Proceeding of the NAVC
North American Veterinary Conference
Jan. 8-12, 2005, Orlando, Florida

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WHAT WORKS AND WHAT DOESN’T IN TREATING LIVER DISEASE

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THE FIRST STEP:
DEFINING DISEASE CHARACTERISTICS

Definitive histomorphologic characterization of hepatobiliary disease (routine and special stains for fibrosis, metals, infectious agents), combined with tissue culture (aerobic and anaerobic bacterial), cytologic imprints of biopsy specimens (may disclose infectious agents not recognized on histology), and quantitative metal analyses (copper [Cu], iron [Fe], zinc [Zn]) assist in selecting appropriate interventional therapies.

THE SECOND STEP:
CONSIDERING MECHANISMS OF LIVER INJURY

OXIDATIVE MECHANISMS

Recognizing the complexities of cellular and molecular mechanisms leading to liver injury and fibrosis is critical for selecting interventional strategies. Oxidative injury (and formation of reactive oxygen species [ROS]) is a central pathomechanism in most forms of acquired liver injury (consult another presentation at this meeting specific to oxidative injury and therapeutic interventions).

TOXINS, ENDOTOXINS, INFECTIOUS AGENTS

Given the central role of the liver in intermediary metabolism and detoxification processes, its large active resident macrophage population (Kupffer cells = 80% of the fixed macrophage population in the body) and its sentinel position between the splanchnic and systemic circulatory systems, the liver has high risk for toxic, infectious, endotoxin, and oxidant mediated injury. Receiving approximately 75% of its blood flow from the alimentary canal, considered to be the richest source of oxidants, invading bacteria, and toxins in the body, both acute and chronic enteritis are thought to play a significant role in instigating liver injury. Furthermore, pancreatic inflammation (parenchyma, ducts) imposes risk for obstructive cholestasis and hepatothbiliary inflammation. A variety of toxins have been identified that specifically impose liver injury. Among encountered toxins are certain drugs: NSAIDs, acetaminophen, phenobarbital, primidone, diazepam (cat).

CHOLESTATIC LIVER DISEASE encompasses a heterogeneous group of disorders associated with impaired bile flow.

In severe hepatic insufficiency (acquired portosystemic shunting [PSS] secondary to intrahepatic fibrosis and portal hypertension), and in patients with other forms of cholestasis, accumulation of membranocytolytic bile acids (hydrophobic bile acids) perpetuates liver injury. These bile acids damage cell and organelle membranes, induce intracellular structural and functional changes, inflammatory responses, and impair bile flow. Free radicals importantly contribute to cholestatic injury; a central feature of bile acid hepatotoxicity is reduction of mitochondrial glutathione (GSH) resulting in reduced production of cell energy. Polymorphonuclear leukocytes also contribute to tissue injury in many cholestatic conditions and also contribute to oxidant membrane injury. Likewise, hepatocellular Cu retention (secondary to reduced Cu egress in bile) and Fe accumulation (in macrophages as a result of inflammation) also contribute to ROS generation. Sulfation of membranocytolytic bile acids reduces their toxicity by permitting renal elimination; this happens extensively in the cat but is seemingly limited in the dog. Taurine conjugation enhances the physiologic digestive functions of bile acids and slows their passive intestinal absorption (small bowel) and attenuates (to some degree) hepatocellular bile acid toxicity.

IMMUNE- MEDIATED MECHANISMS are believed to perpetuate chronic necroinflammatory / cholestatic liver disorders. Infection, endotoxin, or obstructed bile flow may instigate initial injury. Thereafter, a variety of pathologic immunologic responses perpetuate inflammation that ultimately impose oxidative insult. Phenomena thought to unite infection and “auto”immune responses include molecular mimicry (antigens of the infectious agent that closely resemble self-antigens) or innocent bystander effects (infectious agent exposes or mobilizes self-antigens). These responses may culminate in a learned immune repertoire involving T-cells and B-cells that ultimately target foci on normal structures. Infectious agents also may initiate or aggravate responses through an adjuvant effect, providing co-stimulatory inflammatory signals or by functioning as superantigens capable of broadly activating T cells. Environmental factors and toxins also have been implicated in induction of chronic immune responses thought to demonstrate “auto”immune behavior.

TRANSITION METALS: COPPER (Cu) & IRON (Fe)

While these metals function as important catalysts for enzymes and reactions essential to health, pathologic hepatic accumulation imposes oxidant injury.

HEPATIC IRON

Fe accumulation in macrophages is common in necroinflammatory disorders (80% biopsies). Traditional theory suggests that Fe sequestered in macrophages is unable to catalyze free radical reactions; recent work refutes this dogma. Fe is constantly in flux within and between cells and even the minute hepatocellular pool of free Fe participates in free radical reactions. Excess Fe also initiates/ promotes fibrogenesis in unrelated hepatic injury. In the presence of toxins, hepatotoxicity of Fe is enhanced (e.g. endotoxin imposes a “priming” effect on Kupffer cells).

HEPATIC COPPER (Cu)

While increased liver Cu may derive from genetic (transport/storage) disorders, it is more common secondary to cholestasis. Normally Cu absorbed from the gut circulates bound to transport proteins. After hepatic uptake and binding to cytosolic proteins, Cu is used in enzyme pathways and ultimately stored affiliated with metallothionein. In health, excretion into canalicular bile and enterohepatic circulation of Cu regulates a neutral Cu balance. Cholestasis impedes biliary Cu excretion causing eventual lysosomal loading and damage causing organelle and cell membrane oxidation. Accumulation of Cu storage “granules” (Cu-binding protein) that can be observed on routine histology are commonly “over-interpreted” as Cu storage disease (genetic) prompting recommendation of unnecessary chelation therapy.

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**HOW TO DEFINE TRANSITION METAL ASSOCIATED LIVER INJURY?**

Special stains (Prussian blue for Fe; Rhodanine or Rubeanic acid for Cu) must be coordinated with quantitative metal analysis (ug/gm dry weight tissue). Histologic interpretation defines acinar distribution and cell metal localization (macrophage, hepatocyte). Very small liver biopsies can yield erroneous measured metal values and histologic interpretations; e.g. biopsy of only a regenerative nodule may misrepresent tissue activity engaged in the disease process (will appear low relative to active disease). Quantitative tissue Cu measurements define the need for chelation therapy or Zn supplementation and antioxidants (high Cu or Fe liver concentrations indicate a need for Vit. E and thiol donor).

**THE THIRD STEP: CATEGORIZING THE DISORDER (COMMON) & LINKING PATHOMECHANISMS WITH INTERVENTIONAL**

<table>
<thead>
<tr>
<th>Necroinflammatory</th>
<th>chronic hepatitis (CH, canine); cholangiohepatitis (CCHS, cat, supplicative or nonsupplicative); extrahepatic bile duct occlusion (EHBD); lobular dissecting hepatitis; toxin induced injury.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rx</strong></td>
<td>immunomodulation, antioxidants, UDCA, possibly antifibrotics (based on histology).</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>parenchymal disorders associated with hyperbilirubinemia or high bile acids bile duct focused disorders: CCHS, cholangitis, EHBD, biliary mucocele. hepatocellular dysfunction / canicular collapse: hepatic lipodisosis (HL, cats), severe vacuolar hepatopathy (VH, dogs)</td>
</tr>
<tr>
<td><strong>Rx</strong></td>
<td>correct mechanical obstruction, relieve mucocele, provide UDCA, SAMe (antioxidant, other effects), antioxidants.</td>
</tr>
<tr>
<td>Metal Associated</td>
<td>inflammatory / cholestatic disease associated with excess Cu or Fe, +/- Zn depletion</td>
</tr>
<tr>
<td><strong>Rx</strong></td>
<td>If high Cu: chelation, restrict Cu intake, antioxidants, Zn supplementation not concurrent with chelation; Zn depletion: supplemental Zn (PSS, common in disorders requiring protein restricted diets, such feeding may augment Zn depletion)</td>
</tr>
<tr>
<td>Fibrogenesis</td>
<td>non-inflammatory as in juvenile fibrosing hepatitis; inflammatory as in CH, chronic CCHS, chronic EHBD</td>
</tr>
<tr>
<td><strong>Rx</strong></td>
<td>Usually polymodal therapy used: antioxidants, immunomodulation, Silibinin, polyunsaturated phosphatidylcholine or colchicine.</td>
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<tr>
<td>Hepatotoxicity</td>
<td>multiple toxins and drug toxicities have been described. Rule out infectious disorders: titers</td>
</tr>
<tr>
<td><strong>Rx</strong></td>
<td>Suspend toxin exposure, provide enteric removal (emesis, colonic lavage, activated charcoal) as appropriate, discontinue suspect drugs, research toxic mechanisms, use appropriate antidotes. Do not give prednisone, rather remove the agent and make metabolic adjustments to facilitate toxin excretion / removal (e.g. acetaminophen: administer cimetidine to slow biotransformation to toxic adduct, give NAC IV for several days as antioxidant (as above) and SAMe PO; mushroom toxicity (amanita, death cap) give silibinin, penicillin (impairs cell uptake of toxin), antioxidants. Generally, use hepatoprotectants and antioxidants: NAC, SAMe, silibinin, Vit. E.</td>
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<tr>
<td>Portosystemic Vascular Anomaly (PSVA)</td>
<td>congenital macroscopic portal shunting “around” the liver; congenital microvascular intrahepatic shunting (microvascular dysplasia, MVD). No necroinflammatory or cholestatic pathomechanisms involved.</td>
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<tr>
<td><strong>Rx</strong></td>
<td>PSVA: surgical ligation or medical management of hepatic encephalopathy, Zn supplementation. MVD: usually requires no therapy. UDCA NOT indicated in most, antioxidant NOT indicated in most.</td>
</tr>
<tr>
<td>Biliary Mucocele</td>
<td>Inspissated biliary material (gallbladder [GB], “kiwi” fruit pattern on ultrasound, associated with GB cystic mucosal hyperplasia, GB hypokinesis, hyperlipidemia, VH, and sometimes cholecystitis and ruptured GB; may also affect common ducts and hepatic ducts.</td>
</tr>
<tr>
<td><strong>Rx</strong></td>
<td>Remove inspissated biliary material, GB resection may be indicated), hydrocholeresis achieved by maintaining good hydration, UDCA, and SAMe. Always submit cultures for aerobic and anaerobic culture and carefully inspect cytologic preparations of inspissated biliary material for bacteria. May see bacteria cytologically that fail to grow in culture (antibiotic therapy preceding sample acquisition). UDCA is proposed to promote bile flow and aid in elimination of other substances normally excreted in bile, mechanism is complex involving up-regulation of canicular transport stimulating ductal secretion (bicarbonate). SAMe may augment bile flow through increased bile GSH concentrations (an important osmotic drive fueling bile flow). UDCA: 15 mg/kg PO SID, SAMe: 20 mg/kg PO enteric coated tablets on empty stomach. Appropriate antibiotics, fat restricted diet if hyperlipidemia associated with VH, Vitamin E if inflammatory lesions.</td>
</tr>
</tbody>
</table>
INTERVENTIONAL STRATEGIES:

Nutrition

Balanced nutritional support is critical including vitamin supplements (avoid Cu supplement if high tissue Cu). Only restrict protein in patients showing signs of HE (may be vague, may be indicated by ammonium biurate crystalluria, cannot depend on blood ammonia determinations). However, most animals with acquired hepatobiliary disease DO NOT require protein restriction, especially cats. In fact, cats with HL may succumb to this disorder subsequent to dietary protein restriction.

Antioxidants

Approximately 65% of dogs and cats with necroinflammatory liver disorders develop subnormal hepatic GSH concentrations. A number of toxins / adverse drug effects impose liver injury related to oxidant mechanisms. Since oxidant injury is better inhibited than reversed, preemptive therapy early in necroinflammatory and cholestatic liver disease is thought to be most effective. Antioxidant therapy should be combined with disease appropriate immunomodulatory / anti-inflammatory / antifibrotic medications to achieve a synergistic effect. For example, glucocorticoids intervene in membrane liberation of arachidonic acid that initiates production of inflammatory eicosanoids. Since these play a crucial role in inflammatory membrane oxidation, concurrent use of glucocorticoids and antioxidants may yield a synergistic benefit.

DIRECT THIOL / GLUTATHIONE DONORS - N-ACETYL-Cysteine, S-ADENOSYMETHIONINE, WHEY PROTEIN ?, SILIBININ (MILK THISTLE)

N-Acetylcysteine (NAC)

Used IV for crisis intervention, especially during the first few days in cats with HL, and in animals suspected of hepatotoxicity. Dose: 140 mg/kg IV (dilute at least 1:4 with saline or 5% dextrose, give via 0.25 µm micron nonpyrogenic filter), administer over 20-min NOT as constant rate infusion (CRI). Follow-up dosing: 70 mg/kg IV given 2-4 times daily as clinically indicated.

S-Adenosylmethionine (SAME)

For necroinflammatory / cholestatic liver disease / VH / HL:

Broad metabolic benefits derived in liver disease, may have important metabolic implications as a GSH donor and for methylation reactions (including l-carnitine availability) in HL (low vitamin B12 may contribute to SAME and GSH deficiency [compromised methionine availability for transsulfuration pathway]). Dose: 20 mg/kg enteric coated tablets. Be particular about source, Denosyl-SD4™, Nutramax, Inc has proven bioavailability. Dog size tablets used in healthy cats: significantly increased liver GSH.

Whey Protein


Vitamin E (α-Tocopherol)

"Last line of membrane defense" as lipid peroxidation chain terminator. In membranes exists in low molar ratio:phospholipids (especially PUFA) that are highly susceptible to oxidation (1,000-2,000 PUFA:1 Vit. E). Efficient recycling such that oxidized (tocopheroxyl radical) is reduced to its antioxidant form via an interactive group of reductant antioxidant cycles (CoQ10, ascorbate, GSH). Large doses without interactive antioxidants may fail to provide expected antioxidant protection and may be pro-oxidant (accumulated tocopheroxy radical). Vit. E also modulates cellular responses to oxidative stress through signal-transduction pathways (protein kinase C) providing anti-inflammatory and antifibrogenic effects. Since it is not synthesized in vivo it must be ingested (diet or supplementation). Dose: 10 IU/kg PO / day (α-tocopherol acetate); higher dosing for bile duct occlusion or cats with severe sclerosing cholangitis (fat malassimilation due to disrupted bile acid enterohepatic circulation). Water-soluble (α-tocopheryl succinate polyethylene glycol 1000 [TPGS]) preferred if compromised fat uptake (TPGS forms micellar solutions at low concentrations obviating need of bile acids for Vit. E uptake). Toxic effects of Vit E if very large doses: may potentiate oxidant injury and interferes with Vit. K activity (bleeding tendencies).