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Expansion of GPIIB/IIIA Inhibitors Drug Product Range: A Methodological Approach

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Abstract:

Integrin complex GPIIB/IIIA found on platelets plays an important role in thrombogenesis and is a key component of platelet aggregation process. Currently, few inhibitors of GPIIb/IIIa platelet receptors with limited indications and several side effects are used in clinical practice for depression of platelet aggregation. Present study is devoted to development of scientific and methodological approach to expansion of GPIIb/IIIa inhibitors drug product range by means of information analysis methods. Use of peptide compounds as platelet glycoprotein IIb/IIIa inhibitors appears to be most promising. Systematic analysis of natural and synthetic peptide GPIIb/IIIa inhibitors nomenclature allows authors to express modern view on the problem of pharmaceutical development of novel antiplatelet drug products. The authors conclude, that modern approach to pharmaceutical development of peptide antiplatelet drugs for GPIIb/IIIa inhibition should be based on advanced methods, i.e. computer drug design.

Keywords: antiplatelet drugs, antiaggregants, peptide therapeutics, platelet glycoprotein IIB/IIIA inhibitors, novel drug discovery.

INTRODUCTION

For the last 15 years cardiovascular diseases remain #1 cause of death globally [1]. Atherotrombosis – a pathological formation of blood clots due to increase in platelet aggregation activity - is the underlying cause of coronary and brain blood circulation disruption. Platelet glycoprotein IIb/IIIa receptors play one of the fundamental roles in thrombus formation [2-4].

Platelet glycoprotein IIb/IIIa inhibitors (GPIIb/IIIa inhibitors) are powerful antiplatelet drugs. However, only few drug products with limited indications and several side effects are currently available: Abciximab (Reopro), Ruciromab (Monafram), Eptifibatide (Intergrilin), Tirofiban (Aggrastat) [5-8].

Despite its topicality, development of innovative safe and effective GPIIb/IIIa inhibitors remains time-consuming and labor-intensive process. Use of peptide compounds in this case represents a promising approach [9-11], since they are characterized by great physical, chemical, and conformational diversity, and have low toxicity and immunogenicity [12].

Therefore, **the aim** of this study was to develop methodological approach to expansion of antiplatelet (peptide inhibitors of GPIIb/IIIa platelet receptors) drug product range.

MATERIALS AND METHODS

Development of sound methodological approach to assessment of prospects for creation of novel antiplatelet drugs based on GPIIb/IIIa inhibitors was performed using information analysis methods. It involved systemic analysis of peptide GPIIb/IIIa inhibitors nomenclature of natural and synthetic origin.

RESULTS

Thrombocytes, also called platelets, play important role in coronary artery disease pathophysiology, thrombus formation mechanism, and inflammation process [3,4,13-15]. Antiplatelet drugs, currently used in clinical practice, are aimed at suppression of platelet aggregation function and affect different activation pathways. Diversity of antiplatelet drug products is governed by large number of different glycoprotein receptors located on platelet membrane. The main point of application for antiplatelet drugs is the receptors activation of which results in specific process [6].

GPIIb/IIIa complex is the most common platelet receptors which belongs to the integrin family (integrin αIIb/β3). It represents a heterometic protein which acts as a surface receptor for fibrinogen (Fg), von Willebrand factor (vWF), fibronectin (FN), and vitronectin (VN) binding. During aggregation process, platelets interact with each other by means of activated GPIIb/IIIa receptors, independently of their activation pathway [16].

Up to 80 000 GPIIb/IIIa receptors are located on platelet membrane; their activated state is determined by confomational change which is a key component of platelet aggregation process. Therefore, GPIIb/IIIa inhibitors demonstrate rapid and powerful antiaggregation action, mainly due to blocking of fibrinogen bridges formation between adjacent activated GPIIb/IIIa platelet receptors [14, 17, 18].

Due to their advantages, GPIIb/IIIa inhibitors are indicated for patients with acute coronary syndrome as an antithrombotic support drugs during percutaneous coronary interventions to prevent life threatening or thrombotic complications. Recommendations for GPIIb/IIIa

prescription correspond to the C level of evidence and IIa class of recommendation [5, 19].

Activity of GPIIb/IIIa platelet receptors is mediated by their ability to recognize characteristic amino acid sequences: Arg-Gly-Asp (RGD) and Lys-Gln-Ala-Gly-Asp-Val (KQAGDV). RGD sequence occurs in VN, FN, Fg, vWF, whereas KQAGDV sequence is located on the carboxyl-terminal region of fibrinogen γ-chain [16].

Discrete role of GPIIb/IIIa platelet receptors allows for selection of compounds which can regulate their activity. Peptides, which contain RGD and KQAGDV amino acid sequences, are actively studied as candidates for novel plateled aggregation inhibitors [9, 14, 17, 18]. Peptide compounds have several undeniable advantages: they have imperceptible side effects, are easily metabolized into non-active and non-toxic derivatives, possess affinity for integrins (peptide receptors), which mediate most important physiological and pathological processes in human body [12, 20, 21].

Natural GPIIb/IIIa inhibitors containing RGD sequence are of particular interest. They are primarily represented by disintegrins – peptides extracted from viper venoms, e.g., accutin (from *Formosan Agkistrodon acutus* venom), trigramin (from *Trimeresurus gramineus* venom), etc. All disintegrins are homologous, demonstrate rapid and strong inhibition of RGD-dependent processes. However, their use as antiplatelet drugs is limited because of expressed antigenicity. Disintegrin structure was in during pharmaceutical development of synthetic peptide GPIIb/IIIa inhibitors (cyclic peptide Eptifibatide and peptidomimetic Tirofiban) [5, 6, 10, 11].

Linear RGD-containing peptides have low activity and low stability, due to rapid enzymatic cleavage in blood plasma. Cyclic peptides are more stable because of their rigid 3D structure [20].

Some natural peptides (barbourin from snake venom, disagregin from tick saliva), which contain KQAGDV amino acid sequence, have high GPIIb/IIIa platelet receptor specificity [10,11].

During peptide construction, different modifications of Arg-Gly-Asp key sequence are used most commonly. It is feasible to use D-amino acids and replace arginine with amidino- and benzamidino-containing groups in order to decrease peptide susceptibility to enzymatic cleavage. Replacement of arginine with lysine increases aggregation activity of synthetic peptides, since it increases peptide specificity to GPIIb/IIIa complex [9, 22, 23].

Hayashi Y. et al. (1998) studied different amino acid modifications in RGD sequence and established structure-activity relationship. Synthetic peptides with antiaggregation properties should preferably include the following sequence:

$$P\text{-}X_1\text{-}X_2\text{-}X_3\text{-}D\text{-}X_4$$

where X is an amino acid: X_1 and X_3 – small, e.g. glycine; X_2 – large, e.g. isoleucine or cyclic, e.g. proline; X_4 – amino acids with aromatic side chain. Moreover, N-terminal prolin residue is required for GPIIb/IIIa receptor recognition, and asparagine acid residue is needed for antiaggregation properties enhancement [24].

It was found, that number and order of amino acid residues affect affinity between peptide and integrin receptor. Five- to seven-membered peptides with Arg-Gly-Asp-Ser or Arg-Gly-Asp-Phe amin oacid sequences are the most effective thrombogenesis inhibitors. Such peptides and commonly obtained by solid phase synthesis. Vasilyeva et al. (2006) describe biotechnological enzymatic synthesis of peptides with antiaggregation properties, which contain trifunctional amino acids (Asp and Arg). Highest antiaggregation activity was found in synthetic peptides with ARGDS-NH_2 and RGD-dFK sequences [20].

Belushkina N. et al. (2009) and Lotorev D. et al. (2012) have performed computer modeling of binding activity of GPIIb/IIIa platelet receptors with RGD peptides. A dose-dependent antiaggregation activity was established for all peptides under study [9, 22]. Maximal platelet inhibitory effect was demonstrated for Arg-Gly-Gly-Asp-Trp pentapeptide. Peptides with Lys amino acid on the N-terminal were least effective, whereas peptides with arginine on the N-terminal and Gly-Gly sequence showed highest antiaggregation activity. It is possible, that Gly-Gly sequence provides peptide bond strain which results in a more rigid conformation and higher biological activity of peptides [23].

Alekseev A. et al. (2012) have synthesized novel peptide GPIIb/IIIa platelet inhibitors and assessed their antiaggregation activity. It was shown, that Phe-IIa-Ala-Asp-Thru pentapeptide possesses highest activity [25]. In a similar study, the authors have noticed positive effect of asparaginic acid residue on binding between peptide and GPIIb/IIIa platelet receptors, due to ability of peptide to form ionic bond with magnesium ion at the active site. His-IIe-Gly-Asp-Asp pentapeptide showed highest antiaggregation activity [26].

DISCUSSION

At present, high importance of integrins for almost every physiological process in the human body has been confirmed; this stipulates development of novel peptide drugs – integrin inhibitors. One of the strategies for search of such novel drugs is described by Ley K. et al. (2016). In their work leukocyte and thrombocyte integrins, especially glycoprotein IIb/IIIa complex which plays important role in platelet aggregation rpocess, are considered as the most promising pharmacological targets [21, 22].

Antiplatelet drugs acting as platelet aggregation function depressants by affecting different activation pathways are widely used in clinical practice. Different aspects, including interaction of thrombocytes between each other, and interaction with other cells and blood vessel walls, should be taken into account during elaboration of approaches to atherotrombosis prevention and treatment. Novel antiplatelet drugs should selectively inhibit most important atherotrombosis pathways, for example – inhibit GPIIb/IIIa platelet receptors [4, 13].

Use of peptide compounds as GPIIb/IIIa inhibitors represents one of the most promising approaches to development of novel antiplatelet drugs [9, 20, 22, 23, 25, 26]. This is promoted by the fact, that peptides commonly have low toxicity and immunogenicity, they can be easily

metabolized into non-active derivatives, and their diversity allows to select ones that are best fitted to inhibit integrin receptors, which mediate important physiological and pathological processes. Pharmaceutical development of peptide-based drug products is devoted to biological and chemical peptide screening, increasing their stability, bioavailability, and cell membrane permeability, use of novel drug delivery technologies, etc. [10, 11, 12, 20].

Sequence of amino acid residues and their number can significantly alter peptide affinity to GPIIb/IIIa platelet receptors. Different fragments which mimic residues required to bind with integrin, such as Arg-Gly-Asp (RGD) and Lys-Gln-Ala-Gly-Asp-Val (KQAGDV), are usually used in the design of GPIIb/IIIa inhibitors.

Arg-Gly-Asp sequence can be found in VN, FN, Fg, and vWF. Most integrins can recognize RGD sequence. Therefore, RGD-containing peptides should be considered as potential inhibitors of interaction between FN and GPIIb/IIIa platelet receptors. Lys-Gln-Ala-Gly-Asp-Val sequence is specific only for Fg molecules and is located on C-terminus of its γ-chains; it is possible, that Fg binds with GPIIb/IIIa receptors via this part of the molecule [16, 17].

Influence of amino acid sequence modification on the antiaggregation activity of GPIIb/IIIa inhibitors has been thoughtfully studied. Cyclic peptides are more stable than linear since they possess rigid 3D structure and more resistant to enzymatic cleavage. Prospectivity of work in the field of novel RGD-peptids synthesis for development of new antiplatelet drugs - GPIIb/IIIa inhibitors – is confirmed by high exploratory activity in this field [9, 17, 20, 21].

Traditionally, short-chained peptide GPIIb/IIIa inhibitors are obtained by solid phase synthesis or in combination of such synthesis and biotechnological approach [20]. Computer drug design represents modern approach to pharmaceutical development of antiaggregants based on GPIIb/IIIa inhibitors [22, 23, 25, 26]. This approach allows to optimize expensive and time-consuming process of novel drugs pharmaceutical development, and to minimize possible risks.

CONCLUSION

Platelets play a key role in the inflammation process, including atherogenesis. GPIIb/IIIa platelet receptors participate in thrombus formation and are key component of platelet aggregation process.

The search for novel GPIIb/IIIa inhibitors is relevant and aimed at differentiation of antiaggregation drugs by their mode of action at different points of application, and at development of drugs effective at the platelet aggregate formation stage.

Utilization of peptide compounds as GPIIb/IIIa inhibitors is promising, due to their physical, chemical and conformational diversity, low toxicity and immunogenicity, few side effects, and good affinity to integrins.

Modern approach to pharmaceutical development of novel antiplatelet drugs based on GPIIb/IIIa inhibitors includes use of innovative technologies based on computer drug design

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