Circadian hematotoxicity of the antiepileptic valproic acid in mice

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Summary

Antiepileptic drugs may have varying toxicity or efficacy depending on administration time. VPA administration could be associated with a great deal of hematological toxicity and can cause aplastic anemia or peripheral cytopenia affecting one or more cell lines. The objective of this study is to experimentally verify if VPA-induced hematological toxicity in mice varied according to drug administration time in the 24h scale. Different groups of mice received 620 mg/kg of VPA by i.p. route at four different circadian stages. The obtained results showed that VPA treatment induced a significant decrease in the hematological parameter rates (cytopenia) depending on the circadian time; otherwise the Cosinor analysis showed that each hematological variable followed a significant blood type circadian physiological rhythm in controls and in treated mice. The highest significant hematological toxicity illustrated by the leucopenia index and thrombocytopenia was observed in the middle of the dark-activity phase (19 HALO). The chronotherapy may play an important role to better control seizures and limit VPA treatment adverse effects. Indeed, the obtained data indicate that the optimal hematological tolerance is observed when VPA was injected in the middle of the light-rest span of mice, which is physiologically analogous to the end of the activity of diurnal phase in human patients.

Key words: valproic acid; chronotoxicity; murine; circadian rhythm; hematology
INTRODUCTION

The mammalian body is characterized by a complex time structure of biological rhythms in different frequencies which are found at all levels of living systems ranging from subcellular particles to cell and tissue cultures and to the organism as a whole (Berger 2008b). Chronobiology is the study of biological rhythms and the mechanisms of biological timekeeping. It is clearly relevant to the fields of medicine, pharmacology, and drug delivery (Smolensky and Peppas 2007, Berger 2011). The concept of homeostasis in therapy implies that the kinetics and dynamics of medications are comparable whatever the time of the day, day of menstrual cycle, and month of year of their administration. However, there are arguments about the validity of this assumption. The time with reference to circadian rhythms of drug administration can affect their outcome, sometimes markedly (Ohdo 2010).

Both the time-dependent changes in the pharmacokinetics of an agent (chronopharmacokinetics) and the time-dependent changes in the host response (chronopharmacodynamics) must be considered in the design of a chronotherapeutic treatment schedule. The timing of treatment according to the stages of a sensitivity or resistance cycle may attain optimal effects with a minimal dose of an agent and/or the desired effects with minimal toxicity (e.g., cancer chemotherapy) (Smolensky and Peppas 2007). These possibilities have been widely explored in animal experiments and have also been recently applied to clinical medicine where they are expected to gain a much wider use in the near future. Chronotherapeutics is the delivery of medications in unequal amounts over time, for example, during the 24 h scale to determine the drug-delivery pattern dose, and administration times to optimize desired and/or minimize adverse effects (Smolensky and Peppas 2007, Ohdo 2010).

The rhythmically changing physiological state of the organism determines the response to environmental stimuli like physical exercise, pain perception, mental stress, toxic substances, bacterial and viral infections, antigenic stimulation, and drugs used in clinical medicine.

Rhythm identification in animal models helps provide an optimal dosing-time and suggested guidelines to a potential chronotherapy (Ramgopal et al. 2013). The dosing of a medication at the proper biological time with reference to circadian rhythms can result in modulation of its efficiency or its toxicity as demonstrated, in particular for the anticancer agents used in human chemotherapy (Lemmer 2000, Kwiatkowski and Lévi 2003).

Epilepsy is an abnormal functional state of the central nervous system that is characterized by uncontrolled nerve cell activity and clinically by convulsive seizure with or without loss of
consciousness (Brodie 2010). The chronopharmacological studies of antiepileptic drugs are of considerable importance for optimizing therapeutic tolerance in patients and reducing these drugs’ important adverse effects (Quigg 2000, Loddenkemper et al. 2011). Valproic acid (VPA) is a branched-chain carboxylic acid similar to endogenous fatty acid in structure. It was approved for the treatment of epilepsy either as monotherapy or in combination with other anticonvulsant drugs. It is also used in the treatment of a variety of neuropsychiatric illnesses such as mania, bipolar affective disorder, migraine, headache, prophylaxis and several anxiety disorders (Perucca 2002).

Some severe side effects are associated with VPA treatment as hyperammonenemia, hepatotoxicity, thrombocytopenia, platelet aggregation leucopenia, aplastic anemia and pancreatitis (Acharya and Bussel 2000, Alluin et al. 2011). Circadian rhythmicity in physiological processes in animals has been described for a multitude of variables (Haus and Smolensky 1999, Korf and Von Gall 2006). A better understanding of the mechanisms involved in VPA toxicity should help to further optimize the chronotherapy of epilepsy. In this context the present work is aimed at investigating whether murine hematological VPA toxicity varies according to circadian dosing-time.

MATERIAL AND METHODS

Animals and synchronization
A total of 80 male Swiss albino mice aged 6 to 8 weeks and weighing 23–27 (SIPHAT, Tunisia) were used throughout this study. The animals were housed five per cage and acclimated to conditions of our animal research facility for at least 3 weeks prior to the beginning of experiments. During this period, the mice were entrained in two air-conditioned rooms specially designed for chronobiological investigations under a lighting regimen consisting of an alternation of 12 hr of light (L) and 12 hr of darkness (D) (LD 12:12). The light-dark cycle regimen was inversed between the 2 rooms (Room 1: L from 7 to 19; Room 2: L from 19 to 7) in order to allow the exploration of circadian times during the day (Lévi and Roulon 1987).

The room temperature was maintained at 22 ± 2°C and the relative humidity was about 50–60%. During all experiments, a standard diet (Purina Rat Chow; SICO, Sfax 3000, Tunisia) and water were provided ad libitum. The animals were randomly assigned to one of four
groups of 20 mice in order to explore four circadian stages (1, 7, 13 and 19 Hours After Light Onset (HALO)).

In the present study, the entrainment was assessed by the circadian rhythmicity in rectal temperature, the acrophase (peak time) was used as a marker rhythm index and the rectal temperature was measured with a digital thermometer (OMRON Ecosmart, Holt 55005).

All experiments were performed according to the guidelines of care and use of laboratory animals (Touitou et al. 2004).

Study design
The VPA solution was freshly prepared prior to each study by adding an adequate volume of sterilized physiological saline with a few drops of Tween 80. Each dose was administered intraperitonally (i.p.) to mice in a fixed fluid volume (10 ml/kg, body weight).

Twenty control mice (5 mice/time point) received sterile distilled water mixed with Tween 80. Sixty treated mice (15 mice/time point) received a single dose of 620 mg/kg, b.w. by i.p. route at either of the four circadian stages (1, 7, 13 and 19 HALO).

Blood cell counts
Taking into account the circadian injection time, the animals were carefully removed from the cage and were rapidly killed by decapitation. All sacrifices were carried out ± 10 min from the various circadian time-points. Blood was collected in EDTA-treated tubes for hematological examination at the time of sacrifice and immediately frozen on dry ice. The blood samples were taken 24 hours after injection time since the previous study showed that it corresponds to the maximum lethal toxicity (Ben-Cherif et al. 2012).

The count of red blood cells, white blood cells and platelets was performed using a Hematology Analyzer (Medonic CA 650). Hemoglobin concentrations and hematocrit rate were calculated automatically (Dridi et al. 2013).

Statistical analyses
All results were expressed as the mean values ± SD. Statistical significance was calculated by the one way-ANOVA at the significance level $2\alpha=0.05$. Animal synchronization and time series were analyzed for 24-hour rhythm with the Cosinor method based on the least-squares method (Nelson et al. 1979). A rhythm is validated by the rejection of the hypothesis whose amplitude is null. A rhythm was characterized by three parameters: the mesor (M: rhythm-
adjusted mean), the amplitude (A: half of the difference between minimum and maximum cosine function) and the acrophase (Ø: time of maximum, with light onset as phase reference) (Halberg 1969, Nelson et al. 1979). If a statistically significant rhythm was detected, the three parameters were computed with their respective 95% confidence limits (Dridi et al. 2013). The significance of both conventional and chronobiological statistics was needed to qualify temporal changes as rhythms (Refinetti et al. 2007).

RESULTS

Synchronization of mice
The rectal temperature was used as a marker of circadian synchronization of animals. In this study, a statistically significant circadian rhythm in rectal temperature (computed for the combined data of the different HALO groups) was validated by the Cosinor analysis on day 1 before treatment. The acrophase of this 24h rhythm occurred near the mid-half of the dark-activity span (Ø = 19.7 HALO ± 0.3 h). The characteristics of the 24 h pattern in rectal temperature confirmed the physiological entrainment of mice to the environmental LD schedule. Such an entrainment allows the use of the circadian acrophase of rectal temperature as a marker for the dosing time-dependent differences in VPA-associated toxicity.

VPA effect on circulating white blood cell count
The 24h-mean circulating white blood cell count dropped significantly from 7.2±1.73 10^9/l in control mice to 3.56±0.92 10^9/l in treated mice (Fig. 1).

A significant time-related effect was documented in VPA-treated mice. The highest and the lowest mean white blood cell counts were observed in the groups treated at 7 HALO (4.62±0.82 10^9/l) and at 19 HALO (2.58 ± 0.8 10^9/l) respectively.

A circadian rhythm in white blood cell count was demonstrated and statistically validated in control mice both by the t test between values at peak (8.9±1.05 10^9/l at 7 HALO) and trough (4.8±0.53 10^9/l at 13 HALO) and by the cosinor (mesor = 7.15±0.25; double amplitude = 3.7±0.7; acrophase = 3.46 HALO ± 0.726 h) (Table 1). White blood cells in treated mice also exhibited a significant circadian rhythm with an acrophase located at 5.8 HALO±0.095 h.

Because of the physiological circadian rhythm in circulating total white blood cell count, data from treated mice were also expressed as percentages of the corresponding mean time qualified control values. A statistically significant leucopenia was demonstrated in groups
treated at 1, 13 and 19 HALO. The leucopenia index was approximately twice higher in the group treated at 19 HALO (–65.41%) as compared to that injected at 7 HALO (–32.05%) (Fig. 2).

**Effect of VPA treatment on platelet count**

The 24hr-mean platelet count decreased significantly from 870.5±132.7 $10^9$/l in control animals to 528.25±85.07 $10^9$/l in treated ones. However, platelet count varied largely according to the circadian dosing-time (Fig. 3). A circadian rhythm in platelet count was demonstrated and statistically validated in control mice (mesor = 862±13.7; double amplitude = 304±38.4; acrophase = 22.11 HALO±0.485 h). VPA treatment induced an alteration of this rhythm in treated mice. Indeed, the circadian rhythm in platelet count remained present but there was a 12h shift of the circadian acrophase location (9.53 HALO±0.46 h).

Data from treated mice were also expressed as percentages of the corresponding mean time qualified control value to determine the thrombocytopenia index (Fig. 4). Thrombocytopenia varied significantly according to VPA dosing-time. It was minimal (–17.05%) in the group injected at 7 HALO [light (rest) span] and maximal (–58.44%) in the group treated at 19 HALO, and peak-trough differences were statistically significant.

**Effect of VPA treatment on red blood cell count, hemoglobin concentration and hematocrit rate**

Fig. 5 summarizes the number of red blood cells, hemoglobin concentration and hematocrit rate in controls and VPA-treated mice. One-way ANOVA indicates significant decreases in the 24hr-means of the three parameters in treated mice when compared to the control group whatever the time of VPA injection.

Table 1 recapitulates the chronobiological parameters of the different hematological variables which followed a significant circadian rhythm ($\tau = 24$) in controls and treated mice. All acrophases are located in the second half of the resting phase. VPA treatment did not alter those rhythms since there are no significant differences between the chronobiological parameters of controls and treated mice.

In controls, red blood cell counts varied significantly according to VPA injection time; the maximum (6.37±0.1 $10^{12}$/l) and the minimum (4.86±0.23 $10^{12}$/l) were located at 7 and 19 HALO respectively. VPA injection induced a significant decrease in red blood cell count. Indeed the 24hr-mean were 5.69±0.6 $10^{12}$/l and 4.65±0.7 $10^{12}$/l in treated mice and controls respectively. In treated mice, red blood cell count also exhibited a significant circadian
rhythm almost similar to that in controls. In controls, the acrophase was located at 9 HALO±0.24 h while in treated mice it was located at 8.33 HALO±0.64 h.

Hemoglobin concentration varied significantly according to VPA injection time. VPA administration induces a significant decrease in hemoglobin concentration whatever the injection time. Maximum (−21.4 %) and minimum (−13.5 %) hemoglobin decreases were observed when VPA was administered at 19 HALO and 7 HALO respectively.

Hematocrit varied significantly in controls since the lowest (0.39±0.01) and the highest (0.42 ± 0.01) rates corresponded to drug dosing at 19 and 7 HALO respectively.

VPA administration induces a significant decrease in hematocrit rate whatever the dosing-time. Indeed, the 24hr-mean hematocrit rate decreased from 0.40 ± 0.01 in controls to 0.36±.01 in treated animals. The variation of hematocrit rate according to VPA circadian dosing-time is shown in Fig. 5 (C).

**DISCUSSION**

Numerous studies on animals as well as on humans have provided convincing evidence that drugs' side effects can be modified by the circadian time and/or the timing of drug administration within 24 h of the day (Reinberg and Ashkenezi 2003, Smolensky and Peppas 2007, Berger 2008a). The choice of the most appropriate time of the day for drug administration (i.e., the circadian phase at which the drug is administered) may help to achieve rational chronotherapeutics of epilepsy (Ramgopal et al. 2013), as it was the case for other diseases such as asthma, cancer and hypertension in both experimental and clinical studies (Smolensky and D’alonzo 1988, Lemmer et al. 1993, Lemmer 2000, Kwiatkowski et al. 2005, Smolensky et al. 2007, Gery and Koeffler 2010).

VPA is a widely used antiepileptic drug and is also clinically effective as a mood stabilizer in the treatment of manic depression. However, it may induce a variety of side effects, such as bone marrow toxicity, which may lead to life-threatening complications (Kishi et al. 1994). VPA hematological toxicities are common, vary in onset and severity, are recurrent, transient, or persistent, and usually occur with high serum VPA levels. Hematological toxicity was early recognized with isolated thrombocytopenia but has become both more prevalent and more varied at higher serum concentrations needed for adequate seizure control (Acharya and Bussel 2000). A wide spectrum of hematological abnormalities can result, ranging from transient immune thrombocytopenia to leucopenia, red cell aplasia, and bone marrow failure.
Several cases of these adverse effects have been noted in the literature (Barr et al. 1982, Watts et al. 1990, Acharya and Bussel 2000, Vesta and Medina 2003, Chateaubriand 2011).

The circulating elements in the peripheral blood show highly reproducible circadian rhythms (Haus et al. 1983). As in human subjects, circadian rhythms in the number of circulating elements in the blood of laboratory animals, such as mice and rats, have been described by numerous investigators (Haus et al. 1983, Haus and Smolensky 1999, Berger 2004).

The administration of antiepileptic drugs may be associated with a great deal of hematological toxicity (Winkler and Luer 1998, Arroyo and De la Morena 2001, Perucca and Gilliam 2012). Some previous studies in VPA toxicity showed the importance of the circadian time in the experimental studies in mice. Indeed, adverse effects (mortality and weight loss) showed high values at the end of the dark active span. On the other hand, the best tolerance was achieved in the middle of the light-rest span (Ben-Cherif et al. 2012). The present work aims to investigate whether murine hematological toxicity induced by VPA i.p. administration varied according to circadian dosing-time.

As chronobiological studies require animal synchronization with L/D: 12/12 alternation, the rectal temperature was used as a marker of circadian rhythm in mice. The temperature circadian rhythm constitutes one of the most important toxicity end-points in animal experiments with reference to its reliability (Khedhaier et al. 2003, Sani et al. 2011). Like the findings of earlier reports, the peak in rectal temperature in the present study occurred almost in the middle of the dark (activity) span of mice. This peak location coincides with the highest physical activity in mice, i.e. during the dark span (Khedhaier et al. 2003, Dridi et al. 2013).

The results of the present study show some alterations in the hematological variables associated with VPA administration. All the studied hematological variables exhibited a significant circadian rhythm in control mice. The optimal hematological tolerance to VPA was achieved when this drug was given in the second half of light-rest span of mice.

Over all treatment times, VPA treatment caused a significant decrease in white blood cell number. This finding is in accordance with several studies which showed that VPA induced leucopenia (Watts et al. 1990, Vesta and Medina 2003, Rahman et al. 2009). VPA treatment induced a significant leucopenia index at 1, 13 and 19 HALO, with the highest leucopenia index being noted at 19 HALO (–65.41%). It is important here to note that the maximum of mortality observed in mice after VPA i.p. administration is also located in the middle of the dark-active span (17 HALO) (Ben-Cherif et al. 2012). The results also showed that the number of WBC in controls and treated animals followed a circadian rhythm with a peak localized at
the rest period and a trough at the active phase. In controls and treated animals the acrophase is located at 3.46 HALO±0.726 h and at 5.8 HALO±0.095 h respectively. Such rhythm characteristics in control mice were similar to those already reported by Haus et al (1983).

It is well established that the circadian variation in circulating white blood cells is consistent and highly reproducible as group phenomena (Haus et al. 1983, Haus 1996, Haus and Smolensky 1999). The numbers of circulating white blood cells in the human body show high-amplitude circadian variation with a peak localized at the time of the light-dark transition and a trough in the middle of the active period, otherwise, several studies showed that white blood cells count in nocturnal mice exhibit a circadian rhythm with a peak at the beginning of the light period; (Haus et al. 1983, Haus 1996, Ohkura et al. 2007). This was in accordance with our findings.

There are several hormones which have a significant impact on hematological characteristics. Melatonin is an internal synchronizer of circadian oscillations in mammals. The pineal gland synthesizes high levels of melatonin at the rest span (Berger 2008b). Rhythmic variations of these hormone levels play an integrative role in synchronizing various oscillations, especially in endocrine organs. Several studies confirm that melatonin administered in vivo increases leukocyte counts (Moore et al. 2000, Brennan et al. 2002).

VPA is chemically unique among anticonvulsant drugs, being a branched chain carboxylic acid, and it is structurally similar to the fatty acid constituents of cell membranes (Owens and Nemeroff 2003). The structural similarity between VPA and fatty acid constituents of cell membranes may lead to an increased incidence of immune thrombocytolysis (Mallet et al. 2004). VPA is reported as one of the drug-induced immune thrombocytopenia. Indeed, decreased platelet count is the most common and best-recognized hematological effect of VPA. Its incidence varies from 5% to 40% (Nasreddine and Beydoun 2008, Alluin et al. 2011). Thrombocytopenia associated with VPA administration may be severe. It is probably due to antibody-mediated platelet destruction (Morris et al. 1981). It was demonstrated by previous studies that VPA high doses bind to a macromolecule, producing an immunogenic structure. The antibody is directed against platelets because of their membrane fatty acids, which are chemically and configuratively similar to those of VPA (Sandler et al. 1979). Some previous studies showed that the number of platelets circulating in the peripheral blood shows a statistically highly significant circadian variation with in acrophase located in the middle of the active phase in humans (Haus et al. 1983). This is in accordance with our study which showed that in control animals, the number of platelets followed a circadian rhythm with an acrophase located at the end of the dark-active phase (Ø = 22.11 HALO±0.48h).
VPA treatment altered this rhythm and a phase shift of 12 h was noted between the acrophase of platelets number in control mice and the acrophase of platelets number in treated ones (Ø = 9.53 HALO±0.463 h). The study of variation in platelet count has revealed a significant decrease over all treatment times. The index of low platelets (thrombocytopenia) depends on VPA injection-time. However, the highest (−58.44 %) and lowest (−17.05 %) thrombocytopenia were observed in mice treated at 19 and 7 HALO respectively. This is in agreement with several other studies which have shown that VPA may induce severe thrombocytopenia (Ko et al. 2001, Zaccara et al. 2007, Vasudev et al. 2010).

Supporting an immune-mediated hypothesis, Barr et al (1982) demonstrated that 82% of thrombocytopenia cases were associated with an increased platelet-associated immunoglobulin IgG level, and that the platelet count was inversely correlated to the level of platelet-associated IgG (Sandler et al. 1978, Chong 1995, Stasi 2011). The concentrations of serum proteins that are important for immune function, including the immunoglobulins undergo circadian periodic changes. In healthy subjects, the peak concentrations of all three major classes of immunoglobulins occurred in the early to late afternoon (Haus and Smolensky 1999).

Our results have also shown that VPA administration resulted in a variation in the rates of the erythroid line parameters and caused a significant decrease in red blood cells count, hemoglobin concentration and hematocrit rate between controls and treated animals whatever the circadian time. VPA treatment caused a significant decrease in the mean values of those three parameters.

Previous studies in humans showed that the number of circulating red blood cells, hemoglobin concentration and hematocrit rate followed highly reproducible and regular but low amplitude circadian rhythms (Haus 1994). These parameters follow a circadian rhythm with nocturnal acrophases in the horses (Piccione et al. 2005).

The results obtained during the experimental period indicate the existence of a nycthemeral rhythm in these hematological variables in controls and treated mice. The circadian rhythms in hemoglobin concentration, hematocrit rate, and red blood cell number in the nocturnal mouse exhibit their acrophase nearly at the middle of the light-rest span.

Some studies are not in agreement with such results and have not revealed any circadian rhythm in the red blood cell counts. The corresponding authors supposed that it was due to the long erythrocyte life span. This life span seems to be too long to allow a detectable influence of the circadian rhythm in erythropoiesis (Berger 2004). Many other studies confirm the rhythmicity of red blood cells, and validate that circulating red blood cells in diurnally active
Humans undergo obvious circadian fluctuations with the peak at the end of the active span (light) (Haus 1983, O’Neill and Reddy 2011). The literature also confirms that the acrophase of the red blood cell rhythm is nearly at the light-dark transition in nocturnal mice (Haus 1996), and in rats (Dridi et al. 2013). Our study is in accordance with this fact and shows that the acrophase of red blood cell counts, hemoglobin concentration and hematocrit rate are located at the end of the resting phase in both controls and treated mice. VPA treatment did not alter the circadian rhythmicity of the erythroid line parameters.

VPA treatment caused a significant anemia at 19 HALO, which is in accordance with several previous studies that showed that VPA may induce aplastic anemia (The et al. 2004, Zaccara et al. 2007). Our results could underline the importance of exogenous factors, primarily light/dark cycle, on the rhythmic pattern of the hematological variables, and could suggest that their circadian pattern is under light/dark control. Previous studies on the rhythmic pattern of hematological variables in rodents showed that the acrophase of white blood cell count, red blood cell, hemoglobin concentration and hematocrit rate occurred in the light-rest span. This was confirmed by the results obtained in our study in both controls and treated mice.

Although severe hepatic damage is the best known and most feared complication of VPA therapy, hematological side effects may also have serious consequences.

The most important finding of our study is that hematological chronotoxicity is maximized when VPA is administered during nearly the end of the dark-active span and minimized when administered during the last half of the animals’ rest phase. This result is very important since VPA is a drug which is always administered during the diurnal phase in humans (activity span). It seems necessary here to remember that Swiss albino mice are nocturnal rodent, being more active and awake during the dark phase when maintained on a light/dark cycle, which is the case in our study. Nevertheless, the usual phase difference of about 12hr between circadian rhythms in diurnally active human subjects and nocturnally active rodents does not necessarily apply to all circadian periodic functions. Indications about the timing of circadian rhythms obtained in animal experiments cannot be applied to humans without qualification and will have to be reconfirmed in Man in their phase relation to environmental and social synchronizers.

In conclusion, VPA administration may be associated with a great deal of hematological toxicity (anemia, leucopenia, and thrombocytopenia) that could be predictable in time, coinciding with the time of VPA toxicity. A safe administration of antiepileptic drugs requires
therapeutic drug monitoring and chronopharmacology may limit the adverse effects of antiepileptic medications.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Adel Rdissi for proof reading this article. This work was supported by “Le ministère de l’Enseignement Supérieur et de la Recherche Scientifique”.

REFERENCES


Fig. 1. Effect of VPA (620 mg/kg i.p.) on white blood cells count of controls and treated at mice 1, 7, 13 and 19 HALO. * Statistically significant as compared with controls.

Fig. 2. Variation of leucopenia index in mice treated with VPA (620 mg/kg i.p.) at 1, 7, 13 and 19 HALO (n=15 in each circadian stage). Symbols as in Fig. 1.
Fig. 3. Effect of VPA (620 mg/kg i.p.) on the number of platelets in controls and treated mice at 1, 7, 13 and 19 HALO. Symbols as in Fig. 1.

Fig. 4. Variation of thrombocytopenia index in mice treated with VPA (620 mg/kg i.p.) at 1, 7, 13 and 19 HALO (n=15 in each circadian stage). Symbols as in Fig. 1.
Fig. 5. Effect of VPA (620 mg/kg i.p.) on red blood cells count (A) hemoglobin concentration (B) and hematocrit rate (C) in controls and treated mice at 1, 7, 13 and 19 HALO. Symbols as in Fig. 1.
Table 1. Circadian characteristics following valproic acid treatment.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Amplitude</th>
<th>Acrophase (HALO)</th>
<th>Mesor</th>
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<tr>
<td>WBC (10^9/l)</td>
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<tr>
<td>Control</td>
<td>1.85 ± 0.35</td>
<td>3.46 ± 0.726*</td>
<td>7.15 ± 0.25</td>
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<td>Treated</td>
<td>1.14 ± 0.02</td>
<td>5.8 ± 0.095*</td>
<td>3.95 ± 0.02</td>
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<td>PLT (10^9/l)</td>
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<tr>
<td>Control</td>
<td>152 ± 19.2</td>
<td>22.11 ± 0.48*</td>
<td>862 ± 13.7</td>
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<td>Treated</td>
<td>105 ± 1.28</td>
<td>9.53 ± 0.463*</td>
<td>538 ± 1.4</td>
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<td>RBC (10^{12}/l)</td>
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<tr>
<td>Control</td>
<td>0.806 ± 0.05</td>
<td>9 ± 0.24*</td>
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<td>Hg (g/l)</td>
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<td>0.016 ± 0.001</td>
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<td>0.36 ± 0.00</td>
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* Statistically significant