INTRODUCTION
Clopidogrel bisulfate, chemically $S$ (+) - 2-(2-chlorophenyl)-6, 7-dihydrothiene [3,2-C]pyridine-5(4H)-acetic acid methyl ester sulphate is a potent antiplatelet and anti-thrombotic drug (at C-7 clopidogrel has an absolute $S$ configuration). Clopidogrel has been approved and marketed globally with the primary indication being to reduce atherosclerotic events in patients with several co-morbid conditions due to stroke, myocardial infarction and cardiovascular disease. Clopidogrel displays a selective binding on the platelet surface to adenylate cyclase-coupled ADP receptors, which is unique as compared with aspirin and other related drugs. The mechanism of action of aspirin or other related agents involves inhibition of arachidonic acid metabolism, inhibition of cyclo-oxygenase enzyme system or the inhibition of prostaglandin synthesis. Reports have suggested that clopidogrel does not have a direct effect on the enzyme systems cAMP phosphodiesterase, thromboxane A2, adenylate cyclase, as well no effects on the synthesis of prostacyclin [1].

PHARMACOKINETIC ASPECTS
General
Clopidogrel is an inactive pro-drug that requires oxidation to its active thiol metabolite. The active metabolite inhibits platelet aggregation irreversibly by blocking platelet P2Y12
receptors, resulting in reduced adenosine 5′-diphosphate (ADP)-mediated activation of the glycoprotein GPIIb/IIIa complex. About 85% of clopidogrel is hydrolysed via esterases to an inactive carboxylic acid derivative and only about 15% undergoes hepatic cytochrome P450 (CYP)-catalysed metabolism to a 2-oxoclopidogrel intermediate that is subsequently oxidized to the active metabolite, a thiol derivative of clopidogrel [2].

Absorption/Distribution
The absorption of clopidogrel is >50% and is rapid after oral administration. Bioavailability is unaffected by food. Both the parent compound and the main metabolite bind reversibly in vitro to plasma protein (98% and 94% respectively). After oral administration, aspirin is rapidly absorbed from the stomach and proximal small intestine. The gastric mucosa is permeable to the non-ionised form of aspirin, which passes through the stomach wall by a passive diffusion process. Aspirin is distributed throughout most body fluids and tissues. Concentrations in the brain are usually low and are minimal in feces, bile and sweat. The bioavailability of clopidogrel was increased by food (as measured by 9-fold increase in the AUC value), and elimination half-life (t½) doubled (5 h vs. 2.5 h in the fasted status) [3].

Metabolism/Elimination
Clopidogrel is extensively metabolised by the liver. It undergoes rapid hydrolysis into its carboxylic acid derivative; gluconoridation also occurs. The elimination half-life of the main circulating metabolite is 8 hrs with 50% excreted in the urine and 46% in the feces 5 days after dosing. Clopidogrel is an enantiomer carboxylic ester or methyl ester (localized at carbon 10) of S-configuration (whose R-enantiomer is devoid of antiplatelet activity) [4]. The majority of clopidogrel oral dose is rapidly hydrolyzed in vivo by hepatic carboxylesterase (CES 1) into an inactive carboxylic acid form or clopidogrel carboxylate representing about 85% of the clopidogrel-related compounds present in plasma [5]. More recently, in vitro evidence has indicated that clopidogrel carboxylate is not a P450 substrate and does not affect platelet aggregation [6]. However, such an inactive metabolite can serve as a surrogate endpoint to obtain information on the absorption and elimination of the drug [5] and on the compliance of patients to drug use. Via another metabolic pathway, approximately 15% of ingested clopidogrel is metabolized in the liver by several P450 drug metabolizing enzymes to generate a pharmacologically active metabolite that irreversibly blocks the binding of ADP to platelet P2Y12 receptor [7].

DRUG INTERACTIONS
Drug Interaction with Atorvastatin

Atorvastatin enhances the systemic bioavailability of clopidogrel. This might be due to the inhibition of intestinal P-gp function by atorvastatin and so clopidogrel efflux was reduced. This might have led to increased concentration of clopidogrel in systemic circulation. Therefore, concomitant use of clopidogrel with atorvastatin may require close monitoring for potential drug interactions [8]. Ketoconazole (a non-selective, strong, CYP3A inhibitor), sulfaphenazole (a CYP2C9 inhibitor) and omeprazole (a potent CYP2C19 inhibitor) reduced the generation of clopidogrel active metabolite by 38%, 36% and 31%, respectively. Obviously, both CYP2C19 and CYP2B6 are involved in the whole process of clopidogrel active metabolite formation [9].

Other Drug Interactions Linked To High-Risk Pharmacokinetics
The concern about a clopidogrel-omeprazole interaction is the latest in a long line of serious adverse drug reactions based on the principles of high-risk pharmacokinetics [10]. Building on the recent finding that cigarette smoking is an inducer of clopidogrel metabolism leading to higher degrees of platelet inhibition with clopidogrel, show that cigarette smoking, in turn, is associated with differences in the clinical effectiveness of clopidogrel [11].

Drug Interaction with Proton Pump Inhibitor (PPI)
The potential interaction between clopidogrel and proton pump inhibitors (PPI) in patients with acute coronary syndrome raises serious concerns for cardiologists. But there is no conclusive evidence of an increase in adverse events when used in combination. The Pharmacologic and Pharmacodynamic perspectives proved that a real interaction between clopidogrel and PPIs because of competitive inhibition of CYP2C19 isoenzyme which is required for biotransformation of clopidogrel to its active metabolite. The consequent decrease in availability of this active metabolite leads to attenuation of antiplatelets efficacy of clopidogrel. In several observational trials, it was shown that decreased antiplatelets effect of clopidogrel due to PPIs may translate into poor cardiovascular outcomes. However, an incomplete RCT (COGENT) and a post hoc analysis of two large trails showed no significant adverse cardiovascular events with combination. Caution is however needed in patients who are hypometabolizer of clopidogrel putting them at higher risk of adverse coronary events. Since 3% of patients are likely to be hypometabolizers of clopidogrel, routine combination of clopidogrel with PPIs should have advisory warnings. Patients who are at very high risk of developing gastrointestinal (GI) clopidogrel with selected choices such as Pantaprazole may be helpful. Serious consideration should be given to H2 receptor Antagonists or antacids. By
not compromising Cardioprotective effect of antiplatelets agents, the gastroprotective benefit of PPIs should be strongly considered in patients who need both. Health care providers remain alert to more outcomes of data [12]. In addition to that, selected substrates, inducer and inhibitor respectively shown in Table 1.

PHARMACODYNAMIC ASPECTS

Clopidogrel selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet receptor and the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex, thereby inhibiting platelet aggregation. Biotransformation of clopidogrel is necessary to produce inhibition of platelet aggregation, but an active metabolite responsible for the activity of the drug has not been isolated. Clopidogrel also inhibits platelet aggregation induced by agonists other than ADP by blocking the amplification of platelet activation by released ADP. Clopidogrel does not inhibit phosphodiesterase activity. Clopidogrel acts by irreversibly modifying the platelet ADP receptor. Consequently, platelets exposed to clopidogrel are affected for the remainder of their lifespan. Dose dependent inhibition of platelet aggregation can be seen 2 hours after single oral doses of 75 mg Plavix per day inhibit ADP-induced platelet aggregation on the first day, and inhibition reaches steady-state between Day 3 and Day 7. At steady state, the average inhibition level observed with a dose of 75 mg Plavix (Clopidogrel) per day was between 40% and 60%. Platelet aggregation and bleeding time gradually return to baseline values after treatment is discontinued, generally in about 5 days [13].

Adenosine diphosphate (ADP) activates, among other things, the G-protein-coupled purine receptor P2Y12 on the platelet surface. The clopidogrel metabolite blocks this receptor and thereby prevents the ADP binding as well as the subsequent activation of the glycoprotein (GP) IIb/IIIa complex and the subsequent binding of fibrinogen on the GP IIb/IIIa-receptor. The decrease of platelet aggregability depends on the completeness of receptor blocking. A number of experimental and clinical reports point out the inter individual variability of efficacy of clopidogrel (as with ASA). Numerous reasons are under discussion, for example compliance problems, insufficient absorption, incomplete metabolism by cytochrome P450 isoenzyme 2B6 and 3A4, and to a lower extent by 1A1, 1A2 and 2C19 to the pharmacologically effective metabolite in the liver [13]. Molecular levels changes with and without clopidogrel shown in figure no. 2.

Resistance of clopidogrel

Clopidogrel resistance now has been drawing a world-wide attention. Clopidogrel resistance is a widely used term that remains to be clearly defined. So far, it has been used to reflect failure of clopidogrel to achieve its anti-aggregatory effect. Here, the extrinsic and intrinsic mechanisms are intensively illustrated including drug-drug interaction, genetic polymorphisms of the P2Y12 receptor and of the CYPs [14].

Studies in pregnancy

Reproduction studies performed in rats and rabbits at doses up to 500 and 300 mg/kg/day, respectively (65 and 78 times the recommended daily human dose, respectively, on a mg basis), revealed no evidence of impaired fertility or fetotoxicity due to clopidogrel. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of a human response. Clopidogrel has not been found to cause significant fetotoxicity in animal studies at high doses, but there are no adequate and well-controlled studies in pregnant women so far [15].

Nursing Mothers

Studies in rats have shown that clopidogrel and/or its metabolites are excreted in the milk. Literature doesn’t reported that drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the nursing woman [14].

ANIMAL MODELS

In the animals given clopidogrel (as one or daily intravenous injections), the most striking finding was a significant modification in thrombus architecture. After clopidogrel, the base of the thrombus was broader, denoting increased platelet adhesion; furthermore, the thrombus was looser and less compact, being composed of platelets with few contact points. These findings indicate decreased thrombus cohesion and stability. Thrombus density was significantly decreased in the pigs given clopidogrel. These results are in accordance with previous findings in humans given clopidogrel and in a patient with a congenital disorder characterized by a reduced and reversible platelet response. These results are in accordance with previous findings in humans given clopidogrel and in a patient with a congenital disorder characterized by a reduced and reversible platelet response. Researcher reported that qualitative and quantitative changes in thrombus formation observed in pigs treated with clopidogrel correlate with stent patency in injured arteries [16]. Clopidogrel inhibited ADP-induced platelet aggregation in healthy dogs and may be a viable antiplatelets agent for use in dogs. Pharmacodynamic effects of clopidogrel in dogs were similar to effects reported in humans; clopidogrel may be
useful in studies involving dogs used to investigate human disease [17].

**TOXICOLOGICAL ASPECTS**

Clopidogrel can be metabolized by CYP3A4, role of metabolite formation in the toxicity towards hMPCs (human myeloid progenitor cells). At least for clopidogrel, it has been shown that the thiol group-containing, active metabolite R-130964 is present in the systemic circulation after oral ingestion of this drug. Due to its reactivity, this metabolite could possibly be associated with myelotoxicity. Studies show that both the non-metabolized compounds and metabolites are potentially important for the myelotoxicity of ticlopidine and clopidogrel. Both non-metabolized compounds were associated with concentration-dependent toxicity towards hMPCs, which was accentuated by pre-incubation with CYP3A4 or neutrophil granulocytes. Our study showed that non-metabolized clopidogrel is toxic to hMPCs, starting at a concentration of 10 M. By comparison, peak serum clopidogrel concentrations reached 38 g/L (approximately 0.1 M) in healthy in human volunteers who had ingested a clopidogrel loading dose of 600 mg. The half-life of non-metabolized clopidogrel was approximately 1 h in this study. Taking into account that a normal maintenance dose is 75 mg per day and the short half-life of clopidogrel, the human exposure to non-metabolized clopidogrel appears to be too low to be associated with myelotoxicity. Even if the possible production of toxic metabolites from clopidogrel by MPO in bone marrow is taken into consideration, the clopidogrel exposure of the bone marrow appears to be too low to be toxicologically relevant. The myelotoxic element of clopidogrel is therefore most likely to be its major metabolite clopidogrel carboxylate. While clopidogrel carboxylate itself showed no toxicity on hMPCs in our experiments, it was converted to toxic metabolites by MPO. Both the non-metabolized compounds and metabolites of clopidogrel and ticlopidine are toxic for human MPCS [18].

**CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY**

There was no evidence of tumorigenicity when clopidogrel was administered for 78 weeks to mice and 104 weeks to rats at dosages up to 77 mg/kg per day, which afforded plasma exposures >25 times that in humans at the recommended daily dose of 75 mg. Clopidogrel was not genotoxic in four in vitro tests (Ames test, DNA-repair test in rat hepatocytes, gene mutation assay in Chinese hamster fibroblasts, and metaphase chromosome analysis of human lymphocytes) and in one in vivo test (micronucleus test by oral route in mice) [18].

**CLINICAL TRIALS WITH CLOPIDOGREL**

The Clopidogrel vs Aspirin in Patients at Risk of Ischemic Events (CAPRIE) trial was based on the premise that atherothrombosis is a systemic condition that affects all arterial beds with a common pathophysiology. CAPRIE was thus unique by incorporating patients with three distinct indications into a single trial. The study was designed to compare clopidogrel 75 mg daily with aspirin 325 mg daily for prevention of vascular events in patients with stroke within 6 months, myocardial infarction within 35 days, or peripheral vascular disease. A total of 119-185 patients were followed for a mean of 1.9 years. The primary outcome was stroke, myocardial infarction or vascular death. The annualized event rates were 5.32% for clopidogrel and 5.83% for aspirin, an 8.7% relative reduction favoring clopidogrel. This reduction was similar to that seen in the TASS trial when Ticlopidine was compared with aspirin. Relative risks were a 7.3% reduction for the stroke group, a 3.7% increase for the myocardial infarction group, and a 23.8% reduction for the peripheral arterial disease group. Most of the benefit seen in the peripheral arterial disease group was due to a reduction in myocardial infarction. In contrast to earlier ticlopidine trials, the improved results with clopidogrel were not offset by excess side-effects. The rate of treatment discontinuation was the same in aspirin and clopidogrel groups. Side-effects occurred in 11.4% of patients, 9.3% of which were hemorrhagic events with acute coronary syndromes. The Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial was designed to compare the safety and efficacy of combined aspirin-clopidogrel therapy with aspirin alone in high-risk coronary disease patients. The trial randomized 12,562 patients with non-ST-elevation acute coronary syndromes within 24 hour of symptom onset. All patients received aspirin (75–325 mg daily) and were randomized to receive either clopidogrel (300 mg loading dose then 75 mg daily) or placebo for 3–12 months. The composite endpoint was cardiovascular death, nonfatal myocardial infarction and stroke. The endpoint occurred in 11.4% of placebo patients and in 9.3% of clopidogrel patients, a relative risk reduction of 20%. Major bleeding was more frequent in the clopidogrel group, and within groups, bleeding risk was aspirin dose-dependent. In patients undergoing coronary artery bypass surgery, bleeding was significantly increased if clopidogrel had not been discontinued 5 or more days before surgery [19].
**Figure 1.** Structural representation of clopidogrel, clopidogrel carboxylic acid metabolite and the active metabolite. [Correction made in the figure after initial online publication.]

**Figure 2.** Signal transduction by ADP in platelets and its modification by clopidogrel. (Abbreviations: ADP, adenosine diphosphate; P2X1, principal platelet ADP receptor (ADP-liganded ionic channel); AC, adenylate cyclase; cAMP, cyclic adenosine-3¢5¢-monophosphate; IP3, inositol triphosphate; DAG, diacylglycerol; PLC, phospholipase C; Ca++, calcium; PIP2, phosphatidylinositol 4,5-bisphosphate; TP, thromboxane receptor; IP, prostacyclin receptor; TXA2, thromboxane A2; Gq, Gs, Gi, G proteins; P2T, purinergic platelet ADP receptor (high-affinity, G-protein-coupled heptahelial receptor); GP IIb/IIIa, glycoprotein IIb/IIIa; ATP, adenosine triphosphate; PGI2, prostacyclin.) [12].
Table 1. Selected substrates, inhibitors and inducers of cytochrome P450 3A4 and 2C19

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<th>Substrates</th>
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CONCLUSION

For most of Cardiac patients with acute coronary syndrome (ACS) or other condition requiring antiplatelets therapy. Clopidogrel with Proton pump inhibitors (PPIs), Diltiazem, Ketoconazole, Clarithromycin and some other (such as Cimetidine, Efavirenz and Ticlopidine) should be avoided. Almost all PPIs lost their efficacy when combine with Clopidogrel, so if given individual it will be more effect for antiplatelets therapy with some exception. In this review we discussed more about Pharmacokinetics and Pharmacodynamics effect of Clopidogrel in Vascular patients.

ABBREVIATIONS

ADP – Adenosine Diphosphate

CAMP – Cyclic Adenosine Monophosphate

AUC – Area Under Curve

hMPCs – Human Myeloid Progenitor Cells

CURE – Clopidogrel in Unstable Angina to Prevent Recurrent Events

CAPRIE – Clopidogrel Vs Aspirin In Patients at Risk Of Ischemic Events

REFERENCES


