Original Article

# Effects of botropase on clotting factors in healthy human volunteers

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**Abstract**

**Objective:** To evaluate the effects of botropase on various clotting factors in human volunteers. **Materials and Methods:** It was a prospective open label study conducted on human healthy volunteers. After the baseline screening, subjects fulfilling inclusion criteria were enrolled. On the study day, 1 ml of botropase was administered intravenously and after an hour same dose of botropase (1 ml) was given by intramuscular (IM) route. The efficacy and safety parameters were monitored up to 72 h from the time of intravenous (IV) administration. **Results:** A total of 15 volunteers, belonging to 24-35 years of age were included in the study. Botropase significantly reduced the plasma level of fibrinogen and fibrin degradation products after 5 min of IV administration ($P < 0.05$). In addition, factor X was observed to reduce constantly by botropase administration suggesting enhanced turnover between 5 and 20 min of IV administration. Although botropase reduced clotting and bleeding time in all the volunteers, the data remains to be statistically insignificant. **Conclusion:** Present study demonstrated the safety and efficacy of botropase in human healthy volunteers. The study has shown that it is a factor X activator and reduces effectively clotting and bleeding time.

**Key words:** Botropase, clotting factors, fibrinogen, hemocoagulase

## INTRODUCTION

The snake venoms that have been shown to induce defibrinogenation include: Ancrod from the venom of *Calloselasma rhodostoma* (formerly known as *Agkistrodon rhodostoma*), batroxobin from the venom of *Bothrops atrox* and *B. moojeni*, and crotalase from the venom of *Crotalus adamanteus*. The purified fractions of ancrod, batroxobin, and crotalase possess coagulant, proteolytic and esterolytic properties; although their primary mechanism of action is a proteolytic effect on circulating fibrinogen. Ancrod cleaves only the A-fibrinopeptides, but not the B-fibrinopeptides, from fibrinogen; this contrasts with thrombin, batroxobin, and crotalase, which cleave both fibrinopeptides A and B.[1]

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Botropase is a hemocoagulase preparation used to arrest bleeding of different etiology. It is an enzyme preparation with hemocoagulase activity which is attributable to the protein batroxobin. The enzyme clots pure fibrinogen like thrombin, releasing fibrinopeptide A from fibrinogen. The enzyme possesses all the typical characteristics of serine proteases and has a molecular weight of 27,000 Da and its isoelectric point is around 7.5.[2,3] Botropase is said to have actions like thrombin. However, there are many differences between the two agents. Botropase is both systemic and

local hemocoagulant unlike thrombin. Botropase induced clot is not structurally similar to thrombin clot. Botropase is not absorbed by clot like thrombin. It appears that even in the absence of calcium, botropase can cleave the fibrinogen into fibrin. Antithrombin III does not interfere with botropase hemocoagulant action. Over 6 decades, different preparations of botropase have been used clinically all over the world as a hemocoagulant. However, results have been difficult to interpret, and additional trials are needed to better define the optimum role of ancrod and batroxobin.[4-7] There are no data available about the effects of botropase on clotting factors. Hence, the study was undertaken to evaluate the effects of botropase on various clotting factors.

## MATERIALS AND METHODS

It was a prospective open label study conducted in human healthy volunteers. Fifteen male healthy volunteers aged between 18 and 35 years were enrolled into the study after obtaining their written informed consent. The study was approved by institutional ethics committee. Subjects with history of smoking, alcohol, and drug addiction were excluded. Subjects with history of systemic illness, thromboembolic or hypercoagulability of blood, jaundice, and blood donation in the past 3 months were also excluded. The baseline investigations including coagulation parameters, blood sugar, liver function tests, renal function tests, X-ray, and electrocardiography (ECG) were performed. After the initial screening, subjects with abnormal bleeding time, clotting time, and prothrombin time were excluded from the study. Eligible subjects were admitted to intensive care unit of a tertiary care hospital for a period of 36-48 h. Food and water intake was standardized during the study period. On the study day, 1 ml of botropase was administered intravenously and after an hour of same dose of botropase (1 ml) was given by intramuscular (IM) route. The efficacy and safety parameters were monitored up to 72 h from the time of intravenous (IV) administration. Fifteen blood samples were taken at regular intervals to evaluate the effects of botropase on different hematological parameters and coagulation cascade in particular. At the end of the study baseline investigations, X-ray and ECG were repeated. All the observed clinical signs and symptoms were documented and analyzed.

### Statistical analysis

Statistical analysis was performed by using Statistical Package for Social Sciences (SPSS) version 11.0. Student's *t*-test and analysis of variance (ANOVA) were used for analysis. $P < 0.05$ was considered significant. For the analysis of efficacy of IM botropase, the values at 60$^{th}$ min after IV botropase were taken as basal value.

## RESULTS

Their mean age was 29.33 ± 3.94 years. Botropase has significantly reduced the plasma fibrinogen soon after 5 min of IV administration ($P < 0.05$). The average initial value of fibrinogen level was within widely accepted range (200-400 mg/dL). There was significant alterations in fibrin degradation products soon after 5 min of IV injection ($P < 0.05$). The basal value of factor X was 120.3 ± 18.52, which was reduced constantly following botropase administration. Although botropase produced reduction in clotting and bleeding time in all the volunteers, the data remains to be statistically insignificant [Table 1]. The prothrombin and activated partial thromboplasin time (aPTT) were not altered by botropase administration [Table 2].

Parenteral botropase has significantly altered fibrin degradation products level soon after 5 min of IV injection ($P < 0.05$) [Table 2]. This is well-correlated with its action on fibrinogen. However, in two volunteers at two point of time dependent observations have revealed variable results.

IM administration of botropase also decreased the levels of fibrinogen and factor X, but the decrease was statistically not significant [Table 3].

## DISCUSSION

Different botropase preparations have been used to arrest bleeding of different etiology. The hemocoagulase action of botropase is attributable to the protein

**Table 1: Effect of botropase on bleeding and clotting time at different time points (*n*=15)**

| Time points | Bleeding time (s) | | | Clotting time (s) | | |
|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean±SD | Minimum | Maximum | Mean±SD |
| Baseline | 1.45 | 3.30 | 2.41±0.57 | 2.45 | 6.15 | 4.57±1.06 |
| 15 min IV | 1.15 | 2.45 | 2.14±0.35 | 3.15 | 5.30 | 4.23±0.55 |
| 30 min IM | 2.00 | 3.00 | 2.22±0.29 | 3.45 | 5.00 | 4.33±0.52 |
| 24 h | 2.00 | 2.45 | 2.23±0.17 | 4.00 | 6.25 | 4.94±0.80 |
| 72 h | 1.15 | 3.25 | 1.97±0.54 | 2.30 | 5.45 | 3.95±0.96 |

SD=Standard deviation, IV=Intravenous

**Table 2: Effect of intravenous administration of botropase on coagulation parameters at different time points (*n*=15)**

| Coagulation parameter | Botropase IV | | | | | |
|---|---|---|---|---|---|---|
| | 0 (baseline) | 5 min after IV | 10 min after IV | 15 min after IV | 20 min after IV | 40 min after IV |
| Fibrinogen (mg/dL) | 222.2±78.79 | 173.8±44.67* | 150.88±56.73 | 191.53±65.79 | 223.31±154.16 | 265.2±161.33 |
| Factor X (%) | 120.3±18.52 | 121.5±28.33 | 114.27±17.22* | 115.4±19.48* | 115.47±21.95 | 115.07±23.8 |
| aPTT (s) | 34.16±7.46 | 33.08±7.86 | 34.05±8.23 | 33.85±6.20 | 32.69±5.29 | 34.29±7.90 |
| PT (s) | 14.53±3.58 | 13.74±1.58 | 14.09±1.73 | 13.74±1.21 | 13.79±1.61 | 14.11±2.19* |

*Paired sudent's *t* test, significant as compared to baseline (*P*<0.05). aPTT=Activated partial thromboplastin time, PT=Prothrombin time, IV=Intravenous

**Table 3: Effect of intramuscular administration of botropase on coagulation parameters at different time intervals**

| Coagulation parameter | Botropase IM | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 h after IM | 2 h after IM | 4 h after IM | 6 h after IM | 12 h after IM | 24 h after IM | 36 h after IM | 48 h after IM | 72 h after IM |
| Fibrinogen | 194.83±70.09 | 176.75±73.28 | 171.69±84.08 | 196.38±33.83 | 184.85±94.38 | 241.08±94.27 | 222.47±66.36 | 269.07±100.67 | 196.25±33.83 |
| Factor X | 118.73±22.94 | 122.2±21.73 | 117.27±26.13 | 123.5±18.89 | 125.57±20.5 | 121.2±18.82 | 126.92±21.76 | 126.75±19.9 | 122.67±25.83 |
| aPTT (s) | 33.06±5.45 | 33.88±8.28 | 32.97±6.51* | 35.18±12.41 | 34.75±9.91 | 32.38±4.94 | 33.07±7.24 | 31.93±4.45 | 30.50±1.61 |
| PT (s) | 13.95±2.02 | 13.95±1.80 | 15.03±2.89* | 14.65±3.22 | 14.04±1.81 | 13.94±2.07 | 14.38±1.63 | 13.87±1.37 | 13.51±1.23 |

*Paired student's *t* test, *P*<0.05. aPTT=Activated partial thromboplastin time, PT=Prothrombin time

batroxobin. Batroxobin has been investigated in patients with stroke, deep-vein thrombosis, myocardial infarction, peripheral arterial thrombosis, priapism, and sickle-cell crisis. In the coagulation laboratory, batroxobin may be used as reagents to perform coagulation studies on specimens of blood that contain heparin. This can be substituted for thrombin in performing the thrombin time and in removing fibrinogen from plasma for accurate determination of fibrinogen-fibrin degradation products.

The present study has generated scientific data, which is similar to observed clinical effects of botropase hitherto. Distinctly, the findings of this study offer a rational outlook for IV administration of botropase in human beings. It is known, that the thrombin should not be given by IV route, botropase stands a suitable alternative drug for IV hemocoagulant effect.

Our study shows that IV administration of 1 ml of botropase significantly reduced fibrinogen level rapidly. This action appears to be instantaneous and actually provide a basis for its use to arrest bleeding. Further, the estimation of fibrin degradation products in plasma correlate well with observed effects of botropase on fibrinogen. Interestingly, the clotting time and the bleeding time remain reduced up to 72 h after administration of botropase. Within minutes of administration of batroxobin, there is a significant reduction in plasma fibrinogen levels, and these remain exceedingly low with repeated administration (once or twice daily). The rapid fall in plasma fibrinogen levels is accompanied by a slightly delayed but marked rise in the level of fibrinogen-fibrin degradation products. Plasminogen levels are decreased and blood viscosity is reduced, but formed elements in the circulating blood remain unaltered.[2]

Botropase significantly reduces factor X level in plasma, which is pronounced soon after 10 min of IV administration and persisted up to the 20$^{th}$ min. This evinces that botropase is a factor X activator. Activation of factor X triggers the formation of prothrombin, which converts into thrombin. Thus, the liberated thrombin acts on fibrinogen and produces a stable clot. This is an additional indirect mechanism by which botropase reinforces clot formation to arrest bleeding. Overall, botropase injection was well-tolerated and there were no serious adverse events. There was no evidence of thromboembolic episode.

Factor Xa is believed to be a stimulator of mitogenic response. Senden *et al.*, have shown that exposure of human vascular endothelial cells to factor Xa stimulates the production of cytokines such as interleukins 6 and 8 and the expression of various other proteins.[8] It is also recognized that factor Xa causes a striking upregulation of platelet-derived growth factor (PDGF) gene expression. As already alluded, botropase is a prohealer.[3] Perhaps by activation of factor X, botropase enhances chemotaxis, cell migration, interleukin production, and promotes the action of growth factors like PDGF. This may contribute improved quality of wound repair following botropase administration. However, further research is necessary in this regard.

Botropase preparations contains cluster of proteins. There is an urgent need to isolate and deduce the structure of all proteins in this hemocoagulant preparation.

Hopefully, this will pave new indications for each protein present in botropase formulation.

To conclude, present study demonstrates the therapeutic efficacy and safety of botropase as a hemocoagulant. It is a factor X activator. Botropase did not alter prothrombin time (PT) and aPTT in healthy volunteers. Clotting and bleeding time were reduced by botropase. These findings suggest that botropase is efficacious and safe for human use.

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