



Latin Comparative Pathology Group

The Latin Subdivision of the CL Davis Foundation

Diagnostic Exercise

Case #: 45 Month: June Year: 2014

Answer Sheet

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Clinical History: Sprague–Dawley Rats. Toxicology study for a small molecule pharmaceutical. Clinical observations consisted of decreased food consumption and decreased body weight gain. Several rats found dead prior to the conclusion of the study. Animals found that had jaundice and abdominal distension. Generally greater severity and incidence in females than males.

For clinical pathology, macroscopic as well as microscopic findings, please see the first part of this exercise.

Diagnosis: Regenerative, hemolytic anemia and consumptive thrombocytopenia, hypercholesterolemia, hypotriglyceridemia, hyperbilirubinemia.

Likely mechanism: drug-induced alterations in lipid profile and lipid content of RBC, platelet membranes resulting in extravascular hemolysis

Clinical pathology interpretation:

- Anemia: Decreased RBCs. Regenerative as evidenced by brisk reticulocytosis, increased MCV, slightly decreased MCHC. Likely due to hemolysis (schistocytes, acanthocytes, hyperbilirubinemia)
- Thrombocytopenia: Decreased platelets. Increased platelet size suggests the process is likely consumptive (as opposed to lack of production)
- Dyslipidemia: (hypercholesterolemia, hypotriglyceridemia)

- Blood smear: Marked anisocytosis and polychromasia, macrocytosis, poikilocytosis, acanthocytes, spherocytes, schistocytes

EM: RBCs with long, pointed processes (acanthocytes), platelets with long processes similar to processes on acanthocytes

Findings in spleen: Extramedullary hematopoiesis, increased macrophages, hemosiderin, phagocytosed erythrocytes and platelets

Differential diagnosis: Immune-mediated hemolytic anemia and thrombocytopenia, increased RBC and platelet fragility due to abnormal lipid membrane content.

Other data which would be useful/next steps (and results):

- Anti-RBC/plt Ab determination: no evidence of IgG or IgM on RBCs or platelets (this finding makes an immune-mediated mechanism less likely, as does the incidence and dose response. The degree of thrombocytopenia also suggests the cause is not immune-mediated, as immune-mediated mechanisms usually result in more profound drops in platelet counts)
- RBC osmotic fragility test: Increased fragility
- Evaluation of membrane lipid content: Alterations in phosphatidylcholine, cholesterol and/or sphingomyelin of RBC membranes

Typical Gross findings: Enlarged spleen, jaundice if severe enough

Typical microscopic findings:

- Bone marrow: Hypercellularity, increased M/E ratio, megakaryocytosis, phagocytosis of erythrocytes and platelets
- Spleen: Increased macrophages, hemosiderin, phagocytosed erythrocytes and platelets, extramedullary hematopoiesis (most often seen in rodents)

Discussion: The structure of RBC membranes is extremely important for red blood cell resilience and survival in circulation, and having the appropriate proportions of the various lipid components is critical for maintaining membrane structure. Therefore, alterations in lipid membrane content and red blood cell morphology have the potential to shorten erythrocyte lifespan due to early removal by phagocytes. The presence of acanthocytes is highly suggestive of a perturbation of RBC membrane lipid, and has been described with naturally occurring diseases which alter serum/plasma lipids. The mechanism for the test article-related dyslipidemia in this case is unknown.

References:

- Poulet FM et al. Drug-induced hemolytic anemia and thrombocytopenia associated with alterations of cell membrane lipids and acanthocyte formation. *Toxicologic Pathology*, 38:907-922, 2010 (and references therein).

Please send your comments/questions to the whole LCPG list by hitting "reply to all".

A final document containing this material with answers and a brief discussion will be posted on the C. L. Davis website by the end of the current month (http://www.cldavis.org/lcpg_english.html).