UNIL | Université de Lausanne
Faculté de biologie et de médecine

Mémoire de Maîtrise en médecine n° 3347

Rythme circadien de la réponse plaquettaire in vitro au Ticagrelor (Brilique®) chez le volontaire sain

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Lausanne, novembre 2016

Original Research

Circadian variation of platelets inhibition with ticagrelor

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Word count (Total): 2045
Word count (Abstract): 312
Disclosure : This study was financed by AstraZeneca.

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Background

Circadian increased platelet aggregability during the early morning hours is well demonstrated but whether this has an impact on antiplatelet therapy is poorly documented.

Purpose

To observe if Ticagrelor- induced inhibition of platelet aggregation follows a circadian rhythm.

Methods

25 healthy volunteers (11 female; 14 male) were included in the study. Physical activity, food intake and sleep were standardized. Blood samples were collected every 4 hours from an antebrachial venous catheter. Ticagrelor- induced inhibition of platelet aggregation were performed in vitro avoiding thus biais regarding circadian gastro-intestinal absorption. Platelets aggregability test was then performed using VerifyNow System Platelet Reactivity Test®. Diurnal changes in platelets total count, percentage of platelets inhibition, von Willebrand antigen concentrations as well as volunteers' physiological parameters were analyzed by fitting individual data to a sine curve with a 24-h period. A circadian rhythm was deemed to be present if the amplitude of the fitted curve was significantly different from 0 (Wald test).

Results

Volunteers physiological parameters (heart frequency [bpm], systolic/diastolic blood pressure [mmHg] and body temperature [Celsius] followed a significant circadian pattern [amplitude (p-value)] of 6 bpm (<0.001), 5 mmHg/7 mmHg (<0.002) and 0.3° (<0.001) respectively. Platelets inhibition with ticagrelor was significantly lower at 01 PM (38.4%) as compared to any other time (45.2%) (p=0.018). Percentage of inhibition plotted against time followed a circadian pattern (p<0.001) with minimal and maximal values observed respectively at 01 PM and 02 AM. Similarly, von Willebrand antigen concentrations followed a circadian pattern (p<0.001) with an amplitude of 12.24 U/dL. Its concentration was maximal at 12 AM. These differences were not explained by total platelets count (minimal values in the morning).

Conclusion

Ticagrelor- induced inhibition of platelet aggregation follows a circadian rhythm with lower values achieved in the early morning. These results deserve further studies investigating the role of Ticagrelor- induced inhibition of platelet aggregation circadian variation in patients with coronary artery disease.

BACKGROUND

Large registry-based multicenter clinical studies have demonstrated that myocardial infarction incidence follows a circadian rhythm with higher number of cases occurring in the morning [1, 2]. Different factors explaining this phenomenon have been proposed such as a morning increase in catecholamine and platelet activity [3-5]. Indeed, in a study based on healthy volunteers where platelet activity at 3-hour intervals was measured, authors demonstrated a higher aggregability in the early morning [6].

Effect of aspirin intake on this intrinsic phenomenon has then been studied. In the Physicians' Health Study [4] - a randomized double-blind placebo-controlled trial of alternate-day aspirin intake – even the group treated with aspirin showed a circadian variation in onset of myocardial infarction suggesting therefore a circadian variation of aspirin absorption or a circadian variation of platelets' resistance to aspirin. Same observations have been made on patients with more powerful and more recent antiplatelet agent such as clopidogrel or prasugrel. In a study based on STEMI patients treated with aspirin and clopidogrel, authors demonstrated that platelet inhibition continued to be less strong in the midmorning hours [7]. Similarly, in a post hoc analysis of the TRITON-TIMI 38 trial [8], authors demonstrated that even with a regimen of prasugrel, the timing of stent thrombosis exhibited a significant diurnal variation with a highest incidence in the early morning [9]. However, no data exist regarding the circadian variation of platelets aggregability with ticagrelor, which is now the antiplatelet agent of choice in the context of acute coronary syndrome according to the 2012 guidelines of the European society of cardiology [10].

This study was therefore designed to assess a potential circadian variation of Ticagrelor-induced inhibition of platelet aggregation on healthy volunteers and in vitro, avoiding thus circadian variation of ticagrelor absorption [11, 12].

METHOD

Ethics and Disclosures

This study complied with the Declaration of Helsinki regarding investigations on humans and was approved by the University Hospital Center of Lausanne's Institutional Ethics Committee, Switzerland. All participants provided written informed consent. The authors had full access to the data and take responsibility for their integrity. This protocol was financed by AstraZeneca.

Trial Design

Prospective trial.

Inclusion Criteria

The 2 inclusion criteria were (1) to be older than 18 years and (2) to have signed the inform consent with discernment.

Exclusion Criteria

The study exclusion criteria included (1) anemia, (2) stage II kidney disease or more, (3) previous of cardiovascular event, cerebrovascular accident, peripheral vascular disease, chronic inflammatory, metabolic, autoimmune, rheumatic, endocrine disease or any other condition requiring daily medication.

Protocol

To investigate if the platelets inhibition with ticagrelor was circadian, 25 participants were recruited by using advertisements. An antecubital venous catheter was placed on every

participant (non-dominant arm) in order to sample every 4 hours (**Figure 1**). During the day of the experiment, standardized meals validated by a dietary team were provided at 09:00 AM, 13:00 PM and 07:00 PM. The physical activity was composed of 10 minutes' walk before and after every meal. During the rest of the day, participants were free in the hospital but forbidden to eat and to have sport activities. In order to avoid disturbance of their sleep, during the night, blood was sampling in the dark with special material (extension lines and low intensity torches). Participants were asked to be in bed at 9:00 PM and lights were off at 10:00 PM. The quality of their circadian rhythms was assesses by measures of body temperature, heart rhythm and

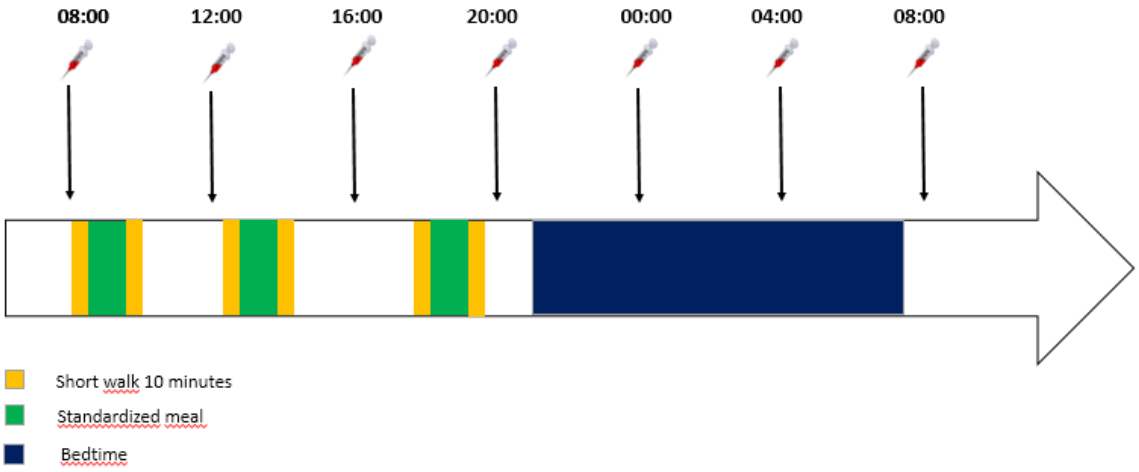


Figure 1: *Schema of the study day*

Laboratory

After every blood sample, ticagrelor was mixed in vitro with volunteer's blood according to his previously determinated IC 50. Between 30 minutes and 1 hour after acquisition, von Willebrand antigen concentrations was analyzed. Percentage of platelet inhibition (relative to a control channel containing thrombin receptor activating peptide) and platelet reaction units (PRU) were calculated using the Ultegra rapid platelet function analyser and the VerifyNow P2Y12 assay cartridges, (Accumetrics Inc, USA). According to the recommendations of the manufacturer, all samples were analyzed between 30 minutes and 2 hours after acquisition. Platelet count was measured at first blood sample for every volunteers and every 4 hours for a subgroup of 6 volunteers.

Statistical Analysis

Statistical analysis was conducted using SPSS version 20 (IBM SPSS Statistics, IBM Corporation, Armonk, New York) and figures were generated by using Graphpad Prism v.6.0 (Graphpad Software). Descriptive results were expressed as number of participants and (%), as average ± standard deviation (SD) or as median and interquartile range (IQR). To determine if arterial blood pressure, heart frequency, body temperature, percentage of platelet inhibition, von Willebrand antigen concentrations and platelet count were dependent on time of sampling, periodic sinusoidal regression analysis were performed using the following equation: $f(t) = a + b * \sin(2\pi * (t-c)/24)$ as previously proposed by Reiter et al. [13] with "a" indicating the midline estimating statistic of rhythm, a measure of the rhythm-adjusted mean value, "b" indicating the amplitude, and "c" indicating the origin of the curve. Circadian rhythm was deemed to be present if the amplitude of the fitted curve was significantly different from 0 (Wald test).

RESULTS

Baseline clinical characteristics

Among the 25 volunteers, 11 were female and 14 were male. Mean age was 24.3 +/- 2.6 years.

Physiological parameters

Body temperature, heart rate, systolic arterial blood pressure and diastolic blood pressure according to sampling time with their fitted sinusoidal regression are presented in **Figure 2**. Statistically significant circadian rhythms were found for all studied parameters (body temperature: $p<0.001$, systolic arterial blood pressure: $p<0.002$; diastolic arterial blood pressure: $p<0.001$; body temperature: $p<0.001$). Mesor/Amplitude were respectively 36.2°/0.26°; 65.18 bpm / 5.9 bpm; 118.1 mmHg/5.18 mmHg and 66.06 mmHg / 7.19 mmHg. Nadir and maximal values – based on the sinusoidal regression – were respectively observed at 5 AM/ 8 PM (body temperature); 12 AM/ 2 PM (heart frequency); 1 AM/ 2 PM (systolic arterial blood pressure) and 1 AM/ 1 PM (diastolic arterial blood pressure).

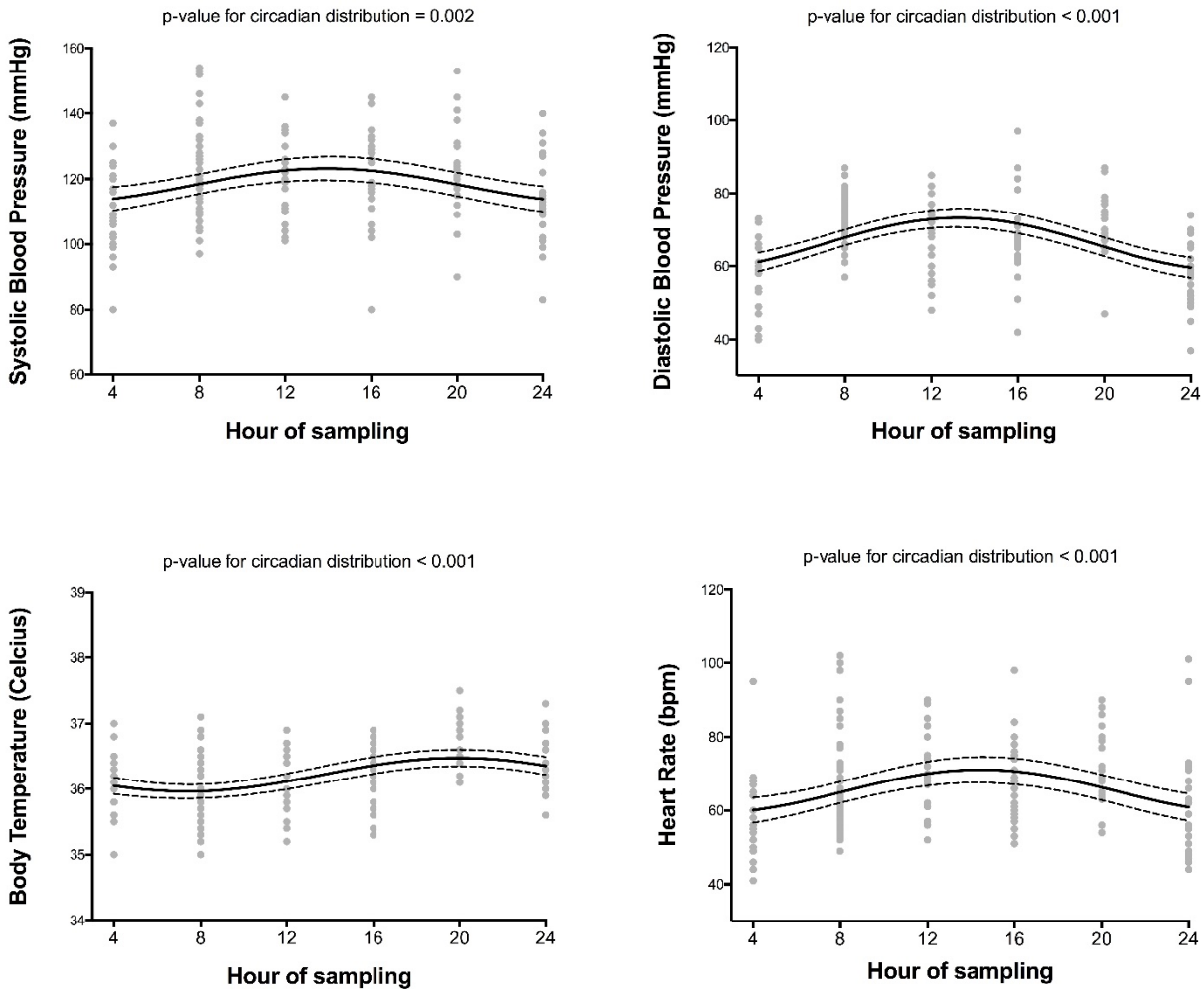
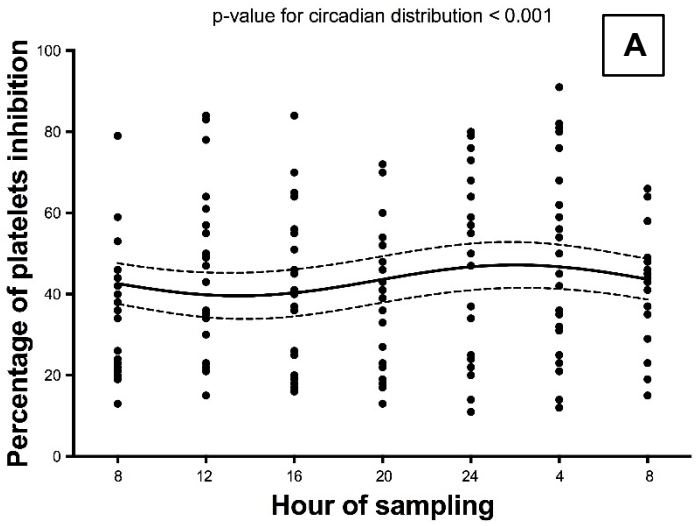


Figure 2:
Body temperature, heart rate, systolic and diastolic blood pressure according to sampling time.

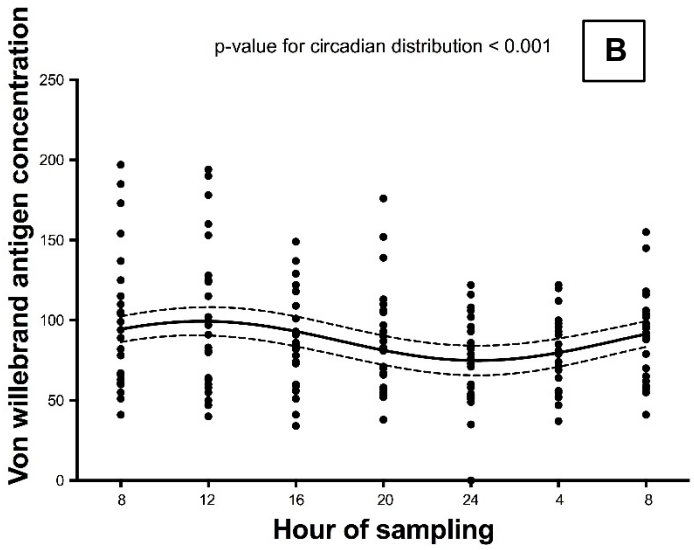
Platelets count inhibition

Percentage of platelet inhibition, von Willebrand antigen concentrations and platelet count according to sampling time with their fitted sinusoidal regression are presented in **Figure 3 A, B and C**, respectively.

Percentage of inhibition plotted against time followed a circadian pattern (p<0.001). The amplitude was 3.8% and the mesor 43.39%. Accordingly, this variation represented 8.76% between the minimum and the maximum . Based on the fitted sinusoidal regression, nadir and maximal values of percentage of platelet inhibition were observed at 01 PM and at 02 AM.



Similarly, von Willebrand antigen concentrations followed a circadian pattern (p<0.001) with an amplitude of 12.24 U/dL and a mesor of 87.12. Based on the fitted sinusoidal regression, nadir and maximal values of percentage of von Willebrand antigen were observed at 12 PM and at 12 AM.



A circadian variation of platelet count was observed (p<0.001) with an amplitude of 21.65 (G/l) and a mesor of 270.4. Nadir and maximal values of percentage of platelet count were observed at 06 AM and at 08 PM.

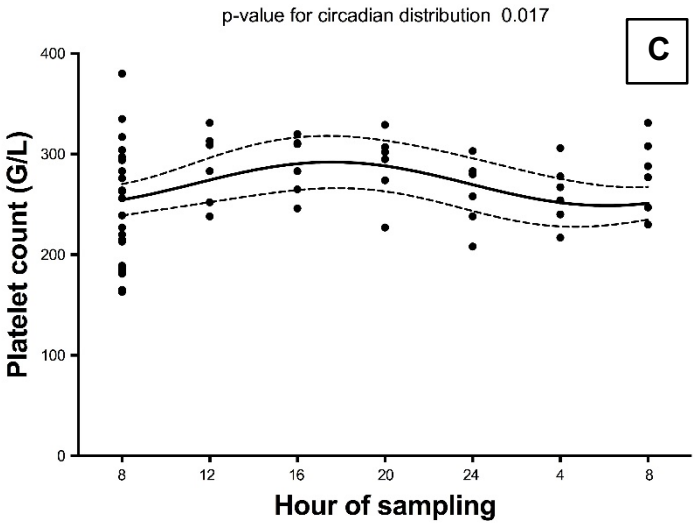


Figure 3 A, B, C: *Percentage of platelet inhibition, von Willebrand antigen and platelet count according of the sampling time.*

DISCUSSION

To the best of our knowledge, the present study demonstrates for the first time a circadian variation of Ticagrelor- induced inhibition of platelet.

Chronotherapy of antiplatelet agents such aspirin has previously been studied. In a recent study based on 133 patients that were randomized to take 100 mg aspirin on awakening or at bedtime, bedtime aspirin intake did reduce platelet reactivity during morning hours [14]. In another recent randomized open-label crossover study comprising 30 outpatients with cardiovascular disease aiming to assess platelet activity according to different timing of aspirin intake, authors also conclude that the level of platelet inhibition was statistically different when comparing morning and evening regimens [15]. However, the data of these studies do not allow to make the difference between a potential circadian variation of aspirin absorption and a circadian variation of platelet's resistance to aspirin. Furthermore, as the composition of the meals are not the same during the day, factors related to concomitant food ingestion could also lead to differences in aspirin absorption [16]. One of the unique feature of the present study is that its design exclude bias due to food interaction or due to potential circadian variations of ticagrelor absorption. Furthermore, the specific design of this study not only allows to compare 2 timing of administration, but also the efficacy of this medication at any time of the day, allowing therefore a real circadian analysis. Here, we observed that platelets aggregbility continues to exhibit its circadian rhythm even after reaction with ticagrelor.

Different authors have suggested that the existence of a morning peak of myocadial infarction frequency should be taken into account to determinate the best timing of medication administration [17]. Indeed, certain authors [15] suggest that aspirin intake in the evening might provide better protection against ischemic events. We feel that this conclusion is probably precipitated. Indeed, the differences that are observed are small, as in the present study, and at this date, no data indicate that such small differences could influence the occurrence of myocardial infarction or more importantly, impact the outcomes. Furtheremore, in contrast to aspirin or other antiplatelet agents such as clopidogrel, ticagrelor is administrated twice a day

and therefore, the question is not to determinate the timing of administration but rather to investigate if a higher morning dose would allow a more stable platelet inhibition through the day. Further research in this direction remains to be done.

LIMITATIONS

In parallel to the circadian variation of platelet agregability, different studies demonstrated that platelet count also exhibits a significant circadian rhythm [18, 19] which could be a source of bias. However, as in the present study, the highest number of platelets are observed in the afternoon and therefore, we cannot suppose that less ticagrelor was administered to the platelets in the morning. The number of subjects involved in the present study is small and the subjects are not representative of a population with a high probability of acute coronary syndrome. However, all the subjects were in good health and the robustness of this study design allowed a physiological observation without any bias.

CONCLUSION

Ticagrelor- induced inhibition of platelet aggregation follows a circadian rhythm with lower values achieved in the early morning. These results deserve further studies investigating the role of Ticagrelor- induced inhibition of platelet aggregation circadian variation in patients with coronary artery disease.

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