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Original article

Circadian-time dependent tolerance and haematological toxicity to isoniazid in murine

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ABSTRACT

Introduction: Isoniazid (INH) is a widely used drug in the prophylaxis and treatment of tuberculosis. In the present study, isoniazid (INH)-induced toxicity was investigated according to the dosing-time in the 24-h scale in mice.
Methods: Two studies were carried out on a total of 180 male Swiss mice synchronized for 3 weeks to 12-hour light (rest) and 12-hour dark (activity) cycle (L/D: 12/12). In the first study a potentially lethal dose of INH (180 mg/kg) was administered by intraperitoneal (*i.p.*) route at six different circadian-times: 1, 5, 9, 13, 17 and 21 hours after light onset (HALO). In the second one, a sublethal dose (120 mg/kg) was administered at three circadian-times (1, 9 and 17 HALO) in order to evaluate the variation of haematological toxicity. Rectal temperature, body weight loss, survival (study 1) and complete cell count (study 2) were determined as toxicity endpoints. The Cosinor and ANOVA methods were used for the data statistical analysis.
Results: The Cosinor analysis of rectal temperature time series prior to treatment validated a circadian rhythm, which demonstrates that mice were well synchronized. Following INH injection, rectal temperature increased in all the six circadian stages at days 2 and 3. Body weight loss varied from −12% at 1 HALO to −7% at 13 HALO ($P < 0.001$). The 24-h mean of mortality induced by INH was 38%. Such lethal toxicity varied according to the circadian dosing-time. Maximum (60%) and minimum (20%) survival rates were observed when INH was administered at 9 and 1 HALO respectively. The highest survival time (25 days) occurred when INH was injected at 9 HALO while the lowest survival time (7 days) occurred when INH was given at 1 HALO. The decrease of haematological variables (cytopenia) was dependent on the circadian dosing-time ($P < 0.001$). The least haematological toxicity illustrated by leukopenia index, anaemia and thrombocytopenia was observed in the middle of the second half of the light-rest phase (9 HALO).

1. Abbreviations

INH	isoniazid
L	light
D	dark
HALO	hours after light onset
SCN	suprachiasmatic nucleus
LD_{50}	50% lethal dose
NaCl	sodium chloride
EDTA	ethylenediaminetetraacetic acid

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RBC red blood cells
WBC white blood cells
PLT platelets
HGB haemoglobin
HCT haematocrit
M mesor
A amplitude
ϕ acrophase

2. Introduction

Chronobiology investigates the biological rhythms that are involved in the organization of living organisms [1]. Biological rhythms consist of variations of biological phenomena that are periodic and foreseeable in time [2]. The temporal variations in cycles of light-dark, rest-activity, fasting-eating, and other environmental conditions, defined as synchronizers, give the organism temporal markers and thus impose their period on these biological rhythms. Circadian rhythms (with a period Γ = 24 h) are present in neuronal and non-neuronal cells. The suprachiasmatic nucleus (SCN) of the mammalian hypothalamus is one of the major central pacemakers or "master clocks" capable of synchronizing the circadian rhythms of the major physiological functions [3]. The circadian rhythms of the haemato-immune system seem to be synchronized by two clocks, the first of which is endogenous, based on clock gene activity in the suprachiasmatic nucleus. The second one is exogenous based on environmental immune stimuli [4]. The circadian changes in circulating blood cells have been recognized in various species and age [5]. The circadian rhythms in the number of mammalian leucocytes can reflect oscillations in haematopoietic proliferation activity in the bone marrow [6]. The circadian rhythmicity of leucocytes, erythrocytes and erythroprotein is influenced by the different genetic backgrounds. The periodic changes in the number of peripheral blood cells can influence their functions. The fact that the autonomous nervous system and the neuroendocrine system have been shown to modulate leucocyte physiology supports the concept that circadian timing is an important aspect of the hypothalamo-immune communication [7]. The circadian-time structure has been studied and showed a great importance for clinical practice and pharmacotherapeutics. Taking into account the circadian rhythms for medical treatment by choosing the right time of the day for drug administration is called chronotherapy. Consequently, drug efficacy can be optimized and/or toxicity can be reduced. Chronopharmacology was investigated with several drugs such as anticancer agents and allowed to realise chronotherapy protocols [8]. *Mycobacterium tuberculosis*-induced tuberculosis remains one of the world's leading infectious causes of death in developing countries; about 80% of the population in many Asian and African countries compared to only 5–10% of the US population [9]. Isoniazid, known as INH (isonicotinylhydrazide), is one of the most effective antituberculosis drugs discovered in 1952 [10]. It is an organic compound, which is used as the first line medication in the prevention and treatment of tuberculosis [11]. INH, often associated with other antituberculosis drugs (rifampicin, pyrazinamid, ethambutol), is the basic chemotherapy approach to clinical tuberculosis control. However, the treatment of tuberculosis continues to be a problem in patients who do not tolerate these drugs if serious side effects do occur and this treatment might present a higher risk of failure and relapse [12]. One of the most common adverse effects of INH is peripheral neuropathy [13] and hepatotoxicity [14]. The latter can be related to the metabolite monoacetyl hydrazine. In addition, the central nervous system effects such as dysarthrea, seizures and dysphobia have been reported with the use of INH. Other adverse effects are hypersensitivity reactions, haemolytic anaemia, arthralgia and thrombocytopenia [15]. Subsequently, the differences in INH toxicity were attributed to genetic variability in N-acetyltransferase, a cytosolic phase II conjugation enzyme primarily responsible for INH deactivation [16].

Chronopharmacology was investigated in order to reduce INH toxicity in patients. The present study was conducted to evaluate whether INH-induced toxicity varies according to drug dosing-time. Three endpoints of INH toxicity were investigated: rectal temperature, body weight change and survival (study 1) and haematological toxicity (study 2).

3. Material and methods

3.1. Animals and synchronization

Two studies were carried out on a total of 180 male Swiss Albinos mice aged 8 to 10 weeks from October to December 2014. The animals were purchased from the Société des industries pharmaceutiques de Tunisie (SIPHAT, Tunis, Tunisia). They were housed 5 per cage and synchronized at 12 h light and 12 h dark regimen (L/D: 12/12) for 3 weeks. The temperature and humidity in the room were respectively 23 ± 1 °C and 60 ± 10%. Food and water were available ad libitum [17]. The rectal temperature was used as a circadian rhythm marker for synchronization in mice. All experiments were performed according to the guidelines of care and use of laboratory animals [18].

3.2. Drug

Isoniazid is a white crystalline powder. It was provided by laboratories Pharmascience (Montreal, Canada). It was freshly prepared prior to each study by adding an adequate volume of sterilized physiological saline. The used doses for study 1 and study 2 were respectively 180 and 120 mg/kg. Each dose was administered to mice by *i.p.* route in a fixed fluid volume (10 ml/kg, body weight).

3.3. Study design

In study 1, controls and treated animals were divided into six groups, which corresponded to the 6 circadian dosing-times (1, 5, 9, 13, 17 and 21 hours after light onset [HALO]). The used 50% lethal dose (LD_{50}) of INH was 180 mg/kg b.w. previously determined by the Miller and Tainter method. This dose was injected in mice by *i.p.* route at each of the six circadian stages (15 mice/circadian-time). The controls (5 mice/circadian-time) received a sterile NaCl 0.9%. In study 2, a total of 60 male mice were treated at 3 different circadian stages (1, 9, 17 HALO). Forty-five mice (15 mice/circadian-time) received a sublethal dose (120 mg/kg) and 15 controls received a sterile NaCl 0.9% (5 mice/circadian-time; Table 1).

In the first study, three toxicity endpoints were investigated: rectal temperature, body weight change and survival for 40 days. Mortality was recorded daily. Rectal temperature was measured with a digital thermometer (OMRON Ecosmart, Holt 55005). Body weight was measured by a high-precision balance (RAD-WAG, WPS 360/C/2). Body weight loss was computed as the percentage relative to the initial body weight prior to treatment.

3.4. Blood cell count

In the second study, blood samples were withdrawn 24 hours following INH injection in both treated mice and controls. All blood samples were collected under anaesthesia by cardiac puncture and