Doxycycline and meloxicam can treat neuroinflammation by increasing activity of antioxidant enzymes in rat brain

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Abstract: The aim of this study is to determine the effects of alone or combined usage of doxycycline and meloxicam on brain superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and matrix metalloproteinase (MMP)-9 levels of lipopolysaccharide (LPS)-induced brain inflammation. Totally 78 rats were divided into 5 groups; Healthy control (n=6), LPS (n=18, 0.05 µg/µL/rat, intracranially), LPS+D (n=18, LPS 0.05 µg/µL/rat, intracranially and doxycycline 40 mg/kg, intraperitoneally), LPS+M (n=18, LPS 0.05 µg/µL/rat, intracranially and meloxicam 2 mg/kg, intraperitoneally), LPS+Combination (n=18, LPS 0.05 µg/µL/rat, intracranially and simultaneously both drug combination) groups. Animals were euthanized at 1, 3 and 6 hours following injections and the brains were removed. Brain SOD, CAT, MDA and MMP-9 levels were determined by ELISA reader. Parameters of LPS groups generally different from Healthy control group. When compared to LPS group, increased SOD level of LPS+D at 3 hours and CAT levels of LPS+M and LPS+D groups were determined (P<0.05) at 3 and 6 hours, respectively. In addition, all treatments statistically significantly (P<0.05) decreased MMP-9 levels at 6 hours. In conclusion, doxycycline and meloxicam may show antioxidant effect via increasing antioxidant enzyme production in the brain; however combined usage of drugs may show more beneficial effect for neuroinflammation.

Keywords: Antioxidants, doxycycline, meloxicam, neuroinflammation.

INTRODUCTION

Neuroinflammation is important in the healing and progression of nervous system diseases. It is the key for many neurodegenerative diseases (Lima et al., 2012). Neuroinflammatory diseases are modeled on experimental animals for the diagnosis and treatment of neurodegenerative diseases such as Parkinson and Alzheimer’s diseases (Amor et al., 2010). Adaptive and innate immune systems in the central nervous system (CNS) limit the tumor and neurotropic viral infections, bacterial diseases, and regenerate the brain tissue after inflammation. Bacterial and viral infections also play an important role in the development of neuroinflammation. Neuroinflammation is necessary for to promote regeneration and repair (Amor et al., 2014). In the treatment of neuroinflammation, the main target is the reduction of cytokines, matrix metalloproteinase (MMP)-9 levels and oxidative stress (Boulamery et al., 2017).

In general, inflammation, oxidative stress and mitochondrial dysfunction are the main cause of neurodegenerative diseases. Free radicals are one of the most important factors that cause death of neurons (Buendia et al., 2016). Reactive oxygen radicals interact with carbohydrates, proteins, lipids and nucleic acids, leading to cellular dysfunction. The brain tissue is more susceptible to oxidative damage due to its high polysaturated fat content, high oxygen consumption, and inadequate antioxidants (Valko et al., 2007). Astrocytes produce antioxidants and free radical scavengers that protect the brain from oxidative stress. However, different signal molecules, mainly cytokines and neurotransmitters, trigger reactive astrogliosis. If inflammation cannot be controlled with anti-inflammatory agents, activated astrocytes release several inflammatory factors. Increased neuronal inflammatory apoptosis lead to increased oxidative damage markers. As a result, in the development of various neurodegenerative diseases, amyloid deposits and glial scarriing occurs when astrocytes lose their function (Agostinho et al., 2010).

Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases and glutathione reductase have antioxidant effects in the body. Hence they may inhibit neuronal inflammation (Mosley et al., 2006). Various anti-inflammatory and antioxidant agents prevent the progression of neuroinflammations with these mechanisms (Agostinho et al., 2010; Valko et al., 2007). MMP-9 releases from microglia, macrophages, and infiltrating neutrophils MMP-9 is activated by the formation of free radicals in neuroinflammation. MMP has a multitude of roles in inflammation and tissue repair. It contributes to the damage of the tissue and the later recovery phases (Yang et al., 2015).

Doxycycline is a broad-spectrum tetracycline antibiotic. The tetracycline group antibiotics have anti-inflammatory effect and they function by decreasing NF-κB activity.
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(Bastos et al., 2012). Doxycycline inhibits microglial activation by decreasing interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α). However, doxycycline may inhibit MMP depending in a dose dependent manner (Jantzie et al., 2010). Doxycycline inhibits neuroinflammatory diseases by modulating lipopolysaccharide (LPS)-induced p38 MAP kinase and NF-κB pathways (Santa-Cecilia et al., 2016). Doxycycline chelates the zinc ion present in the catalytic site of MMPs and inhibits MMP activity. It also has antioxidant properties (Antonio et al., 2014). While it reduces malondialdehyde (MDA) and total oxidative status (TOS) levels, it increases total antioxidant status level (Yagan et al., 2014). Doxycycline antagonizes free radicals, lipid peroxidation and glutamate excitotoxicity and prevents neuronal degeneration (Nogueira et al., 2011).

Meloxicam is a preferential COX-2 inhibitor and protects the brain from damage (Jun-Qing et al., 2006). Oxidative stress in the brain triggers inflammation by COX-2 enzyme. The effect of meloxicam on COX-2 reduces inflammation. Also, this effect can increase the antioxidant effect by reducing oxidative stress (Hakan et al., 2010). It is recommended as an neuroprotective agent by reducing lipid peroxidation and increasing endogenous antioxidant enzymes (Goverdhan et al., 2012). Meloxicam inhibits neuronal death by regulating increased MDA and decreased SOD levels in neuroinflammation (Yu et al., 2014). It has been reported that this effect alone is not enough, despite the antioxidant activity of meloxicam. It can change the level of glutathione and superoxide enzymes (Khan et al., 2017). However, oxidative stress and antioxidant balance may vary depending on the duration of meloxicam administration (Khan et al., 2014). In the acute phase of the disease and with the treatment of meloxicam, it can limit the enhanced activity of various MMPs and prevent the progression of inflammatory diseases (de Grauw et al., 2009).

The aim of this study was to determine the effects of doxycycline and meloxicam treatment on activities of SOD, CAT and levels of MDA and MMP-9 in lipopolysaccharide (LPS)-induced brain inflammation.

**MATERIALS AND METHODS**

**Experimental design**

Seventy-eight male Wistar Albino rats (10-12 weeks old, 250-300 g) were housed and allowed free access to food and water. All procedures were approved by Selcuk University Experimental Medical Practice and Research Center Ethic Committee.

Doxycycline (Doksims 100 ml, Mistav, Ankara, Turkey) and meloxicam (Maxicam X4, Sanovel, Istanbul, Turkey) were supplied. Lyophilized Lipopolysaccharide (LPS, *Escherichia coli* 0111: B4, Sigma-Aldrich Chemie, USA) was diluted with 0.9% saline for experimental neuroinflammation model. The rats were divided in the following groups for experiment. Control Group (C) (Control, n=6) were used as healthy controls. The healthy control group received 10 µL 0.09 % saline solution, intracranially. LPS Group (n=18) animals received 0.5 µg/rat LPS within 10 µL, intracranially. The microinjection was applied intracranially through a 10 µL Hamilton syringe by stereotaxic surgery (Tsai et al., 2003). The application was performed according to the coordinates (0.8 mm posterior to bregma, 1.5 mm lateral to sagittal suture by Hamilton microsyringe). LPS + D Group (n=18) animals received 0.5 µg/rat LPS within 10 µL, intracranially and simultaneously 40 mg/kg doxycycline intraperitoneally. LPS + M Group (n=18) animals received 0.5 µg/rat LPS within 10 µL, intracranially and simultaneously 2 mg/kg meloxicam intraperitoneally. The last, LPS+ Combination Group (n=18) animals received 0.5 µg/rat LPS within 10 µL, intracranially and simultaneously 40 mg/kg doxycycline combination with 2 mg/kg meloxicam intraperitoneally. The rats were anesthetized [20 mg/kg thiopental sodium, intraperitoneally (Pental 1 g; Ulagay, Istanbul, Turkey)] before the drug application and later they were euthanized. The brain tissues were immediately removed at 1, 3 and 6 hours after the last administration in all experiment groups. The brain tissues were separately put on ice.

**Analysis of parameters**

The brain of rats were homogenized in PBS and activities of SOD (Superoxide Dismutase Assay Kit, Item No. 706002, Cayman Chemical Company, USA), CAT (Catalase Assay Kit, Item No. 707002, Cayman Chemical Company, USA) and levels of MDA (Malondialdehyde ELISA kit, Catalog No: E-EL-0060, Elabscience Biotechnology Co. Ltd., China), MMP-9 (Rat matrix metalloproteinase 9 ELISA kit, Catalog No: YLA0585RA, YL Biotech Co., Ltd. China) were measured by ELISA reader (Bio-Tek Instruments Inc., MWGt Lambda Scan 200).

**STATISTICAL ANALYSIS**

All data obtained from brain tissues are defined as mean ± standard error of the mean (SEM). The data were analyzed using ANOVA and Duncan test as a post hoc test (SPSS 22.0). In all parameters, p < 0.05 was the criterion for statistical significance.

**RESULTS**

The effects of doxycycline, meloxicam and combination of both on levels of MDA, MMP-9 and activities of SOD, CAT at different times of neuroinflammation were presented in figs. 1, 2, 3 and 4.
While MDA levels didn’t change in any groups, SOD activity of LPS+D was statistically significantly higher from LPS+ Combination and Control groups at 1 hour. Additionally, SOD activity of LPS+ Combination group was statistically significant higher than Control group at 1 hour. The SOD activity of LPS+D group was statistically higher than all experiment group and LPS+M and LPS+ Combination groups were statistically significant higher than Control group at 3 hours. SOD activities of LPS+D and LPS+M groups were statistically higher than Control group.

![Fig. 1: MDA levels of the brain at different sampling times (mean ± SEM). No statistical difference was found (P > 0.05).](image1)

![Fig. 2: SOD activity of the brain at different sampling times (mean ± SEM). Different letters are statistically significant (P<0.05).](image2)

Higher CAT activities were determined in LPS+M and LPS+ Combination groups than Control group at 1 and 6 hours, whereas CAT activity of LPS+M group was higher than all groups except LPS+ Combination group at 3 hours (p<0.05). CAT activity of LPS+D group was higher than in LPS and Control groups at 6 hours.

![Fig. 3: CAT activity of the brain at different sampling times (mean ± SEM). Different letters are statistically significant (P<0.05).](image3)

![Fig. 4: MMP-9 levels of the brain at different sampling times (mean ± SEM). Different letters are statistically significant (P<0.05).](image4)

MMP-9 levels statistically significantly decreased in LPS+M and LPS+ Combination groups when compared to LPS group at 1 hour, while its level was highest in LPS group from all other groups at 6 hours (p<0.05).

**DISCUSSION**

Many neuroinflammatory diseases have proved to be the underlying cause of oxidative stress associated with inflammation (Gustaw-Rothenberg et al., 2010; Negi et al., 2011). Although MDA is considered a potential biomarker for neuroinflammatory diseases such as dementia and Alzheimer (Gustaw-Rothenberg et al., 2010), some authors think that this situation is controversial (Sekler et al., 2008). Although there is no difference at the MDA level in some periods of the disease (Sekler et al., 2008; Zafrilla et al., 2006), there is a difference in the advance phase. Researchers have reported different neurotoxic reactions and oxidative responses to aldehydes in different regions of the brain such as the frontal and occipital lobes (Zafrilla et al., 2006). In the present study, MDA level was not significantly different among the groups at any sampling times (fig. 1), and this result may be derived from sampling time.

SOD is the first protective defensive line against oxidative stress. Increased SOD level in neurons is related to various oxidative stress-associated neurodegenerative and neuroinflammatory diseases (Schreibelt et al., 2007). Oxidized and misfolded SOD causes mitochondrial dysfunction and disease progression in neuronal diseases (Shvil et al., 2018). In the current study, the SOD level increased with neuroinflammation in the first 3 hours but the endogenous SOD increases in the neuroinflammatory (LPS) group was not enough and decreased at 3 hours. On the contrary, it increased at 6 hours in the treatment
groups and the LPS + D group at 3 hours were statistically higher than the LPS group (fig. 2). In the present study, doxycycline may have prevented oxidative stress via inhibiting SOD degradation and protected against apoptosis from oxidative stress or inflammation (Rhieu et al., 2014), hence this effect increases the activity of SOD as microglia and astrocytes cells protect against apoptosis. Although meloxicam does not pass enough amounts to the brain. Nanoparticle forms of meloxicam may pass to the brain and show anti-inflammatory and antioxidant effects via inhibiting COX-2 (Ianiski et al., 2012). In the current study, insufficient antioxidant effects of meloxicam may not have passed enough to the brain.

CAT activity decreases in systemic LPS induced neuroinflammation. However, brain function can improve by reducing inflammation and triggering antioxidant enzymes (Salmani et al., 2018). Increased catalase activity protects against oxidative stress and neuroinflammation (Godinho et al., 2018). In the neuroinflammation induced by LPS for 1 week, the oxidative stress occurred and the level and activity of antioxidant enzymes such as catalase decreased (Khajevand-Khazaei et al., 2018). In the present study, the increase in CAT activity of the LPS-administered group may be an antioxidant response against inflammation (fig. 3). The level and activity of antioxidant enzymes may not have decreased because endogenous antioxidant enzymes do not cause exhaustion due to LPS dose. Doxycycline is thought to be effective in the treatment of neuropsychiatric disorders such as schizophrenia by increasing SOD and CAT activity (Ben-Azu et al., 2018). Meloxicam does not cause a change in CAT activity in human erythrocytes (Burak Cimen et al., 2003), while it induces the activity in the renal injury (Hassan et al., 2014). The findings of the present study showed that the antioxidant effects (CAT activity) of doxycycline at 6 hours and meloxicam at 3 hours can prevent neurodegenerative diseases by reducing oxidative and inflammatory damage.

LPS induce oxidative stress, matrix metalloproteinase-9 (MMP-9), cytokines and chemokines by damaging microglia cells (Lee et al., 2015; Mayer et al., 2016). Excessive increase in MMP level accompanies pathological, neuroinflammatory brain disorders (Yang et al., 2017). The reduction of the MMP-9 level modulates neuroinflammation and it plays a neuroprotective role (Lee et al., 2015). Doxycycline inhibits cellular damage by suppressing MMP-9 in ischemia and neuronal damage, at the same time it repairs neurovascular matrix degradation, hemorrhage, edema, blood-brain barrier breakdown and neuroinflammation (Lee et al., 2009). Meloxicam diminishes the level of MMP-9 by inhibiting the enzyme COX-2. It has also important role for extracellular matrix remodeling (Kim et al., 2008). In the present study, MMP-9 level was induced by LPS administration. Especially at the beginning of neuronal damage, the level of MMP-9 increased later. COX-2 inhibitor meloxicam treatment, early MMP-9 levels may have regulated the healing process by decreasing MMP-9 levels in the early stages. In the later stages of neuroinflammation, anti-inflammatory and antioxidant doxycycline may have inhibited by neuronal degeneration and MMP-9 expression.

CONCLUSION

The combination of doxycycline and meloxicam are not significantly different when applied alone on oxidative status. However, the combination of drugs may be effective for anti-inflammatory purposes and their different doses could be suitable for the treatment of neuronal diseases.

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