Chemico-Biological Interactions 192 (2011) 46–55

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Handling reactive metabolite positives in drug discovery: What has retrospective structure–toxicity analyses taught us?

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ARTICLE INFO

Article history:
Received 1 July 2010
Received in revised form 30 August 2010
Accepted 2 September 2010
Available online 15 September 2010

Keywords:
Drug
Reactive metabolites
Cytochrome P450
ADRs
Toxicity
Glutathione
Covalent binding

ABSTRACT

Because of the inability to predict and quantify the risk of idiosyncratic adverse drug reactions (IADRs) and because reactive metabolites (RMs) as opposed to the parent molecules from which they are derived are thought to be responsible for the pathogenesis of some IADRs, procedures (RM trapping/covalent binding) are being incorporated into the discovery screening funnel early-on to assess the risk of RM formation. Utility of the methodology in structure–toxicity relationships and scope in abrogating RM formation at the lead optimization stage are discussed in this article. Interpretation of the output from RM assessment assays, however, is confounded by the fact that many successfully marketed drugs are false positives. Therefore, caution must be exercised in deprioritizing a compound based on a positive result, so that the development of a useful and potentially profitable compound won't be unnecessarily halted. Risk mitigation strategies (e.g., competing detoxication pathways, low daily dose, etc.) when selecting RM positives for clinical development are also reviewed.

1. Introduction

The formation of electrophilic reactive metabolites (RMs) is considered to be an undesirable trait of drug candidates on the grounds of evidence linking this liability with drug–drug interactions [1], genotoxicity [2], end-organ toxicity and possibly immune-mediated adverse drug reactions [3–6]. While the potential for drug–drug interactions and genotoxicity can be examined directly from *in vitro* assays (e.g., mechanism-based inactivation of cytochrome P450 (CYP) enzymes, *Salmonella* Ames assay, *in vitro* micronuclei induction, cytogenetics studies, etc.), the same does not often hold true for end-organ and immune-mediated toxicities. Although much of the safety-related attrition occurs in the course of preclinical safety evaluation, some adverse events fail to manifest in animals. Such unpredictable toxicological outcomes (often referred to as idiosyncratic adverse drug reactions (IADRs)) constitute a rare and sometimes life-threatening reaction (e.g., hepatotoxicity, skin rashes, agranulocytosis, and aplastic anemia) in drug-treated patients. IADRs are unrelated to known drug pharmacology, and are generally dose-independent (a notable exception is the dose-dependent hepatotoxicity caused by acetaminophen). Because the incidence of IADRs is very low (1 in 10,000 to 1 in 100,000), these reactions are often not detected, until the drug has gained broad exposure in a large patient population [7]. The concept of xenobiotic metabolism to RM's that covalently modify protein components leading to organ toxicity has its basis in the field of chemical carcinogenicity and the pioneering work by the Millers [8–10], who demonstrated the carcinogenic and hepatotoxic activity of aminoazo dyes to arise from their bioactivation. The extension of these concepts to human drug-induced hepatotoxicity was provided from studies on the anti-inflammatory agent and hepatotoxin acetaminophen [11–13]. Mechanistic studies which established the CYP-mediated oxidation of acetaminophen to a reactive quinone-imine intermediate (NAPQI) [14], capable of depleting levels of the endogenous anti-oxidant glutathione (GSH) and/or binding covalently to liver macromolecules has served as a paradigm for drug toxicity assessment over the decades.

2. RM formation – structure–toxicity relationships and downstream consequences

An understanding of the biochemical basis for drug toxicity has aided to replace the vague perception of a chemical class effect with a sharper picture of individual molecular peculiarity. There are several instances of prototype drugs associated with IADRs that also form RM(s) and elimination of RM liability in follow-on successor agent(s) markedly improves the safety pro-

Abbreviations: Rm, reactive metabolite; IADR, idiosyncratic adverse drug reaction; CYP, cytochrome P450; GSH, glutathione; NSAID, nonsteroidal anti-inflammatory drug; NAT 2, *N*-acetyltransferase 2; HLM, human liver microsomes; PK/PD, pharmacokinetic/pharmacodynamic.

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0009-2797/$ – see front matter © 2010 Published by Elsevier Ireland Ltd.
doi:10.1016/j.cbi.2010.09.003

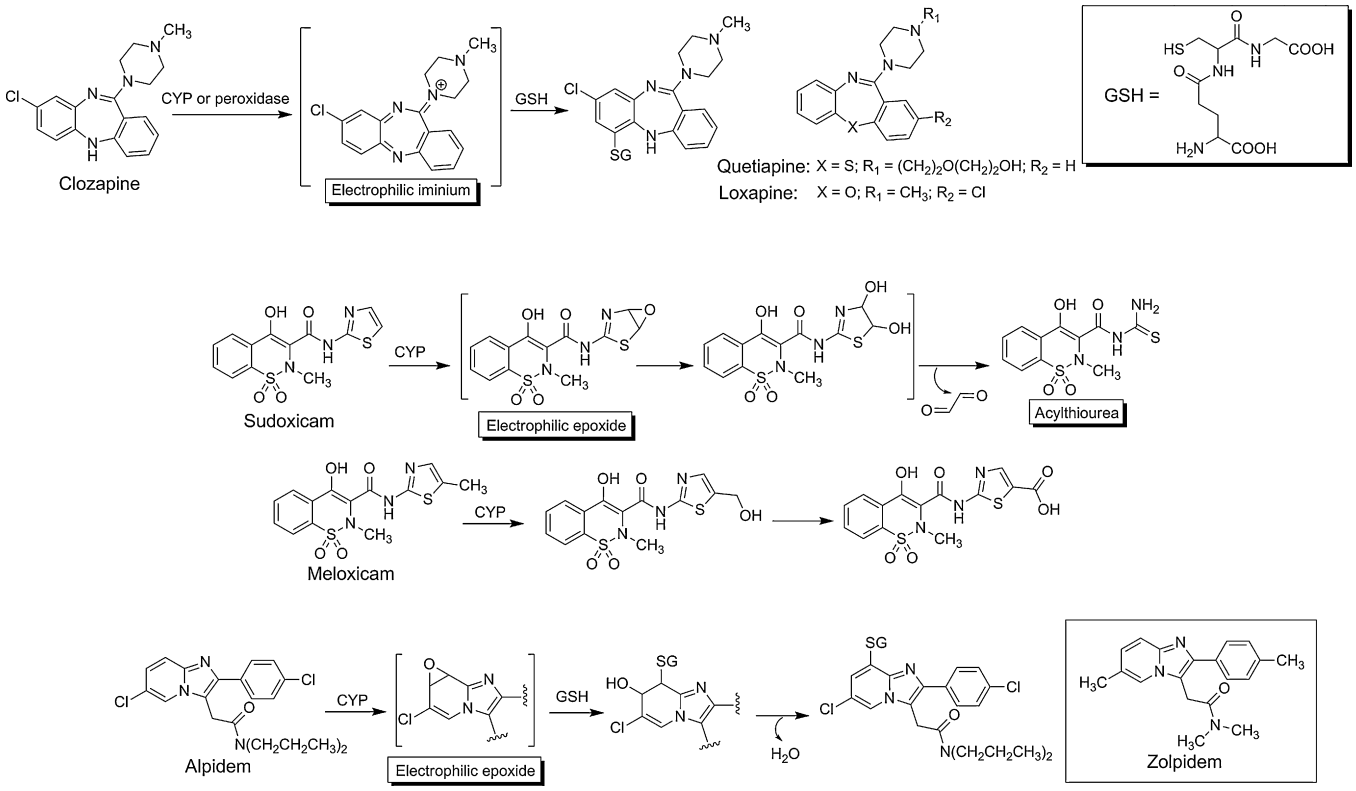

Fig. 1. Illustration of structure–toxicity relationships.

file. Although the evidence is most often anecdotal in nature, a compelling case for chemotype-based toxicity can be inferred from such structure–toxicity analyses. For instance, while clozapine use is limited by a high incidence of agranulocytosis, hepatotoxicity and myocarditis, quetiapine and loxapine are not associated with these adverse events. Clozapine exhibits covalent binding to human neutrophil proteins *in vitro*, via myeloperoxidase-mediated oxidation of the dibenzodiazepine ring to a reactive iminium ion, which covalently binds to the target tissues and also reacts with GSH (Fig. 1) [15,16]. Proteins covalently modified with clozapine have been observed in neutrophils of patients being treated with clozapine, which reaffirms the relevance of the *in vitro* studies [17]. In the case of quetiapine and loxapine, the bridging nitrogen atom is replaced with a sulfur or oxygen atom (see Fig. 1); consequently these drugs cannot form a reactive iminium [18]. Another retrospective analysis involves comparison of the anxiolytic agents alpidem and zolpidem. Alpidem was withdrawn from commercial use within the first year of its introduction due to several cases of severe hepatotoxicity [19]. In contrast, the structural analog and commercial blockbuster drug zolpidem does not possess the hepatotoxic liability. A key structural difference in the two drugs is the replacement of the two chlorine atoms in alpidem with two methyl groups in zolpidem (Fig. 1). In alpidem, the chloro-imidazopyridine ring is metabolized by CYP to a reactive epoxide that adducts with GSH; the detection of thiol conjugates in human excreta provides evidence for the existence of this pathway *in vivo* (Fig. 1) [20]. In contrast, zolpidem is metabolized via oxidation of both methyl groups to the corresponding alcohol and carboxylic acid metabolites and is not subject to RM formation [20]. In the case of the nonsteroidal anti-inflammatory drug (NSAID) sudoxicam, hepatotoxicity observed in the clinic that led to its suspension in clinical trials has been attributed to thiazole ring scission yielding a reactive acylthiourea metabolite capable of oxidizing GSH and proteins (Fig. 1) [21]. The structurally-related NSAID meloxicam does not possess the hepatotoxic liability associated with sudoxicam. Although introduction of a methyl group at the C5 position on the thiazole ring in meloxicam is the only structural difference, the change dramatically alters the metabolic profile such that oxidation of the C5 methyl group to the alcohol (and carboxylic acid) metabolite(s) constitutes the principal metabolic fate of meloxicam in humans (Fig. 1) [21]. It is noteworthy to point out that the successor drugs in these examples have not evolved from specific tactics to eliminate RM liability in predecessor agents. For example, in meloxicam, the C5 methyl group on the aminothiazole ring was essential for selective cyclooxygenase-2 inhibitory potency and was not specifically introduced to eliminate RM formation [22]. However, such retrospective analyses imply that by avoiding functional groups subject to RM formation (also referred to as structural alerts/toxicophores) in lead chemical matter, one would lessen the odds that a drug candidate will lead to idiosyncratic toxicity due to RM formation.

Although it is now possible, in most cases, to identify structures of RMs of drug candidates through *in vitro* and *in vivo* "trapping" studies with nucleophiles, it is not possible to predict *a priori* which (or any) of these electrophilic species would ultimately lead to toxicity, since the downstream biochemical consequences of RM formation remain unclear. The hapten hypothesis proposes covalent modification of protein(s) by a RM followed by cleavage to peptide fragments by a proteasome. These peptides are transported to the cell surface after binding to the major histocompatibility complex class I (MHC-I), and presented to the immune system. It is believed that abnormal peptides, when recognized by cytotoxic T-lymphocytes as non-self peptides, induce the toxic immune reaction finally leading to the cell death [23]. Idiosyncratic anaphylaxis associated with penicillin use, which is one of the best understood IADRs, is mediated via specific IgE antibodies against the drug [24]. The haptenization process involves non-enzymatic β-lactam ring scission by cysteinyl and/or terminal lysine residue(s) in proteins, leading to the acylation of amino acid nucleophiles [25].