COMPLEMENT ACTIVATION BY ACTIVATED CARBON ADSORBENTS

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Introduction

Acute renal failure (ARF) is a condition that affects up to 20% of patients on intensive care units (ICU). Although the most common causes of ARF are sepsis and septic shock [1], acute loss of renal function may also occur following surgery or as a result of trauma, burns or the use of nephrotoxic drugs [2]. Conventional treatment of ARF includes drug therapy in combination with extracorporeal renal replacement therapies such as continuous haemodialysis and haemofiltration. Such extracorporeal therapy consists of a circuit containing a filter through which blood is passed for the removal of low molecular weight uraemic toxins. The retained components of the blood, such as cells and proteins, are rehydrated with isotonic replacement solutions before being returned to the body [3,4,5]. A combination of the conventional filtration-based therapies with adsorptive technology may allow more efficient removal of molecules and complexes that are unable to pass through conventional membranes. However, contact between blood and foreign surfaces such as carbon adsorbents may result in further activation of the body's defence systems. One important component of this defence mechanism is the complement cascade which in turn leads to secondary inflammatory events [6]. The haemoperfusion columns currently available use adsorbents coated with a semi-permeable layer of polymer such as cellulose to improve their biocompatibility. However this coating strongly reduces the adsorptive properties of the material for molecules in the high molecular weight and middle molecule range as it acts as a barrier for the free diffusion of molecules to the carbons [7]. It is clear, therefore, that the development of bio/haemocompatible uncoated adsorbents, which show superior adsorption capacity for a range of molecules, would significantly improve the capabilities of current renal replacement therapies.

This study compares the propensity of a cellulose-coated commercially available adsorbent with three uncoated carbon adsorbents to activate the complement system. An increase in complement would indicate the initiation of an inflammatory response to the carbons. An enzyme immunoassay was used to detect the complement fragment iC3b, an inactivated form of C3 that forms part of both the classical and the alternative complement activation pathways.

Experimental

Preparation of platelet poor plasma

30 ml of blood drawn from healthy donors was added to tubes containing 15 mg of heparin (Sigma, Dorset, UK). Platelet poor plasma (ppp) was prepared by centrifugation of the blood at 2000 g for 15 minutes at room temperature (Multifuge 3S, Heraeus, Germany).

Carbon adsorbents

The following carbon adsorbents were used in this study: SCN activated carbon (produced by pyrolysis of vinyl pyridine-divinyl benzene copolymer, Academy of Science, Ukraine), Adsorba® 300C (a commercially available, cellulose coated Norit RBX1 peat carbon adsorbent, Gambro Dialysatoren GmbH & Co. KG, Hechingen, Germany), MAST 00C and MAST 60C (non-activated [0% burn-off] and CO_2 activated [60% burn off] respectively, phenol formaldehyde resin based pyrolysed carbons, both produced by MAST Carbons Ltd., Surrey, UK). The carbons were sterilized by autoclaving prior to use. The carbon samples (0.1 ml) were placed in 0.5 ml sterile phosphate buffered saline [PBS] (Dulbecco A, Oxoid, Basingstoke, U.K) for approximately 24 hours (room temperature) before the complement activation assay was performed. Microcentrifuge tube plastic (polypropylene) was included as a negative control.

Preparation of Zymosan A (positive control)

Zymosan A (Sigma, Dorset, UK) was used as a positive control for complement activation. It consists of protein-carbohydrate complexes prepared from the cell wall of the yeast *Saccharomyces cerevisiae*. Particles (10 mg) were suspended in 1 ml of PBS and boiled for 15 minutes. The particles were washed and finally re-suspended in 10 ml PBS. Aliquots (0.05 mg) were frozen at –20ºC until required. Defrosted samples were centrifuged at 4500 rpm (Biofuge pico, Heraeus, Germany) for 8 minutes to pellet the particles.

Exposure of platelet-poor plasma to samples

Exposure of samples to platelet-poor plasma was carried out in sterile microcentrifuge tubes. A 0.25 ml volume of PBS was removed from the carbon samples and the controls and an addition of 0.25 ml ppp made, giving a final volume of 0.5 ml of 50% ppp. The samples were incubated in the presence of 50% ppp at 37ºC/5% CO_2 for 30 minutes.

Assessment of iC3b complement fraction in platelet-poor plasma

The iC3b complement fraction was assayed using an enzyme immunoassay (Quidel®, San Diego, USA). The microplate assay consists of a three-step process. The iC3b fraction present in the sample binds to monoclonal anti-human iC3b bound to the assay plate. The antibody is specific and does not bind to other C3 or C3b fragments. Addition of HRP-conjugated goat anti-human iC3b binds to the attached iC3b. Finally a chromogenic substrate is added, which subsequently reacts with the HRP-conjugate resulting in the formation of a green colour, the intensity of which is proportional to iC3b

present in the samples. All reagents and standards were prepared as per instructions. Following incubation the samples were centrifuged briefly (2 minutes at 4500 rpm, room temperature). Aliquots of 50% ppp were removed and diluted with specimen diluent (1:40 for adsorbent samples and negative control, 1:100 for Zymosan positive control). Duplicate wells were prepared for each standard and sample. The assay was performed as per the instructions supplied with the assay. After colour development, optical absorbances were read at 405 nm using a spectrophotometric plate reader (Titertex® Multiskan, Labsystems, Finland). The assay was performed on blood obtained from 3 different donors and one sample of each adsorbent type tested on each occasion.

Results and Discussion

Results for iC3b fraction in the fluid phase have been calculated to represent undiluted plasma and are given in Figure 1. Statistical analysis (2-way ANOVA) demonstrated that there was no significant variation between donors. Plastic (control) and the adsorbents SCN, MAST 00C and MAST 60C induced the production of low levels of iC3b (< 11 $\mu g\ ml^{-1}$) in platelet poor plasma following a 30 minute exposure period. Results for the commercially available, cellulose-coated adsorbent Adsorba 300C (mean value 15.5 $\mu g\ ml^{-1}$) were significantly higher than for the uncoated adsorbents and the plastic control ($p<0.05$, Tukey's pairwise comparison).

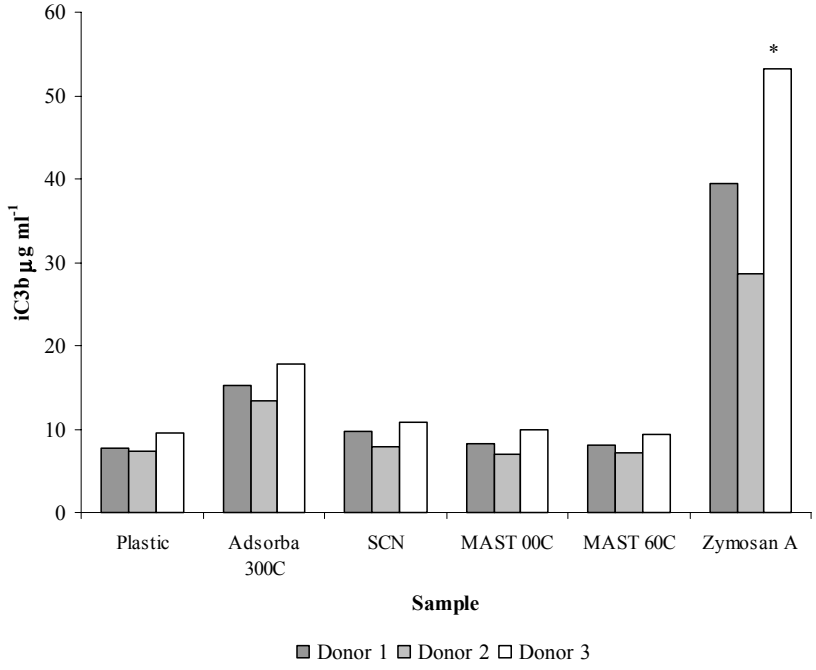

Figure 1. Levels of iC3b complement fraction present in the fluid phase following a 30 minute contact time with test materials (each result is the average of duplicate wells [* = value/2]). Results are expressed for undiluted plasma.

From the preliminary results presented, SCN and MAST carbon adsorbents were found to elicit an iC3b complement response similar to that induced by the plastic control and compared favourably with the commercially available coated adsorbent Adsorba® 300C. These results are consistent with previous studies that have suggested that pyrolytic carbon coatings may improve whole blood compatibility of a medical polymer polyethylene terephthalate (PET) by limiting complement activation [8]. The opportunity to use uncoated carbon adsorbents in extracorporeal devices, without stimulating an excessive immune response, is highly desirable. These materials show improved adsorption characteristics for a wider range of molecules as access to the adsorbent surface is unrestricted by a semi-permeable membrane. iC3b fragment may also be bound to the adsorbent surface due to the nature of the material. Protein adhesion data would, therefore, be useful to support the data presented in this study. It is also known that the iC3b complement fragment is produced as a result of C3b inactivation (by Factor I with cofactor H) which is formed through the splitting of C3 into C3a and C3b. The possibility must also be considered, therefore, that some C3b which is not inactivated may go on to participate in further activation of the cascade to the terminal sequence. Analysis of complement fragments representing this portion of the cascade may also be beneficial.

Conclusion

The results obtained in this study suggest that SCN and MAST carbons 00C and 60 C may be suitable candidates for extracorporeal adsorptive therapies requiring direct blood contact.

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References

[1] Wan L, Bellomo R, Di Giantomasso D, Ronco C. The pathogenesis of septic acute renal failure. Current Opinion in Critical Care 2003; 9; 496-502.
[2] Gabriel, R. Acute Renal Failure. *In* Renal Medicine, third edition, pp 180-193. Baillière Tindall, London, 1988.
[3] Mikhalovsky, SV. Microparticles for hemoperfusion and extracorporeal therapy. *In* Microspheres and Microcapsules and Liposomes, Volume 2, pp 133-163. Ashady, R (editor). Citrus books, London, 1999.
[4] Ronco, C, Bellomo, R. Basic mechanisms and definitions for continuous renal replacement therapies. The International Journal of Artificial Organs 1996; 19 (2); 95-99.
[5] Flynn, C G M. A review of continuous renal replacement therapy. Irish Journal of Medical Science 1994; 163 (7); 331-340.
[6] Kazatchkine, M D, Carreno, M P. Activation of the complement system at the interface between blood and artificial surfaces. Biomaterials 1988;9.
[7] Mikhalovsky, SV. Emerging technologies in extracorporeal treatment: focus on adsorption. Perfusion 2003; 18: 47-54.

[8] Cenni E, Granchi D, Ciapetti G, Stea S, Verri E, Gamberini S, Gori A, Pizzoferrato A, Zucchelli P. *In vitro* complement activation after contact with pyrolytic carbon-coated and uncoated polyethylene terephthalate. Journal of Materials Science: Materials in Medicine 1997;8:771-774.