Emerging science of chronotherapy offers big opportunities to optimize drug delivery

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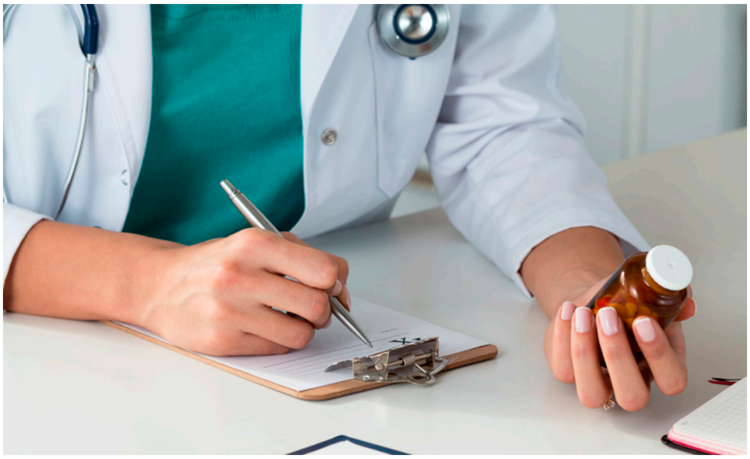
A growing number of biomedical researchers advocate a simple way to improve the effectiveness of medications: administer them based on what studies say is the ideal time. "Almost every drug that's out there probably could be optimized in terms of the time of day it's delivered," says Erik Herzog, a biologist at Washington University in St Louis, MO. It's an approach called chronotherapy, and if Herzog and others are right, the drug treatment implications could be profound.

The activities of the human body, from metabolism to gene expression, don't run at the same speed or intensity throughout the day. Biochemical reaction rates cycle through peaks and low troughs, driven in large part by the body's internal clock as well as individual clocks in different tissues. Buoyed by recent findings, the notion of timing drug treatments for maximum effect is gaining acceptance—even while various nuances of the approach are the focus of continued study. Chronotherapy is not a panacea and isn't applicable for all medications or ailments. But it may offer an important avenue for honing and even improving many therapies.

Finding a Rhythm

Chronotherapy (sometimes called *chronomedicine*) dates back to the 1970s when researchers noticed how mice with cancer responded better to treatment given in decreasing doses over a 24-hour cycle (1). Further experiments in animals showed circadian cycles were important in other diseases too (2). In the past decade, molecular techniques have revealed the mechanism. Triggered by changes in external light, the activity of key genes runs on a daily cycle and so does the physiology they influence (3).

Take heart disease. In a study published last year in *The Lancet*, researchers in France found that patients who have heart surgery in the afternoon are less likely to suffer complications, such as tissue damage, than are those who have the same operation in the morning (4). Analysis of biopsy samples revealed that a circadian rhythm correlated with how well the patients' heart cells could survive the temporary loss—and then restoration—of oxygen during the surgery. The researchers traced this effect to the cyclical activity of a protein called Rev-Erbα, expressed levels of which were high in the morning and low in the afternoon.



Many drug therapies could be better tailored to patients by understanding the times of day the drugs are most effective, a field of research called chronotherapy or chronomedicine. Image credit: Shutterstock/Idutko.

Rev-Erbα inhibits the transcription of another protein, CDKN1a/p21, which has been shown to protect heart cells in animal studies by preventing apoptosis, or cell death. The more of that protective protein there is around in the patients' hearts, as in the afternoon, the less likely the trauma of surgery is to kill cells off.

Many of the 200 million people around the world who take daily statins to reduce levels of cholesterol already benefit from chronotherapy—as long as they take their medication in the evening as directed. That's because levels of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase that statins work against are known to peak in the nighttime hours; it makes sense to synchronize delivery of the drug to the abundance of its target. It likely makes sense for other drugs as well. But the benefits are yet to be proven in trials. That's partly because medical systems don't generally prioritize dosing time.

"It stands to reason that if you give the drug when there's not a lot of the target around then it's not going to do anything," says John Hogenesch, a

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chronobiologist at Cincinnati Children's Hospital Medical Center in OH. Given that about 80% of approved drugs in the United States hit targets known to rise and fall according to predictable circadian rhythms, that's both a problem and an opportunity.

But the chances are slim that a new drug will join statins and the handful of other approved medicines that are optimized to circadian cycles any time soon. Just one is currently being tested in a US clinical trial—by Herzog and his colleagues as a way to better treat a rare brain cancer called glioblastoma. And there's not much incentive for drug companies to investigate what time of day optimizes existing medicines because better timing won't increase sales. In fact, the opposite could be true: one goal of chronotherapy is to find a way to reduce the amount of some drugs given to patients, especially those that carry unpleasant side effects.

Better Measures

Still, the emerging findings are hard to ignore. Herzog's team has now tested their chronotherapeutic approach on about 30 patients and want to recruit another 20 before they report the findings next year. The early results, he says, suggest that asking the cancer patients to take their pills in the morning rather than the evening spurs significant improvement. That's because the researchers have found a cycle of time-sensitivity within the tumor tissue itself, specifically in rates of apoptosis in cancer cells that have their DNA disrupted by the drug temozolomide. "At some times of the day it looks like the drug does almost nothing," Herzog says.

> "We're very likely undertreating pain in the afternoon and even overtreating it in the morning."
> —John Hogenesch

Researchers can help advance chronotherapy approaches, says chronotherapy researcher Amita Sehgal at the University of Pennsylvania in Philadelphia, by adding the time of day to the variables they measure and record routinely whenever they perform an experiment or a clinical study. Not doing so may even explain why some results don't seem to replicate. In one noted example from the early 1990s, researchers at Columbia University in New York working on the signal transduction pathway cAMP in mutant *Drosophila* fruit flies showed the outcome of experiments could differ if data were gathered during the day or night because cAMP levels fluctuated (5). Measuring during more typical daytime hours led to misleading findings.

Another big problem, Hogenesch says, is that previous clinical studies of chronotherapy have frequently used too few patients and dosing time points, weakening statistical power. He's just published an analysis of every trial he could find in the medical literature in which time of day was tested—all 106 of them (6). "They went at it without any idea of what the mechanism might be," he says. "They were shooting in the dark."

Researchers have now built a much better picture of how circadian rhythm affects physiology, Hogenesch says, and this should enable more precise and reliable trials. Lab studies have built a solid evidence base relating to, for instance, which drug targets and metabolizing enzymes are clock regulated. One example is argininosuccinate synthase, expressed in the liver to enable a key reaction in the metabolic chain that makes urea. "We know which ones they are now and in what tissue they are," Hogenesch says. The best drugs to investigate for chronotherapy, he says, are those that act on molecules and pathways that show the largest variations in activity with time.

Tissue Time

Emerging evidence shows that external time is not the only important indicator. The circadian rhythm within distinct tissues can be out of step with the time of day. Earlier this year, Sehgal published a study that shows how a tissue-specific circadian clock gone haywire—such as from chronic jet lag or shift work—can drive disease (7). Working with cultures of human cancer cells and in mice, her group found that using the hormone dexamethasone to artificially advance daily cycles changed the expression of genes, including those associated with cell division. Circadian rhythm disruption increased expression of a protein called cyclin D1. And this, in turn, activated another protein called CDK4/6, a protein that triggers cell division by increasing production of DNA.

The change was important for treatment. The researchers tested the effectiveness of one drug (PD-0332991) that usually works against cancer because it inhibits CDK4/6 activity. Disrupting the circadian rhythms of both the cells and mice made the drug less effective because cell proliferation was harder to control.

Francis Lévi, a biomedical researcher who specializes in chronotherapy at the University of Warwick in the United Kingdom, says assessing the state of an individual's circadian clock and how it differs from external time—such as in their tumor—is an essential part of truly personalized medicine. "If you want to time chronotherapy then you must do it according to the timing of the person you are going to treat," he says. Just as drug doses are adjusted for body weight, therapies could be tailored to differences in internal timing. That's not always a popular message, Lévi says, because it complicates the idea that better medicines administered at a particular time will help a patient population.

A common way to assess the circadian rhythm of distinct tissues and how it relates to the external clock is to measure gene expression patterns in samples taken throughout the day. But Lévi says that's not a realistic strategy for patients. Instead, he and colleagues have developed a tool to track internal time from a single tissue sample. TimeTeller is a computer algorithm that analyzes gene transcription based on the measured expression levels of 10 to 15 genes and can distinguish between healthy and diseased tissue based on the state of its circadian cycle (8).

Lévi's team has tested the approaches by using single samples taken from patients with breast cancer and found the internal clock in the tumor cells could be up to 12 hours out of sync. Using the algorithm to measure this disruption to the circadian cycle, they say, indicated which patients would respond better to treatment.

Other less-invasive technologies in the offing could make chronotherapy approaches more feasible—for example, wearable sensors that track and record the daily rhythms in sleep and temperature. Lévi sees such technology as one means of taking a crucial step toward feasible treatments. "Chronotherapy has to be given outside the hospital," he says. Hospitals are well placed to treat short-term emergencies, he says, but they are not set up to deliver the best treatment for chronic diseases, not least because they operate on their own, artificial, daily cycles.

Indeed, Hogenesch and colleagues analyzed the distribution of 12 drugs to almost 1,500 hospital inpatients and found that nearly a third of all the medicines were ordered (and hence, likely given) in a narrow time slot between 8 AM and noon (9). That's based on staffing patterns—typically a shift change at 7 AM—rather than clinical need, he says. Among those medicines are some that are known to work better in the evening, such as hydralazine, which is given for high blood pressure. They saw the same pattern with morphine: usually distributed in the morning while patients often report that their pain is worse at night. "We're very likely undertreating pain in the afternoon and even overtreating it in the morning," he says.

Still, chronotherapy won't work for all drugs. Medicines that break down very slowly in the body—such as the Alzheimer drug donepezil—can't be made more effective by dosing at a set time because they tend to provide a stable concentration over a 24-hour period. That's why most chronotherapy efforts focus on drugs with a half-life of less than 15 hours. (Donepezil has a half-life of 70 hours.)

And beyond the regimes of hospitals and drug regimen nuances, chronotherapy has another more fundamental challenge. "I don't think people know what chronotherapy is," Hogenesch says. A greater awareness and a fuller picture of the mechanisms at play could make chronotherapy a field whose time has come.

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