

Glucono-Delta-Lactone: An In Vitro Inhibitor of Hyperglycemia-Induced Coagulation

Charles R Spillert* and Irene N Ojini

Rutgers University, New Jersey Medical School, Department of Surgery, Newark, NJ, USA

*Corresponding author: Charles R Spillert, Rutgers University, New Jersey Medical School, Department of Surgery, 185 South Orange Avenue, MSB G502, Newark, NJ 07103, USA, Tel: 973 972 4530; E-mail: spillecr@njms.rutgers.edu

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Abstract

Diabetes is a disease in which premature development of conditions which increase blood coagulation are the leading cause of death. A reduction in the consequences of elevated glucose levels is the main focus of clinical treatment. It has been determined that the addition of glucose to anti-coagulated human blood, followed by incubation for 24 hours results in the generation of a hypercoagulable state, which is evaluated by reduction in whole blood clotting time. Incubation for 2 hours did not show the same change in clotting. Furthermore, the addition of glucono-delta-lactone, a low-toxicity food additive mitigated the hypercoagulable state which may, in part, be produced by tissue factor generated by up-regulated monocytes. This demonstration of in vitro glucose toxicity may broaden our clinical knowledge of the diabetic state. Also, glucono-delta-lactone warrants additional studies to confirm its anticoagulant properties.

Keywords: Glucose toxicity; Diabetes; Whole blood coagulation; Hypercoagulable; Glucono-delta-Lactone; Tissue factor; Anticoagulant

Introduction

There are presently over 20 million diabetic individuals in the U.S. which costs about \$245 billion per year, of which \$176 billion is medical in nature and \$69 billion in other economic loss. Diabetic care is the major cost to the Medicare system [1].

Hyperglycemia in the absence of diabetes was found to enhance clot formation and inhibit the fibrinolytic pathway of clot dissolution. Up to 50% of all ST-segment elevation in heart attacks is present with hyperglycemia and 25% are diabetic. Elevated glucose level is a poor prognostic indicator for these patients with or without diabetes [2].

The hemostatic pathways are altered by diabetes. Increases in procoagulant factors are accompanied by decreasing fibrinolysis resulting in faster clot formation and lower tendency for the clot to dissolve once formed. Tissue Factor (TF), the initiator of the extrinsic clotting cascade is elevated in diabetes as well as glucose levels [3].

Patients with elevated glucose levels or diabetic patients have poorer outcomes after acute ischemic stroke. A possible explanation put forth was related to increase TF-PCA (TF procoagulant activity) levels. The elevated glucose level may be the nidus for the observed outcomes [4].

Increased TF levels are found to be a major cause of cardiovascular complications in diabetes. The monocyte, an immune cell, when activated can generate TF, a potent procoagulant [5,6].

Since elevated glucose levels and monocyte activation to yield TF are in excess in the diabetic state, one could consider the effect of adding glucose to human blood and determining whether increased TF is generated. Furthermore, evaluation as to whether the addition of a possible anticoagulant, glucono-delta-lactone (GDL) can mitigate these phenomena would be of importance [7].

It has been shown that the addition of endotoxin to human blood increases monocyte TF activity and can be mitigated by treatment with GDL during or after incubation as measured by determining the changes in the whole blood clotting time [8].

GDL and its derivatives are naturally occurring small molecules (178 molecular weight units). In humans about 450 mg/kg are generated from endogenous sources, as it is the first oxidation product of glucose. The LD50 after oral administration to rats is above 6000 milligrams/ kilogram. The conference recommendation was based on safety studies; the chemicals in this category are currently of low priority for further work.

That GDL when added to human blood showed significant anticoagulant properties was unexpected. It is low toxicity, stable, and almost 100,000 tons are produced yearly for food and other purposes.

Experimental Design

Refrigerated citrated whole blood samples (CWB) were obtained from University Hospital's clinical laboratory with prior Institutional Review Board (IRB) approval. These CWB samples were treated with exogenous glucose (6.0 or 12.0 mM) to yield a hyperglycemic condition and then incubated. The incubation times were 2 hours and 24 hours. After incubation aliquots of the sample had their clotting time (seconds) determined using a Diagnostica Stago ST4 Coagulation Analyzer. This instrument is sensitive to increasing viscosity of the clotting sample and determines the clotting time. This study was repeated with the addition of GDL at final blood concentrations of 3 and 4 mg/ml.

Paired-t test was used for statistical analysis.

Results

Does exogenous glucose decrease the clotting time of blood?

Effect of incubation time on glucose induced changes in clotting time at 2 hours versus 24 hours.

The addition of glucose to CWB and incubating the sample followed by the determination of the clotting time shows that at 2 hours (n = 6) incubation glucose at 6.0 mM had a Clotting Time (CT) of 328 ± 70 seconds versus the control of 279 ± 70 seconds. No significant reduction in CT. When incubated for 24 hours (n = 30) the glucose sample had a CT of 165 ± 65 seconds compared to the control value of 215 ± 89 . Glucose initiated a significant reduction in CT (p < 0.01).

Does GDL prolong the clotting time of a glucose-induced clotting time reduction?

To determine whether GDL shows anticoagulant properties on blood samples in which glucose (12.0 mM) was incubated for 24 hours alone and with GDL.

The test samples (n = 18) contained

1. 0 glucose, 0 mg/ml GDL
2. 12.0 mM glucose, 0 mg/ml GDL
3. 12.0 mM glucose, 3 mg/ml GDL
4. 12.0 mM glucose, 4 mg/ml GDL

The clotting times were A) 236 ± 79 , B) 201 ± 71 , C) 243 ± 70 , and D) 334 ± 107 seconds.

Two-tailed p values are as follows: A and B p < 0.05, C vs. B p < 0.01, D vs. B p < 0.001.

Thus GDL mitigates the hypercoagulable state induced in human blood incubated with glucose.

Conclusion

Since elevated glucose levels and monocyte activation to yield TF are in excess in the diabetic state, one wonders the effects of adding glucose to human blood and determining whether increased TF is generated. Also, whether the addition of glucono-delta-lactone (GDL) can mitigate the phenomena could be of interest.

It has been shown that the addition of endotoxin to human blood increases monocyte TF activity and this can be mitigated by treatment with GDL during and after this incubation before determining the changes induced in the whole blood clotting time [7,9].

GDL was found to inhibit the activation of human blood clotting induced by tissue factor and thrombin independently. In addition, GDL significantly prolonged the clotting times of these bloods when a combination of procoagulant TF and thrombin were utilized. The significant increase in clotting time induced by GDL for TF and thrombin individually as well as when TF and thrombin were combined was p < 0.001 [10].

Glucose toxicity arises from a reduction of insulin secretion due to chronic hyper-glycemia. This poor control of glucose levels leads to cardiovascular complications, the major cause of death in diabetes [11].

It has been found that in the diabetic population there is a reduction in the blood CT in both inactivated blood and endotoxin-activated samples. This hypercoagulable condition evaluated in a lab, may in

part lead to the many prothrombotic clinical complications in this disease syndrome.

In this study, the measurement of "glucose Toxicity" in vitro is evaluated by monitoring clotting changes induced in human CWB. Exogenous glucose is added to CWB in the amount necessary to have a hyperglycemic condition. The samples were then incubated at varying glucose levels and time frames after which calcium ion was added to initiate clotting in these citrated samples.

Glucono-delta-lactone (GDL), a possible anticoagulant candidate was also added in different concentrations to evaluate clotting changes.

Our findings are that at the concentrations of exogenous glucose added to CWB samples produced no clotting change with a 2 hour incubation period. However, at 24 hours incubation a significant reduction in clotting time was found in the glucose containing aliquot. In other words, a hypercoagulable state was generated. GDL when added to these glucose-activated samples was found to increase the clotting time.

The major findings of this study show that, firstly, glucose when added to human CWB and incubated for 24 hours induced a hypercoagulable condition in the CWB. Secondly, GDL, a small low toxicity molecule, has the ability to reverse the hypercoagulable state initiated by glucose in vitro.

References

1. American Diabetes Association1 (2013) Economic costs of diabetes in the U.S. in 2012. *Diabetes Care* 36: 1033-1046.
2. Undas A, Wiek I, Stępien E, Zmudka K, Tracz W (2008) Hyperglycemia is associated with enhanced thrombin formation, platelet activation, and fibrin clot resistance to lysis in patients with acute coronary syndrome. *Diabetes Care* 31: 1590-1595.
3. Alzahrani S, Ajjan RA Coagulation and fibrinolysis in diabetes. *Diabetes and Vascular Disease Research*.
4. Gentile NT, Vaidyula VR, Kanamalla U, et al. (2007) Factor VIII and tissue factor procoagulant activity in diabetes mellitus after acute ischemic stroke: Impact of hyperglycemia. *Thromb Haemost* 98: 1007-13.
5. Bogdanov VY, Osterud B (2010) Cardiovascular complications of diabetes mellitus: The Tissue Factor perspective. *Thrombosis Research* 125: 112-118
6. Singh A, Boden G, Homko C, Gunawardana J, Rao AK (2012) Whole-blood tissue factor procoagulant activity is elevated in type 1 diabetes: Effects of hyperglycemia and hyperinsulinemia. *Diabetes Care* 35: 1322-1327.
7. Kaufer M, Spillert CR (2011) Glucono-delta-lactone inhibits E. coli induced-upregulated coagulation. *New Jersey Medical School Summer Abstracts* 41-44
8. SIDS Initial Assessment Report for SIAM 18-Gluconic Acid and its derivatives. Paris, France 20-23 April 2004. Conference to determine effects of gluconates on diseases like SIDS.
9. Ngo G, Spillert CR (2012) Glucono-delta-lactone (GDL) inhibits tissue factor and thrombin during human blood coagulation. *NJ Medical School Summer Research Abstracts* 30-34.
10. Kawahito S, Kitahata H, Oshita S (2009) Problems associated with glucose toxicity: Role of hyperglycemia-induced oxidative stress. *World J Gastroenterol* 15: 4137-4142.
11. Spillert CR, Milazzo VJ, Pastena JA, Lazaro EJ (1989) Prediction of clinical course in diabetes using a simple coagulation test. *J Natl Med Assoc* 81: 81-83.