Evaluation of systemic absorption and renal effects of topical ophthalmic flurbiprofen and diclofenac in healthy cats

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Abstract

Objective To investigate systemic absorption and renal effects of topically applied ophthalmic flurbiprofen and diclofenac in healthy cats.

Animals studied Twelve domestic shorthair cats.

Procedures Cats were randomly assigned to two treatment groups (n = 6) and administered one drop (approximately 40 μL) of either flurbiprofen 0.03% or diclofenac 0.1% in both eyes four times daily (6 am, 12 pm, 6 pm, and 12 am) for 14 days. Blood samples were collected on days 0, 4, 8, 14, 16, and 17 and analyzed by liquid chromatography and mass spectrometry for flurbiprofen and diclofenac plasma concentrations. A complete blood count (CBC), serum chemistry, and urinalysis were analyzed at the beginning of the study (Day 0) and at the end of topical drug administration (Day 15).

Results Both drugs demonstrated systemic absorption. Flurbiprofen was detected (mean ± SD) at day 4 (237 ± 65 ng/mL), day 8 (396 ± 91 ng/mL), day 14 (423 ± 56 ng/mL), day 16 (350 ± 66 ng/mL), and day 17 (270 ± 62 ng/mL), and diclofenac was detected (mean ± SD) at day 4 (130 ± 44 ng/mL), day 8 (131 ± 25 ng/mL), day 14 (150 ± 36 mg/mL), and sporadically on day 16. Flurbiprofen plasma concentration decreased slowly over 48 h after the last dose. No clinically significant abnormalities were noted in the serum blood urea nitrogen, creatinine, or urine specific gravity at the end of topical drug administration compared to the beginning of the study.

Conclusions Flurbiprofen and diclofenac were systemically absorbed after topical administration four times daily to both eyes of healthy cats. Flurbiprofen reached higher plasma concentrations compared to diclofenac.

Key Words: diclofenac, feline, flurbiprofen, nonsteroidal anti-inflammatory, renal

INTRODUCTION

Topical nonsteroidal anti-inflammatory drugs are commonly used in veterinary medicine to treat inflammation in the anterior segment of the eye. A portion of a topically applied ophthalmic medication will be absorbed systemically through the conjunctiva or episclera1,2 and nasolacrimal system. Dexamethasone was detected in the serum and urine of horses after treatment of one eye with dexamethasone 0.1% ophthalmic ointment four times daily for 8 days.3 Although absorption takes place, serum levels of topically applied drugs are typically low. However, even with minimal absorption, systemic side effects have been reported.4-7 Suppression of the hypothalamic–hypophyseal–adrenal axis was noted in dogs following application of topical prednisolone acetate 1% four times daily to both eyes for 2 weeks.4,5 In a study evaluating topical application of dexamethasone 0.1% four times daily to both eyes in Beagles (average dose of 0.03 mg/kg of body weight/day), the adrenal glands were suppressed and histopathologic changes were noted in the liver.6 Systemic effects associated with the use of topical NSAIDs are rarely reported. A recent study8 evaluated systemic absorption, adverse ocular effects, and systemic effects of topically applied diclofenac 0.1% administered to healthy cats four times a day for 7 days.8 That study found a decrease in glomerular filtration rate in the second phase of the crossover study, which the authors hypothesized, may have been due to volume depletion attributed to multiple blood draws.8 In humans, systemic absorption of topical ophthal-
mic NSAIDs has been documented to exacerbate bronchial asthma.7

The mechanism of action of NSAIDs involves the inhibition of prostaglandin synthesis by antagonism of the cyclooxygenase (COX) enzymes, of which there are two main isoforms. In general, COX-1 is responsible for homeostatic prostaglandin production such as gastric mucosal protection, platelet adherence, and regulation of renal blood flow.9 The COX-2 isoform produces prostaglandins in response to inflammation, but also produces homeostatic prostaglandins in many tissues.

Flurbiprofen and diclofenac are both considered nonselective COX inhibitors, which means that they inhibit both isoforms indiscriminately.10,11 Side effects of systemic NSAID administration can include renal disease,12 gastrointestinal irritation and ulceration,13,14 and platelet dysfunction.15 Cats do not tolerate systemic NSAIDs as well as other species, which may be due to increased sensitivity or decreased ability for glucuronide conjugation for some NSAIDs.16,17 Meloxicam and robenacoxib are the only NSAIDs licensed in the United States for use in cats. Meloxicam can be administered once perioperatively as a subcutaneous injection, and robenacoxib can be administered once per day as an oral pill for no more than three consecutive days, according to drug label specifications. Therefore, topical application of NSAIDs is more appropriate for prolonged use and for optimal delivery when treating anterior uveitis.

Nonsteroidal anti-inflammatory drug use is controversial in patients with chronic kidney disease due to the potential nephrotoxic effects of NSAIDs. Chronic kidney disease is the most common renal disorder affecting cats, and the prevalence of the condition has increased in recent decades. Studies have compared daily, low-dose (0.02 mg/kg) administration of meloxicam to cats with and without chronic kidney disease and found no statistically significant difference in adverse renal effects or life span.18,19 However, as systemic absorption is known to occur with topically applied ophthalmic drugs, it remains important to evaluate systemic absorption of topically applied NSAIDs in cats.

The purpose of this study was to evaluate the systemic absorption and renal effects of topically applied ophthalmic flurbiprofen and diclofenac in healthy cats.

MATERIALS AND METHODS

Animals

Twelve domestic shorthair cats were used in this study. The cats were obtained from the Kansas State University Comparative Medicine Group. Following completion of the study, they were returned for eventual adoption. A complete ophthalmic examination, including slit-lamp biomicroscopy (Kowa SL-15; Kowa Co Ltd, Torrance, CA), fluorescein staining (BioGlo Hub Pharmaceuticals LLC, Rancho Cucamonga, CA), rebound tonometry (TonoVet9 Helsinki, Finland), and indirect ophthalmoscopy (Keeler Vantage Plus; Keeler Instruments Inc., Broomall, PA), was performed prior to the study. All of the cats had normal ophthalmic and physical examinations prior to inclusion in the study. They were housed in a temperature-controlled environment and exposed to an automated 12-h light/dark cycle (light phase from 7 am to 7 pm, dark phase from 7 pm to 7 am). The Institutional Animal Care and Use Committee at Kansas State University approved this study.

Topical ocular drug administration

The cats were randomly assigned into two treatment groups (n = 6) via a coin toss. One drop (approximately 40 μL) of either flurbiprofen sodium ophthalmic solution 0.03% (Bausch & Lomb Inc. Tampa, FL) or diclofenac sodium ophthalmic solution 0.1% (Alcon Inc. Lake Forest, IL) was administered to both eyes of each cat every 6 h (6 am, 12 pm, 6 pm, and 12 am) for fourteen days (days 1–14) by the same investigator (RL).

Sample collections

Cats were sedated by intramuscular injection with a combination of 3 mg/kg ketamine (mean dose ± SD 11.17 ± 1.47 mg) and 0.006 mg/kg of dexmedetomidine (mean dose ± SD 0.02 ± 0.01 mg) for all sample collections. The dexmedetomidine was reversed after sampling was complete with 0.06 mg/kg atipamezole (mean dose ± SD 0.23 ± 0.05 mg) injected intramuscularly. Approximately 2 mL of whole blood was collected in lithium heparin tubes on days 0, 1, 4, 8, 14, 16, and 17 between 12:30 and 1:00 pm and frozen at −80 °C until sample analysis was performed. A complete blood count (CBC), serum biochemistry, and urinalysis were performed at the beginning of the study (day 0) and at conclusion of drug administration (day 15). Blood and urine samples were collected via jugular venipuncture and cystocentesis, respectively, between 12:00 and 1:00 pm.

Plasma drug analysis (flurbiprofen and diclofenac)

Plasma concentrations of flurbiprofen were determined with high-pressure liquid chromatography with triple quadrupole mass spectrometry (API 3000, Applied Biosystems, Foster City, CA, USA). Flurbiprofen plasma samples and standards (0.1 mL) were added to 0.4 mL internal standard solution (flurbiprofen d5 in 50% methanol with 1% formic acid). The samples were vortexed and centrifuged for 5 min at 15 000 × g, and supernatant was evaporated to dryness under and stream of air. The samples were reconstituted with 0.2 mL of 10% methanol and centrifuged for 5 min at 15 000 × g, and the supernatant was transferred to injection vials with 0.05 mL as the injection volume. The mobile phase consisted of acetonitrile (A) and 0.1% formic acid with a total run time of 6.5 min. A fluorophenyl propyl column (Allure PFP propyl, 50 × 2.1 mm, 5 μm particle size, Restek, Corp. Bellefonte, PA), and indirect ophthalmoscopy (Keeler Vantage Plus; Keeler Instruments Inc., Broomall, PA), was performed prior to the study. All of the cats had normal ophthalmic and physical examinations prior to inclusion in the study. They were housed in a temperature-controlled environment and exposed to an automated 12-h light/dark cycle (light phase from 7 am to 7 pm, dark phase from 7 pm to 7 am). The Institutional Animal Care and Use Committee at Kansas State University approved this study.

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PA, USA) maintained at 40 °C achieved separation. The qualifying and quantifying ions for flurbiprofen were m/z 243.029 and 199.00, respectively, using negative ion mode. The qualifying and quantifying ions for flurbiprofen d5 were m/z 247.957 and 203.90, respectively, using negative ion mode. Standard curves in feline plasma were linear from 25 to 1000 ng/mL with 25 ng/mL being the lower limit of quantification. The accuracy of the assay on replicates of three for each concentration was 110% for 25 ng/mL, 94% for 100 ng/mL, and 98% for 1000 ng/mL. The coefficients of variation of the assay on replicates of three for each concentration was 10% for 25 ng/mL, 7% for 100 ng/mL, and 4% for 1000 ng/mL.

Plasma concentrations of diclofenac were determined with high-pressure liquid chromatography (Shimadzu Prominance, Shimadzu Scientific Instruments, Columbia, MD, USA) with triple quadruple mass spectrometry (API 2000, Applied Biosystems, Foster City, CA, USA). Diclofenac plasma samples and standards (0.2 mL) were added to 0.8 mL internal standard solution (meclofenamic acid 62.5 ng/mL in acetonitrile). The samples were vortexed and centrifuged for 5 min at 15 000 × g, and the supernatant was evaporated to dryness under and stream of air. The supernatant was transferred to injection vials with 0.05 mL as the injection volume. The mobile phase consisted of acetonitrile and 0.1% formic acid with a total run time of 6.5 min. A phenyl column (Thermo Hypersil, 150 × 3 mm, 5 μm particle size, Fisher Scientific, Pittsburgh, PA, USA) maintained at 40 °C achieved separation. The qualifying and quantifying ions for diclofenac were mass to charge ratio (m/z) 296.12 and 215.00, respectively, using positive ion mode. The qualifying and quantifying ions for meclofenamic acid were m/z 296.07 and 243.00, respectively, using positive ion mode. Standard curves in feline plasma were linear from 10 to 1000 ng/mL with 10 ng/mL being the lower limit of quantification. The accuracy of the assay on replicates of three for each concentration was 110% for 25 ng/mL, 94% for 100 ng/mL, and 105% for 1000 ng/mL. The coefficients of variation of the assay on replicates of three for each concentration was 10% for 25 ng/mL, 7% for 100 ng/mL, and 4% for 1000 ng/mL.

**RESULTS**

**Animals**

The 12 cats included five castrated males and seven intact females weighing 2.95–4 kg (mean ± SD 3.71 ± 0.55 kg) and ranging in age from 9 to 13 months (mean ± SD 10.08 ± 1.51 months). There was no statistical difference in weight (P = 0.37) or age (P = 0.86) between treatment groups.

**Plasma drug levels**

Flurbiprofen and diclofenac were detected in the plasma of treated cats. Flurbiprofen was detected (mean ± SD) on day 4 (237 ± 65 ng/mL), day 8 (396 ± 91 ng/mL), day 14 (423 ± 56 ng/mL), day 16 (350 ± 66 ng/mL), and day 17 (270 ± 62 ng/mL). Diclofenac was detected (mean ± SD) on day 4 (130 ± 44 ng/mL), day 8 (131 ± 25 ng/mL), and day 14 (150 ± 36 ng/mL) (Table 1). The flurbiprofen plasma concentrations were greater at all time points when compared to diclofenac. Flurbiprofen was detected in the plasma 48 h after cessation of drug administration and had decreased by less than half from the last dose, but diclofenac was only detected (>10 ng/mL) in 3 of 6 animals at 24 h and in none of the animals at 48 h after the last dose (Table 1).

**Complete blood count and serum biochemistry**

Complete blood counts were within normal limits before (day 0) and immediately after (day 15) drug administration for all cats. Blood urea nitrogen and creatinine were within normal limits before and after drug administration. There was a statistically significant decrease in BUN on day 15 compared to day 0 for the flurbiprofen (P = 0.05) and diclofenac (P = 0.01) groups (Table 2).

**Urinalysis**

The mean ± SD USG in the flurbiprofen and diclofenac groups is reported in Table 2. There was a statistically significant decrease in USG in the diclofenac (P = 0.04) group on day 15 when compared to day 0. All study cats had urine analyzed for protein via reagent pad ‘dipstick’ and sulphosalicylic acid (SSA) turbidimetric method on day 0 and 11 of 12 cats had protein detected in the urine.

<table>
<thead>
<tr>
<th>Flurbiprofen (ng/mL)</th>
<th>Diclofenac (ng/mL)</th>
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<tbody>
<tr>
<td>Day 0</td>
<td>ND</td>
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<tr>
<td>Day 4</td>
<td>237 ± 65</td>
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<tr>
<td>Day 8</td>
<td>396 ± 91</td>
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<tr>
<td>Day 14</td>
<td>423 ± 56</td>
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<tr>
<td>Day 16</td>
<td>350 ± 66</td>
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<tr>
<td>Day 17</td>
<td>270 ± 62</td>
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</table>

ND = none detected, NR = not reported due to <6 cats having detectable levels of drug.

All cats had protein detected in the urine by either test on day 15. Individuals that tested positive for proteinuria with one method did not always test positive with the other. The urine protein values yielded too little data to assign a probability less than 0.1. Therefore, for this data, the probability would always be $P > 0.1$ regardless of the number of negative or positive signs.

**DISCUSSION**

This study demonstrates that systemic absorption occurs after topical flurbiprofen and diclofenac are administered to both eyes of healthy cats four times daily for 14 days. Despite statistical significance, systemic absorption did not have a clinically relevant impact on renal function as measured by serum BUN, creatinine, and USG. In fact, the change in measured parameters occurred in the direction opposite of what is expected when evaluating for evidence of renal disease. However, the measured parameters are relatively insensitive measures of renal function as USG is expected to be decreased when approximately 67% of renal function is lost. Creatinine and BUN are expected to decrease when approximately 75% of renal function is lost.

Our study is in accordance with a recently published report of systemic absorption of topically applied diclofenac in healthy cats. In that study, cats were administered topical diclofenac 0.1% four times a day for 7 days and plasma concentrations of diclofenac were determined on days 1 and 7. Detectable levels of diclofenac were found with accumulation of the drug over 7 days. There was a significant difference in the median $C_{\text{max}}$ between days 1 (59.7 ng/mL) and 7 (106.0 ng/mL). In that study, cats had a decreased glomerular filtration rate after the second phase of the crossover design only in the diclofenac-treated group and the authors hypothesized this was possibly associated with hypovolemia from the multiple blood draws. No significant difference in serum biochemical variables including BUN and creatinine was found in that study.

In the current study, flurbiprofen and diclofenac were detected in the plasma at the first sampling time point 4 days after starting drug administration. While it would have been more precise to administer each drug with a micropipette, our rationale was to mimic the clinical setting. Therefore, the drugs were administered directly from the commercially available dropper bottles. The volume of one drop was measured at approximately 40 μL, and this volume was used to calculate approximate drug dosages. Assuming complete absorption, the mean dose per body weight of flurbiprofen and diclofenac administered to each cat was 0.03 mg/kg/day ($\pm 0.01$ SD) and 0.09 mg/kg/day ($\pm 0.02$ SD), respectively. While the daily dose of flurbiprofen was roughly one-third that of diclofenac, flurbiprofen consistently reached higher plasma concentrations compared to diclofenac. The highest plasma concentrations of flurbiprofen and diclofenac were detected at day 14 (mean $\pm$ SD 423 $\pm$ 56 and 150 $\pm$ 36 ng/mL, respectively). The flurbiprofen plasma concentration was almost three times higher than that of diclofenac at the same time point. Flurbiprofen was detected up to 48 h after discontinuation of topical medication (day 17), and diclofenac was only detected in 3 of 6 cats 24 h after discontinuation of therapy and 0 of 6 cats 48 h after drug discontinuation. These findings suggest that even though more diclofenac was administered on a mg/kg/day basis, flurbiprofen reached higher plasma concentrations and persisted in circulation for a greater amount of time. There are several possible explanations for this observation.

Flurbiprofen may have a longer terminal half-life when compared to diclofenac, resulting in a greater drug accumulation with multiple doses. Drug accumulation can be predicted by the ratio of the terminal half-life to dosing interval. As the ratio increases (i.e., the terminal half-life exceeds the dosing interval), greater accumulation will occur. As the dosing intervals were the same for each of the drugs, greater accumulation would be due to a longer terminal half-life of flurbiprofen. As seen with Table 1, flurbiprofen concentrations continued to increase through day 14, but little increases in diclofenac plasma concentrations were observed from day 4 to 14. Unfortunately, the half-lives of diclofenac and flurbiprofen have not been reported in cats. Our data suggest a longer terminal half-life of flurbiprofen (>48 h) because the plasma concentrations between the last dose (day 14) and the last sample (day 17, 48 h after the last dose) decreased by less than half. In contrast, the plasma concentrations of diclofenac decreased rapidly and within 24 h were only detected (>10 ng/mL) in 3 of 6 cats and 0 of 6 cats at 48 h. A longer terminal half-life for flurbiprofen could be due to decreased drug elimination, decreased rate of absorption from the eye, or a larger volume of distribution. However, flurbiprofen appears to remain in the aqueous humor for a shorter time period with a mean resident time of 3.7 h.

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### Table 2. Mean ± SD BUN, creatinine, and USG at the beginning (Day 0) and after drug administration (Day 15). BUN normal range from 16 to 32 mg/dL. Creatinine normal range from 0.8 to 2.1 mg/dL.

<table>
<thead>
<tr>
<th></th>
<th>Flurbiprofen</th>
<th>Diclofenac</th>
<th>Flurbiprofen</th>
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<th>Flurbiprofen</th>
<th>Diclofenac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>23.2 ± 4.7</td>
<td>22.5 ± 2.4</td>
<td>1.1 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.040 ± 0.01</td>
<td>1.031 ± 0.02</td>
</tr>
<tr>
<td>Day 15</td>
<td>19.7 ± 4.5</td>
<td>19.5 ± 1.6</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.1</td>
<td>1.043 ± 0.01</td>
<td>1.045 ± 0.01</td>
</tr>
<tr>
<td>P value</td>
<td>0.05</td>
<td>0.01</td>
<td>0.79</td>
<td>0.10</td>
<td>0.35</td>
<td>0.04</td>
</tr>
</tbody>
</table>
compared to diclofenac’s 7.4 h in humans. This suggests decreased clearance or larger volume of distribution for flurbiprofen, which may be the cause of the persistence of plasma drug concentrations. Future intravenous pharmacokinetic studies of flurbiprofen and diclofenac are needed to document the true clearances, volumes of distribution, and terminal half-lives.

Another factor that may contribute to the higher plasma concentrations for flurbiprofen compared to diclofenac in our study is a greater extent of absorption for flurbiprofen (i.e., greater bioavailability) compared to diclofenac. Rabbits receiving 225 µg of flurbiprofen topically had 74% of the administered drug in circulation shortly after administration; however, the extent of absorption has not been reported in cats. Differences in volume of distribution were also illustrated in a study evaluating a single application of either flurbiprofen or diclofenac to 165 humans, where the aqueous humor drug concentration–time profiles were evaluated. Flurbiprofen may have less tissue distribution (smaller volume of distribution) in cats and therefore more opportunity to be measured in plasma, while diclofenac may have a greater distribution into the tissues. The differences in terminal half-life, bioavailability, and volume of distribution are possible explanations for the greater and longer detection of flurbiprofen compared to diclofenac in our study, but further studies are needed to document the persistence of flurbiprofen.

Proteinuria was detected in 91.7% (11/12) of cats before and in 100% (12/12) of cats after drug administration. Both methods of urine protein measurement (dipstick and SSA) used in our study have limitations and subjectivity, especially when considering analysis of cat urine. The reagent pad (dipstick) is most sensitive for albumin, but can detect other proteins, especially if the urine concentration is elevated. The mean ± SD USG on day 0 was 1.035 ± 0.01. Reagent pads are made for use in humans, whose average urine concentrating ability is lower compared to cats; in theory, more concentrated cat urine could yield false-positive results.

Physiologic proteinuria is documented in dogs and cats. It is attributed to changes in activity level and stress (prolonged inactivity in an active individual or after strenuous activity in an inactive individual). A study comparing proteinic and nonproteinic dogs found that exercise-restricted, hospitalized dogs confined to a cage had higher urine protein to creatinine ratios than the nonhospitalized dogs. Our study cats lived the majority of their lives in a research setting with limited exercise. They were also not accustomed to the restraint and injections required for sedation before sample collection. Another source of physiologic proteinuria is cauxin. This major urinary carboxylesterase protein is secreted from the renal tubular cells in normal domestic cats. Cauxin excretion takes place exclusively in feline species and is age-dependent, starting at approximately 3 months of age in both males and females. The mean ± SD age of the cats in our study was 10.1 ± 1.5 months. The persistent proteinuria identified in our study cats may have been influenced by one of these factors.

A limitation to our study was the lack of additional testing, specifically urine protein: creatinine ratios, to further investigate proteinuria. The diagnostics performed in this study were clinically relevant, but not the most sensitive for renal injury. Whole blood assays that screen for renal prostaglandins, thromboxane, and cyclooxygenase would be preferable, as well as measuring glomerular filtration rate. These tests were beyond the scope of our study, but could be considered in future studies that evaluate the long-term effect of topical NSAIDs on renal function. An additional limitation in the present study was lack of a negative control group. As a randomized complete block crossover study was not possible, a control group would have increased animal use in the study by an additional six cats. Although the control group would add no information to the drug absorption, exposure, and persistence of these NSAIDs, it may have helped interpret some of the renal testing parameters. It is possible effects compared to a control group could have occurred. However, at the start of the study, it was unknown if either of these medications would even be absorbed systemically and if renal effects would not be expected, then additional cats would have been enrolled in the study without benefit. Based on the results of this study, further studies including a negative control are warranted to investigate the possible effects of topical NSAIDs on renal function.

Given our results, the significant cumulative effect of flurbiprofen compared to diclofenac should be taken into consideration when treating cats. Further increases in plasma drug concentrations using flurbiprofen could occur with longer treatments, producing greater systemic effects. The purpose of the study was to measure plasma concentrations of flurbiprofen and diclofenac in cats after topical dosing, not to describe the terminal half-life. However, future studies investigating flurbiprofen and diclofenac can use this estimate as a guide for study design.

Based on the results of our study, flurbiprofen and diclofenac are systemically absorbed after topical administration to both eyes of healthy cats four times daily for 14 days. No clinically relevant effects on measured renal values were detected after 14 days of drug administration. However, future studies using a negative control group and more sensitive renal testing are suggested based on this study documenting low, but systemic exposure of both topical NSAIDs in cats.

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