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Structure of Purinergic P2Y₁₂ Receptors and some aspects of their biochemistry

Estructura de los receptores purinérgicos de P2Y₁₂ y algunos aspectos de su bioquímica

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ABSTRACT:

The goal of this study is to develop a more rigorous understanding of the P2Y₁₂ receptor first described in blood platelets where it plays a central role in the complex processes of activation and aggregation. P2Y₁₂ receptors are 7-membrane-spanning proteins coupled to G proteins, which are activated by nucleotides, extracellular signaling molecules that are released from damaged cells or secreted via non-lytic mechanisms during inflammatory, ischemic, and hypoxic conditions. It is the drug targets for inhibition of platelet aggregation. We paid attention to structure and properties that provide essential insights for the development of improved P2Y₁₂R ligands and allosteric modulators as drug candidates.

KEYWORDS: P2Y₁₂ receptor, G-protein coupled receptors (GPCR), platelets, inhibitors of the P2Y₁₂ receptor.

RESUMEN:

El objetivo de este estudio es desarrollar una comprensión más rigurosa del receptor P2Y₁₂ descrito por primera vez en las plaquetas de la sangre, donde desempeña un papel central en los complejos procesos de activación y agregación. El receptor P2Y₁₂ es un receptor de siete dominios transmembranales acoplado a proteína G, que se activa mediante nucleótidos que se liberan de las células dañadas o se secretan a través de mecanismos no líticos durante condiciones inflamatorias, isquémicas e hipóxicas. Son los blancos farmacológicos para la inhibición de la agregación plaquetaria. Prestamos atención a la estructura y las propiedades que

proporcionan información esencial para el desarrollo de ligandos P2Y₁₂R mejorados y moduladores alostéricos como candidatos a fármacos.

PALABRAS CLAVE: Receptor P2Y₁₂, receptores acoplados a proteínas G (GPCP), plaquetas, inhibidores del receptor P2Y₁₂.

INTRODUCTION

In recent decades over 800 G-protein coupled receptors (GPCP) have been identified in human cells. Human GPCRs are divided into four subfamilies: rhodopsin - like receptors (class A), secretin and adhesion receptors (class B), glutamate receptors (class C), and frizzled/taste2 receptors (class F), based on their structural similarities and properties. These receptors are activated by the extracellular stimuli, including, ions, neurotransmitters, lipids, chemokines, and hormones, and then couple to G proteins and initiate signaling networks, resulting in a broad range of biochemical processes¹. New methods and technologies in membrane protein engineering and crystallization have been developed to facilitate GPCR structure determination which allowed solving the structure of the P2Y₁₂ receptor.

Structure of P2Y₁₂ receptor

There have been several studies in the literature reporting about cell types that can express it. It is present in macrophages², endothelial cells, platelets, glial cells, and vascular smooth muscle cells³, in yet unspecified leukocytes⁴, on several immune cells including dendritic cells⁵, in osteoclasts⁶ and breast cancer cell lines⁷. It was also shown that P2Y₁₂ is not expressed by human endothelial cells of cerebral, aortic, or cardiac origin⁸. Still, the expression and function of P2Y₁₂ in other cell types remain poorly investigated. P2Y₁₂ is expressed on the plasma membrane of the platelet with about 400 copies per cell⁹. There are published studies describing various radioligands that have been used to characterize and quantify the platelet P2Y₁₂ receptor. Thus, the number of P2Y₁₂ receptors was measured in intact platelets and membrane preparations by using [³H]PSB-0413 selective P2Y₁₂ that is bound to 425 ± 50 sites/platelet¹⁰.

The receptor contains 342 amino acid residues, including 4 extracellular Cys residues at positions 17, 97, 175, and 270: Cys 97 and Cys 175, which are linked by a disulfide bridge and are important for receptor expression; 2 potential N-linked glycosylation sites at its extracellular amino-terminus may modulate its activity¹¹. P2Y₁₂ exists as homo-oligomers situated in lipid rafts and they are disrupted into nonfunctional dimers and monomers¹². In 2014, Zhang et al. reported the 2.6 Å resolution crystal structure of human P2Y₁₂R in complex with a non-nucleotide reversible antagonist ethyl 6-(4-((benzyl-sulfonyl)carbamoyl)piperidine-1-yl)-5-cyano-2-methyl nicotinate (AZD1283) and also identified three structures of P2Y₁₂R in complex with an antagonist AZD1283 and two agonists 2-methylthio-adenosine-5' -diphosphate (2MeSADP, a close analog of endogenous agonist ADP) and derivative 2-methylthio-adenosine-5' -triphosphate (2MeSATP)^{13,14}.

It is known that extracellular loops of receptors play important roles in shaping the entrance to the ligand - binding pockets. In 2016, Mengjie and Beili¹⁵ described that two ligand - binding pockets exist in the receptor. The agonist 2MeSADP and the antagonist AZD1283 occupy one of the pockets, while the other one remains available, suggesting that P2Y₁₂R may simultaneously bind to two different ligands. It was suggested that the endogenous ligand ADP can also bind to this allosteric site and serves as an inhibitor of the receptor¹⁵.

The 7TM regions of GPCRs play important roles in signal transduction. The overall fold of the P2Y₁₂R structure consists of a canonical seven-transmembrane bundle of α -helices and a carboxy-terminal helix VIII that is parallel to the membrane bilayer¹⁶.

Another study indicates the involvement of Arg256, Tyr259, and, possibly, H253 (transmembrane region TM6) amino acid residues in the function of the human P2Y₁₂. Arg256 appears to play a role in the recognition of nucleotide agonists and the non-nucleotide antagonist reactive blue-2, but no role in the recognition of the nucleotide antagonist cangrelor¹⁷.

Deficiency of P2Y₁₂ in the platelets

Genetic variations in GPCR genes can disrupt receptor function in a wide variety of human genetic diseases, including platelet bleeding disorders¹⁸. Some studies have primarily concentrated on P2Y₁₂ congenital deficiency results in bleeding disorders characterized by a platelet impaired response to ADP. Deficiencies of P2Y₁₂ are associated with nucleotide deletions in the open-reading frame, frameshifts, and early truncation of the protein, or with a nucleotide substitution in the transduction initiation codon¹⁹.

In 2016, Li et al. studied the methylation of the P2Y₁₂ promoter of patients with ischemic cerebrovascular disease that is associated with higher platelet reactivity and increased risk of ischemic events. Hypermethylation of promoter DNA is related to transcriptional silencing of gene expression resulting in decreased protein activity. Methylation analysis of peripheral blood samples might be a novel molecular marker to help early identification of patients at high risk for clinical ischemic events^{20,21}. The study by Su et al., reported the association of methylation levels of P2Y₁₂ promoter DNA and the risk of clopidogrel resistance in coronary artery disease patients²². Polymorphisms of the P2Y₁₂ receptor have been proposed to be associated with an increased risk of cardiovascular disease²³.

Transduction of the P2Y₁₂R signal

As it is known purinergic signaling can regulate hemostasis, thrombosis, and inflammation through the co-stimulation of various cell types, including platelets, leukocytes, endothelial, and vascular smooth muscle cells²⁴. Adenine nucleotide mediated cell activation is an important mechanism in the biochemical steps of hemostasis (including thrombosis), and it involves P2Y₁₂ receptor^{25,26}.

ADP binding to the P2Y₁₂ receptor causes a conformational change in the receptor allowing it to act as a guanine - nucleotide factor and activate the membrane - associated heterotrimeric G - protein of the G_i - family. The active GTP - bound G_{αi} - subunit binds to adenylate cyclase leading to decrease cAMP synthesis. G_i triggers the phosphatidylinositol 3 - kinase (PI3-kinase) pathway, which has substrate phosphatidylinositol 4, 5 - bisphosphate (PI4,5P.)²⁷. The enzyme PI3-kinase consists of a catalytic subunit associated with a regulatory subunit [28]. By this enzyme, PI4,5P. is converted to phosphatidylinositol 3, 4, 5 - trisphosphate (PI(3,4,5)P3) at the plasma membrane. It leads to increase calcium level and the activation of the kinase Akt leading to inducing downstream of the Von Willibald Factor receptor, glycoprotein Ib-IX-V, the collagen receptor glycoprotein VI and inhibition of Rap1GAP RASA3^{29,30,31}. Battram et al., characterized the RAP1GAP RASA3 as a major PI(3,4,5)P3-binder and PI3-kinase regulated protein in human platelets. These changes initiate platelet aggregation by altering the ligand-binding properties of the glycoprotein IIb/IIIa³². On the final stage of platelet activation IIb-IIIa receptor binds to fibrin and ensures platelet aggregation resulting in the formation of a thrombus^{33,33}.

Activation of P2Y₁₂ leads to inhibition of vasodilator-stimulated phosphoprotein, which restrains either secretory or adhesive events in platelets. Vasodilator-stimulated phosphoprotein phosphorylation flow cytometry assay is used to monitor platelet responsiveness to P2Y₁₂ targeted antiplatelet therapy^{34,35,36}. P2Y₁₂ G_i signaling leads positive regulation of other intracellular pathways including extracellular-signal-regulated kinase, myosin light chain kinase, and Src family kinases as well as to membrane lipid shifts toward a pro-coagulant state such as phosphatidylserine and P-selectin exposure. ADP also contributes to the release of several agonists such as TXA₂ by acting on P2Y₁₂^{37,52}. P2Y₁₂ has also been shown to regulate the migration of vascular smooth muscle cells (VSMCs). In these cells, ADP via P2Y₁₂ G_α activation inhibited cAMP/

PKA signaling pathway resulting in cofilin dephosphorylation, actin disassembly, and as a consequence, an increase in VSMCs motility and migration³⁸⁻⁴⁰.

Gratacap et al., studied P2Y₁₂ supporting of thrombin generation by amplifying membrane exposition of phosphatidylserine, platelet-derived microparticle formation, and collagen-induced exposure of tissue factor. It contributes to leukocyte activation induced by surface P-selectin exposure and the formation of platelet-leukocyte aggregates^{41,42}.

P2Y₁₂ receptor activation leads also to reduce platelets NO responsiveness and reinforces the production of reactive oxygen species (ROS). ROS can further activate platelets, enhance platelets-leukocytes interactions, and accelerate lipids oxidation and inflammation processes. In endothelial cells and the activation of the P2Y₁₂ receptor decreases the intracellular cAMP concentration, with negative effects on endothelial barrier functions, promoting VSMC contraction and vasoconstriction⁴³.

Drugs as inhibitors of P2Y₁₂ receptor

P2Y₁₂ receptor is involved in the central pathological process in atherothrombosis of the coronary, cerebral, or peripheral arteries that can lead to coronary syndromes, stroke ischemic attack, and acute limb ischemia is platelet activation.

During last year's much more information has become available on applying inhibitors of P2Y₁₂ receptors in platelet inhibition. Dual-antiplatelet therapy with aspirin and a P2Y₁₂ receptor inhibitor is the standard treatment for patients undergoing percutaneous coronary intervention, acute myocardial infarction^{44,45}. There are two main classes of oral P2Y₁₂ inhibitors: thienopyridines (ticlopidine, clopidogrel, and prasugrel) and non-thienopyridine (ticagrelor) agents. The availability of clopidogrel, prasugrel, ticagrelor with varying levels of potency has enabled physicians to contemplate individualized treatment regimens, which may include escalation or de-escalation of P2Y₁₂-inhibiting therapy. Clopidogrel and prasugrel are oral prodrugs requiring hepatic metabolism to generate an active metabolite that irreversibly inhibits the P2Y₁₂ receptor and it has long been the gold standard but has major pharmacological limitations such as a slow onset and long duration effect⁴⁶. Ticagrelor is a direct-acting (no metabolism required) oral agent that reversibly inhibits the P2Y₁₂ receptor. Cangrelor is a direct-acting intravenous agent that reversibly inhibits the P2Y₁₂ receptor. Ticagrelor binds reversibly to the P2Y₁₂ receptor at a site that is distinct from the ADP-binding site^{47,53}. Two novel P2Y₁₂ receptor antagonists vicagrel and selatogrel analogs of clopidogrel with the enhanced and more efficient formation of its active metabolite^{48,49}. Of the P2Y₁₂ inhibitors, ticagrelor seems to be associated with a lower incidence of *de novo* cancer during follow-up comparison with prasugrel and clopidogrel, regardless of the duration of dual antiplatelet therapy⁵⁰.

CONCLUSION

P2Y₁₂-mediated nucleotide signaling is now considered to be a critical player in the development of cardiovascular diseases. Recently it was found that it has the primary role in the inflammatory response⁵¹. P2Y₁₂ is expressed in many cells and not just has a function in the platelets. In addition to its antithrombotic properties, P2Y₁₂ inhibitors can, therefore, be considered to have valuable pharmacological targets for inflammation. More information on the interaction of different substances with P2Y₁₂ would help to establish a greater degree of accuracy on this matter.

REFERENCES

1. Venkatakrishnan, A. J., Deupi, X., Lebon, G., Tate, C. G., Schertler, G. F., et al., 2013. Molecular Signatures of G - Protein - Coupled Receptors. *Nature*, 494: 185–194.

2. Kronlage, M., Song, J., Sorokin, L., Isfort, K., Schwerdtle, T., Leipziger, J., Robaye, B., Conley, P., Kim, C.H., Sargin, S., et al. 2010. Autocrine Purinergic Receptor Signaling is Essential for Macrophage Chemotaxis. *Sci. Signal.*, 3: 55.
3. Rauch, B.H., Rosenkranz, A.C., Ermler, S., Böhm, A., Driessen, J., Fischer, J.W., Sugidachi, A., Jakubowski, J.A., Schrör, K., 2010. Regulation of Functionally Active P2Y₁₂ ADP Receptors by Thrombin in Human Smooth Muscle Cells and the Presence of P2Y₁₂ in Carotid Artery Lesions. *Arterioscler. Thromb. Vasc. Biol.*, 30: 2434–2442.
4. Gachet, C., 2012. P2Y₁₂ Receptors in Platelets and Other Hematopoietic and Non-Hematopoietic Cells. *Purinergic Signal.*, 8(3): 609–619.
5. Addi, A., Cammarata, D., Conley, P.B., Boeynaems, J.M., Robaye, B., 2010. Role of the P2Y₁₂ Receptor in the Modulation of Murine Dendritic Cell Function by ADP. *J. Immunol.*, 185: 5900–5906.
6. Su X., Floyd D.H., Hughes A., Xiang J., Schneider J.G., Uluckan O., Heller E., Deng H., Zou W., Craft C.S., et al., 2012. The ADP receptor P2RY₁₂ regulates osteoclast function and pathologic bone remodeling. *J. Clin. Invest.*, 122:3579–3592.
7. Ballerini, P., Dovizio, M., Bruno, A., Tacconelli, S., Patrignani, P., 2018. P2Y₁₂ Receptors in Tumorigenesis and Metastasis. *Front. Pharmacol.*, 9: 66.
8. Reiner, M.F., Akhmedov, A., Stivala, S., Keller, S., Gaul, D.S., Bonetti, N.R., Savarese, G., Glanzmann, M., Zhu, C., Ruf, W., et al., 2016. Ticagrelor, but not Clopidogrel, Reduces Arterial Thrombosis via Endothelial Tissue Factor Suppression. *Cardiovasc. Res.*, 113: 61–69.
9. Haberstock-Debic, H., Andre, P., Mills, S., Phillips, D.R., Conley, P.B., 2011. A Clopidogrel-Insensitive Inducible Pool of P2Y₁₂ Receptors Contributes to Thrombus Formation: Inhibition by Elinogrel, a Direct-Acting, Reversible P2Y₁₂ Antagonist. *J. Pharmacol. Exp. Ther.*, 339: 54–61.
10. Ohlmann, P., Lecchi, A., El-Tayeb, A., Müller, C.E., Cattaneo, M., Gachet, C., 2013. The Platelet P2Y₁₂ Receptor Under Normal and Pathological Conditions. Assessment with the Radiolabeled Selective Antagonist [(3)H]PSB-0413. *Purinergic Signal.*, 9(1): 59-66.
11. Zhang, J., Zhang, K., Gao, Z.G., Paoletta, S., Zhang, D., et al., 2014. Agonist - Bound Structure of the Human P2Y₁₂ Receptor. *Nature.*, 509: 119–122.
12. Cattaneo, M., 2011. The Platelet P2Y₁₂ Receptor for Adenosine Diphosphate: Congenital and Drug - Induced Defects. *Blood.*, 117: 2102–2102.
13. Zhang, K., Zhang, J., Gao, Z. G., Zhang, D., Zhu, L., et al., 2014. Structure of the Human P2Y₁₂ Receptor in Complex with an Antithrombotic Drug. *Nature.*, 509:115–118.
14. Zhang, D., Gao, Z. G., Zhang, K., Kiselev, E., Crane, S., et al., 2015. Two Disparate Ligand - Binding Sites in the Human P2Y₁ Receptor. *Nature.*, 520: 317–321.
15. Bach, P., Antonsson, T.T., Bylund, R., Björkman, J.-A., Österlund K., et al., 2013. Lead Optimization of Ethyl 6-Aminonicotinate Acyl Sulfonamides as Antagonists of the P2Y₁₂ Receptor. Separation of the Antithrombotic Effect and Bleeding for Candidate Drug AZD1283. *J. Med. Chem.*, 56:7015–7024.
16. Masyuk, A. I., Gradilone, S. A., Banales, J. M., Huang, B. Q., Masyuk, T. V., Lee, S. O., LaRusso, N. F. (2008). Cholangiocyte primary cilia are chemosensory organelles that detect biliary nucleotides via P2Y₁₂ purinergic receptors. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 295(4), G725-G734.
17. Kawaguchi, A., Sato, M., Kimura, M., Ichinohe, T., Tazaki, M., & Shibukawa, Y. (2015). Expression and function of purinergic P2Y₁₂ receptors in rat trigeminal ganglion neurons. *Neuroscience research*, 98, 17-27.
18. Hoffmann, K., Sixel, U., Di Pasquale, F., Von Kügelgen, I., 2008. Involvement of Basic Aminoacid Residues in Transmembrane Regions 6 and 7 in Agonist and Antagonist Recognition of the Human Platelet P2Y₁₂ - Receptor. *Biochem. Pharmacol.*, 76: 1201–1213.
19. Fontana, P., Dupont, A., Gandrille, S., Bachelot-Loza, C., Reny, J.-L., Aiach, M., Gaussem, P., 2003. Adenosine Diphosphate-Induced Platelet Aggregation is Associated with P2Y₁₂ Gene Sequence. Variations in Healthy Subjects Circulation, 108(8): 989-995.
20. Cattaneo, M., 2011. Molecular Defects of the Platelet P2 Receptors. *Purinergic Signal.*, 7(3): 333-339.

21. Li, X.-G., Ma, N., Wang, B., Li X.-Q., Mei, S.-H., Zhao, K., Wang, Y.-J., Li, W., Zhao, Z.-G., Sun, S.-S., Miao Z.-R., 2016. The Impact of P2Y₁₂ Promoter DNA Methylation on the Recurrence of Ischemic Events in Chinese Patients with Ischemic Cerebrovascular Disease. *Scientific Reports*, 6:34570.
22. Su, J. et al., 2014. Association of P2Y₁₂ Gene Promoter DNA Methylation with the Risk of Clopidogrel Resistance in Coronary Artery Disease Patients. *Biomed. Res. Int.*: 450814.
23. Cavallari, U., Trabetti, E., Giovanni, M., Biscuola, M., Girelli, D., Olivieri, O., [et al.], 2007. Variations of the Platelet P2Y₁₂ Receptors are Associated with Coronary Artery Disease. *BMC Med Gene*, 5(8):59.
24. Burnstock G, Ralevic V., 2014. Purinergic Signaling and Blood Vessels in Health and disease. *Pharmacol Rev.*, 66(1):102-192.
25. Baqi Y., Müller, C.E., 2019. Antithrombotic P2Y₁₂ receptor Antagonists: Recent Developments in Drug Discovery. *Drug Discov. Today*, 24(1): 325-333
26. Kupka, D., Sibbing, D., 2018. P2Y₁₂ Receptor Inhibitors: An evolution in drug design to prevent arterial thrombosis. *Expert Opin. Drug Metab. Toxicol.*, Mar; 14(3): 303-315.
27. Vanhaesebroeck, B., Leever, S.J., Panayotou, G., Waterfield, M.D., 1997. Phosphoinositide 3-kinases: A Conserved Family of Signal Transducers. *Trends in Biochemical Sciences*, 22(7): 267–272.
28. Burke, J. E., Williams, R. L., 2015. Synergy in Activating Class I PI3Ks. *Trends in Biochemical Sciences*, 40(2): 88–100.
29. Guidetti, G.F., Canobbio, I., Torti, M., 2015. PI3K/Akt in platelet integrin signaling and implications in thrombosis. *Adv. Biol. Regul.*, 59: 36–52.
30. Battram, A. M., Durrant, T. N., Agbani, E. O., Heesom, K. J., Paul, D. S., Piatt, R., Hers, I., 2016. The Phosphatidylinositol 3,4,5-trisphosphate (PI(3,4,5)P₃) Binder Rasa3 Regulates Phosphoinositide 3-kinase (PI3K)-dependent Integrin α IIb β 3 Outside-in Signaling. *Journal of Biological Chemistry*, 92(5), 1691–1704
31. Stefanini, L., Paul, D. S., Robledo, R. F., Chan, E. R., Getz, T. M., Campbell, R. A., Bergmeier, W., 2015. RASA3 is a Critical Inhibitor of RAP1-dependent Platelet Activation. *Journal of Clinical Investigation*, 125(4): 1419–1432.
32. Battram A.M., Durrant T.N., Agbani E.O., et al., 2017. The Phosphatidylinositol 3, 4, 5 - trisphosphate (PI(3,4,5)P₃) Binder Rasa3 Regulates Phosphoinositide 3 - kinase (PI3K) - dependent Integrin AlphaIIbeta3 Outside - in Signaling. *J. Biol. Chem.*, 292: 1691–1704.
33. Laine, M., Paganelli, F., Bonello L., 2016. P2Y₁₂-ADP receptor antagonists: Days of future and past. *World J. Cardiol.*, 8(5): 327–332.
34. Danese, E., Fava, C., Beltrame, F., Tavella, D., Calabria, S., Benati, M., et al., 2016. Relationship between pharmacokinetics and pharmacodynamics of clopidogrel in patients undergoing percutaneous coronary intervention: comparison between vasodilator-stimulated phosphoprotein phosphorylation assay and multiple electrode aggregometry. *J. Thromb. Haemost.*, 14: 282–293.
35. Hechler, B., Gachet, C., 2017. The P2 Receptors. *Platelets in Thrombotic and Non-Thrombotic Disorders*. Springer; Cham, Switzerland: Pathophysiology, Pharmacology and Therapeutics: An Update: 187–202.
36. Laine, M., Panagides, V., Frère, C., Cuisset, T., Gouarne C., Jouve B., Thuny F., Paganelli F., Alessi M.C., Mancini J., et al., 2019. Platelet reactivity inhibition following ticagrelor loading dose in patients undergoing percutaneous coronary intervention for acute coronary syndrome. *J. Thromb. Haemost.*, 17: 2188–2195
37. Gachet, C., 2012. P2Y(12) receptors in platelets and other hematopoietic and non-hematopoietic cells. *Purinergic Signal.*, 8: 609–619.
38. Cattaneo, M., Schulz, R., Nylander, S., 2014. Adenosine-Mediated Effects of Ticagrelor: Evidence and Potential Clinical Relevance. *J. Am. Coll. Cardiol.*, 63: 2503–2509.
39. Gao, Y., Yu, C., Pi, S., Mao, L., Hu, B., 2019. The Role of P2Y₁₂ Receptor in Ischemic Stroke of Atherosclerotic Origin. *Cell. Mol. Life Sci.*, 76: 341–354.

40. Niu, X., Pi, S.L., Baral, S., Xia, Y.P., He Q.W., Li, Y.N., [et al.], 2017. P2Y(12) promotes migration of vascular smooth muscle cells through cofilin dephosphorylation during atherogenesis. *Arterioscler. Thromb. Vasc. Biol.*, 37: 515–524.
41. Gratacap, M.P., Guillermet-Guibert, J., Martin, V., Chicanne, G., Tronchère, H., Gaits-Iacovoni, F., Payrastre, B., 2010. Regulation and roles of PI3K β , a major actor in platelet signaling and functions. *Enzym. Regul.*, 51: 106–116.
42. Holinstat, M., 2017. Normal Platelet Function. *Cancer Metastasis*. 36(2): 195–198.
43. Parker, W.A.E, Storey, R.F., 2019. Pharmacology and Potential Role of Selatogrel, a Subcutaneous Platelet P2Y12 Receptor Antagonist. *Expert Opinion on Emerging Drugs.*, 25(1):1-6.
44. Jose, R., Rivas, R., Francesco F., Fabiana R., Dominick, J., 2018. Diabetes and Antiplatelet Therapy: from Bench to Bedside. *Cardiovasc. Diagn Ther.*, 8(5): 594–609.
45. Wells, G.A., Elliott, J., Kelly, S., Bai, Z., Boucher, M., Skidmore, B., So, D., Laplante, S., Lee, K., 2019. Dual antiplatelet therapy following percutaneous coronary intervention: clinical and economic impact of standard versus extended duration. Ottawa: Canadian Agency for Drugs and Technologies in Health;78.
46. Cunningham, M. R., Aungraheeta, R., Mundell, S.J., 2017. Pathophysiological Consequences of Receptor Mistraffic: Tales from the Platelet P2Y12 Receptor. *Mol. Cell. Endocrinol.*, 5(449): 74-81.
47. Sun, J., Xiang, Q., Li, C., Wang, Z., Hu, K., Xie, Q., Cui, Y. 2017. Efficacy and Safety of Novel Oral P2Y12 receptor inhibitors in patients with stegment elevation myocardial infarction Undergoing Pci: A Systematic Review and Meta-Analysis. *J Cardiovasc Pharmacol.*, 69(4): 215-227.
48. Hamilos, M., Petousis, S., Parthenakis, Fragiskos. (2018). Interaction between Platelets and Endothelium: from Pathophysiology to New Therapeutic Options. *Cardiovasc. Diagn. Ther.*, 8(5): 568–580.
49. Ziegler, M., Wang, X., Peter, K., 2019. Platelets in cardiac ischemia/reperfusion injury: a promising therapeutic target. *Cardiovasc. Res.*, 115(7): 1178–1188.
50. Joseph, J., Brandon F., Rami, C., Khouzam N., 2019. P2Y12 Inhibitors: Do They Increase Cancer Risk? *Ann. Transl. Med.*, 7(17): 409
51. Mansour, A., Bachelot-Loza C., Nessler N., Gaussem P., Thibault I., 2020. P2Y12 Inhibition beyond Thrombosis: Effects on Inflammation. *Int. J. Mol. Sci.*, 21(4):1391.
1. Rezapour-Nasrabad R. 2020 agility in organizational processes: a new approach to creating competitive advantage. *International Journal of Psychosocial Rehabilitation*; 24(6): 9616-9621
52. Hammami, F., Gargouri, L., Ayed, H. B., Koubaa, M., Rekik, K., Jemaa, T. B., ... & Jemaa, M. B. (2019). Hydatid disease among adults and children: it is time to worry!. *Electronic Journal of General Medicine*, 16(6).
53. Hejdeman, B. (2004). Studies on medical and immunological intervention in HIV-1 infection. Mikrobiologiskt och Tumörbiologiskt Centrum (MTC)/Microbiology and Tumor Biology Center (MTC).