A Report on "The Impact of Circadian Rhythm Disruption on Oxaliplatin Tolerability and Pharmacokinetics in Cry1$^{-/-}$*Cry*2$^{-/-}$ Mice Under Constant Darkness" by Akyel et al. (2025)

Reviewer 2

February 11, 2026

v1

isitcredible.com

Disclaimer

This report was generated by large language models, overseen by a human editor. It represents the honest opinion of The Catalogue of Errors Ltd, but its accuracy should be verified by a qualified expert. Comments can be made here. Any errors in the report will be corrected in future revisions.

> I am wiser than this person; for it is likely that neither of us knows anything fine and good, but he thinks he knows something when he does not know it, whereas I, just as I do not know, do not think I know, either. I seem, then, to be wiser than him in this small way, at least: that what I do not know, I do not think I know, either.
>
> Plato, *The Apology of Socrates*, 21d

> To err is human. All human knowledge is fallible and therefore uncertain. It follows that we must distinguish sharply between truth and certainty. That to err is human means not only that we must constantly struggle against error, but also that, even when we have taken the greatest care, we cannot be completely certain that we have not made a mistake.
>
> Karl Popper, 'Knowledge and the Shaping of Reality'

Overview

Citation: Akyel, Y. K., Seyhan, N. O., Gül, Ş., Çelik, M., Taşkın, A. C., Selby, C. P., Sancar, A., Kavakli, I. H., and Okyar, A. (2025). The Impact of Circadian Rhythm Disruption on Oxaliplatin Tolerability and Pharmacokinetics in Cry1-/-Cry2-/- Mice Under Constant Darkness. *Archives of Toxicology*. Vol. 99, pp. 1417–1429.

URL: https://doi.org/10.1007/s00204-025-03968-7

Abstract Summary: This study investigated the effects of oxaliplatin on $Cry1^{-/-}Cry2^{-/-}$ knockout (Cry DKO) mice under constant darkness, finding that circadian rhythm disruption significantly reduced oxaliplatin tolerability and altered its pharmacokinetics, likely due to dysregulation of detoxification genes.

Key Methodology: In vivo experiments using wild-type and Cry DKO mice, single and repeated oxaliplatin dosing at two circadian times (CT8 and CT16), body weight monitoring, pharmacokinetic analysis (ICP-OES) of plasma and liver, and liver RNA-sequencing (RNA-seq) for transcriptomic analysis.

Research Question: How does the disruption of the circadian rhythm affect the tolerability and pharmacokinetic properties of the anticancer drug oxaliplatin?

Summary

Is It Credible?

Akyel et al. present a study investigating the "impact of circadian rhythm disruption on oxaliplatin tolerability" using a genetic mouse model (p. 1417). The authors claim that *Cry1*-/-*Cry2*-/- (DKO) mice, which lack a functional molecular clock, exhibit severe intolerance to the chemotherapy drug oxaliplatin regardless of the time of administration, unlike wild-type mice which show time-dependent toxicity. They further propose that this intolerance is driven by a "dysregulation in detoxification pathways," specifically the downregulation of Glutathione S-transferase (*Gst*) genes in the liver (p. 1417). While the phenotypic observation of increased sensitivity in the knockout mice appears robust, the study's mechanistic conclusions are undermined by conflicting pharmacokinetic data in the single-dose phase and a confounded experimental design.

A central tension exists between the study's initial toxicity findings and its pharmacokinetic (PK) analysis. In the single-dose experiment, the DKO mice experienced significantly greater body weight loss than wild-type mice, yet the PK data reveal that these sensitive animals actually had lower systemic drug exposure and faster clearance rates (p. 1422). This creates an unresolved paradox for the single-dose phase: the animals with the least drug in their system suffered the most severe initial weight loss. While the authors successfully demonstrate drug accumulation and severe toxicity in the repeated-dose study, they do not adequately explain the initial hypersensitivity observed in the single-dose phase where accumulation had not yet occurred. This discrepancy suggests that the initial intolerance may be driven by intrinsic tissue sensitivity (pharmacodynamics) rather than the pharmacokinetic accumulation mechanism emphasized by the authors. Without resolving this, the claim that toxicity is simply a result of "high concentrations of oxaliplatin" is an over-

simplification that ignores the contradictory single-dose data (p. 1417).

The molecular mechanism proposed—that altered *Gst* gene expression drives toxicity—is also difficult to accept due to the timing of the RNA-sequencing analysis. Liver tissue was collected 24 hours after the third dose of oxaliplatin, at a point when the DKO mice were already experiencing severe weight loss and toxicity (p. 1419). Consequently, it is impossible to distinguish whether the observed downregulation of detoxification genes is the cause of the drug intolerance or merely a downstream symptom of severe liver injury and cellular stress. Furthermore, the RNA-sequencing analysis compared treated wild-type mice to treated DKO mice but lacked vehicle-treated control groups for the drug effect itself. Without these controls, it is difficult to determine if the gene expression differences are a specific response to the drug or constitutive differences between the genotypes. The authors also leap from transcriptomic data to functional conclusions, stating that gene upregulation led to "increased enzyme activity" without providing any protein quantification or enzymatic assays to support this assertion (p. 1427).

Finally, the study's exclusive focus on the liver represents a significant blind spot. Oxaliplatin is primarily cleared via renal excretion, yet the authors did not analyze kidney function or renal gene expression. Given the observed differences in clearance, ignoring the primary organ responsible for drug elimination limits the credibility of the liver-centric mechanistic explanation. Additionally, the statistical approach for the physiological data relies on multiple t-tests rather than a two-way ANOVA, which is the standard for detecting the gene-by-time interactions central to the study's hypothesis. While the observation that circadian disruption worsens oxaliplatin tolerability is likely valid, the specific molecular and pharmacokinetic mechanisms proposed by Akyel et al. are not fully supported by the data presented.

The Bottom Line

The study provides credible evidence that *Cry*-deficient mice are significantly more sensitive to oxaliplatin toxicity than wild-type controls. However, the authors' explanation for this intolerance—that it is driven by liver drug accumulation and *Gst* gene downregulation—is weak. The single-dose data contradict the accumulation hypothesis by showing faster clearance in sensitive mice, and the gene expression analysis is confounded by severe toxicity at the time of sampling.

Potential Issues

Unresolved paradox in single-dose toxicity and pharmacokinetics: A central tension exists between the study's toxicity and pharmacokinetic (PK) data following a single dose of oxaliplatin. The results show that circadian-disrupted *Cry* double-knockout (DKO) mice are significantly more sensitive to the drug's toxicity, as measured by body weight loss, compared to wild-type (WT) mice. However, the single-dose PK data show that these more sensitive DKO mice have substantially lower systemic drug exposure. For instance, at circadian time 16 (CT16), where the toxicity difference was most pronounced, DKO mice had a plasma area under the curve ($AUC_{0-\infty}$) of 72.42 μg h/ml, while WT mice had a much higher exposure of 119.69 μg h/ml. Correspondingly, plasma clearance was faster in the sensitive DKO mice (p. 1422). This finding creates a paradox for the single-dose results, as the animals with lower drug exposure experienced greater weight loss. While the repeated-dose study does show drug accumulation that aligns with the severe toxicity observed later, the mechanism for the initial single-dose intolerance remains unexplained. This suggests that a pharmacodynamic (i.e., intrinsic tissue sensitivity) rather than a pharmacokinetic mechanism may be dominant in the early stages, a possibility the study does not adequately explore.

Confounded design of the RNA-sequencing analysis: The study's primary mechanistic evidence is derived from an RNA-sequencing analysis that appears to be confounded by the experimental design. Liver tissue for gene expression analysis was collected 24 hours after the third and final dose of oxaliplatin, a time point at which the DKO mice were already experiencing severe toxicity and significant body weight loss (p. 1419). This design makes it impossible to distinguish whether the observed changes in gene expression, such as the downregulation of Glutathione S-transferase (GST) genes, are the underlying cause of the drug intolerance or merely a downstream consequence of accumulated drug-induced liver injury, inflammation,

and cellular stress. Furthermore, while the analysis compared treated WT mice to treated DKO mice, it lacks vehicle-treated control groups for the drug effect itself. Without these controls, it is difficult to determine whether the reported differences in gene expression reflect a differential response to the drug or pre-existing, constitutive differences between the two genotypes. This temporal mismatch and lack of appropriate controls weaken the foundation of the article's molecular explanation for oxaliplatin intolerance.

Liver-centric mechanistic investigation omits primary clearance pathway: The study's mechanistic investigation is focused almost exclusively on the liver, with RNA-sequencing performed only on liver tissue. This approach focuses on hepatic detoxification while overlooking that oxaliplatin and its platinum-containing metabolites are primarily cleared from the body via renal excretion. The observed differences in drug clearance and accumulation could plausibly be driven by unmeasured differences in kidney function, renal blood flow, or the expression of drug transporters in the kidney between WT and DKO mice. By not analyzing kidney tissue or function, the study fails to investigate what is arguably the most important organ for the drug's disposition. This omission represents a significant limitation, as the conclusions about a liver-based metabolic mechanism are drawn without considering a major alternative explanation related to the primary route of drug elimination.

Inappropriate statistical analysis of factorial design: The study utilizes a 2x2 factorial design, comparing two genotypes (WT vs. DKO) at two different circadian times (CT8 vs. CT16). However, the physiological and pharmacokinetic data were analyzed using multiple pairwise Student's t-tests instead of the more appropriate two-way analysis of variance (ANOVA) (p. 1420). This statistical approach is suboptimal for two main reasons. First, conducting multiple t-tests without correction increases the probability of Type I errors (false positives). Second, and more importantly, this method fails to formally test for a statistical interaction between genotype

and time. The existence of such an interaction is central to the study's hypothesis that the effect of dosing time on toxicity depends on the integrity of the circadian clock. While the authors use appropriate methods (DESeq2) for their transcriptomic analysis, the failure to apply similar rigor to the primary toxicity and PK data represents a methodological weakness that affects the statistical certainty of the conclusions.

Unsupported interpretation of gene expression data: The article's central mechanistic conclusion—that altered detoxification capacity drives oxaliplatin intolerance—is based on an inferential leap from mRNA transcript levels to protein function. The authors conclude that "upregulation in expression of *Gstm2*, *Gstm3*, and *Gstm7* genes in WT mice... leading to increased enzyme activity, may have been related to enhanced detoxification" (p. 1427). This claim of "increased enzyme activity" is based solely on RNA-sequencing data and is not supported by any direct measurement of GST protein levels (e.g., via Western blot) or functional enzymatic activity. It is well established that mRNA abundance does not always correlate with protein levels or function. While transcriptomic data is valuable for generating hypotheses, presenting these inferences as established functional changes in the conclusion is an over-interpretation of the evidence provided.

Limited generalizability due to single fixed-dose level: The study's conclusions are based on experiments using a single dose of oxaliplatin (12 mg/kg), which was selected as the maximum tolerated dose (MTD) for the more sensitive DKO mouse strain (p. 1420). While this approach is common in toxicology to maximize the observable effect, it limits the generalizability of the findings. It is unclear whether the observed differences in toxicity and gene expression would persist at lower, potentially more clinically relevant doses, or if they are specific to this high-toxicity context. Furthermore, using the MTD of the sensitive strain for both groups means the more resistant WT mice received a sub-MTD dose, which may create a "floor effect" that exaggerates the magnitude of the difference in tolerability. The lack of a dose-response analysis means the robustness of the findings across a range of exposures

remains unknown.

Transparency and reporting deficiencies: The manuscript contains several reporting issues that reduce clarity and confidence in the data. First, the article states that "Hematological evaluation was performed," a key measure for oxaliplatin toxicity, but no hematological data are presented or discussed, leaving a significant gap in the toxicity assessment (p. 1419). Second, the authors state that the 12 mg/kg dose was "determined to be the maximum tolerated dose (MTD) for *Cry* DKO mice" but provide no methods, data, or citation for the dose-finding study that established this critical parameter (p. 1420). Third, sample sizes are reported inconsistently; for example, the Methods section states "n=3–4" for the single-dose study (p. 1419), while Table 1 reports "n=4" (p. 1422), and the caption for Figure 2 reports both "n=5" and "n=3–4" for the same data (p. 1423). Finally, a minor calculation error appears in the text, which states that the C_{max} in WT mice at CT16 was "2.1-fold higher" than in DKO mice, when the data in Table 1 (5.25 vs. 2.10 μg/ml) show it to be exactly 2.5-fold higher (p. 1422). While some of these are minor clerical issues, the omission of the hematology and MTD data represents a more significant lack of transparency.

Future Research

Investigation of renal clearance mechanisms: Future work should investigate the role of the kidney in the altered pharmacokinetics of oxaliplatin in circadian-disrupted models. Since oxaliplatin is renally excreted, analyzing renal blood flow (GFR) and the expression of renal drug transporters in *Cry* DKO mice could explain the clearance differences that the current liver-focused study failed to address.

Validation of enzymatic activity: To substantiate the claims regarding detoxification pathways, future studies must move beyond mRNA quantification. Researchers should perform Western blots and functional enzymatic assays for GSTs in liver tissue. This would determine whether the observed transcriptomic changes actually result in the "increased enzyme activity" claimed by the authors.

Time-course analysis of gene expression: To resolve the cause-and-effect relationship between gene expression and toxicity, future experiments should analyze tissue samples at early time points, prior to the onset of severe physiological decline. Comparing vehicle-treated and drug-treated animals of both genotypes would clarify whether the downregulation of detoxification genes is a pre-existing vulnerability or a reaction to the drug.

Pharmacodynamic sensitivity assessment: Given the paradox of high weight loss despite low drug exposure in the single-dose study, future research should investigate intrinsic tissue sensitivity. This could involve *in vitro* toxicity assays using hepatocytes or other relevant cell types from DKO and wild-type mice to assess cellular survival rates when exposed to identical concentrations of oxaliplatin, thereby isolating pharmacodynamics from pharmacokinetics.

isitcredible.com