SHORT COMMUNICATION

Synthesis of drug metal complexes and their influence on human platelet aggregation

Mansoor Ahmed[1], Mohsin Ali[2]*, Seyed Abdulmajid Ayatollahi[3,4], Marjan Esfahanizadeh[3], Farzad Kobarfard[3,5], Ameer Hasan[1], Khalid Mohammed Khan[6] and Syed Moazzam Haider[6]

[1]Department of Pharmaceutical Chemistry,Faculty of Pharmacy and Pharmaceutical Sciences,University of Karachi, Karachi, Pakistan
[2]Department of Chemistry, University of Karachi, Karachi, Pakistan
[3]Phytochemistry Research Center, Shahid Beheshti university of Medical Sciences, Tehran, Iran
[4]Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5]Department of Medicinal Chemistry, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Iran
[6]HEJ Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan

Abstract: During the past few decades the emergence of inorganic medicinal chemistry has been developed novel therapeutic agents. Researcher's perseverance in this branch of chemistry has led them to explore further valuable chemical spaces by synthesizing metal complexes already known pharmacological agents for their potential use. However, it is in its early stage, this methodology has demonstrated metal complexes with better bioactivities than the parent ligand molecules. In this study, transition metal complexes of pyrazinamide (PZ), isoniazid (INH), fluconazole (FCZ), metformin (dimethylbiguanide, DMBG) and losartan potassium (LS-K) were selected to evaluate for their possible anti-platelets aggregation in the light of reports on divalent and trivalent cations like calcium, copper, manganese, magnesium, and cadmium may influence the process of thrombocytic activity and aggregation. The required evaluation was carried out on human plasma through an APACT 4004 platelet aggregation analyzer. Arachidonic acid (ADP) was used to gauge any alteration in platelet shape and aggregation process. The parent drugs showed some anti-platelets aggregation, however, their metal complexes demonstrated better efficacy.

Keywords: Synthesis, Transition metal complexes, clinically in use molecules, Anti-platelets aggregation, Thrombosis.

INTRODUCTION

The platelet aggregating mechanism is the basic integral process of cardiovascular system that helps in homeostasis regulation of the body in response to various endothelial injuries from both internal and external stimuli (Davì and Patrono, 2007, Varga-Szabo *et al*., 2008). A protective mechanism is exist in body, nonetheless, if not regulating properly it can be the aggravating factor for the progress of thrombus formation inside the vascular channels of the body, resulting in atherosclerosis. It is reported that atherosclerosis is the most frequent underlying cause of both cardiac as well as other coronary artery peripheral vascular diseases (Jennings, 2009, Kottke-Marchant, 2009).

Atherosclerosis commonly described as a hardening of vessels which is rarely a fatal condition, however, the superimposing thrombosis formed on a ruptured or eroded atherosclerotic plaque can precipitate the life-threatening clinical events like acute coronary syndromes like myocardial infarction due to ischemic injury and ischemic stroke (Naghavi *et al.*, 2003, Spagnoli *et al.*, 2004).

Corresponding author: e-mail: mohsin.ali@uok.edu.pk

Numerous research works have been carried out for studying the mechanisms involved in platelet aggravation. Rupture of an atheromatous plaque surface by some stimulus is subsequently followed by hemorrhage into the plaque which is controlled by the platelet aggregation at the site of rupture causing the initial flow obstruction. As in ischemic diseases, disorders in this highly efficiently regulated coagulation system also have a link with hypertension (Lip and Beevers, 1994, Oikawa *et al*., 1997).

In hypertensive, a thrombotic stage is developed by atherosclerosis and renin angiotensin system plays a contributing role in its genesis (Lip and Blann, 2000, Strawn *et al*., 2000). Therefore, antiplatelet therapy plays a key role in the management of patients with thrombotic or thromboembolic disorders (Buch *et al*., 2010, Linden and Jackson, 2010). Currently available antiplatelet agents work through different mechanisms of action (Amidi *et al*., 2013, De Meyer *et al*., 2008, Maree and Fitzgerald, 2007). Among the most commonly used agents such as aspirin (by impairing thromboxane A2 synthesis through irreversible inhibition of cyclo-oxygenase I), clopidogrel (an irreversible antagonist of platelet ADP (adenosine diphosphate) receptor, P2Y12), and glycoprotein (GP) IIb–IIIa antagonists like abciximab AngiotensinII (AngII)

promotes the production and secretion of plasminogen activator inhibitor type 1 (PAI-1) from vascular endothelial cells (Brown and Vaughan, 1996) which acts as a stimulus for thrombocytes aggregation. Among the tested AT1-receptor antagonists, the inhibitory effect of losartan on platelet adhesion and aggregation is higher than that of valsartan (Li *et al*., 1998) while no such action is reported for candesartan (Montón *et al*., 2000).

Continuous research and its resultant findings in inorganic medicinal chemistry have now demonstrated that divalent and trivalent cations like calcium, copper, manganese, magnesium, and cadmium (Lages and Weiss, 1981) can influence the process of thrombocytic activity and aggregation. It has been reported that divalent ions of transition elements (like zinc or manganese) greatly influenced the aggregation than the alkaline earth metals ions like magnesium or calcium (Penglis and Michal, 1969).

During the past decade inorganic metal ion complexes have displayed better bioactivities as anticancer, antacids, and antirheumatic agents (van Rijt and Sadler, 2009, Zhang and Lippard, 2003). The aim of this study was to appraise antiplatelet aggregating effects of some metal complexes (PZ, INH, FCZ, DMBG, and LS-K) to find out whether these complexes have more therapeutic value as compared to the clinically in use agents.

MATERIAL AND METHODS

General preparation of metal complexes

Pyrazinamide (PZ), isoniazid (INH), fluconazole (FCZ), metformin (DMBG) and losartan potassium (LS-K) drugs were interacted with metal salts in methanol. The quantities of drugs solution were taken two folds higher than the metal salts solution, however, the same concentration of both reactants were prepared initially. The mixture of the drug and the metal salt was kept on water bath at 80°C for 3-4h and the solution was stirred occasionally. A solid material of the complex starts to appear that indicate the transformation of ligand into complex, the solid was filtered and washed with hot methanol.

Elemental (CHN & Metal) analysis

Elemental analyses were performed with CHN analyzer whereas quantitative measurements of metal in the three PZ complexes were performed by AA spectrophotometer (PerkinElmer A Analyst 700) for confirming the synthesis of complexes. The results found are very close to theoretical values and the ratio between metal to ligand was found to be 1:2 for all metals. All the results obtained are shown in table 1.

In-vitro evaluation of antiplatelet aggregation activity

The antiplatelet aggregation activity of the transition metal complexes of selected drugs was assessed by using human plasma on an APACT 4004 platelet aggregation analyzer according to literature protocol (Amidi *et al*., 2013). Fresh blood samples of non-smoker healthy volunteers with no history of consumption of any drug up to 15 days prior to the test were collected after taking their consents. Whole blood was collected in sodium citrate (9:1 by volume) which yielded platelet-rich plasma (PRP) after centrifugation (Hermle Z200 A) at 1000 rpm for 8 min. After removing PRP from blood and sodium citrate mixture the remaining part was centrifuged at 3000 rpm for 15 min and platelet-poor plasma. PPP was collected from the above layer which was used as the test blank. PRP was diluted with appropriate amount of PPP in order to set platelet count at 250000 plts/mL. To PRP samples, test compounds previously dissolved in DMSO were added to the prepared PRP samples and the mixture was subsequently incubated for 5 min at 37 °C. To determine the antiplatelet aggregation activity, ADP (5 µM) or arachidonic acid 1.35 (mM) was added to the final mixture and the sample was monitored for 5 min to record any alteration in platelet shape and aggregation process. Dimethyl sulfoxide (DMSO) was used as negative control while indomethacin and aspirin were used as standard drugs. Following formula was used to calculate platelet aggregation inhibition (%):

Inhibition % = [1 – (D/S)] X 100

Where, D = platelet aggregation in the presence of test compounds and S = platelet aggregation in the presence of solvent.

Compounds were screened at the primary concentration of 1mM. The complexes that exhibited higher than 50% inhibitory activity were further diluted to calculate their IC_{50}.

Ethical approval has been granted by the Ethical Review Committee of Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi.

RESULTS

Inorganic metal complexes of five drugs consisting of pyrazinamide (PZ), isoniazid (INH), fluconazole (FCZ), metformin (DMBG) and losartan potassium (LS-K) were evaluated for possible antiplatelet aggregating capability by arachidonic acid and ADP receptor inhibition assay as compared to reference drugs aspirin and indomethacin results are incorporated in tables 1-6.

DISCUSSION

Although all of synthetic transition metal complexes precede over the pure pyrazinamide in exerting an inhibitory effect on AA with the exception of Fe(III) complex, the Mn(II)-PZ complex showed the maximum inhibition of 16.5%. On the other hand, inhibition of ADP was higher with the pure PZ as compared to its metal

Table 1: Comparison of percent elemental composition in metal complexes

Compound	C (%) Theoretical / Practical	H (%) Theoretical / Practical	N (%) Theoretical / Practical	Metal (%) Theoretical / Practical
a[Cu(PZ)$_2$(Cl)2]	31.53 / 30.49	2.62 / 1.98	22.07 / 21.11	16.69 /16.41
b[Mn(PZ))$_2$(Cl)$_2$]	32.26 / 32.83	2.68 / 1.97	22.58 / 22.93	14.77 / 14.81
c[Co(PZ))$_2$(Cl)$_2$]	31.92 / 31.83	2.66 / 1.84	22.34 / 22.50	15.67 / 15.56

M. Wt. = [a] 380.77 g/mole, [b] 372.16 g/mole and [c] 376.16 g/mole

Table 2: Antiplatelet activities of PZ and PZ metal complexes

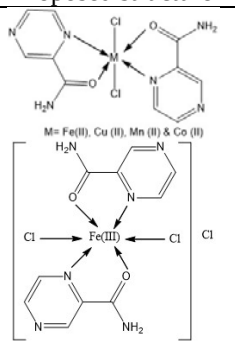
Compound	Inh. A.A (%) (at 1 mM)	Inh. ADP (%) (at 1 mM)	Proposed structure
PZ	1.1	12.3	
Fe(II)-PZ	3.8	4.6	
Fe(III)-PZ	0	6.3	
Cu(II)-PZ	4.6	7.1	
Co(II)-PZ	4.7	1.2	
Mn(II)-PZ	16.5	27.9	
Indomethacin	3 (IC_{50} μM)	℘100 (IC_{50} μM)	
Aspirin	30.3 (IC_{50} μM)	℘100 (IC_{50} μM)	

Table 3: Antiplatelet activities of INH and INH metal complexes

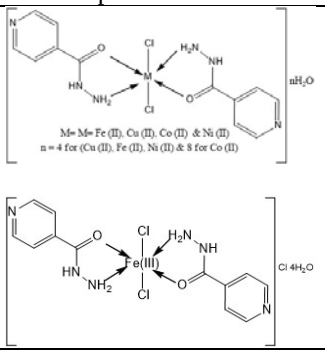
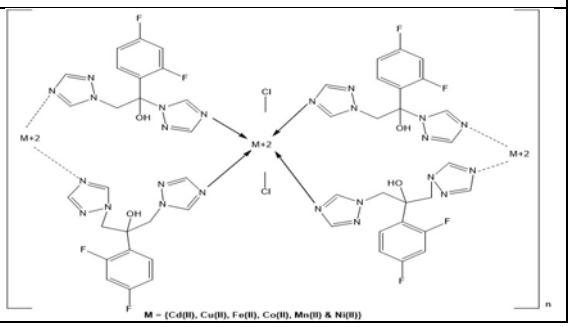
Compound	Inh. A.A (%) (at 1 mM)	Inh. ADP (%) (at 1 mM)	Proposed structure
INH	0	3.6	
Fe(II)-INH	3.2	2.4	
Fe(III)-INH	93.9	2.1	
Cu(II)-INH	2.2	3.2	
Co(II)-INH	7.9	0	
Ni(II)-INH	0	0	

Table 4: Antiplatelet activities of FCZ and FCZ metal complexes

Compound	Inh. A.A (%) (at 1 mM)	Inh. ADP (%) (at 1 mM)	Proposed structure
FCZ	0	9.1	
Fe(II)-FCZ	0	5.3	
Mn(II)-FCZ	0	0	
Cu(II)-FCZ	0	2.1	
Co(II)-FCZ	0	0	
Ni(II)-FCZ	0	0	
Cd(II)-FCZ	9.1	13.3	

complexes excluding the Mn (II)-PZ complex which illustrated more potency with 27.9% inhibition in comparison to the 12.3% of the pure ligand; the observed values for each complex are depicted in table 2.

Table 5: Antiplatelet activities of LS-K and LS-K metal complexes

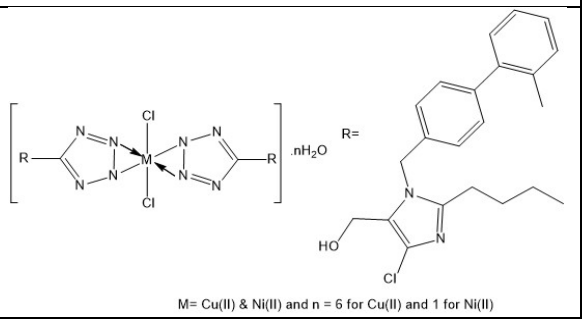
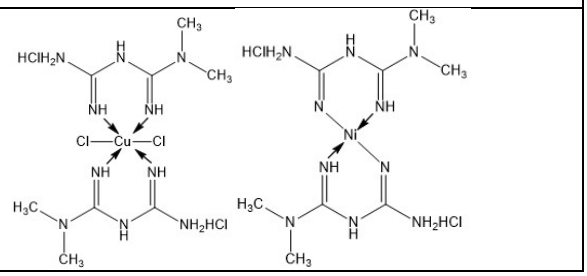
Compound	Inh. A.A (%) (at 1 mM)	Inh. ADP (%) (at 1 mM)	Proposed structure
LS-K	3.08	14.4	
Cu-LS	19.3	27.2	
Ni-LS	3.2	7.3	

Table 6: Antiplatelet activities of DMBG and DMBG metal complexes

Compound	Inh. A.A (%) (at 1 mM)	Inh. ADP (%) (at 1 mM)	Proposed structure
DMBG	5.3	1.3	
Cu(II)-DMBG	7.2	9.5	
Ni(II)-DMBG	2.1	1.1	

Amongst five transition metal complexes of isoniazid three showed inhibitory effects on both A. A and ADP out of which Fe (III) complex exhibited greatest inhibition on A.A with 93.9 percent inhibition at a concentration of 1 mM. The cobalt complex was inhibitory only for A.A demonstrating 7.9% inhibition of this substrate whereas nickel complex was completely inactive on both of our target substrates. Observed values for each complex are shown in table 3.

The results of evaluation of the inhibitory activity potential of metal complexes of fluconazole on A.A and ADP were demonstrated no significant effect on A.A except that of Cd complex. Although inhibitory activity of some complexes was noticed for ADP but it was inconsiderable table 4.

The copper and nickel complexes of losartan demonstrated inhibitory effects on both archidonic acid and ADP with maximal inhibition of Cu (II)-LS on ADP (where a 27.2% inhibition was observed.) Inhibitory effect on A.A was more prominent with copper metal complex as compared to the nickel complex of this ligand which was the minimal one among all the complexes in table 5.

Metformin has inhibitory effects on both A.A and ADP. However, it was observed that the copper complex of this drug possesses higher inhibitory potential in comparison to the parent drug. The nickel complex demonstrated insignificant effects with only 2.1% and 1.1% inhibition of A.A and ADP, respectively table 6.

CONCLUSION

Continuously advancing research in inorganic medicinal chemistry has opened up the new avenues in the field of synthesis of new metal complexes with high therapeutic values. While platinum and ruthenium complexes have established their roles as antineoplastic agents and gold complexes are being used in the treatment of arthritis, new complexes with better efficacy and less/no adverse effects are being sought out by the application of new methodologies. Metal complexes of the drugs used in this study were evaluated for potent antiplatelet activity but no promising results were achieved, however, new information have been obtained by the current study that iron complex with closely structurally similar ligands may be attractive targets to synthesize metal complex. Future research on complexes formed by the ligands which are already in clinical use as antiplatelet aggregating agents will hopefully prove beneficial in the development of inorganic metal complexes as chemical spaces.

REFERENCES

Amidi S, Kobarfard F, Bayandori MA, Tabib K and Soleymani Z (2013). Electrochemical synthesis of novel 1, 3-indandione derivatives and evaluation of their antiplatelet aggregation activities. *Iranian J. Pharm. Res.,* **12**: 91-103.

Brown NJ and Vaughan DE (1996). The renin-angiotensin and fibrinolytic systems: Co-conspirators in the pathogenesis of ischemic cardiovascular disease. *Trends Cardiovasc. Med.,* **6**: 239-243.

Buch MH, Prendergast BD and Storey RF (2010). Antiplatelet therapy and vascular disease: An update. *Ther. Adv. Cardiovasc Dis.,* **4**: 249-275.

Davi G and Patrono C (2007). Platelet activation and atherothrombosis. *N. Engl. J. Med.,* **357**: 2482-2494.

De Meyer SF, Vanhoorelbeke K, Broos K, Salles II and Deckmyn H (2008). Antiplatelet drugs. *Br. J. Haematol.,* **142**: 515-528.

Jennings LK (2009). Mechanisms of platelet activation: Need for new strategies to protect against platelet-mediated atherothrombosis. *Thromb Haemost.,* **102**: 248-257.

Kottke MK (2009). Importance of platelets and platelet response in acute coronary syndromes. *Cleve Clin. J. Med.,* **76**: S2-S7.

Lages B and Weiss H (1981). Dependence of human platelet functional responses on divalent cations: aggregation and secretion in heparin-and hirudin-anticoagulated platelet-rich plasma and the effects of chelating agents. *Thromb Haemost.,* **45**: 173-179.

Li P, Ferrario CM and Brosnihan KB (1998). Losartan inhibits thromboxane A2-induced platelet aggregation and vascular constriction in spontaneously hypertensive rats. *J. Cardiovasc Pharmacol.,* **32**: 198-205.

Linden MD and Jackson DE (2010). Platelets: Pleiotropic roles in atherogenesis and atherothrombosis. *Int. J. Biochem. Cell Biol.,* **42**: 1762-1766.

Lip G and Beevers D (1994). Abnormalities of rheology and coagulation in hypertension. *J. Hum. Hypertens.,* **8**: 693-702.

Lip GY and Blann AD (2000). Does hypertension confer a prothrombotic state? Am. Heart Assoc.

Maree AO and Fitzgerald DJ (2007). Variable platelet response to aspirin and clopidogrel in atherothrombotic disease. Circulation, **115**: 2196-2207.

Monton M, Jimenez A, Nunez A, Lopez BA, Farre J, Gomez J, Zalba LR, De Miguel LS, Casado S and Lopez FA (2000). Comparative effects of angiotensin II AT-1-type receptor antagonists in vitro on human platelet activation. *J. Cardiovasc Pharmacol.,* **35**: 906-913.

Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanadis C, Moreno P and Pasterkamp G (2003). From vulnerable plaque to vulnerable patient. *Circulation,* **108**: 1664-1672.

Oikawa T, Freeman M, Lo W, Vaughan DE and Fogo A (1997). Modulation of plasminogen activator inhibitor-1 in vivo: a new mechanism for the anti-fibrotic effect of renin-angiotensin inhibition. *Kidney Int.,* **51**: 164-172.

Penglis F and Michal F (1969). The induction of blood platelet aggregation by divalent cations. *Cell. Mol. Life Sci.,* **25**: 745-746.

Spagnoli LG, Mauriello A, Sangiorgi G, Fratoni S, Bonanno E, Schwartz RS, Piepgras DG, Pistolese R, Ippoliti A and Holmes DR (2004). Extracranial thrombotically active carotid plaque as a risk factor for ischemic stroke. *Jama,* **292**: 1845-1852.

Strawn WB, Chappell MC, Dean RH, Kivlighn S and Ferrario CM (2000). Inhibition of early atherogenesis by losartan in monkeys with diet-induced hypercholesterolemia. *Circulation,* **101**: 1586-1593.

Van Rijt SH and Sadler PJ (2009). Current applications and future potential for bioinorganic chemistry in the development of anticancer drugs. *Drug discovery today.,* **14**: 1089-1097.

Varga SD, Pleines I and Nieswandt B (2008). Cell adhesion mechanisms in platelets. *Arterioscler Thromb Vasc Biol.,* **28**: 403-412.

Zhang CX and Lippard SJ (2003). New metal complexes as potential therapeutics. *Curr. Opin. Chem. Biol.,* **7**: 481-489.