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Effect of doxycycline and meloxicam on cytokines, brain-derived neurotrophic factor, matrix metalloproteinase-3, tissue inhibitor of metalloproteinase-3 and cyclooxygenase-2 in brain

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ABSTRACT

Objective(s): Prevention of inflammation in early stages will be useful in maintaining vitality of the organism. The objective of this study was to evaluate the effects of doxycycline (DOX) or meloxicam (MLX) monotherapy and combination therapy on the levels of inflammatory mediators in the brain tissues of rats with *Escherichia coli* lipopolysaccharide (LPS)-induced brain inflammation.

Materials and Methods: Seventy-eight rats were divided into the following groups: control (n=6), LPS (0.5 µg/10 µl intracranial) (n=18), LPS (0.5 µg/10 µl intracranial)+DOX (40 mg/kg intraperitoneal) (n=18), LPS (0.5 µg/10 µl intracranial)+MLX (2 mg/kg intraperitoneal) (n=18) and LPS (0.5 µg/10 µl intracranial)+DOX (40 mg/kg intraperitoneal)+MLX (2 mg/kg intraperitoneal) (n=18) groups. Brain tissues were harvested from all rats in the control group and from six rats each in the four experimental groups at 1, 3 and 6 hr under anaesthesia. The levels of tumor necrosis factor α (TNFα), interleukin 4 (IL-4), IL-6, IL-10, IL-17, brain-derived neurotrophic factor (BDNF), matrix metalloproteinase 3 (MMP-3), tissue inhibitor of metalloproteinase 3 (TIMP-3) and cyclooxygenase 2 (COX-2) in the brain tissues were measured using ELISA kits with ELISA device.

Results: LPS administration increased proinflammatory cytokines (TNF, IL-6, IL-17), and MMP-3 levels and decreased anti-inflammatory cytokines (IL-10, IL-4), and BDNF levels. The lowest TNFα levels were detected in the LPS+MLX group ($P<0.05$). All the drug treatment groups showed decreased IL-17 and COX-2 levels compared to the LPS groups.

Conclusion: DOX or MLX monotherapy exerts neuroprotective effects against brain inflammation by decreasing proinflammatory cytokine levels and by increasing anti-inflammatory cytokines levels.

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Introduction

Neuroinflammation is an important factor in the pathogenesis and progression of neurodegenerative diseases. Patients' brains with neurodegenerative diseases showed microglia activation, marked astrocytosis and increased proinflammatory cytokine levels (1).

Lipopolysaccharide (LPS), an immunogenic component of gram-negative bacteria, is widely used to develop experimental models of neuroinflammation (1). LPS administration affects the levels of various inflammatory markers, including inflammatory cytokines, brain-derived neurotropic factor (BDNF), matrix metalloproteinase (MMP), tissue inhibitor of metalloproteinases (TIMP) and cyclooxygenase (COX) (2-4). Cytokines play important roles in inflammation and are divided into two groups as proinflammatory [Tumor necrosis factor (TNF) α, interleukin (IL)-6, IL-17, etc.] and anti-inflammatory (IL-4, IL-10, etc.) cytokines (5, 6). Increased TNF-α, IL-10 and IL-6 levels have been detected in the brain and cerebrospinal fluid of patients with acute infections of nervous system or in LPS- or *Streptococcus pneumonia*-infected models (7-10). COX-2 plays a central role in inflammation (11) and is overexpressed in neurodegenerative disorders such as Parkinson's disease (12). Moreover, LPS administration increases COX activity (13). BDNF, a member of neurotrophin family, promotes the viability and differentiation of neurons (14), and BDNF level decreases during inflammation and in LPS-administrated rats (15). MMPs are proteolytic enzymes secreted in inactive form. These inactivate enzymes are activated during neuroinflammation by the action of free radicals, cytokines and other enzymes and are rapidly inactivated by TIMPs (16).

Tetracycline antibiotics exert bacteriostatic effect by inhibiting bacterial protein synthesis (17); moreover, they exert anti-inflammatory and neuroprotective effects (18). Doxycycline (DOX), a semi-synthetic tetracycline antibiotic, was administered intravenously and showed high lipophilicity and long half-life (18). Besides, DOX increased anti-inflammatory cytokine levels and decreased proinflammatory cytokine levels in an *in vitro* study (19). Meloxicam (MLX), a nonsteroidal anti-inflammatory drug, is often used for treating infections because of its potent analgesic, antipyretic and anti-inflammatory effects (20). Because MLX exerts a more portent effect on COX-2 than on COX-1, it is more effective for treating infections (21). Moreover, MLX exerts neuroprotective effects because of its antioxidant and anti-cytokine activity (21, 22).

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Anti-inflammatory effects of MLX and DOX were associated with reduction of the proinflammatory cytokines and inhibition of the MMP in the brain and the experimental autoimmune neuritis (21, 23). DOX and MLX are two drugs that do not interact with each other and are used together (24). Because both MLX and DOX exert anti-inflammatory effects in nervous system, it was hypothesised that these drugs strongly inhibit the inflammatory mediators of brain inflammation. Therefore, the present study determined the effects of DOX (40 mg/kg intraperitoneal) (23) or MLX (2 mg/kg intraperitoneal) (25) monotherapy and combination therapy on the levels of TNF-α, IL-4, IL-6, IL-10, IL-17, BDNF, MMP-3, TIMP-3 and COX-2 in the brain tissues of rats with brain inflammation induced by intracranial LPS administration.

Materials and Methods

Animal and study design

The study procedure was approved by the Ethics Committee of Experimental Medical Practice and Research Center of Animal Experiments of Selcuk University, Konya, Turkey (2016-38). This study included 78 male Wistar rats that were divided into the following groups: control (n=6), LPS (0.5 µg/10 µl intracranial) (n=18), LPS (0.5 µg/10 µl intracranial)+DOX (40 mg/kg IP, Doksimis 100 ml, Mistav) (n=18), LPS (0.5 µg/10 µl intracranial)+MLX (2 mg/kg IP, Maxicam X4, Sanovel) (n=18) and LPS (0.5 µg/10 µl intracranial) + DOX (40 mg/kg IP)+MLX (2 mg/kg IP) (n=18) groups. LPS, DOX and MLX were diluted by physiological saline solution. Brain tissues were harvested from all the six rats in the control group and from six rats each in the four experimental groups at 1, 3 and 6 hr. Acute phase response against acute infections develops especially within 6 hr in the body. Hence, we chose first 6 hr for determination of cytokine networks and other inflammatory mediators (26, 27). The rats were anaesthetised (70 mg/kg, thiopental, IP) and placed in a stereotaxic apparatus for intracranial LPS administration according to coordinates (0.8-mm posterior to the bregma and 1.5-mm lateral to the sagittal suture, 2.0 mm ventral to the skull surface) by using a Hamilton microsyringe. Next, the rats were intraperitoneally injected with the indicated drugs. The brain tissues were harvested by euthanising the anaesthetised rats through cervical dislocation and were stored at -80 ˚C until further analyses.

ELISA measurement

The levels of TNFα, (Rat TNF-α ELISA kit, Catalog No: E-EL-R0019, Elabscience Biotechnology Co. Ltd., USA), IL-4 (Rat IL-4 ELISA kit, Catalog No: E-EL-R0014, Elabscience Biotechnology Co. Ltd., USA), IL-6 (Rat IL-6 ELISA kit, Catalog No: E-EL-R0015, Elabscience Biotechnology Co. Ltd., USA), IL-10 (Rat IL-10 ELISA kit, Catalog No: E-EL-R0016, Elabscience Biotechnology Co. Ltd., USA), IL-17 (Rat Il-17 ELISA kit, Catalog No: E-EL-R0566, Elabscience Biotechnology Co. Ltd., USA), BDNF (Rat BDNF ELISA kit, Catalog No: E-EL-R1235, Elabscience Biotechnology Co. Ltd., USA), COX-2 (Rat COX-2 ELISA kit, Catalog No: E-EL-R0792, Elabscience Biotechnology Co. Ltd., USA), MMP-3 (Rat MMP-3 ELISA kit, Catalog No: E-EL-R0619, Elabscience Biotechnology Co. Ltd., USA) and TIMP-3 (Rat TIMP-3 ELISA kit, Catalog No: E-EL-R0986, Elabscience Biotechnology Co. Ltd., USA) in homogenised brain tissue samples (0.5 g) were determined using commercial ELISA kits and an ELISA reader (MWGt Lambda Scan 200, USA) following manufacturer's instructions.

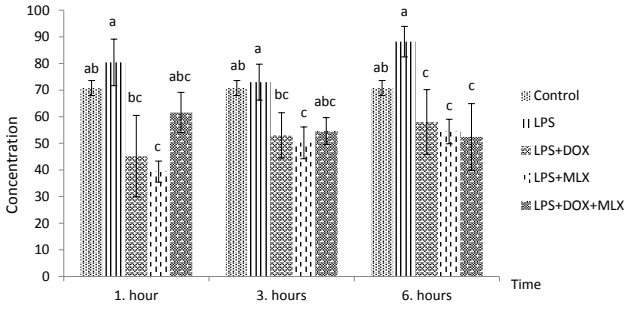

Figure 1. Effect of doxycycline and meloxicam administrations on brain tissue level of TNF-α (pg/ml/0.5 g tissue) in intracranial LPS-induced brain inflammation (mean±SE)
[a, b, c] The different letters are statistically significant (P<0.05)
LPS: Lipopolysaccharide, DOX: Doxycycline, MLX: Meloxicam, TNFα: Tumor necrosis factor α

Statistical analysis

Data are presented mean±SE and were evaluated using ANOVA and Duncan's *post-hoc* test. All statistical analyses were performed using SPSS version 22.0, and P<0.05 was considered to be statistically significant.

Results

The effects of DOX or MLX monotherapy and combination therapy on the levels of TNFα, IL-6, IL-17, IL-10, IL-4, COX-2, BDNF, MMP-3 and TIMP-3 in the brain tissues of the rats with LPS-induced brain inflammation are shown in Figure 1-9, respectively.

DOX and MLX effect on TNF-α level in rat brain

Increased TNF-α levels were determined in LPS group, but it was not statistically significant from control group in all the sampling times. However, TNF-α levels in the LPS+DOX and LPS+MLX groups were determined lower compared to the LPS group at all the sampling

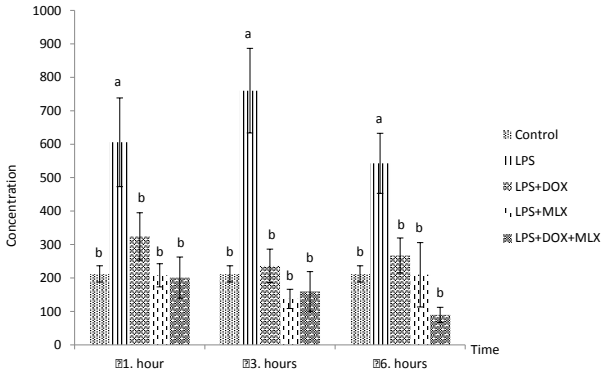

Figure 2. Effect of doxycycline and meloxicam administrations on brain tissue level of IL-6 (pg/ml/0.5 g tissue) in intracranial LPS-induced brain inflammation (mean±SE)
[a, b] The different letters are statistically significant (P<0.05)
LPS: Lipopolysaccharide, DOX: Doxycycline, MLX: Meloxicam, IL-6: Interleukin 6

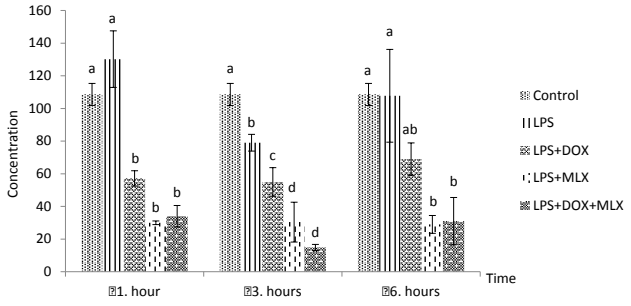

Figure 3. Effect of doxycycline and meloxicam administrations on brain tissue level of IL-17 (pg/m/0.5 g tissue) in intracranial LPS-induced brain inflammation (mean±SE)
[a, b, c, d] The different letters are statistically significant (P<0.05)
LPS: Lipopolysaccharide, DOX: Doxycycline, MLX: Meloxicam, IL-17: Interleukin 17

times (P<0.05) but only decreased at 6 hr in the rats of the LPS+DOX+MLX group (P<0.05) (Figure 1).

DOX and MLX effect on IL-6 level in rat brain

LPS administration significantly increased IL-6 levels at all the sampling times compared to the control group (P<0.05). This LPS-induced increase in IL-6 levels was inhibited in LPS+DOX, LPS+MLX and LPS+DOX+MLX groups at all the sampling times (P<0.05, Figure 2).

DOX and MLX effect on IL-17 level in rat brain

LPS increased IL-17 level, but it was not statistically significant from control group at 1 hr. IL-17 level decreased in the LPS+DOX, LPS+MLX and LPS+DOX+MLX groups (P<0.05) compared to the LPS group (Figure 3).

DOX and MLX effect on IL-10 level in rat brain

LPS administration resulted in a decrease in IL-10 levels for the first 3 hr, especially at 1 hr. Statistically significant changes were determined in the IL-10 levels in LPS+DOX, LPS+MLX and LPS+DOX+MLX compared to the LPS group at all the sampling times (Figure 4).

DOX and MLX effect on IL-4 level in rat brain

IL-4 is an anti-inflammatory cytokine, and LPS administration decreased its level. Drug treatments could not prevent this reduction at all the sampling

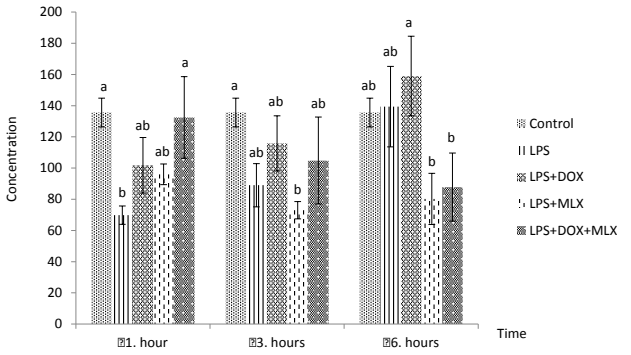

Figure 4. Effect of doxycycline and meloxicam administrations on brain tissue level of IL-10 (pg/ml/0.5 g tissue) in intracranial LPS-induced brain inflammation (mean±SE)
[a, b] The different letters are statistically significant (P<0.05)
LPS: Lipopolysaccharide, DOX: Doxycycline, MLX: Meloxicam, IL-10: Interleukin 10

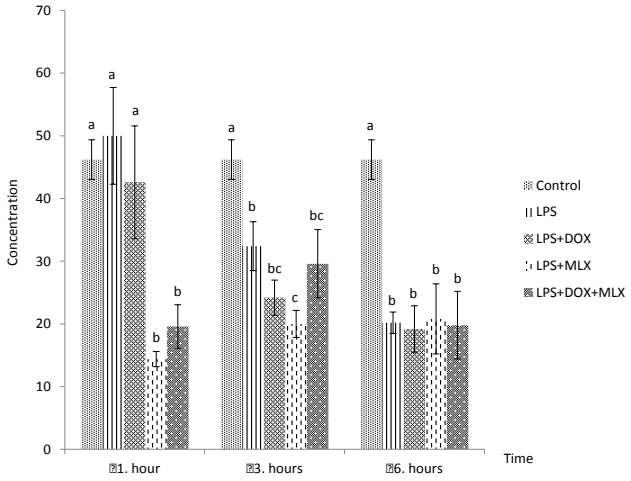

Figure 5. Effect of doxycycline and meloxicam administrations on brain tissue level of IL-4 (pg/ml/0.5 g tissue) in intracranial LPS-induced brain inflammation (mean±SE)
[a, b, c] The different letters are statistically significant (P<0.05)
LPS: Lipopolysaccharide, DOX: Doxycycline, MLX: Meloxicam, IL-4: Interleukin 4

times (Figure 5).

DOX and MLX effect on COX-2 level in rat brain

LPS increased COX-2 level at 1 hr, and the levels of COX-2 decreased at 3 and 6 hr, but these were not statistically significant from control group. COX-2 level decreased in the LPS+DOX, LPS+MLX and LPS+DOX+MLX groups (P<0.05) compared to the LPS group (Figure 6).

DOX and MLX effect on BDNF level in rat brain

LPS increased BDNF level at 1 hr and the levels of BDNF decreased at 3 and 6 hr, but these were not statistically significant from control group. The rats in the LPS+DOX group showed increased BDNF levels at 6 hr (P<0.05) compared to the rats in all the other groups. Moreover, BDNF levels increased in the LPS+DOX and

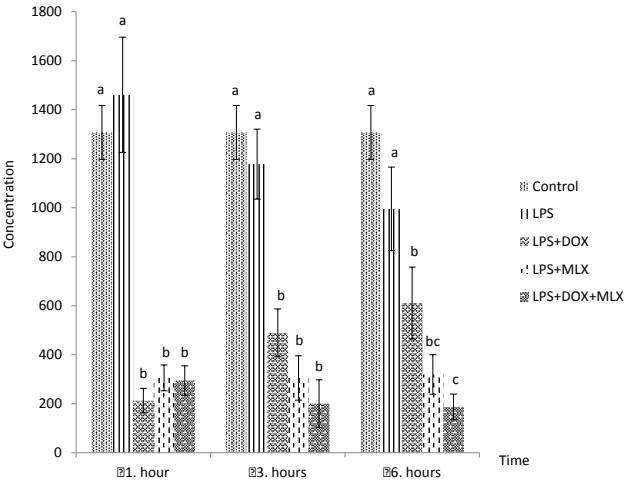

Figure 6. Effect of doxycycline and meloxicam administrations on brain tissue level of COX-2 (pg/ml/0.5 g tissue) in intracranial LPS-induced brain inflammation (mean±SE)
[a, b, c] The different letters are statistically significant (P<0.05)
LPS: Lipopolysaccharide, DOX: Doxycycline, MLX: Meloxicam, COX-2: Cyclooxygenase-2

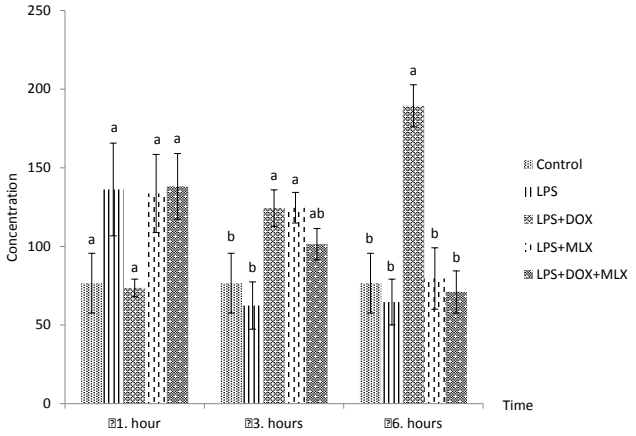

Figure 7. Effect of doxycycline and meloxicam administrations on brain tissue level of BDNF (pg/ml/0.5 g tissue) in intracranial LPS-induced brain inflammation (mean±SE)
[a, b] The different letters are statistically significant (P<0.05)
LPS: Lipopolysaccharide, DOX: Doxycycline, MLX: Meloxicam, BDNF: Brain-derived neurotrophic factor

LPS+MLX groups at 3 hr compared to the LPS group (P<0.05, Figure 7).

DOX and MLX effect on MMP-3 level in rat brain

MMP-3 levels increased in the LPS group after 6 hr (P<0.05) and decreased in the LPS+MLX group after 3 hr (P<0.05, Figure 8).

DOX and MLX effect on TIMP-3 level in rat brain

TIMP-3 levels increased in the LPS group after 3 hr (P<0.05) compared to control group. LPS+DOX and LPS+MLX caused decreased of TIMP-3 levels compared to LPS group after 3 hr (P<0.05) and LPS+DOX increased its level after 6 hr (P<0.05) (Figure 9).

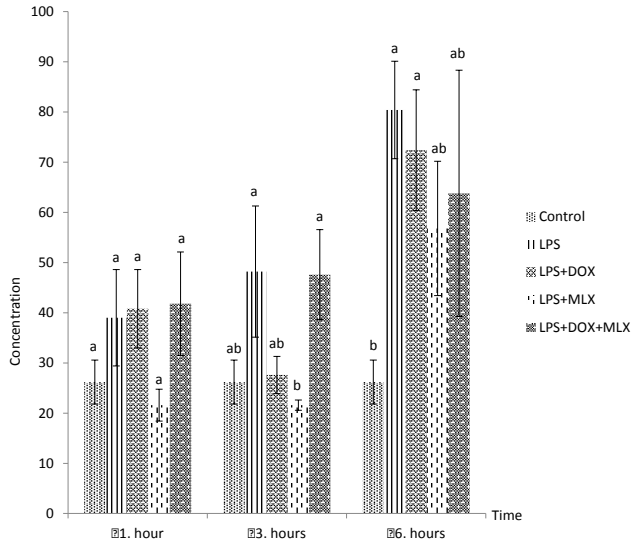

Figure 8. Effect of doxycycline and meloxicam administrations on brain tissue level of MMP-3 (ng/ml/0.5 g tissue) in intracranial LPS-induced brain inflammation (mean±SE)
[a, b] The different letters are statistically significant (P<0.05)
LPS: Lipopolysaccharide, DOX: Doxycycline, MLX: Meloxicam, MMP-3: Matrix metalloproteinase 3

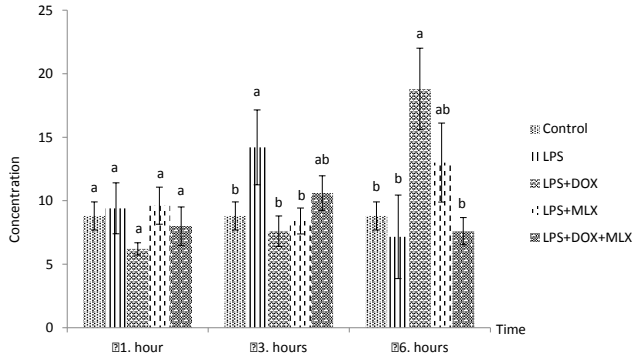

Figure 9. Effect of doxycycline and meloxicam administrations on brain tissue level of TIMP-3 (ng/ml/0.5 g tissue) in intracranial LPS-induced brain inflammation (mean±SE)
[a, b] The different letters are statistically significant (P<0.05)
LPS: Lipopolysaccharide, DOX: Doxycycline, MLX: Meloxicam, TIMP-3: Tissue inhibitor of metalloproteinase 3

Discussion

LPS, a component of the cell wall of gram-negative bacteria, is a potent inducer of inflammation and exerts different effects on immune system cells (28). DOX and MLX are used in the human and veterinary medicine as antibacterial and anti-inflammatory drugs, respectively (20, 29). Although both the drugs have potent neuroprotective properties (18, 22), the neuroprotective effects of the combination of these drugs have not been investigated.

We use a positive control group in our studies with one sampling time. However, since there is not a single sampling time in this study (three sampling time), a positive control group should be formed at each sampling time. As a result, 18 animals would be used for 3 sampling times. For a total of 96 animals, the cost would double for each parameter. There is not enough budget in our projects, we could not add positive group. For this reason, as in our previous studies, due to the sterile study, the negative effects that may be related to the application were eliminated in this study. Hence, we used only one control group. In the present study, intracranial LPS administration increased the levels of TNF-α, IL-6 and IL-17, which are the main proinflammatory cytokines. In experimental studies, LPS is frequently used to develop models of brain inflammation (30). Intracranial LPS administration induces the secretion of inflammatory mediators from glial cells (28). Therefore, several studies have investigated the inflammatory mediators that play important roles in brain inflammation (30). Intracerebroventricular LPS administration increases IL-6 and TNFα levels in the brain tissue or cerebrospinal fluid (3, 31). Moreover, high IL-17 levels have been detected in the central nervous system during inflammation (5). Inhibition of proinflammatory cytokines during inflammation may help in alleviating pathological conditions (5). In the present study, DOX or MLX single drug administration (monotherapy) and combination therapy inhibited the LPS-induced increase in the levels of proinflammatory cytokines (TNFα, IL-6 and IL-17). Previous studies have also reported that DOX (23, 32) and MLX (21) decrease the levels of proinflammatory cytokines (TNFα, IL-6 and IL-17). In

the present study, statistically significant changes were observed in levels of anti-inflammatory cytokines IL-10 and IL-4 (P<0.05). Generally, all the drug treatments increased IL-10 levels during the experimental period; however, a statistically significant increase in IL-10 levels was only observed in the rats of the LPS+DOX+MLX group after 1 hr. However, LPS decreased IL-4 level after 3 and 6 hr (P<0.05). The treatment groups were similar to the LPS group, except the LPS+MLX group. IL-4, an anti-inflammatory cytokine, in the LPS+MLX group decreased further after 3 hr. Similarly, some studies have reported unchanged cerebral IL-10 and IL-4 levels in patients with cerebral infections (33) and decreased cortical IL-10 levels in subjects treated with LPS (3). Some studies have reported increased cortical IL-10 levels after minocycline administration (3) and unchanged *Chlamydia trachomatis*-induced IL-10 expression after DOX administration (32). The anti-inflammatory effects of DOX and MLX may be associated with their depressor effects on nuclear factor-kappa B (34, 35). Nuclear factor-kappa B, a transcriptional factor, plays the main role in the activation of inflammatory mediators, including cytokines and chemokines, in the cell nucleus (36).

In the present study, COX-2 levels were lower in the rats in the LPS+DOX, LPS+MLX and LPS+DOX+MLX groups than in the LPS group, whereas the levels of COX-2 in the control and LPS groups were similar (P<0.05). On the contrary to our result, increased COX-2 expression in the brain has been reported after LPS administrations (37, 38). This discrepancy in results may be associated with differences (such as animal kind, doses of drugs and/or sampling time) in the experimental design of the present and the previous studies. Decreased COX-2 levels have been reported after MLX (39, 40) and DOX (41) treatments. Because activated nuclear factor-kappa B stimulates COX-2 expression (42), the depressor effects of DOX and MLX on COX-2 synthesis may be explained by their inhibitory effect on nuclear factor-kappa B.

The LPS+DOX group showed significantly increased BDNF levels after 6 hr compared to the other groups (P<0.05). However, BDNF levels in the rats of the LPS+DOX and LPS+MLX groups were higher than the LPS group after 3 hr (P<0.05). Studies have reported decreased BDNF mRNA expression after intracerebral LPS administration (43) and increased BDNF levels after DOX and minocycline treatments (3, 44). In addition, MLX increases BDNF protein expression (31). In contrast, one study reported that DOX and minocycline treatments had no effect on decreased BDNF levels (45). Therefore, it can be suggested that DOX and MLX exert irregular effects on BDNF levels based on study conditions.

Although MMP-3 levels were similar in all the groups after 1 hr following the treatments, these levels increased in the LPS group after 6 hr. Moreover, MMP-3 levels in the brains of the treatment groups were similar to those in the LPS group but decreased in the rats of the LPS+MLX group after 3 hr. TIMP-3 levels increased in the rats of the LPS and LPS+DOX groups after 3 and 6 hr, respectively. MMPs play a role in neuronal physiology, cell viability and inflammation (46). In the central nervous system, the levels of most MMPs are negligible or low (46). However, some kinds of MMPs increase in glioma, viral infection, neuroinflammation, multiple sclerosis, Alzheimer's disease, brain trauma and HIV-related neurological diseases (46). Active MMPs are rapidly inactivated by TIMPs (16), and MMP inhibition may prevent the progression of neuroinflammatory diseases (47). In addition, cytokines (TNF-α and IL-6) released from glial cells in infected areas increase MMP-3 and TIMP-3 expression (46, 48). The effect of DOX on MMP levels is controversial. Although DOX suppresses MMP-3 overexpression (49) and exerts a neuroprotective effect by decreasing MMP-3 levels in LPS-exposed microglial BV-2 cells (50), Lazzarini *et al.* (51) reported that DOX did not affect MMP-3 expression. The unimpressive effects of DOX and MLX on MMP-3 and TIMP-3 levels may be related to the doses of these drugs in the present study.

Conclusion

Our results suggest that DOX and MLX exert neuroprotective effects by decreasing proinflammatory cytokine (TNF-α, IL-6 and IL-17) levels and COX-2 synthesis in brain inflammation. In addition, the DOX and MLX combination therapy does not exert higher beneficial effects than their monotherapy.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

1. Catorce MN, Gevorkian G. LPS-induced murine neuroinflammation model: main features and suitability for pre-clinical assessment of nutraceuticals. Curr Neuropharmacol 2016; 14: 155-164.
2. Yazar E, Bulbul A, Avci GE, Er A, Uney K, Elmas M, *et al.* Effects of enrofloxacin, flunixin meglumine and dexamethasone on disseminated intravascular coagulation, cytokine levels and adenosine deaminase activity in endotoxaemia in rats. Acta Vet Hung 2010; 58: 357-367.
3. Zheng X, Liang Y, Kang A, Ma SJ, Xing L, Zhou YY, *et al.* Peripheral immunomodulation with ginsenoside Rg1 ameliorates neuroinflammation-induced behavioral deficits in rats. Neuroscience 2014; 256: 210-222.
4. Lee EJ, Ko HM, Jeong YH, Park EM, Kim HS. β-Lapachone suppresses neuroinflammation by modulating the expression of cytokines and matrix metalloproteinases in activated microglia. J Neuroinflammation 2015; 12: 133.
5. Waisman A, Hauptmann J, Regen T. The role of IL-17 in CNS diseases. Acta Neuropathol 2015; 129: 625-637.
6. Minciullo PL, Catalano A, Mandraffino G, Casciaro M, Crucitti A, Maltese G, *et al.* Inflammaging and anti-inflammaging: The role of cytokines in extreme longevity. Arch Immunol Ther Exp (Warsz) 2016; 64: 111-126.
7. Romero LI, Schettini G, Lechan RM, Dinarello CA, Reichlin S. Bacterial lipopolysaccharide induction of IL-6 in rat telencephalic cells is mediated in part by IL-1. Neuroendocrinology 1993; 57: 892-897.
8. Lehmann AK, Halstensen A, Sørnes S, Røkke O, Waage A. High

levels of interleukin 10 in serum are associated with fatality in meningococcal disease. Infect Immun 1995; 63: 2109-2112.
9. Granert C, Raud J, Waage A, Lindquist L. Effects of polysaccharide fucoidin on cerebrospinal fluid interleukin-1and tumor necrosis factor alpha in pneumococcal meningitis in the rabbit. Infect Immun 1999; 67: 2071-2074.
10. Offner H, Subramanian S, Parker SM, Afentoulis ME, Vandenbark AA, Hurn PD. Experimental stroke induces massive, rapid activation of the peripheral immune system. J Cereb Blood Flow Metab 2006; 26: 654-665.
11. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, *et al.* Cyclooxygenase in biology and disease. FASEB J. 1998; 12: 1063-1073.
12. Laube M, Kniess T, Pietzsch J. Development of anti-oxidant COX-2 ınhibitors as radioprotective agents for radiation therapy-A hypothesis-driven review. Antioxidants (Basel) 2016 ;5:14.
13. Kalmar B, Kittel A, Lemmens R, Környei Z, Madarasz E. Cultured astrocytes react to LPS with increased cyclooxygenase activityand phagocytosis. Neurochem Int 2001; 38: 453-461.
14. Hempstead BL. Brain-derived neurotrophic factor: Three ligands, many actions. Trans Am Clin Climatol Assoc 2015; 126: 9-19.
15. Hachisu M, Konishi K, Hosoi M, Tani M, Tomioka H, Inamoto A, *et al.* Beyond the hypothesis of serum anticholinergic activity in Alzheimer's disease: Acetylcholine neuronal activity modulates brain-derived neurotrophic factor production and inflammation in the brain. Neurodegener Dis 2015; 15: 182-187.
16. Yang Y, Rosenberg GA. Matrix metalloproteinases as therapeutic targets for stroke. Brain Res. 2015; 1623: 30-38.
17. Yazar E. Chemotherapeutics, In: Yazar E, editor. Veterinary Drug, Istanbul, Nobeltip, 2018. p. 85-224.
18. Bastos LF, de Oliveira AC, Watkins LR, Moraes MF, Coelho MM. Tetracyclines and pain. Naunyn Schmiedebergs Arch Pharmacol 2012; 385: 225-241.
19. Bostanci N, Akgül B, Tsakanika V, Allaker RP, Hughes FJ, McKay IJ. Effects of low-dose doxycycline on cytokine secretion in human monocytes stimulated with aggregatibacter *Actinomycete mcomitans*. Cytokine 2011; 56: 656-661.
20. Tras B, Elmas M. Analgesic, antipyretic and anti-inflammatory drugs. In: Yazar E, editör. Veterinary Drug, Istanbul, Nobeltip, 2018. p. 311-334.
21. Desmarais G, Charest G, Fortin D, Bujold R, Mathieu D, Paquette B. Cyclooxygenase-2 inhibitor prevents radiation-enhanced infiltration of F98 glioma cells in brain of Fischer rat. Int J Radiat Biol 2015; 91: 624-633.
22. Yu L, Jiang R, Su Q, Yu H, Yang J. Hippocampal neuronal metal ion imbalance related oxidative stress in a rat model of chronic aluminum exposure and neuroprotection of meloxicam. Behav Brain Funct 2014; 10: 6.
23. Yi C, Zhang Z, Wang W, Zug C, Schluesener HJ, Zhang Z. Doxycycline attenuates peripheral inflammation in rat experimental autoimmune neuritis. Neurochem Res 2011; 36: 1984-1990.
24. Spiess BM, Pot SA, Florin M, Hafezi F. Corneal collagen cross-linking (CXL) for the treatment of melting keratitis in cats and dogs: A pilot study. Vet Ophthalmol. 2014;17:1-11.
25. Takahashi M, Kawaguchi M, Shimada K, Nakashima T, Furuya H. Systemic meloxicam reduces tactile allodynia development after L5 single spinal nerve injury in rats. Reg Anesth Pain Med 2005; 30 :351-355.
26. De Groot CJ, Langeveld CH, Jongenelen CA, Montagne L, Van Der Valk P, Dijkstra CD. Establishment of human adult astrocyte cultures derived from postmortem multiple sclerosis and control brain and spinal cord regions: Immunophenotypical and functional characterization. J Neurosci Res. 1997; 49: 342-354.
27. Ward JL, Harting MT, Cox CS Jr, Mercer DW. Effects of ketamine on endotoxin and traumatic brain injury induced cytokine production in the rat. J Trauma. 2011;70:1471-1479.
28. Espinosa-Oliva AM, de Pablos RM, Herrera AJ. Intracranial injection of LPS in rat as animal model of neuroinflammation. Methods Mol Biol 2013; 1041: 295-305.
29. Capone ML, Tacconelli S, Di Francesco L, Sacchetti A, Sciulli MG, Patrignani P. Pharmacodynamic of cyclooxygenase inhibitors in humans. Prostaglandins Other Lipid Mediat 2007; 82: 85-94.
30. Liu WC, Ding WL, Gu HY, Chen MF, Hu JJ. Lipopolysaccharide-induced cerebral inflammatory damage and the therapeutic effect of platelet activating factor receptor antagonist. Neurosci Bull 2007; 23: 271-276.
31. Chen G, McCuskey RS, Reichlin S. Blood interleukin-6 and tumor necrosis factor alpha elevation after intracerebroventricular injection of *Escherichia coli* endotoxin in the rat is determined by two opposing factors: Peripheral induction by LPS transferred from brain to blood and inhibition of peripheral response by a brain-mediated mechanism. Neuroimmunomodulation 2000; 8: 59-69.
32. Mpiga P, Mansour S, Morisset R, Beaulieu R, Ravaoarinoro M. Sustained interleukin-6 and interleukin-8 expression following infectionwith *Chlamydia trachomatis* serovar L2 in a HeLa/THP-1 cell co-culturemodel. Scand J Immunol 2006; 63: 199-207.
33. de Miranda AS, Lacerda-Queiroz N, de Carvalho Vilela M, Rodrigues DH, Rachid MA, Quevedo J, *et al.* Anxiety-like behavior and proinflammatory cytokine levels in the brain of C57BL/6 mice infected with *Plasmodium berghei* (strain ANKA). Neurosci Lett 2011; 491: 202-206.
34. Zhang Z, Yang WZ, Zhu ZZ, Hu QQ, Chen YF, He H, *et al.* Therapeutic effects of topical doxycycline in a benzalkonium chloride-induced mouse dry eye model. Invest Ophthalmol Vis Sci 2014; 55: 2963-2974.
35. Hassan MH, Ghobara MM. Antifibrotic effect of meloxicam in rat liver: Role of nuclear factor kappa B, proinflammatory cytokines, and oxidative stress. Naunyn Schmiedebergs Arch Pharmacol 2016; 389: 971-983.
36. Barichello T, Generoso JS, Milioli G, Elias SG, Teixeira AL. Pathophysiology of bacterial infection of the central nervous system and its putative role in the pathogenesis of behavioral changes. Braz J Psychiatr 2013; 35: 81-87.
37. Brian JE, Jr Moore SA, Faraci FM. Expression and vascular effects of cyclooxygenase-2 in brain. Stroke 1998; 29: 2600-2606.
38. Cao C, Matsumura K, Ozaki M, Watanabe Y. Lipopolysaccharide injected into the cerebral ventricle evokes fever through induction of cyclooxygenase-2 in brain endothelial cells. J Neurosci 1999; 19: 716-725.
39. Teismann P, Ferger B. Inhibition of the cyclooxygenase isoenzymes COX-1 and COX-2 provide neuroprotection in the MPTP-mouse model of Parkinson's disease. Synapse 2001; 39: 167-174.
40. Han L, Ren Q. Protective effect of meloxicam against acute radiation-induced brain injury in rats. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2014; 30: 375-378.
41. Crawford YG, Gauthier ML, Joubel A, Mantei K, Kozakiewicz K, Afshari CA, *et al.* Histologically normal human mammary epithelia with silenced p16 (INK4a) overexpress COX-2, promoting a premalignant program. Cancer Cell 2004; 5: 263-273.
42. Luo C, Zhang H. The Role of Proinflammatory Pathways in the Pathogenesis of Colitis-Associated Colorectal Cancer. Mediators Inflamm 2017; 2015:5126048.
43. Eimerbrink MJ, White JD, Pendry RJ, Hodges SL, Sadler LN, Wiles JD, *et al.* Administration of the inverse benzodiazepine agonist MRK-016 rescues acquisition and memory consolidation following peripheral administration of bacterial endotoxin. Behav Brain Res 2015; 288: 50-53.
44. Mello BS, Monte AS, McIntyre RS, Soczynska JK, Custodio CS, Cordeiro RC, *et al.* Effects of doxycycline on depressive-like behavior in mice after lipopolysaccharide (LPS)

administration. J Psychiatr Res 2013; 47: 1521-1529.
45. Lai AY, Todd KG. Hypoxia-activated microglial mediators of neuronal survival are differentially regulated by tetracyclines. Glia 2006; 53 :809-816.
46. Bruschi F, Pinto B. The significance of matrix metalloproteinases in parasitic infections involving the central nervous system. Pathogens 2013; 2: 105-129.
47. Mandal, M, Mandal, A, Das, S, Chakraborti T, Sajal C. Clinical implications of matrix metalloproteinases. Mol Cell Biochem 2003;252: 305-29. ,
48. Giraudon P, Buart S, Bernard A, Belin MF. Cytokines secreted by glial cells infected with HTLV-I modulate the expression of matrix metalloproteinases (MMPs) and their natural inhibitör (TIMPs): possible involvement in neurodegenerative processes. Mol Psychiatry 1997; 2: 107-110.
49. Zou X, Wu Z, Huang J, Liu P, Qin X, Chen L, *et al.* The role of matrix metalloproteinase-3 in the doxycycline attenuation of intracranial venous hypertension-induced angiogene. Neurosurgery 2018; 83: 1317-1327.
50. Cho Y, Son HJ, Kim EM, Choi JH, Kim ST, Ji IJ, *et al.* Doxycycline is neuroprotective against nigral dopaminergic degeneration by a dual mechanism involving MMP-3. Neurotox Res 2009; 16: 361-371.
51. Lazzarini M, Martin S, Mitkovski M, Vozari RR, Stühmer W, Bel ED. Doxycycline restrains glia and confers neuroprotection in a 6-OHDA Parkinson model. Glia 2013; 61: 1084-1100.