Effect of Smoking on Pharmacokinetics of Clopidogrel, an Antiplatelet Drug

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Abstract

Purpose: To assess the influence of smoking cigarettes on the pharmacokinetics of the antiplatelet drug, clopidogrel.

Methods: Thirty four male patients, mean age and weight of 59.3 years and 81.1 kg, respectively, who underwent percutaneous coronary intervention (PCI), took part in the study. Each subject received an oral loading dose of 600 mg clopidogrel eight tablets, each 75 mg). Clopidogrel carboxylate plasma level was measured and non-compartmental analysis was used to determine peak plasma concentration (Cmax), time to achieve peak plasma concentration (Tmax), elimination half-life (t1/2e), and area under the curve (AUC0–∞). Other parameters measured include gamma-glutamyltransferase enzyme (GGT), low density lipoprotein cholesterol (LDL-cholesterol), blood urea nitrogen (BUN) and platelet count.

Results: Nineteen patients were smokers (55.9 %). Smokers had higher levels of GGT compared to non-smokers (31.73 ± 14.42 vs. 21.63 ± 11.41 IU/L, p = 0.08) as well as higher levels of LDL-cholesterol (116.79 ± 42.08 vs. 87.07 ± 27.34 mg/dl, p = 0.041, respectively). Smokers had shorter half-life (smokers: 3.47 ± 1.9 h vs. non-smokers: 5.83 ± 4.09 h, p = 0.012). Smoking behavior had no influence on Cmax (p = 0.16), AUC0–∞ (p = 0.65) or Tmax (p = 0.91). In general, the pharmacokinetic parameters were characterized by considerable inter-individual variation (Cmax = 23.2 ± 8.79 µg/ml, coefficient of variation (CV) = 37.9 %), (Tmax = 1.71 ± 1.15 h, CV = 67.2 %), (AUC0–∞ = 120.97 ± 44.4 µg.h/ml, CV = 36.7 %) and (t1/2e = 4.57 ± 3.15 h, CV = 68.9 %).

Conclusion: Smoking behavior may not be a significant determinant of the pharmacokinetics of clopidogrel following oral administration of 600 mg dose in patients undergoing PCI.

Keywords: Antiplatelet, Clopidogrel, Pharmacokinetics, Smoking, Cigarette

INTRODUCTION

Clopidogrel is an antiplatelet agent which selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet receptor (P2Y12) and blocks the subsequent platelet aggregation [1]. Clopidogrel is particularly important in prevention of coronary thrombosis and highly valuable in patients undergoing coronary revascularization [2,3]. Recently published studies revealed a marked inter-individual variability to clopidogrel inhibition of ADP-induced platelet aggregation [4]. It is estimated that laboratory clopidogrel non-responsiveness can be found in 20 % of patients undergoing percutaneous coronary intervention (PCI). This is alarming as the consequences of stent thrombosis are grave with high rates of
death, myocardial infarction (MI), and repeat vascularization [5].

The causal link between smoking and premature coronary arterial disease (CAD) is well demonstrated [6]. Smoking cessation is the single most important intervention for primary and secondary prevention of CAD [7]. Furthermore, a recent large meta-analysis of prospective double blind randomized controlled trials of paclitaxel-eluting stents found that early stent thrombosis (< 30 days) was strongly and independently associated with smoking with an overall mortality rate of 41.2 % [5].

A phenomenon termed “Smoking Paradox” had been noticed with smokers on clopidogrel therapy, in which smoking enhances clopidogrel pharmacodynamics, and smoker patients on clopidogrel had lower platelets reactivity [8]. Additionally it showed a lower incidence of all-cause mortality and that treatment is more effective at reducing the rate of cardiovascular death, myocardial infarction, or urgent revascularization through 30 days among those who smoked ≥ 10 cigarettes/day compared with those who did not [8-10]. Other evidence shows that current smokers (≥ 10 cigarettes/day) had low platelet reactivity [11] and lower active GP IIb/IIIa expression [12] compared with non-smokers. This finding contradicts the very well established notion that smoking is an independent risk factor for coronary vascular disease (CVD) [13].

Our objective is to assess the effect of tobacco smoking, on the pharmacokinetics of clopidogrel among patients receiving 600 mg clopidogrel and undergoing PCI.

EXPERIMENTAL

Study population

Patients (men) who were admitted to Jordan University Hospital for catheterization and underwent PCI would be eligible for the entry into this study if they were given 600 mg of Plavix® (clopidogrel), and consented to participate. Patients were excluded if they were known to have hepatitis B infection or carrier of respective antigen; donated blood within last 2 months; have allergic diathesis or any significant allergic disease; have GI diseases or hepatic disease; were pregnant; have creatinine clearance < 60ml/min; or diagnosed with heart failure with NYHA class 4.

The study was approved by local Research Ethics Committees of the Jordan University Hospital (approval reference number M/C/A/111/1519) and informed consent was obtained from all participants after having been informed verbally by the medical supervisor about the need to withdraw extra blood samples for pharmacokinetic analysis. The decision to give or not to give 600 mg clopidogrel was solely the responsibility of the treating cardio-surgeon performing the PCI. Various laboratory tests were conducted prior to the procedure for each patient. These include serum creatinine, blood urea nitrogen and sodium; liver function (total proteins, albumin, aminotransferases, and alkaline phosphatase); lipid profile and complete blood count.

To be included in the study, smokers were defined as those who smoked least 3 cigarettes per day for at least one year. Non-smokers were defined as patients who never smoked cigarettes or water pipe. Patients who used to smoke and quit (i.e., ex-smokers) were not included in the study irrespective of the period of smoking cessation.

Sample size calculation

The sample size needed to evaluate the influence of smoking on C_max of clopidogrel carboxylic acid metabolite was calculated using equation 1:

\[ N = 2 \times (SD/\Delta)^2 \times (Z_{1-\alpha/2} + Z_{1-\beta}) + 0.25(Z_{1-\alpha/2}) \] ……… (1)

where SD is the estimated standard deviation; \( \Delta \) is the practical significant difference of the mean (33 % of mean); \( Z_{1-\alpha/2} \) is the level of significance (\( \alpha \) level = 0.05); and \( Z_{1-\beta} \) is the power of the study (\( \beta \) level = 0.2). We assumed AUC₀-₉₆ (198.6 ± 52.4 \( \mu \)g.h/ml) based on previously published data [14]. The minimum sample size to detect a difference of at least 60 \( \mu \)g.hr /ml in AUC₀-₉₆ was calculated to be 12. The sample size was further increased to 15 to account for potential data points below lower limit of detection (0.5 \( \mu \)g/ml) over the observation period (8 h).

Intervention and sample collection, chromatographic conditions and pharmacokinetic calculations

The blood sampling, condition of HPLC and pharmacokinetics parameters were reported in previous work [15]. In summary, a whole blood samples from patients were drawn into heparinized test tubes at different time points. Plasma samples were separated and immediately stored at -80 °C until analysis. The
plasma level of clopidogrel carboxylic acid were determined by reverse-phase high-performance liquid chromatographic method, where the separation achieved using isocratic mobile phase. A Dionex® HPLC autosampler system was used. All separations were performed at room temperature. Detection was monitored at 220 nm.

The pharmacokinetic parameters of clopidogrel carboxylic acid metabolite were estimated by standard non-compartmental methods using KineticaTM 2000 Version 4.2 (Innaphase, Philadelphia, PA, USA).

Laboratory parameter measurements

LDL, BUN, GGT and other parameters were measured by standardized and validated methods adopted by Jordan University labs.

Data analysis

Statistical analysis was performed using SPSS® software (version 16.0; SPSS, Inc, Chicago, IL). Data were expressed as mean ± SD. Coefficient of variation (CV) was calculated by as (mean/SD)*100. Normal probability distribution of pharmacokinetic parameters was determined visually by the probability plot (P-P) and quantile-quantile (Q-Q) plot and examining the Kolmogorov-Smirnov-Lilliefors test (K-S test). Equality of variance was assessed by levene’s test. The effect of smoking on pharmacokinetic parameters (Cmax, Tmax and AUC0–∞) was assessed by independent Student t-test. However, Mann-Whitney was utilized to test the effect of smoking on t1/2e. Linear association between pharmacokinetic parameter, t1/2e, and laboratory data (LDL, platelet, BUN and GGT) was tested with Pearson’s correlation coefficient.

RESULTS

Patients’ characteristics

Thirty four male patients received 600 mg clopidogrel loading dose in the study. Patients had an average age of 59.3 ± 9.0 years (range: 44 - 82 years), mean height of 173.7 ± 7.0 cm (range: 155 - 187 cm), average weight of 81.4 ± 12.6 kg (range: 65 – 116 kg), and mean BMI of 26.96 ± 4.25 kgm-2 (range: 21.7 – 38.0 kgm-2) (Table 1).

With regard to laboratory results, patients had normal kidney function (serum creatinine, blood urea nitrogen and sodium); liver function (total proteins, albumin, aminotransferases, and alkaline phosphatase); lipid profile and complete blood count (Table 2).

More than one half of participants were smokers (n = 19). All smokers except two smokers smoked more than 10 cigarettes per day with a mean of 35.6 (range: 3 - 80) cigarettes per day. Smokers and non-smokers had similar medical conditions, prior to admission medications, demographic characteristics, laboratory and physical examination except for one parameter LDL-Cholesterol. Smokers had higher levels of LDL-cholesterol (116.79 ± 42.08 vs. 87.07 ± 27.34 mg/dl, p = 0.041).

Table 1: Demographic and clinical characteristics of recruited patients

| Parameter                  | Smokers (N = 19) | Non-smokers (N = 15) |
|----------------------------|------------------|---------------------|
| Age (years, ± SD)          | 56.7±10.2        | 62.6±5.8            |
| Body mass index (kg/m², ±SD)| 29.3±3.5         | 27.9±5.08           |
| Concomitant disease (N)    |                  |                     |
| Hypertension               | 12               | 12                  |
| Diabetes mellitus          | 8                | 9                   |
| Dyslipidemia               | 5                | 4                   |
| Coronary artery disease    | 8                | 11                  |
| Pre-admission medication history |            |                     |
| Aspirin                    | 13               | 14                  |
| Beta adrenergic receptors blockers | 8      | 12                  |
| ACEIs / ARBs              | 9                | 8                   |
| HMGCo-reductase inhibitors | 11               | 12                  |
| Proton pump inhibitors     | 5                | 3                   |

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; HMGCoA-reductase inhibitor, Hydroxy-methyl-glutaryl-CoA reductase inhibitor
Table 2: Biochemical, hematological and renal profile of patients included in the study

| Parameter               | Mean ± SD     |
|-------------------------|---------------|
| **Kidney function**     |               |
| Na conc. (mEq/L)        | 139±2.9       |
| K conc. (mEq/L)         | 4.64±0.53     |
| Scr (mg/dl)             | 0.80±0.19     |
| BUN (mg/dl)             | 16.1±4.5      |
| **Liver function**      |               |
| Total protein (g/L)     | 6.54±0.44     |
| Albumin (g/L)           | 3.85±0.34     |
| ALT (IU/L)              | 26.92±16.07   |
| AST (IU/L)              | 26.71±12.04   |
| GGT (IU/L)              | 27.46±13.9    |
| LDH(IU/L)               | 371.18±106.56 |
| **Complete blood count**|              |
| RBC (10^6/mm^3)         | 4.64±0.53     |
| WBC (10^3/mm^3)         | 8.8±2.36      |
| Platelets (10^3/mm^3)   | 222.07±62.7   |
| Hgb (g/dl)              | 13.1±1.7      |
| **Other tests**         |               |
| Total Cholesterol (mg/dl) | 160.7±42.9  |
| LDL-cholesterol (mg/dl) | 102.48±38.2   |
| HDL-cholesterol (mg/dl) | 32.05±6.92    |
| Triglycerides (mg/dl)   | 167.85±91.25  |

Scr, Serum creatinine; BUN, blood urea nitrogen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, Gamma-glutamyl transferase; LDH, lactate dehydrogenase; RBC, red blood cell; WBC, white blood cell; Hgb, hemoglobin

Another two laboratory parameters were also different between two groups of study, where smokers had higher GGT levels (31.73 ± 14.42 vs.21.63 ± 11.41, p = 0.08) but lower level of blood urea nitrogen (12.94 ± 2.6 vs. 15 ± 3.06 mg/dl, p = 0.073) (Table 3).

None of these differences is expected to be clinically relevant as they were all within normal range. Additionally, none of these differences is expected to influence the pharmacokinetic parameter values.

**Pharmacokinetic parameters**

The primary pharmacokinetic parameters of clopidogrel carboxylic acid metabolites are reported in table 4. Independent of smoking status, the plasma levels were characterized with significant variability (C_{max} = 23.21 ± 8.79 µg/ml, CV = 37.9 %), (T_{max} = 1.71 ± 1.15 h, CV = 67.2 %), (AUC_{0-∞} = 120.97 ± 44. 4 µg.h/ml, CV = 36.7 %), and (t_{1/2e} = 4.57 ± 3.15 h, CV = 68.9 %).

Regarding the two groups of study, smokers had shorter half-life (p = 0.012). However, smoking behavior had no statistically significantly influence on C_{max}, AUC_{0-∞} nor on T_{max} (Table 4).

Since differences between smokers and non-smokers were found for variables such LDL, GGT and BUN, it was necessary to analyze the possible contribution of these co-variables to the explanation of the significant differences found for t_{1/2e}.

Accordingly, a correlation study was done for t_{1/2e} against these variables (Table 5).

There was no statistically significant correlation between t_{1/2e} and LDL-Cholesterol, GGT or BUN (Table 5). The only significant correlation was with smoking status that decreases t_{1/2e} by 2.36 hr on average.

**DISCUSSION**

Clopidogrel is an important antiplatelet agent that has application in the primary and secondary prevention of cardiovascular complications especially among patients unable to take aspirin. In patients who undergo PCI, dual antiplatelets consisting of aspirin and clopidogrel is the regimen of choice to prevent thrombotic complications [2,16]. Following oral administration, clopidogrel is rapidly absorbed and undergoes extensive hepatic metabolism [17]. The parent drug is inactive and hepatic biotransformation is necessary for its antiplatelet activity [18]. The inactive carboxylic acid metabolite is the most abundant species found in plasma at high concentrations [17].
Table 4: Pharmacokinetic parameters of Clopidogrel carboxylic acid in patients after taking 600 mg clopidogrel

| Parameter        | Mean±SD | Non-smokers | Smokers | P-value |
|------------------|---------|-------------|---------|---------|
| C<sub>max</sub> (µg/ml) |         |             |         |         |
| Mean±SD          | 23.21±8.79 | 20.38±6.48 | 25.44±9.85 | 0.16    |
| Min-Max          | 7.7-46  | 8.9-30.13   | 7.7-46  |         |
| Median           | 22.30   | 22.30       | 24.96   |         |
| CV%              | 37.87%  | 37.87%      | 38.71%  |         |
| T<sub>max</sub> (h) |         |             |         |         |
| Mean±SD          | 1.71±1.15 | 1.91±1.50  | 1.57±0.8 |         |
| Min-Max          | 0.75-6  | 0.75-6      | 0.75-4.06 | 0.91    |
| Median           | 1.075   | 1.03        | 1.13    |         |
| CV%              | 67.25%  | 78.53%      | 50.96%  |         |
| AUC<sub>0-∞</sub> (µg.hr/l) |         |             |         |         |
| Mean±SD          | 120.97±44.4 | 129.7±56.19 | 113±34.7 |         |
| Min-Max          | 64-282.2 | 67-282.2    | 64-191  | 0.65    |
| Median           | 113.17  | 118.60      | 118.60  |         |
| CV%              | 36.70%  | 43.32%      | 30.17%  |         |
| t<sub>1/2</sub> (h) |         |             |         |         |
| Mean±SD          | 4.57±3.15 | 5.83±4.09  | 3.47±1.9 |         |
| Min-Max          | 0.52-10.48 | 2.87-10.48 | 0.52-9.02 | 0.012   |
| Median           | 3.89    | 4.47        | 3.17    |         |
| CV%              | 68.92%  | 70.15%      | 54.75%  |         |

Table 5: Pearson correlation coefficient between elimination half-life and patients’ biochemical data

| Parameter         | Pearson  | p-value |
|-------------------|----------|---------|
| BUN               | 0.293    | 0.123   |
| LDL-cholesterol   | -0.17    | 0.424   |
| GGT               | -0.083   | 0.703   |

BUN, blood urea nitrogen; LDL-Cholesterol, low density lipoprotein; GGT, Gamma-glutamyl transferase

Pharmacokinetic studies of clopidogrel have been performed by measuring plasma levels of either the parent drug [19] or the carboxylic acid metabolite as an indirect approach [17,20]. It has been proposed that information on the absorption and elimination of clopidogrel after oral administration can be derived from the pharmacokinetics of carboxylate metabolite [17].

This research does not address the interaction of smoking behavior with pharmacodynamics of clopidogrel as there are numerous papers published with this regards [11,12,21]. On the other hand only one publication was found on the interaction at the pharmacokinetics level of 75mg clopidogrel in patients [8].

The current study aimed to evaluate the effect of smoking on pharmacokinetic parameters of clopidogrel loading dose of 600 mg in CAD patients planned for PCI. Influence of smoking is important to study as smoking has been associated with platelet hyperactivity, increased cardiovascular risk [6] and it has been found recently to alter the pharmacokinetics parameters of 75 mg clopidogrel in healthy volunteers [22], as well as in patients [8].

The current study revealed that smoking affected at least one pharmacokinetics parameters of clopidogrel carboxylate in smokers. Smokers had lower AUC<sub>0-∞</sub> by 11.5 % (p = 0.65) and had shorter half life by 40.5 % (p = 0.012). It has been proposed that smoking may change drugs’ pharmacokinetics through higher gastric secretion [23] and decreased gastric blood flow [24]. It is unlikely that the effects of smoking on stomach acidity and gastric blood supply contributed to the observed difference on half life as C<sub>max</sub> and AUC<sub>0-∞</sub> were not different between smokers and non-smokers. On the other hand, smoking has been found to interfere with a variety of CYP isoforms; to induce CYP1A2 [25,26], to interact with CYP2C19 [27], and to increase the expression of CYP3A5 [28,29]. Alternatively, smoking was proposed to increase renal clearance [30] by induction of a renal transport protein[30], thus may decrease the elimination half-life as it has been noticed in the current study and in a previous work [22].

The finding of current study parallel our previous work that linked smoking behavior with clopidogrel pharmacokinetics in healthy male volunteer [22], where smokers had shorter half-life of carboxylic acid metabolite after...
administration of a 75 mg single dose clopidogrel by 35%. Smokers also had lower Cmax and AUC compared to non-smokers [22], this also was noticed by the PARADOX study, in which smokers on Clopidogrel therapy had lower Cmax and AUC compared to non-smokers, and had higher exposure to clopidogrel active metabolite compared to non-smokers that can be explained by higher activation of CYP1A2 enzyme as mentioned earlier [8].

It was established that smoking is one of the major risk factors for cardiovascular diseases, dyslipidemia, diabetes, cancers, respiratory diseases and other diseases [31]. Paradoxically it has been found recently that smoking potentiates the effect of clopidogrel [8,10,11], where current smokers on clopidogrel therapy showed lower platelet activity and greater platelet inhibition compared to non-smokers. Also, it has been found that smokers show lower active glycoprotein Ib/IIa expression compared to non-smokers [12] that potentiates antiplatelet effect. This may be because of the way that platelet reactivity is being evaluated, where the same number of platelet is used to assess platelet inhibition post any type of intervention to be studied.

The finding of no effect of smoking on pharmacokinetics of clopidogrel is of importance as there is strong evidence that indicates a publication bias against negative findings. Assessment of the number of meeting-abstracts that turn onto full papers revealed that a trial showing a benefit of a drug or device has much greater chance of full publication than does a trial showing no benefit [32].

Of interest is the observed differences between smokers and non-smokers in relation to the level of LDL-cholesterol [33,34], GGT [35] and blood urea nitrogen [36].

Reasons behind these differences are not yet well known. Liver enzyme GGT is higher in smokers because cigarette smoking might induce liver injury by enhancing lipid membrane peroxidation and cause cell destruction that is responsible for the liver enzyme leakage to systemic circulation.

Lipid profile seems to be altered among smokers. Lower HDL level and higher LDL level have been noticed among different ethnic groups. The high LDL level among smokers has been linked to increase lipolysis by stimulation of the adrenal system, increasing catecholamine, and hence increasing the TG, free fatty acids and VLDL-cholesterol. Nicotine is one of the main constituents of cigarette that has been found to cause increase in triglyceride, cholesterol and VLDL-cholesterol levels and to decrease HDL-Cholesterol levels. Also it has been found that nicotine increases the circulatory pool of atherogenic LDL-cholesterol via accelerated transfer of lipids from HDL-cholesterol and impaired clearance of LDL-cholesterol from plasma compartment therefore it increases the deposition of LDL-cholesterol.

Many factors could cause a lower level of serum urea compared to non-smokers, of these; smokers had been found to consume less quantity of dietary proteins compared to non-smokers which may cause lower amount of urea level and lower level to be excreted [36].

**Limitations of the study**

The findings of this paper are limited by the small sample size included in the study. The needed sample size was calculated based on previously published pharmacokinetics parameters of 600 mg clopidogrel [14]. The difference to be detected between smokers and non-smokers was set at 33 % as a clinically significant difference was hypothesized to equal or to be larger than intrinsic variability reported in the pharmacokinetics behavior of clopidogrel [14,17,20,22]. The AUC calculated for the 34 patients was lower than anticipated in the sample size calculation, and our study would not have been able to detect the specified difference in AUC between smokers and non-smokers.

The recruited patients suffered from multiple medical conditions and were on a large list of medications with the potential to confound the finding of current study. It is practically difficult to select patient who are not on medications that may interfere with pharmacokinetics of clopidogrel as all of them are elders with multiple diseases. Still, the study population is reflection of actual medical practice.

The study was limited to male gender, further research has to include women as gender differences may have a more pronounced effect on cytochrome (CYP) 3A4 [26].

Moreover, the sampling time in the current study was short as it was limited to 8 h post treatment. More time is needed (24-48 h) to have a better estimation of terminal half-life. Unfortunately, this is not feasible in real life situation where real patients are being recruited and most of them are discharged after less than 24 h post stenting. It should be noted that several other studies terminated sampling by 8 h or even less [14,37].
Additionally, the study assumed that changes in clopidogrel carboxylate mirror changes in the active thiol metabolite. Clearly, more research is needed to clarify the effect of smoking on the pharmacokinetics of the active thiol metabolite of clopidogrel.

CONCLUSION

Findings from this study reveal that smoking affects at least one clopidogrel carboxylate pharmacokinetic parameter in smokers, but it may not be a significant determinant of the pharmacokinetics of 600 mg clopidogrel in patients undergoing PCI.

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