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 stimulated with 1000 ng/ml of 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.

Introduction

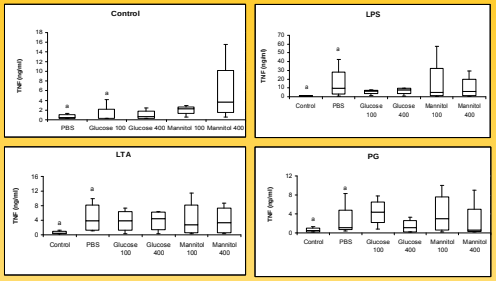
Hyperglycemia is a common condition in critically ill cats, including cats with sepsis, and can develop from endogenous hormones, metabolic dysfunction, or iatrogenic administration of glucose. Hyperglycemia in critically ill human patients promotes a pro-inflammatory state which has shown to exacerbate tumor necrosis factor (TNF) and interleukin (IL)-1β production, impair phagocytosis and leukocytes mobility, and to increase the expression of adhesion molecules on endothelial cells.

Tight glycemic control in critically ill humans improves the patient's outcome and could become a potential strategy for dampening inflammation in critically ill cats as well. However, no studies have evaluated the immunomodulating properties of glucose in cats. The first step to determine the importance of glucose homeostasis during sepsis in cats is to evaluate if glucose promotes the production of pro-inflammatory mediators, *ex vivo*.

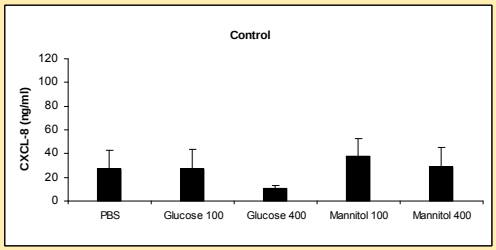
Hypothesis

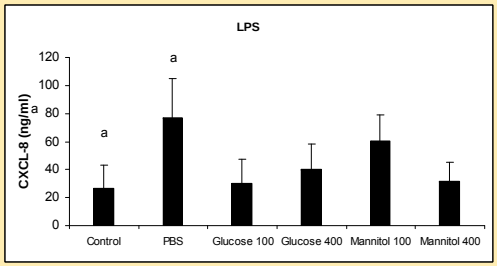
We hypothesized that glucose would amplify pathogen associated molecular pattern-induced production of TNF, IL-1β and CXCL-8 from feline whole blood.

Results

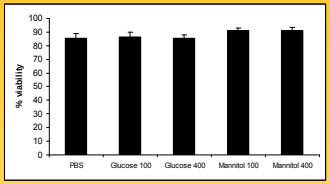


TNF activity in whole blood culture- Comparison of TNF production between glucose, mannitol and PBS after stimulation with a control solution (control), LPS, LTA and PG. The upper and lower edges of the box represent the 75th and 25th percentiles respectively, whereas the line within the box is the median value. Whiskers represent the largest and smallest values. In the absence of PAMPs (control), glucose and mannitol did not stimulate significant TNF production. Stimulation of feline whole blood with LPS, LTA, and PG resulted in significant TNF production compared to control ([a] $p<0.01$). Addition of glucose or mannitol did not significantly alter PAMP-induced TNF production.





CXCL-8 activity in whole blood culture-Comparison of group mean±SE CXCL-8 production between glucose, mannitol and PBS after stimulation with a control solution (control) or LPS. In the absence of PAMPs (control), glucose and mannitol did not stimulate significant TNF production. Stimulation of feline whole blood with LPS resulted in significant CXCL-8 production compared to control ([a] $p=0.04$). Addition of glucose or mannitol did not significantly alter CXCL-8 production in the presence of LPS. Stimulation of feline whole blood with LTA and PG did not significantly stimulate CXCL-8 production nor did glucose or mannitol alter CXCL-8 production (data not shown).



Cell Viability- Comparison of group mean±SE leukocyte viability between glucose, mannitol and PBS. To insure 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 blood was stimulated with 1000 ng/ml of various PAMPs (LPS, LTA, PG) or control (PBS) and incubated again at 37°C for 24 hours. After 24 hours, the plates were centrifuged (400g x 7 minutes) and the supernatant was collected. Samples were stored at -80°C until analyses.

Assays:
Tumor necrosis factor (TNF) activity— Supernatant TNF activity was measured using a cytotoxicity bioassay. Feline rTNF was used to construct a standard curve to determine the concentration of TNF activity in the test wells.
Interleukin-1β activity— Supernatant IL-1β activity was measured using a cytotoxicity bioassay. Feline rIL-1β was used to construct a standard curve to determine the concentration of IL-1β activity in the test wells. These results are pending.
CXCL-8 – Supernatant CXCL-8 concentration was measured using a feline specific ELISA (R and D systems, Minneapolis, MN). The assay was run in triplicate according to the manufacturer's instructions.

Leukocyte Viability - Blood was collected from 5 healthy adult male cats, diluted 1:2 with D-MEM (100mg/dl glucose) and plated on 12 well plates. Blood was incubated at 37°C for 24 hours with mannitol (100 or 400 mg/dl), glucose (100, or 400 mg/dl) or PBS. After 24 hours, ACK Lysis buffer was added to the plates to lyse the red blood cells in the whole blood. The cells were centrifuged (300g x 6 minutes) and the supernatant removed. The cells were washed and resuspended in PBS. Trypan blue was then added to the mixture. One-hundred cells were counted comparing % alive vs. % dead for each treatment.

Statistics- Data were analyzed using commercially available software. For data that were normally distributed a repeated measures one way analysis of variance with post-hoc Tukey multiple comparison procedure was used. Nonparametric data were analyzed using a repeated measures one way analysis of variance on ranks with post-hoc Student-Newman-Keuls Method. A p-value of < 0.05 was considered statistically significant.

Conclusions

Acute glucose exposure did not promote TNF or CXCL-8 production from feline whole blood stimulated with LPS, LTA and PG, respectively compared to the osmotic and non-osmotic control. One possible explanation for the lack of a glucose induced pro-inflammatory shift is a change in leukocyte viability after glucose exposure, *in vitro*. To determine if a high glucose concentration environment resulted in leukocyte death and thus a relative lack of TNF and CXCL-8 production, leukocyte viability was assessed after incubation with glucose 100 and 400 ng/ml and the osmotic and non-osmotic controls used in this study. Glucose did not alter leukocyte viability thus the lack of difference between treatments was not related to leukocyte death. Thus, it appears that exposing leukocytes to a glucose rich 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.

Acknowledgements:

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