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# Rofecoxib exerts no effect on platelet plug formation in healthy volunteers

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**Key words:** Rofecoxib, platelet function, PFA-100, randomized, controlled trial.

**ABSTRACT**

**Objective.** *Although rofecoxib has very high selectivity for cyclo-oxygenase 2 (COX-2), supratherapeutic rofecoxib concentrations (> 1000 mg) inhibit purified human COX-1* in vitro *and $TXB_2$ formation* in vivo. *It is therefore possible that higher doses of rofecoxib may affect platelet function. This could be important if rofecoxib is given to thrombocytopenic patients. In these cases, already moderate inhibition of platelet function could precipitate bleeding complications. We therefore set out to investigate the influence of rofecoxib on platelet function in healthy volunteers.*

**Methods.** *We set up a balanced-randomised, double-blind, placebo-controlled, two way cross-over study. Peripheral blood was withdrawn from 42 healthy volunteers before and 3 hours after intake of 50, 250, 500 mg of rofecoxib or placebo (n=14 per group). Platelet function was assessed by a platelet function analyzer (PFA-100) which measures collagen-epinephrine induced closure time (CEPI-CT) under shear stress.*

**Results.** *CEPI-CT increased by 14% (p = 0.002) and 11% (p = 0.003) three hours after intake of placebo and rofecoxib at dosages of up to 500 mg, respectively. The increase in CEPI-CT versus baseline was not significantly different in the placebo period compared with the active treatment periods (n = 42, p > 0.05).*

**Conclusions.** *Rofecoxib does not impair platelet function. Thus, rofecoxib appears to be a valuable analgetic and antipyretic agent in the therapy of patients at risk for bleeding.*

## Introduction

There is evidence that inhibition of cyclooxygenase-2 (COX-2) is responsible for the anti-inflammatory and analgesic effects of non-steroidal anti-inflammatory drugs (NSAID) (1,2). Inhibition of the isoenzyme COX-1 adversely affects platelet function due to inhibition of thromboxane formation.

Among the NSAIDs currently available on the market, rofecoxib was suggested to exert the highest selectivity for COX-2 (3,4). Coxibs are a new therapeutic class for the symptomatic treatment of osteoarthritis and rheumatoid arthritis with improved gastrointestinal tolerability (5). Athough rofecoxib has the highest selectivity for COX-2, it also caused inhibition of purified human COX-1 *in vitro* with an IC50 value of 26 ± 6 µM ($C_{max}$ after multiple doses of 25 mg rofecoxib average 1.2 µM) (6,7). *In vivo* plasma rofecoxib concentrations of > 5 µM inhibit $TXB_2$ formation by 0-50% (7). In addition, it has not been ruled out that rofecoxib may directly affect platelet function by an intrinsic effect. This could be important if rofecoxib is given to patients with bleeding tendencies. In these cases, already moderate inhibition of platelet function could precipitate bleeding complications.

We therefore set out to investigate the influence of therapeutic and "supratherapeutic" dosages of rofecoxib on platelet function in healthy volunteers. Platelet function was assessed with the platelet function analyzer PFA-100, which is a novel established tool to detect drug-induced platelet dysfunction (8-10).

## Materials and methods

The design was a balanced-randomised, double-blind, placebo-controlled, two-way cross-over study and was approved by the Institutional Ethics Committee. Healthy non-smokers (21 men and 21 women) aged 20-39 years with a BMI ranging from 18-29 kg/m$^2$ were included in this study after their written consent was obtained. Volunteers were randomly assigned to take either placebo or active drug (50, 250 or 500 mg rofecoxib (VIOXX®, MSD, Vienna, Austria)). After a washout period of 7 days volunteers received the alternative regimen, i.e. placebo or one of the dosages of rofecoxib so that all subjects received placebo on one occasion. In order to maintain the double-blind conditions, the study medication was prepared by staff members who did not participate in the data acquisition. The study medication was placed in placebo capsules and filled with cornstarch. The three different medications and placebo were identical in appearance. The study medication was taken under the supervision of a staff member.

Blood was collected into tubes containing 0.129 M buffered citrate (Vac-

uette®, Greiner Labortechnik, Vienna, Austria) before and 3 hours after rofecoxib intake, because the time to reach maximal rofecoxib concentrations has been reported to be 2-3 hours post dosing (4). Recently, a number of new assays testing platelet function including PFA-100 have been introduced (10). The PFA-100 system utilises disposable test cartridges where a platelet plug occludes a microscope aperture cut into a membrane coated with collagen and epinephrine (CEPI). The time required for occlusion (closure time, CT) is indicative of platelet function and primary hemostasis capacity (10, 11). CEPI-CT values exhibit a 9% intra-individual day-to-day variability (12). All PFA-100 measurements were done within 4 hours after blood collection. To test the validity and sensitivity of the PFA-100 device to detect COX-1 inhibition, we analysed blood from our staff members after intake of 500 mg of aspirin and documented closure times > 300 s.

All data is expressed as median and 95% confidence interval (CI). All statistical comparisons were performed using the Wilcoxon matched pairs test and Kruskal Wallis ANOVA with the Mann-Whitney U-test for post hoc comparisons. A two-tailed p-value < 0.05 was considered significant.

**Results**

Baseline values of collagen-epinephrine induced closure time (CEPI-CT) were not significantly different between treatment groups ($p > 0.05$, Fig. 1).

CEPI-CT increased by 14%, 25%, 4% and 20% versus baseline three hours after oral administration of placebo, 50, 250 and 500 mg of rofecoxib, respectively ($p < 0.01$ for placebo and $p = 0.02$ for 500 mg rofecoxib). The increase in CEPI-CT versus baseline was not significantly different in the placebo arm compared with the three active treatment groups ($p > 0.05$).

No significant differences between treatment groups were detected and thus data from all three rofecoxib dosages were pooled (n = 42) in order to detect even small differences between the placebo and treatment groups (13). In this case a significant effect on CEPI-CT versus baseline was observed in the treatment groups (n = 42, $p = 0.003$) and in the placebo period ($p = 0.002$). For the pooled rofecoxib data, the CEPI-CT increased significantly by 11% versus baseline, which was not different from the increase in CEPI-CT values in the placebo period (14%, n = 42, $p = 0.82$).

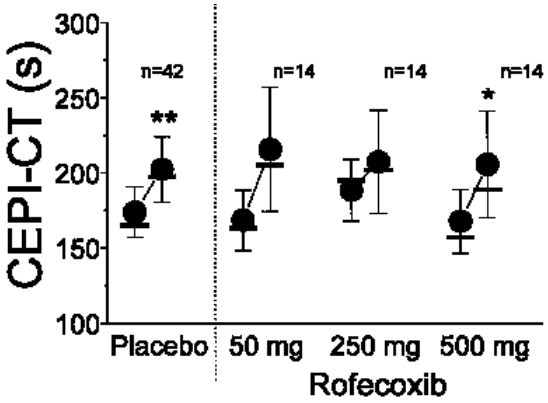


**Fig. 1.** Effect of placebo and rofecoxib on the collagen epinephrine-induced closure time (CEPI-CT) as measured by a platelet function analyzer (PFA-100). Platelet function was assessed before and 3 hrs after a single dose of placebo or 50 mg, 250 mg and 500 mg of rofecoxib in healthy volunteers (n = 42). The mean is shown as circles,the 95% confidence intervals as error bars, and medians as horizontal lines. *$p < 0.05$ and **$p < 0.01$.

**Discussion**

The use of NSAID is problematic in patients with thrombocytopenia or those treated with coumarin derivatives because of the risk of potentially severe bleeding. This problem may be overcome by coxibs such as rofecoxib because of improved gastrointestinal tolerability and the presumed absence of adverse effects on platelet function. As the latter issue has been insufficiently investigated, we carried out the present study to test the intrinsic influence of rofecoxib on platelet function in healthy volunteers.

The major finding of our study is that the effect of rofecoxib on CEPI-CT, even at the highest dosage of 500 mg, was similar to the effect of placebo. The effect of rofecoxib on CEPI-CT was not significantly different between the three treatment groups ($p = 0.12$). For the pooled rofecoxib data CEPI-CT significantly increased by 11% versus baseline, which was not significantly different from the increase in CEPI-CT values in the placebo group (14%, n = 42, $p = 0.82$). As no significant differences were observed between the placebo and active treatment groups, we postulate that this effect is related to a small diurnal increase in CEPI-CT (14) (drug effects were assessed 3 hours after baseline measurements of platelet function) rather than an effect of rofecoxib administration.

Our data is corroborated by a recently published study which showed no changes in *in vivo* bleeding times (Simplate II) after intake of multiple doses of 375 mg rofecoxib in healthy volunteers (7). While the PFA-100 is a high shear stress system, the bleeding time is a low shear stress system. Thus, rofecoxib does not affect platelet function as measured by completely different methods. However, the PFA-100 device is highly sensitive for COX-1 inhibition (10-12) and superior to the bleeding time in this respect.

Further, therapeutic doses of rofecoxib had no effect on platelet aggregation induced by arachidonic acid or collagen (15,16). In another paper, supratherapeutic doses of rofecoxib (up to 1000 mg) did not affect platelet COX-1 activity (2).

In addition, our results are in good agreement with a previous study showing that therapeutic doses of the relatively selective COX-2 inhibitor meloxicam (15 mg/day) prolong CEPI-CT time by only 10% (8). In contrast, intake of 25 mg indomethacin b.i.d., a preferential COX-1 inhibitor, over a period of 7 days increased CEPI-CT by 123% (8) and intake of 100 mg of aspirin over a period of 11 days increased CEPI-CT by 80% (12). More importantly, even single oral doses of 300 mg aspirin induced non-closure in 50% of treated subjects after 2 hours, whereas the other 50% of individuals had an approximately 2-fold prolongation in CEPI-CT (17). This indicates that even single oral doses of COX-1 inhibitors

prolong the CEPI-CT within 2 hours, whereas the COX-2 inhibitor rofecoxib has only placebo-like effects on platelet function. All of these studies unequivocally showed that PFA-100 is a sensitive method to detect effects of different COX-1 inhibitors. Thus, we did not include any positive control in the current study.

The present study is in accordance with previous reports indicating that measuring CEPI-CT with the PFA-100 analyzer offers a simple and reproducible alternative to $TXB_2$ measurements (8, 12). In addition, the PFA-100 allows for measurement of the potential intrinsic effects of NSAIDs, including coxibs, on platelet function.

In conclusion, the effect of rofecoxib on platelet function was similar to placebo. Thus, rofecoxib appears to be a valuable analgetic and antipyretic agent in the therapy of patients at high risk for bleeding.

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