

Screening for Reactive Drug Metabolites During Early Pharmaceutical Drug Discovery

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The concept that reactive drug metabolites could play a role in deleterious in-vivo toxicity events such as tissue necrosis, mutagenicity and teratogenicity is based on research conducted over the past three decades.¹ Although there has yet to be identified a direct mechanistic link between reactive drug metabolite formation and the onset of certain toxicities, there exist a number of cases where the toxicity finding, especially those considered "idiosyncratic", is accompanied by reactive drug metabolite formation by the drug compound.² Furthermore, there is mounting evidence to suggest that protein covalent binding brought about by the inadequate detoxification of reactive metabolites could be a precursor event in the mechanistic cascade leading to immune mediated toxicity in-vivo.³

The possibility that reactive metabolite formation could lead to toxicity has prompted pharmaceutical companies to implement reactive metabolite screening as part of their overall ADMET screening strategy. Although there can exist differences between companies in when and how this process takes place it is generally accepted that the earlier in drug discovery this can occur the better the chance that medicinal chemistry efforts can abrogate the potential liability through structural modification. The reactive metabolite screening strategy employed in our laboratory includes a first tier high throughput, high compound capacity assay that is used during early drug discovery to rapidly and cost effectively identify compounds that form reactive metabolites. The assay is based on a standard microsome stability assay with the addition of the nucleophilic conjugating agent glutathione ethyl ester.⁴ The data from this assay is provided back to project teams in a time period in-line with synthesis cycles to provide an opportunity to design next-round synthesis based on reactive metabolite structure activity relationship (SAR) along with ADME and potency.

In the event efforts to block or abrogate reactive metabolite formation fail and a compound or series shows promise in terms of drug-like properties, the situation becomes slightly complex. Without a direct link to toxicity and only the potential to cause toxicity standing in the way of a potentially beneficial drug compound, next step decision-making turns to assessing risk as quickly as possible. A component of a reactive metabolite risk assessment follow-up plan is to quantitatively determine the level(s) of protein covalent modification or glutathione conjugate formation. A common method to quantify the level of protein covalent modification is to use radiolabeled substrate.⁵ Recent literature reports have also described novel methods for quantifying the levels of glutathione conjugation.⁶ Considering resource utilization (i.e. costs), green chemistry initiatives and our reactive metabolite screening paradigm, we sought to develop an alternative non-radiolabeled approach to quantitatively determine the levels of glutathione conjugation using a novel quaternary ammonium glutathione conjugating agent.⁷ This approach could prove to be useful as a tier I screen follow-up to help assess risk in the event a project moves forward in light of reactive metabolite findings.

This presentation will focus on three areas of reactive metabolite screening; (i) Overview and rationale for screening for reactive metabolites during early drug discovery, (ii) the high throughput, high compound capacity reactive metabolite assay currently used in our laboratory and (iii) development and use of a novel method to semi-quantitatively determine reactive metabolite conjugate levels as a follow-up "risk assessment" approach to our high throughput assay.

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