A Novel Biomaterial for the Targeted Removal of Advanced Glycation End Products from Blood

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Introduction

Vascular complications in patients with chronic kidney failure lead to an increased risk of mortality. Elevated serum concentrations of advanced glycation end products (AGEs) have been implicated in the inflammatory response that leads to vascular dysfunction in renal failure patients. The receptor for advanced glycation end products (RAGE) is the primary receptor for AGEs, and RAGE activation leads to an NFKb-induced inflammatory response. As a strategy to reduce the impact of AGEs on the vasculature, we developed a novel bioadsorbent to selectively remove AGEs from human blood.

Methods

Fabrication of the bioadsorbent: The soluble form of the receptor for advanced glycation end products (sRAGE) was covalently immobilized onto agarose beads (the bioadsorbent) via cyanogen bromide activation chemistry to create the bioadsorbent. *Small scale AGE removal studies:* Bioadsorbent or unmodified beads (control) were incubated with 1) pooled human serum from patients with chronic kidney disease (CKD) or healthy volunteers (NL); and 2) PBS spiked with glycoaldehyde-modified BSA (GBSA) or GBSA conjugated to horseradish peroxidase (GBSA-HRP). *Large scale kinetic AGE removal studies*: PBS (500 ml) spiked with GBSA or freshly isolated human whole blood was recirculated through a hollow fiber device loaded with bioadsorbent. Samples were collected at specific time points, and plasma was isolated for analysis. *AGE detection:* AGE removal was quantified by the Bradford Assay, competitive enzyme-linked immunosorbent assay (ELISA), and direct ELISA on the bioadsorbent or control.

Results

The bioadsorbent bound GBSA with high specificity. Only the bioadsorbent but not agarose beads (control) removed native AGEs from serum samples of healthy and renal failure patients, as quantified via a competitive enzyme-linked immunosorbent assay (ELISA) and a direct ELISA performed on the bioadsorbent (**Figure 1**). Furthermore, pre-incubation of bioadsorbent with NL or CKD-derived serum reduced the binding sites for GBSA-HRP on the bioadsorbent, confirming consumption of binding sites measured via direct ELISA. Finally, whole blood circulated through a hollow fiber device loaded with bioadsorbent showed rapid removal kinetics that support further testing of this approach in a clinical setting.

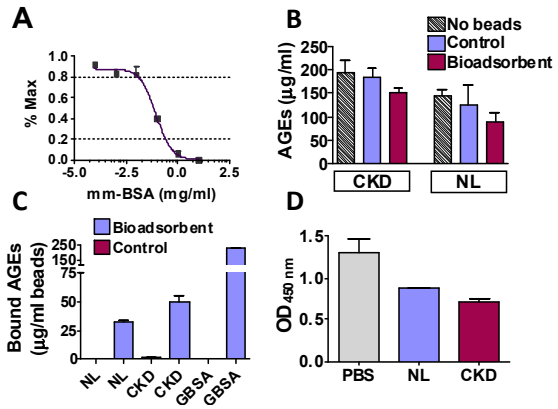

Figure 1. *Bioadsorbent selectively removed native AGEs from the serum of normal and kidney failure patients.* A) Standard curve of competitive ELISA. B) Competitive ELISA of serum samples after incubation with bioadsorbent. C) Direct ELISA on bioadsorbent after incubation with serum. D) Direct ELISA of GBSA-HRP bound to bioadsorbent.

Conclusions

The specificity of the bioadsorbent for native AGEs and rapid removal kinetics in the larger scale study suggest the potential for successful extracorporeal removal of AGEs in patients with renal failure. Future experiments will assess the ability of the bioadsorbent to inhibit native AGE-induced inflammatory response *in vitro*.

References

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