Impaired bioavailability of clopidogrel in patients with a ST-segment elevation myocardial infarction


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Chapter 1

ABSTRACT

Recent data has indicated that interindividual variability of intestinal absorption is an important determinant of the wide response variability to clopidogrel. We hypothesised that the physiological state of STEMI influences the intestinal absorption of clopidogrel. To evaluate this, we determined the pharmacokinetic response to a high loading dose of clopidogrel and the absolute ADP induced change in aggregation from baseline in STEMI patients and healthy volunteers. We found a significantly impaired bioavailability in STEMI patients as compared to healthy volunteers and a strong correlation between the reduction in platelet aggregation and the maximal plasma concentration of the active metabolite of clopidogrel. Although large clinical trails have clearly demonstrated the effectiveness of clopidogrel in the setting of STEMI, this small observational study encourages further research based on clinical endpoints to define the optimal dosing of clopidogrel in STEMI patients.
INTRODUCTION

The combination of clopidogrel with aspirin has demonstrated its effectiveness in reducing recurrent ischemic events and absolute mortality in patients with acute coronary syndromes and after stent implantation [1-4]. Clopidogrel is a prodrug that after oral ingestion is absorbed in the intestine. About 85% of the drug is hydrolyzed by esterases to an inactive carboxylic acid derivative. A small fraction is converted by hepatic cytochrome P450 (CYP3A4) monooxygenase to the active thiol metabolite that irreversibly binds to the adenosine diphosphate (ADP) P2Y12 receptor resulting in a partial inhibition of platelet aggregation. However, a considerable interindividual variability in platelet response to clopidogrel has been reported [5-8]. Several mechanisms are associated with this phenomenon but definite evidence of the impact of each contributing factor is still lacking [9]. Recently, Taubert and colleagues found a strong association between the peak plasma concentration of unchanged clopidogrel (and the active metabolite) with the degree of inhibition of platelet aggregation, suggesting that interindividual variability of intestinal absorption may be an important determinant of response variability to clopidogrel [10,11]. However, in ST-elevated myocardial infarction (STEMI) patients, few data exists about the onset of action and magnitude of platelet inhibition after a 600 mg loading dose of clopidogrel. Theoretically, it is possible that the physiological state of STEMI influences the intestinal absorption of clopidogrel. In order to investigate this hypothesis, we designed the present pilot study. Plasma concentrations of clopidogrel and its metabolites were serially measured after administration of a 600 mg loading dose of clopidogrel in STEMI patients and compared with the plasma concentrations of clopidogrel and its metabolites in healthy controls [10].

MATERIALS AND METHODS

**Study population**

Patients with STEMI presenting at our catheterisation laboratory for primary percutaneous coronary intervention (PCI) were eligible. The inclusion criteria were the presence of chest pain for more than 30 minutes, ST-segment elevation detected on a 12-lead ECG of 0.1 mV in 2 or more limb leads, or 0.2 mV in two or more contiguous precordial leads and the ability to perform primary PCI within 6 hours after the start of symptoms. Patients with cardiogenic shock or patients who were unable to swallow clopidogrel or vomited after intake were excluded. Immediately before coronary angiography, all patients received a bolus of 70 IU/kg of unfractionated heparin.
intravenously (i.v.) together with aspirin (900 mg Aspegic i.v.) and a 600 mg loading
dose of clopidogrel. Oral informed consent was obtained in the emergency room. After
PCI, written informed consent was obtained. The study was conducted according to the
principles of the Declaration of Helsinki and with the laws and regulations applicable in
the Netherlands. The local institutional review board approved the protocol.

**Blood sampling**

Blood samples for liquid chromatography tandem mass spectrometry (LC-MS/MS)
were collected from the anticubital vein in tubes containing K₃-EDTA at the following
time points: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6 and 24 hours post-dose. Plasma was obtained
by centrifugation at 1500 g for 10 minutes and stored at –80°C. Blood samples for light
transmission aggregometry (LTA) were collected in tubes containing sodium citrate
(3.2%) at the following time points: pre-dose, 1, 2, 3, 4, and 24 hours post-dose.

**Platelet function studies**

The maximum level of platelet aggregation after the addition of 5 and 20 μmol/L ADP
was quantified on a four-channel light transmission aggregometer (LABiTec, Germany)
as previously described [10]. The measurements were performed within 2 hours from
blood sampling. Results are expressed as absolute change from baseline (Δaggregation).
Importantly, we performed a correlation and validation study with both the Chronolog
aggregometer (that was used for the controls) and the APACT 4004 aggregometer (that
was used for the STEMI patients) [10]. Head-to-head comparison resulted in an excellent
correlation (r=0.94, p<0.001).

**Liquid chromatography tandem mass spectrometry**

Measurements of the plasma concentration of clopidogrel, the inactive carboxyl
metabolite and the active thiol metabolite were performed in triplicate using a Surveyor
HPLC system (Thermo Electron, Dreieich, Germany) coupled to a TSQ Quantum triple-
quadruple mass spectrometer (Thermo Electron) operating in positive electrospray
ionization (ESI+) mode with selected reaction monitoring (SRM) with a slightly modified
method as previously described [10,11]. Of note, because the method of LC-MS/MS
has been modified slightly throughout the years, all blood samples of the previously
described healthy volunteers [10] were re-measured in triplicate.

**Data analysis**

Statistical analysis was carried out with SigmaStat software (Jandel, San Rafael, CA
USA). Data obtained from STEMI patients were compared with the data obtained from
healthy volunteers. Data are presented as means ± SD. Significance of the differences
between the healthy volunteers and STEMI patients was assessed by the unpaired \(t\)-test for comparison of the pharmacokinetic plasma parameters and by unpaired \(t\)-test with Holm-Sidak correction for comparison of platelet aggregation. Plasma concentration versus time data of each participant was fitted by a one-compartment first order model (Bateman regression). From the individual regression fits the following pharmacokinetic parameters were calculated: maximal plasma concentration (\(C_{\text{max}}\)), time to reach the maximal plasma concentration after drug intake (\(T_{\text{max}}\)), the area under the plasma concentration time curve from intake (\(t=0\)) extrapolated to infinity parameters (AUC\(_{0\rightarrow\infty}\)). To determine reproducibility, the intra assay and inter assay coefficients of variation for unchanged clopidogrel were 5.8% and 8.9% respectively. For the active metabolite, these respective coefficients were 6.9% and 9.9%, and for the inactive carboxyl metabolite 5.5% and 8.4%.

During the inclusion phase of the present study, the national ambulance guidelines were changed into a strategy that all STEMI patients were immediately loaded with 600 mg of clopidogrel in the ambulance. This amendment had a major influence on inclusion rate and we were forced to end the study.

Based on the abovementioned coefficients of variation, a post hoc power calculation was performed using a 2-tailed \(t\)-test for unpaired samples. For the given sample size (\(n=11\) and \(n=10\), respectively) and the given effect size of 1.45, an achieved power (1-beta) of 0.88 was calculated at an alpha error of 0.05, which was sufficient to detect the actually existing difference between the group means.

RESULTS

Patient Demographics
Data were obtained form eleven consecutive STEMI patients and compared with ten healthy controls [10]. The baseline characteristics of STEMI patients and healthy controls are depicted in Table 1.
Table I. Baseline Characteristics of the two study groups

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls (n=10)</th>
<th>STEMI patients (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>35 ± 8</td>
<td>55 ± 15</td>
</tr>
<tr>
<td>Women</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177 ± 8</td>
<td>177 ± 10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70 ± 11</td>
<td>80 ± 15</td>
</tr>
<tr>
<td>Active Smoker</td>
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<td>4</td>
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<tr>
<td>Hypercholesterolemia</td>
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<td>3</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Family History of CVD</td>
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<td>4</td>
</tr>
<tr>
<td>Anterior Infarction</td>
<td>N/A</td>
<td>5</td>
</tr>
</tbody>
</table>

Data are presented as the absolute number of patients or mean value ± SD.

CVD = Cardiovascular disease
N/A = Not Applicable

Pharmacokinetic efficacy of clopidogrel: determination of its metabolites

Pharmacokinetics of clopidogrel were measured in all STEMI patients and all healthy controls. Serial measurements of platelet aggregation were determined in 9 of 11 STEMI patients (No measurements were performed in two STEMI patients who received additional abciximab therapy).

Plasma concentrations of unchanged clopidogrel, its inactive carboxyl metabolite and its active thiol metabolite in controls and STEMI patients are given in figure 1. The mean ± SD of all plasma concentrations (Cmax) of unchanged clopidogrel (11.48±11.85 ng/ml versus 37.98±22.89 ng/ml; P=0.003), its inactive carboxyl metabolite (9864±6.017 ng/ml versus 43588±16.327 ng/ml; P<0.001) and the active thiol metabolite (1.49±1.28 versus 8.37±3.62 ng/ml; P<0.001) were significantly lower in STEMI patients as compared to controls.

There was a trend for a prolonged time-to-maximum concentrations (Tmax in minutes) for unchanged clopidogrel (156±110 versus 84±40; P=0.067), its inactive carboxyl metabolite (171±108 versus 93±55; P=0.023) and the active thiol metabolite (137±100 versus 55±32; P=0.050) in STEMI patients as compared to controls.

The area under the curve (AUC in ng x min/ml) for the plasma concentrations of unchanged clopidogrel (3456±2758 versus 7470±3602; P=0.010), its inactive carboxyl metabolite (3689413±1880068 versus 11610969±3023831; P<0.001) and the active thiol metabolite (387.70±249.97 versus 1139.72±464.76; P<0.001) were significantly lower in the STEMI patients as compared to controls.

The ratio of conversion of unchanged clopidogrel into its inactive carboxyl metabolite and its active thiol metabolite (represented by AUC data) was not significantly different between STEMI patients and healthy volunteers (P=0.368 and P=0.376 respectively).

20
Fig 1: Comparison of pharmacokinetic plasma parameters ($C_{\text{max}}$, $T_{\text{max}}$, and $AUC$) of clopidogrel, its active thiol metabolite and its inactive carboxyl metabolite between STEMI patients (n=11) and healthy controls (n=10) after administration of a 600 mg clopidogrel loading dose. Depicted are box plots representing the arithmetic mean (dashed line), the median and interquartile range (straight lines); whiskers show the $10^\text{th}$/$90^\text{th}$ percentiles.
**Platelet aggregation studies**

A significant decrease in maximal absolute platelet aggregation was demonstrated in both groups. In the STEMI group (figure 2), Δaggregation was (7±8 % for 5 μmol/L ADP and 6±7 % for 20 μmol/L ADP (at 4 hours) and 25±6 % for 5 μmol/L ADP and 23±9 % for 20 μmol/L ADP (at 24 hours). However, this reduction in platelet aggregation was significantly lower (p<0.001 for 5 μmol/L ADP and p<0.001 for 20 μmol/L ADP) as compared to the reduction in healthy volunteers at 6 hours post loading dose (Δaggregation was 56±13 % for 5 μmol/L ADP and 45±25 % for 20 μmol/L ADP).

**Fig 2:** Comparison of changes in platelet aggregation induced by 5 μmol/l (A) and 20 μmol/l ADP (B) between STEMI patients (n=9) and healthy controls (n=10) after administration of a 600 mg clopidogrel loading dose. Individual values are expressed as Δaggregation of pre-dose minus post-dose aggregation in %. Depicted are box plots representing the arithmetic mean (dashed line), the median and interquartile range (straight lines); whiskers show the 10th/90th percentiles.
Correlation between the reduction in aggregation and pharmacokinetics

Linear regression analysis showed a strong and statistically significant correlation between the reduction in platelet aggregation at 4 hours and the $C_{\text{max}}$ values of unchanged clopidogrel ($r=0.753$ for 5 μmol/L ADP; $P=0.019$ and $r=0.683$ for 20 μmol/L ADP; $P=0.048$), its inactive carboxyl metabolite ($r=0.744$ for 5 μmol/L ADP; $P=0.022$ and $r=0.741$ for 20 μmol/L ADP; $P=0.035$) and its active thiol metabolite ($r=0.823$ for 5 μmol/L ADP; $P=0.006$ and $r=0.791$ for 20 μmol/L ADP; $P=0.011$) in STEMI patients. For the controls, regression analysis revealed linear correlations between maximal antiplatelet effect and peak plasma concentrations ($C_{\text{max}}$) of unchanged clopidogrel ($r=0.76; p=0.01$), of the carboxyl metabolite ($r=0.70; p=0.03$), and of the thiol metabolite ($r=0.73; p=0.02$), as well as linear correlations between $C_{\text{max}}$ values of clopidogrel and its metabolites.

DISCUSSION

To our knowledge, this is the first study comparing the pharmacokinetics and platelet response of a high loading dose of clopidogrel in STEMI patients and healthy controls. The present study demonstrated that the bioavailability of clopidogrel is impaired in STEMI patients, resulting in suboptimal platelet inhibition as compared to healthy controls.

Recently, several studies demonstrated that the effectiveness of clopidogrel therapy is affected by several determinants [9]. These include dosing, drug-drug interactions, polymorphisms of the receptors involved in the process of arterial thrombosis and haemostasis, cytochrome P450 (CYP 3A4 and CYP3A5) activity, intestinal absorption and baseline platelet reactivity. However, all these studies were performed in stable angina pectoris patients and/or patients with a non-STEMI. STEMI is characterised by decreased cardiac output and compensatory physiological mechanisms such as an increased sympathetic drive, shunting of blood and vasoconstriction of peripheral arteries to maintain sufficient perfusion of vital organs. It is likely that the pathophysiological state of STEMI has a significant influence on the pharmacological properties of clopidogrel.

Absorption

Little is known about gastric emptying, intestinal motility and subsequent gastrointestinal drug-absorption in STEMI. Our results demonstrate that the absolute plasma concentrations of unchanged clopidogrel ($C_{\text{max}}$, AUC) are significantly lower in STEMI patients as compared to healthy volunteers which probably indicates an impaired intestinal absorption in STEMI patients. Furthermore, there is a trend towards a delayed
time-to-maximum plasma concentration in STEMI patients. Impaired pharmacokinetics of clopidogrel in STEMI can possibly be explained by several compensatory mechanisms. Selective shunting of blood to vital organs decreases gastro-intestinal perfusion. Since intestinal mucosa has an intensive metabolic activity, it is extremely vulnerable to hypoperfusion [12-14]. Also, the hemodynamic effects of STEMI may result in elevated venous pressure which stimulates the release of atrial natriuretic peptide (ANP) [15-16]. In turn, ANP inhibits gut permeability and intestinal motility by modulating autonomous nervous activity and by directly interfering with smooth muscle contractility [17-20]. Finally, impaired gastric emptying may also be a mediator in the altered clopidogrel bioavailability in STEMI.

**CYP3A4 and CYP3A5 activity**

Our results demonstrate that the conversion ratio of unchanged clopidogrel into its inactive carboxyl metabolite and its active thiol metabolite is not significantly different between STEMI patients and healthy volunteers, indicating that CYP3A4 activity in general is not impaired in STEMI patients.

**Elevated on-treatment platelet reactivity**

Although it is commonly known that STEMI patients have an enhanced tendency to form platelet aggregates (high pre-treatment platelet reactivity) which seriously affects the rapidity of the time-dependent inhibition of platelets after a 600 mg loading dose of clopidogrel, it is also likely that the lower plasma concentrations of the active thiol metabolite are the predominant cause of the lower inhibition of platelet aggregation in STEMI patients as compared to healthy volunteers [21, 22]. Moreover, two studies have recently demonstrated the causal relationship between the absolute plasma concentration of the active thiol metabolite and the level of inhibition of platelet aggregation [10, 11]. Therefore, the lower levels of the active thiol metabolite together with a higher pre-treatment reactivity may both have added to the relatively small magnitude of platelet inhibition in response to a 600 mg loading dose of clopidogrel in STEMI patients.

**Study Limitations**

There are several notable limitations to the present study. First, previous published data obtained from healthy controls [10] were used for the comparison of pharmacokinetics of clopidogrel with STEMI patients. Importantly, because the method of LC-MS/MS has been modified slightly throughout the years, all blood samples of the previously described healthy volunteers [10] were re-measured in triplicate. Second, the two groups are (per definition) not similar with respect to baseline characteristics. As expected, age
Antiplatelet therapy in myocardial infarction

and the prevalence of (multiple) cardiovascular risk-factors were much higher in STEMI patients. These differences could have affected the results. However, we believe that it is unlikely that this has contributed to the finding of a reduced pharmacokinetics of clopidogrel in STEMI patients. Moreover, multivariate analysis in a previous study has demonstrated that interindividual variability in response to clopidogrel is not influenced by age, hypertension or family history of CAD [23].

Third, there was a difference in time-points at which the blood-samples for LTA were drawn between the two groups. These different time-points were chosen to allow inclusion of STEMI patients 24 hours a day without compromising the processing capacity of our lab. However, these differences in time-points do not alter the conclusion that can be drawn from our LTA data since several studies in healthy volunteers and/or stable angina pectoris patients have demonstrated no significant differences in inhibition of platelet aggregation between 4-h and 6-h post loading dose [23, 24]. Furthermore, the inhibition of platelet aggregation in STEMI patients remained significantly lower at 24-h as compared to healthy volunteers at 6-h post loading. A fourth limitation includes the fact that two different models of optical aggregometers were used. However, head-to-head comparison between both instruments resulted in an excellent correlation (r=0.94, p<0.0001).

In conclusion, despite the small sample size, the present study demonstrates that the bioavailability of clopidogrel is impaired in STEMI patients, resulting in suboptimal platelet inhibition as compared to healthy controls.
Chapter 1

REFERENCE LIST


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Chapter 1