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Pharmacokinetics and anti-nociceptive effects of the soluble epoxide hydrolase inhibitor *t*-TUCB in horses with experimentally induced radiocarpal synovitis

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Abstract

This study determined the pharmacokinetics, anti-nociceptive and anti-inflammatory effects of the soluble epoxide hydrolase (sEH) inhibitor *t*-TUCB (*trans*-4-{4-[3-(4-Trifluoromethoxy-phenyl)-ureido]-cyclohexyloxy}-benzoic acid) in horses with lipopolysaccharide (LPS)-induced radiocarpal synovitis. A total of 7 adult healthy mares (n=4–6/treatment) were administered 3 μg LPS into one radiocarpal joint and *t*-TUCB intravenously (i.v.) at 0 (control), 0.03, 0.1, 0.3 and 1 mg/kg in a blinded, randomized, crossover design with at least 3 weeks washout between. Two investigators independently assigned pain scores (at rest, walk and trot) and lameness scores before and up to 48h after *t*-TUCB/LPS. Responses to touching the joint skin to assess tactile allodynia, plasma and synovial fluid (SF) *t*-TUCB concentrations were determined before and up to 48h after *t*-TUCB/LPS. Blood and SF were collected for clinical laboratory evaluations before and up to 48h after *t*-TUCB/LPS. Areas under the curves of pain and lameness scores were calculated and compared between control and treatments. Data were analyzed using repeated measures ANOVA with Dunnett or Bonferroni post-test. $P<0.05$ was considered significant. Data are mean±SEM. Compared to control, pain, lameness and tactile allodynia were significantly lower with 1 mg/kg *t*-TUCB, but not the other doses. For 0.1, 0.3 and 1 mg/kg *t*-TUCB treatments, plasma terminal half-lives were 13±3, 13±0.5 and 24±5 h, and clearances were 68±15, 48±5 and 14±1 mL/h/kg. The 1 mg/kg *t*-TUCB reached the SF at high concentrations. There were no important anti-inflammatory effects. In conclusion, sEH inhibition with *t*-TUCB may provide analgesia in horses with inflammatory joint pain.

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fatty acid; pain; arthritis; lameness; musculoskeletal

INTRODUCTION

Cyclooxygenase (COX) inhibition is a common analgesic approach in horses because metabolism of arachidonic acid (ARA) via the COX pathway lead to production of pro-nociceptive lipids, although risk of adverse effects is often a limiting factor (Guedes, 2017). As shown in figure 1, an alternative metabolic pathway via cytochrome P450 epoxygenases results in production of lipid intermediates known as epoxy-fatty acids (EpFAs). These EpFAs, which are largely anti-nociceptive, are inactivated to the respective diols by the downstream enzyme soluble epoxide hydrolase (sEH) (Chacos et al., 1983; Wagner et al., 2014; Spector & Kim, 2015). As predicted, studies in rodent models of inflammatory and neuropathic pain have shown that pharmacologic inhibition of sEH prevents EpFA degradation, resulting in anti-nociception as well as prevention of intestinal ulcers associated with COX inhibitors. Anti-nociception seems to occur via peripheral as well as central effects, with specific mechanisms involving both transcriptional (i.e., repression of COX-2 expression, up-regulation of neurosteroid-producing genes) and non-transcriptional (i.e., opioid) effects (Inceoglu et al., 2006; Inceoglu et al., 2007; Inceoglu et al., 2008; Wagner et al., 2011a; Wagner et al., 2011b; Inceoglu et al., 2012; Wagner et al., 2013; Wagner et al., 2014; Goswami et al., 2016; Wagner et al., 2017). This literature in rodents along with preliminary results in severely laminitic horses (Guedes et al., 2013; Guedes et al., 2016) supports further studies to better understand the potentials and limitations of sEH inhibition for equine pain management.

There are important variations in the anti-nociceptive action of different EpFAs. In rats, the strongest anti-hyperalgesic efficacy is obtained with several of the docosahexaenoic acid (DHA)-derived EpFAs (i.e., epoxydocosapentaenoic acid or EpDPE), followed by those derived from ARA and EPA (i.e., epoxyeicosatetraenoic acid or EpETE). The DHA-derived EpFAs are present in considerable amounts in the central nervous system and are also the preferred sEH substrates, except for the 19(20) regioisomer that is slowly metabolized (Morisseau et al., 2010). Also in rats, ARA-derived EpFAs (i.e., eicosapentaenoic acid or EET) showed biphasic dose-response anti-nociception during LPS-induced inflammatory pain and were slightly pro-nociceptive in the absence of LPS-induced pain (Inceoglu et al., 2006). Finally, sEH-deficient mice developed prolonged mechanical hyperalgesia to zymosan-induced peripheral inflammation via mechanisms involving ARA-derived EpFAs (Brenneis et al., 2011) and linoleic acid-derived EpFAs in the lipoxigenase pathway can be algogenic via activation of transient receptor potential (TRP) channels (Patwardhan et al., 2010). These studies suggest that the predominant EpFA profile, which could be both species- and diet-dependent, as well as specific pain phenotypes could have important influence in the anti-nociceptive effect of sEH inhibitors.

The main goal of this study was to investigate the potential anti-nociceptive effect of a dose range of the sEH inhibitor *t*-TUCB in an experimental model of joint pain in horses. On the

basis of the above rodent literature and the preliminary results in laminitic horses, it was hypothesized that *t*-TUCB would produce anti-nociceptive effects without significant adverse effects.

MATERIALS AND METHODS

Animals and study design

This was a prospective, randomized, crossover, blinded, vehicle-controlled experimental study using 7 adult mares (4 Thoroughbred, 2 Quarter Horse, 1 Dutch Warmblood) aged 13±2 years and weighing 534±15 kg. The actual number of horses per treatment group varied due to unanticipated issues with animal availability during the study. There was at least 3 weeks washout between each treatment. Horses were healthy based on physical and lameness examinations, complete blood cell counts and serum biochemical analyzes performed before and at the end of each experiment. Horses were allowed to acclimatize to appropriately sized stalls for 24–48 h prior to each experiment, received water *ad libitum*, and were fed grass hay once daily in the evening (after outcome measurements and blood sampling; see below). The University of California-Davis Institutional Animal Care and Use Committee reviewed and approved the study.

Synthesis and preparation of t-TUCB

Multi-gram scale syntheses of *t*-TUCB were performed according to established methodology as previously described in detail (Jones et al., 2006; Morisseau et al., 2006; Rose et al., 2010; Tsai et al., 2010). The day before each experiment, *t*-TUCB was dissolved in dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA) to final concentrations of 3, 30 or 90 mg/mL, was filter-sterilized with 0.2 μm pore size sterilizing-grade membranes and placed into sterile 10 mL silicone-coated glass tubes. These tubes were kept upright at room temperature in the laboratory until usage the next day. The solubility of *t*-TUCB in DMSO at room temperature was confirmed via liquid chromatography-mass spectrometry (LC-MS).

Baseline data collection and instrumentation

The day before the experiment, the hairs over the dorsal aspect of both carpi were clipped, and baseline data were collected. After overnight fasting, both jugular veins were aseptically catheterized with 14-gauge, 5.25-inch long over-the-needle catheters (Angiocath, Becton Dickinson Infusion Therapy Systems, Inc., UT, USA) following skin desensitization with 1.5 mL 2% lidocaine. One catheter was for administering *t*-TUCB and was subsequently removed, while the other was for blood sampling and administering sedatives. This catheter was removed 24 hours after beginning the experiment.

Pain model

The LPS-induced radiocarpal synovitis was selected because it has long been used as an inflammatory pain model in analgesic studies in horses (Palmer & Bertone, 1994; Owens et al., 1996; Todhunter et al., 1996; Smith et al., 1998; de Grauw et al., 2009; Santos et al., 2009; Lindegaard et al., 2010b; van Loon et al., 2010) and because intraplantar LPS was used in several of rodent pain models designed to study the role of sEH inhibitors in anti-nociception (Inceoglu et al., 2006; Schmelzer et al., 2006; Inceoglu et al., 2008; Liu et al.,

2010). Horses were sedated with 0.2–0.5 mg/kg xylazine (AnaSed, Akorn, Inc., IL, USA) i.v. and the skin over both carpi was surgically scrubbed using povidone-iodine antiseptic soap. One radiocarpal joint was injected with 3 μg of lipopolysaccharide (LPS) from *E. coli* O55:B5 (catalog number L5418, Sigma-Aldrich, St. Louis, MO, USA) (Lindegaard et al., 2010b) freshly prepared sterile to 1.5 μg/mL in 0.9% NaCl. The first injected joint was randomly assigned; subsequent injections alternated between joints. During each experiment, the contralateral joint was not injected but arthrocenthesis was performed for SF collection.

Treatments

One individual not participating in outcome assessments randomized and prepared syringes containing the test solution for individual horses. To ensure that all horses received the same volume of DMSO and for blinding purposes, all syringes were prepared to contain the same volume of solution (0.009 mL/kg). Treatments consisted of 0.009 mL/kg DMSO (vehicle control) or 0.03, 0.1, 0.3 or 1.0 mg/kg *t*-TUCB. Immediately after LPS injection, 10 mL of blood was aspirated from the jugular vein catheter and the test solution was administered i.v. over 30–45 seconds. The catheter was then flushed with the aspirated blood and then with 5 mL of heparinized saline to ensure administration of the full dose.

Outcome assessments and rescue analgesia

Outcomes were assessed before (baseline) and at 2, 4, 8, 12, 24, 36 and 48 hours after LPS/treatment always in the same sequence (blood sampling, physical exams, pain and lameness scoring, and synovial fluid collection). Physical exam included heart (HR) and respiratory (RR) rates, mean arterial pressure (MAP) via oscillometric technique at the base of tail, and rectal temperature. Carpal joint circumference was determined with measuring tape positioned at the level of the accessory carpal bone. Although several outcomes were assessed, the main variables of interest determined a priori were pain and lameness scores.

Two investigators blinded to treatment allocation independently assigned pain and lameness scores in real time at the pre-determined time points. Pain scores were assigned with a visual analog scale (VAS) for three different conditions (at rest in the stall, walking and then trotting in a straight line) and then averaged to form a final score. The VAS corresponds to a 100 mm line representing the range of possible pain (0 = ‘no pain’ on the left and 100 = ‘worst possible pain’ on the right). The evaluator places a mark on this line corresponding to the pain severity, and the distance from the left extreme to this mark corresponds to the VAS score. Observations of general demeanor (facial expression, position of ears, interest in surroundings) and weight bearing on the LPS-injected leg formed the basis for the VAS scoring. The VAS was shown to be highly reliable when assessing lameness in horses, especially when used by experienced individuals (Vinuela-Fernandez et al., 2011). Horse’s reaction to the tape measure during determination of joint circumference was noted as positive or negative based on whether or not the horse lifted the limb as the tape was applied around the carpus. Limb lift before the tape contacted the skin was not considered a positive response. Lameness was scored according to the American Association of Equine Practitioners guidelines with half-scores permitted on a flat soft ground.

At the 12h evaluation time point, horses with VAS > 50 mm at rest and walk received 4 mg/kg phenylbutazone (Equi-Phar phenylbutazone injection; Vedco Inc., St Joseph, MO, USA) i.v. for rescue analgesia. The cut-off for rescue analgesia was similar or slightly more stringent to that previously reported in this same pain model (Lindegaard et al., 2010b). The dose of phenylbutazone was selected primarily on the basis of its clinical use (Johnson et al., 1993; Raekallio et al., 1997), although pain relief was not readily demonstrated in a Freud adjuvant's synovitis model in horses (Toutain et al., 1994).

Blood and synovial fluid sampling

At each outcome assessment time point, 5-mL blood samples were drawn into EDTA-containing tubes for determining plasma *t*-TUCB concentration. The samples were centrifuged immediately and the plasma was harvested and stored at −80°C until assayed for *t*-TUCB concentrations (see below). After outcome assessments at the baseline, 12 and 24 hours time points, synovial fluid (SF) samples (1–2 mL) were collected aseptically via bilateral radiocarpal arthrocentesis in xylazine-sedated horses. Samples were placed in chilled EDTA-containing tubes and aliquoted for *t*-TUCB concentration, and for determining protein concentration and leukocyte numbers. Aliquots destined for *t*-TUCB concentration were centrifuged immediately, the supernatant harvested and stored at −80°C until assayed.

Determination of t-TUCB concentrations and pharmacokinetic calculations

Concentrations of *t*-TUCB in plasma (all doses) and SF (1 mg/kg dose only) were determined via LC–MS/MS analyses with electrospray ionization following HPLC in the positive mode at 1.0kV capillary voltage as described in detail previously (Tsai et al., 2010). Non-compartmental analysis was used for calculating pharmacokinetic parameters using commercially available software (Phoenix WinNonlin Version 6.2, Pharsight, Cary, NC, USA). The area under the curve and area under the moment curve were calculated using the log-linear trapezoidal method and were extrapolated to infinity using the last measured plasma concentration (C_{last}) divided by the terminal slope (λ_z).

Statistics

Statistical calculations were performed with commercially available software (GraphPad Prism version 5.0f for MAC; GraphPad Software Inc., San Diego, CA, USA). Continuous data not normally distributed according to D'Agostino and Pearson omnibus or the Shapiro-Wilk Normality Test were log-transformed before testing. Data from the 0.03 mg/kg *t*-TUCB treatment were excluded from all comparisons because this treatment resulted in undetectable or very low plasma concentrations (see results section). Areas under the curve (AUC) for pain and lameness scores were calculated for the first 12 h as well as for the entire study period (0–48 h) by the trapezoidal method and compared between control and each treatment with repeated measures one-way ANOVA and Dunnett post-tests. Reaction to the tape measure (i.e., tactile allodynia) was compared between control and treatments with chi-square tests. Data are expressed as mean ± SEM unless otherwise indicated. Significance level was set as $P \leq 0.05$.

RESULTS

Animals

Horses completed the study without overt complications. Two horses were removed prematurely from the study at different time points for reasons unrelated to this study (to participate in separate teaching protocols) such that number of horses per treatment varied. As such, 6 horses were studied in treatments 0, 0.03 and 0.1 mg/kg, 4 horses in treatment 0.3 mg/kg and 5 horses in treatment 1 mg/kg.

Pain and lameness

As shown in Figure 2, the 1 mg/kg *t*-TUCB treatment, but not the others, was associated with significantly lower AUC for pain and lameness compared to control. This was the case for both the first 12 h as well as for the entire 48 h. Proportions of positive-to-negative responses to touch for control, 0.1, 0.3 and 1 mg/kg *t*-TUCB treatments were 1.7, 0.8, 1.3 and 0.2, respectively. Compared to control, the proportion of positive responses was significantly lower with 1 mg/kg treatment but not with the others. Phenylbutazone rescue analgesia was administered to 2/6, 2/6, 2/6, 1/4 and 2/5 horses for control, 0.03, 0.1, 0.3 and 1 mg/kg *t*-TUCB treatments.

Plasma and SF concentrations of t-TUCB

Plasma (all doses) and SF (1 mg/kg dose) concentrations of *t*-TUCB are shown in Figure 3, and the calculated pharmacokinetic parameters detailed in Table 1. There were apparent dose-dependent changes in terminal half-life, clearance and volume of distribution at steady state. When dose-corrected, terminal half-life, clearance and volume of distribution were significantly lower with 0.3 and 1 mg/kg compared to 0.1 mg/kg dose. The difference between the 0.3 and 1 mg/kg doses was not statistically significant. The SF *t*-TUCB concentration was significantly higher in the inflamed compared to the non-inflamed joint.

Physical exam variables

These results are shown in Figure 4. There were significant effects of time, but not of treatment, on RR, HR and MAP that was evident as early as 2 h, peaked between 4–8 h and returned to baseline by 24 h post-LPS. Rectal temperature increased only slightly after LPS administration but was without statistical or clinical significance.

Synovial fluid cytology and protein

The SF protein concentration and leukocyte numbers increased markedly and statistically significantly after LPS administration in all treatments at the 12- and 24-hour evaluation time points compared to baseline (Figure 5). There were significant increases in % of neutrophils and a significant decreases in % of small and large mononuclear cells after LPS injection, but there was no significant *t*-TUCB treatment effect as compared to control. Carpus circumference increased slightly less with the 0.3 and 1 mg/kg *t*-TUCB compared to the remaining treatments, reaching statistical significance at 36 h with the 1 mg/kg *t*-TUCB treatment compared to control.

Hematology and serum biochemistry

The hematology and serum biochemistry results for all treatments (not shown) were within the normal laboratory reference range. Small albeit statistically significant differences as compared to baseline, observed in a few occasions, were deemed not clinically relevant.

DISCUSSION

This study used an inflammatory joint pain model (Lindegaard et al., 2010a; Lindegaard et al., 2010b) to assess the anti-nociceptive and anti-inflammatory effects as well as the plasma distribution profile of a range of doses of the pharmacologic sEH inhibitor *t*-TUCB. In this model, 1 mg/kg *t*-TUCB produced significant anti-nociceptive effects, as indicated by decreased tactile allodynia (i.e., response to touch) as well as in pain and lameness scores. There was negligible anti-inflammatory activity as evaluated by the effects on inflammatory cell numbers, joint effusion and protein concentration in this model. The 1 mg/kg *t*-TUCB treatment resulted in both plasma and SF concentrations several fold higher than its *in vitro* IC_{95} for at least 48 hours. No adverse effects were observed on physical and laboratory examinations, in accordance with previous observations in laminitic horses (Guedes et al., 2013; Guedes et al., 2016). These results indicate that pharmacologic inhibition of sEH may represent a viable strategy for managing inflammatory joint pain in horses.

The pharmacokinetic results, although preliminary, indicated that the 0.1 and 0.3 mg/kg *t*-TUCB doses were characterized by linear or first order plasma kinetics, but the highest dose may have approached a non-linear or zero order kinetics. The half-life estimates indicate that one should see dose accumulation toward a near steady state level with several days of administration of *t*-TUCB. This suggest that after a loading dose a much smaller maintenance dose could be used (Guedes et al., 2013; Guedes et al., 2016) in similar fashion as the COX inhibitor firocoxib (Burkett et al., 2016). Although future studies will be necessary to better define the pharmacokinetics of *t*-TUCB in horses, it is possible that its plasma concentrations may not be useful guide to therapeutic efficacy at any given time as is the case for COX inhibitors in horses (Lees & Higgins, 1985). It is worth noting that the levels of *t*-TUCB were significantly higher in the SF of the inflamed joint compared to the non-inflamed contralateral joint. Finally, how and to what extent *t*-TUCB is metabolized is not known at present. It is also not known if and how exposure to other drugs such as xylazine and the presence or absence of pain may influence the disposition of *t*-TUCB.

In rats, *t*-TUCB doses as low as 0.1 mg/kg significantly attenuated mechanical hyperalgesia to intra-plantar LPS (Wagner et al., 2013) and, in chronic laminitic horses with refractory pain, adding 0.1 mg/kg *t*-TUCB to therapy significantly improved pain-associated behaviors (Guedes et al., 2013; Guedes et al., 2016). These results are in contrast with the lack of significant anti-nociception with 0.1 mg/kg *t*-TUCB in the present study. Although *in vitro t*-TUCB is approximately 3-fold more potent against equine sEH (Guedes et al., 2016) compared to rat sEH (Wagner et al., 2013), the anti-nociceptive effects of sEH inhibitors are mediated not by the drug, but by the stabilizing effects on endogenously produced EpFAs. As a consequence, experimental paradigm (species, pain phenotype, diet, health status, concurrent COX inhibitors) may have profound influence on EpFA profile and thus in the response to sEH inhibitors (Morisseau et al., 2010) (Schmelzer et al., 2006). This makes

direct comparisons between studies difficult. Financial limitations prevented us from determining the EpFA profile in the present study, but further work is warranted to understand the profile and spectrum of effects of EpFAs under different conditions. This knowledge should facilitate optimization of the EpFA profile to the desired outcome.

The current study has several potential limitations to be considered. First, the small sample size for the 0.3 mg/kg *t*-TUCB treatment could have produced a false-negative result (type II error) in pain and lameness scores. Second, the cut-off point for rescue analgesia (VAS > 50 at rest and walk) was arbitrarily selected, although is similar to a previous study (VAS >60) using this same model (Lindegaard et al., 2010b). With the intent of adding robustness to the criteria, it was decided a priori that horses had to meet VAS cut-off both at rest and at the walk, reasoning that if a horse appeared painful at rest, it would be at least as painful at the walk. However, unexpectedly, some horses improved their VAS at the walk compared to the VAS at rest. If only the VAS > 50 at rest had been used, the number of horses qualifying for rescue analgesia would had been 5/6, 5/6, 4/6, 1/4 and 2/5 horses for treatments 0, 0.03, 0.1, 0.3 and 1 mg/kg *t*-TUCB, which would have been in line with a dose-dependent effect of *t*-TUCB. It is unlikely that the rescue analgesia with phenylbutazone at the 12-hour time point was a significant confounding factor since the pain and lameness findings were the same whether the data were analyzed for the first 12 hours (i.e., before rescue analgesia) or for the entire 48 h period. In horses, the analgesic effects of phenylbutazone (4 mg/kg i.v.) could not be demonstrated in a carpal Freund's adjuvant arthritis model (Toutain et al., 1994), and it produced < 12 h of post-surgical analgesia in clinical cases (Johnson et al., 1993; Raekallio et al., 1997). Lastly, xylazine sedation likely did not affect pain and lameness scores at early time points (2 and 4 h) given its short duration of action (England et al., 1992), especially at the low doses used in this study.

In conclusion, our results indicate that inhibition of sEH may have a role in decreasing inflammatory joint pain and lameness in horses. Future studies to expand the pharmacokinetic understanding of *t*-TUCB, including its oral bioavailability, and exploring the role and mechanisms of sEH and EpFAs in joint pain are warranted.

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A. G. P. GUEDES, C. MORISSEAU, S-H. HWANG and B. D. HAMMOCK are authors of composition of matter and/or use patents in this area. B. D. HAMMOCK is the founder of EicOsis. This company is moving sEH inhibitors through clinical trials for treating pain, hypertension, inflammation, and other disorders. However, this study is independent from the company.

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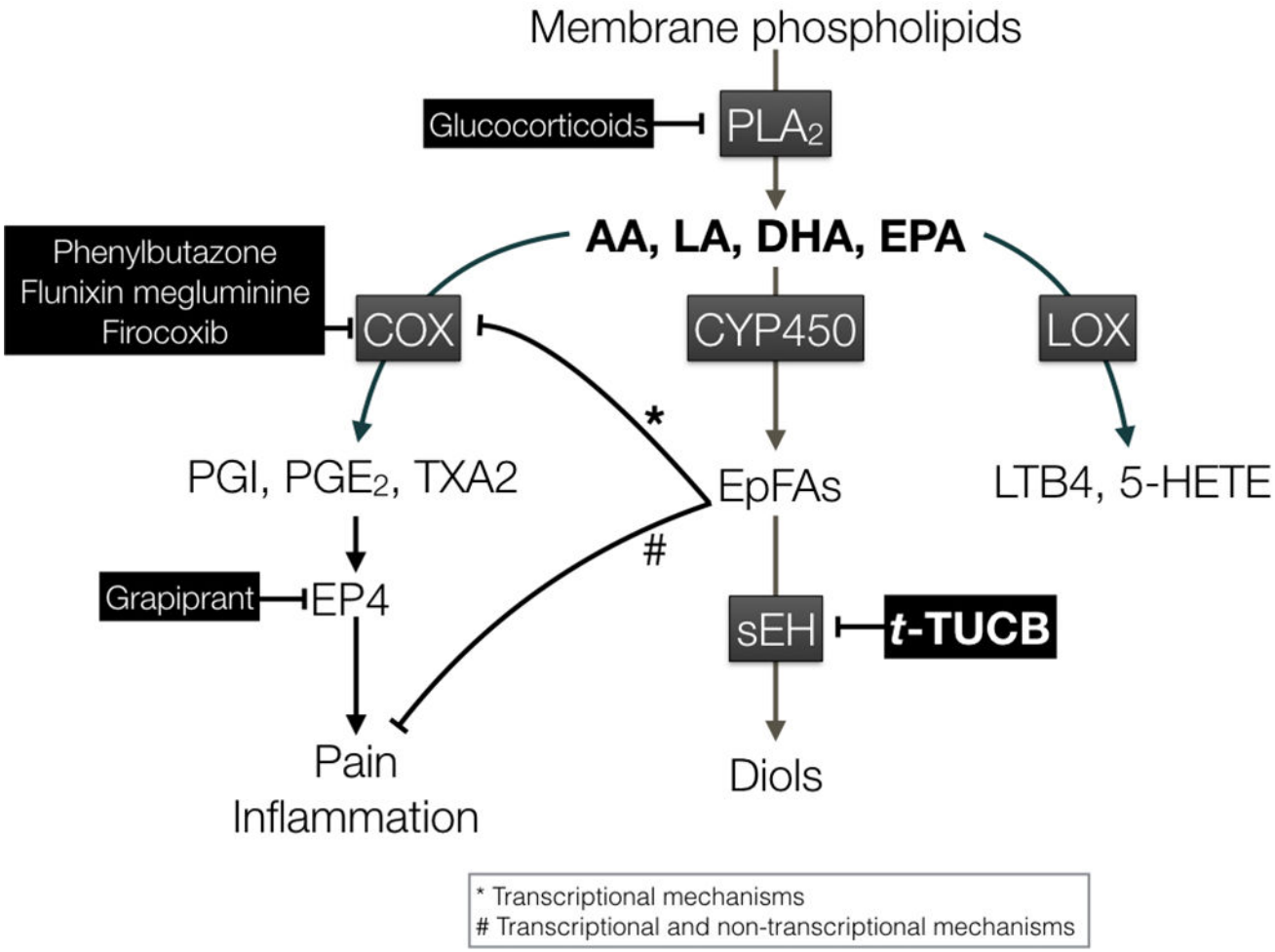

Fig. 1.

Simplified depiction of the 3 major pathways of polyunsaturated fatty acid (PUFA) metabolism. Upon tissue trauma or insult, membrane phospholipids containing the n-6 series arachidonic (AA) and linoleic (LA) acids, and the n-3 series docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids are freed from the cell membrane via the action of phospholipase enzymes (PLA_2 and others) and made available for downstream metabolism. Metabolism via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways gives rise to largely pro-inflammatory and pro-algesic metabolites. PGE_2 produces pain and inflammation by activating at least one of 4 prostanoid receptors (EP). Metabolism via the cytochrome P450 (CYP450) family of enzymes results in anti-inflammatory lipid mediators collectively known as epoxy-fatty acids (EpFAs), which are rapidly metabolized by the soluble epoxide hydrolase enzyme (sEH) to their corresponding diols. Inhibitors of sEH block this degradation and stabilize EpFA levels in vivo. The EpFAs have both transcriptional (suppress COX-2 transcription, thus affecting the AA cascade, stimulate neurosteroid synthesis in the central nervous system, thus activating GABAergic neurons) and non-transcriptional (via opioidergic pathways) mechanisms that result in anti-nociceptive effects. Several clinically available analgesics and/or anti-inflammatory drugs can affect lipid metabolism and/or effect, but the small molecule sEH inhibitor *t*-TUCB is

being investigated as a potential novel strategy for pain management. Key COX metabolites (prostacyclin, PGI; prostaglandin E_2, PGE_2; thromboxane A_2, TXA_2) and LOX metabolites (5-hydroxyeicosatetraenoic acid, 5-HETE; leukotriene B4, LTB4) are shown.

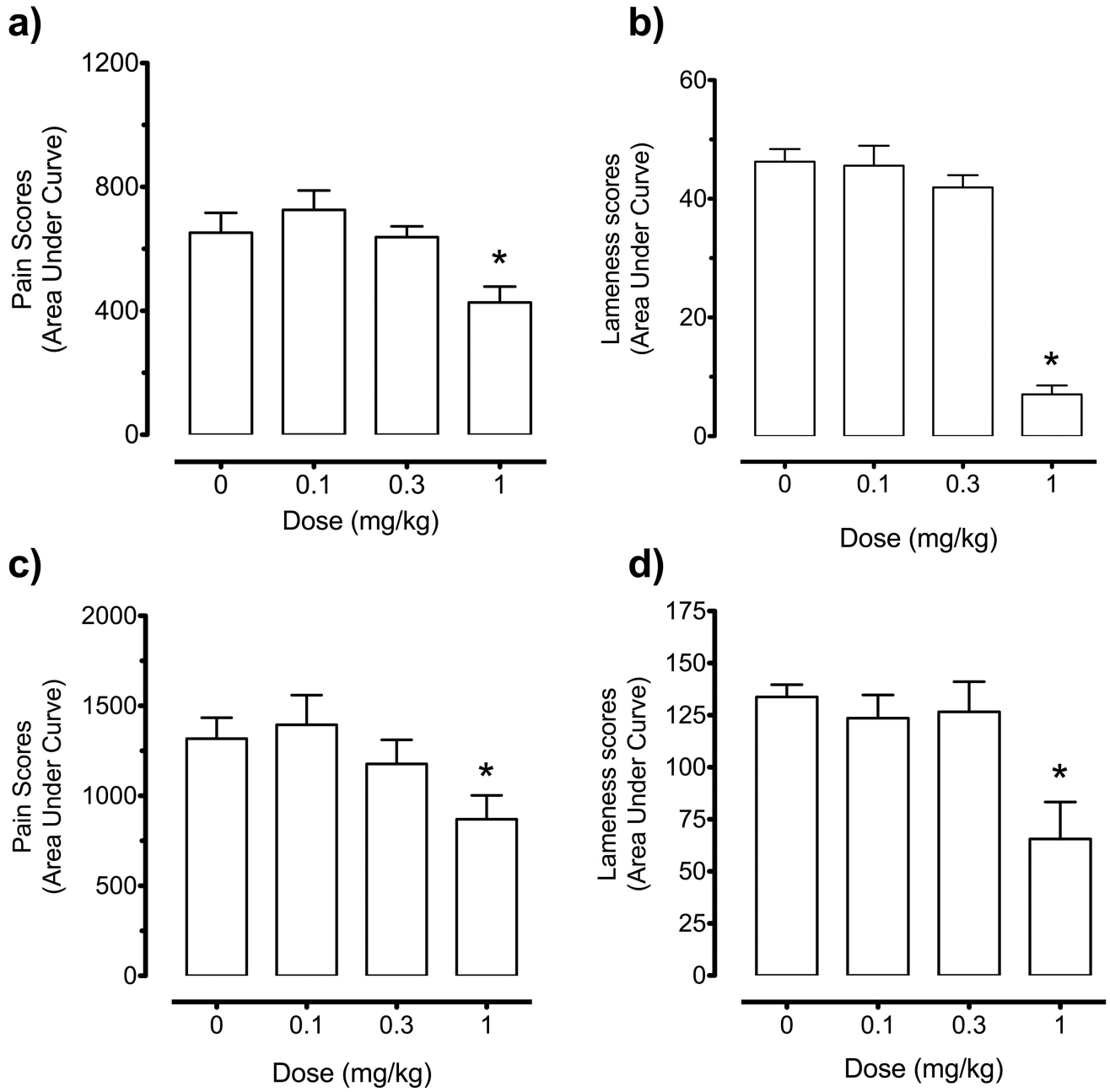

Fig. 2.

Calculated areas under the response curves for visual analog pain scores and American Association of Equine Practitioners lameness scores during 0–12 h (panels a and b, respectively) or 0–48 h (panels c and d, respectively) periods following intravenous administration of a range of doses of the pharmacologic soluble epoxide hydrolase inhibitor *t*-TUCB in horses with lipopolysaccharide-induced radiocarpal synovitis (n=4–6/group). An asterisk indicates that the mean value is significantly difference compared to 0 mg/kg (control) treatment ($P \leq 0.05$). Data are mean ± SEM.

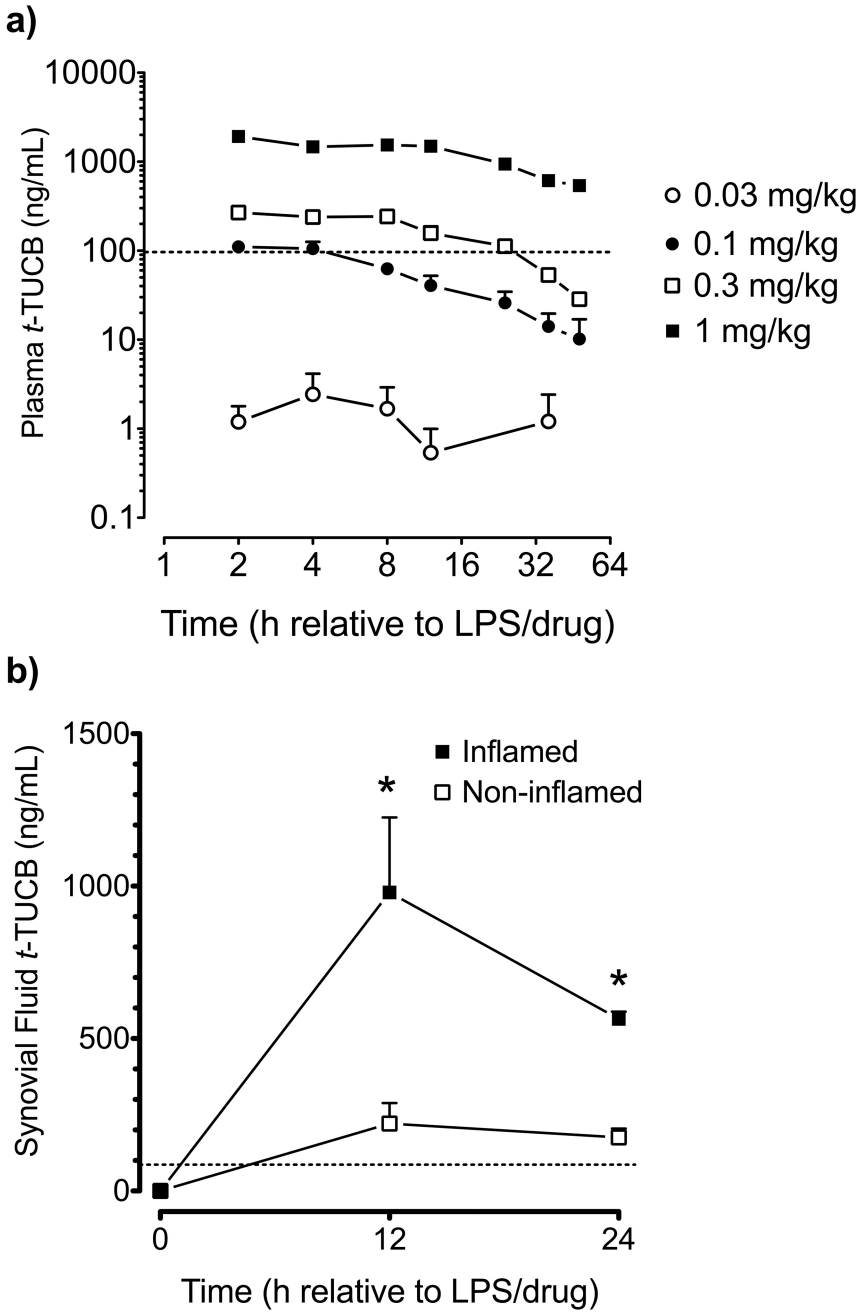

Fig. 3.

Plasma (panel a) and synovial fluid (panel c) concentrations, with respective relationship with the *in vitro* 95% inhibitory concentration (IC_{95} = 96.4 ng/ml, dashed line) for the equine soluble epoxide hydrolase (sEH) of a range of doses of the pharmacologic sEH inhibitor *t*-TUCB in horses with lipopolysaccharide-induced radiocarpal synovitis (n=4–6/group). Synovial fluid concentrations in inflamed and non-inflamed joints are for the 1 mg/kg dose only. An asterisk indicates that *t*-TUCB concentrations are significantly different between joints at each of the time points (P≤0.05). Data are mean ± SEM.

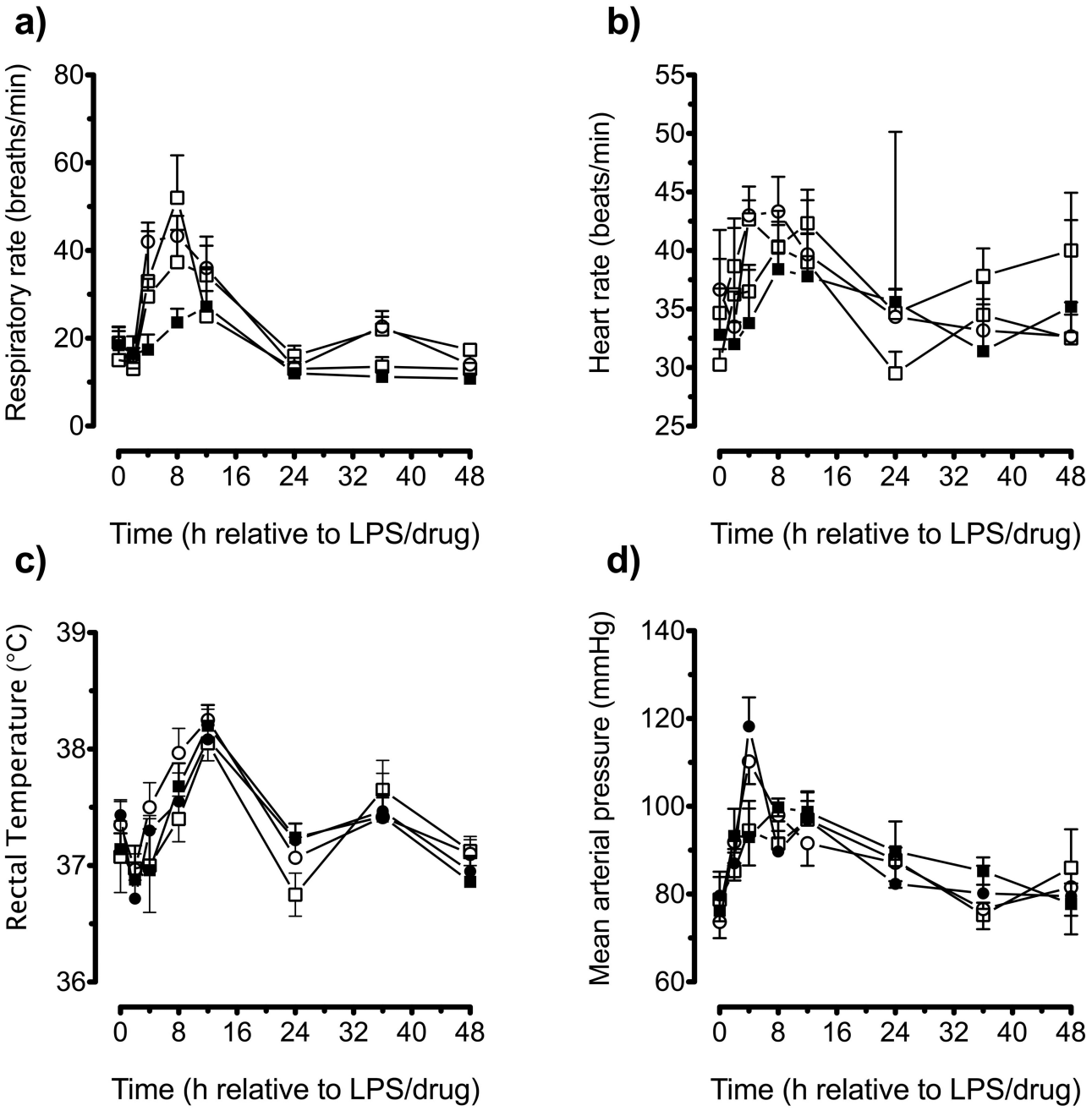

Fig. 4.

Physiologic variables in horses with lipopolysaccharide-induced radiocarpal synovitis (n=4–6/group) and following intravenous administration of a range of doses of the pharmacologic soluble epoxide hydrolase inhibitor *t*-TUCB (0, 0.1, 0.3 and 1 mg/kg; open circles, filled circles, open squares and filled squares, respectively). Baseline data were collected before LPS/*t*-TUCB administration and are denoted as time 0 hours. Data are mean ± SEM.

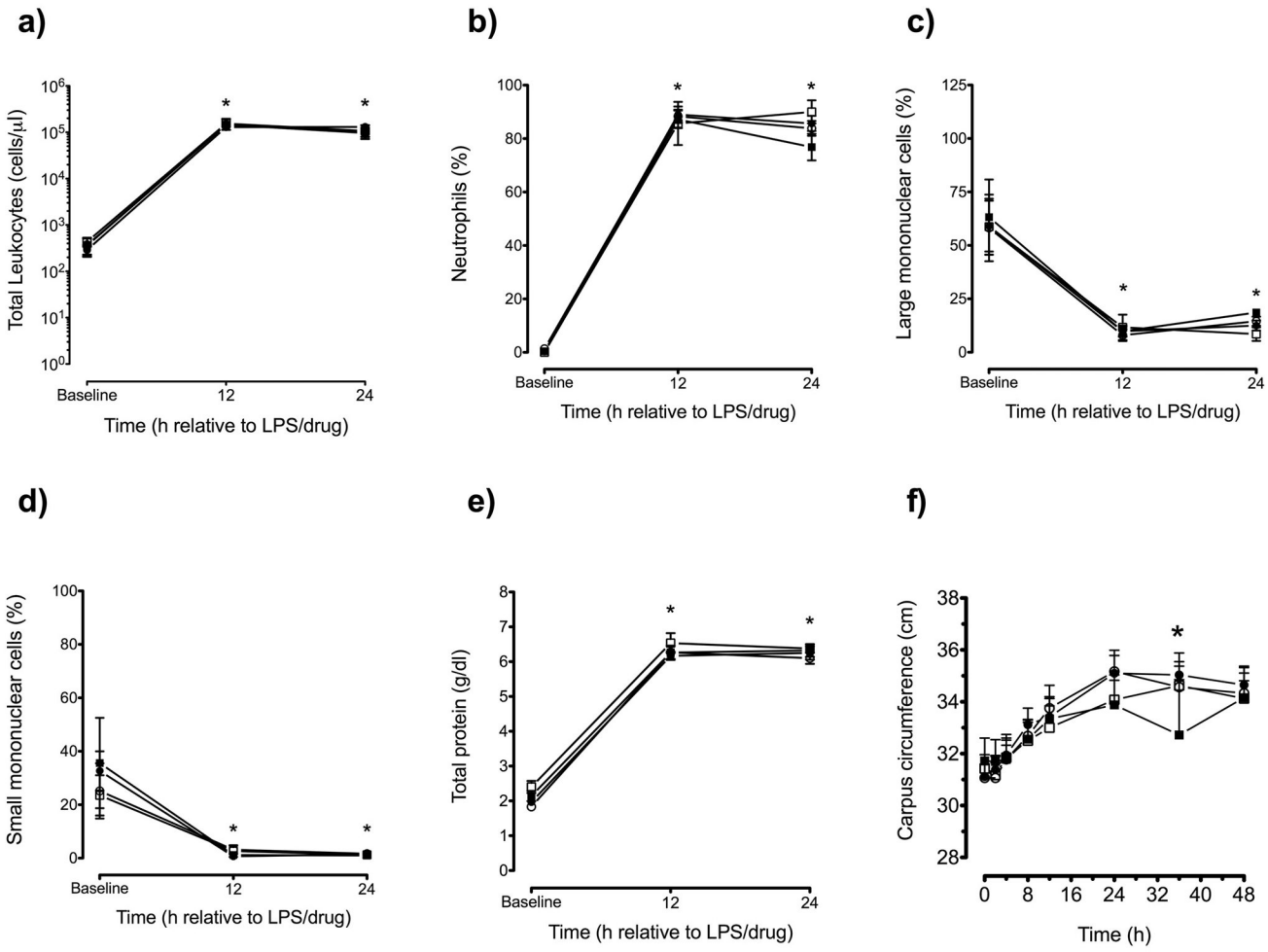

Fig 5.

Synovial fluid inflammatory cell numbers and total protein concentrations (panels a–e) and carpus circumference (panel f) in horses with lipopolysaccharide-induced radiocarpal synovitis (n=4–6/group) and following intravenous administration of a range of doses of the pharmacologic soluble epoxide hydrolase inhibitor *t*-TUCB (0, 0.1, 0.3 and 1 mg/kg; open circles, filled circles, open squares and filled squares, respectively). Baseline data were collected before LPS/*t*-TUCB administration (denoted as 0 hours in panel f). Data are mean ± SEM. In panel f, an asterisk indicates that the mean value is significantly difference between 1 mg/kg and 0 mg/kg (control) treatments at the 36 h time point. For all other panels, an asterisk indicates statistically significant differences between the indicated time point and the respective group baseline value ($P \leq 0.05$).

Table 1

Pharmacokinetic parameters after intravenous administration of 0.1, 0.3 and 1 mg/kg *t*-TUCB, a pharmacologic inhibitor of soluble epoxide hydrolase, in horses with lipopolysaccharide (LPS)-induced radiocarpal synovitis (n=4–6/group). For each parameter, means without common superscript letters are significantly different (P≤0.05). Parameters with values without superscript letters were not compared statistically. Data are mean ± SEM unless otherwise noted.

Parameter	Treatment (mg/kg)		
	0.1	**0.3**	**1**
λ_z (1/h)	0.06±0.004[a]	0.05±0.005[a]	0.03±0.005[a]
$\lambda_{z\ lower}$ (h)	8±4	11±5	14±5
$\lambda_{z\ upper}$ (h)	38±5	48±0	48±0
Terminal half-life λ_z (h)	13±3[a]	13±0.5[a]	24±5[a]
T_{last} (h)	38±5	48±0	48±0
C_{last} (ng/mL)	17±5	29±3	538±76
AUC_{last} (h*ng/mL)	1607±360	5840±488	50139±5604
$AUC_{0-\infty}$ (h*ng/mL)	2005±530	6368±526	71084±5775
Clearance (mL/h/kg)	68±15[a]	48±5[ab]	14±1[b]
V_{ss} (mL/kg)	1252±340[a]	956±96[a]	571±104[a]
Terminal half-life λ_z (h)/dose	148±27[a]	43±3[b]	24±5[b]
Clearance (mL/h/kg)/dose	680±153[a]	161±15[b]	14±1[b]
AUC_{last} (h*ng/mL)/dose	16066±3595[a]	19467±1625[a]	50139±5604[b]
V_{ss} (mL/kg)/dose	12521±2986[a]	3197±318[b]	571±104[b]

Abbreviations: λz = terminal rate constant; T_{last} = time of last measured plasma concentration; C_{last} = last measured plasma concentration; AUC0–∞ = area under the plasma concentration time curve; V_{ss} = volume of distribution at steady state.