

Dosing-Time Dependence of Lethal Toxicity Induced by Nitroprusside in Young Mice

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Abstract

Sodium nitroprusside (SNP) is a potent vasodilator and is commonly used as an antihypertensive agent in post-operative cardiac surgical patients. In this study, we investigated whether the SNP-induced lethality is influenced by the dosing-time. Mortality was induced by administering SNP in different doses (4.9, 5.4, 6.0, 6.6 mg/kg, *i.p.*) to Swiss albino mice, 2 to 8 weeks old, synchronized for at least 2 weeks by 12 h light (rest)/12 h dark (activity) span. Each dose was administered to comparable groups of animals ($n=6$) at six different circadian times: 1, 5, 9, 13, 17, and 21 hours after light onset (HALO). Both χ^2 and cosinor methods were used to analyze the time series data. Statistically significant dosing time-dependent changes were validated in the daily scale with the males more sensitive than females. A ultradian ($\tau = 12$ h) rhythm was detected by cosinor ($P < 0.002$) for each of the three administered doses (4.9, 5.4, 6.0 mg/kg) in 2 week old mice of both genders. Moreover, in addition to the ultradian rhythm, a significant ($P < 0.004$) circadian ($\tau = 24$ h) component had been detected in 6 mg/kg SNP-treated male mice. However, there was only a circadian rhythm detected in 4 and 8 weeks old mice after acute SNP (6.0, 6.6 mg/kg) treatment, with peak time located at ≈ 21 and ≈ 17 HALO, respectively. In conclusion, tolerance to nitroprusside varies not only according to the circadian time but also according to the dose, age, and gender of animal.

Keywords: Acrophase; Age; Circadian rhythm; Mice; Ultradian rhythm; NPS, Sodium nitroprusside

Introduction

Rhythmicity is a fundamental property of all living organisms. Biological rhythms are regular and periodic incident phenomena in living organisms. They can affect sensitivity to drugs, symptoms of various diseases and many other interactions between an organism and its environment. In most rodents and other mammals, rhythms are controlled and/or regulated by the suprachiasmatic nucleus [1-3]. Circadian rhythms are an evolutionary adaptation to day/night alternations, that is, an adaptation to the environmental changes caused by the Earth's rotation. Circadian rhythms in both the desired and undesired (toxic) effects of chemical and physical agents are known [4-7] and thus, the dosing of a medication at the proper biological time with reference to circadian rhythms can result in modulation of its toxicity [8-10].

Sodium nitroprusside (SNP), the potent vasodilator [11,12], is clinically used as an excellent drug for treating cardiac emergencies and lowering blood pressure [13-17]. However, as many drugs, toxic effects of SNP have been reported [18] and ascribed to various decomposition products such as nitric oxide, cyanide (CN^-), thiocyanate and nitrite. It's generally agreed that SNP in humans and many other mammalian species undergoes *in vivo* to release free CN^- [19,20]. It has been shown in poisoned mice and rats that death after overdoses of SNP is a result of the accumulation of lethal levels of free CN^- [19]. The mechanism and site for this biotransformation reaction, however, have remained controversial. Under aerobic conditions *in vitro* SNP reacts with mammalian oxyhemoglobin (HbO_2) in solution to generate the oxidized and unreacted HbO_2 , the methemoglobin (MetHb). As a result of this reaction, the reduced SNP product is unstable and releases free CN^- . The lethal effects of CN^- have been known for more than a century and HCN, cyanide salts or cyanogenic compounds have been used in suicides, homicides and chemical warfare. Most of cyanide's

toxic effects have been attributed to histotoxic anoxia produced by reversible inhibition of cytochrome *c* oxidase, the terminal enzyme of the mitochondrial respiratory chain [21]. It's generally believed that *in vivo* the primary pathway for CN^- detoxification is its metabolic oxidation to thiocyanate mainly catalysed by the mitochondrial enzyme rhodanese [22]. However, the toxic and lethal effects of CN^- are very rapid and treatment of its poisoning needs antidotes, which act very rapidly. Thus, the release of CN^- from SNP, a potent antihypertensive medicine particularly used in hypertension crises, during the course of therapy is also life-threatening problem [23, 24].

A major aim of chronotherapy is minimizing drug-induced side effects by a better optimization of drug at the adequate time-of-day. The purpose of this study was to assess the administration-time effects on SNP-induced lethality in 2, 4, and 8 week old mice.

Materials and Methods

Animals and synchronisation

Male and female *Swiss albino* mice of different age (2, 4, and 8 weeks) were used in this study. The animals were obtained from the Central Animal House (SIPHAT, 2013 Foundouk-Choucha, Tunisia), and experiments lasted from February to July 2007. They were

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acclimatized for at least 2 weeks prior to and during each study [25], to controlled conditions of 12 h light alternating with 12 h darkness (L:D: 12:12), temperature (22 ± 2 °C), and relative humidity (50-60%). All animals were weaned at 21 days old and housed in groups of four or five in cages for each gender. During all experiments, standard food (ALMES, TN) and water were available *ad libitum*. Time series of the rectal temperature were used as marker rhythms to ensure the animals were (chrono-) physiologically healthy and synchronized to the LD entraining cycle. Time is expressed as hours after lights on (HALO). Animals of each gender and age were divided randomly into various groups of six mice, each for using at one of six different circadian stages denoted as 1, 5, 9, 13, 17, and 21 HALO. This study was conducted at the FSB Toxicometry and Chronobiometry Laboratory, Bizerte, Tunisia in compliance with current Good Laboratory Practice standards and in accordance with current guidelines [26].

Drug and lethal toxicity

Sodium nitroprusside [$\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$] brown-red powder was kindly supplied by the National Laboratory of Drug Control (1006 Tunis, Tunisia). The solution was freshly prepared each experiment day by adding an adequate volume of sterile distilled water to obtain the desired concentration. Four different doses of SNP were prepared following a geometric progression with a ratio of 1.1 between each two successive doses (4.9, 5.4, 6.0, and 6.6 mg/kg). Each mouse of each group (6mice/time point) was treated once with a single lethal intraperitoneally (i.p.) dose of SNP. Different groups of 6 mice were injected at the six pre-selected different circadian stages. Three doses (4.9, 5.4, 6.0 mg/kg) were used for 2 week old mice, whereas 4-8 weeks old mice received two doses (6.0, 6.6 mg/kg). Lethality was recorded thereafter 1 h, and the mortality rate (estimated as the percentage of dead animals) was determined for each circadian time at the end of experiment.

Statistical analysis

In this study, time-point data (% of mortality rate) were computed as mean \pm standard error of the mean (SEM) for each experiment. Differences in mortality rates were tested by the χ^2 method. Time series were analyzed by the cosinor method using trial periods (τ) of 24 and 12 h to determine the best fitting cosine function by the method of least squares [27]. For a given trial period, a rhythm is validated by the rejection of the hypothesis of which the amplitude is null (no rhythm [i.e., the rhythm's amplitude= 0]). In principle, a rhythm is detected when A is different from zero (non-null amplitude as verified by F-test of the variance accounted by the fit of the time series data to the cosine curve of a given period vs. that accounted by a straight line to time series data at $P \leq 0.05$). The rhythm is then characterized by other parameters in addition to A: the mesor (M: the 24 h rhythm-adjusted mean) and acrophase (Φ : the peak time of the cosine function, with light onset 0 HALO as Φ_0 reference). A, M, and Φ are given with their 95% confidence limits when rhythm detection is statistically significant. If the confidence limits of the acrophase for a given trial period (e.g., $\tau = 24$ h, 12 h) are greater than ± 2 h, the quantification of both acrophase and amplitude is questionable [28] because the curve pattern of the time series differs too greatly from a cosine function [29]. Therefore, the significance of both conventional and chronobiologic statistics was required to validate the temporal changes as rhythms.

Results

At each experiment, time series of rectal temperature were analyzed by cosinor test the day before treatment. This test revealed a significant

($P < 0.0001$) ultradian rhythm (with the first $\Phi = 2.8$ HALO ± 36 min) in 2 week old mice. However, the cosinor detected significant ($P < 0.0005$) circadian rhythms both in 4 and 8 weeks old mice. As expected, the acrophase (Φ) was located in the first half of the dark (activity) span ($\Phi = 15.9$ HALO ± 78 min) in young-grow-up mice (8 week old), while the Φ was detected nearly at the middle of light (rest) span ($\Phi = 5.2$ HALO ± 90 min) in 4 week old young mice. These findings in rectal temperature confirmed the physiological synchronization of animals to the environmental LD cycle at the time of the SNP experiments.

The administration of SNP to mice caused a short period of calmness followed by increased respiration and convulsions, which started 3-6 min (depending on the dose) following SNP injection. The severity of convulsions was dependent on the dose of SNP, the

Dose (mg/kg)	Gender	Period τ (h)	Mesor \pm SEM (% of lethality)	Amplitude \pm SD (% of lethality)	Acrophase \pm SD (min) ^b	P ^a
4.9	Male	12	61 \pm 5	18 \pm 17	9.0 \pm 120	0.0003
		24	61 \pm 5	6	14.6	NS
	Female	12	40 \pm 4	16 \pm 15	9.6 \pm 120	0.0004
		24	40 \pm 4	7	23.5	NS
5.4	Male	12	60 \pm 4	26 \pm 16	9.8 \pm 74	0.0004
		24	60 \pm 5	12	16.4	NS
	Female	12	60 \pm 4	28 \pm 15	10.4 \pm 60	0.0001
		24	61 \pm 4	14	1.4	NS
6.0	Male	12	69 \pm 4	20 \pm 13	9.1 \pm 79	0.001
		24	72 \pm 4	15 \pm 14	14.0 \pm 258	0.003
	Female	12	66 \pm 5	25 \pm 17	10.0 \pm 82	0.001
		24	66 \pm 5	10	1.7	NS

^aFrom an F test of the rejection of the null amplitude hypothesis.

^bTime of maximum in fitted cosine function, in HALO.

NS: Not significant

Table1: Parameters of ultradian and circadian lethality in 2 week old mice.

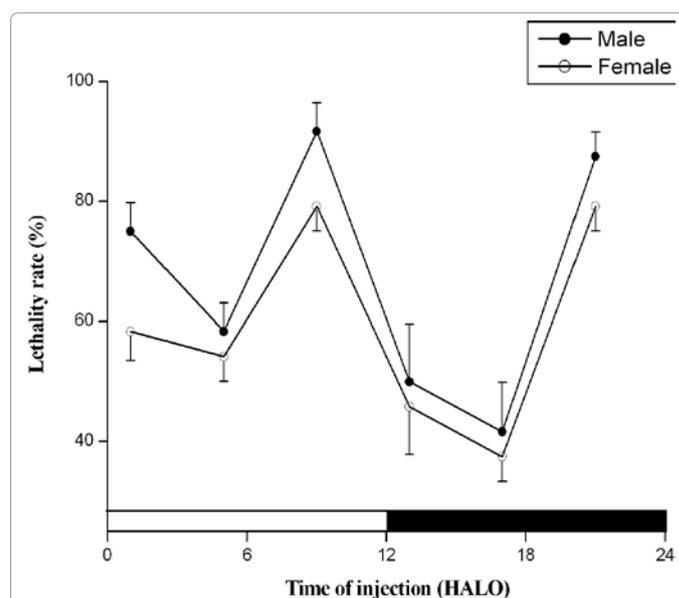


Figure 1: Variations in tolerance to sodium nitroprusside (6 mg/kg, i.p.) as assessed by the percent of mortality at each of the six circadian dosing times (1, 5, 9, 13, 17, and 21 HALO) in a total of 72 Swiss albino male and female mice aged 2 weeks. Each point represents the mean \pm SEM of four independent experiments. The black bar corresponds to the dark period. Maximums of lethality correspond, irrespective to the gender, to drug dosing at 9 and 21 HALO.

higher the dose the stronger the convulsions. After SNP administration at lethal doses, in addition to the behavioural toxicity, we observed jumps, slow respiration, diving without breathing apparatus, brief and involuntary muscular contraction, stiffness of the tail, tonic paralysis of limbs, calmness, and death. The mean survival times of these mice depend both upon the time of day of SNP administration, and upon the dose of SNP administered. As expected, the greater the SNP dose the shorter the survival time. The mice that survived to lethal doses of SNP returned to a calm state after a period (20-45 min) of convulsions, depending on the dose. The number of dead animals was recorded 1 hour following SNP treatment. The 24-h mean mortality

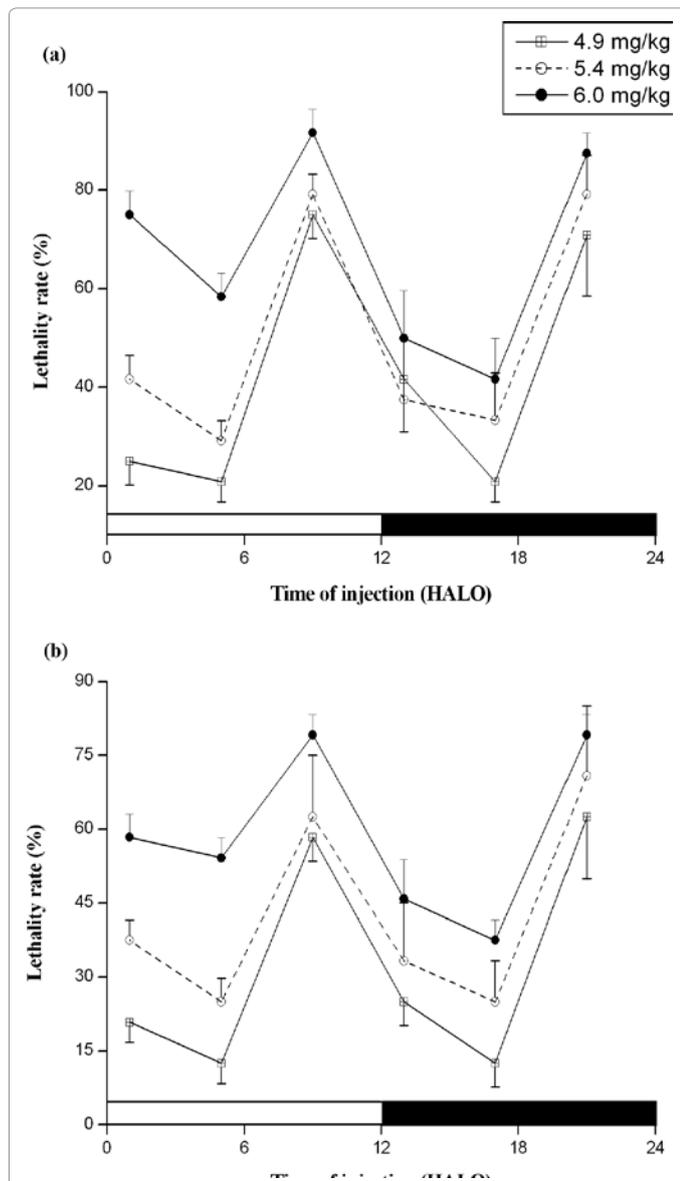


Figure 2: Variations of mortality according to the circadian stage (1, 5, 9, 13, 17, and 21 HALO) at various doses (4.9, 5.4, and 6 m/kg, *i.p.*) of sodium nitroprusside in 2 week old male (a) and female (b) mice. Each point represents the mean \pm SEM of four independent experiments. The black bar corresponds to the dark period. Maximums of lethality correspond, irrespective to the gender, to drug dosing at 9 and 21 HALO. The χ^2 test detected statistically significant differences in mortality rate related both to dosing time ($P \leq 0.0001$) and dose ($P < 0.0003$).

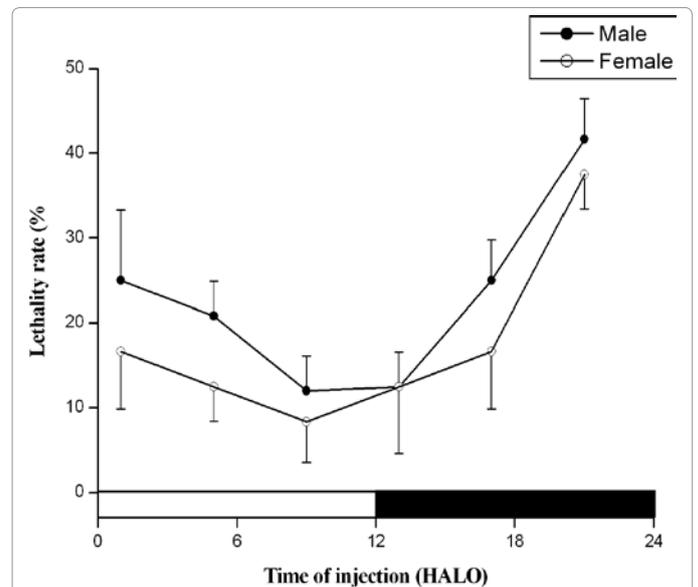


Figure 3: Variations in tolerance to sodium nitroprusside (6 mg/kg, *i.p.*) as assessed by the percent of mortality at each of the six circadian dosing times (1, 5, 9, 13, 17, and 21 HALO) in a total of 72 Swiss albino male and female mice aged 4 weeks. Each point represents the mean \pm SEM of four independent experiments. The black bar corresponds to the dark period. Best drug tolerance occurred, irrespective to the gender, when the drug was injected in the light phase (during the animal's rest span).

rate corresponds to the average of the mean values of the six dosing times. Mortality rates were dose-, gender-, and age-dependent (χ^2 test, $P < 0.01$).

SNP-induced lethality was essentially similar regardless of the circadian time of administration. There were significant (χ^2 test, $P < 0.0001$) differences between the different circadian dosing times in mortality rate. At the age of 2 weeks, statistically significant (Cosinor, $P \leq 0.001$) ultradian (12 h) rhythms were validated in male and female mice (Table 1). No rhythm was detected with the trial τ of 8 h. The first Φ_s of the 12 h rhythms (during the 24 h) occurred at $\approx 9-10.4$ HALO ± 60 min (Table 1), with the subsequent peaks (during the 24 h) occurring at 9 HALO and 21 HALO (Figures 1 and 2). In addition, the young male mice treated with SNP 6 mg/kg exhibited a statistically significant circadian ($\tau = 24$ h; $P < 0.004$) rhythm with $\Phi \approx 14.0$ HALO ± 258 min (Table 1). Survival rates were greater (χ^2 test, $P < 0.01$) in females than in males. The chronograms of mortality rate at age 4 weeks (Figures 3-4 and Table 2) showed the curve patterns of the circadian rhythms validated by both χ^2 test ($P \leq 0.0001$) and cosinor analysis ($P \leq 0.0005$). The circadian peak time was located at 21 HALO and the trough time at 9 HALO. The gender-related difference in mortality rates persisted until age 8 weeks. Figures 5-6 show the circadian rhythm patterns of lethal rate in young-grow-up mice of both genders. Peak and trough times are located at 17 and 5 HALO, respectively. Significant circadian rhythms (Table 3) were validated by χ^2 test ($P < 0.0001$) and cosinor analysis ($P \leq 0.0007$).

Discussion

Chronobiological studies require animal synchronization. This is why, in our study, we used the L/D: 12/12 alternation as a synchronizer and temperature as a marker of circadian rhythms in mice. The present study revealed the existence of ultradian rhythms of rectal temperature in 2 week young mice. The detection of prominent ultradian rhythms

in the early stages of life in mammals, including man, is actually a common phenomenon [30-32]. In fact, the permanent presence of rhythmicity in rectal temperature from the birth in young mice revealed the existence of a particular rhythm genesis concerning this endogenous marker rhythm [33]. The data also demonstrate that the postnatal development of biological rhythms in mice proceeds gradually. Food intake represents a potent synchronizing cue for biological oscillations in adult animals. When restricted to a usual daytime, food intake may rapidly synchronize some daily oscillations independent of the suprachiasmatic nucleus (SCN) [34]. Restricted feeding and wheel running can even induce circadian rhythms that are not displayed during ad libitum feeding [35]. After delivery, the mouse mother nurses her newborns periodically, predominantly in her rest time, i.e., during the day, and leaves them so that she may feed herself

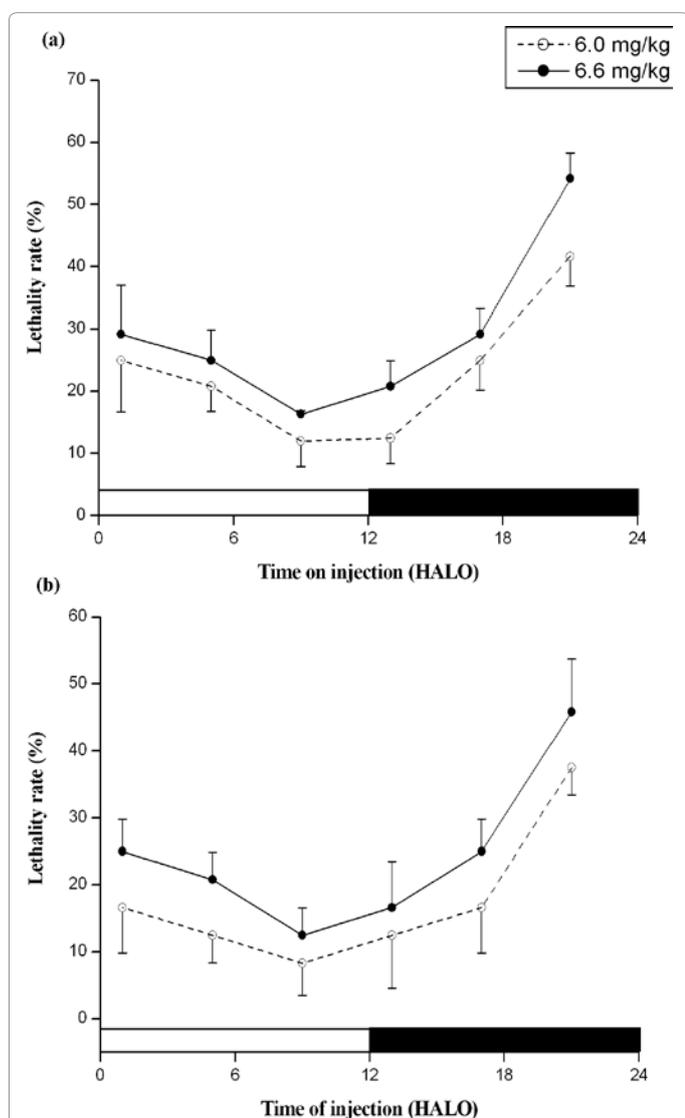


Figure 4: Variations of mortality according to the circadian stage (1, 5, 9, 13, 17, and 21 HALO) at various doses (6.0 and 6.6 mg/kg, *i.p.*) of sodium nitroprusside in 2 week old male (a) and female (b) mice. Each point represents the mean \pm SEM of four independent experiments. The black bar corresponds to the dark period. Best drug tolerance occurred, irrespective to the gender, when the drug was injected in the light phase (during the animal's rest span). The χ^2 test detected statistically significant differences in mortality rate related both to dosing time ($P < 0.0002$) and dose ($P < 0.02$).

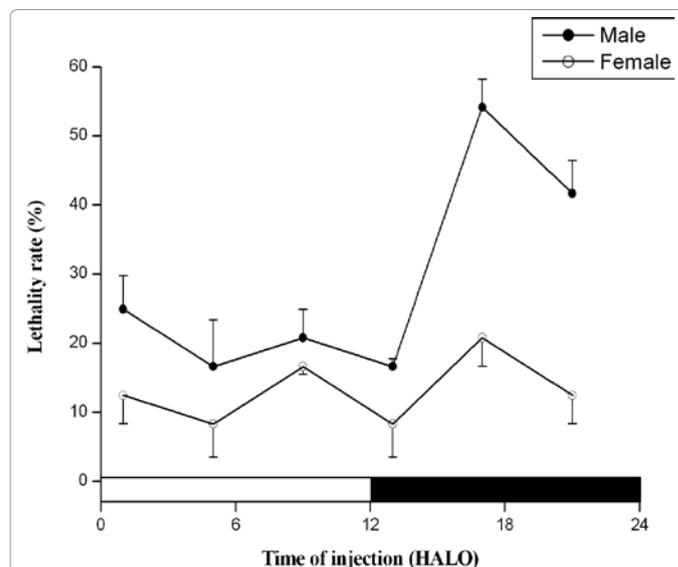


Figure 5: Variations in tolerance to sodium nitroprusside (6 mg/kg, *i.p.*) as assessed by the percent of mortality at each of the six circadian dosing times (1, 5, 9, 13, 17, and 21 HALO) in a total of 72 Swiss albino male and female mice aged 8 weeks. Each point represents the mean \pm SEM of four independent experiments. The black bar corresponds to the dark period. Best drug tolerance occurred, irrespective to the gender, when the drug was injected in the light phase (during the animal's rest span).

during the night. The feeding regimen of the pups is thus mostly in opposite phase to those of adults. The partly restricted feeding of the suckling pups to the daytime might directly entrain the biological clock. Since the second week of life, the mouse pups open their eyes and start to change their feeding habits, i.e., to consume solid food (in addition to milk) during the night hours. After the time of weaning (21 days), until 4 weeks when pups gradually become independent of maternal care, the SCN controls rhythmic ad libitum feeding that mostly takes place during nighttimes.

To the best of our knowledge, little is known about the influence of circadian time on the toxicity of SNP [36]. The toxicity of SNP and its major metabolite, cyanide (CN^-), was previously explored in non-chronobiological investigations in rodents and others mammals [37-39]. The subcutaneous administration of SNP to mice produced similar signs of toxicity described above, that were revealed by others authors when studying CN^- toxicity in mice [39-40].

The purpose of the present study was to determine whether SNP tolerance varies over the course of the day, and if so, to investigate the potential influence of the age. Here, we expose dramatic time-of-day-dependent variations in SNP-induced mortality, which are also depending on the age of animal. Our findings revealed 12 h periodicities in the toxic effects of SNP in term of lethality rate in pre-weaned 2 week old mice. In one of our recent publications we [36] reported that the neurotoxic doses of SNP induced daily variations of ataxia with two peaks in young mice of the same age. The optimal circadian tolerance for SNP in term of survival rate was at 17 HALO with regard to the raw data depicted in the chronograms. However, at the neurotoxic, but nonlethal dose levels, SNP tolerance was observed at 9 HALO [36]. The difference in phases (by 8 h) of the two curve patterns indicates that the mechanisms involved in neurotoxicity might probably differ from those bound to the SNP-induced lethality. In fact, the distribution of SNP at a low and nonlethal dose through tissues would probably be

unimportant. It has been demonstrated that the neuronal toxicity might be related to SNP-induced oxidative effects [41]. On the other hand, the distribution becomes very important at lethal doses [19]. In that case, SNP is rapidly transformed in metabolites such as NO and CN⁻. These last ones are then quickly biotransformed in the body to a much less toxic thiocyanate (SCN⁻) by the rhodanese [42, 43]. Cosinor analysis detected the presence of both a ultradian (12 h) period and circadian (24 h) component in 2 week old male mice treated with SNP 6 mg/kg. The ultradian period was found to be more prominent (i.e., had a larger amplitude and accounted for a greater percentage of variance) than the circadian period (Table 1). In addition, the large confidence limits (> 2 h) of circadian acrophase ($\Phi = 14.0 \text{ HALO} \pm 258 \text{ min}$) and raw data (Figure 1) both confirm the fact that the 12 h period is considered as the “fundamental”. The coexistence of both circadian and ultradian rhythms in 6 mg.kg⁻¹ SNP-treated male mice can be explained by their high sensitivity to cyanide as compared to females [44]. These data suggested that, among other things, both dose and animal sensitivity may also influence the waveform of drug tolerance.

At the age of 4-8 weeks, circadian variations were found in the lethality rates of SNP with similarity in both genders (Figures 3,4,5, and 6). The present results indicate that SNP chronotolerance and chronotoxicity are circadian time-dependent, though the animals were kept in a constant environment (standard regimens of lighting and temperature). These findings are consistent with other results showing that daily changes of drug toxicity and tolerance, including antihypertensive agents, are not necessarily sustained by variations of the photoperiod or thermoperiod along the day [45] and that the “biological clock” is under of genetic factors [46]. As a methodological

Dose (mg/kg)	Gender	Period τ (h)	Mesor \pm SEM (% of lethality)	Amplitude \pm SD (% of lethality)	Acrophase \pm SD (min) ^b	P ^a
6.0	Male	12	28 \pm 5	10	11.9	NS
		24	28 \pm 5	15 \pm 14	21.4 \pm 120	0.0004
	Female	12	25 \pm 4	4	8.5	NS
		24	26 \pm 4	14 \pm 13	22.5 \pm 120	0.0003
6.6	Male	12	44 \pm 4	4	0.5	NS
		24	44 \pm 4	20 \pm 13	20.9 \pm 120	0.0005
	Female	12	36 \pm 4	8	8.7	NS
		24	35 \pm 4	15 \pm 14	20.7 \pm 120	0.0003

^aFrom an F test of the rejection of the null amplitude hypothesis.

^bTime of maximum in fitted cosine function, in HALO.

NS: Not significant

Table2: Parameters of ultradian and circadian lethality in 4 week old mice.

Dose (mg/kg)	Gender	Period τ (h)	Mesor \pm SEM (% of lethality)	Amplitude \pm SD (% of lethality)	Acrophase \pm SD (min) ^b	P ^a
6.0	Male	12	23 \pm 4	6	1.8	NS
		24	25 \pm 4	13 \pm 12	18.8 \pm 120	0.0004
	Female	12	16 \pm 3	6	5.9	NS
		24	15 \pm 3	12 \pm 10	17.3 \pm 120	0.0001
6.6	Male	12	33 \pm 4	10	4.2	NS
		24	32 \pm 4	17 \pm 13	18.0 \pm 120	0.0007
	Female	12	30 \pm 4	10	4.8	NS
		24	30 \pm 4	15 \pm 14	18.7 \pm 120	0.0003

^aFrom an F test of the rejection of the null amplitude hypothesis.

^bTime of maximum in fitted cosine function, in HALO.

NS: Not significant

Table3: Parameters of ultradian and circadian lethality in 8 week old mice.

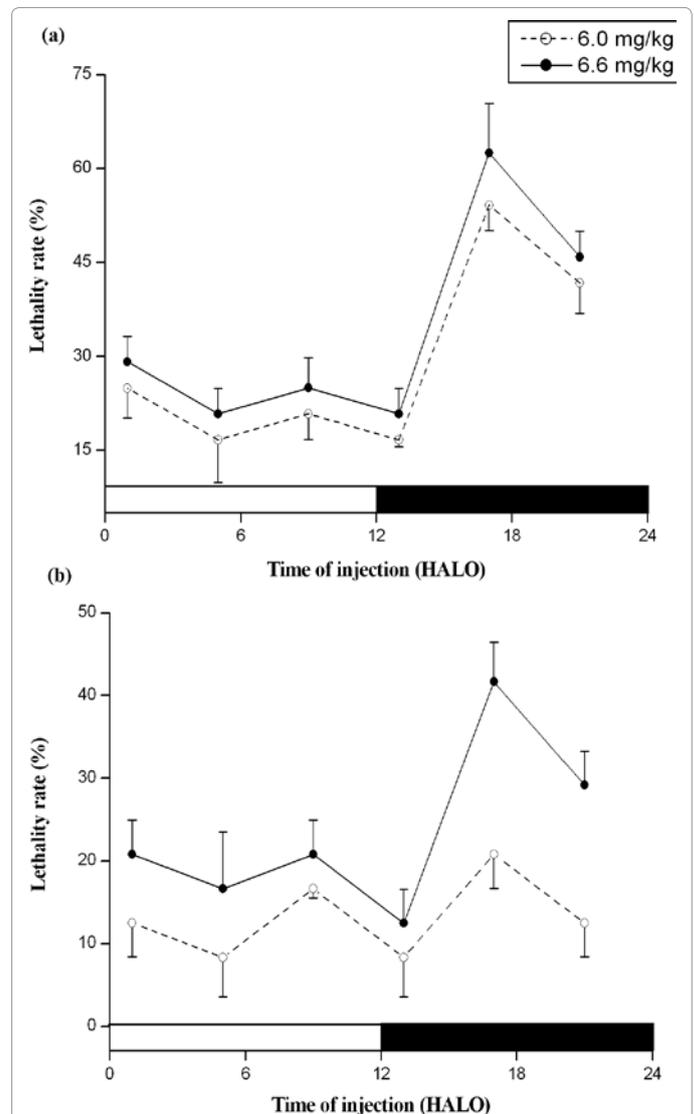


Figure 6: Variations of mortality according to the circadian stage (1, 5, 9, 13, 17, and 21 HALO) at various doses (6.0 and 6.6 m/kg, *i.p.*) of sodium nitroprusside in 2 week old male (a) and female (b) mice. Each point represents the mean \pm SEM of four independent experiments. The black bar corresponds to the dark period. Best drug tolerance occurred, irrespective to the gender, when the drug was injected in the light phase (during the animal's rest span). The χ^2 test detected statistically significant differences in mortality rate related both to dosing time ($P < 0.0001$) and dose ($P < 0.02$).

tool, constant environment conditions are used to demonstrate the endogenous origin of a rhythm [25]. The present study addressed mice of the same strain that were subjected to the same relative humidity, LD cycle, and environment temperature. Therefore, it is likely that the circadian rhythm in (chrono) tolerance of the mice to SNP reflects an endogenous mechanism presumably related to the rhythmicity in drug susceptibility of the target cells. Significant differences in the 24 h mean lethality rate were observed between 2 and 4 weeks old mice ($\chi^2 = 36,51; P < 0.0001$) and between 2 and 8 weeks old animals ($\chi^2 = 31,54; P < 0.0001$), while no statistically significant difference was observed between 4 and 8 weeks old mice. Toxic effects of SNP have been linked to cyanide poisoning [47, 48]. Cyanide gradually released is converted to thiocyanate [49]. Rhodanese, the free cyanide-specific mitochondrial enzyme catalysing this process, utilizes thiosulfate ions

as sulfur donors [50]. The high sensitivity of the young pre-weaned mice to SNP would be due to their less ability to mobilize thiosulfate stores despite increasing cyanide concentrations, leading to accelerated toxicity [51]. In contrast, normal young-weaned mice can detoxify SNP using existing (dietary provided) sulfur stores. Assuming normal rhodanese activity, onset of toxicity may be minutes to hour depending on the above-mentioned sulfur stores. However, there is considerable controversy regarding the true incidence of clinically significant cyanide toxicity. Thus, a correlation would be expected between the sensitivity of mice to lethal doses of SNP and rhodanese activity if the assertions concerning the role of this enzyme in the detoxication of CN are true. However, our previous study [52] showed that there was no correlation between the hepatic enzyme and SNP-induced mortality. These results indicate that neither hepatic rhodanese nor CN detoxification are determining in modelling of SNP-induced lethality. Therefore, a systematic search would be necessary for the true locus of that biotransformation reaction. At this stage, the question that arises is when SNP should be administered in the time scale to increase its efficiency while decreasing its toxicity.

The overall findings of this study suggest that SNP presents level dosing-time (biological rhythm) dependent lethal toxicity. This must be considered when this medication is recommended to patients to manage the toxic side effects of the drug. In conclusion, based on the significant time-of-day effect, the results of this study favour a clinical optimization through SNP chronotherapy. In addition, significant age- and gender-related variations revealed in this study suggest that, in both clinical and scientific settings, appropriate comparison of toxicity level can only be achieved based on data obtained from animals of the same age and gender.

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Declaration of Interest Statement

The authors declare that they have no competing interests.

References

- Moore RY, Eichler VB (1972) Loss of circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Res* 42: 201-206.
- Moore-Ede MC, Sulzman FM, Fuller CA (1992) *The Clock that Times Us*. Cambridge MA: Harvard University Press 448.
- Fukada Y, Okano T (2002) Circadian clock system in the pineal gland. *Mol Neurobiol* 25: 19-30.
- Granda TG, Levi F (2002) Tumor-based rhythms of anticancer efficacy in experimental models. *Chronobiol Int* 19: 21-41.
- Haus E (2002) Chronobiology of the mammalian response to ionizing radiation. Potential applications in oncology. *Chronobiol Int* 19: 77-100.
- Lemmer B (1989) *Chronopharmacology: Cellular and Biochemical Aspects of Interactions*. New York, USA, Marcel Dekker.
- Reinberg A (1992) Concepts in chronopharmacology. *Annu Rev Pharmacol Toxicol* 32: 51-66.
- Hrushesky WJM, Roemeling VR, Sothorn BR (1989) Circadian chronotherapy: from animal experiments to human cancer chemotherapy. *Chronopharmacology: Cellular and Biochemical Aspects of Interactions*. New York, USA: Marcel Dekker 439-473.
- Rich TA, Shelton CH III, Kirichenko A, Straume M (2002) Chronomodulated chemotherapy and irradiation: an idea whose time has come? *Chronobiol Int* 19: 191-205.
- Scheving LE, Tsai TH, Feuers RJ, Scheving LA, Lemmer B (1989) Cellular mechanisms involved in the action of anticancer drugs. *Chronopharmacology: Cellular and Biochemical Aspects of Interactions*. New York, USA: Marcel Dekker 317-369.
- Kreye VAW, Scriabine A (1980) *Sodium nitroprusside*. Pharmacology of antihypertensive drugs, Raven Press New York 373-396.
- Chatterjee K, Ports TA, Wilkerson RD (1981) Physiological and pharmacological basis for the use of vasodilators in heart failure. *Cardiac Pharmacology*, Academic Press, New York 149-205.
- PAGE IH, Corcoran AC, Dustan HP, Koppanyi T (1955) Cardiovascular actions of sodium nitroprusside in animals and hypertensive patients. *Circulation* 11: 188.
- Taylor TH, Styles M, Lamming AJ (1970) Sodium nitroprusside as a hypotensive agent in general anaesthesia. *Br J Anaesth* 42: 859-864.
- Eppens H. (1973). Sodium nitroprusside in hypotensive anaesthesia. *Br J Anaesth* 45: 124.
- Gifford RW Jr (1959) Current practices in general medicine, treatment of hypertensive emergencies including use of sodium nitroprusside. *Proc Staff Meet Mayo Clinic* 34: 387.
- Moffett BS, Price JF (2008) Evaluation of sodium nitroprusside toxicity in pediatric cardiac surgical patients. *Ann Pharmacoth* 42: 1600-1604.
- Smith RP (1973) Cyanate and thiocyanate: acute toxicity. *Proc Soc Exp Biol Med* 142: 1041-1044.
- Smith RP, Kruszyna H (1974) Nitroprusside produces cyanide poisoning via a reaction with haemoglobin. *J Pharmacol Exp Ther* 191: 557-63.
- Leeunwenkamp OR, Van Bennekom WP, Van Der Mark EJ, Bult A (1984) Nitroprusside, antihypertensive drug and analytical reagent, review of (photo) stability, pharmacology and analytical properties. *Pharm Weekbl Sci Ed* 6: 129-40.
- Solomonson LP, Vennesland B, Conn EE, Knowles CJ, Westley J (1981) Cyanide as a metabolic inhibitor. *Cyanide in Biology*, Academic Press, New York, 11-28.
- Himwich WA, Saunders JP (1948) Enzymatic conversion of cyanide to thiocyanate. *Am J Physiol* 153: 348-354.
- Davis DW, Griess L, Kadar D, Stewart DJ (1975) A sudden death associated with the use of sodium nitroprusside for induction of hypotension during anaesthesia. *Can Anaesth Soc J* 22: 547-52.
- Vesey CJ, Cole PV (1985) Blood cyanide and thiocyanate concentrations produced by long-term therapy with sodium nitroprusside. *Br J Anaesth* 57: 148-55.
- Reinberg A, Smolensky MH (1983) Investigative methodology for chronobiology. In: Reinberg A, Smolensky MH, eds. *Biological Rhythms and Medicine*. *Cellul Metabol Physiopathol And Pharmacol Aspects*. Springer-Verlag New York 24-46.
- Touitou Y, Portaluppi F, Smolensky MH, Rensing L (2004) Ethical principles and standards for the conduct of human and animal biological rhythm research. *Chronobiol Int* 21 : 161-70.
- Nelson W, Tong YL, Lee JK, Halberg F (1979) Methods for cosinor-rhythmometry. *Chronobiologia* 6: 305-23.
- De Prins J, Waldura J (1993) Sightseeing around the single cosinor. *Chronobiol Int* 10: 395-400.
- Reinberg A, Le Fur I, Tschacher E (1998) Problems related to circadian rhythms in human skin and their validation. *J Invest Dermatol* 111: 708-709.
- Rensing L (1965) Circadian rhythms in the course of ontogeny. In *Circadian Clocks*, Aschoff J, ed. North-Holland Publishing Co: Amsterdam 401-405.
- Le Fur I, Reinberg A, Lopez S, Mechkouri M, Tschachler E (2001) Analysis of circadian and ultradian rhythms of skin surface properties of face and forearm of healthy women. *J Invest Dermatol* 117: 718-724.
- Reinberg A (2003) *Chronobiologie Médicale, Chronothérapeutique*. Flammarion Médecine-Sciences: Paris 298.

33. Cuigini P (1993) Chronobiologie : Principes et Méthodes. *Sémiologie Médicale et Méthodologie. Annali Instituto Superiore Sanita* 29: 483-500.
34. Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, et al. (2000) Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev* 14: 2950-2961.
35. Van der Veen DR, Minh NL, Gos P, Arneric M, Gerkema MP, Schibler U (2006) Impact of behavior on central and peripheral circadian clocks in the common vole *Microtus arvalis*, a mammal with ultradian rhythms. *Proc Natl Acad Sci USA* 103: 3393-3398.
36. Sani M, Sebai H, Boughattas NA, Ben-Attia M (2011) Time-of-day dependence of neurological deficits induced by sodium nitroprusside in young mice. *J Circadian Rhythms* 9: 5.
37. Schulz V, Gross R, Pasech T, Busse J, Loeschke G (1982) Cyanide toxicity of sodium nitroprusside in therapeutic use with and without sodium thiosulfate. *Klin Wochenschr* 60: 1393-1400.
38. Niknahad H, O'Brien PJ (1996) Antidotal effect of dihydroxyacetone against cyanide toxicity in vivo. *Toxicol Appl Pharmacol* 138: 186-191.
39. Niknahad H, Ghelichkhani E (2002) Antagonism of cyanide poisoning by dihydroxyacetone. *Toxicol Lett* 132: 95-100.
40. Rutkowski JV, Boebuck BD, Smith RP (1985) Effects of protein-free diet and food deprivation on hepatic rhodanese activity, serum proteins and acute cyanide lethality in mice. *J Nutr* 115: 132-137.
41. Fukushina T, Koide M, Ago Y, Baba A, Matsuda T (2006) T-817MA, a novel neurotrophic agent, improves nitroprusside-induced mitochondrial dysfunction in cortical neurons. *Neurochem Int* 48: 124-130.
42. Buzaleh AM, Vazquez ES, Battle AM (1990) The effect of cyanide intoxication on hepatic rhodanese kinetics. *Gen Pharmacol* 21: 219-222.
43. Calabrese EJ (1983) Cyanide toxicity. In : *Principles of Animal Extrapolation*, Calabrese EJ, ed, New York: John Wiley and Sons 278-281.
44. Chanas B, Wang H, Ghanayem BI (2003) Differential metabolism of acrylonitrile to cyanide is responsible for the greater sensitivity of male vs female mice: role of CYP2E1 and epoxide hydrolases. *Toxicol Appl Pharmacol* 193: 293-302.
45. Haus E (1999) Chronobiology of the immune system. In the Committee on Military Nutrition Research, Institute of Medicine. *Military Strategies for Sustainment of Nutrition and Immune Function in the Field: Nutrition and Immune Function*. Washington DC: National Academy Press 437-496.
46. Lincoln GA, Andersson H, Hazlerigg D (2003) Clock genes and long-term regulation of prolactin secretion: Evidence for a photoperiod/circannual timer in the pars tuberalis. *J Neuroendocrinol* 15: 390-397.
47. Rindone JP, Sloane EP (1992) Cyanide toxicity from sodium nitroprusside: risks and management. *Ann Pharmacother* 26: 515-518.
48. Robin ED, McCauley R (1992) Nitroprusside-related cyanide poisoning. Time (long past due) for urgent, effective interventions. *Chest* 102: 1842-1845.
49. Lang K (1933) Die rhodanbildung im Tierkörper. *Biochem Z* 259: 243-256.
50. Ivankovich AD, Miletich DJ, Tinkerb JH (1978) Sodium nitroprusside metabolism and general considerations. *Int Anesthesiol Clin* 16: 1-29.
51. Ivankovich AD, Braverman B, Stephens TS, Shulman M, Heyman HJ (1983) Sodium thiosulfate disposition in humans: relation to sodium nitroprusside toxicity. *Anesthesiology* 58: 11-17.
52. Sani M, Gadacha W, Boughattas NA, Reinberg A, Ben-Attia M (2006) Circadian and ultradian (12 h) rhythms of hepatic thiosulfurtransferase (rhodanese) activity in mice during the first two months of life. *Chronobiol Int* 23: 551-563.