

Anticoagulants: An Overview of Natural and Synthetic Therapeutic Anticoagulants

Chandrasekhar Chanda*, Ranganadha Reddy Aluru

Received: 03 December 2020 / Received in revised form: 16 March 2021, Accepted: 09 March 2021, Published online: 14 March 2021

Abstract

Any substance which prevents blood from clotting or prolongs the clotting time is called an anticoagulant. Some of them are isolated from natural sources like heparin, hirudin, anti-thrombin, urokinase, and streptokinase and some are synthetic *e.g.*, Warfarin, acenocoumarol, brodifacoum. Several proteins with anticoagulant properties isolated from natural sources are currently employed in treating thrombotic disorders. Heparin and Warfarin are the two most widely used natural and synthetic anticoagulant drugs respectively, for the treatment of thrombotic disorders like deep vein thrombosis, acute myocardial infarction, thromboembolism, prevention of systemic embolism in patients with atrial fibrillation. This review article focuses on currently available and newer classes of natural and synthetic anticoagulants, their mechanism of action, and therapeutic potential. Pubmed databases was used for articles selection, papers on natural and synthetic anticoagulants were obtained and reviewed. This review article helps in better understanding the underlying pathways of currently available natural and synthetic anticoagulants and will be useful in designing/discovering new classes of drugs.

Keywords: Natural anticoagulants, Synthetic anticoagulants, Antiplatelet agents, Snake venom anticoagulants.

Introduction

The Natural Mechanism of Blood Clotting

To understand the mechanism of action of anticoagulants one should have precise knowledge about the natural blood-clotting mechanism i.e., hemostasis, and the roles of various clotting factors involved. Hemostasis is a mechanism of forming clots on the walls of damaged blood vessels. Hemostasis occurs in three stages 1) Vasoconstriction: Constriction of blood vessels at the site of injury to reduce blood loss. This phenomenon occurs mainly

because of the activation of the sympathetic nervous system by epinephrine and release of vasoconstrictors like Thromboxane, Angiotensin, ATP, thrombin, etc. by the platelets and the endothelial cell at the site of injury. 2) Formation of platelet plug: Exposure of collagen and release of Von Willebrand factor from the endothelial cells leads to adhesion of platelets at the site of damaged blood vessels.

The activated platelets release various chemicals, hormones, and clotting factors into the blood vessels and further activating the surrounding platelets leading to the formation of the platelet plug. 3) Blood coagulation: This is called secondary hemostasis which occurs through two pathways i) Contact activation (intrinsic) pathway and ii) Tissue factor (extrinsic) pathway. The extrinsic pathway starts when the endothelial cells get damaged and release factor VII into the bloodstream. The intrinsic pathway gets activated when the collagen from the damaged blood vessels get exposed to blood clotting factor XII. Conversion of prothrombin to thrombin is the vital step in the coagulation cascade which involves various clotting factors and enzymes. Thrombin thus formed converts fibrinogen to fibrin which leads to the polymerization of fibrin and forming a mesh-like structure binding to the platelet plug and preventing the loss of blood. Normal endothelium (when there is no injury) secretes prostacyclin's, thrombomodulin's, nitric oxide, and other coagulation inhibitors which either inhibit platelet aggregation or prevent the clotting factor aiding in blood coagulation (Di Minno *et al.*, 2017; Weitz & Harenberg, 2017; Karimi *et al.*, 2018; Abd Elwahaab *et al.*, 2019; Adiga, 2019; Andrusenko *et al.*, 2020; Tsai, 2020). Anticoagulants and antiplatelet drugs also work similarly by targeting a specific step in the coagulation pathway (Adams & Bird, 2009; Hilal-Dandan & Brunton, 2014; Chan *et al.*, 2016; Samuelson & Cuker, 2017).

Figure 1.

Chandrasekhar Chanda*

Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, Andhra Pradesh, India 522502.

Ranganadha Reddy Aluru

Department of Biotechnology, Vignans foundation for science, Technology, and Research, Vadlamudi, 522213, India.

*Email: chandrasekharchanda02@gmail.com



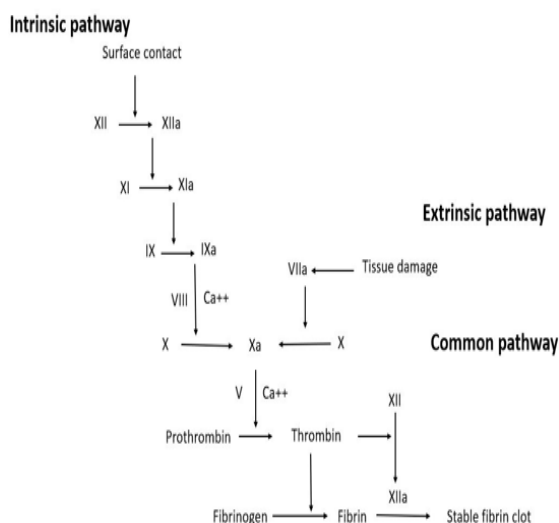


Figure 1. Blood coagulation cascade with three major pathways
1) Intrinsic pathway 2) Extrinsic pathway 3) Common pathway

Synthetic Anticoagulants

Most widely used anticoagulants are the structural derivatives of coumarin a phytochemical isolated from the seeds of *Dipteryx odorata* in the 1820s. Later several anticoagulants were synthesized based on coumarin structure which includes warfarin, acenocoumarol, phenprocoumon, etc. All these coumarin structural derivatives were found to have the ability to suppress vitamin k dependent synthesis of coagulation factors in the liver and prevent blood coagulation (Ansell *et al.*, 2008; Hylek *et al.*, 2012; Bracey *et al.*, 2018; Levy *et al.*, 2018).

Warfarin

Warfarin (3-(α -acetylbenzyl)-4-hydroxycoumarin) is the most widely used oral anticoagulant medicine which is synthesized by adding benzal acetone to 4-hydroxycoumarin in the presence of pyridine. Warfarin is commonly used in treating pulmonary embolism, deep vein thrombosis, and to prevent stroke in people having valvular heart diseases and atrial fibrillation (Francis & Crowther 2010). Warfarin prevents blood clotting by blocking vitamin k epoxide reductase, an enzyme that reactivates vitamin k (Agnelli *et al.*, 1998). Prevention of vitamin k reactivation suppresses the synthesis of clotting factors II, VII, IX, and X and other proteins involved in blood clotting (Rydel *et al.*, 1991). Although warfarin is the most widely used oral anticoagulant it has common side effects like bleeding and tissue damage (Persson *et al.*, 2001; Molteni & Cimminiello 2014; Chan *et al.*, 2020; Chaudhary *et al.*, 2020).

Acenocoumarol

Acenocoumarol is structurally analogous to warfarin where *p*-nitrobenzalacetone is used in chemical synthesis instead of benzal acetone (Van & Lane 1997; Mekaj *et al.*, 2015). Acenocoumarol also blocks the enzyme called vitamin k reductase and prevents the synthesis of vitamin k dependent blood clotting factors.

Phenprocoumon

Phenprocoumon is 4-hydroxycoumarin which is substituted at position 3 by 1-phenyl propyl. Phenprocoumon is chemically synthesized by acylation followed by alkaline hydrolysis and decarboxylation reactions. Phenprocoumon is a structural derivative of coumarin and it is considered a long-lasting oral anticoagulant drug. Phenprocoumon has a similar mechanism of action to warfarin and acenocoumarol and is used in the treatment of thromboembolic disorders (Prochaska *et al.*, 2015).

Natural Anticoagulants

Several anticoagulants have been purified from natural sources and some synthesized chemically are currently used in the treatment of blood vascular disorders. These anticoagulants either prevent blood from clotting or prevent existing blood clots to grow. Some of the anticoagulants purified from natural sources and their mechanisms of action are discussed below.

Heparin

Heparin is a highly sulfated glycosaminoglycan (Mucopolysaccharide) with an average molecular weight of 12-15 kDa. It is one of the naturally occurring anticoagulants produced by basophils and mast cells at the site of injury. Heparin binds to Antithrombin III (thrombin inhibitor) and changes its conformation leading to the activation of Antithrombin III. The activated Antithrombin III inactivates the thrombin and other proteases involved in the blood clotting. Heparin is very effective in preventing deep vein thrombosis and pulmonary emboli in patients (Agnelli *et al.*, 1998; Francis & Crowther 2010; Nutescu *et al.*, 2016).

Hirudin

Hirudin is a naturally occurring peptide present in the salivary gland of leaches. Hirudin functionally similar to Antithrombin III by inhibiting the activity of thrombin (Rydel *et al.*, 1991). Hirudin is a thrombolytic drug as it can inactivate the fibrin-bound thrombin. This is one of the advantages of Hirudin over Heparin as the latter cannot inactivate fibrin-bound thrombin and dissolve the blood clot.

Anti-Thrombin

Anti-thrombin belongs to a family of serine protease inhibitors produced by the liver containing just 432 amino acids. Anti-thrombin inactivates many of its physiological targets like thrombin, factor XI, factor IX, factor X, and factor XII and other serine proteases involved in the coagulation cascade (Francis & Crowther, 2010). Inherited or acquired (liver malfunctioning) antithrombin deficiencies lead to malfunctioning of the coagulation pathway. Anti-thrombin is currently employed as a potential therapeutic drug for the prevention of clots before or during the surgery and in patients with anti-thrombin disorders (Joseph & Kini, 2004).

Urokinase

Urokinase is a serine protease with 411 amino acid residues first isolated from human urine. Its main physiological function in the blood is to activate tissue plasminogen. Plasmin thus formed digests the fibrin clots and other plasma proteins like fibronectin, thrombospondin, laminin, etc. Urokinase is used as a thrombolytic agent in the treatment of severe deep vein thrombosis, pulmonary embolism, and myocardial infarction.

Streptokinase

Streptokinase is an enzyme produced by several species of streptococci that can bind and inactivate human plasminogen. Streptokinase is a relatively inexpensive thrombolytic agent that is currently used in the treatment of myocardial infarction and pulmonary embolism (Meneveau *et al.*, 1997; Sikri & Bardia, 2007). One of the major limitations of these anticoagulants is their adverse side effects including hemorrhage, necrosis, osteoporosis, induced thrombocytopenia. Also, most of the anticoagulants cannot act directly on fibrin clot except a class of anticoagulants (i.e., tissue plasminogen activators) which takes the help of plasmin to digest fibrin clots. Search for the newer class of anticoagulants with lower side effects and capable of digesting existing blood clots gained importance during the past two decades. Snake venom was found to be one of the richest natural sources for anticoagulant factors, mostly in the form of snake venom metalloproteinases (SVMP's), three-finger toxins (3FTx's) C type lectin-like proteins (CLP's), and phospholipases (Kini, 2006). Ability to proteolyze fibrinogen as well as fibrin clot and the ability to act as a potent antiplatelet agent by blocking the platelet surface receptors shows their immense therapeutic potential.

Snake Venom Anticoagulants

The blood vascular system is targeted by several snake venom proteins. Many proteins from snake venoms with anticoagulant properties were purified and characterized (Kornalik, 1985; Hutton & Warrell, 1993; Markland, 1998; Lu *et al.*, 2005) and some are under clinical trials in combinational therapy (Sherman *et al.*, 2000). Snake venom anticoagulants can affect the platelet aggregation process also they can act as very strong thrombolytic agents. Most of the snake venom anticoagulants belong to the class of snake venom metalloproteinases. Sizes of these proteins may exist from 24 kDa up to 300 kDa (Joseph & Kini, 2004). Snake venom anticoagulants inhibit the clotting of blood by several mechanisms. Some of them exhibit enzymatic activities like phospholipase and metalloproteinase and prevent blood clotting and some exhibit antiplatelet activity by binding to integrin present on the platelet surface. However, we still need to understand the detailed mechanism of action of several anticoagulant proteins from snake venoms.

Conclusion

Thrombosis is one of the most dreaded diseases of mankind. Both oral as well as parental anticoagulants are currently employed for

treating thrombotic disorders. Bleeding is one of the adverse side effects of most of the vitamin K antagonists as well as parental anticoagulants. Oral anticoagulants showed adverse side effects in the form of tissue bruising, gastrointestinal bleeding, and intracranial hemorrhage whereas parental anticoagulants are having side effects in the form of thrombocytopenia and thromboembolism due to antibody-mediated platelet aggregation. Present drugs to counter the disease conditions are expensive and give rise to harmful side effects. Hence, there is a need for discovering newer classes of anticoagulants with minimal side effects. This review focuses on the mechanism of action of currently available major natural and synthetic anticoagulants which helps in better understanding the underlying pathways and helps in designing/discovering new classes of drugs.

Acknowledgments: The authors would like to thank management Koneru Lakshmiiah Education Foundation Vaddeswaram, Guntur, for helping us with necessary resources

Conflict of interest: None

Financial support: None

Ethics statement: None

References

- Abd Elwahaab, H. A., Rahmy, A. F., Hagag, A. A., Fares, H. M., & Fouad, S. A. (2019). Effect of aerobic exercises on blood coagulation and fibrinolysis factors in elderly hypertensive patients. *Journal of Advanced Pharmacy Education and Research*, 9(1), 44-48.
- Adams, R. L., & Bird, R. J. (2009). Coagulation cascade and therapeutics update: relevance to nephrology. Part 1: overview of coagulation, thrombophilias and history of anticoagulants. *Nephrology*, 14(5), 462-470.
- Adiga U. (2019). Serum indices – A tool to measure interfering substances in blood samples. *International Journal of Pharmaceutical and Phytopharmacological Research*, 9(2), 43-46.
- Agnelli, G., Piovella, F., Buoncristiani, P., Severi, P., Pini, M., D'Angelo, A., Beltrametti, C., Damiani, M., Andrioli, G. C., Pugliese, R., et al. (1998). Enoxaparin plus compression stockings compared with compression stockings alone in the prevention of venous thromboembolism after elective neurosurgery. *New England Journal of Medicine*, 339(2), 80-85. doi:10.1056/NEJM199807093390204.
- Andrusenko, S. F., Denisova, E. V., Anfinogenova, O. I., Melchenko, E. A., Kadanova, A. A., & Brycina, I. E. (2020). Changes in the concentration of ethyl alcohol in the blood when it is stored in different temperature conditions. *Pharmacophore*, 11(3), 75-77.
- Ansell, J., Hirsh, J., Hylek, E., Jacobson, A., Crowther, M., & Palareti, G. (2008). Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians evidence-based clinical practice

- guidelines. *Chest*, 133(6), 160S-198S. doi:10.1378/chest.08-0670.
- Bracey, A., Shatila, W., & Wilson, J. (2018). Bleeding in patients receiving non-vitamin K oral anticoagulants: clinical trial evidence. *Therapeutic Advances in Cardiovascular Disease*, 12(12), 361-380. SAGE Publications Ltd. doi:10.1177/1753944718801554
- Chan, N. C., Eikelboom, J. W., & Weitz, J. I. (2016). Evolving treatments for arterial and venous thrombosis: role of the direct oral anticoagulants. *Circulation Research*, 118(9), 1409-1424. doi:10.1161/CIRCRESAHA.116.306925
- Chan, N., Sobieraj-Teague, M., & Eikelboom, J. W. (2020). Direct oral anticoagulants: evidence and unresolved issues. *The Lancet*, 396(10264), 1767-1776. doi:10.1016/S0140-6736(20)32439-9
- Chaudhary, R., Sharma, T., Garg, J., Sukhi, A., Bliden, K., Tantry, U., Turagam, M., Lakkireddy, D., & Gurbel, P. (2020). Direct oral anticoagulants: a review on the current role and scope of reversal agents. *Journal of Thrombosis and Thrombolysis*, 49(2), 271-286. doi:10.1007/s11239-019-01954-2
- Di Minno, A., Frigerio, B., Spadarella, G., Ravani, A., Sansaro, D., Amato, M., Kitzmiller, J. P., Pepi, M., Tremoli, E., & Baldassarre, D. (2017). Old and new oral anticoagulants: food, herbal medicines and drug interactions. *Blood Reviews*, 31(4), 193-203. doi:10.1016/j.blre.2017.02.001
- Francis, C. W., & Crowther, M. (2010). Chapter 23. Principles of Antithrombotic Therapy. In Lichtman MA, Beutler E, Kipps TJ, et al. *Williams manual of Hematology (8th ed.)*. Access Hem Onc ISBN 978-0-07-162144-1
- Hilal-Dandan, R., & Brunton, L. L. (2014). *Goodman and Gilman's Manual of Pharmacology and Therapeutics*. 2nd ed. McGraw Hill Professional. pp. 523-543.
- Hutton, R. A., & Warrell, D. A. (1993). Action of snake venom components on the haemostatic system. *Blood reviews*, 7(3), 176-189. doi:10.1016/0268-960X(93)90004-N.
- Hylek, E. M., Palareti, G., Ageno, W., Gallus, A. S., Wittkowsky, A., & Crowther, M. (2012). Oral anticoagulant therapy: antithrombotic evidence-based clinical practice guidelines: american college of chest physicians therapy and prevention of thrombosis. *Chest*, 141, e44S-e88S. doi:10.1378/chest.11-2292.
- Joseph, J. S., & Kini, R. M. (2004). Snake venom prothrombin activators similar to blood coagulation factor Xa. *Current Drug Targets-Cardiovascular & Hematological Disorders*, 4(4), 397-416. doi:10.2174/1568006043335781.
- Karimi, A. A., Jahanpour, F., Mirzaei, K., & Akeberian, S. (2018). The effect of swaddling in physiological changes and severity of pain caused by blood sampling in preterm infants: randomized clinical trial. *International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR)*, 8(5), 1-5.
- Kini, R. M. (2006). Anticoagulant proteins from snake venoms: structure, function and mechanism. *Biochemical Journal*, 397(3), 377-387. doi:10.1042/BJ20060302.
- Kornalik, F. (1985). The influence of snake venom enzymes on blood coagulation. *Pharmacology & Therapeutics*, 29(3), 353-405. doi:10.1016/0163-7258(85)90008-7.
- Levy, J. H., Douketis, J., & Weitz, J. I. (2018). Reversal agents for non-vitamin K antagonist oral anticoagulants. *Nature Reviews Cardiology*, 15(5), 273. doi:10.1038/nrcardio.2017.223
- Lu, Q., Clemetson, J. M., & Clemetson, K. J. (2005). Snake venoms and hemostasis. *Journal of Thrombosis and Haemostasis*, 3(8), 1791-1799. doi:10.1111/j.1538-7836.2005.01358.x.
- Markland, F. S. (1998). Snake venoms and the hemostatic system. *Toxicon*, 36(12), 1749-1800. doi:10.1016/S0041-0101(98)00126-3.
- Mekaj, Y. H., Mekaj, A. Y., Duci, S. B., & Miftari, E. I. (2015). New oral anticoagulants: their advantages and disadvantages compared with vitamin K antagonists in the prevention and treatment of patients with thromboembolic events. *Therapeutics and Clinical Risk Management*, 11, 967. doi:10.2147/TCRM.S84210
- Meneveau, N., Schiele, F., Vuilleminot, A., Valette, B., Grollier, G., Bernard, Y., & Bassand, J. P. (1997). Streptokinase vs alteplase in massive pulmonary embolism: a randomized trial assessing right heart haemodynamics and pulmonary vascular obstruction. *European Heart Journal*, 18(7), 1141-1148. doi:10.1093/oxfordjournals.eurheartj.a015410.
- Molteni, M., & Cimminiello, C. (2014). Warfarin and atrial fibrillation: from ideal to real the warfarin affaire. *Thrombosis Journal*, 12(1), 1-7. doi:10.1186/1477-9560-12-5.
- Nutescu, E. A., Burnett, A., Fanikos, J., Spinler, S., & Wittkowsky, A. (2016). Pharmacology of anticoagulants used in the treatment of venous thromboembolism. *Journal of Thrombosis and Thrombolysis*, 41(1), 15-31. doi:10.1007/s11239-015-1314-3
- Persson, E., Nielsen, L. S., & Olsen, O. H. (2001). Substitution of aspartic acid for methionine-306 in factor VIIa abolishes the allosteric linkage between the active site and the binding interface with tissue factor. *Biochemistry*, 40(11), 3251-3256. doi:10.1021/bi001612z.
- Prochaska, J. H., Göbel, S., Keller, K., Coldewey, M., Ullmann, A., Lamparter, H., Jünger, C., Al-Bayati, Z., Baer, C., Walter, U., et al. (2015). Quality of oral anticoagulation with phenprocoumon in regular medical care and its potential for improvement in a telemedicine-based coagulation service—results from the prospective, multi-center, observational cohort study thrombEVAL. *BMC Medicine*, 13(1), 14. doi:10.1186/s12916-015-0268-9.
- Rydel, T. J., Tulinsky, A., Bode, W., & Huber, R. (1991). Refined structure of the hirudin-thrombin complex. *Journal of Molecular Biology*, 221(2), 583-601. doi:10.1016/0022-2836(91)80074-5.
- Samuelson, B. T., & Cuker, A. (2017). Measurement and reversal of the direct oral anticoagulants. *Blood Reviews*, 31(1), 77-84. doi:10.1016/j.blre.2016.08.006
- Sherman, D. G., Atkinson, R. P., Chippendale, T., Levin, K. A., Ng, K., Futrell, N., Hsu, C. Y., & Levy, D. E. (2000). Intravenous anidrol for treatment of acute ischemic stroke:

- the STAT study: a randomized controlled trial. *Jama*, 283(18), 2395-2403.
- Sikri, N., & Bardia, A. (2007). A history of streptokinase use in acute myocardial infarction. *Texas Heart Institute Journal*, 34(3), 318.
- Tsai, H. O. N. (2020). Pharmacological Review of Anticoagulants. In *Anticoagulation Drugs - the Current State of the Art*. IntechOpen. doi:10.5772/intechopen.88407
- Van Boven, H. H., & Lane, D. A. (1997, July). Antithrombin and its inherited deficiency states. In *Seminars in hematology*, 34(3), 188-204.
- Weitz, J. I., & Harenberg, J. (2017). New developments in anticoagulants: Past, present and future. *Thrombosis and Haemostasis*, 117(7), 1283-1288. doi:10.1160/TH16-10-0807.