**ABSTRACT**

Isotretinoin (Accutane) is a therapeutic drug used for the treatment and prevention of severe acne. A previous study done on the effects of isotretinoin concluded that patients taking isotretinoin showed a significantly prolonged activated partial thromboplastin time (aPTT). aPTT is a timed, clinical test to detect abnormalities in the intrinsic pathway of the coagulation cascade. An irregular or prolonged aPTT indicates at least one factor deficiency or inhibition in the intrinsic pathway. The objective of this study is to investigate which factor(s) of the pathway is being inhibited. Normal patient plasma was collected, treated with a standardized concentration of isotretinoin, and an aPTT was performed. The collected plasma, was then pooled into one solution and underwent a factor assay. The factor assay comprised of reagent factor deficient plasmas (XII, XI, IX, and VIII). The pooled patient plasma will be mixed with each reagent plasma and an aPTT will be measured. The factor assay was performed with the pooled plasma as a control and then treated with isotretinoin. Results indicate the factor assay show statistically significant ($p=0.001$) prolonged aPTT times in factor deficient XI and IX when treated with isotretinoin. There were no significant prolonged times for factor deficient XII and VIII, suggesting that isotretinoin had no inhibitory affect on these coagulation factors. In conclusion, isotretinoin prolongs aPTT times by inhibiting factors XI and IX in vitro in the intrinsic pathway of the coagulation cascade.

**RESULTS**

Parallel testing was performed to ensure the reagents used in preparation showed no significant effects on aPTT times. An ANOVA was performed to determine if there are significant differences among the means of the groups, and a $F$ value of 0.704 and a $P$ value of 0.59. With a set alpha level of 0.001 this indicates that the groups are not statistically significant.

Kruskal-Wallis Rank Sum Test was performed to determine if there are statistically significant differences between one or more of the groups. The Kruskal-Wallis Rank Sum Test computed a $P$ value of 2.2 x 10^{-6}. The $P$ value is less than our alpha level (0.001) this test indicates that the means of groups are statistically significant.

Factors XI, and IX show delayed aPTT times when treated with isotretinoin. To compare the means of the control and treated groups, a Mann Whitney U test was performed for factors XI and IX. The test computed a $P$ value of 1.215 x 10^{-5} for factor XI, and a $P$ value of 5.775 x 10^{-6} for factor IX. Both $P$ values are less than the alpha level (0.001) indicating the treated groups show statistically elongated aPTT times.

**INTRODUCTION**

Internal bleeding occurs when a blood vessel becomes damaged. To prevent further bleeding, there is a multistep reaction known as the coagulation cascade. The coagulation cascade plays a critical role in clotting to prevent further internal bleeding from damaged vessels. As a blood vessel is damaged, platelets adhere to the damaged area and begin to form an aggregated plug. Platelets provide an area for the assembly and instigation of coagulation complexes to generate thrombin. Thrombin converts fibrinogen into fibrin, and the fibrin strands combine with the aggregated platelets to form the platelet-fibrin hemostatic plug.

The coagulation cascade can be separated into three different pathways: common, intrinsic, and extrinsic. Activation of either intrinsic or extrinsic pathway will lead to the activation of the common pathway which results in the development of thrombin and the platelet-fibrin hemostatic plug.

In the intrinsic pathway, there are four main coagulation factors involved: XII, XI, IX, & VIII. All factors circulate in the blood as inactive enzymes until they are activated by contact from a damaged blood vessel or contact with another activated factor.

**MATERIALS & METHODS**

Patient plasma was collected from normal healthy individuals not prescribed with isotretinoin. Blood samples were collected in Sodium Citrate tubes, centrifuged and the plasma was separated and frozen until testing. All testing was performed on the ACL Elite Coagulation Instrument.

An initial aPTT time was performed to establish a control group. The patient plasma samples were then treated with a standardized concentration of isotretinoin. Following the addition of isotretinoin, and another aPTT was performed.

The factor assay was comprised pooling the patient samples to control for varying aPTT times of each patient and how it may react with factor deficient plasma. Using the ACL Elite, the instrument will mix together an aliquot of pooled plasma and an aliquot of one of the factor deficient plasmas, and an aPTT will be measured. The concept of mixing the plasmas is the pooled plasma will compensate for the factor deficient plasma thus continuing the clotting process. This process was performed with factor deficient plasmas: VIII, IX, XI, and XII. After initial times were established, the pooled plasmas were then treated with a standardized concentration of isotretinoin, and the factor assay with each of the factor deficient plasmas was performed. It is hypothesized that if isotretinoin inhibits one of the intrinsic factors, then the treated pooled plasma will be unable to compensate for the factor deficient plasma resulting with a longer aPTT time.

**DISCUSSION**

With the addition of different treatments, the patient plasma did not show any statistical significance or delay. However, the group treated with 1000 ng/ml of isotretinoin show higher median aPTT times. Although the slight difference is not statistically significant, it may be clinically significant towards the patient.

The factor assay resulted with two factors, XI and IX, showing statistically elongated aPTT times for the treated groups vs their controls. Thus the researchers reject the null hypothesis stating the factor assay would show no significance comparing the means for each group. The objective for this study was to investigate what factor(s) are inhibited due to isotretinoin. Showing statistical significance, isotretinoin inhibits coagulation factors in the intrinsic pathway of coagulation, specifically Factors XI and IX.

Further studies upon this topic can perform an *in vivo* study, investigating patients prescribed with isotretinoin and its effects on the intrinsic coagulation pathway.

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