

Results of thyroid function tests and concentrations of plasma proteins in dogs administered etodolac

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Objective—To determine the effects of etodolac administration on results of thyroid function tests and concentrations of plasma proteins in clinically normal dogs.

Animals—19 healthy random-source mixed-breed dogs.

Procedure—Blood samples for measurement of serum thyroxine (T_4), 3,5,3'-triiodothyronine (T_3), free T_4 (fT_4), and endogenous canine thyroid stimulating hormone (cTSH) were measured twice before as well as on days 14 and 28 of etodolac administration (mean dosage, 13.7 mg/kg, PO, q 24 h). Plasma total protein, albumin, and globulin concentrations and serum osmolality were measured once before as well as on days 14 and 28 of etodolac administration.

Results—Etodolac administration did not significantly affect serum T_4 , T_3 , fT_4 , or cTSH concentrations or serum osmolality. Significant decreases in plasma total protein, albumin, and globulin concentrations were detected on days 14 and 28 of administration.

Conclusions and Clinical Relevance—Results of thyroid function tests are not altered when etodolac is administered for up to 4 weeks. Therefore, interpretation of results of these tests should accurately reflect thyroid function during etodolac treatment. Plasma total protein, albumin, or globulin concentrations that are less than the respective reference range in a dog administered etodolac for ≥ 2 weeks may be an effect of treatment rather than an unrelated disease process. A decrease in plasma protein concentrations may reflect subclinical injury of the gastrointestinal tract. (*Am J Vet Res* 2002;63:1492–1495)

Nonsteroidal anti-inflammatory drugs (NSAIDs) can influence results of thyroid function tests in dogs and other species.^{1-3,a} Decreased concentrations of thyroxine (T_4), free T_4 (fT_4), 3,5,3'-triiodothyronine (T_3), and thyroid stimulating hormone (TSH) have been reported in humans and rats administered salicylate, salicylate, and other NSAIDs.^{1,4,5} Proposed mechanisms for the alterations of thyroid function include impaired protein binding of thyroid hormones, impaired hepatic uptake of thyroid hormones, and decreased deiodination of thyroid hormones.⁶⁻⁹ The effect of NSAIDs on results of thyroid function tests varies depending on the drug.¹

Etodolac preferentially inhibits cyclooxygenase

(COX)-2 to a greater degree than it inhibits COX-1 in humans in vitro and ex vivo.^{10,11} However, studies^{12,13} that used canine cells suggest that etodolac may preferentially inhibit COX-1 in dogs. It is an effective treatment for dogs with osteoarthritis and has few adverse effects on the gastrointestinal tract during short-term administration.^{14,15} Similar to most other NSAIDs, etodolac is highly bound to plasma proteins.¹⁶ Because approximately 99.9% of T_4 and 99% of T_3 are bound to plasma proteins, any factor that reduces protein binding could have a substantial impact on serum T_4 and T_3 concentrations. Competition between thyroid hormones and etodolac for binding sites on plasma proteins such as albumin could cause a reduction in serum T_4 and T_3 concentrations, leading to confusion when interpreting results of thyroid function tests. Indeed, administration of the NSAID carprofen to dogs decreases serum T_4 but not fT_4 concentrations.^b The purpose of the study reported here was to evaluate the effects of etodolac on results of thyroid function tests in clinically normal dogs.

Materials and Methods

Animals—Nineteen random-source mixed-breed dogs (3 males, 16 spayed females) of unknown age were included in the study. Body weight ranged from 9.6 to 22.6 kg (mean, 15.1 kg). Dogs were considered to be healthy on the basis of results of physical examination, CBC, and serum biochemical analysis. Each dog had a normal response (serum T_4 concentration > 35 nmol/L at 6 hours after TSH administration) to IV administration of bovine TSH^c (0.1 IU/kg). Dogs were housed in indoor runs with a 12-hour light:12-hour dark cycle and were fed a maintenance dry food^d once per day.

Experimental protocol—Dogs were allowed to acclimate for at least 2 weeks prior to initial administration of etodolac. During the acclimation period, a CBC, serum biochemical analysis, and TSH response test were performed.

Each dog was administered etodolac^e (dosage range, 12.1 to 15.6 mg/kg; mean dosage, 13.7 mg/kg, PO, q 24 h) for 4 weeks. Blood samples for measurement of serum concentrations of T_4 , T_3 , fT_4 , and canine TSH (cTSH) were obtained 7 days before administration was initiated, immediately before administration of etodolac on the day administration was initiated (day 0), and on days 14 and 28 after initiation of etodolac administration. Blood samples for hormone analyses were obtained by jugular venipuncture and allowed to clot in siliconized glass tubes. Samples were centrifuged (2,000 \times g for 15 minutes) within 30 minutes after collection. Serum was harvested and stored frozen at -70°C until analysis.

Similar to the samples collected for serum harvest, blood samples were collected on days -7 , 14, and 28 into glass tubes containing lithium heparin. Plasma was harvested within 60 minutes after blood collection. Plasma total protein and albumin concentrations were determined within 2 hours after collection of blood samples on days -7 , 14, and

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28. In addition, serum osmolality was measured on days -7, 14, and 28.

Analysis of samples—Serum T_4 ¹ and T_3 ⁸ concentrations were assayed by use of radioimmunoassays validated for use in dogs.¹⁷ Serum concentrations of cTSH were measured by use of a commercial radioimmunometric assay^h validated by the manufacturer for use in dogs. Concentrations of fT_4 were measured by use of a validated¹⁹ equilibrium dialysis radioimmunoassay.¹ All samples were assayed in duplicate. Intra-assay coefficient of variation (CV) was determined by serial measurement of 10 samples of pooled canine serum that contained low, medium, and high concentrations of hormones. Interassay CV was determined by measuring hormone concentrations of pooled serum that contained low, medium, and high concentrations of hormones on 4 days. Intra-assay CV for T_4 was 7.5, 6.3, and 7.3% for low (10.3 nmol/L), medium (24 nmol/L), and high (53 nmol/L) pools, respectively. Intra-assay CV for T_3 was 5.2, 4.4, and 4.9% for low (0.62 nmol/L), medium (1.62 nmol/L), and high (2.33 nmol/L) pools, respectively. Intra-assay CV for fT_4 was 7.6, 9.2, and 7.1% for low (10 pmol/L), medium (27 pmol/L), and high (79 pmol/L) pools, respectively. Intra-assay CV for cTSH was 6.2 and 5.6% for low (0.19 ng/mL) and high (1.14 ng/mL) pools, respectively. Interassay CV for T_4 was 8.2, 6.8, and 6.9% for low, medium, and high pools, respectively. Interassay CV for T_3 was 6.6, 5.4, and 5.4% for low, medium, and high pools, respectively. Interassay CV for fT_4 was 6.3, 7.4, and 8.2% for low, medium, and high pools, respectively. Interassay CV for cTSH was 5.8 and 7.1% for low and high pools, respectively.

Plasma protein and albumin concentrations were measured by use of standard methods in the clinical laboratory of our facility. Values were determined by use of an automated chemistry analyzer.¹ Serum osmolality was measured in triplicate by use of freezing-point depression on an osmometer.^k

Statistical analysis—Data were expressed as mean \pm SEM. The mean of the 2 hormone concentrations determined before administration of etodolac (ie, baseline value) was used for comparison with subsequent measurements. A repeated-measures ANOVA was performed by use of a statistical program.¹ Comparison of mean values for days 14 and 28 with the baseline value was accomplished by use of the Dunnett test. A value of $P < 0.05$ was considered significant.

Results

We did not detect adverse effects of the administration of etodolac in the dogs, and all dogs tolerated the administration of etodolac well. There was not a significant effect of etodolac administration on any of the variables measured by the thyroid function tests (Table 1).

Plasma total protein concentration was significantly decreased on days 14 and 28 of etodolac administration, compared with the concentration before administration (Table 1). Plasma albumin concentration was significantly decreased on days 14 and 28 of administration, compared with the concentration before administration. Plasma globulin concentration was also significantly decreased on days 14 and 28, compared with the concentration before administration. Before etodolac administration, 1 dog had plasma concentrations of total protein and globulin that were higher than the respective reference ranges, and plasma albumin concentration in another dog was higher than the reference range. The concentration of 1 or more plasma proteins was less than the respective reference ranges 14 or 28 days after beginning administration of etodolac in 9 of 19 dogs. At day 14 of administration,

Table 1—Mean \pm SEM serum concentrations of thyroxine (T_4), 3,5,3'-triiodothyronine (T_3), free T_4 (fT_4), and canine thyroid stimulating hormone (cTSH), concentrations of plasma proteins, and serum osmolality in samples obtained from dogs before and on days 14 and 28 after initiation of administration of etodolac (mean dosage, 13.7 mg/kg, PO, q 24 h) for 28 days

Variable	Before	Day 14	Day 28
T_4 (nmol/L)	28 \pm 1.3	27 \pm 1.6	27 \pm 2.0
T_3 (nmol/L)	1.39 \pm 0.08	1.20 \pm 0.12	1.49 \pm 0.15
fT_4 (pmol/L)	24 \pm 1.5	23 \pm 1.6	26 \pm 2.4
cTSH (ng/mL)	0.24 \pm 0.03	0.20 \pm 0.03	0.23 \pm 0.03
Total protein (g/dL)	6.51 \pm 0.09	5.74 \pm 0.18*	5.71 \pm 0.19*
Albumin (g/dL)	3.49 \pm 0.07	3.04 \pm 0.09*	3.07 \pm 0.10*
Globulin (g/dL)	3.02 \pm 0.13	2.69 \pm 0.17*	2.63 \pm 0.17*
Serum osmolality (mOsm/L)	313 \pm 1.5	313 \pm 0.7	312 \pm 1.5

*Within a row, value differs significantly ($P < 0.05$) from value obtained before onset of administration.

plasma total protein concentration was less than the reference range in 6 dogs and higher than the reference range in 1 dog, plasma albumin concentration was less than the reference range in 4 dogs, and plasma globulin concentration was less than the reference range in 1 dog and higher than the reference range in 2 dogs. On day 28 of administration, plasma total protein concentration was less than the reference range in 6 dogs and higher than the reference range in 2 dogs, plasma albumin concentration was less than the reference range in 2 dogs, and plasma globulin concentration was less than the reference range in 4 dogs and higher than the reference range in 2 dogs. There was not a significant effect of etodolac administration on serum osmolality.

Discussion

Effects of a number of NSAIDs on thyroid function have been evaluated in humans. Of the many NSAIDs used in humans, salsalate and salicylate have the most consistent and extensive effect on results of thyroid function tests.^{4,5} The main effect of NSAIDs on thyroid function is through impairment of hormone binding to plasma transport proteins. Administration of the NSAID salsalate to humans results in rapid displacement of T_4 and T_3 from plasma proteins, resulting in an acute decrease in serum T_4 and T_3 concentrations and presumably an increase in fT_4 and free T_3 concentrations.^{4,5} Serum TSH concentration decreases in response to the increase in concentrations of free thyroid hormones.⁴ Salsalate and salicylate impair binding to all major thyroid hormone transport proteins in humans.⁶ Effects of other NSAIDs on thyroid function have been less extensively studied, and we are aware of only 1 study¹⁸ in which investigators evaluated the effects of etodolac on results of thyroid function tests. In that study, results for humans that were receiving etodolac to treat various arthralgias were compared with a control population that was not treated with an NSAID. There was not a significant difference in serum concentrations of T_4 , fT_4 index, fT_4 measured by use of an analogue assay, T_3 , and TSH between control patients and patients receiving etodolac. Other NSAIDs that can alter 1 or more indices of thyroid function in humans include nabumetone, ketoprofen, diclofenac, and naproxen.^{1,18} In horses, phenylbutazone reduces concentrations of T_4 and fT_4 .²

On the basis of results of the study reported here, administration of etodolac for 4 weeks to clinically normal dogs does not alter results of thyroid function tests. Because the effects of NSAIDs on results of thyroid function tests in humans are dependent on the particular drug, this outcome is not surprising. Carprofen is the only other NSAID that the authors are aware of that was investigated for its effects on thyroid function tests. Preliminary results revealed slight decreases in serum T_4 and cTSH concentrations after administration of carprofen for 2 and 5 weeks.^b Serum fT_4 concentration and the fraction of T_4 that was free were not altered. An in vitro study^a of canine serum that used equilibrium dialysis to enable investigators to evaluate the effects of various drugs on protein binding of T_4 revealed that the NSAID flunixin meglumine inhibited protein binding, thus increasing the free fraction of T_4 in the dialysate. In that same study, investigators failed to detect a similar effect for phenylbutazone. It is perhaps surprising that there was not a reduction in serum T_4 and T_3 concentrations in the study reported here, because concentrations of plasma proteins decreased, but the decrease in plasma proteins was slight (12 to 13%) and may not have been of sufficient magnitude to reflect decreased protein-bound thyroid hormone concentrations. Reduction in plasma concentrations of thyroid transport proteins would be expected to result in changes similar to those that occur with impaired protein binding.

Although impaired activity of 5'-deiodinase, the enzyme responsible for metabolizing T_4 to T_3 and degradation of 3,3',5'-triiodothyronine (reverse T_3), is apparent in rats treated with salicylate, the effects of NSAIDs on this enzyme in humans appears to be small or nonexistent.^{4,5,8} We did not detect evidence of altered thyroid hormone metabolism in the study reported here.

According to the package insert,^e hypoproteinemia occurs in some dogs treated with etodolac. Hypoproteinemia does not appear to be a reported complication in humans treated with etodolac.¹⁹ The cause of the hypoproteinemia in the study reported here is unclear, although the concurrent decrease in albumin and globulins is most consistent with gastrointestinal loss or dilution by water retention rather than lack of production or renal loss. Renal loss seems unlikely, because etodolac administration did not increase renal excretion of albumin in clinically normal humans after 14 days of treatment.²⁰ Dilution of plasma proteins secondary to impaired excretion of water is another possible cause of hypoproteinemia. Inhibition of prostaglandin production during NSAID administration increases vasopressin secretion, enhances antidiuretic effects of vasopressin, and decreases urinary excretion of sodium.²¹⁻²³ These changes increase fluid retention and lead to dilutional hypoproteinemia. Because serum osmolality was not altered during etodolac administration, retention of water is unlikely to be the cause of the hypoproteinemia found in the study reported here.

Toxic effects on the gastrointestinal tract that result in gastric and duodenal erosions and ulcers are evident during treatment with many NSAIDs. However, it is believed that the small intestine may be

a more common site for the adverse effects of NSAIDs, compared with the stomach.²⁴ Enteropathy induced by NSAIDs may range from mild to severe and may include bleeding and protein loss.^{24,25} Lesions associated with NSAID-induced enteropathy are often found in the middle part of the small intestine and, thus, are not detected during routine gastrointestinal endoscopy.

Tests of gastrointestinal permeability are necessary to document alterations in mucosal integrity. Tests of gastrointestinal permeability, including measurement of fecal excretion of various carbohydrates or ⁵¹Cr-EDTA have been used to document increased permeability during NSAID treatment.²⁴ The proposed mechanism of increased permeability includes an effect of NSAIDs on mitochondrial activity in epithelial cells in addition to inhibition of COX.^{24,25} Similarly, intestinal inflammation, measured by the use of radiolabeled leukocytes or fecal calprotectin, is increased in patients administered NSAIDs.²⁵ Similar testing would be necessary to explore the cause of the hypoproteinemia recognized in dogs in the study reported here.

^aFerguson DC. Influence of common drugs on the free thyroxine fraction in canine serum (abstr). *J Vet Intern Med* 1989;3:120.

^bFerguson DC, Moore GE, Hoenig M. Carprofen lowers total T_4 and TSH, but not free T_4 concentrations in dogs (abstr). *J Vet Intern Med* 1999;13:243.

^cBovine TSH, Sigma Chemical Co, St Louis, Mo.

^dCanine Dry Science Diet Maintenance, Hill's Pet Nutrition Inc, Topeka, Kan.

^eEtoGesic, Fort Dodge Animal Health, Fort Dodge, Iowa.

^fCoat-A-Count Canine T_4 , Diagnostic Products Corp, Los Angeles, Calif.

^gCoat-A-Count Canine T_3 , Diagnostic Products Corp, Los Angeles, Calif.

^hCoat-A-Count Canine TSH IRMA, Diagnostic Products Corp, Los Angeles, Calif.

ⁱFree T_4 by equilibrium dialysis, Nichols Institute Diagnostics, San Juan Capistrano, Calif.

^jOlympus AU400 automated chemistry analyzer, Olympus America, Melville, NY.

^kModel 5004 automatic osmometer, Precision Systems Inc, Matick, Mass.

^lSAS, version 8.01, SAS Institute Inc, Cary, NC.

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