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Effect of Administration of Neurokinin B Agonist (Senktide) and Antagonist (SB222200) on Hematological and Coagulation Parameters in Adult Male Rats

Faiqah Ramzan,[1] Muhammad Waqas Luqman,[2] Tahira Atta,[3] Muhammad Haris Ramzan[3]

1. FVAS, Gomal University, Dera Ismail Khan, Pakistan
2. Khyber Medical University Institute of Medical Sciences (KMU-IMS), Kohat, Pakistan / The University of Texas, USA
3. Khyber Medical University Institute of Medical Sciences, Kohat, Pakistan

ABSTRACT

Background: Neurokinin B is known as a regulator of reproduction, however its chronic effects on hematological and coagulation parameters in adult male mammals are not well understood
Objective: To explore the effects of Senktide (Neurokinin B agonist) and SB222200 (Neurokinin B antagonist) administration on hematological and coagulation parameters in adult rats.
Materials and Methods: This laboratory-based experimental study was conducted at Gomal University and KMU Institute of Medical Sciences from February 2019 to April 2021. Healthy adult male *Sprague Dawley* rats were randomly assigned to three groups (n=5 animals/group). They were administered intraperitoneally with Senktide (1µg) and SB222200 (1µg) consecutively twice daily for 12 days. Control rats received distilled water injections. The animals were sacrificed after 12-day treatment. Blood samples were collected directly from the left ventricle to analyze hematological and coagulation parameters.
Results: Senktide-treated rats showed significantly prolonged bleeding time (111±17.10sec) compared to SB222200 (27±12.55sec) and control (33±12.55sec). Clotting time was also higher (Senktide: 96±17.10sec; SB222200: 39±8.22sec; control: 42±12.55sec), as were prothrombin time [PT] (Senktide: 90±14.58sec; SB222200: 15.80±3.90sec; control: 15±4.12sec), activated partial thromboplastin time [APPT] (Senktide: 92±25.25sec; SB222200: 16.20±3.19sec; control: 19.40±2.97sec) and international normalized ratio (Senktide: 6.60±1.07; SB222200: 1.16±0.29; control: 1.10±0.30) in Senktide-treated rats. Mean platelet volume (Senktide: 8.88±0.33fl; SB222200: 7.92±0.29fl; control: 8.04±0.48fl) and leukocyte count (×10³/µl) (Senktide: 12±0.79; SB222200: 8.89±0.97; control: 8.87±1.32) were significantly elevated however platelet count (×10³/µl) (Senktide: 792.20±50.39; SB222200: 968.60±45.33; control: 960.60±58.71) was reduced in Senktide-treated rats.
Conclusion: Senktide administration led to leukocytosis, thromboctopenia, and affected coagulation pathways with prolonged BT, CT, PT and APTT.
Keywords: Activated Partial Thromboplastin Time, Blood Coagulation, Blood Coagulation Tests, Neurokinin B, Platelets, Prothrombin Time, Senktide, SB222200, Tachykinins.

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INTRODUCTION

Neurokinin B, a decapeptide, belongs to the family of peptide hormones called tachykinins.[1] Biological actions of the tachykinins are mediated through three different receptors namely NK1 (Neurokinin-1), NK2 and NK3 which belongs to the G-protein coupled membrane receptor.[1] Tachykinins (Substance P, Neurokinin A and Neurokinin B) preferentially binds to their receptors NK1, NK2 and NK3 respectively. However, these peptides can bind with a lower affinity to each of these receptors.[1] Neurokinin B is produced from the pre-pro-tachykinin-B gene,[1] cleaved enzymatically to form pro-neurokinin B, which is further processed into Neurokinin B.[2] Biological effects of Neurokinin B are primarily mediated through neurokinin receptors, particularly NK3, which are extensively distributed throughout the central nervous system and peripheral tissues.[1]

The critical importance of Neurokinin B was first evidenced by its significant role in reproductive health. Research has demonstrated that mutations in the TAC3 gene, which encodes Neurokinin B, can result in hypogonadotropic hypogonadism.[3,4] The peripheral or central Neurokinin B administration, along with its agonists and antagonists, has produced varying effects on different biological processes. The intracerebral Neurokinin B or its agonist (Senktide) administration, leads to elevated levels of reproductive hormones in male rodents.[5] Antagonists of Neurokinin B exhibit strong suppressive effects on follicular growth and estradiol secretion in females.[6]

Beyond its role in reproduction, Neurokinin B is involved in a spectrum of biological processes. Its activity has been observed in immune responses, inflammation, and even in conditions such as pre-eclampsia, menopause, and cancer.[2,7-9] Tachykinins can activate immune cells and promote the production of inflammatory molecules.[10] Tachykinins (Neurokinin B and Substance P) are linked to the development of certain cancers, including breast and gastric cancer., Blocking NK1 receptors has been shown to reduce platelet aggregation and thrombus size, highlighting the important role of tachykinins in coagulation.[12]

Bleeding disorders, inherited in an autosomal recessive manner, are common worldwide. Pakistan's high rate of consanguineous marriages likely increases

Correspondence: Dr. Muhammad Haris Ramzan
Associate Professor,
Khyber Medical University Institute of Medical Sciences, Kohat, Pakistan
Email: mharisramzan@gmail.com

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the prevalence of coagulation disorders, posing significant public health challenges.[13] However, these disorders often remain undiagnosed due to a lack of specialized diagnostic facilities, low public awareness, and low clinical suspicion.[13] Insights into how Neurokinin B influences blood cell counts and clotting mechanisms could lead to new therapeutic approaches for managing these conditions. Limited information exists regarding impact of Neurokinin B on hematological and coagulation parameters.

While Neurokinin B plays a crucial role in the onset and maintenance of puberty and reproduction, limited information exists regarding its impact on hematological and coagulation parameters. Therefore, the study intended to assess the impact of Senktide (Neurokinin B agonist) and SB222200 (Neurokinin B antagonist) on hematological and coagulation parameters in adult male rats following intraperitoneal administration.

MATERIALS AND METHODS

This laboratory-based experimental study was conducted at Gomal University, Dera Ismail Khan and KMU Institute of Medical Sciences, Kohat from February-2019 to April-2021. Animals were acquired from the National Institute of Health, Islamabad. Healthy adult male *Sprague Dawley* rats having an average weight of 250 – 300 grams were included in the study. Adult male rats that were already on drug therapy, used in any study/experiment within the last month, or on medication for any disease in the past month were excluded. The animals were housed in steel mesh cages during the study. Each steel mesh cage contained five rats. The animals were acclimatized for 10 days before the experiment commenced, maintained on a 12-hour light-dark cycle at a temperature of 25±2°C, with access to food and water *ad libitum*. European Union Regulations for the use of animals in research were followed for animal handling during the experimental period. 14 The present study was approved by the Ethical Committee of Khyber Medical University vide letter # DIR/KMU-EB/EN/000451, dated 19-04-2018.

Senktide – a Neurokinin B agonist was acquired in lyophilized form from Phoenix Pharmaceuticals Inc. (Burlingame, CA, USA), while SB222200 – a Neurokinin B antagonist, was obtained in lyophilized form from MedChemExpress LLC (Princeton, NJ, USA). Both the peptides were separately dissolved in of dimethyl sulfoxide (1 ml) [VWR, USA] to create a stock-solution. Distilled water was then added to these solutions for intraperitoneal (i.p.) injection. The rats were randomly divided into three groups (n=5 animals per group). Control group received intraperitoneal injections of distilled water, while 1 µg of Senktide and 1 µg of SB222200 were administered to peptide treated groups. Intraperitoneal injections were administered twice daily, every 12-hours, for 12-days consecutively.

After 12-days of peptide treatment, the animals were anesthetized with sodium pentobarbital (60 mg/kg body weight i.p.) three hours after the last peptide dose and then sacrificed.

To determine the effect of Neurokinin B treatment on coagulation parameters, blood samples were collected from the left ventricle and transferred into blue top vacutainers containing 3.2% trisodium citrate [for PT (prothrombin time) and APTT (activated partial thromboplastin time) analysis] and purple top vacutainers with EDTA.K3 (for complete blood count analysis). PT and APTT were measured using Soluplastin and APTTest kits (Wiener Lab, Rosario, Argentina), following the manufacturers' instructions and INR (international normalized ratio) was calculated. Complete blood counts were analyzed using an automated hematology analyzer (RT-7600, China). Bleeding time (BT) was measured using a modified Duke's method, with a 3 mm incision on the rat tail and timing until bleeding ceased.[15] Clotting time (CT) was assessed using the capillary tube method, timing until clot formation upon breaking the tube every 15 seconds.

Statistical Package for Social Sciences (SPSS) version 26.0 was used for the statistical analysis. Data were presented as mean ± SD. Statistical significance was assessed using one-way ANOVA followed by post hoc Tukey's test, with $p<0.05$ considered statistically significant.

RESULTS

Throughout the experiment, all peptide-treated rats remained healthy as assessed by their vital signs, overall condition of the body, eating habits, drinking habits, and activity inside the cage. However, a notable exception was observed in the rats treated with Senktide that their incised wounds continued to bleed for more than 30 minutes post-treatment. In contrast, rats that served as control (received distilled water treatment) and those received SB222200 showed no excessive bleeding tendency.

The study found a significant increase ($P<0.001$) in the total leukocyte count in the Senktide-treated group (12±0.79 $\times10^3$/µl) compared to the control group (8.87±1.32 $\times10^3$/µl) and the SB222200 (8.89±0.97 $\times10^3$/µl) (Table 01).

The red blood cell count (RBC) did not show significant differences among the peptide treated and control groups. Parameters such as RDW, hemoglobin levels, MCH, MCHC, MCV and hematocrit did not exhibit significant ($P>0.05$) variations among the groups.

The platelet count showed a significant decrease in the Senktide-treated group (792.20±50.39 $\times10^3$/µl) compared to SB222200 (968.60±45.33 $\times10^3$/µl) and control group (960.60±58.71 $\times10^3$/µl) (Table 01). Mean

platelet volume (MPV) exhibited a significant increase in Senktide-treated animals compared to SB222200-treated and control groups (Table 01).

Bleeding Time (BT) and Clotting Time (CT) were significantly prolonged ($p<0.001$) in the Senktide treated animals (111±17.10 sec and 96±17.10 sec) as compared to SB222200 (27±12.55 sec and 39±8.22 sec) and control group (33±12.55 sec and 42±12.55 sec) (Figure 01). Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) were significantly prolonged ($p<0.001$) in the Senktide group (90±14.58 sec and 92±25.25 sec) as compared to SB222200 group (15.80±3.90 sec and 16.20±3.19 sec) and control group (15±4.12 sec and 19.40±2.97 sec) (Figure 02). International normalized ratio (INR) exhibited significant ($p<0.001$) elevation in Senktide group (6.60±1.07) compared to SB222200 (1.16±0.29) and control group (1.10±0.30).

Table 01. Hematological Parameters of the Control, Senktide and SB222200 Treated Groups

Variables	Control +	SB222200	Senktide
Total Leukocyte Count (103/µl)	8.87±1.32	8.89±0.97	12±0.79***
RBC Count	6.74±0.37	6.79±0.41	6.69±0.40
Hemoglobin (g/dl)	11.72±0.45	11.82±0.44	11.16±0.38
Hematocrit (%)	38.98±1.39	38.58±1.27	35.24±1.49**
Mean Corpuscular Volume [MCV] (fl)	54.84±2.78	55.24±2.15	54.06±1.50
Mean Corpuscular Hemoglobin [MCH] (ρg)	16.68±0.79	16.48±0.76	17.12±0.73
Mean Corpuscular Hemoglobin Concentration [MCHC] (g/dl)	30±0.30	30.60±0.92	31.66±0.48
Red Blood Cell Distribution Width [RDW] (%)	38.90±0.78	34.50±1.11	34.08±1.11
Platelet Count (103/µl)	960.60±58.71	968.60±45.33	792.20±50.39***
Mean Platelet Volume [MPV] (fl)	8.04±0.48	7.92±0.29	8.88±0.33**
Platelet Distribution Width [PDW] (%)	15±0.20	15.06±0.30	15.16±0.31

***P<0.001 and **P<0.01 compared to control; + data published previously[16]

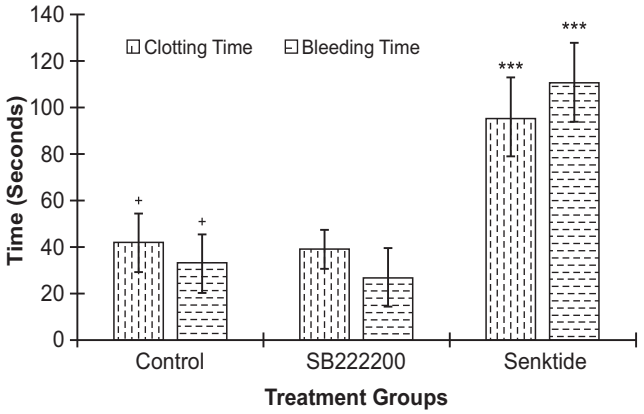


Figure 01. Bleeding time and clotting time of the control and peptide (SB222200 and Senktide) treated groups (Significance of Senktide treated animals as compared to control and SB222200 is shown as *; ***P<0.001); +data published previously[16]

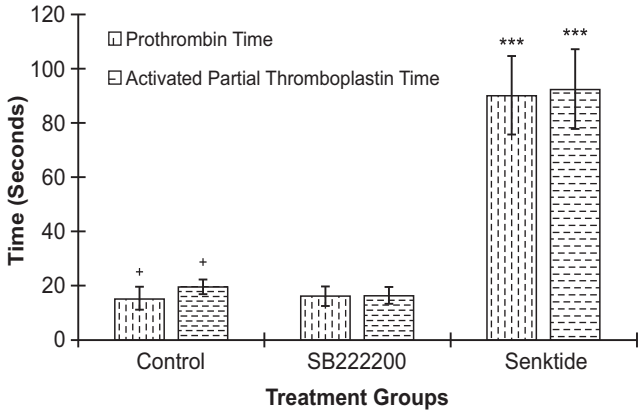


Figure 02. Prothrombin time (PT) and activated partial thromboplastin time (APTT) of the control and peptide (SB222200 and Senktide) treated groups (Significance of Senktide treated animals as compared to control and SB222200 is shown as *; ***P<0.001); +data published previously[16]

DISCUSSION

The present study investigates the anticoagulation effects of intraperitoneally administrated Senktide (Neurokinin B agonist) and SB222200 (Neurokinin B antagonist) in adult male rats. We observed a significantly increased CT and BT with increased PT, APTT, INR and TLC as compared to control and SB222200. Conversely, a decreased platelet count in Senktide treated rats was observed in Senktide treated animals compared to control (Table 01 and Figure 02).

Prolonged bleeding time is linked with the thrombocytopenia, impaired platelet ability to adhere with vascular endothelium, von Willebrand disease and disseminated intravascular coagulation. Increased clotting time is associated with hemophilia, deficiencies of the factors involved in clotting pathways (II, V, XI, XII) and congenital coagulation disorders. Elevated PT reflects the activity of the extrinsic coagulation pathway, and is associated with the deficiency of vitamin K or the acute/chronic liver disease. Conversely, APTT evaluates intrinsic coagulation pathway and it is typically performed when there is unexplained bleeding or clotting.[17,18]

In present study, we observed significantly increased clotting and bleeding times, along with prolonged PT and APTT, suggesting an impact on both intrinsic and extrinsic coagulation pathways. We also noted a marked decrease in platelet count, indicating a disruption in the normal coagulation process. It's worth mentioning that prolonged bleeding time is often associated with thrombocytopenia. In spite of the prolonged BT, PT and APTT, coupled with reduced platelet count, the occurrence of disseminated intravascular coagulation (DIC)[17] as a reason for increased bleeding propensity is highly unlikely. This is supported by the fact that the Senktide treated rats remained active, healthy and survived throughout the study period without displaying any signs of internal hemorrhage, sepsis, infection, or liver damage (data not shown). Therefore, our study suggests that Senktide likely blocks the coagulation pathway at its initial stages.

Prolongation of the PT and APTT was observed in Senktide treated rats which suggests interference with the availability of the vitamin K, however it's important to note that vitamin K deficiency typically does not affect bleeding time or platelet count.[17,18] Therefore, while it is speculative at this stage to conclude that Senktide directly impedes the availability of vitamin K to stimulate its anticoagulation effects. However, the possible role Senktide in down-regulating the vitamin K dependent pathways, cannot be entirely overruled.[17]

Senktide has a short plasma half-life and blood samples were collected at least three hours after the last dose of the peptide and anticoagulation profile was assayed. This suggests that the primary anticoagulation effects might occur shortly after peptide administration. This rapid onset of action is similar to anticoagulants like argatroban and heparin, which also induce anticoagulation effects in a short time frame.[19] Despite using citrated blood for the conduction of PT and APTT tests—standard for all coagulation tests[20]—the observed anticoagulation effect is more likely attributable to Senktide treatment rather than sodium citrate.

The results of the present study demonstrated a negative association between the thrombocytes and MPV. Presence of bleeding disorders or iron deficiency or anticoagulants may present with a similar scenario.[21] Patients having heterozygous thalassemia may have increased MPV. Conversely a decreased MPV has been observed in subjects undergoing chemotherapy. This converse relationship between thrombocyte size and count is valuable in assessing abnormal thrombocyte production.[21]

In present study, marked thrombocytopenia was evident with Senktide treatment. Heparin-induced thrombocytopenia is a serious and often underestimated adverse effect of heparin treatment which occurs in about 2% of individuals when they are exposed to heparin for more than four days.[22] Heparin is commonly prescribed for the treatment of deep vein thrombosis and pulmonary embolism. It is a powerful anticoagulant and it significantly increases the formation of thrombin-antithrombin.[17]

Although Senktide appears to act similarly to heparin, it is a small peptide, and its mechanisms may differ. The exact mechanism behind Senktide-induced thrombocytopenia remains unknown, however the present data suggested a direct action of the peptide on platelets. Discerning reduction in thrombocytes with increased WBC counts, supports the notion of Senktide-induced thrombocytopenia. Additionally, there was no significant change in hemoglobin content and hematocrit, while mean corpuscular hemoglobin (MCH) increased with Senktide treatment (Table 01).

In vivo, Senktide-induced thrombocytopenia might result from modulation of megakaryocyte production. Thrombocyte count can be used as a prognostic index as the production of the thrombocytes was stimulated by many neoplasms through initiation of megakaryocyte production.[23] Although platelet aggregation tests were not conducted in this study, Senktide-induced disaggregation of thrombocytes might be accountable for the observed anticoagulation effects.

With Senktide treatment, we observed a significant reduced thrombocyte count with prolonged anticoagulation profiles. These conditions are typically associated with end-stage liver disease, disseminated intravascular coagulation or massive transfusion reactions.[17,18] Although liver function tests were not performed, necropsy of the peptide-treated rats showed no signs of liver damage. Additionally, the animals enrolled in the present study were healthy throughout the experiments and did not show any signs of sepsis as mentioned earlier. Given that the administered peptides are non-toxic, it is likely that the observed anticoagulation effect was attributable to Senktide.

Senktide may exert its effects by down-regulating thromboplastin, thereby deactivating the factors especially VII, X and IX. Another probability is that

Senktide directly inhibits thrombin, which can subsequently suppress the subsequent events in the coagulation pathways.[17] Anticoagulatory activity of the Senktide may be activated directly by inhibiting the formation or actions of the thrombin or tissue plasminogen activator. Our results suggests that Senktide can be used as an effective thrombolytic therapy. This potential effect of Senktide warrants additional investigation. It could be highly beneficial for treating venous thromboembolism. Current treatment options for thromboembolism include unfractionated and low molecular weight heparins (LMWHs), warfarin and inferior vena cava filters.[24]

Limited data is available on the effect of tachykinins on the hematological and coagulation profile. It has been demonstrated that SP induces platelet aggregation and degranulation. This effect of SP has been supposed to be mediated through tachykinin receptors, specifically NK1 (the receptor for SP) and NK3 (the receptor for Neurokinin B).[9] Another study highlighted the role of tachykinins (SP and Endokinins A and B) in the activation of platelet function and the formation of thrombi.[25] Furthermore, additional research indicated that the pro-thrombotic effects of tachykinins on platelets are mediated via the neurokinin 1 receptor.[12] In another study, it was found that Substance P and Neurokinin A are crucial for neutrophil priming and the release of superoxide anions from neutrophils.[26]

Anticoagulants often use GPCR to mediate their anticoagulant effects and aggregation of platelets.[17,18] Notably, Neurokinin B, its agonists and antagonists also exerts its actions through a GPCR.[1] Therefore, it is plausible that Senktide promotes its anticoagulation effects by using intracellular second messenger pathways.

To our knowledge, this is the initial report which investigated the effects of continuous administration of Senktide and SB222200 on hematological and coagulation parameters following subcutaneous administration of the peptides. Consequently, direct comparisons with other studies are currently not possible.

CONCLUSION

Senktide administration led to increased leukocyte count, altered platelet dynamics with decreased count and increased volume, and affected coagulation pathways with prolonged bleeding and clotting time, PT and APTT. Further research is needed to understand these effects and their clinical significance.

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Ethical Consideration

This study was approved by Institutional Review Board of Institute of Medical Sciences, KMU (DIR/KMU-EB/EN/000451), Kohat, Pakistan.

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Conflict of Interest

Authors declared no conflict of interest.

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DATA SHARING STATEMENT: The data that support the findings of this study are available on request from the corresponding author. The data is not publicly available due to privacy or ethical restrictions.

AUTHORS' CONTRIBUTION

The following authors fulfill authorship criteria as per ICMJE guidelines;

Ramzan F:	Conception and study design, aquisitiion, analysis and interpretation of data, drafting the manuscript, critical review, approval of the final version to be published.
Luqman MW:	Analysis and interpretation of data, drafting the manuscript, approval of the final version to be published.
Atta T:	Analysis and interpretation of data, drafting the manuscript, approval of the final version to be published
Ramzan MH:	Conception and study design, aquisitiion, analysis and interpretation of data, drafting the manuscript, critical review, approval of the final version to be published.

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