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Research Article

In-vitro Drug Interaction of Clopidogrel with Omeprazole and Lansoprazole

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ABSTRACT

Atherosclerotic cardiovascular disease is very common worldwide. Clopidogrel is prescribed for chronic use in these patients. Clopidogrel is a prodrug and a adenosine diphosphate (ADP) receptor inhibitors. Its active metabolite act on P2Y12 Receptor which is a G-Protein coupled receptor and it inhibits the process of initiation of platelet aggregation. Omeprazole and lansoprazole are the proton pump inhibitors which are mostly prescribed in combination with clopidogrel to reduce gastric bleeding. There were no studies done to elucidate the changes in metabolic clearance profile of clopidogrel in combination with either omeprazole or lansoprazole in rat liver microsomes. Pharmacokinetics is the mathematical analysis of ADME. For this study prepared pooled liver microsomes from Sprague Dawley rat for used as a valuable *in-vitro* model to elucidate biotransformation mechanism of new chemical entity and drug-drug interaction by different methods and standardize the prepared rat liver microsomes with respect to commercially available Xenotech's rat liver microsomes. Metabolism, clearance and elimination rate constant of clopidogrel was significantly decreased when it was co-incubated with omeprazole as compared to co-incubation with lansoprazole but half life of clopidogrel was significantly increased when it was co-incubated with omeprazole as compared to co-incubation with lansoprazole. So lansoprazole is better to prescribe as a co-administration therapy with clopidogrel.

Keywords: Clopidogrel, Microsomes, HPLC analysis, CYP3A, CYP2C19 and CYP3A.

INTRODUCTION

Liver is the major organ for drug metabolism and elimination. In this regard liver is the locus for metabolism mediated drug-drug interaction. This occurs due to inhibition or induction of metabolism of one drug by the other drug. Seventy percent of drugs are metabolized through CYP 450 super family. Prediction of drug-drug interaction in lead compound is carried out by different in vitro and in vivo models. (Rodrigues AD *et al*., 2001, Jia L *et al*., 2007).

Clopidogrel is marketed worldwide in nearly 110 countries, with sales of US$..... billion in 2013. It had been the 2nd top selling drug in the world for a few years as of 2012 and was still growing by over 20% in 2012. Clopidogrel is thiopyridine class drug which inhibits platelet activation and it is most potent drugs in this class and most widely prescribed alone or in combination with aspirin. Clopidogrel is a prodrug which is metabolized by two pathways: (1) One mediated by esterases leading to hydrolysis into its inactive carboxylic acid derivative (85% of circulating metabolites). (2) Cytochrome first oxidizes clopidogrel to a 2-oxo-intermediate metabolite. Subsequent metabolism of the 2-oxo-clopidogrel intermediate results in formation of a thiol derivative, an active metabolite of clopidogrel. This metabolic pathway is mediated by CYP3A4, CYP2C19, CYP1A2 and CYP2B6. The active thiol metabolite binds rapidly and irreversibly to receptors, thus inhibiting platelet aggregation(Mega JL *et al*., 2009). Omeprazole and lansoprazole are mainly metabolized by CYP3A, CYP2C19 and CYP3A (Li X *et al*., 2004, Meyer UA., 1996, Unge P 1997).

MATERIALS AND METHODS

Method of analysis of clopidogrel

(Clarke TA *et al*., 2003, Singh SS *et al*., 2005, Pereillo JM *et al.*, 2002, Shin BS *et al.*, 2007)

Sample preparation procedure of clopidogrel

Microsomes test 0 min sample preparation

50 µl of incubation mixture containing clopidogrel was added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH and 200 µl acetonitrile was added and mixed it on vortexer for 1 min and centrifuged the sample at 10,000 rpm for 5 min and transferred the supernatant in vials for HPLC analysis.

Microsomes test 15 min sample preparation

50 µl of incubation mixture containing clopidogrel was added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH was added then incubated at 37 °C at 80 rpm in shaking water bath. 200 µl acetonitrile was added to stop the reaction after 15 min. Mixed on vortexer for 1 min then centrifuged at 10,000 rpm for 5 min and transferred the

supernatant in vials for HPLC analysis.

Microsomes test 30 min sample preparation

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1. Percentage metabolism of clopidogrel at 60 min alone and in the presence of omeprazole and lansoprazole in rat liver microsomes.

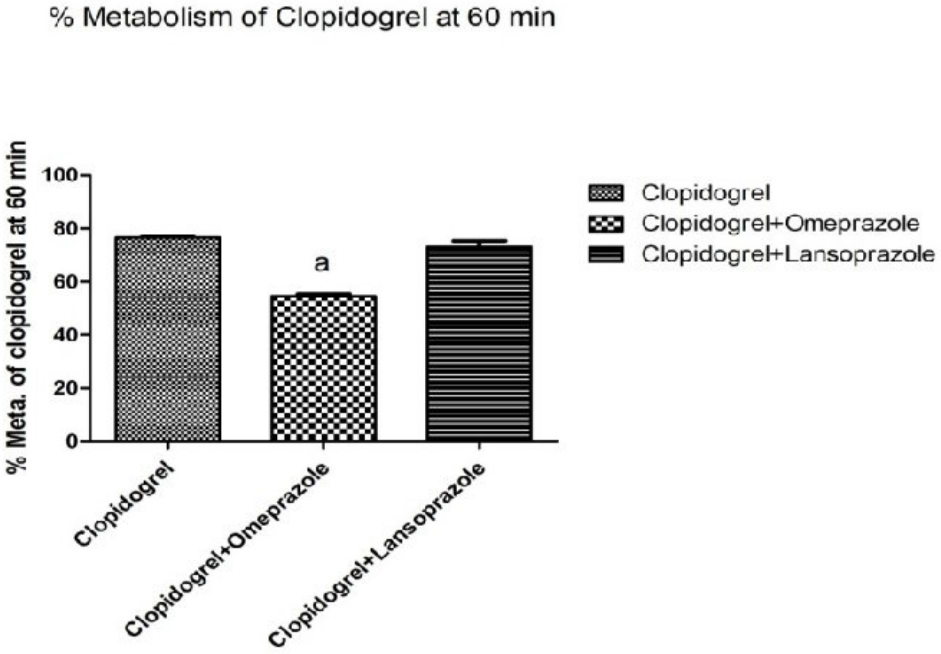

Figure 1: Graph showing % metabolism of clopidogrel at 60 min

Rat liver microsomes were co incubated with clopidogrel alone for 60 min and in the presence of omeprazole and lansoprazole respectively.

Table 1: Percentage metabolism of clopidogrel at 60 min in different groups.

Group	No. of experiments(n)**	Mean % metabolism of clopidogrel at 60 min ±SEM
Clopidogrel	3	76.62 ± 0.23
Clopidogrel in presence of omeprazole	3	3 54.69 ± 0.76[a]
Clopidogrel in presence of lansoprazole	3	73.21 ± 2.26

2. Area of clopidogrel alone and in the presence of omeprazole and lansoprazole at different time points:-

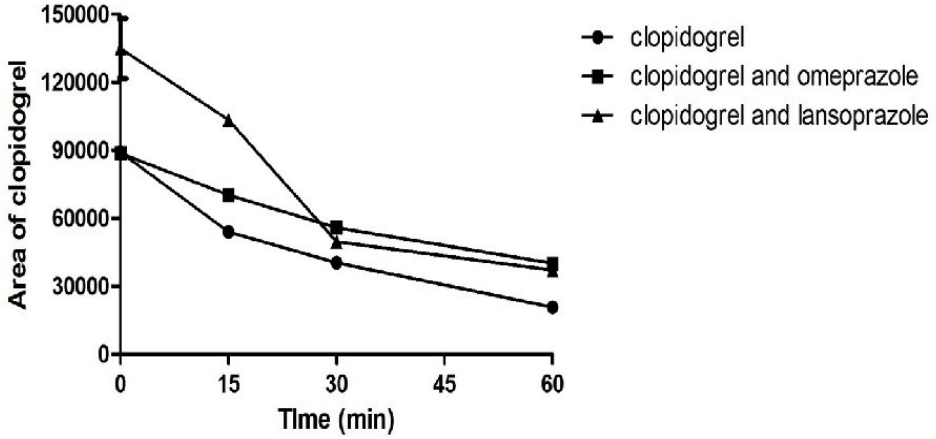

Figure 2: Area of clopidogrel alone and in the presence of omeprazole and lansoprazole.

50 µl of incubation mixture containing clopidogrel was added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH was added then incubated at 37 °C at 80 rpm in shaking water bath. 200 µl acetonitrile was added to stop the reaction after 30 min. Mixed on vortexer for 1 min then centrifuged at 10,000 rpm for 5 min and transferred the supernatant in vials for HPLC analysis.

Microsomes test 60 min sample preparation

50 µl of incubation mixture containing clopidogrel was

3. Metabolic clearance profile of clopidogrel

Table 2: Metabolic clearance parameters of clopidogrel

Group	No. of experiments(n) **	Mean half life (min) ±SEM	Meanelimination rate constant (min^{-1}) ±SEM	Meanintrinsic clearance (ml/min/kg) ±SEM	Scaled plasma clearance (ml/min/kg) ±SEM
Clopidogrel	3	29.41 ±1.24	0.023±8.57	98.02 ± 0.35	35.23 ± 0.046
Clopidogrel in presence of omeprazole	3	52.84 ±1.30[a]	0.013 ±3.32[a]	54.62 ±1.38[a]	27.39 ± 0.34[a]
Clopidogrel in presence of lansoprazole	3	30.24 ±1.61	0.023 ± 1.16	101.27 ±4.32	34.90 ± 0.66

added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH was added then incubated at 37 °C at 80 rpm in shaking water bath. 200 µl acetonitrile was added to stop the reaction after 60 min. Mixed on vortexer for 1 min then centrifuged at 10,000 rpm for 5 min and transferred the supernatant in vials for HPLC analysis.

Sample preparation procedure of clopidogrel in combination with omeprazole

Microsomes test 0 min sample preparation

50 µl of incubation mixture containing clopidogrel and omeprazole was added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH was added and 200 µl acetonitrile. Mixed on vortexer for 1 min and centrifuged at 10,000 rpm for 5 min and transferred the supernatant in vials for HPLC analysis.

Microsomes test 15 min sample preparation

50 µl of incubation mixture containing clopidogrel and omeprazole was added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH was added then incubated at 37 °C at 80 rpm in shaking water bath. 200 µl acetonitrile was added to stop the reaction after 15 min. Mixed on vortexer for 1 min then centrifuged at 10,000 rpm for 5 min and transferred the supernatant in vials for HPLC analysis.

Microsomes test 30 min sample preparation

50 µl of incubation mixture containing clopidogrel and omeprazole was added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH was added then incubated at 37 °C at 80 rpm in shaking water bath. 200 µl acetonitrile was added to stop the reaction after 30 min. Mixed on vortexer for 1 min then centrifuged at 10,000 rpm for 5 min and transferred the supernatant in vials for HPLC analysis.

Microsomes test 60 min sample preparation

50 µl of incubation mixture containing clopidogrel and omeprazole was added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH was added then incubated at 37 °C at 80 rpm in shaking water bath. 200 µl acetonitrile was added to stop the reaction after 60 min. Mixed on vortexer for 1 min then centrifuged at 10,000 rpm for 5 min and transferred the supernatant in vials for HPLC analysis.

Sample preparation procedure of clopidogrel in combination with lansoprazole

Microsomes test 0 min sample preparation

50 µl of incubation mixture containing clopidogrel and lansoprazole was added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH was added and 200 µl acetonitrile. Mixed on vortexer for 1 min and centrifuged at 10,000 rpm for 5 min and transferred the supernatant in vials for HPLC analysis.

Microsomes test 15 min sample preparation

50 µl of incubation mixture containing clopidogrel and lansoprazole was added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH was added then incubated at 37 °C at 80 rpm in shaking water bath. 200 µl acetonitrile was added to stop the reaction after 15 min. Mixed on vortexer for 1 min then centrifuged at 10,000 rpm for 5 min and transferred the supernatant in vials for HPLC analysis.

Microsomes test 30 min sample preparation

50 µl of incubation mixture containing clopidogrel and lansoprazole was added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH was added then incubated at 37 °C at 80 rpm in shaking water bath. 200 µl acetonitrile was added to stop the reaction after 30 min. Mixed on vortexer for 1 min then centrifuged at 10,000 rpm for 5 min and transferred the supernatant in vials for HPLC analysis.

Microsomes test 60 min sample preparation

50 µl of incubation mixture containing clopidogrel and lansoprazole was added in 2 ml eppendorf tube and preincubated the reaction mixture at 37 °C at 80 rpm in shaking water bath. After 5 min 10 µl reduced NADPH was added then incubated at 37 °C at 80 rpm in shaking water bath. 200 µl acetonitrile was added to stop the reaction after 60 min. Mixed on vortexer for 1 min then centrifuged at 10,000 rpm for 5 min and transferred the supernatant in vials for HPLC analysis.

System suitability standard (SST)

5 µl of analyte stock solution and 320 µl of diluent (Acetonitrile: Methanol: Water (4:4:2) were mixed and analyzed by HPLC.

Formulas to calculate metabolic clearance of clopidogrel

4. Half life of of clopidogrel

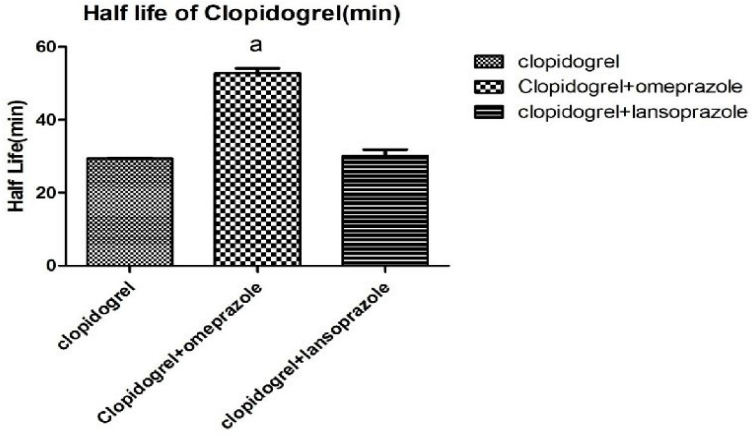

Figure 3: Half life of clopidogrel in different groups

5. Elimination rate constant of clopidogrel:-

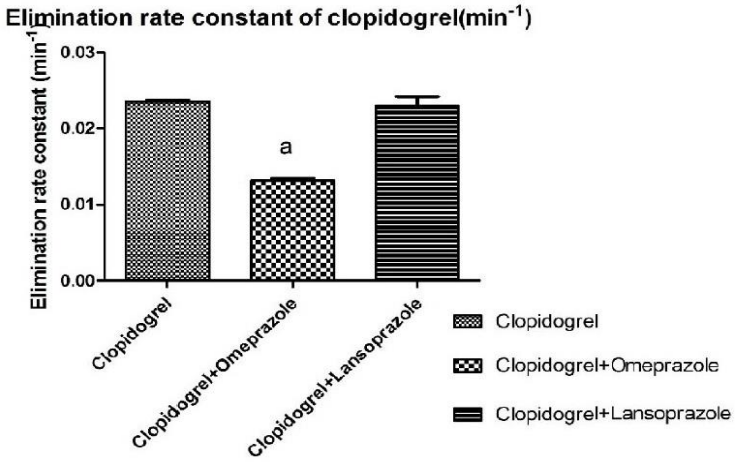

Figure 4: Elimination rate constant of clopidogrel in different groups

6. Intrinsic clearance of clopidogrel:-

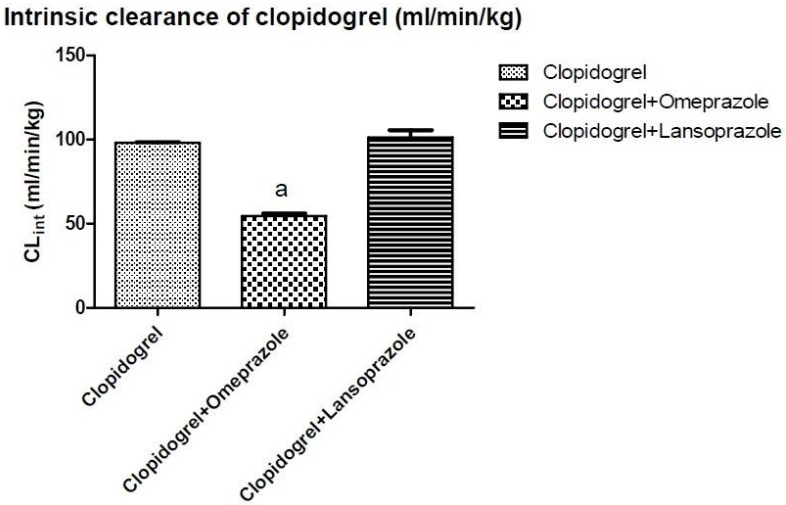

Figure 5: Intrinsic clearance of clopidogrel in different groups

7. Scaled plasma clearance of clopidogrel:-

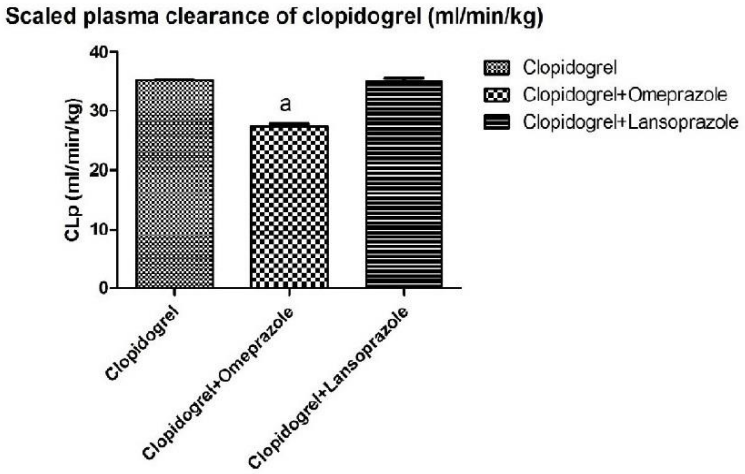

Figure 6: Scaled plasma clearance of clopidogrel in different groups

8.HPLC chromatogram.

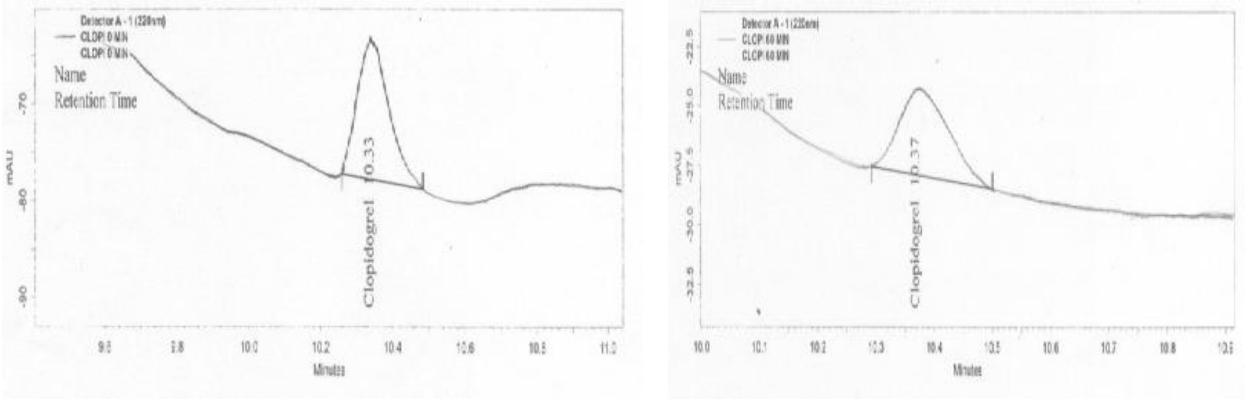

Figure 7, 8: Area of clopidogrel at 0 min and 60 min respectively.

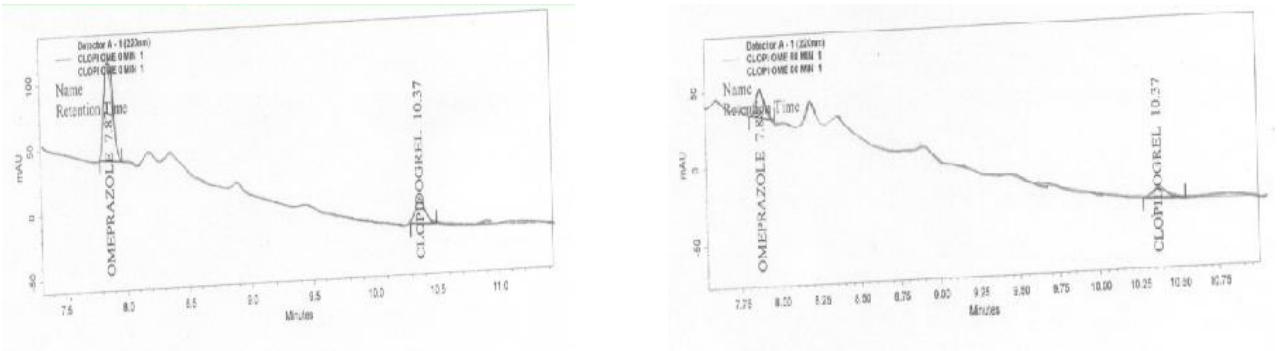

Figure 9, 10: Area of clopidogrel in presence of omeprazole at 0 min and 60 min respectively

Half life ($t_{1/2}$) = -0.693/SLOPE
Slope: Y axis: - Natural logarithm of % remaining
X axis: - Time
Elimination rate constant (Kel) = 0.693/t ½
Intrinsic clearance (CL_{int})
[0.693/t 1/2]× [Total microsomal Proteins (52 mg)/Protein Concentration (0.5 g/ml)] ×
[40 G of liver / 1 kg body weight]
Scaled plasma clearance [CL_{int} (p)]
[(CL_{int} × Hepatic blood flow Q_h) / (CL_{int} + Hepatic blood flow Q_h)]
Where, Qh = Hepatic blood flow (55 ml/min)

RESULTS AND DISCUSSION

Clopidogrel is used for primary and secondary prevention of atherosclerotic cardiovascular disease, acute coronary syndrome either alone or in combination with aspirin for chronic period of time. Proton pump inhibitors like omeprazole and lansoprazole are usually prescribe in

combination with clopidogrel to reduce gastric acidity (Adams RJ *et al*., 2008). CYP3A and CYP2C19 play a major role in clopidogrel active metabolite (thiol form)

Statistical Analysis

Result are expressed as Mean±SEM (standard error of mean) of at least 6 animals per group. Statistical

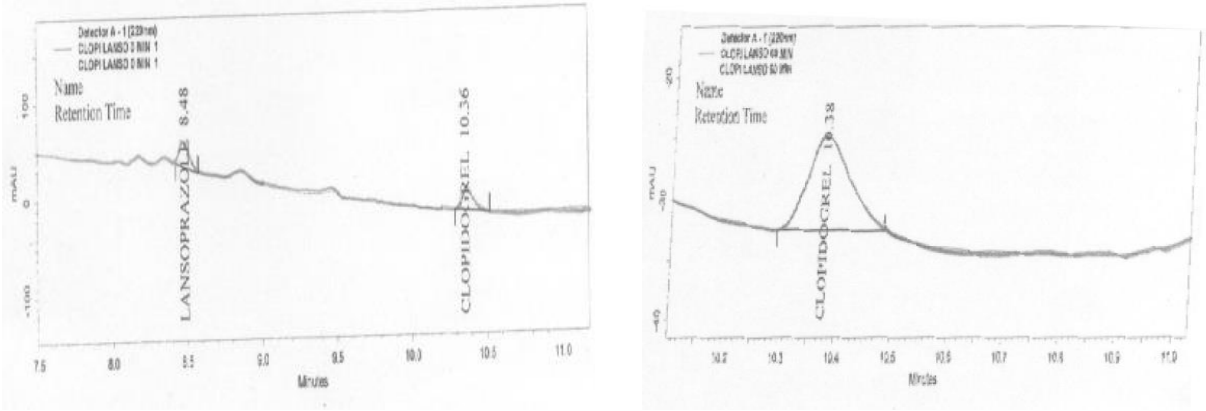

Figure 11, 12: Area of clopidogrel in presence of lansoprazole at 0 min and 60 min respectively

formation which is responsible for anti platelet activity (Clarke TA *et al*., 2003). Omeprazole and lansoprazole are specific CYP2C19 inhibitor so there is a possible inhibition of clopidogrel active metabolite formation (Unge P, 1997, Meyer UA, 1996) In the present study clopidogrel percentage metabolism was checked alone and coincubation with omeprazole and lansoprazole at different time points. The final concentration of substrate (clopidogrel) and inhibitor (omeprazole and lansoprazole) in the reaction mixture was 10 µm. Half life of clopidogrel was significantly increased in the presence of omeprazole as compared to lansoprazole (figure 10). Elimination rate constant of clopidogrel was significantly reduced in the presence of omeprazole as compared to lansoprazole (figure 11). Intrinsic clearance of Clopidogrel was significantly reduced in the presence of omeprazole as compared to lansoprazole (figure 12). Scaled plasma clearance of clopidogrel was significantly reduced in the presence of omeprazole as compared to lansoprazole (figure 13). The above results indicate that omeprazole and lansoprazole inhibit the metabolism of clopidogrel. Clopidogrel metabolism was significantly reduced when it was coincubated with omeprazole as compared to lansoprazole. Intrinsic clearance, elimination rate constant of clopidogrel was significantly reduced when it was coincubated with omeprazole as compared to lansoprazole.

CONCLUSION

Percentage metabolism of clopidogrel was decreased when it was co-incubated with omeprazole as compared to co incubation with lansoprazole Intrinsic clearance and elimination rate constant of clopidogrel was significantly decreased when it was co-incubated with omeprazole as compared to co-incubation with lansoprazole. Half life of clopidogrel was significantly increased when it was co-incubated with omeprazole as compared to co-incubation with lansoprazole. So among both lansoprazole and omeprazole, from safety point of view lansoprazole is better to prescribe as a co-administration therapy with clopidogrel. Schiff base which shows good lipophilic properties with electron rich morpholine ring in Mannich base.

significance of differences between group was determined by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test. ^{a} = $p<0.05$.

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