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ZORA URL: https://doi.org/10.5167/uzh-72883
Conference or Workshop Item

Originally published at:
Kook, P H (2013). Cobalamin in gastrointestinal disease. In: North American Veterinary Conference, Orlando, U.S., 19 January 2013 - 23 January 2013.

COBALAMIN IN GASTROINTESTINAL DISEASE

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Physiology

Cobalamin (Cbl) is a water-soluble vitamin belonging to the B-group (Vit B12). It is an essential cofactor for mammalian enzyme systems, and adequate amounts are required for nucleic acid synthesis and hematopoiesis. The two most important reactions involving Cbl are the conversion of methylmalonyl-coenzyme A (CoA) to succinyl-CoA and the remethylation of homocysteine. Cbl deficiency leads to reduced activity of these enzyme systems resulting in increased concentrations of serum and urinary methylmalonic acid (MMA) and total plasma homocysteine. Therefore, measurements of these metabolites allow the assessment of cellular Cbl availability and are the tests of choice to detect early or mild Cbl deficiency in humans.

Once ingested, dietary protein is partially digested by pepsin and HCl, and Cbl is being released. Next, Cbl immediately binds to a transporter protein called haptocorrin (former R-protein). Haptocorrin is mostly synthesized and secreted by the gastric mucosa. Haptocorrin is in turn digested by pancreatic proteases in the small intestine and free Cbl binds to intrinsic factor. This glycoprotein is produced by the stomach and pancreas in dogs and solely by the pancreas in cats, and the Cbl-intrinsic factor complex is then internalized into the ileal enterocyte by endocytosis. This is mediated by a specific membrane protein receptor on the cell surface termed the cubam receptor.

Dogs and cats are unable to synthesize Cbl and are entirely dependent upon adequate dietary sources, principally organ and muscle tissue. An association exists between diseases of the gastrointestinal tract and hypocobalaminemia in cats and dogs. Gastrointestinal disease may affect the absorptive capacity of the ileal mucosa and chronic enteropathies will lead to serum hypocobalaminemia and ultimately – when all stored cellular Cbl has been used – to Cbl deficiency (identifiable through MMA and/or homocysteine measurements). This differentiation between decreased serum Cbl levels and concurrent decreased cellular Cbl availability is difficult without costly laboratory tests (MMA), but it should be pointed out that some dogs and cats have subnormal serum Cbl concentrations in the face of non detectable urine or serum MMA concentrations.[2,3] Low Cbl values may in turn interfere with intestinal health, because enterocytes belong to the fast dividing cells that are especially dependent on Cbl.[1]

GASTROINTESTINAL DISEASE AS A CAUSE FOR HYPOCOBALAMINEMIA

Hereditary causes

In humans, congenital Cbl deficiency due to selective ileal malabsorption is a rare autosomal recessive disorder appearing initially in early childhood. Hereditary forms of Cbl deficiency have also been reported in different dog breeds. Inherited selective intestinal Cbl malabsorption and deficiency has been described in a familiy of Giant Schnauzer in the early 90s.[4] The known genetic defect is located in the cubilin (*CUBN*) and the amnionless (*AMN*) gene encoding the ileal Cbl-intrinsic factor receptor. Hereditary selective Cbl malabsorption has also been reported in Australian Shepherds and is assumed to be the cause for Cbl deficiency in Beagles. Things may be a bit different in the Border Collie: The recent finding of methylmalonic aciduria in healthy Border Collies with normal serum Cbl concentrations and in Border Collies with clinical signs of Cbl deficiency that had low serum Cbl concentrations is intriguing and indicate different mechanisms.[2] Those healthy Border Collies may have had a defect in the mitochondrial metabolic cobalamin pathway (ie, methylmalonyl-CoA mutase) and those Border Collies with clinical signs of Cbl deficiency may have had selective intestinal malabsorption of Cbl. An ongoing genetic study is currently investigating the cause of hereditary Cbl deficiency in Border Collies in Switzerland. Finally in Shar Peis, a region on chromosome 13 is thought to be responsible for Cbl deficiency.

Clinicopathologic Signs in hereditary Cbl Deficiency

The dogs are usually between 6 and 12 months when they are finally diagnosed. The median age of Border Collies in a recent study was 11.5 months (range 8–42).[5] Cbl-deficient Beagles seen by the author were also 10 to 11 months old. Typical historical complaints are intermittent diarrhea and picky appetite to anorexia with poor body condition (BCS 2-3/9), and failure to grow. Clinically the marked weakness (some dogs seem obtunded) and small growth are the predominant finding, some dogs have difficulties eating and food keeps dropping out of the mouth.[5] Upon inspection of the oral cavity care should be taken to closely examine the tongue. Intermittent impaired swallowing can be due to glossodynia and glossitis (multifocal, painful red erosions on the tongue). These findings point to primary stomatodynia as the cause of dysphagia, rather than a neuromuscular disorder. Glossitis, mucosal ulcerations, glossodynia, stomatodynia, and peripheral paresthesia have all been reported in cobalamin-deficient human infants and adults. Hematologic evaluation can vary considerably (from normal to megaloblastic anemia). A normocytic, normochromic non-regenerative to regenerative mild anemia is often seen. The typical macrocytic (megaloblastic) anemia seen in humans (pernicious anemia) is only rarely observed. Blood smear evaluation shows signs of dyserythropoesis (increased numbers of nucleated red blood cells). Bone marrow cytology of the very first reported case of Cbl deficiency in a Border Collie presenting with very mild normocytic anemia revealed erythroid hyperplasia with erythrophagocytosis.[6] Hematologic abnormalities of Cbl-deficient Giant Schnauzers included occasional macrocytic normoblasts with immature nuclei together with reduced erythroid precursors in a hypocellular bone marrow.[4] These differences may reflect individual or breed-specific variations of cobalamin-deficient states. Subtle hematologic abnormalities may go undetected when only an automated blood cell count is done. These findings indicate that hereditary Cbl deficiency may not be routinely

associated with hematologic changes and normal hematologic results should therefore not preclude serum Cbl assessment.
Findings on biochemistry can include mild hypoproteinemia, mild to moderate hypoglycemia and elevated AST.[6] Hypoglycemic seizures after strenuous exercise/playing are possible. Most clinicians will suspect a portosystemic shunt, and in fact blood ammonia levels can be increased in dogs with hereditary Cbl deficiency.[7] This reversible abnormality is very likely due to an Cbl-dependent urea cycle abnormality. Mild proteinuria (1 + dipstick, UPC 0.35-0.5) is seen on urinalysis. It is believed that absence of the cubam receptor complex in proximal renal tubular cells results in reduced reabsorption of albumin. In the human literature it is reported that mild proteinuria often persists, even after successful treatment with cobalamin is initiated. Results of diagnostic imaging are usually normal and without submitting a Cbl, chances are very high that the actual problem will be missed, and only short-lived to no clinical improvement is seen with symptomatic therapy.

Diagnosis

The diagnosis relies on the combination of low Cbl values (< 150 ng/l), markedly elevated serum or urine MMA, and, most important, dramatic response to Cbl supplementation alone.
All reported dogs responded quickly to parenteral (not peroral!) Cbl, they quickly gain weight and act like normal dogs. However, when diagnosed around 1 year of age, they remain smaller than the breed standard.
Hereditary Cbl deficiency has not been conclusively described in cats. However Vaden et al reported on a kitten with lethargy, anorexia, failure to thrive, anemia, hypoglycemia, hyperammonemia, hypocobalaminemia and methylmalonic aciduria. Even though the clinical picture very much resembles canine hereditary Cbl deficiency, it is unclear whether this cat's Cbl deficiency was primary or the result of some other undiagnosed abnormality.[8]

CHRONIC SMALL BOWEL DISEASE

Any diffuse small bowel or focal ileal disease (inflammatory or neoplastic) will cause reduced enteral Cbl absorption. Overall cats with gastrointestinal disease seem more prone to develop hypocobalaminemia than dogs. In a study from 2001, 61% of 80 cats with clinical signs of gastrointestinal disease were hypocobalaminemic. Definitive diagnoses in 22 cats included inflammatory enteropathy, intestinal lymphoma, cholangiohepatitis or cholangits, and pancreatic inflammation.[9] Serum concentrations of Cbl were particularly low in 6 cats with intestinal lymphoma.[9] We recently looked at Cbl values of 261 cats presenting with gastrointestinal disease, and a total of 108/261 (41.4%) cats had hypocobalaminemia. Cbl concentrations were below the sensitivity of the assay (< 150 ng/l) in 69 (26.4 %) of all cats had and 39 (15 %) had concentrations between 150 - 304 ng/l (reference range 305-1967).[10] Diarrhea was the most common clinical sign in hypocobalaminemic cats and vomiting or anorexia was the most common sign in normocobalaminemic cats. Only cats with both, vomiting and diarrhea were more likely to have hypocobalaminemia than cats with other clinical signs (odds ratio, 2.879; 95 % CI, 1.313-6.310). Serum Cbl concentration was negatively correlated with age of the patient and positively correlated with body condition score. Only Cbl concentrations below the detection limit of the assay affected results of routine bloodwork. Cats with values < 150 ng/l had a significantly higher neutrophil counts, higher MCV and significantly lower hematocrit and albumin concentration than cats with measurable subnormal and normal Cbl values.[10] Among the diagnoses made in 125 cats, lymphoma and inflammatory enteropathy were most common. In 69 cats with Cbl concentrations below the sensitivity of the assay (< 150 ng/l) small cell lymphoma was the most common disease.[10] Intestinal small cell lymphoma in cats is diagnosed much more frequently today than in the past and this infiltrative disease is most often found in the ileum, the location where Cbl is absorbed. The author finds a Cbl < 150 ng/l in a cat with weight loss (+/- diarrhea and vomitus) a danger signal and indicator to pursue further diagnostics (intestinal biopsies), rather than initiating a treatment trial for inflammatory enteropathy.
It is unknown for how long Cbl malabsorption has to persist, before all hepatic stores are depleted. Cbl normally undergoes enterohepatic circulation, but as diseases of the distal small bowel ultimately compromise this cycle, the Cbl half-life will be shortened. While the half-life of Cbl in humans is reported to be a couple of months, the circulating half-life of parenteral cyanocobalamin was 5 days in cats with IBD, and 12.75 days in healthy cats.[9] The rapid depletion of circulating cobalamin in cats suggests that cats may be highly susceptible to Cbl deficiency.
Numbers are a somewhat lower in dogs with chronic enteropathy. In a prospective study from Switzerland 13 out of 70 dogs (18%) with fully worked-up chronic enteropathy had initial hypocobalaminemia with concentrations < 200 ng/l. Seven of 13 dogs with initial hypocobalaminemia were subsequently euthanized because of refractoriness to treatment. Dogs with initial serum Cbl concentration below the cut off value of 200 ng/L had a highly significant higher chance for a negative outcome. In addition, there was a highly significant association between a low serum albumin concentration and a low serum Cbl concentration.[11] A recent study from Texas found a higher prevalence of 36% for hypocobalaminemia in dogs with chronic GI disease. Only 9% of these dogs also had increased serum MMA concentrations, suggesting Cbl deficiency on a cellular level.[12] A study from Liverpool looked at breed predispositions for hypocobalaminemia in 9.997 dogs (40 breeds) with signs of gastrointestinal disease (exocrine pancreatic insufficiency excluded) and the Shar Pei was at highest risk for low Cbl concentrations followed by the Staffordshire Bull Terrier and German Shepherd.[13] Similar results have been reported for the Shar Pei breed in North America.

EXOCRINE PANCREATIC INSUFFICIENCY (EPI)

Three mechanism may lead to hypocobalaminemia in EPI: Firstly the reduced to ceased pancreatic synthesis of intrinsic factor impairs ileal absorption of Cbl. Secondly the reduced concentrations of pancreatic proteases hinder the intestinal digestion of haptocorrin (Cbl enters the small bowel bound to haptocorrin and needs to be split first), and therefore even less Cbl is available that can bind to intrinsic factor. Thirdly the lacking bactericidal pancreatic

juice may result in an overgrowth of Cbl-consuming bacteria (dysbiosis). Batchelor and colleagues showed that 82% of 178 dogs with EPI were hypocobalaminemic, and severeCbl deficiency (< 100 ng/l) was associated with shorter survival.[14] Feline EPI was once considered a rare disease, but most recently feline EPI has been reported and diagnosed more frequently. Until 2012 all reported cases of cats with EPI that had a Cbl measured were hypocobalaminemic. However in a 2012 study from Texas on 150 cases of feline EPI, only 92 of 119 cats (77%) had hypocobalaminemia (median: 149 ng/L; range: 149–1,001 ng/l) and 83 of those cats (70%) had undetectable serum Cbl concentrations (< 149 ng/l).[15]

MISCELLANEOUS CAUSES

Feline hyperthyroidism is commonly associated with hypocobalaminemia and it is assumed that hyperthyroidism directly or indirectly affects Cbl uptake, transport protein-binding, excretion or utilisation in this species.[16] Because hyperthyroidism can mimic gastrointestinal disease, a serum thyroxin concentration should always be measured in a hypocobalaminemic cat > 7 years, before more expensive diagnostics are pursued.

CLINICAL SIGNS

Usually the clinical picture is dominated by the underlying disease causing low hypocobalaminemia and it is difficult to assess the effects separately. Experimentally induced Cbl deficiency in cats lead to progressive anorexia, weight loss, and an unkempt hair coat.[17]

Neurologic complications (progressive spinal ataxia, change in behaviour) may be seen in severe cases of Cbl deficiency with massive methylmalonic acidemia/aciduria. It is striking to observe that Cbl-deficient animals do often not satisfyingly respond to standard treatments until supplemented with Cbl.

DIAGNOSIS

Every cat and dog with a chronic gastrointestinal disease should have its Cbl status evaluated. The blood sample should be shipped in light protected tubes, as Cbl may be readily degradable under the influence of light. It is usually recommended that samples are shipped overnight either frozen or at least with included ice packs to keep them cooled during shipment. Most laboratories measure Cbl with an automated chemiluminescence assay (Immulite, Siemens), because the radioimmunoassay is not anymore available commercially. Ideally every laboratory should establish its own reference range, even when using the same assay, but differences between labs are small and do not seem clinically relevant. For example, the reference range for cats is 290-1500 ng/l in Texas and 305-1967 ng/l in Zurich[10], and for dogs 252-908 ng/l in Texas and 261-1001 in Zurich.[19] MMA measurement (gas chromatography) is required in order to assess the Cbl status on a cellular level. The clinical utility of this costly parameter has not been critically investigated yet. Ruaux et al. showed that only 68.4% of cats with Cbl concentrations below the ref. range had concurrently elevated serum MMA concentrations. [18] Similar results have been reported in dogs. These observations imply that some cats and dogs with subnormal serum Cbl concentrations will be supplemented unnecessarily.

THERAPY

Cbl should be supplemented whenever serum Cbl concentration is below the reference range in both dogs and cats. Most commonly, cyanocobalamin is chosen for supplementation, as it is both widely available and more inexpensive than hydroxycobalamin. Very little evidence-based information about Cbl supplementation in dogs and cats is available. It has been shown in cats with GI disease and severe hypocobalaminemia that parenteral supplementation lead to clinical improvement and biochemical normalization.[20] Cbl deficiency itself may lead to Cbl malabsorption so that Cbl should always be supplemented parenterally. Since Cbl is a water-soluble vitamin, excess Cbl is excreted through the kidneys and clinical disease due to over-supplementation has not been described. The average cat dose is 250 mcg SC weekly, and 250-1500 mcg (depending on body weight) SC weekly in the dog. We recommend injections every 7 days for 6 weeks, then one additional dose after 4 weeks, and retesting 6 weeks after the last dose. If the underlying disease process has resolved and Cbl body stores have been fully replenished, serum Cbl concentration should be supranormal at the time of reevaluation. The author has seen cases where Cbl supplementation fails to keep the serum Cbl in the desired range, although patients are doing clinically fine. This is especially the case in dogs with hereditary Cbl deficiency on long-term supplementation.[5] In dogs, Cbl is mostly bound to two proteins taken up by tissues and bound to enzymes with a long half-life. Thus, it is possible that Cbl remains active in tissues even when serum concentrations are low. Another explanation may be the Cbl formulation. Water-soluble formulations may be excreted too fast, before it actually can be bound to transcobalamin and stored in the liver. In those cases a cyanocobalamin-tannin complex suspended in a sesame-oil may help. The product is more expensive, but it ist he author´s experience that it lasts 6- 8 weeks in dogs needing constant supplementation.

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