Valley International Journals
Open Access Journal
International Journal Of Medical Science And Clinical Inventions
Volume 3 issue 9 2016 page no. 2168-2170 e-ISSN: 2348-991X p-ISSN: 2454-9576
Available Online At: http://valleyinternational.net/index.php/our-jou/ijmsci

# Glucono-delta-lactone Potentiates Anticoagulant Properties of Low Molecular Weight Heparin

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***Abstract: The heparins are a class of drug called anticoagulants. They are generally used to prevent or stop abnormal blood clotting, which can result in thrombosis. The clot, when formed in blood vessels, prevents or interrupts blood flow to vital tissue or organs resulting in injury and even death. Low molecular weight heparin is an effective drug used to prevent many types of clotting episodes. Therefore, agents that increase the anticoagulant properties of this drug could reduce the morbidity and mortality associated with many diseases. Glucono-delta-lactone, when added to human blood in a test tube, was found to significantly increase the clotting time. This is a marker for an agent of potential anticoagulant use.***

**Keywords:** *Glucono-delta-lactone, low molecular weight heparin, anticoagulant, clotting time, tissue factor*

## INTRODUCTION:

Heparin is used to prevent blood clotting and reduce the tendency to form life-threatening clots. It is used primarily to treat clotting disorders, as well as to prevent clots from forming in patients undergoing kidney dialysis, blood transfusions, and vascular surgery. Heparin exists in two medically useful forms: Unfractionated heparin (UFH) and low molecular weight heparin (LMWH). LMWH was developed as a safer alternative to UFH and is capable of preventing some types of blood clots from growing and traveling to distant areas of the body. It is commonly employed postoperatively and used ubiquitously in hospitals. Low molecular weight heparin works by activating antithrombin, a protease inhibitor, which normally works late in the coagulation cascade to inhibit thrombin and mitigate the clotting process (2).

Glucono-delta-lactone (GDL) is a low molecular weight, low toxicity substance that is found naturally in the human body, in many food and cosmetic products, and is on the FDA's Generally Recognized As Safe (GRAS) list. Previous studies have shown that GDL exerts its anticoagulant effect in blood via antithrombin and anti-tissue factor properties (1). The goal of this experiment was to study whether GDL will have a synergistic effect with low molecular weight heparin to increase the clotting times of human citrated whole blood. Prolongation of the clotting times with GDL may prove clinically useful as an adjuvant to heparin therapy.

## METHODS:

Two-day-old human citrated whole bloods (CWB) were obtained from University Hospital's clinical labs according to IRB Protocol. The bloods were pooled into aliquots containing different reagents (n=10) of approximately 500ul each. The aliquots were gently mixed and incubated for 15 minutes at 37°C to allow enough time for the reagents to react. Three hundred microliters of each aliquot was then added to cuvettes containing 32ul of 0.1M $CaCl_2$ (to initiate clotting). Final blood concentrations of reagents are listed below.

The heparin employed was Fragmin (dalteparin sodium) which is utilized for subcutaneous injection to reduce clotting tendency.

Experiment 1 (n=10)


DOI: 10.18535/ijmsci/v3i9.13

1. 0.85% saline (Control)
2. 0.2 units LMWH final concentration
3. 2mg/ml GDL
4. 0.2 units LMWH + 2mg/ml GDL

Experiment 2 (n=10)

1. 0.2 units LMWH + 0.5mg/ml GDL (Control)
2. 0.2 units LMWH + 1mg/ml GDL
3. 0.2 units LMWH + 2mg/ml GDL

The samples were analyzed using the Sonoclot Coagulation Analyzer (Sienco, Wheat Ridge, CO, USA), a mini-viscometer that is sensitive to fibrin clot formation (3). This instrument is currently FDA approved for evaluating human blood coagulation. The Sonoclot was used to measure activated clotting times of the samples.

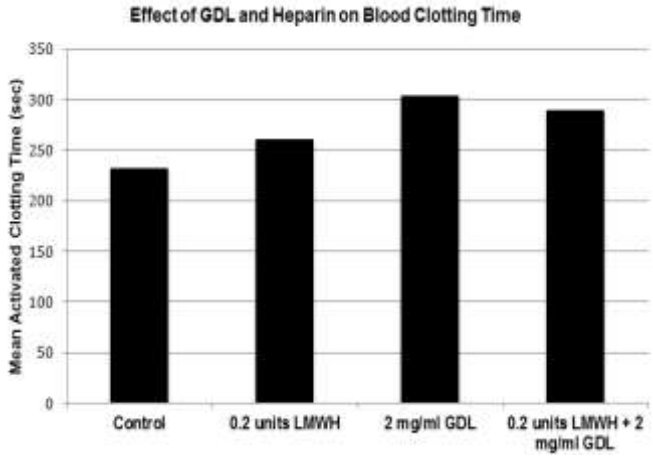
Clotting times were compared using a statistical program to perform paired t-tests and repeated measure analysis of variance (ANOVA). Significance was defined as test values with $p<0.05$.

**RESULTS:**

*Experiment 1* – What is the effect of GDL and heparin on blood coagulation?

**Table 1:** Effect of GDL and heparin on blood coagulation (N=10)

| Sample | Mean activated clotting time (sec) |
|---|---|
| Control | 232 ± 58 |
| 0.2 units LMWH | 262 ± 65 |
| 2mg/ml GDL | 304 ± 79 |
| 0.2 units LMWH + 2mg/ml GDL | 290 ± 72 |

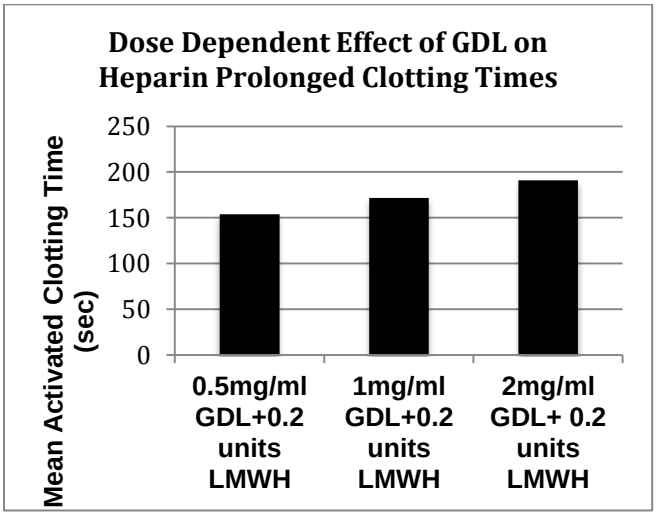



GDL administered at 2mg/ml final concentration combined with 0.2 units heparin was found to significantly lengthen the clotting times of citrated whole bloods compared to heparin alone. Two tailed t-tests indicated a significant increase in clotting time between samples treated with saline versus 0.2 units heparin (p=0.008), 2mg/ml GDL (p=0.0002), and 0.2 units heparin combined with 2mg/ml GDL (p=0.004).

*Experiment 2* – Is there a dose-dependent effect of GDL on heparin during blood coagulation?

**Table 2:** Dose-dependent effect of GDL on heparin during blood coagulation (N=10)

| Sample | Mean activated clotting time (sec) |
|---|---|
| 0.5mg/ml GDL+0.2 units LMWH | 154 ± 52 |
| 1mg/ml GDL+0.2 units LMWH | 172 ± 60 |
| 2mg/ml GDL+ 0.2 units LMWH | 191 ± 53 |




GDL was previously shown to significantly potentiate the anticoagulant properties of heparin on citrated whole blood. To determine whether this increase is dose dependent we tested three increasing concentrations of GDL on 0.2units LMWH in blood. Two tailed t-tests indicated a significant increase in activated clotting times between samples treated with 0.5mg/ml GDL combined with heparin in blood versus 1.0 mg/ml GDL (p=0.026), and versus 2.0 mg/ml GDL (p=0.0005). A significant increase was also


DOI: 10.18535/ijmsci/v3i9.13

detected between the 1.0mg/ml and 2.0mg/ml GDL aliquots (p=0.021)

**DISCUSSION:**

Low molecular weight heparin activates antithrombin, a protease inhibitor. Antithrombin normally works late in the coagulation cascade to inhibit thrombin and Factor Xa. Many anticoagulants are administered at this stage in the coagulation cascade when prothrombin is converted to thrombin. Previous experiments have suggested that GDL may function at this stage by antagonizing thrombin and tissue factor, the exact mechanism of which is still unknown. This mechanism allows GDL to attack more than one portion of the coagulation cascade, unlike LMWH. Because LMWH does not affect tissue factor, GDL has the potential to be a more versatile anticoagulant with a wider spectrum of anticoagulant effects.

This experiment showed that GDL is capable of potentiating the anticoagulant properties of low molecular weight heparin in a dose dependent manner. Further, under the conditions of this experiment we found that 2mg/ml GDL had a significantly prolonged clotting time compared to 0.2units of heparin. The mechanism is possibly based on GDL working synergistically with heparin to potentiate the inhibition of thrombin in addition to GDL inhibiting tissue factor. Patients with clotting disorders may one day benefit from the addition of GDL to heparin therapy, resulting in a more comprehensive inhibition of the coagulant cascade. Future studies will investigate the steps of the coagulation cascade affected by GDL in combination with LMWH.

**Competing Interest:** The authors have received no funding or financing from any organization that may benefit from publication of this manuscript. Charles R. Spillert has been assigned US Patent 8,530,455 B2 by Rutgers University.

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DOI: 10.18535/ijmsci/v3i9.13