Supplemental Text

A sample of one test question and module content is given below. In brief, after the posttest is completed, the incorrect answer explanation along with the correct answer explanation are both given (i.e., if the student selected the wrong answer) or just the correct answer explanation (i.e., if the student selected the correct answer). In addition, each student is also provided a more detailed overview of the disease entity, including relevant pathophysiology with clinical and anatomic pathology details, differential diagnosis, and references for further study. Each module design consists of a clinical vignette, hyperlinks to macroscopic (gross) images with description, detailed explanation of the whole slide image included in the pretest, along with additional descriptions of ancillary slides and tests, as well as description of the disease entity with differential diagnosis. Finally, additional reading materials are also provided by hyperlinks at the end of the module. Thumbnails for the whole slide images are provided on the same online page as the written module description, in order that students can access slides, case descriptions, and additional resources, all from one site launch.

Question Stem

A 17-year-old male presented to an outside hospital with abdominal pain and vomiting. He was found to have very high AST and ALT levels along with elevated bilirubin and alkaline phosphatase levels. The patient quickly developed fulminant hepatic failure and subsequently underwent liver transplantation at a children's hospital.

A section of the native liver explant is provided (H and E stain). What is the most likely cause of this patient's hepatic failure with this type of injury around the central veins?

Answers along with Individual Answer Explanation for Correct and Incorrect Answer Choices

A. Acetaminophen ingestion overdose/toxicity
   a. Correct. Acetaminophen (Tylenol) is detoxified in hepatocytes through sulfation and glucuronidation with a small amount converted to a highly toxic metabolite by the p450 cytochrome oxidation pathway. This toxic metabolite is detoxified by glutathione (GSH) interaction. During times of large dose administration, GSH is depleted and the toxic metabolite accumulates in the hepatocyte destroying macromolecules while covalently binding to proteins and nucleic acids. This combination of effects results in massive liver necrosis in the form of coagulative type necrosis (preservation of basic outline of cell with pink/eosinophilic cell outlines– presumably hypoxic type cell death) for which we see happening in this case extending out around the central vein or "zone 3." Zone 3 is the first area preferentially affected in acetaminophen toxicity as it coincides with the highest concentration of enzymes responsible for detoxifying the drug. While N-acetylcysteine treats acute exposure toxicity, it needs to be administered in the first 16 h after exposure. After 36 h, the damage becomes irreversible with necrosis of the hepatocytes, as we see in this case. Of note, high levels of ferrous sulfate cause a similar pattern of coagulative necrosis, but the periportal hepatocytes are preferentially affected in Zone 1 first.

B. Aspirin ingestion overdose/toxicity
   a. Incorrect. Aspirin (acetylsalicylic acid) is also hepatotoxic in large doses. Biopsy in such cases will show focal necrosis with mild inflammation of the portal tracts and typically a diffuse microvesicular steatosis (Reye syndrome). Other drugs that can cause microvesicular steatosis include tetracycline and valproic acid. The metabolites of aspirin are acyl and phenolic glucuronide conjugates, but under larger dose ingestion, these pathways are saturated leading to accumulation of otherwise minor nontoxic metabolites that may be responsible for resultant hepatic injury. However, the exact mechanism leading to lipid peroxidation, mitochondrial damage, and hydroxyl radical scavenging of the hepatocyte.

C. Ethanol ingestion overdose/toxicity
   a. Incorrect. Alcohol in the form of ethanol is detoxified in the liver via the alcohol dehydrogenase (ADH) pathway in which the major rate limiting factor is the re-oxidation of NADH. The increased NADH to NAD ratio results in lactic acidosis, hypoglycemia, impaired fat metabolism, impaired metabolism of serotonin, and alterations in steroid metabolism. Macrovesicular steatosis is the earliest histologic manifestation of alcoholic liver injury. Over time with continued hepatocellular injury, fibrosis ensures with wide bands of fibrosis encircling regenerating hepatocytes without a recognizable central vein (e.g., alcoholic cirrhosis). In pediatric patients, macrovesicular steatosis is more often associated with nonalcoholic fatty liver disease (NASH) which can lead to steatohepatitis.

D. Hormonal steroid use
   a. Incorrect. Chronic exposure to hormonal steroids can cause various vascular changes in the liver including sinusoidal dilation and large blood-filled cavities (peliosis hepatis) most notable in anabolic steroids and contraceptive steroids. They can also cause hepatocellular adenomas. Rarely, hepatic vein thrombosis and zone 3 necrosis can occur from the thrombogenic effects of contraceptive steroids. This is a rare complication and many of these cases seem to be associated with an overt or latent myeloproliferative disease. In this case there is no evidence of thrombosis in the central veins to explain the zone 3 (perivenular) necrosis.

E. Wilson's disease
   a. Incorrect. Wilson disease is caused by an autosomal recessive mutation (chromosome 13q14.3) encoding a P-type ATPase which transports copper. There are over 280 mutations making it difficult to diagnose solely by genetic testing. It results in copper overload with low serum ceruloplasmin levels (below 5 mg/dL). While most cases present with a more subacute phase with a recurrent chronic hepatitis leading to end-stage fibrosis (cirrhosis), rare cases may present with acute liver failure. Thus, Wilson disease should always be on the differential diagnosis of fulminant hepatic failure in the pediatric population. In these cases, there can be massive to submassive necrosis with hepatocellular dropout and lobular collapse with associated microvesicular steatosis with swollen/enlarged (ballooning) hepatocytes, and pigment-laden Kupffer cells. A copper stain will show increased staining in Kupffer cells (sinusoidal resident macrophages) in addition to periportal hepatocytes. Depending on the extent and time frame, massive cell necrosis may show replacement with a prominent bile ductular reaction. Other laboratory findings that are also helpful for diagnosis including a low serum ceruloplasmin, elevated 24-h urine copper expression (usually >100 ug), and elevated liver copper concentration from the tissue (over 250 μg/g dry weight); however, remember for the latter, a fresh liver core must be sent to the pathologist in a metal-free container!

Detailed Overview of the Disease Entity with Relevant Pathophysiology along with Pathologic Explanation

Acetaminophen ingestion overdose/toxicity is the correct answer. Acetaminophen (Tylenol) is detoxified in hepatocytes through sulfation and glucuronidation with a small amount converted to a highly toxic metabolite by the p450 cytochrome oxidation pathway. This toxic metabolite is detoxified by GSH interaction. During times of large dose administration, GSH is depleted, and the toxic metabolite accumulates in the hepatocyte destroying macromolecules while covalently binding to proteins and nucleic acids. This combination of effects results in massive liver necrosis in the form of coagulative type necrosis (preservation of basic outline of cell with pink/eosinophilic cell outlines – presumably hypoxic type cell death) for which we see happening in this case. The coagulative type necrosis of the hepatocytes extends out around the central vein or "zone 3." Zone 3 is the first area preferentially affected in acetaminophen toxicity as it coincides with the highest concentration of enzymes responsible for detoxifying the drug. The periportal hepatocytes are swollen with injury also but not yet necrotic (although on their way!). While N-acetylcysteine treats acute exposure toxicity, it needs to be administered in the first 16 h after exposure. After 36 h, the damage becomes irreversible with necrosis of the hepatocytes, as we see in this case. Of note, high levels of ferrous sulfate cause a similar pattern of coagulative necrosis, but the periportal hepatocytes are preferentially affected in Zone 1 first. Aspirin (acetylsalicylic acid) ingestion overdose/toxicity is also hepatotoxic in large doses. Biopsy in such cases will show focal necrosis of hepatocytes with mild inflammation of the portal tracts and a diffuse microvesicular steatosis (Reye syndrome). Other drugs that can cause microvesicular steatosis include tetracycline and valproic acid. The metabolites of aspirin are acyl and phenolic glucuronide conjugates, but under larger dose ingestion, these pathways are saturated leading to accumulation of otherwise minor nontoxic metabolites that may be responsible for resultant hepatic injury. However, the exact mechanism leading to lipid peroxidation, mitochondrial damage, and hydroxyl radical scavenging of the hepatocyte membranes is not fully elucidated. Alcohol in the form of ethanol is detoxified in the liver via the ADH pathway in which the major rate-limiting factor is the reoxidation of NADH. The increased NADH to NAD ratio results in lactic acidosis, hypoglycemia, impaired fat metabolism, impaired metabolism of serotonin, and alterations in steroid metabolism. Macrovesicular steatosis is the earliest histologic manifestation of alcoholic liver injury. Over time with continued hepatocellular injury, fibrosis ensures with wide bands of fibrosis encircling regenerating hepatocytes without a recognizable central vein (e.g., alcoholic cirrhosis). In pediatric patients, macrovesicular steatosis is more often associated with nonalcoholic fatty liver disease (NASH) which can lead to steatohepatitis. Chronic exposure to hormonal steroids can cause various vascular changes in the liver, including sinusoidal dilation and large blood-filled cavities (peliosis hepatis) most notable in anabolic steroids and contraceptive steroids. They can also cause hepatocellular adenomas. Rarely, hepatic vein thrombosis and zone 3 necrosis can occur from the thrombogenic effects of contraceptive steroids. This is a rare complication, and many of these cases seem to be associated with an overt or latent myeloproliferative disease. In this case, there is no evidence of thrombosis in the central veins to explain the zone 3 (perivenular) necrosis in this case. Wilson disease is caused by an autosomal recessive mutation (chromosome 13q14.3) encoding a P-type ATPase which transports copper. There are over 280 mutations making it difficult to diagnose solely by genetic testing. It results in copper overload with low serum ceruloplasmin levels (below 5 mg/dL). While most cases present with a more subacute phase with a recurrent chronic hepatitis leading to end-stage fibrosis (cirrhosis), rare cases may present with acute liver failure. Thus, Wilson disease should always be on the differential diagnosis of fulminant hepatic failure in the pediatric population. In these cases, there can be massive to submassive necrosis with hepatocellular dropout and lobular collapse with associated microvesicular steatosis with swollen/enlarged (ballooning) hepatocytes, and pigment-laden Kupffer cells. A copper stain will show increased staining in Kupffer cells (sinusoidal resident macrophages) in addition to periportal hepatocytes. Depending on the extent and time frame, massive cell necrosis may show replacement with a prominent

bile ductular reaction. Other laboratory findings that are also helpful for diagnosis including a low serum ceruloplasmin, elevated 24-h urine copper expression (usually >100 μg), and elevated liver copper concentration from the tissue (over 250 μg/g dry weight); however, remember for the latter, a fresh liver core must be sent to the pathologist in a metal-free container!

Sample Module Design and Description, Related to Question Above

A 17-year-old male presented to an outside hospital with abdominal pain and vomiting. He was found to have very high AST and ALT levels along with elevated bilirubin and alkaline phosphatase levels. The patient quickly developed fulminant hepatic failure and subsequently underwent liver transplantation at Children's Hospital. A section of the native liver explant is provided (click here) for gross image.

Explanation of the slide

Acetaminophen ingestion overdose/toxicity results in massive liver necrosis in the form of coagulative type necrosis (preservation of basic outline of cell with pink/eosinophilic cell outlines – presumably hypoxic type cell death), for which we see happening in this case. The coagulative type necrosis of the hepatocytes extends out around the central vein or "zone 3." Zone 3 is the first area preferentially affected in acetaminophen toxicity as it coincides with the highest concentration of enzymes responsible for detoxifying the drug (see mechanism of action below). The periportal hepatocytes are swollen with injury also but not yet necrotic (although on their way!).

While N-acetylcysteine treats acute exposure toxicity, it needs to be administered in the first 16 h after exposure. After 36 h, the damage becomes irreversible with necrosis of the hepatocytes, as we see in this case. Of note, high levels of ferrous sulfate cause a similar pattern of coagulative necrosis, but the periportal hepatocytes are preferentially affected in Zone 1 first. Aspirin (acetylsalicylic acid) ingestion overdose/toxicity is also hepatotoxic in large doses. Biopsy in such cases will show focal necrosis of hepatocytes with mild inflammation of the portal tracts and a diffuse microvesicular steatosis (Reye syndrome). Other drugs that can cause microvesicular steatosis include tetracycline and valproic acid.

***Mechanism of action: Acetaminophen (Tylenol) is detoxified in hepatocytes through sulfation and glucuronidation with a small amount converted to a highly toxic metabolite by the p450 cytochrome oxidation pathway. This toxic metabolite is detoxified by GSH interaction. During times of large dose administration, GSH is depleted and the toxic metabolite accumulates in the hepatocyte destroying macromolecules while covalently binding to proteins and nucleic acids.*

View all resources-thumbnails (additional slides explanation)

The PASD stain better highlights the outlines of the ballooned hepatocytes and basement membranes and also highlights macrophages within the areas of necrosis. The reticulin stain highlights the fragmentation of the lobular reticulin network (black staining fragmentation) which is disrupted in the areas of hepatocellular necrosis. The trichrome does not show significant (blue) portal or bridging fibrosis.

More resources

(Fontana RJ. Acute Liver Failure Including Acetaminophen Overdose. Medical Clinics of North America 2008;92(4):761-94).