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Biochemistry of Anticoagulant Drugs

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ABSTRACT —

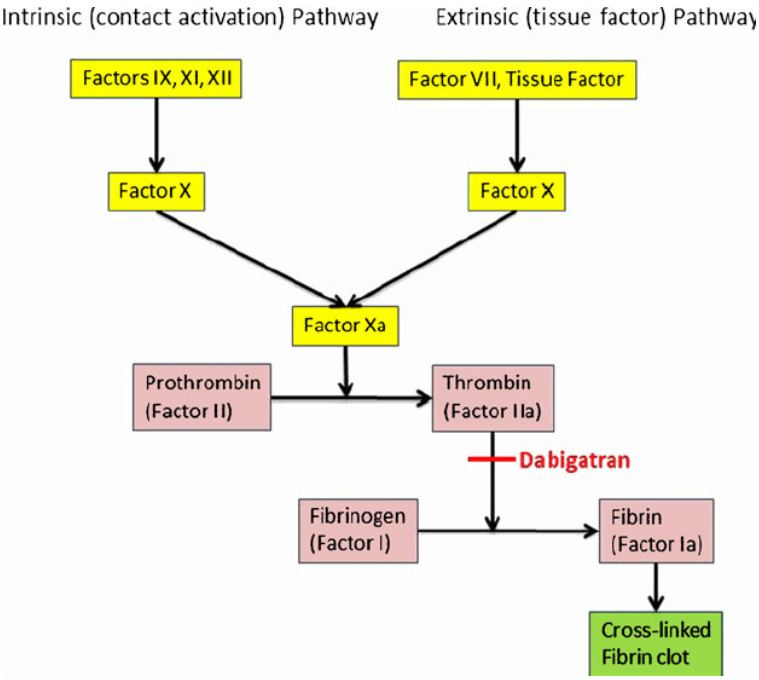
In this review, we want to broadly explore the basic biochemistry of blood coagulation and the mechanism of action used anticoagulant drugs that work by targeting components of the biochemical apparatus of the coagulation pathway. After a comprehensive summary of the biochemical events involved in blood coagulation, the relationship between drug structure and function targets of different drug classes were discussed. The action of vitamin K-dependent inhibitors is responsible for production of incomplete clotting factors, which impairs blood clotting. Synthesis of gamma-carboxyl glutei acid GLA factor domain is mediated by vitamin K peroxide reductant, which is inhibited by warfare and other related molecules. Thrombin is a key enzyme in the blood clotting process, making it a compelling drug target; PACK and Argatroban are those drugs that inhibit the enzyme by covalent and non-covalent binding. Next a direct inhibitor of thrombin is hirudin, an anticoagulant weapon of leeches that has recently been used in clinical treatment, binds bidentally with thrombin. The human body's anticoagulant, ant thrombin, is the drug's target heparin, which provides increased anticoagulant activity only by acting as a bridge between thrombin and antithrombin. The pharmacokinetic properties of representative drugs of each class were also discussed in this review.

Key words: Anticoagulant, Hemostatic, Thrombomodulin, Thrombosis, Warfarin

I. Introduction

The control of proper blood flow is a complex and highly structured biological process with the synchronized action of many complementary and opposing mechanisms of influence. A delicate balance in the blood vessels is achieved for clearanceunrestricted blood flow and at the same time facilitating the immediate formation of a clot at the site of injury. Substances that agents that promote blood coagulation are known as procoagulants, while substances that inhibit blood coagulation are called anticoagulants. Under normal physiological conditions, the effect of anticoagulants suppresses the effect of procoagulantsprevention of unwanted formation of blood clotsPrevention of blood loss, known as hemostasis, is accomplished in a ruptured blood vessel via multistage stage procedure

1). Although blood coagulation in humans and animals evolved as a defense response, some medical conditions and procedures require restriction of blood clotting. There are several life saving surgeries like bypass and heart transplant where the blood passes through an external tube where it eventually clots without any external retarding agent. Thrombophilia, or hypercoagulability, is an abnormal condition where blood vessels are more likely to clot. This condition greatly increases the risk of death such as deep vein thrombosis (DVT) and pulmonary embolism. Under these circumstances, antithrombotics play an indispensable role by counteracting the threats of deadly blood clots. Furthermore, the multifaceted regulatory framework of blood coagulation inherently provides rich opportunities for intervention with medication. Antithrombotics, used to prevent unwanted blood clotting, can work either by slowing down time by coagulation (anticoagulant drugs) or prevention of platelet aggregation (antagonizing drugs). List of currently sold a clinically used anticoagulant drugs are listed below



II. Discussion

1. Biochemistry of the blood clotting process

Blood coagulation is one of the most complex biochemical processes in the human body. To understand the mechanism of actionantithrombotics, it is necessary to discuss the molecular basis of the blood clotting process. So in this section we will provide a brief insight into the molecular biology of blood clot formation and the components required for this process.

1.1. Diagram of blood coagulation pathway

Initiation of blood clotting when a vessel is traumatized can be done by two routes - extrinsic and intrinsic These pathways provide cascades of chemical reactions that consist of proteins called factors (referred to as Roman numerals). Many of these factors in their activated forms have proteolytic properties that further activate another factor [3], [4]. These cascades ultimately lead to the activation of thrombin from prothrombin. The inner track is launched trauma to the blood or exposure of the blood to collagen, which activates factor XII, while the extrinsic pathway is induced when blood comes into contact with extra-vascular tissues, leading to the release of tissue factor. Ultimately, both ways work information about the blood clot at the same time.

The next step in the coagulation pathway is the activation of fibrinogen. Thrombin is a proteolytic enzyme that hydrolyzes fibrinogen to fibrin monomer. These monomers are not linked by any covalent interaction with each other and then covalently cross-linked by fibrin stabilizing factor, followed by attachment of platelets to the fibrin mesh to form a blood clot. So far we have discussed the factors involved in the formation of a blood clot or procoagulants. Discuss the we must look for anticoagulants that are naturally present in the blood to prevent coagulation in the vessel

the most important anticoagulant factors that include

(1) Smoothness of the cell surface, which prevents activation of the intrinsic pathway;

(2) The glycocalyx layer (mucopolysaccharide adsorbed on the surface of endothelial cells) that repels platelets and factors, thereby preventing clot activation;

(3) Thrombomodulin, which is an endothelial membrane-bound protein responsible for binding thrombin and therefore minimizes the possibility of coagulation.

2. Anticoagulant Drugs

The first anticoagulant discovered was hirudin, in 1884 by Haycraft[13], from the saliva of medicinal leech but it was used clinically when it was produced by genetic engineering in 1986. So, first clinically used anticoagulant drug was heparin. Due to the outbreak of world war I, research was directed towards procoagulant studies than anticoagulant studies. In 1916, a medical student at John Hopkins university, McLean who was studying procoagulant activity of alcoholic extracts from brain, liver and heart of the dogs discovered a compound that could inhibit blood coagulation. Two years later, Howell isolated the active compound and named it “Heparin”[14]. In 1933, Charles and Scott purified and

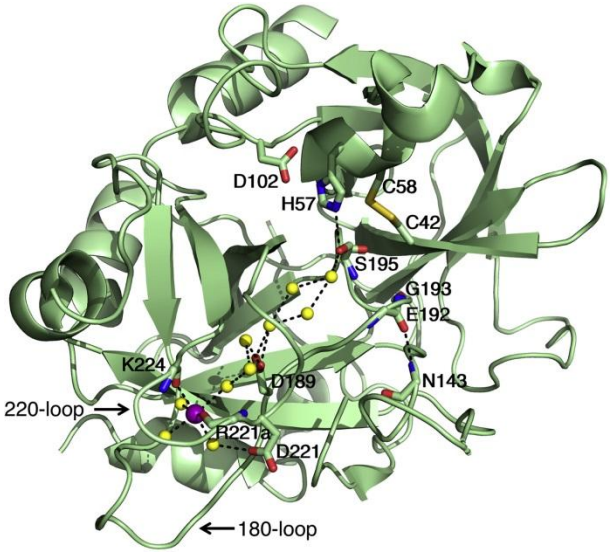
crystalized heparin and started to use it on

Another singular incident of discovery of anticoagulant started at North Dakota and Alberta when cattle suffered a mysterious haemorrhagic disease, later proved to be caused because of the presence of dicoumarol in the spoiled clover. In 1945, Link et. Al. decided to test coumarin derivatives as rodenticide and warfarin was introduced. It was considered extremely toxic for humans but unsuccessful suicide attempt by a navy inductee with 567 mg of warfarin proved that it was not as toxic as believed. This paved the path of commercialization of warfarin[16]. 2.1. Vitamin K dependent Inhibitors As stated previously, the formation of clot involves the interaction between factors and in most of the cases this interaction takes place on the cell surface. GLA domain facilitates theCa2+ mediated protein membrane interaction hence one can say GLA to be an essential feature for blood coagulation.GLA domain is produced in liver by post translational modification of glutamic acid which is carbonylated at the g position by the pathway known as Vitamin K dependent carboxylation system[7]. Carboxylation reaction is accomplished by the enzyme g-glutamyl carboxylase which needs CO2as carboxyl source and O2(Fig. 8). It also requires Vitamin K as a cofactor which is successively converted into an epoxide. But the hydroquinone form needs to be regenerated for the catalytic activity of gamma glutamyl carboxylase, and this is achieved by the oxidoreductase enzyme Vitamin K epoxide reductase[17], this cyclic conversion is known as Vitamin K cycle[18]. Warfarin and other related molecules partially mimic the structure of Vitamin K and hence can bind with vitamin K epoxide reductase.However, the enzyme canno longer act upon those molecules, thereby, leading to the inhibition of the enzyme which further leads to the arrest of blood coagulation.

Vitamin K epoxide reductase (VKOR) is a type IIIntegral membrane protein[19], [20].It has 263 amino acid residues spanning across three transmembrane domains. Topology of the domains[21] are depicted in the following figure(Fig. 9). Active site of this enzyme lies in the CXXC motif[22](which is the characteristic motif of thioredoxin enzyme family) inside the third transmembrane helix located near the lumen. Figure 9: Proposed topology diagram of Vitamin K Epoxide reductase; different sites are marked by hatch marks Mechanism of action of this enzyme was proposed by Silverman[23]. According tothis mechanism, reduction of disulphide bonds is the key for the activation of the enzyme. Further, the epoxide is protonated by a nearby residue which is probably Asp103. Now, due to the instability of the protonated three-member epoxide ring, the nearby sulphidesactupon the stericallyless congested carbon ring and cleaves.After which, the alcohol group is re-protonated and ultimately leaves the system concerted reductive elimination reaction

Warfarin (3-(a-acetonylbenzyl)-4-hydroxycoumarin), the most prescribed oral anticoagulant drug, contains a coumarin moiety within(Fig. 11), which is essential for its activity. It inhibits the enzyme VKOR, thus,discontinuing the regeneration of KH2 from KO,thus, leading tothe starvation of Vitamin K, thereby, causing thegeneration of under-developed factors that are biologically inoperative and consequentially blood coagulation ceases. Figure 11: Structure of Warfarin and Vitamin K derivatives Binding of warfarin to VKOR is very tight and thus thought to be irreversible. Silverman, based on the structural similarity between Vitamin K and warfarin as well as accounting for the mechanism of VKOR activity, proposed a mechanism for irreversible inactivation by warfarin[24]. In his hypothesis, he proposed the binding of deprotonated warfarin to the reduced form of enzyme. Further experimental data suggested that warfarin attaches to the oxidised form rather than the reduced one. It was reported that inhibition of VKOR by warfarin was highest and happens more rapidly, when the enzyme was subjected to warfarin prior to its contact to DTT. However, preincubation of VKOR with DTT, before warfarin incubation, reduces warfarin inhibition of VKOR activity[25]. Most currently, various derivatives of warfarin have been synthesized and their activity on VKOR are being surveyed[26]. It has been observed that, when the -OH in the 4th position is replaced by -SH the activity of enzyme is decreased by 8-fold. Substituents 582 at position 3 have been shown to be important for binding, for example, as natural compound ferulenol, is almost 22 times more inactivating than that of warfarin. Hence, the following features about the mechanism of action of warfarin and related drugs can be inferred. Firstly, warfarin binds to the active site of enzyme VKOR. Secondly, inhibition by warfarin is noncovalent and most importantly, warfarin binds to the enzyme because it mimics the transition state of the reductive elimination step (Fig. 12)[26]. Figure 12:Structural similarity between transition state of reductive elimination (right) and deprotonated form of warfarin Detection of VKOR gene[27], [28] enables in order to further elucidate the binding of warfarin to the enzyme from the molecular perspective. It has been observed that three mutations in the recombinant protein VKORY139C, Y139S, or Y139F,are warfarin resistant[29], [30]. Mutation of Tyr-139 toPhe, makes the VKOR resistant to warfarin, but still retains the actual enzyme activity. Again, it is clear from the topology diagram that, Tyr-139 lies as the same side of the helix that of active cystine residues. So, these observation leads to the conclusion that Tyr-139 or specifically hydroxyl group of tyrosine plays a key role in warfarin binding[30]. The arena of orally administered anticoagulant drug is dominated by warfarin. It is used as a racemic mixture and nobenefit is observed by administrating only one enantiomer. Because of its mechanism of action, the complete pharmacodynamic effect to a dose, takes almost 2-5 days. The main enzyme responsible for the metabolism of warfarin is cytochrome P450 (CYP2C9). It converts (S)-warfarin to its inactivated oxidised form. Warfarin can also be metabolised by reductases which produces compounds with minimum anticoagulant activity. Thus, an entire dose of warfarin can be excreted through urine. 2.2. Synthetic Thrombin Inhibitor Thrombin is the central enzyme in the blood coagulation cascadebecause its converts factor XI, VII, V, XIII to their respective activated form and most importantly thrombin generates fibrin monomer form fibrinogen[31]. Therefore, the development of novel therapeutically-effective drugswere an alluring prospect in targeting thrombin.Drugs, using both covalent and non-covalent mechanism of action, has been designed for inhibition of thrombin. Thrombin is constituted of two polypeptide chains:A chain (36 residues) and B chain (259 residues). These two chains are attached by disulphide bonds between Cys-1 and Cys-122[32]. Previous studies on A chain received little interest in the field of research as it was thought to be an additional appendage to the catalytic B chain. Later it was revealed that A chain plays a vital role for stabilization of B chain as mutations in A chain resulted into severe bleeding[33], [34]. B chain is comprised of two asymmetrically associated six stranded b-barrels which comes together to accommodatethe catalytic triad of the enzyme i.e.,His57, Asp-102, Ser-195. Thrombin is normally represented in Bode orientation (Fig. 13)where small A chain is placed behind the B

chain[35]. Here,the active site hasbeen viewed in the centre, apart from two distinctive Exosites. These sites are rich in positively charged residues like Arg/Lys. Exosite I is situated at the right-hand lower side and interacts with substrates like fibrinogen and thrombomodulin. Exosite II is located at left hand upper side and binds the molecules of heparin, dermatan etc. There are other substrate specific pockets responsible for accommodation of specific ligand groups, thus contributing to the overall specificity of thrombin (sites are designated by S1, S2, S3 etc)[36]. Figure 13:Structure and important sites of thrombin; thrombin is viewed in standard Bode orientation where A chain (blue) is placed be behind the B chain (green); active site residues (red) are situated in the centre between two beta barrels (PDB ID 1ETT) [37]. Thrombin is a chymotrypsin-like serin protease enzyme, its mechanism of action has been reviewed in many resources[38], [39].



Thrombin selectively cleaves the Arg residue (P1 residue) and it is often found that the next residue is Pro (P2 residue). Early anticoagulants based on inhibition of thrombin were designed by imitating endogenous ligand fibrinogen A. Bajusz designed a series of tripeptide aldehyde developed after fibrinogen A peptide cleavage site. One class of these tripeptide i.e., D-Phe-Pro-ArgCHO(Fig. 14)has been found out to be the most efficient in terms of clotting time[40]. This molecule can bind to the active site as it has same structure as that of endogenous substrate, Arg at P1 and Pro next to it. The active serine residue attacks at the aldehyde and the reaction can be said to be arrested at that stage by forming a hemiacetallinkage(Fig. 15), here the aldehydic group acts a "Serine trap". Figure 14: Structures of covalent thrombin inhibitors; most of them utilises the D-Phe-Pro-Arg sequence to bind with the active site Further investigation on this class of drugs led to development of more potent thrombin inhibitor D-Phe-Pro-Argchloromethylketone (PPACK)(Fig. 14). When PPACKsare attached to the thrombin Ser-195 and His-57, both are linked covalently to the drug. His-57 residue is appended to the chloromethylketone trap and Pro, D-Phe are stabilised in the S2 and S4 sites, respectively. In case of boronic acid mediated inhibitors, boronic ester linkage is formed at the active Ser-195 residue[41]. Many drugs of this class portray high anticoagulant activity which is principally attributed to their covalent bond forming mechanism of binding[42]. However, it must be mentioned that serine trap concept has many potential drawbacks. For example, these drugs fail to achieve necessary efficacy as they exhibit slow binding kinetics. Further studies have revealed that slow binding inhibitors are less successful than fast binding inhibitors. Again, nonspecific covalent binding might lead to immunological responses and other side effects. Figure 15:Covalent binding mode of thrombin inhibitor PPACK; phenyl side chain occupies the S4 cleft whereas the proline side chain occupies the S2 cleft. Ser195 and His-57 both are trapped covalently within the molecule Parallel to the 'serin trap approach', another class of drug was developed which are not dependent on covalent bond formation with active serine residues[43]. Two initial examples of this class are NPAP (naphthylsulfonyl-glycyl-4-AmidinoPhenylAlaninePiperidide) and argatroban[44]. Both of these drugshave been designed earlier after the prototype, N- | Volume 7, Issue 7 | ISSN: 2456-3315 IJRTI2207082 International Journal for Research Trends and Innovation (www.ijrti.org) 584 tosyl-arginine methyl ester (TAME). Argatroban is a type of TAME where methyl ester is substituted by amide. Toxicity due to sulfonyl group is counteracted with carboxylic acid group. X-ray structure of argatroban bound thrombin showed that the S1 pocket is occupied by the guanidine side chain, an orientation different from that of serine trap inhibitors or PPACK and consequently guanidine can make an ionic bond with Asp-189[45]. Furthermore, a part of piperidine ring along with methyl appendage is inserted into the S2 pocket tightly and the carboxylic group is pointed towards the oxyanion hole forming hydrogen bonds with Ser-195. Another unique feature of argratroban is that it can bind to both soluble thrombin and clot-bound thrombin[46]. 2.3. Hirudin and related derivatives Hirudin is an anticoagulant[47], extracted from medicinal leechHirudomedicinalis. It prevents coagulation of blood during blood extraction and even after the removal of the leech, it continues to bleed due to the effect of hirudin. It is another example of an inhibitor, which directly targets the active site of thrombin for its anticoagulant activity. Hirudin is a family of around 20 related small proteins or polypeptides having 65 to 66 residues (molecular mass around 7kDa), where the tyrosine at 63 position is O-sulphated. The structure of hirudin[48] is stabilised by three disulphide bridges. There are three distinctive regions in

the three-dimensional structure of hirudin, first one is a central core made of 3-30 residues (in other isoforms 37-46, 56-57), second there is a "finger" of 31-36 residues and a disordered C-terminus loop. Therapeutically used hirudin are produced by recombinant technology using yeast so they lack the sulphate group at Tyr-63 but nevertheless they are highly selective and efficacious towards thrombin[49]. (a) (b) Figure 16: Binding of hirudin with thrombin; (a) schematic diagram showing binding of C-terminal of hirudin at the fibrinogen binding cleft and binding of N-terminal of hirudin at catalytic active site; (b) structure showing the binding of hirudin (blue) at fibrinogen binding site of thrombin (yellow) (PDB ID 1HRT)[50]. Binding of hirudin to thrombin is elucidated by x-ray crystallography. This binding is very tight as it is a result of interaction of hirudin with active site of thrombin as well as fibrinogen binding Exosite I so hirudin can be thought to be a bivalent ligand[51].The long chain of C-terminus of hirudin attaches to the positively charged fibrinogen binding site whereas the N-terminus simultaneously occupies the main active site of the thrombin[52]. Val-1 and Tyr-3 side chains plays an important role in N-terminus binding as they occupy S2 and S3 pockets respectively by making several hydrophobic interactions. The primary substrate-specificity site S1 remains vacant on hirudinbinding[53]. Desirudin is used in hip or knee surgery for prevention of DVT. Lepirudin, another variant of hirudin where Val-Val is substituted by Leu-Thr, is used as a substitute of heparin in HIT patients[54]. Major structural alterations in hirudin lead to the discovery of hirudin like molecules namely, "Hirugens" and "Hirulogs"[55]. The Hirugens are peptide fragments containing only the C-terminal fibrinogen binding domain. "Hirulogs"[56] are the peptide analogues of hirudin where the nonbinding core sequence is eliminated and thrombin binding sequence are attached by Poly-Gly linker. One of the most important examples of hirugen is Bivalirudin. As hirudin is a non-human protein it can trigger the formation of antibodies in body[57]. Another drawback of the hirudin is its strong pharmacokinetics dependency on renal functionwhich makes it difficult in determining the dose for elderly and patients with renal impairments. If a patient surfers minor bleeding due to drug effects, then pausing the drug is suffice but if the bleeding is fatal or the patient has suffered from renal failure, hemofiltration[58] is required to reduce the level of plasma lepirudin[59]. 2.4. Heparin and other related drugs The final class of drugs that will be described over here is heparin. Heparin is the choice of anticoagulant when a rapid therapeutic effect is required. Heparin utilises an indigenous anticoagulant factor antithrombin for this activity. Antithrombin is a glycoprotein present in blood plasma (0.12 mg/ml) and has a life time of 3 days. It contains 432 amino acid residues andits structure is stabilized by three disulphide bonds. Antithrombin also contains four glycosylation sites[60], each of which contains four similar biantennary oligosaccharide chain. Three-dimensional structure of antithrombin[61] shows two noteworthy features .First, it contains a five stranded beta sheet which dominates the structure. Second feature is the presence of a large loop containing the active residues (Arg-393 and Ser-394). This loop is the characteristic of a serine protease inhibitor or serpin and is also present proteins, e.g., antichymotrypsinetc[62], [63]. Structure of Antithrombin (PDB ID-1ATH); reactive bond loop is coloured pink and active residuesare highlighted Antithrombin acts on various type of factors involved in coagulation cascade but its principal effect is discerned by its action on thrombin and Factor Xa[64]. Inactivation by antithrombin is achieved by trapping of thrombin and other protease in an equimolar amount.

The process of inactivation is initiated by the recognition of reactive bonds by protease enzymes. Arg-393 is critical for its recognition as it the P1 residue of the enzyme, the proteinase can bind to the extended loop of the antithrombin[65]. Hence the process of cleavage is ceased in an intermediate stage. Thrombin can cleave the bond in greater than three days followed by the dissociation of thrombin-antithrombin complex, henceforth, setting free the thrombin for action[66]. Heparin, a polysaccharide or precisely a glycosaminoglycan(Fig. 18), is composed of the alternating arrangement of DGlucosaminesanduronic acids. This disaccharide repeats can be 5 units long (molecular weight ~3000) or can go upto 50 units in highest cases (molecular weight ~30,000). The anomer of uronic acid, iduronic acid is present in sulphated form at position 2. Its other anomerglucuronic acid is also present in non-sulphated form but as minor constituent. Glucosamine can be N-sulphated at the position 3, or can be present in N-acylated form in position 6, or both acylatedsulphated modifications can be present in a single glucosamine unit[67]. Numerous structural combinations are possible for formation of heparin by the above-mentioned units but fortunately, when a cell synthesizes, it only manufactures a limited number of features necessary for its biological activity. The commercial preparation of heparin can alter the overall composition of biological heparin but still can retain the activity. Figure 18:Structure of Heparin; only a small portion of the large polymeric chain has been depicted here The rate of antithrombin-thrombin reaction is 1.5-4*107 M-1 s -1 but in presence of heparin it can be increased by 2000 to 4000- fold[68], [69]. The reaction between Factor Xa and antithrombin is accelerated by heparin up to 500 to 1000-fold. Mechanism of this extraordinary acceleration starts with formation of heparin-antithrombin complex. Heparin binds to the antithrombin using first five pentasaccharide which induced the conformational changes[70]. It has been proposed that this conformational change leads to the partial insertion of reactive bond loop into the beta sheet and thereby bringing the reactive loop into active conformation[71]. Now thrombin binds to this newly formed heparin-antithrombincomplex through Exosite-2 by some non-specific interactions between positively charged Lys/Arg residue and strong negatively charged heparin. That is how thrombin comes in the contact with reactive bond loop of antithrombin through the assistance of bridging mechanism of heparin. Then, heparin rapidly dissociates from the overall complex and inhibits another protease, hence we can shed light on the catalytic mechanism of action of heparin inhibition[72]. (a) Mechanism of action of Heparin; (a) Heparin antithrombin (AT) complex is formed by the binding of antithrombin to the heparin chain; then thrombin (T) binds to the heparin chain of the heparin antithrombin complex and consequently the reactive bind is inserted into the active site of thrombin thus it is inhibited. Binding of FXa proceeds in a similar fashion but does not involve binding of FXa to the heparin chain(b) Structure of antithrombin(yellow)-Heparin(pink)-heparin chain(white) complex; small chain of thrombin is depicted in green (PDB ID 1TB6)[73]. Due to the large size and charge on heparin, it cannot be orally administered and has to be injected in the form of intermittent IV. Heparin canbeeliminatedvia two mechanisms, first it can bind to the macrophages and endothelial cells where it can undergo degradative metabolism and second pathway is through renal clearance. First process is saturable whereas the latter process is unsaturable and slow. Pharmacokinetics of heparin is non-linear; therefore,heparin can only be administered in hospital set-up, under

strict invigilation of patients. III. Conclusion Blood coagulation is one of the most complex procedures in mammalian physiology resulting from a joint performance of many well-orchestrated smaller processes. Further research in the field of blood coagulation will give an insight of the process from the molecular level. But the development of anticoagulant drugs is restricted with targeting small number of enzymes. Surely, there are many factorseffect of whose inhibitions are currently unknown and further investigation in this field may lead to development of safe and smoothly administrable anticoagulant compounds. IV.

References

[1] John E. Hall and Michael E. Hall, Guyton and Hall Textbook f Medical Physiology, 14th ed. Philadelphia: Elsevier, 2021.

[2] Donald J Abraham, Burger′s Medicinal Chemistry and Drug Discovery: Drug Discovery and Drug Development, 6th ed., vol. 3. Wiley–Blackwell, 2003.

[3] R. G. Macfarlane, "An Enzyme Cascade in the Blood Clotting Mechanism, and its Function as a Biochemical Amplifier," Nature 1964 202:4931, vol. 202, no. 4931, pp. 498–499, 1964, doi: 10.1038/202498a0.

[4] E. W. Davie and O. D. Ratnoff, "Waterfall Sequence for Intrinsic Blood Clotting," Science (1979), vol. 145, no. 3638, pp. 1310–1312, Sep. 1964, doi: 10.1126/SCIENCE.145.3638.1310.

[5] W. E. Winter et al., "Clotting factors: Clinical biochemistry and their roles as plasma enzymes," Advances in Clinical Chemistry, vol. 94, pp. 31–84, Jan. 2020, doi: 10.1016/BS.ACC.2019.07.008.

[6] B. Furie and B. C. Furie, "The molecular basis of blood coagulation," Cell, vol. 53, no. 4, pp. 505–518, May 1988, doi: 10.1016/0092-8674(88)90567-3.

[7] J. Stenflo, "Contributions of Gla and EGF-Like Domains to the Function of Vitamin K-Dependent Coagulation Factors," Critical Reviews™ in Eukaryotic Gene Expression, vol. 9, no. 1, pp. 59–88, 1999, doi: 10.1615/CRITREVEUKARYOTGENEEXPR.V9.I1.50.

[8] P. J. Lenting, O. D. Christophe, H. terMaat, D. Jasper G Rees, and K. Mertens, "Ca2+ Binding to the First Epidermal Growth Factor-like Domain of Human Blood Coagulation Factor IX Promotes Enzyme Activity and Factor VIII Light Chain Binding," Journal of Biological Chemistry, vol. 271, no. 41, pp. 25332–25337, Oct. 1996, doi: 10.1074/JBC.271.41.25332.

[9] M. J. Page and E. di Cera, "Serine peptidases: Classification, structure and function," Cellular and Molecular Life Sciences 2008 65:7, vol. 65, no. 7, pp. 1220–1236, Feb. 2008, doi: 10.1007/S00018-008-7565-9.

[10] T. J. Schuijt et al., "Factor XA activation of factor v is of paramount importance in initiating the coagulation system: Lessons from a tick salivary protein," Circulation, vol. 128, no. 3, pp. 254–266, Jul. 2013, doi: 10.1161/CIRCULATIONAHA.113.003191. .

[11] J. W. Weisel and R. I. Litvinov, "Fibrin formation, structure and properties," Sub-Cellular Biochemistry, vol. 82, pp. 405– 456, Jan. 2017, doi: 10.1007/978-3-319-49674-0_13/FIGURES/11. © 2022 IJRTI | Volume 7, Issue 7 | ISSN: 2456-3315 IJRTI2207082 International Journal for Research Trends and Innovation (www.ijrti.org) 588 [12] L. Muszbek, Z. Bereczky, Z. Bagoly, I. Komáromi, and É. Katona, "Factor XIII: A coagulation factor with multiple plasmatic and cellular functions," Physiological Reviews, vol. 91, no. 3, pp. 931–972, Jul. 2011, doi: 10.1152/PHYSREV.00016.2010/ASSET/IMAGES/LARGE/Z9J0031125840010.JPEG.

[13] I. S. Whitaker, J. Rao, D. Izadi, and P. E. Butler, "Historical Article: Hirudomedicinalis: ancient origins of, and trends in the use of medicinal leeches throughout history," British Journal of Oral and Maxillofacial Surgery, vol. 42, no. 2, pp. 133–137, Apr. 2004, doi: 10.1016/S0266-4356(03)00242-0.

[14] J. McLEAN, "The discovery of heparin.," Circulation, vol. 19, no. 1, pp. 75–78, 1959, doi: 10.1161/01.CIR.19.1.75.

[15] A. F. Charles and D. A. Scott, "Studies on heparinObservations on the chemistry of heparin," Biochemical Journal, vol. 30, no. 10, pp. 1927–1933, Oct. 1936, doi: 10.1042/BJ0301927.

[16] K. P. LINK, "The Discovery of Dicumarol and Its Sequels," Circulation, vol. 19, no. 1, pp. 97–107, 1959, doi: 10.1161/01.CIR.19.1.97.

[17] R. G. Bell and J. T. Matschiner, "Vitamin K Activity of Phylloquinone Oxide," Archives of Biochemistry and Biophysics, vol. 141, no. 2, pp. 473–476, Dec. 1970, doi: 10.1016/0003-9861(70)90164-5.

[18] D. W. Stafford, "The vitamin K cycle," Journal of Thrombosis and Haemostasis, vol. 3, no. 8, pp. 1873–1878, Aug. 2005, doi: 10.1111/J.1538-7836.2005.01419.X.

[19] J. T. Matschiner, R. G. Bell, J. M. Amelotti, and T. E. Knauer, "Isolation and characterization of a new metabolite of phylloquinone in the rat," Biochimica et BiophysicaActa (BBA) - General Subjects, vol. 201, no. 2, pp. 309–315, Feb. 1970, doi: 10.1016/0304-4165(70)90305-3.

[20] A. Zimmermann and J. T. Matschiner, "Biochemical basis of hereditary resistance to warfarin in the rat," Biochemical Pharmacology, vol. 23, no. 6, pp. 1033–1040, Mar. 1974, doi: 10.1016/0006-2952(74)90002-1.

[21] J. K. Tie, C. Nicchitta, G. von Heijne, and D. W. Stafford, "Membrane Topology Mapping of Vitamin K Epoxide Reductase by in Vitro Translation/Cotranslocation," Journal of Biological Chemistry, vol. 280, no. 16, pp. 16410–16416, Apr. 2005, doi: 10.1074/JBC.M500765200.

[22] L. Goodstadt and C. P. Ponting, "Vitamin K epoxide reductase: homology, active site and catalytic mechanism," Trends in Biochemical Sciences, vol. 29, no. 6, pp. 289–292, Jun. 2004, doi: 10.1016/J.TIBS.2004.04.004.

[23] R. B. Silverman, "Chemical Model Studies for the Mechanism of Vitamin K Epoxide Reductase," J Am ChemSoc, vol. 103, no. 19, pp. 5939–5941, 1981, doi: 10.1021/JA00409A068.

[24] R. B. Silverman, "Model Studies for a Molecular Mechanism of Action of Oral Anticoagulants," J Am ChemSoc, vol. 103, no. 13, pp. 3910–3915, 1981, doi: 10.1021/JA00403A050/ASSET/JA00403A050.FP.PNG_V03.

[25] M. J. Fasco, L. M. Principe, W. A. Walsh, and P. A. Friedman, "Warfarin Inhibition of Vitamin K 2,3-Epoxide Reductase in Rat Liver

Microsomes," Biochemistry, vol. 22, no. 24, pp. 5655–5660, 1983, doi: 10.1021/BI00293A031/ASSET/BI00293A031.FP.PNG_V03.
[26] M. Gebauer, "Synthesis and structure–activity relationships of novel warfarin derivatives," Bioorganic & Medicinal Chemistry, vol. 15, no. 6, pp. 2414–2420, Mar. 2007, doi: 10.1016/J.BMC.2007.01.014.
[27] T. Li, C. Y. Chang, D. Y. Jin, P. J. Lin, A. Khvorova, and D. W. Stafford, "Identification of the gene for vitamin K epoxide reductase," Nature 2004 427:6974, vol. 427, no. 6974, pp. 541–544, Feb. 2004, doi: 10.1038/nature02254.
[28] S. Rost et al., "Mutations in VKORC1 cause warfarin resistance and multiple coagulation factor deficiency type 2," Nature 2004 427:6974, vol. 427, no. 6974, pp. 537–541, Feb. 2004, doi: 10.1038/nature02214.
[29] H. J. Pelz et al., "The Genetic Basis of Resistance to Anticoagulants in Rodents," Genetics, vol. 170, no. 4, pp. 1839– 1847, Aug. 2005, doi: 10.1534/GENETICS.104.040360.
[30] S. Rost, A. Fregin, M. Hünerberg, C. G. Bevans, C. R. Müller, and J. Oldenburg, "Site-directed mutagenesis of coumarintype anticoagulant-sensitive VKORC1: evidence that highly conserved amino acids define structural requirements for enzymatic activity and inhibition by warfarin," ThrombHaemost, vol. 94, no. 4, pp. 780–786, Oct. 2005, doi: 10.1160/TH05-02-0082.
[31] K. G. Mann, K. Brummel, and S. Butenas, "What is all that thrombin for?," Journal of Thrombosis and Haemostasis, vol. 1, no. 7, pp. 1504–1514, Jul. 2003, doi: 10.1046/J.1538-7836.2003.00298.X.
[32] E. di Cera, "Thrombin," Molecular Aspects of Medicine, vol. 29, no. 4, pp. 203–254, Aug. 2008, doi: 10.1016/J.MAM.2008.01.001.
[33] S. Akhavan, E. Rocha, S. Zeinali, and P. M. Mannucci, "Gly319 → Arg substitution in the dysfunctional prothrombin Segovia," British Journal of Haematology, vol. 105, no. 3, pp. 667–669, Jun. 1999, doi: 10.1046/J.1365- 2141.1999.01423.X.
[34] J. B. Lefkowitz et al., "The prothrombin Denver patient has two different prothrombin point mutations resulting in Glu300→Lys and Glu-309→Lys substitutions," British Journal of Haematology, vol. 108, no. 1, pp. 182–187, Jan. 2000, doi: 10.1046/J.1365-2141.2000.01810.X.
[35] W. Bode, D. Turk, and A. Karshikov, "The refined 1.9-A X-ray crystal structure of D-Phe-Pro-Argchloromethylketoneinhibited human alpha-thrombin: structure analysis, overall structure, electrostatic properties, detailed active-site geometry, and structure-function relationships.," Protein Science : A Publication of the Protein Society, vol. 1, no. 4, p. 426, 1992, doi: 10.1002/PRO.5560010402.
[36] W. Bode, "Structure and interaction modes of thrombin," Blood Cells, Molecules, and Diseases, vol. 36, no. 2, pp. 122– 130, Mar. 2006, doi: 10.1016/J.BCMD.2005.12.027.
[37] H. Brandstetter et al., "Refined 2·3ÅX-ray crystal structure of bovine thrombin complexes formed with the benzamidine and arginine-based thrombin inhibitors NAPAP, 4-TAPAP and MQPA: A starting point for improving antithrombotics," Journal of Molecular Biology, vol. 226, no. 4, pp. 1085–1099, Aug. 1992, doi: 10.1016/0022-2836(92)91054-S.. A. Owen, "SERINE PROTEINASES," Encyclopedia of Respiratory Medicine, Four-Volume Set, pp. 1–10, Jan. 2006, doi: 10.1016/B0-12-370879-6/00264-7.
[39] L. Hedstrom, "Serine protease mechanism and specificity," Chemical Reviews, vol. 102, no. 12, pp. 4501–4523, Dec. 2002, doi: 10.1021/CR000033X/ASSET/IMAGES/LARGE/CR000033XF00015.JPEG.
[40] S. Bajusz, E. Barabas, P. Tolnay, E. Szell, and D. Bagdy, "INHIBITION OF THROMBIN AND TRYPSIN BY TRIPEPTIDE ALDEHYDES," International Journal of Peptide and Protein Research, vol. 12, no. 4, pp. 217–221, Oct. 1978, doi: 10.1111/J.1399-3011.1978.TB02889.X.
[41] W. Bode, I. Mayr, U. Baumann, R. Huber, S. R. Stone, and J. Hofsteenge, "The refined 1.9 A crystal structure of human alpha-thrombin: interaction with D-Phe-Pro-Argchloromethylketone and significance of the Tyr-Pro-Pro-Trp insertion segment.," The EMBO Journal, vol. 8, no. 11, pp. 3467–3475, Nov. 1989, doi: 10.1002/J.1460-2075.1989.TB08511.X.
[42] A. K. Ghosh and S. Gemma, "Design of Serine Protease Inhibitors," Structure-Based Design of Drugs and Other Bioactive Molecules, pp. 67–112, May 2015, doi: 10.1002/9783527665211.CH3.
[43] J. Stürzebecher, U. Stürzebecher, and F. Markwardt, "Inhibition of batroxobin, a serine proteinase from Bothrops snake venom, by derivatives of benzamidine," Toxicon, vol. 24, no. 6, pp. 585–595, Jan. 1986, doi: 10.1016/0041- 0101(86)90179-0.
[44] C. Izquierdo, F. J. Burguillo, J. L. Usero, and A. del Arco, "Kinetic study of the inhibition of the amidolytic activity of thrombin by benzamidine and N-dansyl-(p-guanidino)-phenylalanine-piperidide (I-2581)," International Journal of Biochemistry, vol. 19, no. 11, pp. 1105–1112, Jan. 1987, doi: 10.1016/0020-711X(87)90313-2.
[45] S. Okamoto et al., "Thrombin Inhibitors. 1. Ester Derivatives of Nα-(Arylsulfonyl)-L-arginine," Journal of Medicinal Chemistry, vol. 23, no. 8, pp. 827–830, 1980, doi: 10.1021/JM00182A003.
[46] R. Kikumoto et al., "Thrombin Inhibitors. 2. Amide Derivatives of Nα-Substituted L-Arginine," Journal of Medicinal Chemistry, vol. 23, no. 8, pp. 830–836, 1980, doi: 10.1021/JM00182A004.
[47] F. Markwardt, "The comeback of hirudin--an old-established anticoagulant agent.," Folia Haematologica (Leipzig, Germany : 1928), vol. 115, no. 1–2, pp. 10–23, Jan. 1988, doi: 10.1016/0049-3848(87)90022-3.
[48] G. M. Clore, D. K. Sukumaran, M. Nilges, J. Zarbock, and A. M. Gronenborn, "The conformations of hirudin in solution: a study using nuclear magnetic resonance, distance geometry and restrained molecular dynamics," The EMBO Journal, vol. 6, no. 2, pp. 529–537, Feb. 1987, doi: 10.1002/J.1460-2075.1987.TB04785.X.
[49] S. R. Stone and J. Hofsteenge, "Kinetics of the Inhibition of Thrombin by Hirudin," Biochemistry, vol. 25, no. 16, pp. 4622–4628, 1986, doi: 10.1021/BI00364A025/ASSET/BI00364A025.FP.PNG_V03.
[50] J. Vitali et al., "The structure of a complex of bovine alpha-thrombin and recombinant hirudin at 2.8-A resolution.," Journal of Biological Chemistry, vol. 267, no. 25, pp. 17670–17678, Sep. 1992, doi: 10.1016/S0021-9258(19)37095-4.

[51] E. Degryse et al., "Point mutations modifying the thrombin inhibition kinetics and antithrombotic activity in vivo of recombinant hirudin," Protein Engineering, Design and Selection, vol. 2, no. 6, pp. 459–465, Mar. 1989, doi: 10.1093/PROTEIN/2.6.459.

[52] J. B. Lazar, R. C. Winant, and P. H. Johnson, "Hirudin: amino-terminal residues play a major role in the interaction with thrombin.," Journal of Biological Chemistry, vol. 266, no. 2, pp. 685–688, Jan. 1991, doi: 10.1016/S0021- 9258(17)35224-9. [53] A. Betz, J. Hofsteenge, and S. R. Stone, "Interaction of the N-Terminal Region of Hirudin with the Active-Site Cleft of Thrombin," Biochemistry, vol. 31, no. 19, pp. 4557–4562, Feb. 1992, doi: 10.1021/BI00134A004/ASSET/BI00134A004.FP.PNG_V03.

[54] A. Greinacher and N. Lubenow, "Recombinant Hirudin in Clinical Practice," Circulation, vol. 103, no. 10, pp. 1479– 1484, Mar. 2001, doi: 10.1161/01.CIR.103.10.1479.

[55] J. Y. Chang, P. K. Ngai, H. Rink, S. Dennis, and J. M. Schlaeppi, "The structural elements of hirudin which bind to the fibrinogen recognition site of thrombin are exclusively located within its acidic C-terminal tail," FEBS Letters, vol. 261, no. 2, pp. 287–290, Feb. 1990, doi: 10.1016/0014-5793(90)80573-2.

[56] E. Skrzypczak-Jankun, V. E. Carperos, K. G. Ravichandran, A. Tulinsky, M. Westbrook, and J. M. Maraganore, "Structure of the hirugen and hirulog 1 complexes of α-thrombin," Journal of Molecular Biology, vol. 221, no. 4, pp. 1379–1393, Oct. 1991, doi: 10.1016/0022-2836(91)90939-4.

[57] A. Greinacher, P. Eichler, D. Albrecht, U. Strobel, B. Pötzsch, and B. I. Eriksson, "Antihirudin antibodies following lowdose subcutaneous treatment with desirudin for thrombosis prophylaxis after hip-replacement surgery: incidence and clinical relevance," Blood, vol. 101, no. 7, pp. 2617–2619, Apr. 2003, doi: 10.1182/BLOOD-2002-04-1055.

[58] F.-C. Riess et al., "Recombinant hirudin as an anticoagulant during cardiac operations: experiments in a pig model," European Journal of Cardio-thoracic Surgery, vol. 11, pp. 739–745, 1997, Accessed: Jul. 05, 2022. [Online]. Available: https://academic.oup.com/ejcts/article/11/4/739/350220

[59] N. Lubenow, P. Eichler, T. Lietz, and A. Greinacher, "Lepirudin in patients with heparin-induced thrombocytopenia – results of the third prospective study (HAT-3) and a combined analysis of HAT-1, HAT-2, and HAT-3," Journal of Thrombosis and Haemostasis, vol. 3, no. 11, pp. 2428–2436, Nov. 2005, doi: 10.1111/J.1538-7836.2005.01623.X. [60] V. Picard, E. Ersdal-Badju, and S. C. Bock, "Partial Glycosylation of Antithrombin III Asparagine-135 Is Caused by the Serine in the Third Position of Its N-Glycosylation Consensus Sequence and Is Responsible for Production of the βAntithrombin III Isoform with Enhanced Heparin Affinity," Biochemistry, vol. 34, no. 26, pp. 8433–8440, 1995, doi: 10.1021/BI00026A026/ASSET/BI00026A026.FP.PNG_V03..

[61] R. W. Carrell, P. E. Stein, G. Fermi, and M. R. Wardell, "Biological implications of a 3 A structure of dimericantithrombin," Structure, vol. 2, no. 4, pp. 257–270, 1994, doi: 10.1016/S0969-2126(00)00028-9. ©

[62] L. T. Hunt and M. O. Dayhoff, "A surprising new protein superfamily containing ovalbumin, antithrombin-III, and alpha1-proteinase inhibitor," Biochemical and Biophysical Research Communications, vol. 95, no. 2, pp. 864–871, Jul. 1980, doi: 10.1016/0006-291X(80)90867-0.

[63] R. Carrell and J. Travis, "α1-Antitrypsin and the serpins: variation and countervariation," Trends in Biochemical Sciences, vol. 10, no. 1, p. 20, 1985, doi: 10.1016/0968-0004(85)90011-8.

[64] S. T. Olson and J. D. Shore, "Demonstration of a two-step reaction mechanism for inhibition of alpha-thrombin by antithrombin III and identification of the step affected by heparin.," Journal of Biological Chemistry, vol. 257, no. 24, pp. 14891–14895, Dec. 1982, doi: 10.1016/S0021-9258(18)33366-0.

[65] S. T. Olson and I. Bjork, "Regulation of thrombin activity by antithrombin and heparin," Seminars in Thrombosis and Hemostasis, vol. 20, no. 4, pp. 373–409, 1994, doi: 10.1055/S-2007-1001928/BIB.

[66] Å. Danielsson and I. Björk, "Slow, spontaneous dissociation of the antithrombin—thrombin complex produces a proteolytically modified form of the inhibitor," FEBS Letters, vol. 119, no. 2, pp. 241–244, Oct. 1980, doi: 10.1016/0014- 5793(80)80262-6.

[67] J. Choay and M. Petitou, "The chemistry of heparin: a way to understand its mode of action," Med J Aust, vol. 144, pp. 7– 10, 1986.

[68] S. T. Olson and I. Bjork, "Predominant contribution of surface approximation to the mechanism of heparin acceleration of the antithrombin-thrombin reaction. Elucidation from salt concentration effects.," Journal of Biological Chemistry, vol. 266, no. 10, pp. 6353–6364, Apr. 1991, doi: 10.1016/S0021-9258(18)38125-0.

[69] R. E. Jordan, G. M. Oosta, W. T. Gardner, and R. D. Rosenberg, "The kinetics of hemostatic enzyme-antithrombin interactions in the presence of low molecular weight heparin.," Journal of Biological Chemistry, vol. 255, no. 21, pp. 10081–10090, Nov. 1980, doi: 10.1016/S0021-9258(19)70431-1.

[70] L. H. Lam, J. E. Silbert, and R. D. Rosenberg, "The separation of active and inactive forms of heparin," Biochemical and Biophysical Research Communications, vol. 69, no. 2, pp. 570–577, Mar. 1976, doi: 10.1016/0006-291X(76)90558-1.

[71] R. W. Carrell, D. L. Evans, and P. E. Stein, "Mobile reactive centre of serpins and the control of thrombosis," Nature 1991 353:6344, vol. 353, no. 6344, pp. 576–578, 1991, doi: 10.1038/353576a0.

[72] P. G. W. Gettins, B. Fan, B. C. Crews, I. v. Turko, S. T. Olson, and V. J. Streusand, "Transmission of Conformational Change from the Heparin Binding Site to the Reactive Center of Antithrombin," Biochemistry, vol. 32, no. 33, pp. 8385– 8389, Aug. 1993, doi: 10.1021/BI00084A001/ASSET/BI00084A001.FP.PNG_V03.

[73] W. Li, D. J. D. Johnson, C. T. Esmon, and J. A. Huntington, "Structure of the antithrombin–thrombin–heparin ternary complex reveals the antithrombotic mechanism of heparin," Nature Structural & Molecular Biology 2004 11:9, vol. 11, no. 9, pp. 857–862, Aug. 2004, doi: 10.1038/nsmb811