The Importance of Full and Correct Prediction of Human Metabolites in Discovery and Preclinical Development

A HμREL® White Paper

by Ronald E. White, Ph.D., President
White Global Pharma Consultants, LLC
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CONCLUSION: **In this case study, one can see the fundamental importance of having confidence that the selected human *in vitro* metabolite-generation system will provide – during the preclinical phase – a full and correct assessment of the metabolites that will actually be seen later in clinical trials.**

The available evidence indicates that HμREL*human*™ hepatocyte co-cultures are such a system.

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Abstract

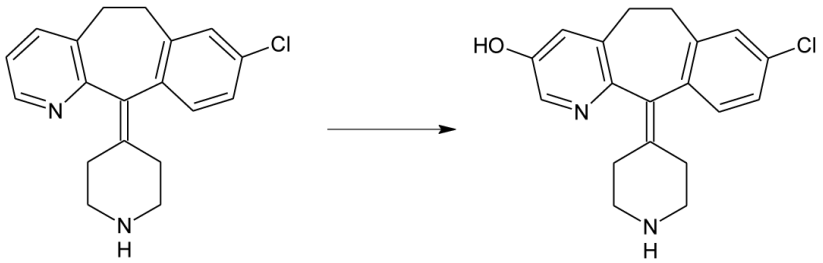
The clinical development history of the non-sedating antihistamine desloratadine (Clarinex®) exemplifies the regulatory challenges that can arise when the lack of metabolically adequate in vitro tools causes an important human metabolite (in this case, 3-hydroxy-desloratadine) to be missed early in pre-clinical development. Recent in vitro studies of the metabolism of desloratadine, performed on HμREL*human*™ primary hepatocyte co-cultures, displayed robust production of 3-hydroxy-desloratadine and demonstrated how the the state-of-the-art HμREL® system can facilitate clinical development and avoid regulatory delays.

Introduction

A common activity in the preclinical phase of development of a new drug candidate is prediction of prominent circulating human metabolites, and there are several reasons for this.

1. To understand the major clearance pathways of the drug in order to anticipate possible drug-drug interactions and polymorphic metabolism
2. To be alerted to the possible production of active metabolites in humans
3. To be alerted to the possible production of problematic metabolites (e.g., structure alerts, reactive intermediates, unique human metabolites)
4. To determine the metabolic similarity of various animal species compared to humans to validate their use for preclinical safety studies

Both the U.S. Food & Drug Administration (FDA) and the European Medicines Agency (EMA) have emphasized the significance of circulating human metabolites in characterization of drug candidates through issued Guidances, including the so-called "MIST" (Safety Testing of Metabolites, Ref. 1) and Drug Interaction (Ref. 2) Guidances.



Desloratadine 3-OH-Desloratadine

The following case history shows how lack of a reliable method for preclinical prediction of human metabolites can have important consequences during the clinical development phase. During clinical development of desloratadine, the standard human ^{14}C-ADME study revealed that the major circulating metabolite was 3-hydroxy-desloratadine (Ref. 3).

This discovery raised interest among regulatory authorities because of four concerns:

1. Mice, rats, and monkeys failed to produce 3-hydroxy-desloratadine (Ref. 4) and were thus essentially not exposed to this metabolite in preclinical safety studies compared to typical human clinical exposures at efficacious doses.

2. 3-Hydroxy-desloratadine has pharmacological activity similar to that of desloratadine, so that the *in vivo* therapeutic effect derives from the combined effects of both the parent drug and the metabolite. Therefore, FDA felt it was felt necessary to assay plasma levels of 3-hydroxy-desloratadine (Ref. 5) to determine its pharmacokinetics and extent of contribution to efficacy.

3. Metabolic conversion of desloratadine to 3-hydroxy-desloratadine was found to be polymorphic in patients, with about 7% of the general population and 20% of African-Americans having very low clearance of desloratadine through this major route of clearance in the normal population (Ref. 6). As a consequence, patients with the "Poor Metabolizer" (PM) phenotype had substantially longer half-lives and six times greater exposure for desloratadine than the normal population, which displayed the "Extensive Metabolizer" (EM) phenotype (Ref. 7).

4. The enzymology of formation of 3-hydroxy-desloratadine was unknown (Ref. 6) due to the inability of animals or available *in vitro* human systems to produce this metabolite. Therefore, neither the mechanism of metabolism nor the biochemical basis for the observed metabolic polymorphism was understood. And, as a matter of practical clinical use, it was not possible to prospectively determine the EM/PM phenotype of particular patients (Ref. 6).

Successful application of HμREL*human*™ hepatocyte co-cultures to the problem

As mentioned above, previous attempts with animal species or *in vitro* human systems (e.g., monoculture hepatocytes, liver microsomes, expressed CYP enzymes) failed to generate 3-hydroxy-desloratadine, making it impossible to ascertain the biochemical source of the metabolite. In contrast, a recent investigation of human metabolism of desloratadine using HμREL® human hepatocyte co-cultures compared to hepatocyte monocultures and liver microsomes showed that the HμREL® system consistently produced more metabolites than at least one and sometimes both of the comparator methods. Most significantly, as shown in Table 1 below, the HμREL® system displayed robust production of the elusive 3-hydroxy-desloratadine.

Table 1. Identification of 3-hydroxy desloratadine

Drug (m/z)	Metabolites (m/z)	Phase 1 or Phase 2	1° or 2° Metabolite	% in Excreta	% of Circulating Radioactivity	Microsomes	Hepatocytes	HuREL®
Loratidine (383.1521)	Hydroxy-Loratadine (399.1460)	1	1			Yes	Yes	Yes
	Hydroxy-Loratadine-glucuronide (575.1899)	2	2			No	Yes	Yes
	Desloratadine (311.1460)	1	1			Yes	Yes	Yes
	5-6/-Hydroxy-Desloratadine (327.1460)	1	2			Yes	Yes	Yes
	3-Hydroxy-Desloratadine-glucuronide (503.1460)	2	2	>50%	>50%	No	No	Yes
	Desloratadine ring cyclized (323.1233)	1	2			No	No	Yes

The intriguing implications

If H*μ*REL*human*™ hepatocyte co-cultures would have been available during preclinical development of desloratadine, the Sponsor would have been provided with an early alert that a major metabolic pathway for desloratadine was not present in the preclinical safety species. Furthermore, the Sponsor would have learned of a major active metabolite requiring bioanalytical monitoring in clinical studies. Importantly, the H*μ*REL® system would have allowed an *in vitro* investigation of the formation of 3-hydroxy-desloratadine, likely resulting in identification of the enzyme system responsible. These three advantages probably would have reduced the exceptionally long regulatory approval period (45 months; Ref. 8) and eliminated a post-approval commitment to investigate the enzymology. It is not possible to say exactly how many resources might have been conserved with this capability, how much disruption of the timeline for the business plan might have been avoided, or how much development costs would have been reduced and future sales revenues augmented, but it is certain that the cost of applying the H*μ*REL® system would have been trivial in comparison.

Conclusion

In this case study, one can see the fundamental importance of having confidence that the selected human *in vitro* metabolite-generation system will provide – during the preclinical phase – a full and correct assessment of the metabolites that will actually be seen later in clinical trials. The available evidence indicates that H*μ*REL*human*™ hepatocyte co-cultures are such a system.

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H*μ*REL® Corporation

675 US Highway One
North Brunswick, NJ 08902
www.hurelcorp.com