Effect of Cardiac Stimulant Drug (MSCD) on Blood Coagulation Cascade and Platelets

Krupa¹ & Sharath Kumar MN^{2*}

¹Guest Lecturer, Department of Biochemistry, Bangalore University, Bangalore-560056, India. ^{2*}Senior Executive, Department of Environmental Microbiological Lab, Sobha Pvt Ltd., Bangalore-560064, India. E-mail: sharathm.nandish@gmail.com*

DOI: <http://doi.org/10.38177/ajast.2022.6102>

Copyright: © 2022 Krupa & Sharath Kumar MN. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Article Received: 14 October 2021

Article Accepted: 07 January 2022

Article Published: 21 January 2022

ABSTRACT

Current study deals with the effect of cardiac stimulant drug (Mephentermine Sulphate Composite Drug) on blood coagulation cascade and plasma platelets. The purity of MSCD was adjudged by GC-MS chromatographic technique. Single sharp peak was evolved in GC-MS by using DB-5 column. Furthermore, MSCD did not alter the clotting time of human citrated plasma in both Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP) at the concentration of 5-50 µg. Moreover, MSCD did not alter ADP and Epinephrine induced platelet aggregation at the concentration of 5-50 µg. Remarkably, MSCD exhibit nontoxic property as it was unable to damage RBC cell membrane.

Introduction

Plasma coagulation cascade and plasma platelets play a pivotal role in the formation of blood clot at the site of tissue injury [1]. Several factors were involved in the coagulation cascade such as factor-I to factor-XIII [2]. Coagulation cascade operates in three pathways namely intrinsic, extrinsic and common pathway [3]. Contrastingly, platelets play a crucial role in the formation of platelet plug at the site of tissue injury which ultimately leads to clot formation by activating the plasma coagulation factors [4]. Inert platelets in the plasma was activated by few platelet agonists namely ADP, collagen, thrombin and arachidonic acid [5]. Regulation of plasma coagulation cascade and platelets in order to prevent oozing out of blood at the site of tissue injury was termed as hemostasis [6]. Alternatively, due to some unknown genetic and environmental factor leads to formation of unusual blood clot in the arteries and veins leads to formation of thrombus (clot) and the process was known to be thrombosis [7]. Similarly, some unknown factors majorly genetic factors lead to be hemophilia disorders [8].

Thus, anticoagulant and antiplatelet agents play a pivotal role in the treatment of thrombotic and hemophilia disorders [9]. Thus, the present study mainly focused on the evaluation of Mephentermine sulphate composite drug on plasma coagulation cascade and plasma platelets.

Materials and Methods

All chemicals used were of analytical grade. Fresh human blood was collected from healthy donors for the platelet rich plasma (PRP). ADP and Epinephrine were purchased from Sigma Chemicals Company.

GC-MS analysis

GC-MS was analyzed as described by Justesen et al., on quadrupole mass spectrometers in the Electron Capture Negative Ion Chemical Ionization (ECNICI) mode with capillary column. Helium gas was used as carrier gas at the flow rate of 1ml/min and the injection volume of 0.5 µl (split ratio of 10:1). Temperature program was set as follows, injector temperature 250°C; ion-illuminator temperature 280°C, oven temperature 110°C. Mass spectrum was taken at 80eV; a scan interval of 0.5sec [10].

Preparation of Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP)

The method of Ardlie and Han [11] was employed for the preparation of human platelet-rich plasma and platelet-poor plasma. The platelet concentration of PRP was adjusted to 3.1×10^8 platelets/ml with PPP. The PRP maintained at 37°C was used within 2hr for the aggregation process. All the above preparations were carried out using plastic wares or siliconized glass wares.

Plasma re-calcification time

The plasma re-calcification time was determined according to the method of Quick et al., [12]. Briefly, the MSCD (0-50µg) was pre-incubated with 0.2ml of citrated human plasma in the presence of 10mM Tris HCl (20µl) buffer pH 7.4 for 1min at 37°C, 20µl of 0.25M CaCl₂ was added to pre-incubated mixture and clotting time was recorded.

Platelet aggregation

The turbid metric method of Born was followed using a Chronology dual channel whole blood/optical lumi aggregation system (Model-700) [13]. Aliquots of PRP were pre-incubated with various concentrations of MSCD (0–50µg) in 0.25ml reaction volume. The aggregation was initiated independently by the addition of agonists, such as ADP and Epinephrine followed for 6min.

Direct hemolytic activity

Direct hemolytic activity was determined by using washed human erythrocytes. Briefly, packed human erythrocytes and phosphate buffered saline (PBS) (1:9v/v) were mixed; 1ml of this suspension was incubated independently with the various concentration of MSCD (0-200µg) for 1hr at 37°C [14]. The reaction was stopped by adding 9ml of ice-cold PBS and centrifuged at 1000g for 10min at 37°C. The amount of hemoglobin released in the supernatant was measured at 540nm. Activity was expressed as percent of hemolysis against 100% lysis of cells due to addition of water that served as positive control and phosphate buffered saline served as negative control.

Results and Discussions

Purity of the compound plays a major role in the research work, if the purity of sample is good then the prediction of research work sounds good.

Fig.1. GC-MS Chromatography of MSCD

Based on the purity of the sample we can predict the result obtained from the output of research work is relatively due to the particular drug. Thus, in order to know the purity of purchased MSCD GC-MS chromatography technique was performed. It shows single sharp peak with some minor spikes in the chromatogram which suggests that the purchased drug is 99% purity and remaining 1% with some adjuvants (Fig.1).

Plasma re-calcification time of MSCD

To evaluate the anticoagulant or pro-coagulant property of MSCD, we performed plasma re-calcification time analysis in invitro study. Captivatingly, it did not exhibit any role on blood coagulation cascade. MSCD stay remain as control in human citrated plasma clotting time. This suggests that MSCD have no role in plasma coagulation cascade as it fails to exhibit neither anti nor pro coagulant property. Anti-coagulant drugs are widely used in the treatment of thrombotic disorders worldwide [15]. Similarly, pro-coagulant drugs are play a major role in the treatment of hemophilia disorders, thus it is widely used to treat hemophilia patients [16]. But unfortunately, MSCD does not exhibit neither anti-coagulant nor pro-coagulant property (Fig.2).

Fig.2. Plasma re-calcification time of MSCD

Role of MSCD on plasma platelets

Platelets plays a pivotal role in blood clotting process [17]. Morphology of the platelets bears few receptors for different agonists to activate the inertia platelets [18]. Furthermore, the activated platelets help in the formation of blood clot at the site of tissue injury [19].

Fig.3. Platelet aggregation of MSCD

Thus, antiplatelet agents play a crucial role in the treatment of thrombotic disorder [20]. In order to know the role of MSCD on platelets, platelet aggregation analysis was performed using ADP and epinephrine as an agonist in lumi aggregometer instrument. Unfortunately, MSCD did not induce or inhibit the platelet aggregation process against ADP and epinephrine induced platelet aggregation (Fig.3).

Non-toxic property of MSCD

MSCD was unable to cleave RBC cell membrane in invitro assay. Thus, it concludes that MSCD profound to be exhibit non-toxic in nature (Fig.4).

Fig.4. Hemolytic activity of MSCD (50-200µg)

Conclusion

In conclusion, MSCD did not exhibit neither anti nor pro coagulant properties in plasma coagulation cascade assay. In addition, it did not profound to display neither platelets aggregation inducer nor platelets inhibitor in ADP and epinephrine induced platelet aggregation assay. Fascinatingly, MSCD found to show non-toxic property as it unable to cleave RBC membrane.

Declarations

Source of Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare no competing financial, professional and personal interests.

Consent for publication

Authors declare that they consented for the publication of this research work.

Bibliography

1. M.B. Marulasiddeshwara, S.S. Dakshayani, M.N. Sharath Kumar, R. Chethana, P. Raghavendra Kumar, S. Devaraja, Facile-one pot-green synthesis, antibacterial, antifungal, antioxidant and antiplatelet activities of lignin capped silver nanoparticles: A promising therapeutic agent, Materials Science & Engineering C (2017).

2. Nandish SK, Kengaiah J, Ramachandraiah C, Shivaiah A, Chandramma, Girish KS, Kempaiah Kemparaju, Sannanigaiah D. Anticoagulant, antiplatelet and fibrin clot hydrolyzing activities of flax seed buffer extract. *Phcog Mag* 2018; 14: S175-183.
3. Ramachandraiah C, Nandish SKM, Kengaiah J, Shivaiah A, Chandramma, Devaraja S. (2017) Evaluation of Anticoagulant and Antiplatelet Activity of Pisum sativum Pod Extract. *J Blood Res Hematol Dis* 2:1.
4. Shashidhar Bharadwaj. S, Boja Poojary, Sharath Kumar M. Nandish, Jayanna Kengaiah, Kirana MP, Anupam J Das, Usha, Madan Kumar, K. Byrappa, Ananda Kulal, Devaraja Sannanigaiah. A simple and efficient synthesis, alpha-glucosidase inhibitory activity and in silico study of Benzimidazole hybrid scaffold with quinolinyl oxadiazoles skeleton: As potential leads for the management of type-II diabetes mellitus. *ACS Omega*, 2018.
5. Chethana Ramachandraiah, Sharath Kumar M Nandish, Jayanna Kengaiah, Chandramma Srinivasa, Ashwini Shivaiah, Girish K S, Kemparaju Kempaiah, Sebastin Santhosh, Devaraja Sannanigaiah. Macrotyloma Uniflorum Seed Extract exhibits strong anticoagulant and antiplatelet properties. *Asian J. of Phar. and Pharmacology*, 2019.
6. Rashmi Hosamani S.S.Dakshayani, M.B. Marulasiddeshwarab, M.N. SharathKumar, G.Ramesh, P. RaghavendraKumar, S.Devaraja. Antimicrobial, anticoagulant and antiplatelet activities of green synthesized silver nanoparticles using Selaginella (Sanjeevini) plant extract. *Int. Journal of Biological Macromolecules*, 2019.
7. Chandramma Srinivasa, Sharath Kumar M Nandish, JayannaKengaiah, Chethana Ramachandraiah, Ashwini Shivaiah, Devaraja Sannanigaiah. Caesalpinia CristaSeed Exhibits Strong Anticoagulant and Antiplatelet Activity. *Journal of Pharmacy And Biological Sciences*, 2019.
8. Ashwini Shivaiah, Jayanna kengaiah, Sharath Kumar M. Nandish, Chethana Ramachandraiah, Chandramma, Sebastien Martin Santhosh, Manohar Shinde and Devaraja Sannanigaiah. Evidences for the presence of proteolytic enzyme in finger millet (eleusine coracana) and its beneficial role in thrombosis. *Asian Journal of Science and Technology*, 2018.
9. G.Ramesha, M.N. Sharath Kumar, P.Raghavendra Kumar, P.A.Suchetan, S.Devaraja, ForoSabine, G. Nagaraju. Synthesis, characterisation, crystal structures, anticoagulant and antiplatelet activity studies of new 2,6-dipyrazinylpyridines with pendant trimethoxyphenyl. *Journal of Molecular Structure*, 2019.
10. Sharath Kumar MN, Kengaiah J, Ramachandraiah C, Shivaiah A, Chandramma, Girish KS, Kempaiah Kemparaju, Sannanigaiah D. Isolated linseed oil from Linum usitatissimum exhibited pro-coagulant property. *International journal of biological sciences*, 2019.
11. Pushpalatha K. C Usha B, Sharath Kumar M Nandish, Shashidhar Bharadwaj. S, Thejaswi N, Devaraja Sannanigaiah. Study on Anti-inflammatory, Anticoagulant, Antiplatelet Activity of Grewia serrulata DC and Grewia heterotricha Mast. *International Journal of Pharmacy and Biological Sciences*, 2019.
12. J. Kengaiah, S. K. M. Nandish, C. Ramachandraiah, Chandramma, A. Shivaiah, G. J. Vishalakshi, M. Paul, M. S. Santhosh, R. L. Shankar and Devaraja Sannanigaiah. Protective Effect of Tamarind Seed Coat Ethanol Extract on Eryptosis Induced by Oxidative Stress. *Biochemistry (Moscow)*, 2020.

13. Chandramma Srinivasa, Jayanna Kengaiah, Sharath Kumar M Nandish, Chethana Ramachandraiah, Sujatha M Hanumegowda, Ashwini Shivaiah, Sebastian Santhosh, Devaraja Sannanigaiah. *Caesalpinia crista* coat extract protects red blood cell from sodium nitrite-induced oxidative stress and exhibits antiplatelet activity. *Blood Coagulation & Fibrinolysis*, 2020.
14. Sharath Kumar MN, Kengaiah J, Ramachandraiah C, Shivaiah A, Chandramma, Sannanigaiah D. Purification and characterization of non-enzymatic glycoprotein (NEGp) from flax seed buffer extract that exhibits anticoagulant and antiplatelet activity. *International journal of biological macromolecules*, 2020.
15. Sharath Kumar MN, Kengaiah J, Ramachandraiah C, Shivaiah A, Chandramma, Sannanigaiah D. Extracted Oil from Flax Seed Inhibited only Thrombin and Collagen Induced Washed Platelet Aggregation. *Int J Ayu Pharm Chem.*, 2020.
16. Sharath Kumar M. Nandish, Jayanna Kengaiah, Chethana Ramachandraiah, Chandramma, Ashwini Shivaiah, Sebastian santhosh, Kesturu S. Girish, Kempaiah Kemparaju, Devaraja Sannanigaiah. A high molecular weight (168 kDa) cysteine protease from Flax seed buffer extract exhibits strong anticoagulant, antiplatelet and clot dissolving property. *Biochemistry Moscow* 85(09), 2020.
17. Sneharani AH, Sachin HR, Sharath Kumar MN, Devaraja S. Anticoagulant and antiplatelet activities of novel serine protease purified from seeds of *Cucumis maderaspatensis*. *3 Biotech.* 11(30), 2021
18. Golla Ramesh, Sharath Kumar MN, Raghavendra P, Devaraja S. Role of 4-substituted 2,6-bispyrazinyl-(4-dimethoxyphenyl) Pyridines on Plasma Re-calcification time and platelet. *Asian Journal of Applied Science and Technology.* 5(2), 2021
19. K.M. Prabhu Kumar, B.C. Vasantha Kumar, M.N. Sharath Kumar, P. Raghavendra Kumar, S. Devaraju, R.J. Butcher, H.D. Revan Siddappa. Synthesis, structural characterization, CT-DNA interaction study and antithrombotic activity of new ortho-vanillin-based chiral (Se,N,O) donor ligands and their Pd complexes. *Inorganica Chimica Acta.* 528 (2021).
20. Sannanigaiah D, Shivaiah A, Kengaiah J, Srinivasa C, Nandish SKM, Ramachandraiah C, Hanumegowda S, Manjappa B, Martin SS, Laxmaiah RK, Shinde M. Sorghum protein extract protects RBC from sodium nitrite-induced oxidative stress and exhibits anticoagulant and antiplatelet activity. *Folia Med (Plovdiv)* 2021; 63(6): 884-94.