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Minireview

Multiple effects of acetaminophen and p38 inhibitors: Towards pathway toxicology

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Abstract The majority of drug-related toxicities are idiosyncratic, with little pathophysiological insight and mechanistic understanding. Pathway toxicology is an emerging field of toxicology in the post-genomic era that studies the molecular interactions between toxicants and biological pathways as a way to bridge this knowledge gap. Using two case studies – acetaminophen and p38 MAPK inhibitors – this review illustrates how a pathway-based perspective has advanced our understanding of compound and target-based toxicities. The advancement of pathway toxicology will be dependent on integrated applications of techniques from basic sciences and a fundamental understanding of the interdependence of multiple biological pathways in living organisms.

Keywords: Systems biology; Mechanisms of drug-induced toxicity

1. Introduction

In the US alone there are 2.1 million drug-related injuries and 100000 deaths per year [1]. The vast majority of these drug-related toxicities are idiosyncratic, with little pathophysiological insight and mechanistic understanding. This apparent gap has triggered us to examine how a new perspective in Toxicology can provide us with greater insight in mechanisms of toxicity. Indeed, the history of Toxicology has evolved from a descriptive to mechanistic science, with new understandings provided by seminal breakthroughs in biology and medicine [2]. In the sixteenth century, Paracelsus promoted the concept of a "toxicon", the toxic agent, as a chemical entity. In the 1940s, Elizabeth and James Miller's work demonstrated that the human body converts the initial toxicon into metabolites, many of which are responsible for initiating the toxic responses in vivo [2]. Many current post-genomic technical advances (gene knockouts, RNA interference, whole genome scans, gene expression analysis, proteomics, metabonomics, and other panomics) provided modern toxicologists an expanded view of multiple interactions among molecular pathways. These breakthroughs provided clear illustrations that while a given toxicon may initially perturb primarily one biological pathway, the interactions and interdependence of molecular pathways almost inevitably leads to downstream perturbation of multiple signaling cascades with concomitant effects on physiologically relevant endpoints in the whole organism (Fig. 1). This has significant implications to our understanding of mechanisms of drug-induced toxicity. In this review, we will use two case examples to illustrate this point.

2. Acetaminophen-induced liver toxicity, from a single mechanism to multiple pathways

Acetaminophen, an non-steroidal anti-inflammatory drug (NSAID), has been widely used for over 50 years in the treatment of pain and fever and provides for the effective relief of these symptoms. It is safe when used at therapeutic doses. However, overdose (either intentional or accidental) can lead to serious and even fatal hepatotoxicity. In the US acetaminophen is responsible for up to 50% of all adult cases of acute liver failure [3].

In the liver, acetaminophen is metabolized to the non-toxic conjugated metabolites acetaminophen glucuronide and acetaminophen sulfate. These detoxification pathways account for over 90% of the acetaminophen metabolism by the liver. Under normal conditions, less than 10% of the acetaminophen is metabolized by the cytochrome P450 enzymes (primarily CYP 2E1, 1A2, and 3A4) to produce a toxic metabolic intermediate called *N*-acetyl-*p*-benzoquinoemine (NAPQI). The small amount of NAPQI formed is normally further detoxified by intracellular glutathione. In the cases of high drug load (such as intentional or accidental overdose), and/or low intracellular glutathione reserve (such as after fasting or alcohol use), NAPQI can covalently modify thiol groups on cellular proteins. The traditional thought is that such covalent protein adducts are the cause of liver cell or hepatocyte injury [3]. However, it was demonstrated that the generation of NAPQI is necessary but not

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Abbreviations: MAPK, mitogen-activated protein kinase; NAPQI, *N*-acetyl-*p*-benzoquinoemine; TNF, tumor necrosis factor; NO, nitric oxide; NSAID, non-steroidal anti-inflammatory drug; IVIVC, invitro invivo correlations; IVT, in vivo toxicology; ADME, absorption distribution metabolism excretion; PPARα, peroxisome proliferator-α; (IL-1), interleukin-1; EGFR, epidermal growth factor receptor; ERK, extracellular regulated kinase; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of MMP; TUDC, tauroursodesoxycholate; BSEP, bile salt export pump

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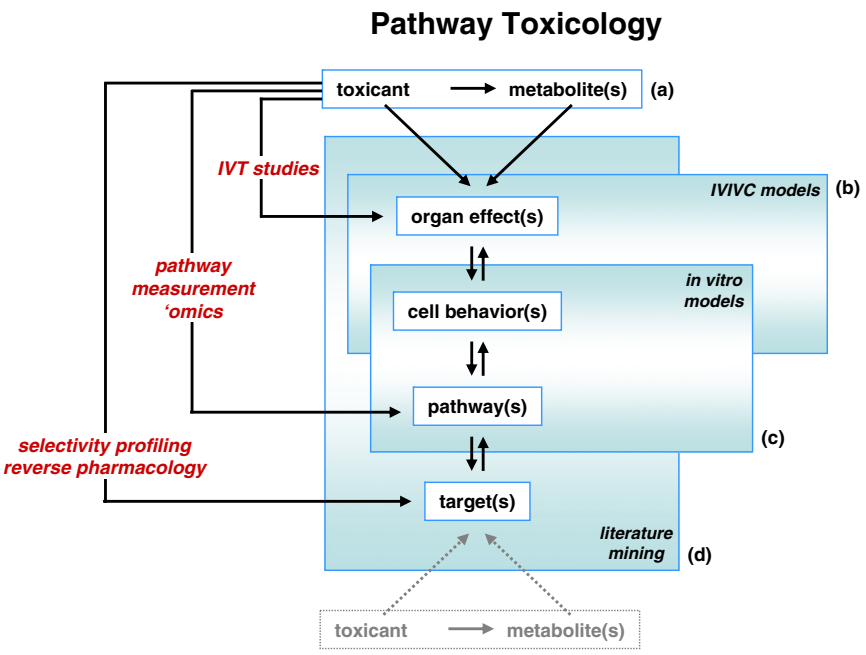

Fig. 1. An integrated view of pathway toxicology. (a) multiplication of toxicons: a toxicant may be converted to metabolites by families of biological enzymes; (b) in vivo toxicology (IVT) studies can uncover initial organ effects; (c) in vitro models and pathway measurements can elucidate cell behaviors and pathways; (d) literature mining, expression, and other omics profiling of the toxicant and its metabolite can dissect the ultimate targets that are responsible for the observed adverse effects on pathways, cell behaviors, and organ effects.

sufficient to account for acetaminophen-induced liver injury. The *m*-hydroxy isomer of acetaminophen, 3′-hydroxyacetanilide, is not hepatotoxic in mice, even though the amounts of covalent binding are almost equivalent [4]. The covalent binding of 3′-hydroxyacetanilide is also located in the centrilobular hepatocytes, the area of the ensuing necrosis and covalent binding by the hepatotoxic isomer acetaminophen [5]. These and other studies raised the possibility that the amount of covalent binding is not a determinant of the final toxicity outcome.

Recently, genomic, metabolomic and proteomic approaches have been applied in the human hepatocyte chimeric mice model to investigate the mechanisms and pathways of acetaminophen induced toxicity [6]. In this animal model, human hepatocytes were transplanted into transgenic mice. The replacement ratio of mouse liver with human liver in chimeric mice was estimated at 75–95%, with human specific metabolic responses to drugs observed. In acetaminophen-treated animals, perturbations in lipid metabolism, fatty acid transport, and glycolysis indicated suppression of the β-oxidation pathways of fatty acids, leading to the depletion of acetyl-CoA. Perturbations in the oxidative stress-related proteins such as peroxiredoxin 1 and catalase, were observed. These findings are in accordance with those observed in normal mice treated with acetaminophen [7]. These data suggest that both intracellular mitochondrial and oxidative stress pathways were perturbed by the treatment of acetaminophen.

The question still remains as to whether it was the parent acetaminophen or the NAPQI metabolite responsible for the observed changes in mitochondrial energy status and oxidative stress. Data from the in vitro treatment of freshly-isolated mouse hepatocytes have provided mechanistic insight [8]. In this in vitro model, acetaminophen-induced cell death occurs in two phases, a metabolic phase and an oxidative phase. The metabolic phase occurs with NAPQI formation, glutathione depletion and covalent protein binding. The oxidative phase occurs with increased oxidative stress, loss of mitochondrial membrane potential, mitochondrial permeability transition, and resulting toxicity. The oxidative phase does not require the presence of either acetaminophen or the NAPQI metabolite, as addition of acetaminophen in the second phase did not alter toxicity [8]. This study raised the interesting possibility that while both acetaminophen and 3′-hydroxyacetanilide can initiate a similar metabolic phase, acetaminophen is unique in its ability to progress to the oxidative phase at least in the mouse hepatocytes. Further pathway studies using these two isomeric chemicals and the mouse hepatocyte model are required to delineate the differences in the triggering events leading the metabolic phase to oxidative phase.

In addition to these in vitro studies, a series of knock out mice were used to investigate their susceptibility to acetaminophen induced hepatotoxicity (e.g., reviewed by Kaplowitz [9]). Not surprisingly, cytochrome 2e1 and 1a2 null mice offered protection against acetaminophen toxicity, indicating the importance of the metabolic phase [10]. Glutathione synthesis and detoxification enzymes are regulated by the transcription factor Nrf2, and Nrf2 null mice are more susceptible to acetaminophen toxicity [11]. Interestingly, several key proteins in the cytokine family and the innate immune system have emerged as important modulators of the progression and severity of organ damage. Interferon, Fas or Fas ligand null mice are resistant, while interleukin-10 and -6 null mice are susceptible to acetaminophen toxicity. In most of these knockout models, alterations in toxicity were not associated with changes in the amount of covalent binding or glutathione depletion (reviewed by Kaplowitz [9]).