Published in final edited form as:

Eur J Pharmacol. 2007 July 12; 567(1-2): 67–76. doi:10.1016/j.ejphar.2007.03.048.

Clinical efficacy of buprenorphine to minimize distress in MRL/lpr mice

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Abstract

MRL/MpJ-Fas^{lpr} (MRL/lpr) mice are an accepted animal model to study human systemic lupus erythematosus. We tested if a commonly used analgesic (buprenorphine hydrochloride) would reduce pain and distress in these mice without impacting the progression of autoimmune disease. Female MRL/lpr mice were randomly separated into four groups. Experimental groups received cyclophosphamide (25 mg/kg i.p. weekly), buprenorphine (0.09 mg/kg/mouse/day via drinking water), or cyclophosphamide + buprenorphine from 11 - 21 weeks of age. Controls received no treatments. Mice were monitored daily by a licensed veterinarian (blinded observer) and assigned a score weekly on parameters associated with pain and distress as well as progression of disease. Proteinuria was measured weekly, and serum anti-dsDNA antibody levels were determined at 11, 15, and 18 weeks of age. At 21 weeks of age, the animals were euthanized and the kidneys and spleens were removed for evaluation. Regardless of the parameter observed, buprenorphine did not significantly decrease distress when compared to the controls. Buprenorphine did not alter the progression of autoimmune disease, based on characteristics of splenic architecture and splenocyte cell profiles, development of lymphadenopathy, or kidney histology as compared to controls. This study indicates that buprenorphine at this dose and route of administration was ineffective in reducing distress associated with disease progression in the MRL/lpr strain. More studies are needed to determine if, at a different dose or route, buprenorphine would be useful as adjunctive therapy in reducing distress in MRL/lpr mice.

Keywords

Buprenorphine; MRL/lpr; distress; systemic lupus erythematosus; pruritus

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1. Introduction

MRL/MpJ-Fas^{lpr} (MRL/lpr) mice spontaneously develop an autoimmune syndrome similar to human lupus erythematosus including autoantibody production and immune complex glomerulonephritis and are commonly used as a murine model to study lupus (Reilly and Gilkeson, 2002). MRL/lpr mice begin showing overt signs of disease by 3 months of age. Along with autoantibody production and immune complex glomerulonephritis, MRL/lpr mice develop polyarthritis, severe lymphadenopathy, hypergammaglobulinemia, proteinuria, skin lesions with alopecia, scab formation, and erythematous lesions of the ear pinna that often become necrotic as the mice age (Andrews et al., 1978; Molano et al., 2003; Okamoto et al., 2004; Reilly et al., 2000; Reilly et al., 2002; Sekine et al., 2001; Watanabe-Fukunaga et al., 1992). MRL/lpr mice also show severe excoriations of the skin due to self-trauma associated with excessive pruritus, which is thought to be similar to the pruritus seen in humans with autoimmune diseases, such as systemic lupus erythematosus and primary biliary cirrhosis (Kapadia and Haroon, 1996) (Umeuchi et al., 2005). These lesions interfere with species specific behaviors, leading to increasing distress in the mice.

Few reports exist concerning methods to minimize pain and distress in MRL/lpr mice that do not interfere with the study of disease progression for research purposes. Many compounds used in laboratory animals to minimize pain and distress have significant disadvantages for treating large colonies of animals, including frequent dosing schedules and/or the need to use injectable routes of administration (Christoph et al., 2005; Cooper et al., 1997; Gades et al., 2000; Gross et al., 2003; Roughan and Flecknell, 2004). These problems contribute significantly to the difficulties in managing pain and distress in murine models of autoimmune disease.

Buprenorphine hydrochloride is a potent opioid analgesic with partial agonistic properties at μ -opioid receptors (Ide et al., 2004; Pick et al., 1997). Buprenorphine has been shown to have an antinociceptive potency 25 to 40 times higher than morphine (Cowan, 2003). Benefits of buprenorphine include a high analgesic potency with a long duration of action (Kogel et al., 2005) and formulation as a water soluble compound that can be administered orally in the drinking water (Cooper et al., 1997; Lindsay et al., 2005). The use of buprenorphine as an analgesic has shown efficacy in rodent models of acute and chronic pain, including visceral, somatic, and inflammatory pain (Christoph et al., 2005; Roughan and Flecknell, 2004). Buprenorphine has also been shown to attenuate self-injurious behaviors in mouse models of psychostimulant addiction and, although there have been no controlled studies of its use in humans for treatment of systemic lupus erythematosus, it is thought to be an appropriate drug of choice in humans suffering from other illnesses that stimulate self-trauma (Mori et al., 2006). A recent study in mice with experimental *Toxoplasma gondii* infection has indicated that clinical signs of pain and distress are reduced when buprenorphine is administered via the drinking water without interfering with mortality rates as compared to untreated controls (Lindsay et al., 2005).

The purpose of this study was to determine if buprenorphine hydrochloride could be used to alleviate pain and distress in MRL/lpr mice without negatively impacting common research parameters, including immune system function, or progression of disease.

2. Materials and Methods

2.1. Animals

Eight week old MRL/lpr female mice were purchased from Jackson Laboratories. The mice were housed in specific pathogen free conditions at the Center for Molecular Medicine and Infectious Diseases animal facility at Virginia Polytechnic and State University. They were

housed in a 12h light:dark cycle and provided food and water ad libitum. All animal protocols were approved by the Institutional Animal Care and Use Committee at the Virginia Polytechnic and State University.

2.2. Reagents

DMEM and RPMI were obtained from Gibco (Gaithersburg, MD). The protein assay kit was purchased from Bio Rad (Hercules, CA). All other reagents were purchased from Sigma (St. Louis, MO).

2.3. Urine Protein Analysis

Urine protein determinations were made using Chemstrip® 2 GP reagent strips from Roche (Indianapolis, Indiana).

2.4. Study Groups

Forty mice were randomly separated into 8 cages, with 5 mice per cage, and 2 cages in each study group for a total of 10 mice per study group. At 11 weeks of age, the mice received cyclophosphamide, buprenorphine, or cyclophosphamide + buprenorphine. The control group received no treatments. Buprenorphine hydrochloride (Abbott Laboratories, North Chicago, Illinois) was administered via the drinking water to deliver 0.09 mg/kg per mouse per day (Cooper et al., 1997; Lindsay et al., 2005). Clean bottles with freshly prepared buprenorphine-treated water were provided every three days as a method to help ensure potency of the drug. Green-tinted water bottles were used to prevent decay of buprenorphine, which is a light sensitive compound. Cyclophosphamide (25 mg/kg BW) was given weekly to the cyclophosphamide and cyclophosphamide + buprenorphine groups via intraperitoneal injection in 40 μ l saline.

2.5. Animal Observations

All study animals were observed daily for evidence of extreme pain and distress by the veterinarian, who was blinded to the treatment regime. Extreme pain and/or distress was defined as pain and/or distress that exceeded what is considered normal for disease progression in MRL/lpr mice and was indicated by any one of the following: 1 – open skin lesions that would not stop bleeding, 2 – evidence of significant respiratory distress such as abdominal breathing and/or cyanosis of the mucous membranes, or 3 – any animal that was non-responsive to stimulation. During the course of the study, two animals were removed due to extreme pain and distress. The first animal was in severe respiratory distress and was euthanized one week prior to the termination of the study. The second animal was euthanized due to uncontrollable bleeding secondary to self mutilation one day prior to termination of the study.

Once weekly, each animal was given a complete physical examination. The physical examination included observation within its cage and palpation once removed from its cage. These observations were scored using a numerical scoring system that was developed from the current animal care policy for MRL/lpr mice housed at Virginia Polytechnic Institute and State University at the time of this study. The scoring criteria are listed below.

2.5.1. Body Condition Scoring—Body condition scoring was done using a 5 point scale as described previously (Ullman-Cullere and Foltz, 1999). A body condition score (BCS) of 3/5 was considered within normal limits for mice on this study. A BCS of 1/5 represented an emaciated animal, 2/5 represented an underweight animal, 4/5 indicated an overweight animal, and 5/5 indicated an obese animal. As an indicator of pain or distress, only a body condition score of 2/5 or less was considered significant. If considered significant, the BCS was added to the combined distress score as follows: 1 = BCS of 2/5, 2 = BCS 1/5.

2.5.2. Mentation Scoring—Mentation was assessed using the following parameters: BAR = Bright, alert, and responsive (BAR indicated normal activity level and mentation for a mouse); QAR = Quiet, alert, and responsive (QAR indicated an animal that was less active than normal while under observation in the cage, but became more active upon stimulation); DR = Depressed, but responsive (DR indicated an animal who would respond to stimulation, but remained huddled on the cage bottom); and NR = Non-responsive (NR indicated a conscious animal that would not respond even with significant stimulation). Each animal was given a score to represent their individual level of mentation. The scoring system used for mentation was as follows: 0 = BAR, 1 = QAR, 2 = DR, 3 = NR.

2.5.3. Characterization of Skin Lesions—Mice were individually examined for evidence of skin disease and self-trauma. The lesions were scored based on the following: 0 = no lesions noted, 1 = mild lesions (alopecia with small amount of scabbing present), 2 = moderate lesions (scabbing over < 5% of the skin surface), and 3 = severe lesions (scabbing over >5% of the skin surface).

2.5.4. Other Common Indicators of Stress—Other common indicators of stress in rodents were noted during the weekly physical exam. These included abnormal respiratory rate and effort, presence of a ruffled haircoat, presence of huddling behavior, and noticeable pruritus. Each of these parameters were evaluated prior to any manipulation and scored using the following criteria. Respiratory rate and effort was scored as follows: 0 = normal respiratory rate and effort, 1 = increased respiratory rate, but normal effort, 2 = increased respiratory rate and effort, 3 = increased respiratory rate and effort with an abdominal component to the breathing. For the ruffled haircoat, huddling behavior, and pruritus parameters, the mice were scored as follows: 0 = absence of the indicator, and 1 = presence of the indicator. These scores were combined together to form a score titled “other indicators of stress”.

2.5.5. Combined Distress Scores—The scores on each animal from the above categories (body condition scoring, mentation, characterization of skin lesions, and other common indicators of stress) were added together to give a combined distress score.

2.5.6. Lymphadenopathy—During the physical exam, each mouse was palpated to determine the extent of lymph node enlargement that was present. Lymph node enlargement was scored as follows: 0 = None, 1 = mild enlargement (palpable, but not easily visible), 2 = moderate enlargement (easily visible, but not interfering with mobility), 3 = severe enlargement (easily visible and interfering with mobility regardless of extent of interference).

2.6. Urine Protein Measurements

Urine was collected weekly and protein levels were measured using reagent strips. Urine protein levels were reported as negative, trace, 30 mg/dl (1+), 100 mg/dl (2+), or 300 mg/dl (3+).

2.7. Sera Collection and Anti-dsDNA Antibody Levels

At 11, 15, and 18 weeks of age, the mice were anesthetized with isoflurane and bled from the retro-orbital sinus. At each time point, anti-dsDNA antibody levels were measured using an ELISA as previously described (Reilly et al., 2006).

2.8. Flow Cytometric Analysis

At the time of sacrifice, the mice were weighed and necropsied. The spleens were removed, weighed, aseptically dissociated, and treated with Tris-ammonium chloridelysis buffer (pH 7.2) to remove erythrocytes. Flow cytometric analysis was performed using fluorescein-

isothiocyanate (FITC)-conjugated CD11c, CD21, CD62L (L-selectin), CD79b, and GL-7; R-phycoerythrin (R-PE)-conjugated CD3, CD4, CD23, CD138; PerCP-CY5.5-conjugated CD25, and Texas-Red-conjugated CD45R/B220 rat anti-mouse monoclonal antibodies (BD Pharmingen, San Diego, CA). Cells were resuspended in 0.1% BSA in PBS and aliquoted (0.25×10^6 cells/100 μ l/well) into 96-well plates. One hundred μ l of PBS including relevant monoclonal antibody equivalent to 1 μ g/ 1×10^6 cells added onto cells. The cells were incubated for 30 min, at 4°C. After 30 min, cells were washed with 0.1% BSA in PBS, and analyzed by flow cytometry (Coulter Epics XL/MXL, Hialeah, FL). For analysis, the cells were gated in forward scatter versus side scatter. The CD4CD25CD62L cells were further gated including the CD25⁺ versus CD62L⁺ T-cell subsets over CD4⁺ T-cells.

2.9. Kidney Pathology

At the time of necropsy, the kidneys were removed and divided into sections. One portion was placed in buffered formalin for subsequent embedding in paraffin, followed by sectioning, H&E and PAS staining. Sections were assessed via light microscopy for glomerular proliferation, glomerular inflammation, glomerular size, number of nuclei per glomerulus, crescents, necrosis and fibrosis. Each of these parameters was graded from 0 to 3+ and an overall glomerular score derived. The pathology and morphometric analysis were performed by a pathologist blinded to the groups (Dr. Arben Santo).

2.10. Statistical analysis

Analysis of variance (ANOVA) was used to evaluate statistical significance. A two-tailed P value of <0.05 was considered significant.

3. Results

3.1. Observation of animals

As MRL/lpr mice age, they develop typical manifestations of autoimmune disease similar to that seen in lupus patients, including alopecia, pruritus, and lymphadenopathy (Fig. 1A). When the scores for individual mice were tabulated, we found no significant differences between the cyclophosphamide, cyclophosphamide + buprenorphine, buprenorphine, or control groups in the following parameters: characterization of skin lesions (Fig. 1B), other indicators of distress (Fig. 2A), body condition scoring (data not shown), or lymphadenopathy (Fig. 2B). Although there was no statistical difference between groups in these four parameters, there was a steady trend seen in the data towards higher pain and distress scores regarding characterization of skin lesions, other indicators of distress, and body condition scoring in the buprenorphine group and control group when comparing to the cyclophosphamide or the cyclophosphamide + buprenorphine group.

For mentation, there was no significant difference between the cyclophosphamide and the cyclophosphamide + buprenorphine groups. Likewise, there was no significant difference between the buprenorphine and the control groups. Pain and distress, as indicated by mentation status, was significantly higher in the buprenorphine and control groups when compared with either the cyclophosphamide or cyclophosphamide + buprenorphine groups (Fig. 3A).

When the scoring systems were tabulated together to form the combined distress score, there was no significant difference between the buprenorphine group and the control group. The combined distress scores for the cyclophosphamide group were significantly lower than the remaining three study groups, including the cyclophosphamide + buprenorphine group. However, the combined distress scores for the cyclophosphamide + buprenorphine group were also significantly lower than either the buprenorphine or control groups, which had the highest scores for combined distress out of the four study groups (Fig. 3B).

3.2. Sera and Urine Disease Indices

As the mice aged, we measured common markers indicating the progression of lupus nephritis including proteinuria and the presence of antibodies to double stranded DNA (Fig. 4). The amount of protein in the urine was low in all groups but increased as the mice aged (Fig. 4A). By 17 weeks of age, the cyclophosphamide and cyclophosphamide + buprenorphine groups showed lower amounts of protein in the urine than either the buprenorphine or the control group ($p < 0.05$).

As MRL/lpr mice age, there is an increase in antibody levels to double stranded DNA. The cyclophosphamide and cyclophosphamide + buprenorphine groups had significantly lower antibodies to double stranded DNA in the sera than the buprenorphine group or the control group (Fig. 4B). These results showed that buprenorphine did not effect antibody production.

3.3. Renal Pathology

To determine the effects of buprenorphine on kidney function, renal tissue was assessed from each mouse following necropsy (Fig. 5). Buprenorphine and control mice showed typical glomerulonephritis, characterized by enlarged glomeruli, proliferation of glomerular cells, infiltration inflammatory cells, increased mesangial matrix and crescent formation (Fig. 5A, 5B). In contrast, mice treated with cyclophosphamide or cyclophosphamide + buprenorphine showed a lesser degree of glomerulonephritis, varying degrees of focal glomerular cell proliferation, occasional inflammatory cells, and a only a slight increase in the mesangial matrix (Fig. 5C, 5D). There was also a substantial reduction in the number of inflammatory cells in the cyclophosphamide and the cyclophosphamide + buprenorphine groups when compared with either the buprenorphine or the control groups, indicating that buprenorphine treatment did not alter renal pathology. When the scores were tabulated, the cyclophosphamide and the cyclophosphamide + buprenorphine groups had significantly less renal disease than the buprenorphine group or control group (Fig. 5E).

3.4. Spleen Pathology

To determine the effect of buprenorphine treatment on splenomegaly in MRL/lpr mice, the total body weight and spleen weight were measured in the mice at 21 weeks of age (Fig. 6). Spleen weights and spleen to body weight ratios were significantly lower in the cyclophosphamide and cyclophosphamide + buprenorphine groups compared to the buprenorphine group or the control group (Fig. 6A, 6B). There was no significant difference in body weights in any of the treatment groups (Fig. 6C). Splenomegaly was significantly reduced in the cyclophosphamide and the cyclophosphamide + buprenorphine group compared to the buprenorphine and the control mice.

To further analyze splenic profiles, flow cytometric analysis was performed (Fig. 7). We sought to characterize the various T cell and B cell populations in the four study groups. CD4⁺ CD25⁺ + regulatory T cells (Tregs), constitute 5–10% of peripheral CD4⁺ T cells in normal naive mice and humans. The subpopulation of CD4⁺CD25⁺CD62L⁺ cells possesses a significantly higher suppressive capacity than the CD4⁺CD25⁺CD62L[−] T cells (Szanya et al., 2002). Percentages of CD4⁺CD25⁺ T cells and CD4⁺CD25⁺CD62L⁺ T cells were slightly (but statistically significant) higher in the cyclophosphamide group compared to the control group (Fig. 7A). The percentages of these two groups were also higher in the cyclophosphamide + buprenorphine group and the buprenorphine group when compared to controls, but the difference was not statistically significant.

We also examined the different B cell populations in the four study groups. The percentages of germinal center B cells (CD45R/B220/CD3/GL-7), marginal zone cells (CD45R/B220/CD23/CD21), and dendritic cells (CD45R/B220/CD11c/Ly6c) were not different between

cells from the four study groups. The plasma cell (CD45R/B220/CD138/CD79b) population was slightly higher in the control group (Fig. 7B).

4. Discussion

The use of MRL/lpr mice has been a widely accepted animal model to study SLE for over 30 years. Although the mice develop disease manifestations similar to humans, concerns exist as to the humane use of older mice since as they age the mice develop severe skin and ear pathologies. Therefore, these studies were conducted to examine if an analgesic could be administered to the mice to alleviate the skin manifestations without interfering with autoimmune disease. Buprenorphine was chosen due to the low incidence of tolerance development, its potency as an analgesic, the long duration of action, the water soluble nature of the compound, and its reported activity in rodent models, including its effects on the immune system.

An important consideration when using opioid analgesics for chronic administration is the development of tolerance. Tolerance refers to the diminution in the response of a patient to a drug after prolonged use. Regarding tolerance development to opioids, as opioid use becomes prolonged a larger amount of drug is required to reach the same plane of analgesia that was reached with the initial dose. Although buprenorphine can cause the development of tolerance, it is thought to be associated with less severe tolerance when compared with other opioids. Tolerance was not one of the parameters evaluated in this study; however, the decreased incidence of tolerance development with buprenorphine compared to other opioids was a consideration for choosing buprenorphine as the most appropriate analgesic for chronic administration. A study by Gringuaz et al. showed tolerance to buprenorphine developed in mice following 14 days of treatment at 1.2 mg/kg given s.c. b.i.d., however the tolerance that developed was the least noticeable with buprenorphine when compared to the other drugs tested, including butorphanol, nalbuphine, and cyclorphan (Gringuaz et al., 2001).

Concerning the effects of buprenorphine on the immune system, Banerjee and Sarkar reported that buprenorphine administration altered immune function in mice by decreasing lymphocyte and monocyte counts and increasing neutrophil counts. However, these effects were transient and resolved within 45 days of drug withdrawal. (Banerjee and Sarkar, 1997) In another study, buprenorphine was shown to have no significant effect on immunity based measurements of lymphoproliferation, natural killer cell activity, interferon-gamma production, and interleukin-2 production (Martucci et al., 2004). Other studies have also indicated that buprenorphine has minimal long term effects on the immune system (D'Elia et al., 2003; Gomez-Flores and Weber, 2000; Lindsay et al., 2005) and does not alter renal perfusion in a rodent model of renal ischemia (Deng et al., 2000).

Evaluating pain and distress in laboratory animals can be challenging. Based on guidelines from the Institute of Laboratory Animal Research, distress can be defined as the state in which an individual is unable to adapt to increasing or persistent stress which results in maladaptive behaviors. Examples of maladaptive behaviors include inappropriate social interactions, self-mutilation, and repetitive stereotypic movements. When initially presented with a stressor, an animal will either adapt to the stress or will develop maladaptive behaviors that will lead to distress. Pain, by comparison, is considered to be a stressor and therefore one of the causes of distress. Some of the common indicators of pain and distress in rodent species have been identified as the following: abnormal vocalizations, self-mutilation, changes in normal group behavior or grooming, depression as indicated by a reluctance to move and decreased activity, abnormal appearance including hunched positioning or ruffled hair coats, or increased respiratory rate and associated grunting or chattering on expiration (Institute of Laboratory

Animal Resources (U.S.). Committee on Pain and Distress in Laboratory Animals.). Committee on Pain and Distress in Laboratory Animals., 1992 #70).

This study was designed to determine if the use of an analgesic would aid in the relief of distress without having an effect on autoimmune disease progression in the MRL/lpr model. Concerning the parameters measured, the distinguishing factor was whether or not the parameter could be altered by treatment with analgesics. For example, any parameter that was suspected to be unaffected by administration of analgesics, but would be changed by progression of disease was used to indicate autoimmune disease progression. These objective measures were presence or absence of proteinuria, auto-antibody production, renal involvement, splenomegaly, splenocyte profiles, and the development of lymphadenopathy. Our hypothesis was that these parameters would not be affected between the buprenorphine treated groups and the non-buprenorphine treated groups. If this hypothesis was correct, then buprenorphine could be used as an analgesic without interfering with common parameters used to indicate autoimmune disease progression.

In this study, there was no evidence to suggest that buprenorphine negatively impacted the progression of autoimmune disease in MRL/lpr mice. In all of the parameters used to indicate disease progression, there was no significant difference between the buprenorphine and control groups, or between the cyclophosphamide and cyclophosphamide + buprenorphine groups. This indicates that the administration of buprenorphine does not interfere with the progression of autoimmune disease in MRL/lpr mice.

Parameters used as indicators of distress in this study included body condition scores, changes in mentation, severity of skin lesions, abnormal respiratory rate, presence of ruffled hair coats indicating a lack of grooming, huddling behaviors, and pruritus. Two of these parameters, skin lesions and pruritus, have been used as measures of autoimmune disease progression in other studies. However, analgesic administration could interfere with the measurement of these two parameters. For example, depending on the underlying cause of pruritus, scratching behavior can be either a cause of pain or a response to pain. If the pruritus seen in MRL/lpr mice is associated with pain that occurs secondary to self-mutilation, then the administration of analgesics could decrease the incidence of pruritus. However, if the pruritus is associated with disease progression and acts only as a cause of pain, analgesic administration would be expected to have no effect on the incidence of pruritus. The severity of the skin lesions seen can be considered in a similar manner. If the lesions develop as a response to pain, analgesic administration would be expected to decrease the incidence of skin lesions whereas if the lesions develop solely due to disease progression, then analgesics would be expected to have no effect. Since the measurement of these two parameters could be influenced by analgesic administration, they were not appropriate indicators of disease progression in this particular study. Therefore, these two parameters were used as indicators of pain and distress rather than as indicators of disease progression.

Based on measurements of the parameters used to indicate distress, this study demonstrated that buprenorphine, when dosed at the previously published dose of 0.09 mg/kg per mouse per day in the drinking water (Lindsay et al., 2005), was not adequate as the sole method of alleviating pain and distress in MRL/lpr mice that have concurrent evidence of autoimmune disease. This was indicated by the lack of decrease in distress scores between the groups treated with buprenorphine and the groups not treated with analgesics.

There are several likely explanations for this finding. One potential consideration is the dose of buprenorphine and the route of administration. Although the dose of buprenorphine used in this study has been cited as being effective (Lindsay et al., 2005), there has been very few pharmacologic trials to determine the most appropriate dose of buprenorphine for analgesia in

mice. Buprenorphine is typically used to treat mild, moderate, and severe pain by varying the dose given (Christoph et al., 2005). Dose ranges for buprenorphine vary from 0.002 mg/kg to 0.5 mg/kg (Christoph et al., 2005; Gades et al., 2000; Lizasoain et al., 1991; Mizoguchi et al., 2003; van Loo et al., 1997). The wide range of doses indicates the lack of controlled studies involving buprenorphine in mice. However, the dose used in this study (0.09 mg/kg/day) falls within the expected therapeutic dose range (Banerjee and Sarkar, 1997; Christoph et al., 2005; Gades et al., 2000; Gross et al., 2003; Lindsay et al., 2005; van Loo et al., 1997).

It is also possible that the administration of buprenorphine in the drinking water may affect the absorption or efficacy of the drug. However, buprenorphine has been demonstrated to be effectively absorbed via administration in the drinking water and provided appropriate analgesic efficacy in rats (Cooper et al., 1997). Due to the close phylogenetic relationship between mice and rats, it is likely that absorption and efficacy of buprenorphine administration in the drinking water of mice would be similar to that seen in rats. Furthermore, Lindsay and colleagues have shown that buprenorphine administered via the drinking water, at the same dose as was used in this study, decreased pain and distress in an experimental murine model of *Toxoplasma gondii* (Lindsay et al., 2005). Because of the lack of pharmacological studies to determine the most appropriate therapeutic range of buprenorphine in mice when administered via drinking water, it is difficult to say whether buprenorphine at a higher dose would provide adequate analgesia to decrease pain and distress in MLR/lpr mice.

A second potential explanation for our findings involves the underlying cause of distress in autoimmune skin diseases. Recently published findings have indicated that spontaneous scratching behavior in MLR/lpr mice may be a response to autoimmune pruritus rather than as a response to pain or distress (Umeuchi et al., 2005). This would suggest that controlling the pain in these animals would not decrease the underlying cause of the scratching, the autoimmune pruritus. In a normal animal, the natural negative feedback mechanism for excessive scratching behavior includes initiating a painful response associated with the self trauma. This painful response inhibits further scratching. Therefore, it could be hypothesized that analgesic administration would decrease the pain sensation caused by self trauma and potentially disrupt the normal negative feedback loop. As a result, interruption of this feedback loop could cause worsening self-trauma in animals treated with analgesics if the underlying cause of the pruritus was not controlled.

Another explanation for the lack of desired effect of buprenorphine could be attributed to its pharmacological properties. Currently, there are four opioid receptors that are thought to be clinically important. Exogenous opioids have variable affinities for these four receptors, resulting in the differing effects of opioid drugs. These four receptors include the three classical opioid receptors (μ , κ , and δ) which are primarily involved in the analgesic response to opioids, and the opioid-receptor-like receptor ORL-1 which is thought to oppose the analgesic effect seen with stimulation of the classical opioid receptors. Exogenous opioids are either agonistic, partial agonistic, or antagonistic at these receptors. Buprenorphine is considered to be an agonist-antagonist. It is a partial agonist at the μ and ORL-1 receptors and an antagonist at the κ and δ receptors. The desired analgesic effects of buprenorphine are associated with its actions as a μ agonist, while its relative lack of negative side effects when compared to other opioids is thought to be due to its antagonistic properties at the κ and δ receptors as well as its agonistic properties at the ORL-1 receptors which moderate the analgesic effect. (Christoph et al., 2005) In addition to their role in analgesia, central μ receptors have been found to play a role in the processing of the itch sensation when using a pruritogen-induced scratching model in mice, while κ receptors are thought to suppress this itch sensation. (Umeuchi et al., 2003). Therefore, while the actions at the μ receptors may be causing sufficient analgesia, stimulation of these receptors may also be assisting in the processing of the itch sensation. As a κ antagonist, buprenorphine would be unlikely to contribute to suppression of this itch sensation as seen

with κ agonists. An example of a κ agonist that has recently been shown to suppress the itch sensation is nalfurafine (Umeuchi et al., 2005). Taken together, the contribution of buprenorphine to the itch cycle could overwhelm the benefits of its analgesic properties.

In conclusion, buprenorphine should still be considered as a potential analgesic of choice for reducing pain and distress in mice. It can also be considered as a compound that does not impact the progression of autoimmune disease in MRL/lpr strains when given in the drinking water at 0.09 mg/kg/mouse/day, based on the criteria measured in this study. However, its potential use to moderate scratching behavior and/or serve as an analgesic for MLR/lpr mice has yet to be substantiated. Further studies are needed to determine if buprenorphine, and opioids in general, can be used effectively to prevent pain and distress associated with the progression of disease in mice suffering lupus-like syndromes.

Acknowledgements

The authors would like to thank the staff of the Teaching and Research Care and Support Service at Virginia Tech for their assistance with this project as well as The Arthritis Foundation for their gracious support of Dr. Christopher Reilly's research.

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Fig. 1.

Characterization of skin lesions was based on a numerical scoring system from 0 to 3 (no lesions to severe lesions, respectively). The animal shown here (A) was scored as a 2 for moderate lesions (defined as scabbing over <5% of the skin surface). (B) Tabulation of skin lesion scores. No significant differences were seen between the four treatment groups based on skin lesions ($P < 0.05$). Each group in Fig. 1B represents the average of at least 8 animals.

Fig. 2.

Tabulation of scores for other common indicators of distress scores and lymphadenopathy. (A) Other common indicators of stress included abnormal respiratory rate and effort, presence of a ruffled haircoat, presence of huddling behavior, and noticeable pruritus. Each of these parameters was scored on the numerical scoring system listed below. Respiratory rate and effort: 0 = normal respiratory rate and effort, 1 = increased respiratory rate, but normal effort, 2 = increased respiratory rate and effort, 3 = increased respiratory rate and effort with an abdominal component to the breathing. Ruffled haircoat, huddling behavior, and pruritus: 0 = absence of the indicator, and 1 = presence of the indicator. These scores were combined together to form a score titled "other indicators of stress". There were no significant differences noted between the four treatment groups based on other indicators of distress ($P < 0.05$). (B) Lymphadenopathy was scored on a numerical scoring system from 0 to 3 (none to severe enlargement, respectively). There were no significant differences noted between the four treatment groups based on the development of lymphadenopathy ($P < 0.05$). Each group in Fig. 2A and 2B represents the average of at least 8 animals.

A **B**

Fig. 3. Tabulation of scores for mentation and combined distress scores. (A) Mentation scores were significantly higher in the buprenorphine and the control groups when compared to the cyclophosphamide and the cyclophosphamide + buprenorphine groups ($P < 0.05$). (B) When the scores were tabulated together into the combined distress scores, the cyclophosphamide group was significantly lower than the other three treatment groups ($P < 0.05$). In Fig. 3A and 3B, each group represents the average of at least 8 animals.

Fig. 4.

(A) Measurements of proteinuria in MRL/lpr mice. Female mice were treated with buprenorphine, cyclophosphamide, cyclophosphamide + buprenorphine or untreated controls for 10 weeks beginning at 11 weeks-of age. Mice were analyzed for proteinuria production by dipstick measurements weekly. (B) Measurement of anti-dsDNA production at 11, 15 and 19 weeks of age. Sera were serially diluted 1:2 beginning at 1:000 dilutions. Optical density (OD) readings are the average from sera from each mouse performed in triplicate. Each graph represents the average of at least 8 animals (* $p < 0.05$).

Fig. 5.

Renal pathology of MRL/lpr mice at 21 weeks of age. Representative picture of a kidney from mouse treated with (A) cyclophosphamide, (B) cyclophosphamide + buprenorphine C) buprenorphine or (D) untreated controls. (E) Graphical tabulation of renal score pathology; the cyclophosphamide and cyclophosphamide + buprenorphine mice had significantly less renal pathology than the buprenorphine or control animals ($p < 0.05$).

Fig. 6.

Splenic weight measurements in MRL/lpr mice at 21 weeks of age. Female mice were treated with buprenorphine, cyclophosphamide, cyclophosphamide + buprenorphine for 10 weeks beginning at 11 weeks of age. Controls received no treatment. (A) Graphical representation of spleen weights to body weight ratio. (B) Spleen weight in grams and (C) body weight measurements in grams. The cyclophosphamide and cyclophosphamide + buprenorphine mice had significantly smaller spleens and spleen to body weight ratios than the buprenorphine or control animals ($p < 0.05$).

Fig. 7.

Flow cytometric analysis of splenic T and B cell activation markers in cyclophosphamide, cyclophosphamide + buprenorphine, buprenorphine only treated, or untreated control MRL/lpr mice. (A) Splenic CD4+ T cells were gated for CD25 and CD62L analysis; (B) Splenic cells were analyzed for different B cell surface markers. Data represents the mean $\pm$ standard deviation of at least five mice at 21 week of age.