**It’s all about timing -**

**Impact of the circadian clock on the development of a novel anti-cancer drug candidate**

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**T**he announcement that JC Hall, M Rosbash and MW Young were awarded the Nobel Prize in Physiology and Medicine for their discovery of the mechanism of the circadian clock triggered high interest in the possible relevance of biological rhythms for sleep disorders, and mental health, as well as neurodegenerative, metabolic and malignant diseases. However, less attention has been paid to the impact of the circadian clock for pharmacology and drug development. This is especially important for anticancer medications, whose attrition rate exceeds 85%.

**Purpose:**  To illustrate the often overlooked role of the circadian clock in pharmacology, I undertook *in vitro* and *in vivo* experiments in order to determine whether circadian timing would alter the pharmacology and toxicity of FY26, a novel osmium-based anticancer candidate developed by P. Sadler at Warwick University.

**Methods:** A first issue was the determination of the most physiological method to reset and synchronise the circadian clocks of cells in culture. First, 50 000 murine heaptocarcinoma cells (Hepa1-6, ATCC® CRL-1830™), transfected with the clock gene Per2*-luc* reporter construct, were subjected to 24 h temperature schedules starting at 37°C for 12 h, from *Time 0* (T0) to T12, then decreasing to 32°C for another 12 h, from T12 to T24/0) a step followed by alternating 12-h plateaus at 37°C and 36°C. The effect of FY26 timing was assessed by determining the circadian changes of half of the inhibitory concentration (IC50) following exposure to 16 µM, 12 µM, 10 µM, 8 µM, 6 µM, 4 µM, 2 µM, 1 µM and 0.5 µM FY26 at one of four circadian times, 6 h apart. Using the same synchronisation method, the possible time-dependent effect of 1 µM of FY26 on the circadian clock was investigated through continuous recording of bioluminescence in the *Per2-luc* reporter construct using the LumiCycle technology, for up to 6 days. *In vivo* correlates were assessed through the administration of a single intraperitoneal injection of 50 mg/kg of FY26 at one of six dosing times 4 h apart to 9 to 10 weeks old C57Bl/6 male mice, whose circadian clocks were synchronised with 12:12 h alternating light dark cycles for 3 weeks. Light onset and offset were defined as *Zeitgeber Time 0* (ZT0) and ZT12, respectively of which *Zeitgeber* is defined as an environmental rhythmic cue e.g. light with which has entraining properties.

**Results**: Both *in vitro* experiments demonstrated best tolerability of FY26 at T16 (4 h after temperature switching from 36°C to 37°C) with an IC50 of 9.6 µM ± 0.1 µM, as compared to 12.3 µM ± 0.5 µM after FY26 exposure at T22 (2 h before temperature switching from 36°C to 37°C (p<0.001).

In vivo experiments revealed that FY26-induced body weight loss, a toxicity marker ranged from 5.1% ± 1.1% following drug dosing at ZT6, near the middle of the rest phase of the mice, as compared to 9% ± 0.9% alter treatment at ZT18 or 22, in the second half of activity phase of the animals (p<0.001).

**Conclusions:** The *in vitro* and *in vivo* experiments clearly demonstrated changes of the tolerability of FY26 which varied significantly both in cells and in mice according to the time of administration. Taken together, the data suggested that the osmium complex could be least toxic following dosing in the early-to-mid rest spans, which could correspond to the first part of the night in humans. Ongoing studies aim at the determination of chronopharmacokinetics/pharmacodynamics mechanisms, the relations of chronotoxicity with anticancer efficacy, and possible sex-related differences.

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