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Glucose-based dialysis fluids inhibit innate defense against *Staphylococcus aureus*

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ABSTRACT

Background: Staphylococcus aureus peritonitis is a serious complication of Chronic Peritoneal Dialysis (CPD) and associated with a higher risk for severe and recurrent infections compared with other bacteria. We have previously shown that complement-mediated effectors essential for optimal opsonophagocytosis of *S. aureus* are inhibited by high glucose concentrations. Since most commonly used peritoneal dialysis (PD) fluids are glucose-based, we hypothesized that glucose-based PD fluids likely inhibit complement host defenses against *S. aureus*.
Methods: Commercially available PD fluids were tested: glucose-based (Dianeal), Dianeal supplemented with amino acids, icodextrin-based (Extraneal) and amino acid-based (Nutrineal). Control PD fluid was generated to simulate Dianeal excluding the glucose. Three commercially available glucose concentrations were tested: Dianeal 1.5% (15gm/1000 ml), Dianeal 2.5% (25 gm/1000 ml) and Dianeal 4.25% (42.5 gm/1000 ml). Complement effectors against *S. aureus* were analyzed including opsonization with C3-fragments, anaphylatoxin generation, and phagocytosis efficiency. We also evaluated clinical strains, including MRSA strains, and specific complement activation pathways.
Results: Glucose-based PD fluids inhibited complement opsonization of *S. aureus* (≥7-fold reduction) and inhibited *S. aureus*-induced generation of anaphylatoxins C3a and C5a (>10-fold reduction) compared to non-glucose based PD fluids. Dianeal 1.5%, 2.5% and 4.25%, all similarly inhibited C3-mediated opsonization. Glucose-based PD fluids showed a ≥4-fold reduction in opsonization of clinical strains of *S.aureus*, including MRSA strains. Decreased opsonization of *S.aureus* in the glucose-based PD fluid compared with non-glucose based fluids correlated with decreased phagocytosis by neutrophils.
Conclusion: Complement-mediated opsonophagocytosis of *S. aureus* and anaphylatoxin generation were severely inhibited in glucose-based PD fluids compared with non-glucose-based PD fluids. By inhibiting complement host defenses, glucose-based PD fluids may increase the risk of and severity of *S. aureus* peritonitis for CPD patients using these fluids.

1. Introduction

As of 2009, almost 400,000 individuals in North America receive dialysis for chronic renal failure (Collins et al., 2012). Dialysis is widely used to manage end stage renal disease (ESRD) either by hemodialysis or peritoneal dialysis. Many patients choose peritoneal dialysis because it can be performed overnight allowing patients to maintain normal daytime activities and thus, improving lifestyle for many populations (Chaudhary et al., 2011; Furth et al., 2001). Hemodialysis poses significant risks for central venous line infections or intravascular infections with the vascular access being implicated in up to 73% of all bacteremia infections in these patients (Nassar and Ayus, 2001). Chronic Peritoneal Dialysis (CPD) increases the risk of peritonitis and is estimated to be 0.35 episodes/patient year (Kerschbaum et al., 2012). In spite of extensive efforts taken to prevent the infectious

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complications associated with peritoneal dialysis, it remains a major reason for discontinuation of peritoneal dialysis and switch to hemodialysis (Klaus, 2005; Piraino et al., 2011). *Staphylococcus aureus* is associated with more severe peritonitis than other bacterial etiologies and is associated with an increased risk of recurrent peritonitis (Leppanen et al., 2006; Szeto et al., 2011).

A major contributor to innate humoral host defenses is the complement system, which is comprised of about 30 proteins that act in an amplification cascade (Lambris JD and Wetsel, 1998). Complement plays critical roles in the immunogical control of *S. aureus* infections via opsonization and the generation of anaphylatoxins (Cunnion et al., 2003a; Lambris et al., 2008; Watts et al., 2005). Complement can be activated via classical, lectin or alternative pathways leading to enzymatic cleavage of the central molecule of the complement system, C3, with resultant opsonization with C3b and iC3b forms (Frank MM, 2001). The anaphylatoxins C3a and C5a are also generated leading to neutrophil recruitment and stimulating degranulation (van Kessel et al., 2014).

Hyperglycemia is well known for increasing the risk of bacterial infection in diabetic patients and this association has largely been attributed to inhibitory effects on neutrophil responses (de Souza Ferreira et al., 2012; Perner et al., 2003). Hyperglycemic conditions have also been associated with increased risk of *S. aureus* infection in humans (Yates et al., 2009) and correlates with impaired killing of *S.aureus* in an animal model (Rich and Lee, 2005). Previous investigators have identified very slow mechanisms by which elevated glucose environments adversely affect complement proteins. Non-enzymatic glycation of up to 20% of C3 molecules may occur over 48 h in hyperglycemic conditions (Austin et al., 1987). The binding of glucose to the biochemically active site of C3 decreases attachment to pathogens (Hostetter, 1990). Our laboratory has demonstrated a much more rapid mechanism whereby glucose added to human serum for less than 5 min dramatically alters C3 interaction with *S. aureus* (Hair et al., 2012a). In high glucose environments (> 6 mM), the central molecule C3 undergoes tertiary structural changes, unrelated to glycosylation, associated with strongly inhibited opsonization of *S. aureus* (Hair et al., 2012a). We have subsequently shown in a diabetic rat model of *S. aureus* peritonitis that complement-mediated host defenses against *S. aureus* were severely inhibited compared with euglycemic controls leading to more severe infection (Mauriello CT et al., 2014). These observations raised the question as to whether glucose-based peritoneal dialysis fluids would also inhibit complement-mediated host defenses against *S. aureus*, potentially contributing to the increased risk of *S. aureus* peritonitis for patients receiving CAPD. The most commonly used peritoneal dialysis (PD) fluids are glucose-based, containing extremely high concentrations of glucose. Dianeal is available in 1.5%, 2.5%, and 4.25% glucose concentrations which correlate with 83 mM, 130 mM and 236 mM glucose, respectively. To our knowledge, no human studies have been published that evaluate whether glucose-based peritoneal dialysis fluids are associated with a different risk of peritonitis compared with non-glucose-based peritoneal dialysis fluids. A clinical trial assessing risk of peritonitis conducted in Turkey was reported, however glucose was present in all of the dialysis fluids that subjects received (Duranay et al., 2007).

Here we report the impact of glucose-based PD fluids on complement-mediated host defenses against *S. aureus* in comparison to non-glucose-based PD fluids, which use amino acids or icodextrin to generate an osmotic gradient. We demonstrate that complement-mediated opsonophagocytosis of *S. aureus* and anaphylatoxin generation are significantly inhibited in glucose-based PD fluids, but not for amino acid- or icodextrin-based PD fluids.

2. Methods

2.1. Ethics statement

After written informed consent was provided, healthy human volunteers donated blood which was used to generate serum that was used as a reagent in the following studies. Eastern Virginia Medical School IRB approved this protocol: 02-06-EX 0216.

2.2. Bacteria

The *S. aureus* laboratory strain, Reynolds was grown overnight in 2% NaCl Columbia broth at 37 °C to post-log phase in all the experiments, unless otherwise stated. Clinical strains of *S. aureus*, 3 methicillin-resistant *S. aureus* (MRSA) and 3 Methicillin-susceptible *S. aureus* (MSSA), were obtained as discarded de-identified isolates from invasive human infections (EVMS IRB 06-04-WC-0040). Speciation and antibiotic susceptibility were performed using CLSI criteria and each strain had a distinctly different PFGE pattern (Hair et al., 2012a).

2.3. Peritoneal dialysis fluids

Icodextrin-based (Extraneal) PD fluid and glucose-based PD fluid (Dianeal PD-2 Peritoneal Dialysis Solution) in varying glucose concentrations: Dianeal 1.5% (PD-2 1.5% Dextrose), Dianeal 2.5% (PD-2 2.5% Dextrose), Dianeal 4.25% (PD-2 4.25% Dextrose), was obtained commercially. Dianeal 1.5 % supplemented with small amounts of amino acids for nutritional support was also obtained commercially (PENtech Infusion). Amino acid-based (Nutrineal) PD fluid was donated by Baxter International. As an additional control, glucose-free PD fluid was generated using the electrolyte composition of Dianeal, but excluding glucose. Another commercially available bicarbonate/lactate based peritoneal dialysis solution with a physiological pH is Physioneal® 40. We were not able to purchase this fluid for testing in our experiments so the composition was replicated to generate the following Physioneal -like PD fluid with and without glucose: Sodium 132 mmol/L, Calcium 1.25 mmol/L, Magnesium 0.25 mmol/L, Chloride 95 mmol/L, Lactate 15 mmol/L, Bicarbonate 25 mmol/L, glucose anhydrous 75.5 mmol/L.

2.4. Serum and complement components

Normal human serum (NHS) was prepared from human blood, pooled, and frozen in small aliquots,

as previously described (Cunnion et al., 2001). Pooled NHS was used as the source of functional complement in these studies. C4-depleted human serum (C4dpl) and purified C3, C3b and iC3b were purchased (Comp. Tech.) and tested for purity and functionality (Cunnion et al., 2004).

2.5. Complement activation assays

Unless otherwise noted, 1×10^9 bacteria were incubated with 5% NHS in each PD fluid for 60 min at 37 °C. Into 95 μl of PD was mixed 5 μl of NHS and then the solution was added to pelleted *S. aureus* to yield a 5% NHS PD fluid suspension. The PD fluids included glucose-based (Dianeal 1.5%), glucose-based with nutritional amino acids (Dianeal + AA), icodextrin-based (Extraneal), amino acid-based (Nutrineal), or control (glucose-free) with only electrolytes. After incubation the samples were sedimented and the supernatant was assayed for C5a and C3a, as described below. The pelleted *S. aureus* was washed with phosphate buffered saline (PBS) twice to remove non-covalently bound molecules.