INTRODUCTION

Intra-articular morphine is widely used in humans for post-operative pain relief following joint surgery. In addition, IA morphine may have anti-inflammatory effects, providing added benefit in joints with postoperative synovitis [1]. While the analgesic efficacy of IA morphine has been widely investigated, very little is known about its effects on the articular cartilage. The aim of the current study was to provide a placebo-controlled in vivo investigation of the effects of IA morphine on synovial fluid (SF) biomarkers of inflammation and articular cartilage turnover.

MATERIAL AND METHODS

All experimental procedures were pre-approved by the Utrecht University Ethical Committee in compliance with Dutch legislation on the care and use of experimental animals. A randomized, blind, placebo-controlled cross-over trial was conducted in n=8 healthy warmblood horses (age 7.1 ± 3.7 yr, weight 589.1 ± 61.6 kg; mean ± SD). At t=0 hrs, synovitis was induced in the right (n=4) or left (n=4) tarsocrural joint by sterile injection of 0.5 ng lipopolysaccharide (LPS) from E.Coli O55:B5. At t=1 hr post-LPS injection, the same joint was randomly assigned to be injected with morphine hydrochloride (0.2 mg/kg; n=4) in 20 ml saline or 20 ml saline only (placebo; n=4). At t=4, t=8, t=28, t=52 and t=168 hrs, serial synovial fluid samples were obtained. Following a 3-week wash-out period, synovitis was induced in the contralateral tarsocrural joint and treatments reversed.

Synovial fluid samples were analyzed for total WBC count and total protein concentration. Prostaglandin E2 concentrations were measured using a commercial ELISA kit (RnDSystems, Minneapolis, MN, USA) following RP-18 extraction [2]. General MMP-activity was measured by means of a fluorimetric assay as previously described [3]. GAG release was quantified by the 1,9-dimethylmethylenedine blue assay adapted for use in microtiter plates [2], while concentrations of CS846 epitope (a putative marker of aggrecan synthesis), carboxypropeptide of type II collagen (CPII, a marker of type II collagen synthesis) and C2C (collagenase-cleavage fragments of type II collagen) were measured by use of commercial ELISA kits (IBEX, Montreal, Quebec, Canada).

RESULTS

Morphine significantly reduced SF WBC counts at t=8 (P<0.001) and total protein concentrations at t=4 (P=0.033) post-LPS compared to placebo (figure 1A and B). Morphine also significantly reduced SF bradykinin (P=0.026; data not shown) and prostaglandin E2 concentrations (P<0.001; figure 2C) compared to placebo treatment at t=4 and t=8, respectively, but there was no treatment effect on substance P levels at any time point (data not shown).

Figure 1 Synovial fluid concentration of leukocytes (A), total protein (B), prostaglandin E2 (C) and general MMP activity (D). Synovitis was induced in the tarsocrural joint of n=8 horses at t=0 by LPS injection, IA morphine (0.2 mg/kg) or placebo was administered at t=1.

Synovial fluid MMP activity was significantly lower in morphine- vs. placebo-treated joints at t=168 hrs post-LPS (P=0.044; figure 1D), and there were no treatment effects on biomarkers of aggrecan turnover (GAG and CS846; figure 2A and B) or type II collagen turnover (C2C and CPII; figure 2C and D).

DISCUSSION

This study confirms and extends the peripheral anti-inflammatory effects of IA morphine by demonstrating reduction of SF WBC counts and total protein concentrations, but also of synovial fluid prostaglandin E2 and bradykinin release, which to our knowledge has not previously been shown in vivo.

The observed modest attenuation of SF MMP activity by IA morphine only reached statistical significance at t=168 hrs. It is unlikely to be due to reduction in leukocyte influx, as WBC by this time had returned to normal. Levels of type II collagen and aggrecan turnover markers after induction of inflammation were comparable in morphine-treated and placebo-treated joints. These data suggest IA morphine to have a neutral effect on short-term cartilage turnover in the presence of inflammation.

There are a few limitations to the current study. Firstly, the LPS model induces acute inflammation that is transient but more severe than that which is likely to be seen postoperatively. Also, the lack of visualization of the articular cartilage and the short-lasting inflammation preclude a direct appreciation of any morphological effects of morphine vs. placebo-treatment. There was consistent joint effusion and morphine-treated joints were less effused; hence, reductions in SF mediator concentrations in these joints will be underestimated rather than overestimated based on these data.

In conclusion, this study demonstrates that a single IA morphine injection in joints with acute synovitis reduces local inflammation without detrimental effects on articular cartilage turnover as evidenced by SF biomarkers profiles.

REFERENCES