ASSESSMENT OF INFLAMMATORY CYTOKINE REMOVAL BY A NOVEL ACTIVATED CARBON DEVICE IN A FLOWING SYSTEM

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INTRODUCTION
Systemic inflammatory response syndrome (SIRS) is a complex syndrome involving disruption of the controlled inflammatory processes which normally maintain haemostasis. It can be a costly complication for both patient and healthcare provider, with over 50% of all ICU patients affected, and is associated with high morbidity and mortality rates despite improved supportive therapies (Robertson et al, 2006). Individual initiators of the inflammatory response have been targeted in the search for effective treatment strategies. However, despite success in animal trials, single target therapies have failed to produce significant clinical benefit possibly because they fail to impact enough of the complex mechanisms which contribute to SIRS (Oda et al, 2004). In light of these findings extracorporeal therapies involving haemoadsorption of inflammatory mediators have been investigated as a broader approach to the treatment of SIRS.

The current study utilizes a novel uncoated, polymer pyrolysed AC module developed specifically for the removal of cytokines and toxins associated with SIRS under technology patented to MAST Carbon. Haemocompatibility and adsorptive capacity for endotoxin and cytokines have been shown in a static system (Sandeman et al, 2005; Howell et al, 2006). The current study investigated cytokine removal from plasma by the device in a flowing system more closely reflecting its clinical application. Any propensity to exacerbate the pro-coagulant state observed in SIRS by excessive removal of plasma proteins or clotting factors or by direct activation of the intrinsic coagulation pathway was also examined.

EXPERIMENTAL

Synthesis
Novel, synthetic pyrolysed carbons were produced with defined porous structure from a number of synthetic organic co-polymers and shaped into three dimensional carbon tubes by MAST Carbon Technologies using patented monolithic carbon technology (patent PCT/GB02/01142).
**Porosimetry**

The porous structure of the activated carbon modules was investigated using an Autosorb-1 micropore gas porosimeter and a PoreMaster mercury porosimeter (Quantachrome Instruments, UK).

**Scanning Electron Microscopy (SEM)**

Fractured transverse samples of the carbon monoliths were viewed on an EVO series scanning microscope (Carl Zeiss SMT Ltd. UK).

**Cytokine adsorption**

The flow modules were primed with sterile phosphate buffered saline (PBS) at a flow rate of 1 ml min⁻¹. A 40 ml volume of fresh frozen human plasma (National Blood Service) was spiked with the recombinant human cytokines TNF, IL-6, IL-8 and IL-1β (BD biosciences) at a concentration of 1000, 2000, 500 and 1000 pg ml⁻¹ respectively. The spiked filtrate was pumped through the module at a flow rate of 500 μl min⁻¹. Samples of filtrate, which passed through the carbon tube and samples of retentate, which passed along the outside of the tube were collected at 10 minute intervals during each cycle. Filtrate and retentate were recombined and pumped back through the module in a second cycle. Retentate and filtrate samples were collected as before. Samples were tested for cytokine concentration by ELISA according to manufacturer’s instructions (Pharminogen).

**Clotting factor adsorption and total plasma protein adsorption**

Four different coagulation assays testing fibrinogen concentration, thrombin (T), prothrombin (PT) and activated partial thromboplastin (APPT) clotting time were used to assess differences in filtrate and retentate plasma clotting characteristics. Cytokine spiked and non-spiked plasma control samples were included in addition to the assay kit normal and abnormal standard controls. The assays were run on a START 4 coagulometer according to manufacturer’s instructions (Diagnostica Stago).

Filtrate and retentate plasma samples were analysed for total plasma protein content using a standard high concentration-sensitivity DC protein assay (BioRad). Results were analysed for statistical significance using Student’s t-test.

**RESULTS**

**Porosimetry**

The mercury pore size distribution plot for the mesoporous flow module clearly shows a mesoporous component in the 10 to 50 nm range and the presence of macropores above 500 nm diameter (figure 1).
Figure 1. A mercury pore size distribution plot showing the presence of mesoporous and macroporous domains within the mesoporous test module and the absence of mesopores in the control module.

SEM

The non-mesoporous carbon module had a more open structure with porous channels of 10 to 30 μm observed. The structure of the mesoporous carbon module was much more compact with the majority of pores visualized at low resolution smaller than 10 μm. At high resolution a large number of mesopores were observed in the 20 to 50 nm range in the mesoporous test module.

Cytokine adsorption

The percentage of cytokines TNF, IL-6, IL-8 and IL-1beta removed from spiked plasma by filtration through the mesoporous carbon flow modules over time is shown in figure 4a. Initially the module removed 90-100% of each of the cytokines measured. IL-8 and IL-1beta removal remained consistently high over...
the 120 minute time course of the experiment. IL-6 removal remained high but dropped slightly to 80% by the end of the 120 minute flow cycle. TNF adsorption dropped significantly with plasma filtration time so that, by the end of the 120 minute cycle, very little removal was observed. Removal of the cytokines TNF, IL-8 and IL-1beta by the non-mesoporous module was negligible.

**Figure 3** Percentage removal of the cytokines TNF, IL-6, IL-8 and IL-1β from spiked human plasma filtered through mesoporous carbon modules over time (mean +/- SEM, n=3)

**Total plasma protein adsorption**

Plasma filtration through the mesoporous carbon flow modules did not alter the total plasma protein concentration. No significant difference in filtrate versus retentate plasma protein concentration was observed (p>0.05). The mean filtrate total plasma protein concentration was 87.7 +/- 5.35 mg/ml throughout the time course of the experiment compared with 85.4 +/- 2.1 mg/ml for the retentate samples.

**Clotting factor adsorption**

The fibrinogen concentration in the initial mesoporous flow module filtrate samples was significantly lower than that in retentate samples at 1.6 g/l compared to 2.6 g/l but was above the abnormal fibrinogen concentration range (0.75 to 1.25 g/l) (p<0.05). However, fibrinogen levels returned to those of the retentate samples by the end of each experiment (figure 4). The initial thrombin and pro-thrombin filtrate clotting times were raised initially but dropped to that of the retentate samples so that over the time course of the experiment no significant difference was found (n=3, p>0.05). The APPT filtrate clotting times were higher than that of the retentate samples for a more prolonged time but returned to retentate levels by the 100 minute sample time point (n=3, 0.01<p<0.05). There was no significant difference in filtrate and retentate fibrinogen concentration or thrombin, prothrombin and APPT clotting times for the non-mesoporous controls (data not shown).
Figure 4 Measurement of fibrinogen concentration of the mesoporous filtrate, retentate and control plasma samples showed initially lower levels of fibrinogen in filtrate samples which returned to normal levels towards the end of each experiment (mean +/- SEM, n=3)

**CONCLUSIONS**

A mesoporous, activated carbon device has been developed with the capacity to remove cytokines TNF, IL-6, IL8 and IL1-β from human plasma in a flowing model system. It did not exacerbate the tendency of plasma to clot or alter total plasma protein levels and holds potential as an extracorporeal adsorbent therapy for the removal of cytokines central to the progression of SIRS. The device makes a significant contribution to the use of adsorbents for SIRS treatment since it is a comparably inert system in which porosity can be manipulated to promote the removal of inflammatory mediators.

**REFERENCES**


