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RESEARCH PAPER

Effects of tramadol and o-desmethyltramadol on canine innate immune system function

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Abstract

Objective Tramadol is a commonly used opioid analgesic in dogs, particularly in dogs with a compromised immune system. An opioid may be selected for its immunomodulatory effects. Consequently, the objective of this study was to investigate the effects of tramadol and o-desmethyltramadol (M1) on the function of canine leukocytes *in vitro.* The hypothesis was that tramadol and M1 would not alter polymorphonuclear leukocyte (PMN) phagocytosis, PMN oxidative burst, or stimulated leukocyte cytokine production capacity of tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-10.

Study design *In vitro* pharmacodynamic study.

Animals Six healthy dogs.

Methods Blood from six dogs was obtained and incubated with various concentrations of tramadol and M1. Phagocytosis and oxidative burst were assessed using flow cytometry, and lipopolysaccharide (LPS), lipoteichoic acid (LTA) and peptidoglycan (PG)-stimulated leukocyte production of TNF, IL-6, and IL-10 were measured using a canine specific multiplex assay.

Results No differences were detected in phagocytosis or oxidative burst with any drug concentration. Tramadol did not alter leukocyte cytokine production, however, M1 significantly blunted IL-10 production.

Conclusions Tramadol and its metabolite M1 were sparing to PMN phagocytosis and oxidative burst in dogs *in vitro*. Tramadol did not alter leukocyte cytokine production, however, M1 blunted IL-10 production at clinically achievable concentrations suggesting that M1 may promote a proinflammatory shift.

Clinical relevance These data suggest that tramadol has minimal effect on phagocytosis and oxidative burst, and may promote a proinflammatory shift. Therefore, tramadol may be an ideal opioid analgesic in dogs at high risk of infection. Further investigation *in vivo* is warranted.

Keywords dog, immunomodulation, tramadol.

Introduction

Tramadol is a centrally acting opioid analgesic commonly used in dogs in the treatment of pain, particularly in situations of sepsis, cancer, and in the post-operative period. The analgesic actions of tramadol are the result of two synergistic

mechanisms - μ opioid receptor agonism and inhibition of neuronal reuptake of norepinephrine and serotonin (Gillen et al. 2000; Grond & Sablotzki 2004). The main active metabolite of tramadol is o-desmethyltramadol (M1), which has a 400-fold higher affinity for the human μ opioid receptor than tramadol (Gillen et al. 2000). Since tramadol is commonly used in dogs in situations of potential immune compromise, and tramadol has immunomodulatory effects in other species, it is of great importance to understand the effects of this analgesic drug on the immune system in dogs.

Macrophages and neutrophils have opioid receptors on their cell surface, and due to receptor interaction certain opioids alter immune function in many species (Sacerdote et al. 2000; Ocasio et al. 2004; Liu et al. 2006; Shirzad et al. 2009; Bosshart 2010). For example, morphine promotes excessive production of inflammatory mediators leading to increased complication rates in the murine model of septic shock (Ocasio et al. 2004) and decreases phagocytosis (Bosshart 2010). In contrast, tramadol is generally considered to spare innate immune system function (Grond & Sablotzki 2004; Liu et al. 2006). Tramadol has no effect on phagocytosis in humans (Beilin et al. 2005) and improves immune function in rats by modulating inflammation through leukocyte cytokine release (Liu et al. 2008). Although immunomodulation by opioids has been studied in rodent models and humans, no studies to date have investigated the effect of tramadol on the innate immune system in dogs.

Differences in the immunomodulatory effects of opioids in other species are related to opioid structure and mechanism of action, and tramadol is suspected to have the least detrimental immunomodulatory effects. An analgesic with minimal immunosuppressive effects on the immune system in dogs at high risk for infection would be ideal to incorporate into pain control protocols. The objective of this study was to evaluate the influence of different concentrations of tramadol and its M1 metabolite on the function of the healthy canine innate immune system *in vitro*. It was hypothesized that tramadol and M1 would not alter polymorphonuclear leukocyte (PMN) phagocytosis, PMN oxidative burst, or stimulated leukocyte cytokine production capacity of TNF-α, IL-6, and IL-10.

Materials and methods

Sample collection

Whole blood was collected from six healthy, adult, client owned dogs following informed owner consent (University of Missouri Animal Care and Use protocol #7334) that were not treated with any medications in the month prior to enrollment, with the exception of routine parasitic preventatives. Six healthy dogs ranging in age from 1 to 7 years were enrolled. Breeds included mixed breed (n = 3), and one each of Great Dane, Golden Retriever, and Boxer. The health status of the dog was confirmed with history, physical examination, complete blood count (CBC), plasma biochemical profile and urinalysis. The biochemical profile parameters measured were glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, albumin, total protein, globulins, anion gap, calcium, phosphorous, cholesterol, total bilirubin, alanine aminotransferase, alkaline phosphatase, and gamma-glutamyl transpeptidase.

Leukocyte viability

Twenty-one milliliters of whole blood was diluted 1:2 with Roswell Park Memorial Institute (RPMI) medium to which was added 200 U mL^{-1} of penicillin and 200 mg mL^{-1} of streptomycin (Gibco, Invitrogen; Life Technologies, NY, USA), then incubated with tramadol hydrochloride salt (Sigma-Aldrich, MO, USA) diluted in saline to a final well tramadol concentration of 20,000 ng mL^{-1}, o-desmethyltramadol base (Sigma-Aldrich) diluted in saline to a final well concentration of 10,000 ng mL^{-1}, or saline for 24 hours on 12 well culture plates at 37 °C and 5% CO_2. Red blood cell lysis was accomplished using ammonium-chloride-potassium lysis buffer. Leukocyte viability was determined using trypan blue exclusion (Welters et al. 2000). The cells from each well were stained with 0.4% trypan blue (Invitrogen, NY, USA). The number of dye-excluding (live) cells and positively stained (dead) cells were counted using a hemocytometer. The assay was performed in duplicate.

Sample incubation

Immediately after collection, and prior to phagocytosis, oxidative burst, or leukocyte cytokine