

# Clinical trial evaluating the effects of a reduced iso-alpha acids (RIAA), rosemary extract, and oleanolic acid supplement on parameters of platelet function and blood coagulation

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## ABSTRACT

Several botanical and therapeutic agents have been reported to have adverse effects on platelet function; therefore, evaluating the effect of a novel anti-inflammatory on platelet function and blood coagulation is an essential step in clinical safety assessment. In this report, we present the findings of a clinical trial assessing the effect of Meta050 (1 week at 2 tablets bid) on parameters of blood coagulation and platelet function. The results indicate Meta050 has no direct effect on blood coagulation. Moreover, Meta050 does not appear to influence platelet function. This observation is consistent with in vitro data that indicates Meta050 does not inhibit the COX enzymes.

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## INTRODUCTION

Non-steroidal anti-inflammatories (NSAIDs) have been reported to induce gastrointestinal bleeding, as well as have adverse effects on platelet function. For example, aspirin is well-known to lengthen platelet closure times, and naproxen has been shown to reduce platelet response to various stimuli.<sup>1</sup> The accepted mechanism underlying these alterations is inhibition of platelet COX-1 activity, which then results in alteration of thromboxane A<sub>2</sub> levels and, thus, affects platelet function.<sup>2</sup> In support of this, COX-2 inhibitors such as valdecoxib and celecoxib have not been associated with alterations in platelet function.<sup>3,4</sup>

Many therapeutics have been noted to modify the hypothermic action of warfarin, including salicylates, barbiturates, antacids, MAO inhibitors, and estrogen contraceptives, among many others. For example, cimetidine has been reported to prolong prothrombin time (PT) in patients with a stable warfarin anticoagulated state, and broad-spectrum antibiotics, in particular second- and third-generation cephalosporins, exert a hypothermic effect. These interferences have led to cautions regarding

co-administration of these agents with warfarin.

Given the myriad of influences of commonly used anti-inflammatories, it seems that evaluating the effect of a novel anti-inflammatory on platelet function and blood coagulation is an essential step in clinical safety assessment. Evaluating blood coagulation is a standard practice and can be performed by monitoring PT and partial thromboplastin time (PTT). Platelet dysfunction can be detected by assessing times to form plugs after platelets are stimulated.<sup>5</sup>

In this summary, we report the findings of a clinical trial with the recently developed natural anti-inflammatory, Meta050, on assessment of blood coagulation parameters and platelet function in healthy subjects.

## METHODS

### SUBJECTS

Subjects were 6 healthy, ambulatory individuals between 18 and 65 years on no medications known to affect platelet function. Subjects were initially screened by phone. Exclusion criteria included allergy to

one or more of the test substances, NSAIDs, or aspirin; current use of the test product, NSAIDs, COX inhibitors, or anti-inflammatories or natural products known to influence platelet function or blood coagulation; history of liver, kidney, or heart disease; uncontrolled hypertension; and history of deep vein thrombosis or pulmonary embolus, peptic ulcer, gastritis, or esophagitis. In addition, subjects were excluded if their regular intake of alcohol was more than 2 drinks per day, or if the screening laboratory indicated abnormal reading.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Candidates who agreed to participate signed Informed Consents prior to the start of the trial (Visit 2). A copy of the Informed Consent was provided to each subject.

#### ***TRIAL DESIGN***

The study was an open-label assessment of platelet function and blood coagulation after 7 days of consuming Meta050. Subjects were initially screened at Visit 1 and a blood draw was obtained for screening laboratories (CBC and CMP). After acceptance into the study, a baseline blood draw was obtained at Visit 2, and subjects were assigned 2 tablets (880 mg) Meta050 twice daily for 7 days. On the 8<sup>th</sup> day, subjects returned for Visit 3, at which time they consumed the morning dose of Meta050, and blood was drawn 4 hours later. A wash-out period of approximately 7 days followed. During this time, blood was drawn for assessment of platelet function and then subjects were administered 325 mg aspirin. Blood was drawn 4 hours later for control assessment of platelet function.

No medications known to affect platelet function, such as NSAIDs or aspirin, were allowed for the duration of the trial. Fish oil capsules and Szechwan mushrooms were also not allowed for the duration of the trial. Subjects were told to continue regular supplementation, diet, and lifestyle.

Compliance with the test product and protocol were assessed by questionnaire at each visit. In addition, tablet containers were collected and tablets were counted at Visit 3.

#### ***LABORATORY AND STATISTICAL ANALYSES***

PT was calculated as International Normalized Ratio (INR), using the International Sensitivity Index (ISI) in the following relationship:

$$\text{INR} = (\text{subject's result/reference range average})^{(\text{ISI})}$$

Blood coagulation and CBC were performed by Northwest Laboratories (Tacoma, WA).

Platelet closure times were assessed using the PFA-100 system, which assesses the time to develop a platelet plug—from platelet attachment, activation, and aggregation to full occlusion of an aperture on a membrane. The platelet function is reported as “closure time.” Platelet closure times were obtained from Puget Sound Blood Center (Seattle, WA).

Statistics were performed using standard analyses on a SAS statistical package (JMP, SAS Institute). Significance was predetermined as  $p < 0.05$ .

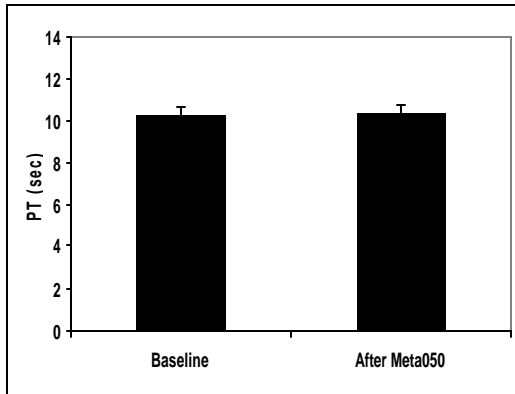
## **RESULTS**

#### ***PROTHROMBIN TIME***

The PT is a standard laboratory measure of blood coagulation. It is used in the evaluation of the extrinsic coagulation system, screening of deficiencies in factors II, V, VII, and X, and to monitor anticoagulant therapy. Because PT can be performed in a variety of ways, different laboratories may find different reference ranges. Therefore, the PT value must be interpreted with respect to the established reference range from the laboratory that performed the assay.

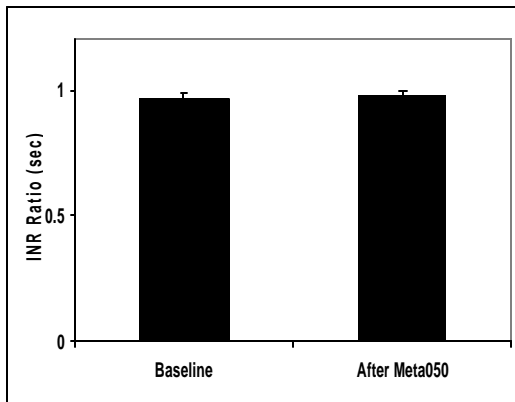
The PT value for the subjects before and after Meta050 is shown in Figure 1. No significant change ( $p = 0.504$ ) was noted between the baseline of 10.2 sec (95% CI,

9.8 to 10.6) and the value after Meta050 of 10.33 sec (95% CI, 9.9 to 10.7).



**Figure 1.** Average ( $\pm$  SEM) prothrombin time (PT) for the 6 subjects at baseline and after treatment with Meta050 (2 tablets (880 mg) bid) for 1 week. The laboratory reference range was given as 9.2-12.8 sec. The p-value between baseline and after Meta050 was 0.504 (two-tailed, paired T-test).

In order to standardize PT readings among different reporting laboratories, the INR is used. The INR for healthy individuals not on oral anticoagulant medications is 0.8 to 1.2 sec, whereas guidelines for oral anticoagulant medication suggest maintenance of an INR between 2.5 and 3.5 sec. The average INR for the 6 subjects before and after Meta050 is shown in Figure 2.

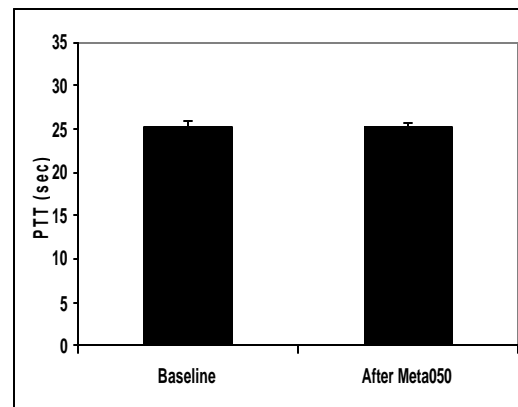


**Figure 2.** Average ( $\pm$  SEM) INR for the 6 subjects at baseline and after treatment with Meta050 (2 tablets (880 mg) bid) for 1 week. The laboratory reference range was given as 0.0-3.5 sec. The p-value between baseline and after Meta050 was 0.504 (two-tailed, paired T-test).

#### **PARTIAL THROMBEOPLASTIN TIME**

The PTT is a common screening test done to evaluate function of the intrinsic clotting system. The activated PTT reports the clotting time of plasma from the activation of factor XII through the formation of a fibrin clot. The reference range for PTT is 21-31 sec, and the therapeutic goal of anticoagulant therapy with heparin is an activated PTT of approximately 1.5 to 2.5 times this value.

A prolonged PTT in non-heparinized patients can occur due to salicylates, inherited or acquired intrinsic clotting factor deficiency or abnormality (XII, XI, IX, VII, V, II, I), massive blood replacement, hemophilia A, or excessive warfarin dosage. A decrease in PTT can occur due to digitalis, tetracyclines, antihistamines, nicotine, elevated factor VIII, tissue inflammation, or trauma. As shown in Figure 3, the PTT remained constant in the subjects from baseline (25.2 sec; 95% CI, 23.8 to 26.6) to after the Meta050 treatment (25.28 sec; 95% CI, 24.6 to 25.9).



**Figure 3.** Average ( $\pm$  SEM) partial thromboplastin time (PTT) for the 6 subjects at baseline and after treatment with Meta050 (2 tablets (880 mg) bid) for 1 week. The laboratory reference range was given as 21-31 sec. The p-value between baseline and after Meta050 was 0.93 (two-tailed, paired T-test).

### PLATELET FUNCTION

Platelet function can be assessed by the closure time assay, which determines the time to develop a platelet plug. Platelet plug formation is a complex process that involves several steps. Initially, platelets are activated by exposure to collagen, which is uncovered at sites of injury. After activation, platelets are stimulated to form a plug; two such stimuli are epinephrine and adenosine diphosphate (ADP). Aspirin inhibits plug formation after epinephrine stimulation, but not in the presence of ADP; therefore, these can be used as controls in the assay.

Table 1 shows the closure times obtained after 1 week of continuous Meta050 consumption, and 4 hours after the morning dose of Meta050 on Day 8. As shown, no significant change was observed after 1 week of Meta050 consumption with either the epinephrine or the ADP stimuli ( $p=0.7$  and  $0.55$ , respectively). In contrast, the control tests with the 4-hour aspirin challenge resulted in a significant increase in closure time with epinephrine ( $p=0.0002$ ). However, as expected, the ADP closure time was not significantly changed ( $p=0.15$ ).

**Table 1.** Assessment of platelet function with Meta050. The average ( $\pm$  SEM) closure times are shown for 6 subjects after 1 week of Meta050 (2 tablets (880 mg) bid). Platelets were activated with collagen and then stimulated to produce a plug by either epinephrine (Epi/Col) or ADP (ADP/Col).

	Closure Times (sec)	
	Epi/Col*	ADP/Col*
Baseline	107.0 $\pm$ 4.53	80.7 $\pm$ 6.25
After Meta050	104.8 $\pm$ 7.8	77.3 $\pm$ 7.2
After Wash-out	114.3 $\pm$ 6.4	76.2 $\pm$ 4.2
Aspirin Control	283.7 $\pm$ 17.3	81.7 $\pm$ 6.8

\*Reference ranges are 83-107 sec for Epi/Col closure time, and 62-104 for ADP/Col closure time.

The data with the aspirin challenge and the epinephrine and ADP control indicate integrity of the assay. In addition, Table 2 shows control values from the complete blood counts, which were also obtained during the trial.

**Table 2.** Average ( $\pm$  SEM) control blood values for total white blood cells (WBC), red blood cells (RBC), platelets (PLT), hematocrit (HCT), and hemoglobin (HGB) initially, after 8 weeks of Meta050 at 2 bid, and after a 1-week wash-out.

	Baseline	After Meta050	After Wash-out
WBC (th/mm <sup>3</sup> )	7.4 $\pm$ 0.6	5.9 $\pm$ 0.5	5.9 $\pm$ 0.8
RBC (mil/mm <sup>3</sup> )	4.5 $\pm$ 0.1	4.6 $\pm$ 0.2	4.5 $\pm$ 0.2
PLT (th/mm <sup>3</sup> )	256 $\pm$ 14	260 $\pm$ 23	247 $\pm$ 17
HCT (%)	41 $\pm$ 1	42 $\pm$ 2	41 $\pm$ 2
HGB (g/dL)	14.1 $\pm$ 0.4	14.2 $\pm$ 0.6	14.0 $\pm$ 0.6

\*Reference ranges are: WBC, 4.0-12.0 th/mm<sup>3</sup>; RBC, 4.0-6.0 mil/mm<sup>3</sup>; PLT, 150-450 th/mm<sup>3</sup>; HCT, 37-54 %; HGB, 12.0-18.0 g/dL.

### SUMMARY

We assessed the effect of Meta050 at a standard recommended dose on platelet function and blood coagulation. The data obtained from this assessment indicated no effect of Meta050 on blood coagulation or platelet function directly. These data are consistent with in vitro mechanistic studies, which have indicated Meta050 does not directly inhibit the COX enzymes, but may modulate the induction signal at sites of inflammation.<sup>6,7</sup> It should be noted, however, that these data do not rule out an affect of Meta050 on hepatic CYP450 enzymes, which can also interfere with anticoagulant therapy.

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## NOTE

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