In Vitro Study of Variables Relevant to Perioperative Care of the Surgical Patient: Glucose, Osmolarity, and Rewarming

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BACKGROUND: We sought to determine the effects of altering osmolarity and the reversibility of the detrimental immunologic effects of hypothermia on human monocyte HLA-DR surface expression and reactive oxygen species (ROS) formation.

STUDY DESIGN: The effects of altering osmolarity on HLA-DR surface expression and ROS formation were assessed using lipopolysaccharide (LPS)-treated samples treated with either saline, glucose, or mannitol, incubated at 37°C for 2 hours. HLA-DR surface receptor expression and ROS formation were determined after incubation. The effects of the reversibility of hypothermia were measured by incubating LPS-treated samples at 34°C, 37°C, and 40°C for 1 hour. The samples were subsequently rewarmed at 40°C for 1 and 2 hours. The effects of rewarming on HLA-DR surface receptor expression and ROS formation were reassessed.

RESULTS: In the osmolarity experiments, there was a 49% decrease in ROS formation in samples treated with mannitol as compared with saline and glucose. Alterations of osmolarity had no significant effect on HLA-DR surface expression. In the rewarming experiments, rewarming for either 1 or 2 hours abolished any significant differences in HLA-DR surface expression and ROS formation between samples preincubated at the different temperatures.

CONCLUSIONS: The presumed inert mannitol was found to significantly decrease ROS formation, but had no effect on HLA-DR surface expression. In addition, the effects of hypothermia on HLA-DR surface expression or ROS formation may be better reversed within 2 hours than in 1 hour. (J Am Coll Surg 2011;212:180–186. © 2011 by the American College of Surgeons)

Aspects of perioperative care remain at the forefront of clinical quality improvement initiatives. We have pursued several such leads at the laboratory bench, attempting to define a biologic basis to confirm some of these often clinically confusing observations. Development of surgical infection, despite the introduction of safer antibiotics and more timely administration, still confounds surgeons today. Consequently, the focus has shifted more toward optimizing innate host defense mechanisms in order to minimize the occurrence of infective sequelae in the surgical patient.[1] Temperature, oxygen, and glucose are factors involved in development of surgical infection by either promoting or attenuating the protective innate immune response.[2-5]

Tight glucose control in the surgical patient was thought to be clearly defined and well understood at the clinical and cellular levels; however, more recent reports on glucose control disagree and remain debatable.[6-8] Just as we are seeing the clinical acceptability of tight glucose control wax and wane, there is also some ambiguity about many related laboratory queries. With respect to glucose, we recently found in our in vitro model an impairment of the formation of reactive oxygen species (ROS) with increasing glucose concentrations.[9] Additionally, with increasing glucose levels, proinflammatory cytokines such as TNF-α were attenuated, and polymorphonuclear cell (PMN) phagocytosis was enhanced. However, monocyte HLA-DR, which has been frequently shown to possess prognostic value, surprisingly failed to demonstrate any differences with increasing glucose concentrations at the early stages of the inflammatory response seen in our model.[9]

An important part of our glucose studies over the past 5 years has been to dissect the impact of osmolarity induced

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Abbreviations and Acronyms

DHR = dihydrorhodamine
EDTA = ethylenediaminetetraacetic acid
LPS = lipopolysaccharide
MCF = mean channel fluorescence
PMA = phorbol myristate acetate
ROS = reactive oxygen species

by high glucose concentrations and secondarily reassess the relative biologic inertness of mannitol. In essence, how many of the adverse effects of hyperglycemia are due to increased osmolarity? We initially used mannitol as an "inert" osmotic agent. Clinically, mannitol is widely used for patients with cerebral edema resulting from closed head injuries in order to reduce intracranial pressure. Although mannitol has long been considered an inert osmotic diuretic, clarification of any previously overlooked biologic effects is important clinically for the ongoing use of mannitol.

Given the strong consensus of data that unintended hypothermia is harmful, one must ask whether and how rewarming can reverse the subcellular effects of hypothermia. The protective effect of fever has been widely accepted for several decades. Hypothermia has been associated with detrimental effects such as increased surgical site infection, prolonged length of hospital stay, and an even higher overall mortality rate.[10-15] Therefore, perioperative temperature monitoring and ultimately, the uniform avoidance of intraoperative hypothermia are recognized as quality improvement measures aimed at improving overall patient care.[16,17] Unlike temperature, the effects of oxygen and glucose on the development of infection are still widely debated at this time. As discussed earlier, the importance of tight glucose control in critically ill patients represents a clinical conundrum without a clear solution in sight. We believe clarification of these effects sets the stage for better clinical understanding and broad application. We hypothesize that 2 separate markers, reactive oxygen species (ROS) and monocyte HLA-DR surface expression, contribute mechanistically to variable osmolarity, hypothermia, and rewarming as host responses to infection.

METHODS

Part 1: Whole blood sampling, reagent preparation and culture conditions

After approval of the University of Louisville Institutional Review Board and written informed consent, 10-mL venous blood samples were taken from 21 healthy, 21- to 41-year-old volunteers, of whom 9 were women and 12 were men. Samples for the ROS assays were collected in sodium heparin BD Vacutainers (Becton-Dickinson and Co) and in ethylenediaminetetraacetic acid (EDTA) Vacutainers for HLA-DR surface expression (Becton-Dickinson and Co). Before blood sample donation, volunteers were required to fast for a minimum of 6 hours. The criteria for exclusion included any history of diabetes mellitus, pregnancy, immunosuppressive disorders, other autoimmune processes, or chronic use of medication.

For osmolarity studies, the following were sterilely prepared or purchased accordingly: mannitol stock solution was prepared and used to achieve a final concentration of 607 mg/dL whole blood (Sigma-Aldrich, Inc), and a 193 mg/dL NaCl final concentration was subsequently prepared and used for our NaCl treatment (Sigma-Aldrich, Inc). A 5% dextrose stock solution was used to adjust the glucose concentration in the blood samples to 600 mg/dL (Baxter Healthcare Corporation). These solutions were prepared to represent equimolar solutions that previously demonstrated a significant effect on ROS formation. A stock preparation of 100 ng/mL lipopolysaccharide (LPS) (Sigma-Aldrich, Inc) was prepared and used for all HLA-DR expression studies. A final LPS concentration of 1 ng/mL in whole blood, a dose confirmed by pilot experiments, provided the endotoxin challenge during incubation for all HLA-DR expression assays.[5] Dihydrorhodamine (DHR) 123 was purchased from Invitrogen. A 60 μg/mL DHR working solution was freshly prepared in PBS immediately before ROS assay. A 1 mg/mL stock of phorbol myristate acetate (PMA) was prepared according to instructions, aliquoted, and stored at -80°C. A 10 μg/mL working solution of PMA in Dulbecco's PBS was prepared immediately before ROS assays. Our previous studies showed that a concentration of 100 ng/mL of PMA, 3μg DHR, and 30 minutes of sampling time is optimal for ROS stimulation and production.[18]

Osmolar culture conditions

Aliquots of whole blood (840 μL) were transferred into 5 mL Falcon polypropylene tubes (Becton-Dickinson and Co). A volume of 100 μL of mannitol, glucose, or NaCl was added to the appropriate culture tubes to obtain equal molar concentrations (3.33 moles) in a final volume of 1 mL. All blood cultures incubated for HLA-DR determinations included 1 ng/mL LPS final concentration, a dose confirmed by pilot experiments. The lipopolysaccharide provided the endotoxin challenge during incubation for all HLA-DR expression assays. Culture tubes with no osmotic additives served as controls for baselines and flow cytometric set up.

Rewarming culture conditions

Aliquots of whole blood (900 μL) were transferred into the appropriate 5-ml Falcon polypropylene tubes. All blood cultures incubated for HLA-DR determinations included