# The Effect of Known Inhibitors and Inducers of Human Cytochrome P450 3A4 on the Platelet Inhibitory Activity of Clopidogrel

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## Introduction

Clopidogrel is a prodrug that requires *in vivo* conversion to an active metabolite in the liver to exert its platelet antiaggregatory effect. Clopidogrel requires metabolic activation by cytochrome P450 (CYP) 1A2 in rats.[1] The enzyme responsible for clopidogrel activation in man has not been determined.

## Methods

### Part 1:

Inhibition of platelet aggregation by clopidogrel was studied after the concomitant administration of clopidogrel and either erythromycin (a CYP3A4 inhibitor) or rifampin (a CYP3A4 inducer). Platelet aggregation was measured with the FDA approved point-of-care Plateletworks™ test platform (Helena Laboratories, Beaumont, TX) and the MI-CROS™ cell counter (ABX Diagnostics, Irvine CA). *Human volunteers were randomized:*

- Group 1 (n=9) received clopidogrel 75 mg/day for six days, followed by a washout period of 14 days, followed by four days of erythromycin 250 mg four times a day, and finally by both clopidogrel and erythromycin for six days.
- Group 2 (n=10) received clopidogrel 75 mg/day for six days, followed by a washout peroid of 14 days, followed by 4 days of rifampin 300 mg twice a day, and finally by both clopidogrel and rifampin for six days.

Platelet aggregation was detemined at 0, 2, 4, 6, 20, 24, and 30 days for both groups.

### Part 2:

An Erythromycin Breath Test (ERMBT, Metabolic Solutions, Inc., Nashua, NH) was used as an *in vivo* probe to measure hepatic CYP3A4 activity. An intravenous dose of [$^{14}$C-N-methyl]-erythromycin (3 mCi, 0.1 mmol of erythromycin) was administered and a single breath sample was then collected after 20 minutes. Quantitation of exhaled $^{14}CO_2$ provided a selective measure of the "instantaneous" hepatic CYP3A activity.[2]

- Baseline platelet aggregation and ERMBT were measured before (0 hours) and two hours after clopidogrel 450 mg oral administration.
- After a 14-day washout period, a single oral dose of troleandomycin 500 mg was administered.
- Clopidogrel 450 mg was administered orally one hour after troleandomycin.
- Platelet aggregation and ERMBT were measured again at 0 and 2 hours after clopidogrel administration.

### Part 3:

Microsomes prepared from genetically engineered human lymphoblasts and containing either CYP3A4 (40 pmoles/mL) or 1A2 (50 pmoles/mL) were tested for their ability to metabolize clopidogrel. Clopidogrel was separated using high performance liquid chromatography and detected spectrophotometrically at 220 nm as described.[3] Disappearance of clopidogrel was measured.

## Results

Figure 1: The Effect of Erythromycin on Platelet Aggregation Inhibition by Clopidogrel

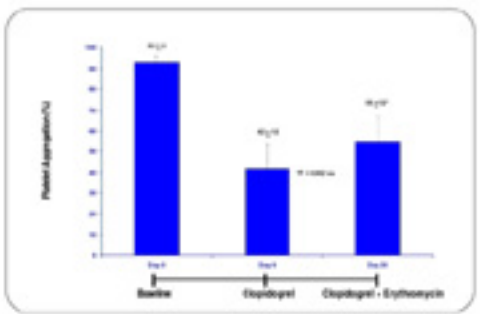



Figure 2: The Effect of Rifampin on Platelet Aggregation Inhibition by Clopidogrel

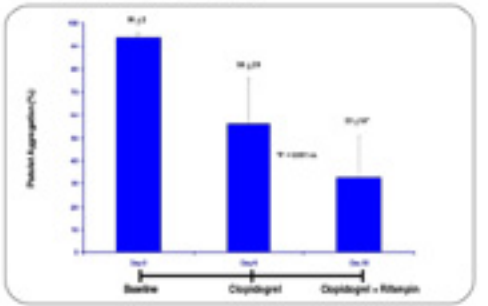



Figure 3: The Effect of Troleandomycin and Clopidogrel on Cytochrome P450 3A4 Activity

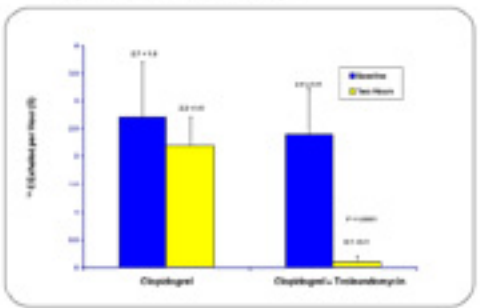



Figure 4: The Effect of Troleandomycin on Platelet Aggregation Inhibition by Clopidogrel

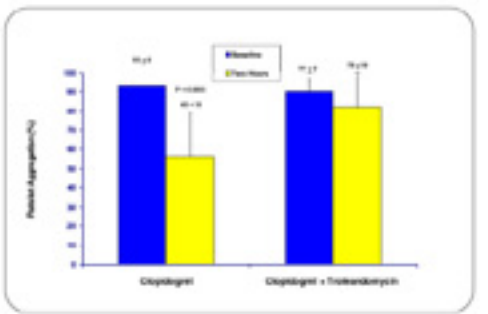
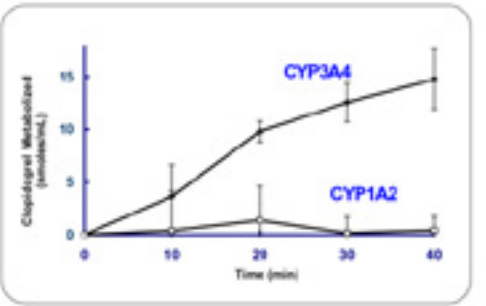



Figure 5: In Vitro Metabolism of Clopidogrel by Cytochrome P450 3A4




## Discussion

The decrease in the antiplatelet activity of clopidogrel by erythromycin and the enhancement of this activity by rifampin supports the concept that the metabolic activation of clopidogrel is primarily catalyzed by CYP3A4. This hypothesis was further substantiated when we used troleandomycin to inhibit CYP3A4. We selected troleandomycin because it is a more potent and selective inhibitor of CYP3A4 than erythromycin.

## Conclusion

- Metabolic activation of clopidogrel in humans is largely catalyzed by CYP3A4.
- Inhibitors and inducers of CYP3A4 may alter the efficacy of clopidogrel.
- It may be important to test that platelet aggregation inhibition targets are met in patients taking clopidogrel.

## References

1. Savi P, et al. Thromb Haemost 1994;72:313-7.
2. Watkins PB. Pharmacogenetics 1994;4:171-84.
3. Savi P, et al. Thromb Haemost 2000;84:891-6.