Effects of carprofen and meloxicam on C-reactive protein, ceruloplasmin, and fibrinogen concentrations in dogs undergoing ovariohysterectomy

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Objective—To evaluate the effects of perioperative oral administration of carprofen and meloxicam on concentrations of 3 acute-phase proteins in dogs undergoing elective ovariohysterectomy (OVH).

Animals—18 healthy adult anestrous female dogs undergoing elective OVH.

Procedures—Dogs were allocated to 3 groups (6 dogs/group). A placebo treatment, carprofen (2.0 mg/kg), or meloxicam (0.2 mg/kg) was orally administered to the dogs of the respective groups. The initial doses were administered 30 minutes before premedication prior to OVH; additional doses were administered once daily for 4 days after surgery. Blood samples were collected 45 minutes before premedication and 4, 8, 12, 24, 36, 48, 72, 96, and 120 hours after the end of OVH; samples were used for measurement of total WBC and neutrophil counts and concentrations of C-reactive protein (CRP), ceruloplasmin, and fibrinogen.

Results—Values did not differ significantly among groups for WBC and neutrophil counts, serum concentrations of CRP and ceruloplasmin, and plasma concentrations of fibrinogen. Concentrations of all inflammatory markers, except serum ceruloplasmin, increased significantly following OVH, but in a similar manner for each group. No significant changes were detected in serum ceruloplasmin concentrations over time.

Conclusions and Clinical Relevance—Perioperative administration of both carprofen and meloxicam did not significantly affect the concentrations of CRP, ceruloplasmin, and fibrinogen in dogs undergoing OVH. Thus, use of carprofen or meloxicam should not affect clinical interpretation of results for these 3 acute-phase proteins. (Am J Vet Res 2013;74:1267–1273)
Moreover, knowledge of the postoperative APR is essential if APPs are to be used for monitoring postoperative infections.

Ovariohysterectomy is one of the most commonly performed surgical procedures in small animal practice. This surgery can cause marked signs of postoperative pain in dogs. Administration of analgesics before and after surgery may result in improved postoperative well-being of ovariohysterectomized dogs. Post-surgical pain control is commonly achieved in dogs by administration of opioid drugs, NSAIDs, local anesthetics, or a combination of these products.

The NSAIDs are among the most widely used drugs in veterinary medicine. They block the activity of COX enzymes and reduce prostaglandin concentrations throughout the body. As a consequence, inflammation, pain, and fever are reduced. Inhibition of COX-2 is believed to mediate the therapeutic actions of NSAIDs, whereas COX-1 inhibition results in adverse effects. The duration (12 to 24 hours) and efficacy of the NSAIDs make them ideal for the clinical management of postoperative pain and inflammation in dogs; however, patients and NSAID selection must be considered prior to NSAID use because of the potential for adverse effects (eg, gastric ulcers, renal injury, decrease in wound healing, and an increase in hemorrhage related to platelet dysfunction). The NSAIDs, including carprofen and meloxicam, have been developed with the aim of enhancing the intended effects of these agents while reducing adverse effects. Investigators in a study found via use of a canine monocyte-macrophage cell line that carprofen, a propionic acid derivative, was only 1.75 times as selective for COX-2 as for COX-1, whereas meloxicam, an enolic NSAID, inhibited COX-2 activity 12 times as effectively as it inhibited the activity of COX-1. Regarding analgesic effects, both carprofen and meloxicam provide satisfactory analgesia for 72 hours after OVH in dogs.

The APPs are believed to have an important role in restricting tissue damage via scavenging and neutralization of oxygen free radicals, proteolytic enzymes, antigens, and microorganisms. They also promote resolution and tissue repair and are believed to enhance nonspecific and specific components of host defenses. However, when this effect of APPs is excessive in magnitude or duration, the beneficial inflammatory process may lead to deterioration, rather than to restoration of homeostasis. Thus, it is important to know whether perioperative treatment with carprofen or meloxicam may regulate the intensity of the APR in dogs undergoing OVH.

Moreover, several factors (eg, breed, pregnancy, environment and housing, administration of vaccines, use of phenobarbital, anthelmintic or glucocorticoid treatment, and assay method) can influence serum concentrations of some APPs. In these situations, it is possible to have false-positive increases in concentrations of APPs. Consequently, preanalytic and analytic factors that influence APP concentrations should be considered to ensure proper and adequate clinical interpretation of the results. Although NSAIDs are widely and successfully used for many clinical situations, information regarding their effects on serum APP concentrations are scarce and variable in the literature. Studies in humans have revealed conflicting results, ranging from negligible effects to a decrease in concentrations, which may have been related to differences in the time of blood sample collection. Administration of carprofen, etodolac, or meloxicam to dogs with induced acute synovitis had no significant effect on CRP concentrations, whereas oral administration of a single dose of acetylsalicylic acid (200 mg/kg) resulted in increases in CRP, serum amyloid A, haptoglobin, and fibrinogen concentrations. Although CRP, ceruloplasmin, and fibrinogen concentrations have been evaluated in animals after OVH, the authors are not aware of any reports on the alteration of APPs in ovariohysterectomized dogs treated with carprofen or meloxicam for postoperative pain control. Therefore, the purpose of the study reported here was to investigate the effects of carprofen and meloxicam administration on concentrations of 3 APPs (CRP, ceruloplasmin, and fibrinogen) in dogs undergoing OVH.

Materials and Methods

Dogs—Eighteen healthy adult anestrous bitches from the Animal Shelter in Aydin, Turkey, were selected to undergo elective OVH. Dogs were of various breeds, weighed between 12 and 20 kg, and were 1.5 to 4.0 years old. Vaginal cytologic examinations were performed to confirm the stage of the estrous cycle of each dog. Dogs had unremarkable results for hematologic and biochemical analyses. The dogs were housed in kennels in identical conditions. Each dog was fed a commercial dog food twice daily throughout the study; the amount of food was determined on the basis of body weight. Water was offered ad libitum. Food and water were withheld for 12 hours before anesthesia. The study protocol was approved by the Animal Ethics Committee of the University of Adnan Menderes.

Study design—The dogs were allocated via a randomization procedure (randomization was achieved by use of a random-number table generated by a computer) into 3 groups (n = 6 dogs/group). Dogs in the placebo group received an empty gelatin capsule (PO, q 24 h) for 5 days (control treatment). Dogs in the meloxicam group received 2 mg of meloxicam/kg (PO, q 24 h) for 5 days. Dogs in the meloxicam group received 0.2 mg of meloxicam/kg (PO, q 24 h) for 5 days. The drug dosages used in the study were the dosages commonly recommended in the veterinary literature.

The first doses were orally administered 30 minutes before injection of preanesthetic medications. Day of OVH was designated as day 0.

OVH—Anesthetic and surgical procedures were standardized for all dogs. All surgeries were performed by the same surgeon (TT), who was experienced with the technique. Dogs were premedicated with atropine sulfate (0.04 mg/kg, IM) and anesthetized via IM injection of xylazine hydrochloride (1.1 mg/kg) and ketamine hydrochloride (22 mg/kg). After dogs were anesthetized, a standard aseptic preparation was performed, which was followed by elective OVH via a routine ventral midline approach.
Duration of surgery did not exceed 60 minutes from the administration of preanesthetic medications. After the surgery was completed, all dogs received 2 doses of long-acting amoxicillin (15 mg/kg, SC, q 48 h). Clinical examinations were performed daily beginning on the day of OVH and continuing until the skin sutures were removed 7 days after OVH.

Collection of blood samples and laboratory analyses—Blood samples (2.5 mL/sample) from all dogs were collected via cephalic venipuncture into 2 evacuated tubes (one that contained EDTA-K3 and another that contained a coagulation activator). Blood samples were obtained 45 minutes before injection of preanesthetic medications (baseline; time 0) and 4, 8, 12, 24, 36, 48, 72, 96, and 120 hours after the end of OVH. An aliquot of each sample was used to determine hematologic variables. A CBC was performed with a blood cell counter2 calibrated for canine blood; WBC and neutrophil counts were used for statistical analysis. Plasma fibrinogen concentration was measured via the Millar technique.24 Hematologic variables and plasma fibrinogen concentrations were determined within 6 hours after sample collection.

Serum was obtained by centrifugation at 3,000 g for 10 minutes. Sera were harvested and stored in a freezer at −20°C; all serum samples were analyzed on the same day after the conclusion of the sample collection period. Serum CRP concentration was measured with a commercially available canine sandwich ELISA kit. The assay was performed in accordance with the manufacturer’s instructions. The serum concentration of ceruloplasmin was determined by measuring p-phenylenediamine oxidase activity as described in another study.25

Statistical analysis—Statistical analyses were performed with a statistical software program.6 A Kolmogorov-Smirnov test was used to assess all variables for normality. For data that were not distributed normally, transformations were applied to normalize the distribution. The effects of time, group (ie, treatment), and group-by-time interaction were assessed via
an ANOVA for repeated measures. When a significant group-by-time interaction was detected, Tukey multiple comparison tests were used to compare treatments within each time period. Within each group, the baseline value was compared with the values at various time points after OVH by use of the Bonferroni correction method. Results were considered significant at values of P < 0.05. Comparisons within and between groups were based on the final statistical model.

**Results**

All dogs remained healthy throughout the study. Slight increases in rectal temperature were detected in the placebo group on days 1 or 2, but the temperature remained within reference limits in all 3 groups for the duration of the study. Dogs in the placebo group developed mild swelling of the abdominal incision (days 1 through 3) and had signs of pain (days 1 and 2); however, these signs of inflammation were not accompanied by fever or purulent discharge from the incision, and all dogs remained bright and alert.

Changes in mean values for serum concentrations of CRP and ceruloplasmin, plasma concentrations of fibrinogen, and WBC and neutrophil counts were determined over the sample collection period for the 3 groups (Figure 1). Values increased significantly over time for CRP (P < 0.001) and fibrinogen (P = 0.018) concentrations and WBC (P < 0.001) and neutrophil (P < 0.001) counts but not for ceruloplasmin concentrations (P = 0.273). The increase was similar among all 3 groups. The repeated-measures ANOVA revealed that there was no significant effect of group for CRP (P = 0.740), ceruloplasmin (P = 0.276), and fibrinogen (P = 0.070) concentrations as well as WBC (P = 0.951) and neutrophil (P = 0.637) counts. Similarly, there was no significant group-by-time effects for CRP (P = 0.255), ceruloplasmin (P = 0.080), and fibrinogen (P = 0.597) concentrations as well as WBC (P = 0.703) and neutrophil (P = 0.646) counts. Neither carprofen nor meloxicam was able to significantly diminish the APR, compared with the effect for the placebo treatment.

Values for all 5 inflammatory markers were similar among groups before surgery. After OVH, serum CRP and plasma fibrinogen concentrations increased in all 3 groups, although with a different time course and magnitude, whereas there were no significant differences in serum ceruloplasmin concentrations between baseline and postoperative times, although the concentration appeared to increase after OVH (Figure 1). Serum CRP concentration increased significantly (P < 0.001), compared with the preoperative baseline concentration, at 4 hours, peaked at 24 hours after OVH, and typically decreased gradually thereafter in all 3 groups. Mean peak CRP concentrations were 16.9-fold, 15.7-fold, and 14.7-fold as high as the preoperative concentrations for the placebo, carprofen, and meloxicam group, respectively. Fibrinogen concentrations began to increase 24 hours after OVH, reached maximum values at 48 hours after OVH (approx 1.5-fold increase), and remained elevated up to 120 hours after OVH in all 3 groups. Mean CRP, ceruloplasmin, and fibrinogen concentrations in dogs receiving carprofen or meloxicam did not differ significantly from concentrations in dogs receiving the placebo treatment at any time after OVH.

Perioperative administration of carprofen and meloxicam had no significant effect on WBC and neutrophil counts in dogs undergoing OVH. In all 3 groups, mean WBC count increased significantly (P = 0.004), compared with the preoperative baseline count, at 8 hours after OVH, reached a maximum 12 hours after OVH, and typically decreased gradually thereafter (Figure 1). Increased WBC counts were mainly attributable to neutrophilia. Similar to WBC counts, the neutrophil count increased significantly (P = 0.002), compared with the preoperative baseline count, at 8 hours after OVH, peaked at 12 hours after OVH (approx 3.5- to 4-fold increase), and then decreased up to 96 hours after OVH in all 3 groups.

**Discussion**

Ceruloplasmin, CRP, and fibrinogen are recognized as positive APPs in dogs. The blood concentrations of these APPs can increase significantly in many inflammatory and tissue-injury conditions; thus, evaluation of these APPs can provide valuable information for use in the diagnosis, prognosis, and monitoring of disease in canine practice. However, this requires that the response not be modulated or induced by drug treatments, which could lead to results that would be incorrectly interpreted. Although NSAIDs are among the most frequently used drugs for conditions associated with inflammation, acute and chronic pain, and fever, data on their effects on APPs are scarce and controversial. Analysis of results of the present study indicated that perioperative oral administration of carprofen and meloxicam has no significant effects on concentrations of CRP, ceruloplasmin, and fibrinogen during the 120-hour period after a bitch undergoes OVH.

One of 7 criteria needed for APP interpretation in companion animals is that the APP profile should include at least 1 major positive APP and 1 moderate positive APP because of differences in the magnitude and time course of increases in concentration. In dogs, serum CRP is considered a major APP because it increases rapidly (10- to 100-fold increase), whereas ceruloplasmin and fibrinogen are classified as moderate APPs because they have only a 2- to 10-fold increase in plasma concentration in response to inflammation.4 In the present study, 1 major positive APP (CRP) and 2 moderate positive APPs (ceruloplasmin and fibrinogen) were selected for use in determining the effects of perioperative oral administration of carprofen or meloxicam to bitches undergoing OVH.

In a review of APPs, investigators tabulated CRP concentrations of 0.22 mg/L to 16.4 mg/L for healthy dogs. However, there are reports in which the concentrations were as high as 23.1 mg/L or 31 mg/L. The CRP concentrations before OVH in all 3 groups of the present study were < 10 mg/L, which is in accordance with results reported by other investigators. Higher values reported with the same assay method indicate physiologic variation of serum CRP concentrations in healthy dogs. Similarly, reference ranges for ceruloplasmin in serum or plasma of dogs may differ widely. The ceruloplasmin concentration determined for healthy dogs has been reported as < 4.93 mg/dL.
Leeleucocytosis and neutrophilia as well as increased se-
dogs is characterized by a number of effects, including
ogen concentrations in all 3 groups were within ranges
shelter than in client-owned or laboratory dogs. Fibrin-
of subclinical disease expected in dogs obtained from a
ment or the source of animals, with a greater degree
have reflected differences in the method of measure-
ment of significant differences between the preoperative and
sresponse to OVH could have been responsible for the lack
of significant differences between the preoperative and postoperative values.

The APR to surgical trauma, including OVH, in
dogs is characterized by a number of effects, including
leukocytosis and neutrophilia as well as increased se-
rum concentrations of CRP and ceruloplasmin and
plasma concentrations of fibrinogen. In the present study, OVH induced increases in WBC and neu-
trophil counts as well as marked increases in CRP and fibrinogen concentrations, compared with preoperative values. These results are indicative of a substantial APR to the surgical trauma. In contrast to CRP and fibrinogen concentrations, ceruloplasmin concentrations did not change significantly after OVH. A number of studies have found a significant increase in ceruloplasmin concentrations in response to surgical trauma and the inflammatory process, although authors of 1 study reported no significant difference in plasma concentrations of ceruloplasmin after OVH. Although ceruloplasmin is slightly more specific than other APPs because it remains stable during treatment with corticosteroids, inflammation in dogs could lead to only a 2- or 3-fold increase or an increase of 50%, which clearly compromises the usefulness of ceruloplasmin concentrations when assessing mild inflammatory conditions. Furthermore, interindividual variation in the ceruloplasmin response to OVH could have been responsible for the lack of significant differences between the preoperative and postoperative values.

The magnitude and time course of the change in concentrations of the APPs differs depending on the type of inflammatory stimulus. In surgical interventions, the severity of the APR is also influenced by the intensity of the surgical procedure and experience of the surgeon. Laparoscopic OVH causes less surgical trauma than does traditional OVH, and there is a smaller increase in serum CRP concentrations in dogs when OVH is performed by an experienced surgeon.

In the present study, all surgeries were performed by the same surgeon, who was experienced with this technique.

Serum CRP concentrations can increase up to 95-
fold, whereas 2- to 3-fold increases in ceruloplasmin concentrations and up to 4-fold increases in fibrinogen concentrations have been reported in response to surgical trauma in dogs. Concentrations of CRP in dogs increase significantly by 4 hours after stimulation, with a maximum peak 24 hours after surgical trauma, including OVH. In contrast, plasma fibrinogen concentrations reportedly increase by 24 hours, and maximal serum concentrations of ceruloplasmin and plasma concentrations of fibrinogen have been detected 2 and 3 days after OVH, respectively. Studies on the APR in healthy bitches after OVH have revealed that serum concentrations of CRP increase significantly from a baseline value of 3.33 mg/L to 8.83 mg/L, from 2.8 mg/L to 103.8 mg/L, and from 3.91 to 36.34 mg/L on the first or second day after OVH. Plasma fibrinogen concentrations also increase significantly from a mean baseline value of 0.27 g/dL to 0.44 g/dL and from 0.15 g/dL to 0.52 g/dL by 24 hours after OVH. Results of the present study were in agreement with findings in other studies. In the study reported here, the serum CRP concentration increased 16.9-fold for the placebo group, 15.7-fold for the car-
profen group, and 14.7-fold for the meloxicam group at 24 hours after OVH, compared with baseline values. The increases in plasma fibrinogen concentration were approximately 1.3-fold for all 3 groups at 48 hours after OVH. The apparent discrepancy regarding the rate of increase for CRP and fibrinogen concentrations among studies may be related to the degree of surgical trauma or experience of the surgeon.

The NSAIDs, including carprofen and meloxicam, are commonly administered for 3 or 4 days in the perioperative management of pain resulting from OVH. Despite the fact administration of NSAIDs is frequently used for pain reduction during the perioperative period, data on the effects of NSAIDs on APPs in surgical patients are limited and contradictory. Most surgical studies in humans have found that NSAIDs have no important effects on the classical stress hormones, APPs, and leukocytosis. However, investigators in 1 study found that serum concentrations of CRP after thoracotomy are reduced by flurbiprofen. Results of a study in clinically normal dogs revealed that administration of carprofen (2.2 mg/kg, PO, q 12 h for 5 days) caused no significant alteration in fibrinogen concentration. Similarly, administration of meloxicam (0.1 mg/kg, PO, q 24 h for 7 days) to healthy dogs resulted in a significant decrease in fibrinogen concentration, but values remained within the reference interval. Meloxicam appears to also have no effect on concentrations of serum CRP and haptoglobin, which are considered important markers of the innate immune system of pigs. Conversely, administration of carprofen to bulls undergoing castration reduced surgery-related increases in fibrinogen and haptoglobin concentrations. Finally, the use of NSAIDs, including carprofen and meloxicam, for pain relief did not significantly alter serum concentrations of CRP in dogs with experimentally induced synovitis. Glucocorticoids, which have eicosanoid-depressing, anti-inflammatory actions similar to those of NSAIDs, do not affect serum concentrations of CRP and ceruloplasmin in healthy dogs, although they generally enhance the stimulatory effects of cytokines on the production of APPs.

The present study revealed that there were no significant effects of perioperative administration of therapeutic doses of carprofen and meloxicam on serum concentrations of CRP and ceruloplasmin and on plasma concentrations of fibrinogen in bitches after OVH. Increased CRP and fibrinogen concentrations in the first few days after surgery were a physiologic phe-
omenon in response to tissue injury caused by OVH.

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because there were no significant differences among the 3 groups at each time point after OVH. To our knowledge, this is the first study conducted to determine the effects of perioperative administration of carprofen and meloxicam on CRP, ceruloplasmin and fibrinogen concentrations with bitches after OVH. Therefore, results of this study were not directly comparable with those of previous studies.

The fact there were no significant effects of carprofen and meloxicam on concentrations of the 3 positive APPs in this study may be explained by the influences of NSAIDs on proinflammatory cytokines. Proinflammatory cytokines (eg, IL-1, IL-6, and tumor necrosis factor-α) play a central role in activation of the APR, which is reflected in the production of CRP and other APPs by the liver. Interleukin-6 is the main cytokine released after routine surgery. It primarily regulates the hepatic component of the APR and induces the production of APPs such as CRP, fibrinogen, α1-antitrypsin, and haptoglobin. Although concentrations of proinflammatory cytokines were not measured in the present study, NSAIDs are not thought to directly block production of IL-6, and there is no convincing in vivo evidence that synthesis of IL-1 is affected by NSAIDs.

It is possible that pathways involving prostaglandin synthesis play only a minor role in eliciting the postoperative APP response. In addition, it is noteworthy that carprofen at therapeutic doses does not inhibit the COX enzymes. On the other hand, there is evidence indicating that NSAIDs administered at suprapharmacologic doses can stimulate the APR as a result of increased gastrointestinal tract permeability. The NSAIDs compromise the integrity of the gastrointestinal barrier, depending on both the compound and dose administered; thus, NSAID-induced gastrointestinal injury releases proinflammatory bacteria or lipopolysaccharide (or both) into the systemic circulation and liver, where such foreign material stimulates an APR.

Investigators in 1 study used an endoscopic scoring system to assess the development of gastroduodenal lesions in dogs treated with orally administered carprofen or meloxicam at therapeutic doses for 28 days; however, compared with results for control dogs, scores for the lesions were not significantly different. In the present study, both carprofen and meloxicam were administered at therapeutic doses for a short-term period, and none of the dogs had clinical signs related to gastrointestinal tract lesions; therefore, stimulation of an APR as a result of gastrointestinal tract injury induced by these NSAIDs was unlikely.

In addition to their usefulness in diagnostic evaluations, positive APPs are used as predictive variables for uncomplicated and complicated surgery in human and veterinary medicine. In various types of uncomplicated surgeries, a marked postoperative decrease in the CRP, ceruloplasmin, and fibrinogen concentrations at different times after an initial increase can be detected. In contrast, an increase or persistently high concentrations of these APPs after surgery have been detected in patients with complicated surgeries. Analysis of data collected from humans during the 6-day period after elective surgery revealed that CRP concentrations slowly decreased after peaking at day 2 after surgery, whereas fibrinogen concentrations initially increased slowly and did not return to within reference limits until up to a week after CRP concentrations had returned to baseline values. Data on CRP and fibrinogen concentrations collected over a 21-day period confirmed the information from earlier reports. When a patient had no postoperative complications, CRP concentrations returned to within reference limits by day 10 after surgery, whereas fibrinogen concentrations returned to within reference limits by day 21 after surgery. Typically, serum concentrations of CRP in dogs gradually decreased over several days after surgery. In dogs that underwent an OVH, CRP concentrations in dogs with no complications during or after surgery began to decrease 3 days after surgery and reached values within reference limits in 3 weeks, whereas CRP concentrations in dogs with postoperative infections remained high after surgery. In the present study, serum concentrations of CRP and plasma concentrations of fibrinogen decreased rapidly after peak concentrations were reached, but the concentrations did not return to preoperative values in all 3 groups because of the short observation period.

For the study reported here, we concluded that perioperative oral administration of carprofen and meloxicam at therapeutic doses did not lead to significant changes in CRP, ceruloplasmin, and fibrinogen concentrations. Therefore, use of carprofen or meloxicam did not affect clinical interpretation of concentrations of these 3 APPs.

References

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