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Circadian Rhythm of Dihydrouracil/Uracil Ratios In Healthy Subjects With Normal And Inverted Sleep/Wake Cycle

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ABSTRACT

Many researches are full of reflections on the significant risk of toxicity of cancer patients under 5-FU chemotherapy with DPD deficiency profile. One of them demonstrated that circadian rhythm of Dihydrouracil/Uracil plasmatic ratio in healthy subjects was observed suggesting that this parameter could be a good biomarker of DPD activity circadian rhythm. However, it is not yet known if subjects with inverted sleep wake cycle keep the same circadian rhythm of DPD activity as subjects with normal sleep pattern. For this purpose, we have collected and analyzed a sample of blood from 33 healthy patients all volunteers randomly selected from the Clinic Centre of CHU Farhat Hached Sousse, Tunisia. 9 of them have an inverted sleep /wake rhythm and the others have a normal sleep pattern. We have proceeded with the chromatographic analysis using HPLC system. Data acquisition and processing were accomplished automatically by computing integrator. More important and without forgetting that human being has a biological clock that can adapt to environmental changes and adjust the body according to the desired rate. For the first time, this study showed the existence of UH2/U plasmatic ratio circadian rhythm in subjects with reversed sleep pattern compared to subjects with normal sleep. A controlled and adjusted time administration of 5-FU according to DPD circadian rhythm of subjects with inverted sleep pattern could be strongly useful for the optimization of 5-FU treatment and could help in the decrease of his side effects .

Keywords: 5-Fluorouracil, Dihydropyrimidine dehydrogenase, Dihydrouracil, Uracil, Circadian rhythm.

INTRODUCTION

Dihydropyrimidine dehydrogenase (DPD) is the first and rate-limiting enzyme in the catabolic path of the anticancer agent 5-fluorouracil (5-FU) but also the endogenous pyrimidines uracil (U) and thymine (TH). In many cancer patients, 5-FU treatment causes toxic side effects and even death in subjects suffering from complete DPD deficiency. It has been demonstrated that DPD activity shows a circadian rhythm [1] which could play an important role in the chronomodulation of the 5-FU administration and then the optimization of the therapeutic index of this drug [2,3]. In a study realized by JIANG H, a significant circadian rhythm

of the dihydrouracil/uracil (UH2/U) plasmatic ratio in healthy subjects was observed suggesting that this parameter could be a good biomarker of DPD activity circadian rhythm [4]. However, it is not yet known if subjects with an inverted sleep cycle keep a same circadian rhythm of DPD activity. In this report, the purpose was to see if DPD activity circadian rhythm of subjects with reversed sleep/wake cycle (night working subjects) keeps the same profile. This information would be useful in the chronomodulation of the 5-FU chemotherapy administration in the case of subjects with a changed sleep pattern.

This important Study was made to see if DPD activity circadian rhythm of subjects with reversed sleep/wake cycle keeps the same profile as subjects with normal sleep cycle to improve the treatment with an adjusted administration of the anti cancer drug 5-FU and probably helps to eliminate the side effects.

MATERIALS AND METHODS

Subjects and method: A total of thirty-three healthy subjects (18 females, 34.94 ± 8.39 years old; 15 males, 34.6 ± 11.52 years old) were randomly selected from the Clinical Center of CHU Farhat-Hached of Sousse (Tunisia). The volunteers were healthy without any characteristic phenotype. A written informed consent was obtained from all the studied subjects. In this study the subjects receive a Mediterranean diet qualitatively very close. In this study, 9 subjects have an inverted sleep-awake rhythm and 24 subjects have a normal sleep pattern. For all the participants sampling was performed on the same day.

Sample preparation: A 5 mL sample of whole blood was collected in an EDTA tube. Centrifugation at 3500 rpm for 15 min was performed and plasma was isolated. Ammonium sulfate (600 mg) was then added and vortex-mixed for 2 min. The addition of 3 mL of the extraction solution (isopropanol–ethyl acetate (15:85, v/v)) was then performed. The sample was vortex-mixed for 3 min and centrifuged for 20 min at 4000 rpm. Two milliliters of the organic phase were collected and evaporated to dryness at 55°C under a flow of nitrogen gas. The dried residue was dissolved in 50 µL of mobile phase (potassium phosphate buffer KH_2PO_4 0.05 M, pH = 3.2). A 5 µL of this sample extract were injected into the HPLC.

HPLC apparatus and chromatographic conditions: The chromatographic analysis was developed using a HPLC system which consisted of an Agilent® series 1200 LC system (Agilent Technologies®, Waldbronn Germany) equipped with a multi wavelength UV detector (diode array detector). Data acquisition and processing were accomplished automatically by computing integrator. Chromatographic separation was achieved by the use of two Hypersil ODS (5 µm, 250 mm x 4 mm and 5µm, 150 mm x4 mm respectively) columns maintained at 33°C and protected by a guard column containing the same packing material. The elution was carried out isocratically with a mobile phase consisting of 0.05 M potassium phosphate buffer (pH = 3.2) at a flow rate of 0.5 mL min^{-1}. For each analysis the UV detection of the UH2, U was monitored at 205 nm.

RESULTS AND DISCUSSION

As illustrated in figures 1 and 2, the plotted points on the curve are the result of an extraction mixture of 10 mL of plasma from a sample of 10 patients, with an average of 0.1 mL plasma of each one.

Circadian rhythm of UH2/U ratios levels in subjects with a normal sleep/wake cycle: The plasmatic analysis performed on healthy subjects with normal sleep pattern showed an important variation of UH2 / U ratio over 24 h illustrated in figure 1. As illustrated in table 1, UH2/U plasmatic ratio reached a minimum value of about 2. 91in the early morning (about 5: 10 am) whereas a maximal peak of 8.12 was observed early in the evening (about 6:30 pm).

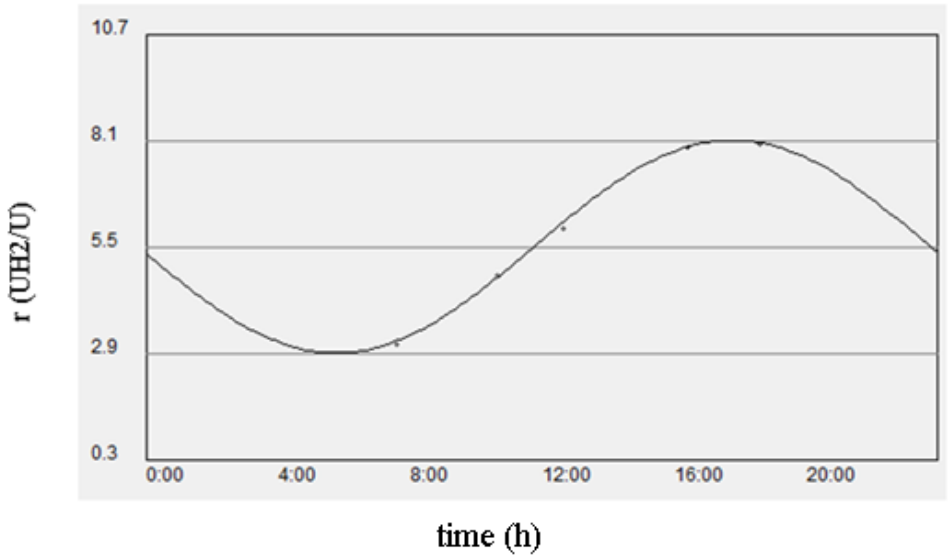

Figure 1: Circadian rhythm of UH2/U ratios in plasma of subjects with a normal sleep/wake cycle.

Circadian rhythm of UH2/U ratios levels in subjects with an inverted sleep-wake cycle: The existence of a circadian rhythm of the UH2/U plasmatic ratio in subjects with a reversed sleep patterns was confirmed here as shown in figure 2. A significant circadian rhythm variation of UH2/U ratio was obtained over 24 h. As well as subjects with a normal sleep pattern, UH2/U ratio in healthy subjects with inverted sleep-wake cycle reached minimum and maximum values as shown in table 1 with less amplitude between the minimum and maximum. Whereas, through the value obtained at 11:20 am and a peak was observed at 10:10 pm which marks a shift of six hours between the UH2/U peaks of subjects with diurnal activity and subjects with nocturnal activity.

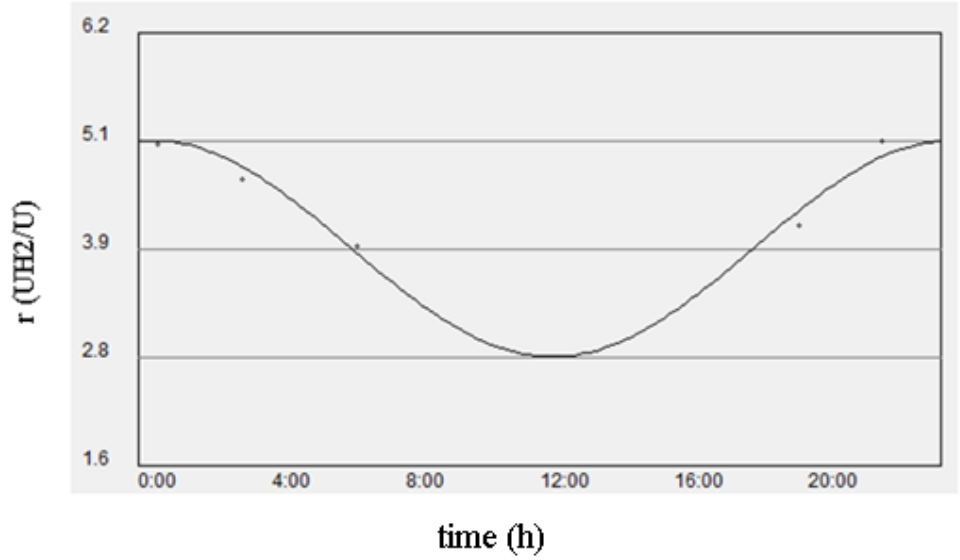

Figure 2: Circadian rhythm of UH2/U ratios in healthy subjects with inverted sleep/wake cycle.

Table 1: Analysis of UH2/U ratios variation in subjects with normal (a) and inverted (b) sleep-wake cycle

	Maximum UH2/U	Minimum UH2/U	Time max (h:min)	Time min (h:min)
(a)	8.12	2.91	18:30	5:10
(b)	5.12	2.8	22:10	11:20

5-FU is a widely used anticancer drug. It has been demonstrated that the risk of patients developing severe toxicity under 5-FU chemotherapy is significant especially in cancer patients with DPD deficiency profile. An inverted relationship was observed between the circadian profile of 5-FU plasma concentration and DPD activity [5] suggesting that the dosing times of chemotherapy influenced host tolerance and treatment effectiveness in cancer patients [6-9]. The study of Jiang H on healthy subjects with normal sleep pattern, it has been demonstrated that UH2/U plasmatic ratio displays a significant circadian rhythm consistent with DPD activity circadian pattern [4]. In this study, we have analyzed the evolution of UH2/U plasmatic ratio, as the more sensitive biomarker of DPD circadian pattern, during 24 h with subjects with normal and inverted sleep-wake cycle. The aim was to show the existence of UH2/U circadian rhythm of subjects with nocturnal activity but also show if changed sleep pattern affects the UH2/U ratio circadian pattern thus, the DPD circadian rhythm. The results obtained during this work clearly showed that, in subjects with a normal sleep-wake cycle, UH2/U plasmatic ratio presents a significant variation over 24 h with a maximum peak obtained in the evening and a minimal value observed early in the morning. These results were in agreement with the findings of Jiang H and al [4] with a slight difference in the timing of the minimal peak. Despite the difference between the two studied populations, the results obtained are similar (the organism has a low UH2/U ratio during the day). These results show that the circadian rhythm of the UH2/U plasmatic ratio is independent from the spatial-temporal context and populations genetic origins. The results also show that the biological rhythms of the organism are not randomly organized, but it is a time scheduling of many metabolic, nervous and endocrine activities. The human organism is naturally diurnal and all biological rhythms, are needed to be adjusted to face mentally and physically its daily activity. Several crucial functions such as muscle activity, heart rate or nervous system function reach their maximum performance during the day at the expense of other less important activities. Temporal organization of the organism activities according to its requirements could explain the low DPD activity during the day. In addition, a study showed that DPD could be a clock control gene which means a controlled DPD gene expression. That could explain the variation of the DPD activity over the 24 h [10].

Here, the monitoring of the evolution of the UH2/U plasmatic ratio over 24 h was performed on healthy subjects having an inverted sleep rhythm. In these subjects professionally active at night, a variation of the UH2/U over 24 h was demonstrated. A circadian rhythm of UH2/U plasmatic ratio was then confirmed. The results demonstrated that healthy subjects with a reversed sleep pattern showed that the peak marking the maximum value of UH2/U ratio was phase shifted by about three hours thirty compared with subjects with normal sleep-wake cycle. In the same group, the peak marking a minimum value of UH2/U was phase displaced by six hours which demonstrates a modification in the circadian rhythm of the UH2/U plasmatic ratio of the healthy subjects with an inverted sleep-wake pattern. Our findings were in perfect agreement with previous studies realized on night shift workers. Numerous studies demonstrated that several biological rhythms (like the circadian rhythm of the secretion of melatonin, a considerable indicator of endogenous circadian body clock statue), are modified in night workers in order to match their sleep-wake cycle [11,12]. The human organism has an internal clock which governs its rhythms depending on the light-dark cycle. It is responsible for synchronizing biological rhythms according to this cycle. This internal biological clock is responsive to any environmental variation thus detecting modification in sleep pattern generates desynchronization of biological rhythms. An internal biological clock adaptation will cause a change in the circadian rhythm of several biological functions (including DPD activity) in order to

adjust the organism biological rhythms to this modified way of life [13]. This phenomenon could explain the phase shift of maximum and minimum peaks registered in subjects with inverted sleep-wake cycle compared with the normal sleep-wake cycle group. In a study of Harris BE et al. [1], times of maximum and minimum peaks of a night shift worker patient were shifted by 12 h compared to other group members. The shift observed in this patient was more important than the shift found in this study but their study result was from only one patient which is not enough to have final conclusion. Also cancer patients treated with 5-FU have disrupted UH2/U circadian rhythm as shown in the study of Jiang H et al. [4] which could influence DPD circadian rhythm.

APPLICATIONS

In many cancer patients, 5-Fluorouracil (5-FU) treatment is toxic and even causes death. Nevertheless, all patients are subjected to a standard therapy regimen because there is no reliable way to identify beforehand those patients who are predisposed to 5-FU induced toxicity. Present study was made to see if DPD activity circadian rhythm of subjects with reversed sleep/wake cycle keeps the same profile as subjects with normal sleep cycle to improve the treatment with an adjusted administration of the anti cancer drug 5-FU and probably helps to eliminate the side effects.

CONCLUSION

In conclusion, in this study it was demonstrated for the first time, to the best of our knowledge, the existence of UH2/U plasmatic ratio circadian rhythm in subjects with reversed sleep pattern. The time maximum and minimum values was desynchronized compared to subjects with normal sleep pattern. Thus, a time adjusted administration of 5-FU according to DPD circadian rhythm of the subjects with inverted sleep pattern could be strongly useful for the optimization of the 5-FU treatment and could help in the decrease of the 5-FU side effects. Further investigations will be realized on healthy subjects with night shift rotating work to determine the statue of the UH2/U plasmatic ratio circadian rhythm. Thus a new question could be helpful when asked to the patient: are you a night worker?

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