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Bioavailability and biochemical effects of diclofenac sodium 0.1% ophthalmic solution in the domestic chicken (Gallus gallus domesticus)

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Objective To determine if topical ophthalmic diclofenac sodium 0.1% solution alters renal parameters in the domestic chicken, and to determine if the drug is detectable in plasma after topical ophthalmic administration.

Animals Thirty healthy domestic chickens

Procedures Over seven days, 6 birds were treated unilaterally with 1 drop of artificial tear solution (group 1), 12 birds were treated unilaterally (group 2) and 12 bilaterally (group 3) with diclofenac sodium 0.1% ophthalmic solution. Treatments were provided for 7 days, every 12 hours in all groups. Pre- and post-treatment plasma samples from all birds were evaluated for changes in albumin, total protein, and uric acid. Post-treatment samples of all birds were also analyzed by HPLC-MS for detection of diclofenac sodium.

Results Changes in pre- and post-treatment plasma albumin were significant ($P < 0.05$) in groups 2 and 3, but not for group 1. Pre- and post-treatment changes in total protein and uric acid pre- and post-treatment were not significant for any group. Diclofenac sodium was not detectable (limit of detection = 0.10 ng/mL) in plasma samples from birds in group 1. Concentration of drug in group 3 was statistically greater than group 2 ($P = 0.0008$).

Conclusions and Clinical Relevance Topical ophthalmic diclofenac sodium 0.1% administered every 12 hours in one or both eyes for 7 days is detectable in systemic circulation in the domestic chicken at 15 minutes post-administration, but did not cause overt changes in parameters used to monitor renal physiology.

Key Words: avian, diclofenac, glomerulonephritis, nonsteroidal anti-inflammatory

Abbreviations:

HPLC-MS, high-performance liquid chromatography with mass spectrometry

NSAID, nonsteroidal anti-inflammatory drug

Diclofenac sodium 0.1% ophthalmic solution is a commercially available NSAID used to treat inflammation associated with ocular disease by inhibiting cyclooxygenase, the enzyme responsible for prostaglandin synthesis during uveitis.[1,2]

Corticosteroids may also be used to treat ocular inflammation, however, topical corticosteroids are used cautiously in ophthalmic diseases due to potential side effects.[3] In birds, topical ophthalmic corticosteroids use is further limited by systemic absorption and consequent potential immunosuppression.[4] Side effects of topical ophthalmic NSAID therapy can include delayed wound healing, keratitis, and keratomalacia, but are generally considered more benign than effects of corticosteroids.[5-10] Thus, topical NSAIDs are often preferred for chronic management of ocular inflammation in birds.

Recently the use of diclofenac in some avian species has been investigated. Multiple studies have evaluated the toxic effects of oral and injectable diclofenac in various avian species after a sharp decline in the population of *Gyps* vultures in South Asia after the birds ingested carrion from diclofenac-treated livestock. These studies reported fatal renal disease and secondary visceral gout formation in the affected birds.[11-20] Topical diclofenac sodium ophthalmic solution has been used in domestic and wild-caught birds for treatment of ocular inflammation without reported negative clinical side effects.[21,22] However, following topical ophthalmic application the drug is absorbed into systemic circulation, creating a potential for systemic side effects.[3,23,24] To the authors' knowledge, no studies have evaluated the bioavailability or hemodynamics of the drug following topical ophthalmic administration in avian species.

The purpose of this study is to determine if topical ophthalmic diclofenac sodium 0.1% solution adversely affects renal function in the domestic chicken, and to determine if the drug is detectable in plasma after topical ophthalmic administration. The authors hypothesized that a

minor amount of diclofenac would reach detectable levels in plasma, but would not cause a significant change in the parameters used to monitor avian renal function.

Materials and Methods

Approval for this study was obtained from the Iowa State University Institutional Animal Care and Use Committee. The study was conducted in accordance with the Association for Research in Vision and Ophthalmology guidelines concerning the use of animals in ophthalmic and vision research.

Thirty female domestic chickens *(Gallus gallus domesticus)* approximately 2 years of age with a mean weight of 1.46 ± 0.21 kg were acquired from a single laying farm. Each bird received a brief ophthalmic examination with slit-lamp biomicroscopy. The wing tag number for each bird was recorded prior to being randomly assigned a study number (1 – 30). The flock was divided into 3 groups: control (Group 1, n = 6), unilateral treatment (Group 2, n = 12), and bilateral treatment (Group 3, n = 12) and allowed 48 hours to acclimate to the study environment. Throughout the study, each group was housed in a 1.2 x 2.4 meter cage that was elevated 30.5 cm from the floor. The atmosphere was maintained at 21°C with a 12-hour light:dark photoperiod. All groups received identical feed and water sources *ad libitum*.

On day 0, all birds were manually restrained for venipuncture of either the jugular or wing vein with a 25 gauge needle and 2.5 mL of whole blood was collected into a sodium heparin tube. Plasma was collected from the tubes after centrifugation (3,300 rpm x 10 minutes). Each plasma sample was divided evenly into two additive-free 1.5mL blood collection tubes. The samples were kept at -80°C until analysis.

The animals were treated every 12 hours with either artificial tear solution (control) or diclofenac sodium 0.1% ophthalmic solution for 7 days. Group 1 received 1 drop of artificial tear solution in both eyes, group 2 received 1 drop of diclofenac sodium 0.1% in the left eye, and group 3 received 1 drop of diclofenac sodium 0.1% in both eyes.

At the end of the treatment period, an additional 2.5 mL of blood was collected, as previously described, 15 minutes after administration of the final treatment. Plasma was collected and stored via the previously described techniques. After venipuncture, the birds were immediately euthanized using 1-2 mL of intravenous pentobarbital sodium (390 mg/mL). Whole kidneys were harvested from each bird and fixed in 10% neutral-buffered formalin. One kidney from each treatment group was chosen randomly for histopathological review. Tissue samples were sectioned into 5 μm cuts and stained with hematoxylin and eosin.

Biochemical Analysis

A commercially available chemical analyzer was used to obtain albumin, uric acid, and total protein levels for each bird from the frozen pre- and post-treatment plasma samples.

Pharmacokinetic Analysis

Detection of diclofenac concentration in the post-treatment plasma samples from each bird was performed using HPLC-MS detection.[a]

Statistical analysis

Due to non-parametric distribution, pre- and post-treatment biochemical parameters (albumin, uric acid, and total protein) within each group were compared using the Wilcoxon signed-rank

test. Statistical outliers were identified when data was interpreted with a boxplot graph. *P* values < 0.05 were considered significant.

Plasma diclofenac concentrations between groups 2 and 3 were compared using a one-way ANOVA. Linear regression diagnostics and leverage values were utilized to identify outliers.

Results

Pre- and post-treatment albumin, uric acid, and total protein means for each group are summarized in Table 1. Initial statistical analysis revealed no significant difference between pre- and post-treatment total protein or uric acid parameters within any group. A significant decrease in albumin levels was present in post-treatment groups 2 (P = 0.036) and 3 (P = 0.003) when compared to pre-treatment values. Two birds (#22 and #23, both from group 3) were determined to be statistical outliers as identified. Omitting the data from these birds resulted in strengthening the significant decrease in post-treatment albumin (P = 0.002). One of these birds (#22) was also considered an outlier when assessing total protein. Omitting the data point for this bird resulted in a significant decrease (P = 0.023) from pre- to post-treatment total protein within group 3.

Post-treatment plasma diclofenac concentrations were undetectable in samples from each bird in group 1. Detectable concentrations of diclofenac were present in all animals from groups 2 and 3. Results are summarized in Figure 1. Bird #24 (group 3) was identified as an outlier, but it was not influential since drug concentrations in group 3 were significantly greater than group 2 with (P = 0.0008) and without (P = 0.0001) including this data point.

Histopathological examination of the 3 kidneys (1 from each group) showed similar changes. Each sample demonstrated hypercellular mesangial regions in one-third to half of the renal corpuscles within enlarged glomeruli. The peripheral capillary loops in the enlarged glomeruli were distended with a thickened basement membrane. Hypertrophied podocytes and occasional adhesions to Bowman's capsule were also present. These changes are consistent with chronic proliferative glomerulonephritis.

Discussion

Topical ophthalmic diclofenac administered twice daily to chickens unilaterally or bilaterally for one week resulted in detectable plasma levels of drug and minor biochemical alterations. Elevation in uric acid and decreases in albumin and total protein have been demonstrated in plasma of domestic chickens after administration of oral diclofenac sodium in a previous study.[11] For comparison, the present study evaluated for biochemical evidence of renal pathology by monitoring plasma albumin, total protein, and uric acid levels after administration of topical ophthalmic diclofenac sodium 0.1% for one week.

After discarding outliers, significant decreases in post-treatment albumin ($P = 0.002$) and total protein ($P = 0.023$) were present within the groups treated with diclofenac (groups 2 and 3). A statistical difference was not detected for uric acid in any of the 3 groups. Uric acid is a more significant indicator of avian renal pathology than albumin or total protein.[25] In the present study, although albumin and total protein decreased post-treatment, they were maintained within previously published reference intervals.[26]

In birds, 90% of uric acid is eliminated via the kidneys and is used in conjunction with clinical signs and other diagnostic testing to assess for renal disease.[27] Fluctuations in uric acid are

possible due to extrarenal causes and other confounding factors (species, diet, age, environment, ovulatory activity, and laboratory methodology for sample processing), therefore sequential evaluation is preferred to evaluate for renal dysfunction.[27,28] Ultimately, single-point biochemical parameters may raise suspicion for renal pathology, but antemortem diagnosis is most accurately made when combining serial clinical pathologic abnormalities with clinical signs (lethargy, polyuria, weakness), urinalysis, abdominal imaging, and endoscopic renal evaluations.[29,30]

Finally, various disease processes decrease albumin while increasing globulin.[31] This potentially results in the cumulative total protein remaining within a normal reference interval. The use of albumin:globulin ratio has more clinical significance.[32] Therefore, the resultant changes in total protein and albumin in the present study may not specifically reflect changes in renal function.

Concentration of diclofenac in plasma was determined using HPLC-MS. Plasma samples were not analyzed for the presence of diclofenac prior to the start of this study due to the history of no previous NSAID administration. Post-treatment samples were negative for diclofenac in control birds. In a review of ophthalmic medications, it was determined that peak plasma concentrations of approximately half of the drugs were reached after 15 minutes of topical ophthalmic administration in humans.[23] Another study reported the time to maximum plasma concentration after ophthalmic administration of diclofenac sodium 0.1% was 15 minutes in rabbits.[33] Although the pharmacokinetic profile of topical ophthalmic diclofenac sodium 0.1% in birds has not been established, the timing of post-treatment sample collection in the current study was determined based on those previous reports. As expected, diclofenac was detected in group 2 (mean = 25.4 ± 9.19 ng/mL) and group 3 (mean = 51.3 ± 21.19 ng/mL).

Biochemical or fatal changes of diclofenac treated birds in previous studies occurred at doses ranging from 0.005 mg/kg to 20 mg/kg with *Gyps* vultures being the most sensitive to effects.[11,12,14,16-20] Of the studies that utilized chickens, a 2 mg/kg dose caused biochemical, but non-lethal, changes.[11] If we assume 100% systemic absorption of the diclofenac sodium ophthalmic drops (~0.04 mg/drop), the mean per treatment dose of diclofenac for birds in the present study would be 0.028 mg/kg and 0.057 mg/kg for the treated groups 2 and 3, respectively. The extent of systemic effects from topically applied ophthalmic diclofenac is unknown, however, given the low plasma concentration detected in the present and previous studies it is unlikely that a toxic level could be reached in plasma.[3,34]

Previous studies demonstrated that sensitivity differences in response to NSAIDs exist between avian species.[12-14,18] For example, one report determined that although chickens can succumb to diclofenac toxicity (50% lethal dose ~10 mg/kg), they are more tolerant of the biochemical effects (abnormal renal parameters and acute renal tubular necrosis) compared to *Gyps* vultures (50% lethal dose ~0.1 mg/kg).[12,14] Other studies reported the Pied crow (*Corvus albus*) and turkey vulture (*Cathartes aura*) are not susceptible to diclofenac-induced renal toxicity.[12,18] Although the present study demonstrated the apparent safety of unilateral or bilateral twice daily ophthalmic diclofenac administration in chickens, it may not be possible to extrapolate the safety of this dose to all avian species.

Chronic proliferative glomerulonephritis was observed histopathologically in one randomly selected sample from each of the three treatment groups. These lesions differ from the glomeruli-sparing acute proximal convoluted tubular necrosis observed in Pakistani vultures (*Gyps bengalensis*) that were naturally and experimentally exposed to diclofenac via ingestion of contaminated meat.[16,17] Acute renal tubular necrosis has been documented in a variety of other

avian species after treatment with diclofenac sodium, including the domestic chicken.[11,13,14] Due to the different region of the kidneys affected (glomeruli versus tubules), and given the control group in the present study was also affected; it is unlikely the lesions were caused by diclofenac administration. Glomerulonephritis reportedly occurs spontaneously in clinically normal chickens, as well as in conjunction with numerous disease processes.[35-41] In general, there are many etiologic causes for avian renal disease, including viruses, bacteria, parasites, mycotoxins, neoplasia, and nutritional imbalances.[30,42] Additional testing to attempt to diagnose the underlying cause of the chronic glomerulonephritis identified with histopathology was not performed in this study and was presumed to be preexisting. No birds exhibited clinical signs of disease throughout the duration of the present study, and visceral gout was not observed during postmortem examinations.

Limitations to the present study include a short duration and infrequency of treatment. When treating clinical disease, ophthalmic NSAID dosing may be required several times daily and several weeks of treatment may be necessary.[4] Increasing the frequency of treatment to four times daily in both eyes would likely result in increased systemic absorption of the medication, thereby increasing the risk of biochemical effects of the drug. Additionally, the exact time to peak plasma concentration of ophthalmic diclofenac sodium in chickens is unknown, therefore higher concentrations of drug may be present before or after the 15 minute collection point used in the present study. Methodology of sample collection and processing must also be considered when interpreting results. As was used in the present study, many laboratories use the bromcresol green method to test albumin levels. This method has not been validated for avian species and uses human albumin for standards and controls.[43] Discrepancies between the bromcresol green method and gel electrophoresis indicate the latter is the preferred method for determination of

avian albumin concentrations.[44,32,45] Urinalysis to assess renal function can be performed by patient catheterization or via collection from fresh droppings.[43,46] These techniques require anesthesia or housing birds individually which was not feasible in this study. Financial limitations prohibited using gel electrophoresis in the present study. Additionally, financial constraints prohibited histopathologic evaluation of additional kidneys collected at necropsy. Finally, a small per group sample size may have led to the abnormally distributed data, which prohibited the comparison of biochemical parameters between groups.

Results of this study indicate topical ophthalmic diclofenac sodium 0.1% was detectable in systemic circulation in the domestic chicken at 15 minutes post-administration when administered every 12 hours in one or both eyes for one week. Although minor biochemical changes were detected, the lack of clinical evidence of renal pathology and lack of histopathologic acute tubular necrosis, it was concluded that ophthalmic diclofenac sodium did not have negative renal effects in the present study. Due to the possibility of different interspecies response to NSAIDs, further investigation of topical ophthalmic diclofenac sodium effects on the more commonly treated domestic bird species (i.e. passerines and pscittacines) is warranted.

FOOTNOTES

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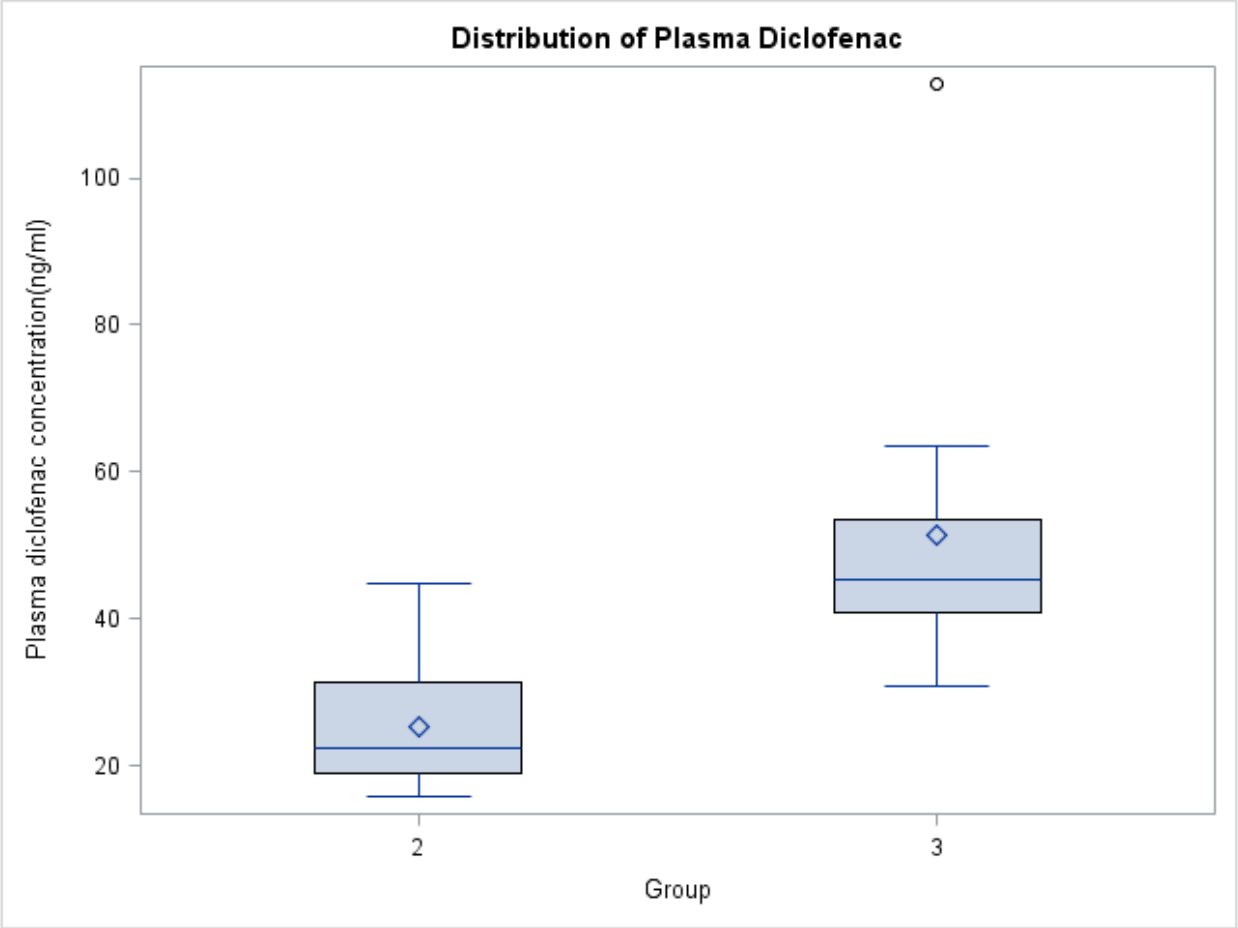
FIGURE LEGENDS

Figure 1. Plasma diclofenac concentrations (ng/mL) in healthy adult chickens (*Gallus gallus domesticus*) after administration of unilateral (group 2) or bilateral (group 3) topical ophthalmic

diclofenac sodium 0.1% solution. Bird #24, the outlier in group 3, is identified by an open circle (°).



TABLES

Table 1. Mean ± SD values (prior to outlier exclusion) for albumin, total protein and uric acid before and after treatment with either artificial tear solution or diclofenac sodium 0.1% ophthalmic solution in healthy adult chickens (*Gallus gallus domesticus*). *Denotes a significant difference from pre-treatment value (P = 0.05). Group 1 = control group receiving artificial tear solution on the left eye twice daily. Group 2 = treatment group receiving diclofenac sodium 0.1%

on the left eye twice daily. Group 3 = treatment group receiving diclofenac sodium 0.1% on both eyes twice daily.

Table 1.

		Group 1 (n = 6)	Group 2 (n = 12)	Group 3 (n = 12)
Albumin (1.3 - 2.8 g/dL)	Pre	1.75 ± 0.105	1.85 ± 0.189	1.95 ± 0.183
	Post	1.7 ± 0.110	1.78 ± 0.185*	1.83 ± 0.154*
Total Protein (3.3 - 5.5 g/dL)	Pre	4.5 ± 0.469	4.84 ± 0.565	5.03 ± 0.563
	Post	4.77 ± 0.408	4.78 ± 0.555	4.8 ± 0.449
Uric Acid (2.5 - 8.1 mg/dL)	Pre	4.03 ± 1.496	6.23 ± 5.910	7.41 ± 5.652
	Post	3.38 ± 1.291	8.17 ± 9.842	11.18 ± 11.420