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SHORT COMMUNICATION

Prolonged Hemocoagulase Agkistrodon Halys Pallas Administration Induces Hypofibrinogenemia in Patients with Hematological Disorders: A Clinical Analysis of 11 Patients

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Abstract Hemocoagulase Agkistrodon Halys Pallas, a well-established hemostatic agent used in China, has widespread applications in various conditions of bleeding and hemorrhages. Although it presents a low risk of vascular thrombosis, the agent can be easily tolerated and does not show any harmful effects, except allergy. However, long-term use of hemocoagulase may induce hypofibrinogenemia in some patients. Herein, we report 11 patients of prolonged administration of hemocoagulase Agkistrodon, with particular attention to the dynamic changes in their coagulation functions. A marked decline in the level of fibrinogen was observed in patients suffering from hematological disorders, following an extended exposure to hemocoagulase. Nevertheless, the low fibrinogen levels increased after withdrawal of the agent. Since the D-dimer level did not show any significant increase, hemocoagulase inducing lower fibrinogen may be considered to be a process of primary fibrinolysis. In conclusion, we suggested that hemocoagulase must be cautiously used in patients with hematological disorders, because of the potential risk of fibrinolysis; and the coagulation functions should be carefully evaluated during the administration of hemocoagulase.

Keywords Batroxobin · Anemia · Aplastic · Fibrinolysis · Afibrinogenemia · Myelodysplastic syndromes

Hemocoagulase Agkistrodon Halys Pallas, hereafter referred to as hemocoagulase, is a well-established hemostatic agent used in China. Currently, it is widely utilized in various situations, such as traumatic bleeding, pediatric pulmonary hemorrhage, joint operations, gastrointestinal bleeding, and other surgeries [1–4]. The main mechanisms underlying the hemostatic action of hemocoagulase are as follows: (1) it divides the α-subunit of fibrinogen into A-peptide and fibrin monomers, which in turn polymerize into insoluble fibrin polymers or fibers under the promotion of factor XIII; (2) it can prevent the release of factor XIII, which markedly decreases the risk of thrombosis [5, 6]. When used for a short period of time, the agent can be well-tolerated and barely shows any adverse effects other than allergies [5, 7]. In China, many physicians are using this agent in patients with low platelet count in order to reduce the risk of bleeding in those patients. However, there is limited data available on the maximum cumulative doses of hemocoagulase that can be used. Furthermore, it remains obscure whether prolonged (defined as longer than 7 days) use of this agent will result in any hemostatic disorder. In order to address these questions, we conducted a retrospective study. In the present study, 11 patients were reported on whom hemocoagulase was administered for a prolonged period of time. Furthermore, side effects of hemocoagulase on coagulation function in these cases were documented and analyzed.

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Materials and Methods

Ethics Statement

This retrospective study was conducted in accordance with the principles of the Declaration of Helsinki [8]. The study protocol was approved by Ethics Committee of the First Affiliated Hospital of Zhejiang Chinese Medical University, Zhejiang, China (No. 2016-LW-007-01). All the patient records were anonymized and de-identified, prior to analysis.

Patients

Eligible patients had a diagnosis of hematological disorder and were all treated with hemocoagulase over a consecutive 7-day. Exclusion criteria were supportive transfusions of plasma, prothrombin complex concentrates, cryoprecipitate and human fibrinogen. 36 patients who received prolonged use of hemocoagulase were screened for this retrospective study. All of them were admitted to Zhejiang Provincial Hospital of TCM from January 2016 to March 2016. 25 patients were ineligible because fresh frozen plasma were introduced in the treatment, which made the interpretation of their coagulation functions elusive. 11 patients (including 7 males and 4 females), were eligible and evaluable. Their median age was 31 years, with a range of 11–68 years. The cumulative dose of hemocoagulase administered to each patient was documented. Weight adjusted dose of hemocoagulase was defined as the cumulative dose of hemocoagulase divided by the patient's weight.

Use of Hemocoagulase

The dosage and duration of the hemocoagulase administration varied and were determined by physicians on personal experience. Generally, hemocoagulase were intravascularly administered to a patient with platelet count less than 20,000/mL at a dose of 2U/d.

Evaluation of Coagulation Functions

The coagulation functions were evaluated 2 times at least, before the initiation of hemocoagulase administration and the next day to the cessation of hemocoagulase administration. A 2–3 mL sodium citrate-anticoagulated peripheral blood was obtained from all the patients. All the samples were obtained around 6 a.m. The coagulation function parameters, viz. prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (FIB), and D-dimer (D-D) were analyzed by an automatic coagulation analyzer (Sysmex CA7000, Japan) according to the manufacturer's instructions. FIB was estimated by coagulation method using Dade Thrombin reagent.

Statistical Analysis

Statistical analyses were performed using SPSS 22.0 (IBM corp., Armonk, NY, USA). Overlay scatterplot were graphed by Prism 6.0c (GraphPad Software, La Jolla, CA, USA). Statistical differences between two arms were assessed by Wilcoxon signed ranks test. A P value of <0.05 (2-tailed) was considered significant.

Results

Clinical Features of the Patients

All the 11 patients included in the present study were primarily diagnosed with hematological disorders. Given the high risk of hemorrhage, all the patients received one prophylactic consecutive administration of hemocoagulase; except for one patient, who received an additional round of the agent following the recovery of her coagulation functions (patient 6 and 7 refer to the same patient). None of them showed evidence of severe hepatic dysfunction. No schistocyte was found in any of their peripheral blood smears. Disseminated intravascular coagulation (DIC) scores were calculated according to the scoring system recommended by International Society on Thrombosis and Haemostasis [9]. No diagnosis of overt DIC was established because all the scores were lower than 5 points. The median cumulative dose of hemocoagulase was estimated at 38 KU (with a range of 20–154 KU); and the median weight adjusted dose was 0.79 KU/kg (ranging from 0.38 to 3.21 KU/kg). Other specific information regarding the clinical features of the patients has been summarized in Table 1.

Coagulation Functions

The data on the coagulation functions before and after hemocoagulase administration are listed in Table 2. A non-parametric paired test (Wilcoxon signed ranks test) was adopted to compare the four coagulation function parameters: PT, APTT, FIB, and D-D in arm 1 and arm 2. No significant differences were found in PT, APTT, or D-D between arm 1 and arm 2 ($P = 0.387$, 0.583, and 0.666, respectively). Notably, FIB in arm 2 was significantly lower than that in arm 1 ($P = 0.003$), as presented in Table 2 and Fig. 1.

Table 1 Clinical features of the 11 patients under study

Patient	Gender	Age	Weight (kg)	Child pugh score	Primary diagnosis	Chemo	Allergy	FFP transfusion	Cumulative dose of hemocoagulase (KU)	Weight adjusted dose of hemocoagulase (KU/kg)	Platelet transfusion	Bleeding	ISTH DIC score
1	M	35	54	Grade A	SAA	No	No	No	40	0.74	Yes	Intracranial hemorrhage	4
2	F	21	55	Grade A	SAA	No	No	No	24	0.44	Yes	No	2
3	F	47	63	Grade A	SAA	No	No	No	68	1.08	Yes	No	3
4	M	17	48	Grade A	SAA	No	No	No	154	3.21	Yes	No	3
5	M	11	23.5	Grade A	SAA (syngeneic HSCT)	Yes	No	No	20	0.85	Yes	No	2
6	F	31	59	Grade A	AML	Yes	No	No	24	0.41	Yes	Petechia	4
7	F	31	59	Grade A	AML	Yes	No	No	32	0.54	Yes	No	2
8	M	68	81	Grade A	MDS	Yes	No	No	68	0.84	Yes	No	3
9	F	50	53	Grade A	MDS	Yes	No	No	68	1.28	Yes	Petechia	3
10	M	24	95	Grade A	NSAA	No	No	No	36	0.38	Yes	No	3
11	M	12	49.5	Grade A	NSAA	No	No	No	44	0.89	Yes	No	3
12	M	59	59	Grade A	MM	Yes	No	No	28	0.47	Yes	Gingival bleeding	3

Patient 6 and 7 refer to the same patient

Chemo Chemotherapy; *FFP* fresh frozen plasma; *ISTH* International Society on Thrombosis and Haemostasis; *DIC* disseminated intravascular coagulation; *M* male; *F* female; *SAA* severe aplastic anemia; *HSCT* hematopoietic stem cell transplantation; *AML* acute myeloid leukemia; *MDS* myelodysplastic syndrome; *NSAA* non severe aplastic anemia; *MM* multiple myeloma

Table 2 Evaluation of coagulation functions before and after hemocoagulase administration

Patient	Arm 1 (before hemocoagulase administration)				Arm 2 (after hemocoagulase administration)			
	PT (second)	APTT (second)	FIB (g/L)	D-D (mg/L)	PT (second)	APTT (second)	FIB (g/L)*	D-D (mg/L)
1	11.70	33.20	3.36	3.33	14.10	29.20	0.58	2.88
2	12.00	23.70	1.01	0.17	11.70	27.90	1.22	0.30
3	10.00	17.00	1.51	0.91	11.70	24.00	0.85	1.03
4	11.30	26.20	3.64	0.70	12.20	30.30	1.23	0.18
5	11.60	35.90	1.91	0.29	11.40	30.30	0.67	0.59
6	12.60	26.50	2.21	12.82	11.60	23.50	0.79	0.78
7	11.90	38.00	1.37	0.36	13.10	35.30	0.70	0.67
8	11.80	26.10	1.90	1.12	12.60	21.40	0.92	1.64
9	11.60	23.90	2.91	0.95	11.30	22.90	0.71	2.40
10	11.10	28.10	2.35	0.70	10.30	24.70	1.12	0.37
11	11.60	34.10	5.17	0.81	10.80	24.20	2.05	1.43
12	13.30	31.00	2.24	0.80	13.60	35.60	0.71	0.81

Patient 6 and 7 refer to the same patient

PT prothrombin time; *APTT* activated partial thromboplastin time; *FIB* fibrinogen; *D-D* D-dimer

* $P = 0.003$ versus the counterpart in arm 1 as analyzed by the Wilcoxon signed ranks test

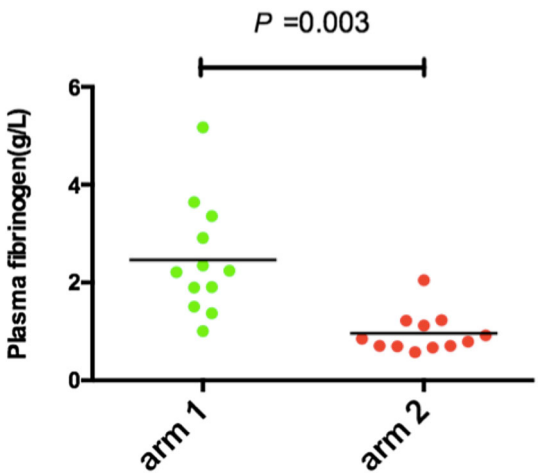

Fig. 1 Plasma fibrinogen levels in arm 1 and arm 2

Discussion

Hemocoagulase is a complex mixture of thrombin-like enzymes derived from the venom of the snake, Agkistrodon. Since the discovery of the first hemocoagulase, batroxobin, in 1954 [10], more than 40 different thrombin-like enzymes have been isolated and characterized. Hemocoagulase catalyzes the last reaction in the process leading to clotting of the blood protein fibrinogen [11], which resembles the physiological function of human thrombin. However, hemocoagulase does not induce or release the factor XIII, a fibrin-stabilizing factor crosslinking fibrin. In addition, the clot induced by hemocoagulase has been confirmed to lyse in urea solution [12]. Hence, hemocoagulase is thought to cause very low risk of thrombosis, in comparison to the human thrombin.

At present, hemocoagulase is widely used in ameliorating traumatic bleeding, pulmonary and gastrointestinal hemorrhages, and in preventing hemorrhages particularly caused by invasive procedures. The cumulative dose of hemocoagulase varies among different cases, depending on its specific use. In our study, the minimal cumulative dose of hemocoagulase reached 20 KU, with a median of 38 KU; while the median weight adjusted dose was assessed as 0.79 KU/kg. The cumulative dose of hemocoagulase in the present study was superior to the amounts reported in previous studies [13–15]. The major adverse drug reaction of hemocoagulase is allergy [5, 14, 16], which is attributed to its nature of allo-protein immunity. In some cases, the anaphylactic reaction induced by hemocoagulase could be fatal [16]. Therefore, patients with a previous anaphylactic reaction to venom from Agkistrodon halys (Pallas) should not be exposed to hemocoagulase again.

However, use of hemocoagulase in patients with hematological disorders still remains a controversy. Moreover, publications related to this issue are relatively meager. So far, no clinical trials or retrospective studies have established that hemocoagulase reduces the risk of hemorrhage in patients with hematological disorders. In this study, patients who mainly presented with bone marrow failures, such as AA and MDS, received prolonged exposures to hemocoagulase. However, physical signs and laboratory tests demonstrated no clues of severe hepatic dysfunction or microangiopathic hemolysis in these cases.

We reevaluated the patients' peripheral blood smears, but no schistocyte was found. And we failed to establish a diagnosis of overt DIC as the ISTH score was lower than 5 points. Other concomitant agents, such as cyclosporine, tarcolimus, androgen mimics, carbazochrome sodium sulfonate, and antibiotics were judiciously excluded from the list of suspicious agents causing hypofibrinogenemia. Fortunately, the low fibrinogen status was reversible and the level of fibrinogen increased after withdrawal of hemocoagulase. In certain severe cases, an aggressive salvage therapy, such as fresh frozen plasma transfusion or human fibrinogen injection was needed. As a result, hemocoagulase is highly suspected to induce the hypofibrinogenemia, on the basis of its fibrinolytic character as well as sporadic reports of hypofibrinogenemia induced by it. Wang et al. [15] reported three patients who showed prolonged PT and APTT results, and developed significant hypofibrinogenemia following long-term administration of hemocoagulase. The extreme hypofibrinogenemia observed in their study could have affected the PT and APTT tests. Although hemocoagulase does activate some coagulative factors [11], it is difficult to conclude that it prolongs or shortens PT and APTT, based on our results. Nevertheless, limited sample size in both the studies may essentially lead to a sampling bias. Therefore, further studies with larger sample size are warranted to elucidate the cumulative dose-effect of hemocoagulase on PT and APTT tests.

Interestingly, the level of D-dimer did not increase significantly. Overall, our findings demonstrated that the lower fibrinogen level induced by hemocoagulase is most likely a process of primary fibrinolysis; this is in accordance with the results of previous experimental studies on hemocoagulase. Nevertheless, this phenomenon could result in unnecessary challenges for physicians [15], or even fatal hemorrhages in some patients, especially when their platelet count remains low. Of particular interest is the application of hemocoagulase in patients with ischemic stroke/transient ischemic attack (TIA) for secondary prevention. A randomized, controlled clinical trial carried out by Xu et al. [17] enrolled 112 ischemic stroke/TIA patients with concomitant hyperfibrinogenemia, out of which 52 were treated with batroxobin and 60 without batroxobin. It was found that the stroke/TIA recurrence in patients without batroxobin was higher than in patients with batroxobin after a 1-year follow-up.

Taken together, our study clinically indicated that prolonged hemocoagulase administration lowers plasma fibrinogen, probably through primary fibrinolysis. However, hemocoagulase should be used cautiously in patients with hematological disorders, because of the potential fibrinolytic risk. Furthermore, frequent monitoring of coagulation function should be prefered during the period of hemocoagulase administration.

Author Contributions Wu Dijiong contributed to the conception of the study. Xu Linglong contributed to data collection, performed the data analyses and wrote the manuscript.

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