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Effective Cancer Treatments Follow the Clock

Oncologists have long thought that cancer treatments tend to be more effective at certain times of day. But they have been unable to turn this knowledge into practice, because they did not understand the phenomenon well enough. Now, researchers have discovered a molecular mechanism that explains why sensitivity to anti-cancer drugs changes with the clock. They said their findings could lead to new drug treatments that may be more effective because they harness the power and precision of the body's internal clock.

The research team, which included senior author Joseph S. Takahashi, a Howard Hughes Medical Institute investigator at Northwestern University, and senior author Marina P. Antoch at the Cleveland Clinic Lerner Research Institute in Cleveland, Ohio, published its findings February 1, 2005, in the early online edition of the *Proceedings of the National Academy of Sciences*.

In experiments, which were conducted in mice, the scientists found that the body's internal biological clock affects the survival of immune cells that are targets of the anti-cancer drug cyclophosphamide (CY).

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- Joseph S. Takahashi

"We became interested in examining this issue because there is a long history of knowledge that chemotherapeutic agents produce different mortality and morbidity at different times of the day," said Takahashi. The initial experiments with normal mice, performed by Antoch during her tenure in Takahashi's lab, confirmed that animals treated with CY survived better when they received treatment in late afternoon than those whose treatments were initiated early in the morning. Antoch further extended these original findings

after she moved to Cleveland and established her research program in the Department of Cancer Biology at the Cleveland Clinic Foundation.

To examine the mechanism for this difference, Antoch and her colleagues used mice that genetically lack different components of the body's internal clock. "Knowing the molecular mechanism of internal clock function lets us make some important predictions of how these mice may respond to drug treatment," said Antoch. "Thus, defects in *Clock* or *Bmal1* genes, which essentially damp the cycles of the internal clock may produce very different effect when compared to defects in *Cryptochrome* gene, which, in contrast, 'jams' the circadian clock at the most active point in its cycle."

Biological clocks function in the brain as well as lung, liver, heart and skeletal muscles. They operate on a 24-hour, circadian (Latin for "about a day") cycle that governs functions like sleeping and waking, rest and activity, fluid balance, body temperature, cardiac output, oxygen consumption and endocrine gland secretion.

In their experiments, the researchers measured the animals' body weight as an indicator of response to the anti-cancer drug. They discovered that *Clock*-mutant and *Bmal1*-knockout mice showed high sensitivity to the drug at any time it was administered—as if the drug were administered early in the morning or late at night. In contrast, the *Cryptochrome* knockout mice showed more resistance to the drug at all times than did normal mice.

The researchers then tested whether this effect might be due to differences in the metabolic activation of the anti-cancer drug, but found essentially none. "This was a real surprise, because some of the enzymes involved in activating CY in the liver show circadian rhythms," said Takahashi. "We thought that the liver might be activating the drug more strongly at some times, or detoxifying it less effectively, or both."

However, when the researchers analyzed the activity of the knockout animals' immune system B cells, they found evidence that the activity of the *Clock* and *Bmal1* genes determined the cells' sensitivity to CY.

"Thus, this paper gives us specific mechanistic insight into the role of circadian rhythms in sensitivity to such drugs," said Takahashi. "This is not some vague metabolic difference between day and night. This is a tangible difference in the immune system that influences sensitivity."

The findings may well extend to the effects of other anti-cancer drugs, as well as to radiation therapy and may provide a rationale for adjusting the timing of chemotherapy to make it less toxic. "There is one more very important clinical application of these findings," Antoch said, "as they provide a rationale for developing drugs that can enhance the therapeutic index through the modulation of the circadian clock. We have already started screening sets of chemical compounds for their ability to affect this function.

We are also planning additional studies to discover the molecular signals from the circadian machinery to the immune system that might prove to be useful drug targets.”