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COMMENTARY

The Challenge of Interpreting Alanine Aminotransferase Elevations in Clinical Trials of New Drug Candidates

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Bardoxolone methyl has shown promise in improving kidney function associated with type 2 diabetes. However, this drug also caused elevations in serum alanine aminotransferase (ALT) in some patients, raising liver safety concerns. In this issue, Lewis *et al*. present data supporting these elevations as an on-target effect of the drug, but the evidence provided will probably not carry much weight with regulators. This example highlights the urgent need for improved liver safety biomarkers.

Although drug-induced liver injury (DILI) remains a leading cause for attrition of new drug candidates, the serum biomarkers used to detect DILI have remained unchanged for half a century. The most sensitive biomarker for hepatocellular injury remains serum ALT, which is abundant in hepatocytes. Following hepatocyte death, ALT is released into circulation causing an elevation in serum. A challenge with utilizing ALT to assess liver safety in clinical trials is that ALT elevations can be caused by drugs that are quite safe for the liver. For example, ALT elevations occur frequently in individuals treated with heparins or cholestyramine, although these drugs carry no liability for serious DILI.[1,2] Owing to this phenomenon, treatment-emergent ALT elevations alone do not generally prompt the US Food and Drug Administration (FDA) to place a hold on clinical trials. Instead, the current FDA guidance for industry[3] recommends continuation of drug treatment in participants with asymptomatic ALT elevations of < 8× the upper limit of normal (ULN) while monitoring for a rise in serum total bilirubin > 2 × ULN indicating functional liver impairment. For the purposes of predicting postmarketing safety, these "Hy's Law cases" (ALT > 3 × ULN concurrent with total bilirubin > 2 × ULN) are assumed to have at least a 10% chance of progressing to liver failure (i.e., 1:1000 Hy's Law cases in a clinical trial predicts a liver failure rate of 1:10,000 postmarketing).[4]

When treatment-emergent ALT elevations are observed in clinical trials, the FDA may request an additional large clinical trial to establish adequate liver safety. However, the "Rule of Three" frequently used by the FDA means that to exclude a Hy's Law case in 5,000 people (or an estimated liver failure in 1:50,000 treated patients), a clinical trial must contain at least 15,000 drug-treated patients and probably a comparable comparator group. It is understandable that sponsors observing treatment-emergent ALT elevations hope to find plausible underlying mechanisms other than hepatotoxicity.

In this issue, Lewis *et al*. describe the experience with the investigational drug candidate, bardoxolone methyl, in a phase III clinical trial. Although bardoxolone methyl showed promise to improve kidney function in this patient population, ALT elevations > ULN were observed in 38% of drug-treated patients compared with 5% of placebo-treated patients. Twelve patients receiving bardoxolone methyl experienced ALT elevations > 5 × ULN, the international consensus criteria for DILI,[5] compared with just one placebo-treated patient. Similar trends were observed in levels of aspartate aminotransferase and gamma-glutamyl transferase supporting a liver source. Six drug-treated patients were discontinued due to aminotransferase elevations, including 2 patients with ALT exceeding 10 × ULN.

Lewis *et al*. present evidence that the liver chemistry elevations observed do not reflect hepatotoxicity or portend a serious DILI liability. They note that, in animals, bardoxolone methyl is hepatoprotective in some instances. In patients, the bardoxolone methyl-induced aminotransferase elevations were asymptomatic and trended toward normal with continued drug treatment, and there were no Hy's Law cases.

Lewis et al. instead propose that the ALT elevations result from an on-target effect of the drug. Bardoxolone methyl activates the KEAP1-Nrf2 pathway by interacting with KEAP1, the Nrf2 repressor, thus allowing translocation of Nrf2 to the nucleus and activating a cassette of genes that reduce oxidative stress. The authors showed that, consistent with previous publications, altering activation of the Nrf2 pathway in mice affects serum ALT levels. They found that relative to wild-type mice, Nrf2-null mice had reduced serum ALT (~ 0.7-fold) and mRNA of the ALT2 isoform in the liver (~ 0.6-fold). Conversely, Keap-1-knockdown mice had increased serum ALT (~ 1.2-fold) and mRNA of the ALT1 isoform in the liver, relative to wild-type mice. Furthermore, the authors found that eight cell lines that express aminotransferases exhibited dose-dependent increases of aminotransferase mRNA expression when treated with bardoxolone methyl. At the highest dose (1,000 nM), the maximum elevations for the expression of the ALT isoforms were modest (e.g., an ~ 4-fold increase in HepG2 cells and an ~ 3-fold change in SK-HEP-1 cells). These findings support that bardoxolone methyl might increase serum aminotransferase levels in patients through enzyme induction as an on-target effect.

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Although Lewis *et al*. present convincing evidence that bardoxolone methyl can induce expression of aminotransferases, these data will not likely persuade regulators that this drug candidate is safe for the liver. It is well-known that most patients who develop ALT elevations from drugs with known serious liver safety liabilities (e.g., isoniazid and troglitazone) will have resolution of these elevations with continued treatment.[4] The observation that some bardoxolone methyl-treated patients similarly adapt does not mean that all patients will. Additionally, although the nonclinical studies support that induction of aminotransferase genes can be an on-target effect of bardoxolone methyl, the magnitude of the effects observed was minimal and inconsistent with the large ALT elevations observed in some patients in the BEACON trial, particularly the 2 patients with ALT > 10 × ULN. Furthermore, the ALT ULN used in this trial was 47 U/L. Current guidelines recommend using standardized ALT ULNs of 33 and 25 U/L for men and women, respectively.[6] When applying these consensus ULNs, the 2 patients with the highest ALT elevations in the BEACON trial experienced ALT > 20 × ULN. Moreover, the ALT increases in the preclinical experiments were expressed as fold baseline. Based on the ALT over time graphs for the two patients (in the supplement), it would appear that each elevation was > 35× baseline. Whereas the biochemical nature and timing of these substantial elevations is consistent with the more minor and transient elevations observed in most patients, it remains possible that had bardoxolone methyl treatment been continued, these two patients would have experienced liver dysfunction. Unfortunately, future clinical trials of bardoxolone methyl will likely have to impose the current FDA guidelines of stopping drug treatment when ALT rises above 8 × ULN, meaning that as this threshold is likely reached by some patients, the hypothesis that these elevations will resolve with continued dosing probably cannot be tested.

What else could be done to bolster the case that bardoxolone methyl-induced ALT elevations do not reflect DILI? If the effect is on-target, it may be possible to associate ALT elevations with a desired pharmacodynamic end point. It would also make sense to determine whether other serum biomarkers associated with liver injury rose along with ALT. For instance, GLDH is a large protein found in the mitochondria. This biomarker is considered more liver-specific than ALT and given its location, it is less likely than the aminotransferases to become significantly elevated in serum without hepatocyte death. A rise in ALT that is not accompanied by a rise in GLDH would support the absence of hepatotoxicity. Interestingly, GLDH rose in individuals that developed heparin-induced and cholestyramine-induced ALT elevations,[1,2] suggesting that transient drug-induced hepatocyte death may not necessarily indicate a liver safety liability.

Additional support for an on-target effect would be to demonstrate that the observed ALT elevations are consistent with Quantitative Systems Pharmacology modeling that includes population variation in liver exposure, drug-target engagement followed by transcriptional activation of ALT expression, and release of ALT into circulation. An on-target effect would be further bolstered by demonstrating that bardoxolone methyl does not interfere with off-target adverse outcome pathways believed to underlie DILI. Along with oxidative stress that might be difficult to detect given the on-target effect, mitochondrial impairment and inhibition of bile acid efflux transporters are mechanisms thought to induce liver injury. An existing Quantitative Systems Toxicology model (DILIsym) integrates data of this type along with population variation in liver exposure to explain or predict a liver safety liability of new drug candidates.[7] The inability of DILIsym to predict ALT elevations due to bardoxolone methyl along with successful prediction by Quantitative Systems Pharmacology would be quite convincing evidence for an on-target mechanism.

In summary, although ALT is a sensitive biomarker for hepatotoxicity, it lacks specificity to identify drug candidates with a serious DILI liability. Because treatment emergent ALT elevations may result in regulatory requirements for additional large clinical trials, and because it has not yet been possible to confidently identify the subset of individuals who will develop DILI from any drug, ALT elevations often lead to the decision to abandon the development of otherwise promising drugs. Bardoxolone methyl has shown potential to improve kidney function in patients with type 2 diabetes but it has also been shown to cause ALT elevations. Lewis *et al*. provide nonclinical data supporting induction of aminotransferases as an on-target effect of the drug. However, the effects are modest and not consistent with the substantial ALT elevations observed in the clinical trials and seem unlikely to convince regulators of the safety of this drug candidate. The bardoxolone methyl experience highlights the urgent need for better DILI biomarkers and some promising new biomarkers have been proposed.[8,9] The key limiting factor in validation of new DILI biomarkers is the lack of serial serum samples prospectively collected and archived from clinical trials that have demonstrated varying degrees of liver safety. This will require standardized sample collection, storage, and retrieval of relevant phenotypic data.[10] Precompetitive efforts need to be accelerated in this direction.

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