

Use of ⁶⁴Copper Measurements to Diagnose Canine Copper Toxicosis

George J. Brewer, MD, William Schall, DVM, Robert Dick, BS,
Vilma Yuzbasiyan-Gurkan, PhD, Michael Thomas, DVM
and George Padgett, DVM

Inherited canine copper toxicosis is a serious problem in Bedlington terriers and West Highland White terriers, and may also be a problem in other less-studied breeds. Affected dogs become ill at midlife with progressive and ultimately fatal liver disease. Treatments for removal of copper and prevention of copper accumulation are available, but are most effective if begun before the dog becomes ill. Until recently diagnosis has not been available until the dog is 1 year of age, and then only by an invasive liver biopsy with determination of liver copper concentration. The authors studied the use of ⁶⁴copper for early diagnosis of canine copper toxicosis. Two procedures were evaluated. The first involved measuring the concentration of ⁶⁴copper in blood 24 hours after oral administration of the radioisotope. At this time, ⁶⁴copper was associated primarily with ceruloplasmin secreted into the blood by the liver. This procedure is useful in the diagnosis of the human counterpart, Wilson's disease. However, the authors found it to be nondiscriminatory between affected and unaffected dogs. In contrast, the second procedure, which involved measuring ⁶⁴copper excreted in stool during 48 hours after an intravenous dose of radioisotope, yielded results that differentiated most affected and unaffected dogs. (*Journal of Veterinary Internal Medicine* 1992; 6:41-43)

COPPER TOXICOSIS (CT) is an autosomal recessive disorder of hepatic copper accumulation, leading eventually to hepatic failure and death if untreated.¹⁻³ It is believed that CT is similar to a human autosomal recessive disorder of copper accumulation called Wilson's disease (WND).⁴ Copper toxicosis is common in Bedlington Terriers (BT) and probably in West Highland White Terriers (WHWT). Breeders have a difficult time trying to minimize the effects of this gene because diagnosis of affected dogs by the current method of measuring he-

patic copper content is delayed to 1 year of age, and requires liver biopsy. Diagnosis of the carrier state has been attempted by serial liver biopsy,⁵ but in our opinion this method is not reliable. Otherwise, diagnosis of the carrier state is impossible except by a tedious and expensive progeny testing program. Thus, the gene frequency remains high and numerous affected dogs suffer the fate of early death from liver failure.

We have applied ⁶⁴copper technology, which has been successful in WND, to CT in dogs. In human patients, both the affected and the carrier state can be diagnosed by the low rate of ⁶⁴copper incorporation into blood ceruloplasmin over 24 to 48 hours.⁶ A previous report indicated that affected BTs also had an abnormally low rate of ⁶⁴copper incorporation into blood ceruloplasmin.⁴ In this study, we investigated use of this procedure for diagnosis of CT affected and carrier animals. In addition, since biliary excretion of copper into the stool is defective in CT, we evaluated stool radioactivity after an intravenous dose of ⁶⁴copper to determine if it is diagnostically reduced in affected dogs.

Methods

Dogs used in this study were all client-owned and were studied after obtaining written informed consent from

From the Department of Human Genetics and Internal Medicine (Brewer), University of Michigan, Ann Arbor, Department of Internal Medicine (Schall), College of Veterinary Medicine, Michigan State University, Lansing, Department of Human Genetics (Dick and Yuzbasiyan-Gurkan), University of Michigan, Ann Arbor, Department of Radiology (Thomas), College of Veterinary Medicine, Michigan State University, Lansing, and Department of Pathology (Padgett), College of Veterinary Medicine, Michigan State University, Lansing, Michigan.

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Reprint requests: George J. Brewer, MD, Department of Human Genetics, University of Michigan, 4708 Med. Sci. II, Box 0618, Ann Arbor, MI 48109-0618.

the owners. The sample consisted of seven unaffected and eight affected BTs and fifteen unaffected and seven affected WHWTs. The diagnosis in each dog was established by liver biopsy and measurement of hepatic copper content. Affected animals had liver copper concentrations of at least 1100 $\mu\text{g/g}$ dry weight of tissue (average = 3341 $\mu\text{g/g}$). Unaffected dogs had liver copper values less than 800 (average = 403). Preliminary data were collected to evaluate whether the test procedures were going to be useful to differentiate heterozygous carrier animals from normal animals. This involved comparing four known heterozygous carrier animals with one progeny proven homozygous normal animal. Results were similar so no further attempt was made to differentiate these genotypes. None of the affected dogs were receiving anticopper therapy or copper dietary restriction at the time of these studies.

The ^{64}Cu studies reported are of two types. The first involved oral administration of 500 microcuries of ^{64}Cu to a fasted dog with blood sampling at 24 and 48 hours. The dose and sampling periods selected were based on extrapolations from human studies. After preliminary data from four dogs indicated that the 48-hour sample was not providing additional information, only the 24-hour blood sample was collected in the remaining studies. Radioactivity in the 24-hour blood sample was determined using a Packard Auto Gamma 5650 gamma counter* and reported as percent of the administered dose.

The second procedure involved intravenous administration of 500 microcuries of ^{64}Cu followed by collection of stools over the next 48 hours (beginning and ending stools determined by a stool dye marker) and determination of stool radioactivity. Radioactivity of the stool was determined by assaying an aliquot of homogenized stool using a Packard Auto Gamma 5650 gamma counter and reported as percent of the initial dose.

For statistical comparisons, an unpaired Students *t*-test was used; if the variances appeared unequal, a log transformation of the data and a *t*-test of the transformed data were also employed.⁷

Results

Results of the plasma study at 24 hours are shown in Figure 1 and Table 1. The mean value for seven unaf-

TABLE 1. Plasma Radioactivity Values 24 Hours After Oral Administration of Radiocopper

Unaffected		Affected	
Bedlington terriers			
Mean	0.64	Mean	0.72
sd	0.26	sd	0.66
N	7	N	6
<i>P</i> = nonsignificant			
West Highland white terriers			
Mean	1.05	Mean	0.58
sd	0.42	sd	0.22
N	15	N	7
<i>P</i> = nonsignificant			

Values are % of administered dose.

affected BTs was 0.64% \pm 0.26 compared with a mean of 0.72% \pm 0.66 for six affected Bedlingtons. The mean for 15 unaffected WHWTs was 1.05% \pm 0.42 compared with 0.58% \pm 0.22 for seven affected WHWTs. This procedure did not discriminate affected from unaffected animals of either breed (Fig. 1).

The stool radioactivity study results are shown in Figure 2 and Table 2. This test shows considerably more discrimination between the affected and unaffected dogs. The mean of seven unaffected BTs was 5.56% \pm 2.30 and for eight affected was 1.66% \pm 0.62. The mean of 15 unaffected WHWTs was 6.01% \pm 2.46 and the mean of seven affected WHWTs was 2.24% \pm 0.87. In both breeds, these results are significantly different (*P* < 0.01, nonpaired *t*-test). Because the variances were not approximately equal in these samples, the data were log transformed and retested by the nonpaired *t*-test. The means of the transformed data were also significantly different.

Discussion

The most exciting result of this study is that the stool ^{64}Cu test shows the capacity to discriminate reasonably well between affected and unaffected dogs of both breeds. For example, from a practical standpoint, if one selected a value of 4% of administered dose as a cutoff point (Fig. 2), all affected dogs in this sample would have been diagnosed (no false negatives). Two of seven unaf-

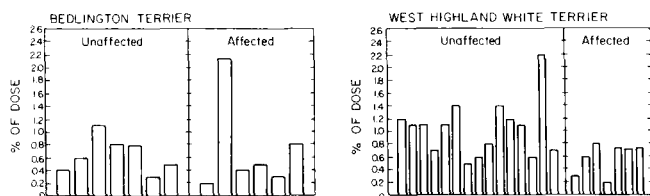


FIG. 1. Graphic portrayal of plasma radioactivity values 24 hours after oral administration of radiocopper (values in % of administered dose).

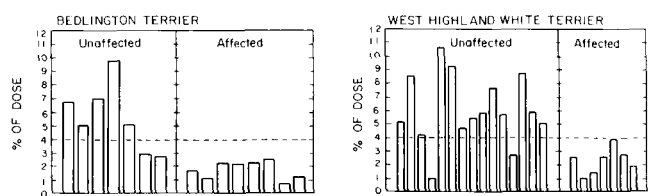


FIG. 2. Graphic portrayal of radioactivity in stools collected over 48 hours after an intravenous dose of radiocopper (values are % of administered dose). A 4% cutoff value is suggested to differentiate affected from unaffected dogs.

* Packard Instrument Co., Downers Grove, IL.

TABLE 2. Radioactivity Values in Stool Collected Over 48 Hours After an IV Dose of Radiocopper

Unaffected		Affected	
Bedlington terriers			
Mean	5.56	Mean	1.66
sd	2.30	sd	0.62
N	7	N	8
			$t = 3.98 P < 0.01$
Log transformed data			
Mean	0.70	Mean	0.22
sd	0.21	sd	0.14
			$t = 5.3 P < 0.001$
West Highland white terriers			
Mean	6.01	Mean	2.24
sd	2.46	sd	0.87
N	15	N	7
			$t = 4.80 P < 0.01$
Log transformed data			
Mean	0.73	Mean	0.31
sd	0.25	sd	0.20
			$t = 3.8 P < 0.01$

affected BTs and two of fifteen unaffected WHWTs would have been labeled as false positives and inappropriately diagnosed as affected. The major requirement from the breeder's standpoint is to have as few as possible false negatives so that affected dogs are not used for further breeding. In that connection, this test procedure seems to have considerable benefit. With more experience, the false negative rate may be reduced further. We need to make certain, for example, that the intravenous dose was delivered substantially into the vein by carrying out a blood radioactivity measurement a few minutes after injection. That type of control was not done in this study. Another aspect to be explored is whether the test is effective in identifying affected dogs at a young age. Such studies are in progress.

In contrast to the useful results of the stool study, the plasma study was negative. This in spite of the fact that a previous report had indicated that affected dogs did have low rates of incorporation of radiocopper into ceruloplasmin and into the blood at 24 hours.⁴ Possible explanations for the differences in results include: 1) The prior study used primarily mongrels as controls (only one unaffected Bedlington was studied), and mongrels might be different than unaffected Bedlingtons with regard to this characteristic; 2) the levels of radioactivity may have been lower in the previous study. Unaffected animals in our study averaged 0.92% of dose at 24 hours compared with 0.56% in the previous study; and 3) results in the previous study indicate an inverse effect of hepatic copper concentrations on plasma radioactivity, suggesting

that the dilution of radioisotope in the liver may influence the results. Possibly our affected dogs had different concentrations of hepatic copper than in the previous study.

In any case, we believe that the present results indicate this plasma procedure is of little value for test purposes. Incorporation of radiocopper into ceruloplasmin and secretion into the blood is uniformly decreased in the human patient with WND. The reason for this difference between the human and the canine disease is not known. In the canine disease, plasma ceruloplasmin is not decreased as it is in the human disease. There are indications that there may be more than one form of ceruloplasmin circulating in some species.⁸⁻¹¹ The major type circulating in the dog may not be the type interacting with the WND or CT genes.

In conclusion, incorporation of radiocopper into circulating plasma ceruloplasmin does not appear to be abnormal in canine CT. However, excretion of radiocopper into stool is abnormally low in CT and could be used as a diagnostic test. The criterion that dogs showing less than 4% of the administered dose in the stool in 48 hours be called positive would lead to few false negatives, but perhaps 15 to 30% false positives.

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