The effects of glucose on pathogen associated molecular pattern (PAMP)-induced inflammatory mediator production in a feline whole blood culture system

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Abstract

In humans, hyperglycemia promotes inflammation, especially in the critically ill. Hyperglycemia is a common finding in cats with critical illness, yet it is unknown if glucose promotes inflammation in cats. We hypothesized that glucose would amplify PAMP-induced production of TNF, IL-1β and CXCL-8 from feline whole blood. Blood was collected from 4 healthy adult male cats, diluted 1:2 with D-MEM and added to 12 well plates. Blood was incubated for 1 hour with glucose (100 or 400 mg/dl), an osmotic control, mannitol (100 or 400 mg/dl), or non-osmotic control, PBS, and then stimulated with 1000 ng/ml lipopolysaccharide (LPS), lipoteichoic acid (LTA), peptidoglycan (PG) or control (PBS) and incubated for 24 hours. Tumor necrosis factor (TNF), interleukin (IL)-1β, and CXCL-8 was measured in supernatant using feline specific assays. Additionally, leukocyte viability was assessed after incubation with either glucose (100 or 400 mg/dl), mannitol (100 or 400 mg/dl), or PBS. Stimulation of feline whole blood with LPS, LTA, and PG resulted in significant TNF production compared to control. Lipopolysaccharide was the only PAMP that stimulated significant production of CXCL-8 production. Addition of glucose or mannitol did not significantly alter PAMP induced TNF and CXCL-8 production. Results for IL-1β are pending. Leukocyte viability was not significantly different between treatments. These data suggest that acute glucose exposure does not promote PAMP induced TNF and CXCL-8 production from feline whole blood.

Results

Tissue viability—Comparison of group mean±SE leukocyte viability between glucose, mannitol and PBS. To show that glucose and mannitol were not toxic to cultured leukocytes, leukocyte viability after a 24 hour incubation with the concentrations of glucose and mannitol used in this study were evaluated. There was no difference in cell viability between treatments (p>0.245).

Methods

Whole blood culture—Blood was collected from 4 healthy adult male cats, diluted 1:2 with D-MEM (100 mg/dl glucose) and plated on 12 well plates. Blood was incubated at 37°C for 1 hour with glucose (100 or 400 mg/dl), an osmotic control (mannitol 100 or 400 mg/dl), or a non-osmotic control (PBS). The lipopolysaccharide (LPS), lipoteichoic acid (LTA), peptidoglycan (PG) or control (PBS) and incubated at 37°C for 24 hours. After 24 hours, the plates were centrifuged (400g at 7 minutes) and the supernatant was collected. Samples were stored at 80°C until analysis.

Conclusions

Acute glucose exposure did not promote TNF or CXCL-8 production from feline whole blood stimulated with LPS, LTA and PG. This supports our initial hypothesis that acute high glucose concentration environment in an acute fashion does not promote PAMP induced TNF or CXCL-8 production. There are several important limitations to this study that prevents the direct application of these data to the clinical patient. First, due to the small sample size of 4 cats, the lack of difference in inflammatory mediator production may simply be a result of type II statistical error. Further testing should be done in a larger population of cats to verify these results. Second, in the clinical patient, hyperglycemia affects multiple cell types and proteins and results in metabolic changes that were not studied in this in vitro model. The interaction between these cells, proteins and metabolic changes found only in vivo may result in a different type of response. Third, we only evaluated acute glucose exposure in this study. In the clinical patient, hyperglycemia is typically more chronic in nature. It is possible that glucose requires a longer period of time to induce a change in leukocyte responsiveness. Nevertheless, based on these data, glucose does not appear to induce a dramatic pro-inflammatory shift in feline whole blood.

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