A toxicologist’s guide to biomarkers of hepatic response

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Biological markers (biomarkers) are used to recognize, characterize and monitor treatment-related responses following exposure to xenobiotics. Biomarkers serve three primary applications in toxicology: 1) to confirm exposure to a deleterious agent, 2) to provide a system for monitoring individual susceptibility to a toxicant, and 3) to quantitatively assess deleterious effects of a toxicant to an organism or individual. Because the liver is a general target for adverse effects of drugs and other chemicals, biomarkers of unidirectional hepatic response to xenobiotics are of particular interest to the pharmaceutical toxicologist. General requirements for the latter category of biomarkers are sample availability, target organ specificity, sensitivity for the toxicity of interest, accessibility, a relatively short half-life, and available detection systems. Biomarkers that can be assayed in biological fluids from both human and animal subjects are particularly desirable. Histologically, acute and subacute hepatic toxicity commonly involves necrosis, steatosis, cholestasis, vascular disorders, or multiple lesions. The purpose of this review is to summarize reported applications using clinical analytes and biochemical indicators of hepatic dysfunction with emphasis on those that show promise of supplementing or improving upon standard laboratory procedures. Liver function markers refer to peripheral indicators of hepatic synthetic and secretory activities, enterohepatic function, or perturbations of the hepatic uptake and clearance of circulating biomolecules. Liver injury biomarkers include various peripheral proteins released in response to a cellular damage or locally, proteins that are significantly altered within the liver. These include both circulating cytosolic, mitochondrial, or canalicul membrane markers, and the up-regulation or depletion of radical scavengers, modulators, and stabilizers of intracellular damage. Subsequent recovery from a toxic insult involves repair, regenerative, and proliferative responses that constitute the third class of biomarkers. Of these, protein markers found either in sera, plasma, or urine either during or just prior to the early manifestation of histological hepatic lesions are of greatest interest. Examples of a number of these markers, their documented applications in humans or animals, and potential advantages as well as limitations are presented. Human & Experimental Toxicology (2002) 21, 253–262.

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Applications in toxicology

Biomarkers serve three distinct toxicological applications. The first involves the assessment of exposure to a potentially deleterious agent. This application is particularly useful in environmental or occupational studies. In the simplest case, biomarkers of exposure can include the substance of interest or its free or conjugated metabolites in biological fluids. Examples include the formation of DNA adducts, protein adducts, the induction of specific sequestering proteins such as the heavy-metal-binding metallothionein, or marked increases for certain serum sialoglycoconjugates during heavy metal toxicity. Formation of a reactive metabolite or binding specificity is often required. Protein adducts are generally more useful than DNA adducts because both cytosolic proteins and circulating proteins such as haemoglobin and serum albumin are generally more accessible to a wide range of chemical reactants and, unlike nuclear DNA, are not affected by repair mechanisms. Protein adducts tend to be stable and relatively long-lived in the body. Recent examples of occupational monitoring of peripheral fluids for specific biomarkers include the detection of haemoglobin adducts of some 1,3-butadiene metabolites, plasma β-glucuronidase of liver origin as a more sensitive and rapid biomarker of organophosphorous insecticide poisoning compared to plasma acetylcholinesterase inhibition, and epichlorohydrin DNA adducts in circulating blood cells during occupational exposure. The induction of hepatic drug-metabolizing

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enzymes indicative of a particular class of xenobiotics, as for example, CYP4A induction following exposure to di-[2-ethyl-hexyl] phthalate can serve as a sensitive indicator of exposure in male rats, which are susceptible.

A second major biomarker application involves monitoring to gauge susceptibility of a subject to the toxicant, especially in genetically heterogeneous populations or species with a high incidence of genetic polymorphism. This category has its greatest utilization in clinical monitoring, either to select representative responders as participants for drug treatment or to monitor high-risk individuals in the postmarketing phase when genetic susceptibility to toxic injury has been clearly established. A high incidence of polymorphism in some human hepatic drug-metabolizing enzymes, for example, can result in substantial variation in susceptibility to the deleterious effects of certain drugs extensively metabolized by the liver. Periodic clinical monitoring of conventional serum markers of hepatic injury such as the transaminases is a routine postmarketing recommendation for some chronic-use drugs including certain HMG-CoA reductase inhibitors, nonsteroidal anti-inflammatories, and antihypertensive agents.

A third application is the use of biomarkers to assess the potential of a therapeutic agent to act as a toxicant in the organism as part of the safety evaluation process. Inherent in this application is the demonstration of a mechanistic correlation between the biomarker and the unintentional biological activity or deleterious effect of the chemical in the liver. A well known example is the formation of the toxic reactive metabolite (N-acetyl-p-benzoquinoneimine) from acetaminophen when detoxification by conjugation is overwhelmed, leading to acute hepatocellular necrosis and elevated levels of the serum biomarker, alanine aminotransaminase. In humans, altered prothrombin time is a prognostic indicator of acetaminophen-induced fulminant hepatic failure. Toxicity indicators can originate at the cellular or molecular level in parenchyma cells, cholangiocytes, endothelial cells, Kupffer cells, or other liver cell types. Biomarkers that can be assayed in biological fluids of both human and animal subjects are particularly useful although even hepatic biomarkers with no peripheral counterpart can be investigated in preclinical species or, in some cases, human hepatocyte cultures (Table 1).

Conventional clinical chemistry markers that include liver predominant or specific enzymes released from damaged liver cells to the blood, altered levels of circulating bilirubin or bile salts normally cleared via hepatic transport systems, or diminished circulating blood levels of transport proteins or clotting factors synthesized by the liver, are used by the clinical pathologist as indicators of deleterious parenchymal or biliary effects. A listing of conventional clinical chemistry, hematology, and urinalysis procedures used for toxicological screening studies can be found elsewhere. This review will briefly summarize currently used biomarkers and emphasize other, less conventional biomarkers that have been described in the recent literature.

### Biomarkers associated with major categories of liver injury

Zimmerman has extensively described drug-induced hepatic injury, classifying acute hepatic injury as either cytotoxic or cholestatic. Cytotoxic lesions include hepatocellular necrosis, steatosis, or a combination of the two. Both categories are characterized by relatively specific biomarkers.

Steatosis or fatty liver results from an excessive accumulation of intracytoplasmic neutral lipid in a microvesicular or macrovesicular pattern within hepatocytes. Based on the number of chemical toxicants known to cause it, microvesicular steatosis is more common in subacute drug-induced liver injury whereas chronic steatosis is primarily macrovesicular in nature. This cellular lesion can appear as either a zonal or diffuse change depending upon the toxicant and exposure conditions. Pathogenesis of fatty liver may be due to increased hepatic synthesis of fatty acids, decreased oxidation or utilization of fatty acids, or can be associated with impaired secretion of hepatic triglycerides due to diminished lipoprotein formation or the diminished release of complete lipoproteins. Although serum AST may be slightly elevated, steatosis is diagnosed by histopathological examination. Specific peripheral

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**Table 1** Six general properties that describe a useful biomarker of drug-induced hepatic toxicity

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Availability</td>
<td>Present in biological fluids such as blood or urine at detectable levels.</td>
</tr>
<tr>
<td>Specificity</td>
<td>Exclusively or predominately of liver origin or quantitatively affected primarily by normal hepatic function.</td>
</tr>
<tr>
<td>Prevalence</td>
<td>Characteristic of similar hepatic condition or function in multiple species including human.</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Substantially, quantifiably, and reversibly altered as the result of liver injury or dysfunction at sublethal, nonfatal drug levels.</td>
</tr>
<tr>
<td>Persistence</td>
<td>Stable under extrahepatic physiological conditions, but with a half-life short enough to permit dose–response and recovery determinations over a period of days or weeks.</td>
</tr>
<tr>
<td>Relevancy</td>
<td>Confirmed to be associated with the same hepatic histological or functional affects by independent studies.</td>
</tr>
</tbody>
</table>

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biomarkers for this lesion remain elusive although circulating lipids can be affected and subsequent lipid peroxidation can produce characteristic markers (see below).

Acute cholestatic injury is termed hepatocanalicular, cholangiolytic, or cholestatic hepatitis when accompanied by portal inflammation and slight hepatocyte injury and termed canalicular, bland, pure, or steroidal cholestasis when accompanied by little inflammation and even less hepatocyte injury. Mechanistically, hepatocellular (intrahepatic) cholestasis refers to a functional defect in hepatocellular bile formation while ductal cholestasis (obstructive or extrahepatic cholestasis) results from a blockage in bile secretion and flow within the bile ducts.

When toxicants produce parenchymal injury, hepatocytes die as the result of apoptosis or necrosis. Depending on the agent, hepatocyte death can occur in a diffuse (focal) pattern or can be found predominantly in specific regions (zonal pattern) as for example, periportal or centrilobular zones (acinar zones 1 and 3, respectively), or can involve major portions of the entire liver (panlobular). Subsequent to initial altered membrane permeability or final disruption that accompanies necrotic death, cytosolic enzymes (e.g., alanine aminotransferase [ALT], sorbitol dehydrogenase [SDH]), and sometimes mitochondrial marker enzymes (e.g., ornithine carbamyltransferase [OCT]) are released into the blood plasma or serum. Mechanisms leading to toxicant-induced liver necrosis vary and include mitochondrial dysfunction, covalent binding of reactive moieties to cell macromolecules, lipid peroxidation, damage to the cytoskeleton, mitochondrial permeability transition, or less commonly, secondary effects involving immune responses.

Hepatic veno-occlusive disease (HVOD) is a potentially serious adverse effect, and occurs as a regimen-related toxicity following high-dose chemotherapy and hematopoietic stem cell transplantation. Pyrrolizidine alkaloid poisoning can also cause HVOD as for example, through the administration of herbal preparations. Risk factors for HVOD are well established, but the biology of the syndrome remains poorly understood. Although HVOD is caused by the disruption of the microcirculation by an as yet unknown mechanism, in vitro studies have suggested that toxins that cause HVOD initially target sinusoidal endothelial cells (SECs) perhaps via profound glutathione (GSH) depletion. The pathogenesis of VOD of the liver appears to be secondary to endothelial damage of terminal hepatic venules, which leads to activation of the coagulation cascade, fibrin deposition, and eventual fibrous obliteration of the hepatic venules. VOD is accompanied with a decrease of natural anticoagulants such as protein C, which serve as useful biomarkers. Clinical diagnosis of HVOD is based on weight gain, hepatomegaly, and jaundice.

Other types of acute and subacute hepatic injury include mixed necrotic and cholestatic lesions, inflammation and infiltration, and vascular disorders other than VOD. Traditional methods for monitoring biomarkers indicative of liver injury include serum biochemistry, cytochemistry, immunochemical methods based on biochemical, histological, morphological, and physiological changes in whole organisms; however, changes at the cellular and molecular levels of organization, especially in nucleic acids and proteins, are increasingly being used to supplement these more traditional biomarkers. A number of these will be described.

Blood serum or plasma biomarkers

Blood represents a very convenient protein pool for repetitive biomarker analysis over time. Serum liver enzymes evaluated in preclinical animal studies may predict adverse liver effects in humans provided all appropriate interspecies differences are taken into consideration. Altered liver synthetic function can be ascertained by treatment-related decreases in transport proteins (e.g., albumin, hemopexin, thyroxine-binding globulin, transthyretin, transferrin), while circulating total bilirubin is used to monitor excretory function. The hepatic synthesis of coagulation proteins can be affected by liver dysfunction. Urea cycle enzymes (e.g., carbamoyl-phosphate synthase and arginosuccinate synthetase) which determine the rate and flux of the cycle, can be suppressed during drug-induced necrosis of the periportal area. Hepatocellular necrotic injury can result in elevated peripheral activities of SDH, ALT, and OCT while 5’nucleotidase (5’NT), gamma-glutamyltransferase (GGT), or alkaline phosphatase (ALP) elevations are indicative of cholestatic injury although the latter two are not liver specific. OCT and GGT may be inducible proteins in some circumstances. Thus, elevations in blood OCT or GGT activities following exposure to xenobiotics can indicate either hepatic microsomal enzyme induction or hepatic injury.

Although a clear association between treatment-related increases in ALT and SDH activities and pathological changes in the liver has been demonstrated, in a study of the relative sensitivity of eight commonly used end-points, SDH had greater positive and negative predictive values than similar changes in ALT in a series of sixty-one 13-week rat toxicity studies. While hepatic ALT is located predominantly in the periportal area, plasma isocitrate
dehydrogenase may be more useful as a marker for centrilobular necrosis.\textsuperscript{30} Other recent clinical findings have suggested serum cystatin C as a potential marker for liver fibrosis,\textsuperscript{31} serum hyaluronic acid for liver cirrhosis,\textsuperscript{32} and serum levels of procollagen III peptide were found to be related to fibrogenesis in chronic hepatitis patients.\textsuperscript{33} Serum levels of the oxidative stress marker, thioredoxin, are reportedly elevated in chronic liver disease.\textsuperscript{34} For markers of cholestasis, the aforementioned study\textsuperscript{29} concluded that, compared to total bile acid concentration, serum alkaline phosphatase may be more useful as an indicator of decreased food intake (decreased activity) than of cholestasis (increased activity). Because diet has a considerable effect on the protein content of plasma in all species, it is critically important to monitor peripheral biomarkers under carefully controlled conditions.

In a study of sequential plasma samples from nine patients with acetaminophen poisoning, glutathione S-transferase and F protein reportedly offered clear advantages over ALT for detecting minor degrees of acute liver dysfunction, particularly when only centrilobular damage may be involved.\textsuperscript{35} Studies have suggested that serum F protein, which is not inducible like some marker enzymes, is an indicator of hepatocellular dysfunction associated with anticonvulsant therapy\textsuperscript{36,37} and may serve as a sensitive specific marker of hepatocellular damage in humans.\textsuperscript{38} Other serum proteins that may be potentially useful as markers of liver injury include malic dehydrogenase elevations,\textsuperscript{39} marked accumulation of asialoglycoproteins,\textsuperscript{40} elevations of the calcium binding protein regucalcin\textsuperscript{41,42} and liver-type arginase (arginase 1), a urea cycle enzyme.\textsuperscript{43,44} All of these latter biomarkers merit further study.

**Biomarkers of detoxification and recovery**

**Regenerative markers**

Conventional serum biomarkers include injury-related release of cellular constituents, attenuated production of liver proteins or the altered release of hepatic lipids (lipoproteins, triglycerides, and cholesterol). Cholestatic liver dysfunction is characterized by impaired extraction, biotransformation, and excretion of bilirubin and/or altered synthesis, intracellular metabolism, and excretion of bile acids leading to altered blood levels that are routinely detected by altered serum chemistries. Beyond these, a number of other potential markers are of current investigational interest to the research toxicologist. These generally signal a protective or regenerative response to the injury. Transient elevations of proteolytic enzymes for tissue remodeling after cell injury or acute phase response proteins for limiting or repairing tissue damage may also function as sensitive biomarkers. Following acute necrotic injury, a compensatory cell proliferative response can result in increasing levels of circulating α-fetoprotein (AFP), GGT, retinol-binding protein (RBP), and des-γ-carboxy-prothrombin (DCP).\textsuperscript{45–47} Chronologically, elevations of these regenerative markers follow the decline of necrotic markers such as ALT or AST and signal recovery. But AFP and DCP can also be elevated by other anomalies such as hepatocellular carcinoma\textsuperscript{48} and plasma levels of RBP are altered by renal toxicity,\textsuperscript{49} which may limit application for chronic toxicity studies. Nevertheless, for short-term toxicity studies, regenerative and proliferative protein markers may provide useful and sensitive indicators when early necrotic markers are no longer elevated.

**Scavengers and modulators**

Another category of defensive biomarkers includes scavengers and modulators. These may increase in response to a toxic insult or decrease during a fulminating response to a toxic insult. For example, as a key component of HDL expressed mainly in the liver, serum paraoxonase (PON1) activity is elevated following exposure to organophosphorus insecticides.\textsuperscript{50} It has been proposed that the PON family of enzymes may protect against several other types of acute and chronic toxicities associated with cellular oxidative damage.\textsuperscript{51} Cellular stress proteins and molecular chaperones are responsive to a variety of stressors and therefore may comprise an ideal set of proteins with the potential to be used as biomarkers of chemical toxicity.\textsuperscript{52} These could be particularly informative in situations where rapid systemic recovery might mask pathophysiological changes due to toxicant exposure, i.e., they may offer enhanced sensitivity following low levels of exposure. But because they are intracellular proteins, their utility as biomarkers may be limited to in vitro systems if they are not released from affected cells in vivo. Stress proteins (Hsps) and other molecular chaperones are highly conserved, participate in protein folding and transport, and promote cell recovery via protein stabilization following damage caused by local or systemic stressors.\textsuperscript{53} At least four heat shock proteins, three glucose-regulated proteins, protein disulfide isomerase, and ER-60 are constitutively expressed at detectable levels in unstrained human and rat liver\textsuperscript{54} and thus can be monitored in liver tissue. Some in vitro models, however, have indicated that stress proteins may be relatively insensitive indicators of compound-induced toxicity compared to more conventional end-points.\textsuperscript{55} The reticuloplasmins GRP94, protein disulfide isomerase (PDI), BiP (GRP75), RP60, and CRP55, which are...
normally retained within the ER, are sensitive to cellular calcium perturbations, which can promote their secretion. Trifluoroacetylated PDI has been identified as one of the immunogens associated with halothane hepatitis. Both PDI and calreticulin, which binds to denatured proteins, have been identified as autoimmune antigens in the Long–Evans Cinnamon rat, which is characterized by hereditary spontaneous hepatitis. This suggests the possible role of reticuloplasmins in immune-mediated liver toxicity.

**DNA and lipid markers**

Studies in rats have demonstrated that, among various lipoproteins, plasma triglyceride and VLDL are more sensitive markers than ALT following exposure to CHCl₃, a known liver toxicant. Oxidative damage to lipid can result in elevated levels of malondialdehyde (MDA), a degradation product of lipid peroxides. MDA can be monitored in blood although it is not specific to liver. Chemically stable F₂ isoprostanes are another class of lipid peroxidation end products that can be measured in urine. Oxidative damage to DNA can result in the production of 8-hydroxy-2-deoxyguanosine (OH8dG). Exposure to dielldrin, which produces oxidative stress in mouse liver, results in increased hepatic and urinary MDA and OH8dG. Thus, biomarkers of liver toxicity are not limited to proteins.

**Acute phase, conjugating, and other proteins**

Within the liver, enzymes that catalyze the conjugation of xenobiotics or their metabolites include glutathione S-transferase and the sulfotransferases ST1A1, ST1C1, and ST2A1 among others. Elevated serum levels of cerebroside sulfotransferase have been reported for patients with hepatocellular carcinoma. Plasma β-glucuronidase of hepatic microsomal origin is reportedly a very sensitive biomarker following acute intoxication by organophosphates and carbamates, although it may not be liver specific. As previously mentioned, cytosolic α-glutathione S-transferase is of particular interest to the toxicologist as a circulating enzyme marker of hepatic damage.

Acute phase reactants (e.g., α₁-acid glycoprotein, C-reactive protein, C₃ component of complement, haptoglobin, α₁-antitrypsin) are produced primarily in liver and are responsible for limiting and repairing tissue damage. Following allyl alcohol intoxication, the hepatic gene expression of ‘positive’ acute phase proteins was elevated and increased with the dose, but the mRNA of the urea cycle enzymes and glutamine synthetase was uniformly reduced.

In general, proteins that act as radical scavengers, modulators or stabilizers of macromolecular or cellular damage, or that participate in a repair or regenerative response can be useful and sensitive biomarkers of moderate liver toxicity. But with notable exceptions, relatively few can readily be found in biological fluids and those that are may be responding to systemic or nonhepatic insults. Further exploration of some of these biomarkers may be restricted to liver biopsies or whole liver studies in nonclinical species while others such as α-GST can be detected peripherally.

**Urine as a source of biomarkers of hepatic response**

In general, physicochemical analysis of urine for biomarkers is a useful tool for assessing renal function and injury, but some recent reports suggest that urine protein analysis may provide information on liver injury as well. For example, agents that interfere with the heme biosynthesis pathway can alter urinary porphyrins. A recent report indicated that eight degradation products of lipid peroxides including MDA were useful noninvasive biomarkers for carbon tetrachloride damage in the rat liver when measured as urinary products. Similar increases in the urinary excretions of lipid peroxide degradation products were also noted in studies with rats treated with diquat although it produced slight nephrotoxicity as well as slight hepatotoxicity. NMR-based metabolomic methods using urine samples from toxin-dosed rats are also promising techniques for biomarker analysis. When H NMR spectroscopy methods were used to investigate biochemical variability in urine from rats treated with hydrazine as a model hepatotoxicant known to produce steatosis, there were elevated urinary levels of taurine and creatine and changes in other biomarkers subsequent to dosing. The latter are markers for hepatotoxicity. Studies with aflatoxin B₁ in rats has confirmed that urinary taurine appears to be a useful, noninvasive marker when hepatotoxicity is extensive. Others have used H NMR spectroscopy of urine combined with pattern-recognition methods of data analysis to investigate the time-related biochemical changes induced in rats by three model hepatotoxins: alpha-naphthyl isothiocyanate (ANIT), D-(+)-galactosamine (GalN), and butylated hydroxytoluene (BHT). Biomarker changes common to all three hepatotoxins included a reduction in the urinary excretion of citrate and 2-oxoglutarate and an increased excretion of taurine and creatine. Increased urinary excretion of betaine, urocanic acid, tyrosine, threonine, and glutamate was characteristic of GalN toxicity. Both GalN and ANIT caused increased urinary excretion of bile acids, while glycosuria was evident in BHT- and ANIT-treated rats.
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Source</th>
<th>Timing</th>
<th>Biomarker properties</th>
<th>Proposed indication</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine aminotransferase</td>
<td>Sera of rat, human, and other species</td>
<td>Rapid but may be transient</td>
<td>1, 3, 4, 5, 6</td>
<td>Periportal hepatocellular necrosis or increased membrane permeability</td>
<td>Originates in periportal cytoplasm and mitochondria. Affected by fasting or glucocorticoids in some species. Specificity varies with species.</td>
<td>23, 29, 30, 75, 76</td>
</tr>
<tr>
<td>Sorbitol dehydrogenase</td>
<td>Rat serum</td>
<td>1–3 weeks</td>
<td>1, 2, 4, 5, 6</td>
<td>Hepatocellular necrosis</td>
<td>A cytoplasmic enzyme. Human serum data is limited. Sensitive predictor of morphological change in rat.</td>
<td>26, 29, 75, 77</td>
</tr>
<tr>
<td>Protein C (factor XIV) or Ornithine carbamyltransferase</td>
<td>Human plasma</td>
<td>Concurrent with lesion</td>
<td>1, 2</td>
<td>Decreased with hepatic veno-occlusive disease</td>
<td>A fibrinolytic protein synthesized in liver.</td>
<td>20, 21</td>
</tr>
<tr>
<td></td>
<td>Sera of rat, human and other species</td>
<td>Within 18 hours</td>
<td>1, 2, 3, 4, 5, 6</td>
<td>Hepatocellular necrosis</td>
<td>Urea cycle enzyme found in mitochondria. Less studied in human. Inducible in rat liver. Very liver specific.</td>
<td>26, 27, 75, 76</td>
</tr>
<tr>
<td>5’Nucleotidase</td>
<td>Sera of rat, human and other species</td>
<td>Fairly rapid</td>
<td>1, 3, 4, 5</td>
<td>Hepatobiliary effects</td>
<td>A membrane enzyme found in kidney, pancreas, and liver, but usually considered liver specific when found in serum.</td>
<td>75, 76</td>
</tr>
<tr>
<td>Gamma glutamyl-transpeptidase</td>
<td>Rat and human sera</td>
<td>Concurrent with lesion</td>
<td>1, 3, 4, 5, 6</td>
<td>Hepatobiliary effects</td>
<td>Originates in canalicular surfaces of hepatocytes and bile duct cells and found in kidney. Inducible in humans and rats. Serum GGT originates in the hepatobiliary system.</td>
<td>27, 28, 75, 76</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>Sera of several species</td>
<td>Concurrent with lesion</td>
<td>1, 4, 5, 6</td>
<td>Hepatobiliary effects</td>
<td>Ubiquitous plasma membrane enzyme; inducible in dogs. Not liver specific but useful.</td>
<td>15, 26, 75, 76</td>
</tr>
<tr>
<td>α-Glutathione S-transferase</td>
<td>Human plasma, rat serum</td>
<td>Within hours</td>
<td>1, 2, 3</td>
<td>Centrilobular hepatocellular injury; early sensitive marker</td>
<td>High cytosolic concentration throughout liver. Short half-life. GST-B is liver specific.</td>
<td>35, 66, 76</td>
</tr>
<tr>
<td>Biomarker</td>
<td>Species and source</td>
<td>Appearance</td>
<td>Hepatic responses</td>
<td>General description</td>
<td></td>
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<tr>
<td>F-protein</td>
<td>Human serum</td>
<td>Within hours</td>
<td>1, 2, 4, 5.</td>
<td>Acute liver dysfunction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malic dehydrogenase</td>
<td>Human and rat sera</td>
<td>Similar to AST</td>
<td>1, 3, 4, 5.</td>
<td>Liver damage; acute hepatitis. Also hepatocellular carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asialoglycoprotein</td>
<td>Human and rat sera</td>
<td>Within days</td>
<td>1, 2, 3</td>
<td>Accumulates during cellular necrosis, but also with liver carcinoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regucalcin</td>
<td>Human and rat serum</td>
<td>10 and 24 hours; similar to ALT and AST</td>
<td>1, 2, 4</td>
<td>Chronic liver injury and subacute injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginase 1</td>
<td>Human and rat sera</td>
<td>Can precede ALT and AST</td>
<td>1, 2, 3, 4, 5.</td>
<td>Hepatitis; chemically induced liver damage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipids including VLDL, triglycerides, cholesterol</td>
<td>Rat plasma</td>
<td>19–32 hours</td>
<td>1, 2, 4</td>
<td>Liver dysfunction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Fetoprotein</td>
<td>Human and rat sera</td>
<td>3–4 days; 6–8 days</td>
<td>1, 3, 4, 5, 6.</td>
<td>Hepatocyte proliferation (regeneration) following liver injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinol-binding protein (RBP)</td>
<td>Human serum</td>
<td>6–8 days</td>
<td>1, 2, 4, 5.</td>
<td>Hepatocellular regeneration following liver injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α1 acid glycoprotein</td>
<td>Rat sera</td>
<td>48 hours</td>
<td>1, 2, 4, 5.</td>
<td>Tissue repair in response to injury (heavy metal toxicity)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malondialdehyde (MDA)</td>
<td>Mouse and rat urine</td>
<td>7 days; 12 hours</td>
<td>1, 5</td>
<td>Oxidative damage to lipid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taurine</td>
<td>Rat urine</td>
<td>48–96, 48–120, and 72–120 hours</td>
<td>1, 4, 5, 6.</td>
<td>Acute hepatic damage, centrilobular necrosis, cholestasis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Representative timing information, proposed indications, and general descriptive information are summarized from the indicated references. These summaries are provided for comparative purposes only. The biomarker properties refer to the six attributes described in Table 1 and are designated here as follows: 1=availability, 2=specificity, 3=prevalence, 4=sensitivity, 5=persistence, and 6=relevancy.
The combination of urinary metabolites, which were significantly altered at various time points, allowed for differentiation between biliary and parenchymal injury. Other recent studies in rats have demonstrated that NMR-based metabonomic techniques can permit visualization of key time periods in the development of toxic injury enabling the identification of lesion-specific, matrix-specific biomarkers of cholestasis and hepatotoxicity. Further work, especially studies using NMR spectroscopy, appears to be promising and can easily be extended to humans in many cases. Using pattern-recognition methods such as principal component analysis, a combination of several biomarkers in peripheral fluids may provide greater sensitivity and selectivity than possible with individual markers.

Summary

A summary of the principal biomarkers surveyed here is found in Table 2. Blood components and urine provide especially rich sources of liver biomarkers including but not limited to proteins indicative of initial cellular damage, altered hepatocellular function, and recovery from cellular damage and restoration of function. Requirements for a robust serum protein biomarker indicative of liver toxicity can be summarized as follows. 1) It should be highly conserved across a range of species to permit extrapolations between species. 2) It should not be significantly altered by fasting, food restriction, or diet. 3) Changes in activity or concentration should be associated with liver histopathological change or other accepted benchmarks of toxicity, either concurrently or subsequently manifested. 4) It should be present in detectable quantities in biologic fluids and proportionally modified in accordance to the degree of toxicity. Biomarkers can also include DNA and protein adducts, lipids, and lipid degradation products. In addition to conventional clinical chemistry end-points, pharmaceutical toxicologists are currently evaluating several categories of biomarkers. These categories include peripheral protein markers released as a result of hepatocellular necrosis or hepatic cholestasis, circulating secretory proteins synthesized by the liver and sensitive to functional impairment, metabolic products or carriers normally cleared during normal liver function, and cellular marker proteins that indicate regeneration, recovery, or act as scavengers, detoxification proteins, modulators, or stress proteins. Although plasma or serum biomarkers have been more frequently reported in the literature, sophisticated methods for urinalysis may produce additional non-invasive biomarkers for both clinical and preclinical toxicology studies. Recent developments for data analysis involving pattern recognition or multivariate methods may increase both sensitivity and selectivity of biomarker analysis in the near future. With further investigation and confirmation, some of these biomarkers may eventually supplement or even replace the more standardized biomarkers used by the clinical pathologist.

References


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