The Influence of Botropase on the Hypercoagulable Plasma State in the Patients with Cancer

Yingwei Zhu[1], Tongbao Feng[2], Xiuwen Zhang[3], Hong Liu[4], Hongying Chao[5], Yiwu Sun[5]

[1]Department of Oncology, [2]Clinical Laboratory, [3]Central Laboratory, [4]Department of pathology and Pathophysiology, Medical School of Southeast University, Nanjing 210009, China, [5]Department of Hematology, Changzhou Second People's Hospital, The affiliated hospital of Nanjing Medical University, Changzhou 213164, China.
yiwu_sun@hotmail.com

Abstract: Botropase posses effect in coagulation by converting plasma fibrinogen into soluble fibrinogen/fibrin. In this retrospective study, a total of 48 cancer patients (stage IV) with abnormal D-dimer (DD) value was treated with Botropase for the local hemorrhage. It was found the average DD level significantly increased ($P<0.01$) by comparing with the level before treatment of Botropase. Thromobine time (TT) and activated partial thromoplastin time (APTT) did not prolong following DD value. There were no significant changes in platelet and red blood cell (RBC) count between before and after administration of Botropase. The above results suggest that Botropase alleviated the hypercoagulable plasma state of the patients.
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Introduction

The coagulation disorder (such as thrombosis and disseminated intravascular coagulation), which is triggered by multiple factors such as the excessive release of thrombin, the circulating TF-positive MPs (micro-particles) derived from tumor and the subsequent formation and deposition of fibrin in the vasculature, is a common hemostatic complication in the patients with solid tumors. [1,2,3,4] This clinical syndrome is characterized by the occurrence of bleeding and/or thrombosis, with evidence of activation of the clotting and fibrinolytic system. [3,5] However, it could presented as chronic disseminated intravascular coagulation or as abnormity of hemostasis test in most of cancer patients. [4] Botropase, athrombin-like enzyme, which induces plasma fibrinogen converting into soluble fibrinogen/fibrin monomer complexes, is used to treating a variety of hemorrhage in clinical practice. [6,7,8] It appears Paradoxical that Botropase is administrated in the patients with tumors, who have been carrying hypercoagulable plasma state. There are no clinical reports in this field so far. we retrospectively investigated 48 patients with cancer, and systematically analyzed their clinical features, laboratory tests variable for hemostasis and peripheral hemogram, evaluating the influence of Botropase on coagulable plasma profiles.

Patient and Methods

Patients: The study was approved by the local ethics committee, which waived the need for informed consent. The patients with different types of cancer were admitted from 2011 to 2015, and were treated with Botropase due to hemorrhage. The authors had access to identifying information during and after data collection. The cancer was defined according to pathological findings. Disease clinical staging depended on the systems which NCCN (National Comprehensive Cancer Network) recommend for different types of cancer. Exclusion criteria were DIC, which was diagnosed on the parameters issued by Chinese hematology society in 1999, and thrombosis. The patients that needed the blood transfusion for their bleeding were also excluded. Other patients who took the medications (such as Heprin) or suffered from severe liver diseases (such as hepatocirrhosis), were not included.

Blood Sampling and Handling: Blood was sampled into sterile sodium citrate or EDTA contained tubes (Greiner bio-one) respectively for analysis of hemostasis-related parameters and thrombin generation measurement, and complete blood count (CBC). Blood was immediately centrifuged at room temperature for 3 minutes at 3500g, and plasma was aliquoted. Routine hemostasis parameters were determined directly with immunoturbidimetry (ITM). Sysmex CA-8000 was used for blood coagulation determination. Complete blood count was performed with Sysmex XE-2100 or XS-800i.

Routine clinical Parameter: All routine hemostasis parameters such as prothrombin time (PT), activated partial thromoplastin time (APTT), fibrinogen (FBG), thromobine time (TT) and D-dimer (DD) were determined using the commercial kits (Sysmex, Japan) including the corresponding normal and standard plasma. The normal range of the

parameters is listed as followings: DD 0.00-0.55mg/L; PT 9-13 second; APTT 20-40 second; TT 14-21 second; FGB 2-4 g/L.

Computed tomography (CT) and Magnetic Resonance Imaging (MRI) were used for disease clinical staging. The status of tumor metastasis was detected by Emission Computed Tomography (ECT) and positron emission tomography (PET CT). The deep vein thrombosis and pulmonary embolism was diagnosed with Colorful Doppler, spiral CT and Venography respectively.

Statistical analysis: All of patients were divided into two groups according to the treatment of Botropase. These unpaired samples were analyzed by *t* test. P<0.05 was considered as statistically significant.

Results

The 48 patients with abnormal DD value distributed among 13 different types of patients with cancer, as shown in Table 1. They suffered from the local bleeding (such as gastrointestinal tract, respiratory tract, genitourinary tract, nasal cavity), and were intravenously given Botropase at a dosage of 1~2 ku/6 hours until arresting bleeding. The routine hemostasis parameters and complete blood count (CBC) were detected in 3 days before and during the treatment of Botropase.

Table 1. Demographics of the 48 patients with solid tumors

		N
Age	Average	58
	Range	27~83
Gender	Male	31
	Female	17
Cancer Stage	IV	48
Type of Cancer	Nasopharyngeal Carcinoma	2
	Lung Cancer	5
	Gastric Cancer	14
	Ovarian Cancer	2
	Melanoma	2
	Cervical Cancer	3
	lymphoma	2
	Pancreatic Cancer	2
	Breast Cancer	3
	Colonal Cancer	5
	Prostate Cancer	2
	Esophageal Cancer	4
	Biliary tract carcinoma	2

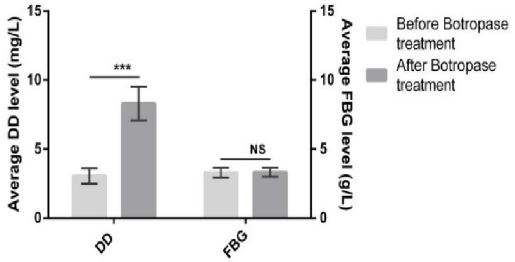

Figure 1. The influence of Botropase on D-Dimer and FBG level. DD increased dromatically after the treatment of Botroposae (p<0.01); P>0.05 between FBG groups. Presented as mean with SEM. DD: D-dimer; FBG: Fibrinogen.

The average D-dimer level were significantly increased following Botropase treatment (p<0.01, Figure 1), in contrast to the level before Botropase treatment, which detected by immunoturbidimetry. Nevertheless, there was no significant difference of FBG between before and after Botropase treatment (p>0.05, Figure 1). Other hemostasis parameters, such as PT, APTT and TT, were also not significantly affected by Botropase administration (p>0.05, Figure 2).

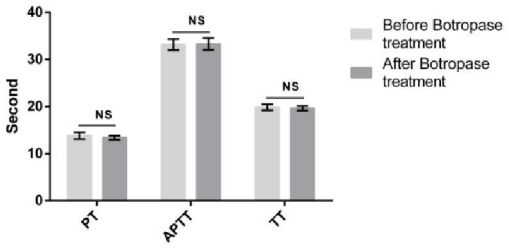

Figure 2. The effect of Botropase on coagulation. No significant difference between before and after the treatment of Botropase (p>0.05). Presented as mean with SEM. PT: Prothrombin time; APTT: Activated partial thromboplastin time; TT: Thromobine time.

The values of white blood cell, red blood cell and platelet before and after Botropase treatment were compared respectively, in consideration of the usual influence of the intravascular coagulation on complete blood count. The Statistical results were listed in Table 2, and indicated no significant difference between two groups (p>0.05).

Table 2. Comparison complete blood count (CBC) between before and after treated with Botropase

	Before treatment (X±SD)	After treatment (X±SD)	*t*	*p*
WBC（x10^9/L）	9.92±5.34	10.35±6.31	0.34	0.738
RBC（x10^{12}/L）	3.32±0.87	3.19±0.94	0.25	0.254
Hb (g/L)	99.47±26.64	95.41±27.78	1.08	0.283
Platelet（x10^9/L）	180.00±122.64	184.82±127.21	0.30	0.765

Results are presented as average value ±SD. Normal value of CBC: White blood cell (WBC) 3.5~9.5x109/L; Red blood cell (RBC) 10~12x1012/L; Hemoglobin (Hb) 130~175g/L; Platelet 125~350x109/L.

Discussion

Botropase is commonly used to arrest bleedings of various etiology. It is believed that Botropase functions by plasma fibrinogen converting into fibrin/fibrin polymer, which triggers the adhesion and cohesion of platelet to produce a clot. [6,7] The patients with cancers, especially in IV phase, usually encounter both the hypercoagulable plasma state and the bleeding tissue. We investigated the effect of Botropase on hemostasis-related profiles in such kind of patients.

Our results demonstrated a hallmark that D-dimer remarkably increased (p<0.01) on the baseline of its abnormally higher level in all 48 patients. This suggested that fibrinogen level decreased and fibrinogen and fibrin degradation products increased after the administration of Botropase. In animal study, Botropase caused not only defibrinogenation but also prolonging of PT and APTT. [9,10] However, Our data did not show any significant extension (P>0.05) in both PT and APTT by comparing their levels before Botropase treatment, which consisted with the reported in normal human. [11] In fact, the average level of the above two parameters showed a slight decrease (P>0.05) after the treatment of Botropase. This suggests that the treatment of Botropase can alleviate the hypercoagulable plasma state of cancer patients by its fibrinolysis. Obviously, the effect of defibrinogenation reduces blood viscosity, and maybe influences DIC nascency in these patients.

In late stages of cancer, patients with a large tumor burden, metastasis and bone marrow infiltration are at risk developing clotting abnormality, which may consume platelet. Some of 48 patients with high D-dimer level recruited in this retrospective study may suffer local tumor-associated microclots. We did not find the any dropping in platelet and red blood count following the increasing of DD after treatment of Botropase. In experimental model of DIC in rats, Botropase could partially inhibit the fall in platelet count. [9,10] In normal healthy human volunteers, Botropase significantly reduced the level of fibrinogen and fibrin degration products but did not alert platelet and red blood count. [11] Interestingly, the treatment of Botropase slightly increased the average level of platelet in our data, but the increase was statistically not significant. This suggested that the administration of Botropase could interrupt the coagulation processing, triggered by tumor associated mediators (e.g. tissue factors, plasminogen activators and vascular permeability-enhancing factors), rather than induce DIC.

The results of this retrospective study reflect the defibrinogenation effect of Botropase. In these 48 patients, Botropase exerted itself on coagulation activation to arrest bleeding, but there were no evidences that it induced microclots formation and even DIC. It may be safe to use Botropase in the cancer patients with hypercoagulable plasma state.

Correspondence to:
Yiwu Sun
Department of Hematology, Changzhou Second People's Hospital, The affiliated hospital of Nanjing Medical University, 68 Gehu Road, Changzhou, Jiangsu, China, 213164.
E-mail: yiwu_sun@hotmail.com

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