hematolog, Russia). The sorption efficacy of IgG and IgM antibodies was tested by ELISA as reported earlier (Selina et al 2008).

Study of microparticle hemocompatibility. Six samples of microparticles based on Alg and various polycations were studied. The hemocompatibility tests were carried out after 15 min incubation of normal human blood with microparticles (50 mg/mL) in sterile conditions at 37°C. Blood hemoreactivity was tested according to ISO10993-4 by: 1) optical microscopy observation of the whole blood (smear) after incubation with microparticles; 2) study of platelet and red blood cell size distribution (Coulter Counter); 3) evaluation of hemolysis according to ASTM (F 756-00) using Drabkin's method; 4) complement activation by the alternative pathway assessed by C3a protein fragment activation (ELISA kit, Beckton Dickinson).

RESULTS AND DISCUSSION

Specific elimination of pathogenic proteins from blood flow is a serious challenge, especially when the protein should be removed from whole blood, without previous plasma separation. Sorbent/device should be hemocompatible. Rydberg et al (Rydberg 2004) showed specific removal of anti-A/B antibodies using ABO immunosorbent with synthetic carbohydrates linked to Sepharose matrix. Nevertheless this technique as well as other presently used require plasma separation from blood cells before to remove antibodies by the immunosorption column. We suggested alginate-chitosan microparticles to be hemocompatible, and therefore they could be proposed for antibody removal directly from whole blood. Earlier we demonstrated the ability of these microparticles to eliminate both IgG and IgM antibodies from serum (Bovin 2009). We also showed that antibody binding capacity of the microparticles was comparable to Sepharose one and can be enhanced with increasing glycoconjugate quantity entrapped. However, the question of microparticle hemocompatibility has not been obvious.

Table 1. A list of microparticle samples studied.

Sample	Polymer composition
1	Alg/Chit
2	Alg/Chit M
3	Alg/DEAE dextran
4	Alg/Chit/Alg
5	Alg/Chit M
6	Alg/DEAE dextran/Alg

In this research we evaluated hemocompatibility of a set of Alg-polycation microparticles (Table 1). Microscopic observation of whole blood after incubation with all microparticle samples did not reveal any changes in cell morphology. There was no alteration in platelets and red blood cells (RBC) size distribution (Fig.1).

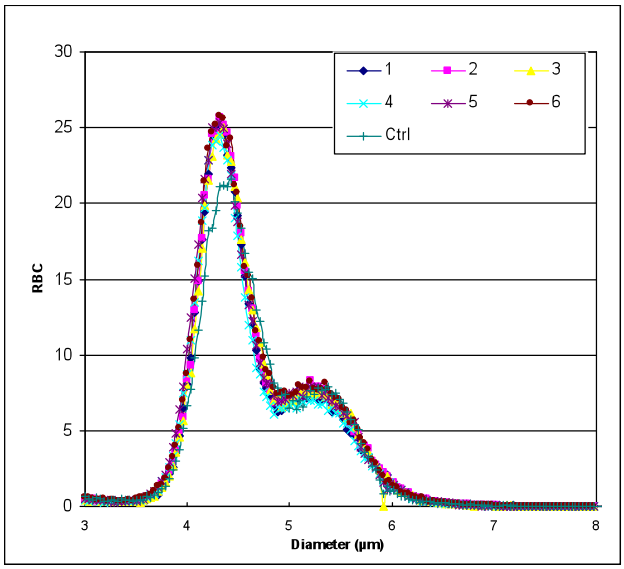

Fig. 1. Red Blood Cell size distribution in whole blood samples after 30 min incubation with microparticles.

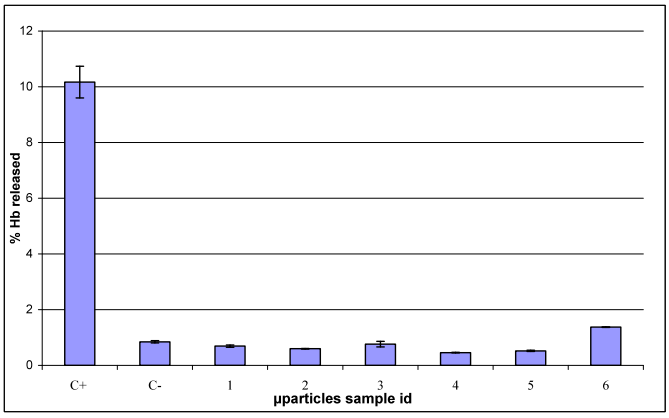

Fig. 2. The hemoglobin release from RBC after whole blood incubation with microparticles. C- is negative control (whole blood+PBS) when RBC damage does not occur. Positive control (C^+) is whole blood mixed with saponin solution (0.2%).

As seen from Fig.2, the highest hemoglobin released level (for sample 6) did not exceed 1.3% while according to ASTM, a hemolysis % inferior to 2 % is considered as non-hemolytic.

To estimate microparticles ability to activate blood complement system through an alternative pathway, C3a protein fragment concentration was tested in the whole blood after incubation with microparticles. The C3a concentration in the whole blood patterns was 1300-1800 ng/ml for all 6 microparticle samples (close to the negative control) while for the positive control C3a concentration was 9000 ng/ml (Fig.3). The positive control relies upon the incubation of Zymozan (polysaccharide suspension from *Saccharomyces cerevisiae*s known to activate blood complement system) with the whole blood.

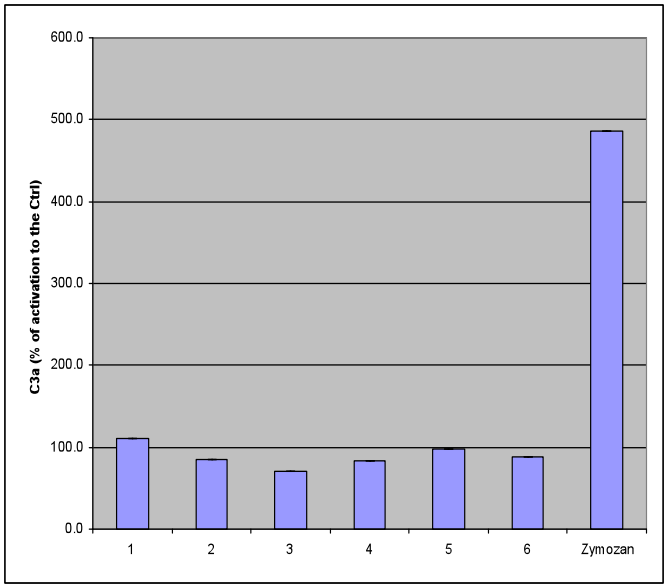

Fig. 3. Concentration of C3a in the whole blood samples, after 15 min incubation with microparticles.

CONCLUSION

The prescreening hemocompatibility tests demonstrated that all Alg-polycation microparticles were hemocompatible and can be considered as good candidates for development of novel immunosorbents to remove antibodies directly from whole blood without previous plasma separation. These tests could be extended at least to the control of the coagulation pathways activation, i.e. intrinsic or extrinsic routes.

REFERENCES

Bovin N., Markvicheva E., Selina O. (2009). *Sorbent for removal of antibodies from the whole blood and the technique for its preparation* Russian patent RU 2360707.

Khraltsova L. et al. (2000) *An enzyme-linked lectin assay for alpha1,3-galactosyltransferase.* Analytical Biochemistry, 280, 250-257.

Shilova N. V., et al. (2005) *High molecular weight neoglycoconjugates for solid phase assays.* Glycoconjugate J., 22 , 43- 51.

Selina O et al. (2008) *Alginate-chitosan microspheres for the specific sorption of antibodies,* Rus J Bioorg Chem, 34, 4, 522-529.

Rydberg I et al (2004) *In vitro assessment of a new ABO immunosorbent with synthetic carbohydrates attached to Sepharose*. Transpl Int, 17, 666-672.