Effect of amlodipine on platelet inhibition by clopidogrel in patients with ischaemic heart disease: a randomised, controlled trial

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ABSTRACT
Background Amlodipine inhibits cytochrome P450 (CYP) enzyme and has the potential to reduce clopidogrel bioactivation in vivo. Reports in previous retrospective studies described greater platelet reactivity in patients on amlodipine.

Objective To evaluate the treatment effect of clopidogrel in patients on amlodipine versus not on calcium-channel blockers (CCBs).

Design and setting Randomised, controlled, open-label trial conducted in a regional acute hospital.

Patients and interventions 98 patients on clopidogrel for ischaemic heart disease were recruited consecutively and randomised to take either amlodipine or drugs with inert CYP effects as controls. The P2Y12 reaction unit (PRU) was measured using whole blood obtained at baseline and on day 28.

Main outcome measures The primary analysis involved the PRU values on day 28. The secondary analyses were percentage of platelet inhibition and poor response to clopidogrel as defined by PRU>235.

Results Both groups experienced comparable day 28 PRUs (amlodipine 227±84 vs control 214±90; mean difference 12.7, 95% CI −22 to +47). Percentage of platelet inhibition (amlodipine 33% vs control 38%, mean difference −4.5%, 95% CI −14% to +5%) and those with poor response on day 28 (amlodipine 49% vs control 45%; p=0.76) did not differ significantly.

Conclusions Concomitant amlodipine has no negative impact on clopidogrel-mediated platelet inhibition in patients with ischaemic heart disease.

INTRODUCTION
Amlodipine and clopidogrel are commonly co-administered in patients with ischaemic heart disease, because amlodipine is effective and relatively free from serious side effect in treating hypertension or angina.1 2

Clopidogrel is a prodrug requiring two-step metabolism by hepatic cytochrome P450 (CYP) to exert its antiplatelet effect. Clopidogrel is first converted to 2-oxo-clopidogrel (a thiolactone), then, in a second step, converted to the pharmacologically active thiol-containing metabolite.

The contribution of CYP1A2, CYP2B6 and CYP2C19 to the formation of 2-oxo-clopidogrel in the first step has been shown to be 35.8%, 19.4% and 44.9%, respectively. The contribution of CYP2B6, CYP2C9, CYP2C19 and CYP3A4 to the formation of the active metabolite was 32.9%, 6.8%, 20.6% and 39.8%, respectively.3

The association between calcium channel blockers (CCBs) and decreased clopidogrel response is postulated to be related to the inhibition of CYP enzymes involved in clopidogrel bioactivation4; but this is controversial, with conflicting data. Some,5–7 but not all,8–10 previous observational studies have found that co-administration of CCBs was associated with a diminished pharmacodynamic response to clopidogrel.

In general, interindividual variability in cytochrome activity can be attributed to inactivation of the enzyme at the molecular level, or regulation of its transcription or polymorphism at the genetic level.11 Mechanisms of CYP enzyme inactivation can be further divided into three categories: reversible, quasi-reversible and irreversible. Among CYP enzymes involved in clopidogrel metabolism, 3A4 and 2B6 may be inactivated by amlodipine in a quasi-reversible mechanism. Amlodipine has relatively stronger inhibition on CYP2B6 and CYP1A1 but weak inhibition on other human CYP enzymes.12–14

However, pharmacokinetic studies suggest significant drug interaction is unlikely because the concentration of amlodipine required to produce a 50% decrease in the activities of CYP enzymes (IC50) was found to be much greater than typical therapeutic concentrations of the drug in plasma.

The plasma concentration of amlodipine after a therapeutic dose has been reported to be 5–50 nM.15 Considering that amlodipine exhibits an IC50 value of 4.9 μM for CYP3A4 and 1.95 μM for CYP2B6, these values were more than 100-fold and 40-fold, respectively, higher than its normal plasma concentration.13

We hypothesised that amlodipine has a minimal effect on clopidogrel metabolism. To measure the potential effect of amlodipine on clopidogrel-mediated platelet inhibition, we quantified the P2Y12 reaction unit (PRU).6 It has been shown to correlate better with in vivo plasma levels of the active metabolite of clopidogrel than other point-of-care tests.16

Fibrinogen-coated microparticles in the VerifyNow assay agglutinate with platelets in whole blood in proportion to the number of expressed glycoprotein IIb/IIIa receptors. A change in optical transparency caused by higher P2Y12-mediated platelet reactivity is expressed by higher PRU. High on-treatment platelet reactivity has an increased...
risk of adverse cardiovascular events. A PRU value >235 correlated with a 6.3% absolute risk increase in non-fatal myocardial infarction at 1 year after PCI. The coefficient of variation for the test precision was less than 8%.24

The aim of this study is to evaluate the treatment effect of clopidogrel in patients on amlodipine versus not on CCBs using PRU as a surrogate outcome.

METHODS
Study design
This randomised, controlled, open-label trial was registered at clinicaltrial.gov (NCT01203696). The study complied with the Declaration of Helsinki and was approved by the local ethics committees; all subjects gave written informed consent before enrolment.

Study population and period
Patients with ischaemic heart disease who were prescribed aspirin and clopidogrel co-therapy were screened at Ruttonjee Hospital between July 2010 and April 2011. The inclusion and exclusion criteria are listed in box 1.

Platelet reactivity measurement
Patients had taken either a 300 mg clopidogrel loading for at least 12 h, or a maintenance dose of 75 mg daily for at least 7 days before blood was sampled for baseline platelet reactivity, using the VerifyNow P2Y12 assay (Accumetrics, San Diego, California, USA). In case the patients had to undergo a percutaneous interventional procedure, blood was collected either before arterial puncture, or 1 week after the procedure, to avoid contamination by platelets activated during these procedures.

Venous blood was collected using 21 G needles, in a one-off successful attempt in a vein which had not been previously punctured, according to the manufacturer’s recommendations. The first 3 ml of the blood drawn into a plain bottle was discarded to avoid contamination by platelets activated in this blood taking process; 2 ml of blood was then collected using a citrate container for the P2Y12 assay. All blood samples were taken by the same operator, who also checked the machine for proper function daily.

Randomisation
All the treatment codes were prepared by an independent ward clerk at the beginning of the study and sealed in blinded envelopes for randomisation with equal allocation. The investigators drew a blinded envelope for each subject. After the first blood sampling, patients were randomised to either amlodipine (Norvasc, Pharmascience, Canada) or a non-calcium-CCB (non-CCB) with inert CYP activity as control. The dose of amlo- dipine was 2.5 mg for patients whose baseline systolic blood pressure (SBP) was 131–140 mm Hg, and 2.5 mg more for each increment of 10 mm Hg; maximum dose was 10 mg for SBP > 161 mm Hg. The choice of non-CCB depended on the indication. For patients who needed additional drug for blood pressure control (defined as SBP > 130 mm Hg or diastolic blood pressure (DBP) > 90 mm Hg), the choice of non-CCB would be either diuretics or hydralazine. Diuretic choices included indapamide (Natrilix SR, Sarbier, France), co-amilozide (Moduretic, Jean-marie-pharmacal, Hong Kong) or furosemide (Lasix, Aventis Pharma, Hong Kong). For patients requiring angina control, the non-CCB would be isosorbide mononitrate (Elantan, Schwarz Pharma, Hong Kong).

Follow-up and primary analysis
Clinical follow-up of patients and the second platelet reactivity were carried out on day 28±3 at a designated outpatient clinic. Drug compliance was assessed by direct questioning and pill count. Telephone contacts were made by data coordinators when follow-ups were missed to enhance completeness of data.

The primary analysis was PRU at day 28. The secondary analyses were the percentage of P2Y12 receptor inhibition on day 28, and the prevalence of poor response to clopidogrel on day 28 (defined as PRU > 235, in line with the previously published consensus cut-off point).23

Patients with either acute coronary syndrome or stable coronary disease were enrolled. Primary outcomes of platelet reactivity were measured on day 28 to minimise the effect of acute coronary syndrome on platelet reactivity. This time frame, although arbitrary, was inferred from observational studies showing that high on-treatment platelet reactivity in the acute setting may settle later on when measured by optical aggregometry with ADP 20 μmol/l as the agonist, although this finding could not be reproduced by measurement with the VerifyNow assay.26 Furthermore, as coronary intervention performed after baseline blood sampling might induce endothelial injury and affect platelet reactivity, delaying the second measurement to day 28 would avoid this effect during hospitalisation.

Statistical analysis
Sample size was based on a previous cohort with similar selection criteria, showing a mean PRU difference of 48 between

Box 1 Selection criteria

Inclusion criteria
Patients who fulfill all the following criteria would be eligible for study:

- Treated with aspirin and clopidogrel co-therapy for ischaemic heart disease
- Persistent angina greater than New York Heart Association (NYHA) II or blood pressure >130/90 mm Hg
- Asian ethnicity
- Informed consent obtained

Exclusion criteria
The presence of any of the followings excluded patients from the study:

- Use of any of the following drugs within 2 weeks:
  - Any calcium-channel blocker
  - Strong inhibitors of cytochrome P450 (CYP) 3A4 enzyme (any anti-HIV protease inhibitors, itraconazole, ketocnazole)
  - Strong inducers of CYP 3A4 or 2B6 enzyme (carbamazepine, phenytoin, rifabutin, ketoconazole)
  - Proton-pump inhibitors except pantoprazole
  - Any calcium-channel blockers
  - Use of any of the following drugs within 2 weeks:
    - Heparin or glycoprotein IIb/IIIa inhibitor within 48 hours
    - Treated with aspirin and clopidogrel co-therapy for stable coronary disease
    - Use of heparin or glycoprotein IIb/IIIa inhibitor within 7 days before blood was sampled for baseline platelet reactivity
    - Amlodipine or any calcium-channel blockers
    - Proton-pump inhibitors except pantoprazole
    - Any calcium-channel blockers
    - Use of any of the following drugs within 2 weeks:
      - Active cancer
      - Pregnant or lactating mothers
CCB and non-CCB group, with an SD of 84 units. A total of 98 patients (49 per randomised group) was the minimum required with a power of 80% and two-sided $\alpha$ value of 0.05, in order to reject the null hypothesis. Assuming 4% dropout during the study, enrolment of 102 patients was expected.

Normal distribution of interval variables was verified by the Kolmogorov–Smirnov test. Continuous variables were expressed as mean±SD and compared with the unpaired two-tailed Student t-test. Comparisons of categorical variables, expressed as counts (percentage), were performed with the $\chi^2$ test or Fisher’s exact test. Statistical analyses were performed using SPSS V.13.0 according to the intention-to-treat principle.

RESULTS
A total of 102 patients were enrolled consecutively. Among the 50 patients in the amlodipine group, one stopped clopidogrel after a normal angiogram refuted the diagnosis of ischaemic heart disease, and three patients among the 52 in the control group did not complete the study. None had crossover of treatment. The final study population consisted of 49 patients in each group (figure 1); 96% of the patients were Chinese.

The mean±SD daily dose of amlodipine was 4.0 mg±2.4 mg. Drugs used as controls were hydralazine (8 patients, 65±22 mg), isosorbide mononitrate (18 patients, 48mg±16 mg) and diuretics (23 patients). Drugs used in the diuretic group were: indapamide (8 patients, 1.5 mg), furosemide (7 patients, 22±7.5 mg) and co-amilozide (8 patients, 2.5/25 mg).

Baseline clinical characteristics and laboratory results of both groups were comparable (table 1). Normal distribution is observed at baseline and day 28 PRUs (Kolmogorov–Smirnov test, with p values ranging from 0.2 to 0.3). The baseline PRU was 206±82 in the amlodipine group and 213±91 in the control group (mean difference 7.0, 95% CI –41 to +28). There was no significant difference in the percentage of P2Y12 receptor inhibition between the two treatment groups (amlodipine 38±23, control 37±21, mean difference –1.0, 95% CI –7 to +10, p=0.78). Mean SBP of both groups on day 28 was similar (amlodipine group 131±17 mm Hg, control group 129±17 mm Hg, 95% CI –1.6 to +12).

Primary analysis
The day 28 PRU was 227±84 in the amlodipine group and 214±90 in the control group (mean difference 12.7, 95% CI –22 to 47); the means of the two groups did not differ significantly (table 2).

Secondary analyses
Percentage inhibition on day 28
The percentage inhibition on day 28 was 33%±22% in the amlodipine group and 38%±25% in the control group (mean difference –4.5%, 95% CI –14 to +5.0%).

Poor responders to clopidogrel on day 28
Twenty-four patients (49%) in the amlodipine group and 22 (43%) patients in the control group were classified as poor
responders. Compared with baseline, when the corresponding
numbers were 20 (41%) and 16 (33%), respectively, there was no
significant difference in the numbers of poor responders
(p=0.76). No patient experienced death, myocardial infarction
or heart failure requiring hospitalisation during the study
period.

DISCUSSION

Our randomised study suggests that amlodipine does not attenuate the efficacy of clopidogrel, when compared with diuretics,
hydralazine or nitrate as control. This result agrees with those from previous studies which indicated that interaction of amloidine and clopidogrel via inhibition of cytochrome metabolism might be clinically insignificant.4-10 Although more potent anti-platelet agents such as prasugrel or ticagrelor are now available, a substantial proportion of the population is taking clopidogrel due to its cost. The absence of significant pharmacological interaction has economic and safety implications.

Several reports in the literature described CCBs as being associated with high on-treatment platelet reactivity.5-7 Harmasz et al, for example, conducted a cross-sectional study of 623 consecutive patients undergoing elective PCI, and reported that poor response to clopidogrel was 2.3 times more likely to occur in patients taking amlodipine than in those without CCB treatment.5 However, users of amlodipine were older than patients in the other groups in their population.

The strength of the current study is validation of similar baseline platelet reactivity in both groups before drug intervention. Moreover, known potential confounders, especially diabetes mellitus (DM), were balanced. The presence of DM was an independent predictor of lower platelet inhibition with clopidogrel.26 Insulin normally inhibits P2Y12 signalling on the platelet surface. Platelets in type 2 DM patients were less responsive to insulin, thereby increasing signalling through the P2Y12 cascade.27-30 In addition, CYP2B6 enzyme is irreversibly inhibited by the active metabolite of amiodarone, and reversibly inhibited by the herbal compound ginkgo biloba. No patients in the current study reported use of these drugs.

Genetic variations leading to change in cytochrome activity may confound our primary outcome of PRU.31-34 The CYP2B6 gene has been mapped to chromosome 19. Exposure to drugs that induce CYP2B6 transcription through nuclear receptors will increase the enzyme activity and hence may neutralise the inhibitory effect of amloidipine.35 Examples of these drugs include phenobarbitone, phenytoin, carbamazepine and rifampicin. Nonetheless, patients on these drugs were excluded from our study. Because of some common mechanism of transcription regulation shared between CYP2B6 and 3A4, a number of drugs can induce transcription of both genes simultaneously.16-38

Although tests on genetic polymorphism were not performed in our study, its inter-ethnic difference was minimised because our population only consisted of ethnic Asians. Single nucleotide polymorphisms of CYP2B6 have been reported. The most frequent variant allele is *6 caused by 516G>T and 785A>G.39 Patients with homozygous (*6/*6) genotype were reported to have 50% reduction in the mean enzyme activity compared with the wild-type (*1/*1) group.40 However, the prevalence of the CYP2B6*6/*6 genotype is reported to be only 2-5% in Chinese.41 As CYP2B6 only plays a minor role in clopidogrel bioactivation, it can therefore be expected that polymorphism of CYP2B6 will have only a modest effect on PRU in our patients. Recent findings that carriage of loss of function *2 allele of CYP2C19 gene only accounts for 5% of the variability

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**Table 1 Baseline characteristics of patients**

<table>
<thead>
<tr>
<th>Risk factors/history, n (%)</th>
<th>Amlodipine group (n=49)</th>
<th>Control group (n=49)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, n (%)</td>
<td>26 (%)</td>
<td>27 (%)</td>
<td>0.31</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>27 (55%)</td>
<td>22 (44%)</td>
<td>0.23</td>
</tr>
<tr>
<td>Hypertension</td>
<td>46 (94%)</td>
<td>48 (98%)</td>
<td>0.31</td>
</tr>
</tbody>
</table>
| Previous myocardal
infarction*                 | 26 (53%)                | 21 (43%)            | 0.31    |
| ACS within 1 month†         | 15 (30%)                | 22 (45%)            | 0.15    |

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**Table 2 Results of analysis on day 28**

<table>
<thead>
<tr>
<th>Results of analysis on day 28</th>
<th>Amlodipine group (mean±SD)</th>
<th>Control group (mean±SD)</th>
<th>Mean difference (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet reactivity unit (primary end-point)</td>
<td>227±84</td>
<td>214±90</td>
<td>12.7 (−22 to +47)</td>
<td>0.47</td>
</tr>
<tr>
<td>Platelet inhibition, % (secondary end-point)</td>
<td>33±22</td>
<td>38±25</td>
<td>−4.5 (−14 to +5.0)</td>
<td>0.34</td>
</tr>
<tr>
<td>No. of poor responder to clopidogrel, n (%) (secondary end-point)</td>
<td>24 (49%)</td>
<td>22 (45%)</td>
<td>–</td>
<td>0.76</td>
</tr>
</tbody>
</table>
of the pharmacodynamic response to clopidogrel are also con-

of this regard.42

This study had several limitations. First, it was not placebo
controlled, thus the control treatment might have influenced
platelet inhibition. However, this design resembled the real-life
situation as diuretics and nitrates were the commonest alter-
catives to CCBs in that context. Second, the study sample size was
relatively small in view of the inherent large variability of
on-treatment platelet reactivity. Surrogate pharmacological end-
points, albeit with clinical implications, were investigated
instead of clinical adverse cardiovascular events. Our results are
hypothesis-generating but open to the possibility of chance find-
ings. Third, the dose of amiodarone was modest and further
studies to confirm a similar effect for higher doses of amlo-
dipine would be helpful. The current result should not be general-
ised to other subclasses of CCBs because of different pharma-
dynamic properties.12,43

In conclusion, amiodarone does not significantly attenuate the
antiplaet effect of clopidogrel in patients with ischaemic heart
disease with respect to pharmacological end-points. The
common practice of concomitant use of these two drugs may be
continued.

Contributors All authors were solely responsible for the design, conduct
and analyses of the study, drafting and editing of the paper and its final contents.

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Competing interest FKLC has served as a consultant for Pfizer, Eisai, Takeda
and Otsuka, and has received lecture fees from Pfizer, Astra Zeneca and Takeda.

Patient consent Obtained.

Ethics approval Ethics Committee of the Hong Kong East Cluster, Hospital
Authority, Hong Kong Special Administrative Region.

Provenance and peer review Not commissioned; externally peer reviewed.

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33. Clinical signi


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