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Impact of Tramadol and Meloxicam on Oxidative Stress in Dental Extractions due to Periodontal Disease in Dogs

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ABSTRACT

Some diseases, including periodontal diseases as well as anesthesia and drugs can increase oxidative stress in the body which causes the destruction of cells. Therefore, the effect of tramadol and meloxicam as the two frequently used analgesic drugs on the induction of oxidative stress was investigated in this study. 30 dogs affected with advanced periodontitis which is candidates for dental extraction under general anesthesia were assigned to two different groups randomly. After induction of anesthesia group A received meloxicam (0.2 mg/kg, IV) and group B received tramadol (3 mg/kg, IV) as an analgesic. Prior to injection, at 30 minutes and 60 minutes, and 3 hours after the induction of anesthesia blood samples were collected to measure indicators of oxidative stress including malondialdehyde (MDA), Superoxide Dismutase (SOD), and Total Antioxidant Capacity (TAC). As a result, an increase in all indicators of oxidative stress was observed at different times measured in both groups. However, the increase was more evident in group B. A significant difference in mean ± SD increase of MDA and decrease of SOD at 30, 60 min, and 3 hours after the injection was observed between the groups. In conclusion, meloxicam provided less imbalances on oxidative stress and therefore, has beneficial effects in dental extractions due to advanced periodontitis in dogs, which is very probable done by regulating the inflammatory and oxidation factors.

Introduction

Oxidation is a natural phenomenon of the body in which different cells produce free radicals.[1] An average balance between free radicals and antioxidants is needed for the body to remain functional. Thus, oxidative stress refers to the imbalance of free radicals and induced amount of free radicals caused by the inability of cells to produce antioxidants, nutritional deficiency, or excess production of reactive oxygen species (ROS). The ROS can cause oxidative damage to proteins, lipids, carbohydrates, and DNA, harming cells' structure and function, and leading to mutation, destruction, and cell death or apoptosis. Peroxidation of membrane phospholipids occurs during oxidation stress that produces malondialdehyde (MDA) which can be used as an indicator of oxidative stress. Other indicators are total antioxidant capacity (TAC) and a group of antioxidant enzymes, including superoxide dismutase (SOD), which can efficiently evaluate blood antioxidants.[1,2]

It has been reported that some diseases, including periodontal diseases, can increase the production of free radicals and, as a result, increase oxidative stress in the body.[3] Also, periodontitis is a destructive disease of periodontal tissues that can cause oxidative stress through the activation of neutrophils that produces free radicals. On the other hand, oxidative stress contributes

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to periodontitis and vice versa.[3,4] Since periodontal diseases are prevalent and advanced tissue injuries, as they progress, they can cause considerable morbidities such as periodontal abscesses, tooth loss, and severe pain in advanced situations.[5] Periodontal therapy and almost all dental procedures are painful, so, they are usually performed under general anesthesia.[5,6] Studies have shown that different drugs used in general anesthesia could lead to oxidative stress, MDA production, and cell injury either directly or indirectly. Therefore, induced oxidative stress might be more harmful in periodontal patients undergoing general anesthesia and analgesic administration.[7] So, in a dog that needs dental extraction due to periodontitis under general anesthesia and pain medication different factors are involved in inducing oxidative stress. Therefore, evaluation of their effective impact on oxidative stress indicators seems necessary and this article aims to discuss some parts of them.

Numerous studies have been conducted on prescribing different painkillers for dental pain. Opioids and non-steroidal anti-inflammatories are often suggested and frequently prescribed for pain relief in small animals. Opioids and opioid-like drugs are believed to control moderate to severe postoperative pain, especially in dogs.[8] Tramadol is commonly used among opioid-like drugs, and due to its relatively easy access, it is widely used in veterinary dentistry in Iran.[9] Although these drugs are somewhat effective and have minimal side effects, several reports discuss the induction of oxidative stress following their use. Some research has found opioids to be effective in reducing oxidative stress and its harmful results, and on the contrary, others have considered them responsible for increased oxidative stress.[8-11]

Other available and effective drugs that are frequently used due to their analgesic properties in controlling pain during and after dental procedures are NSAIDs.[12,13] Studies concluded that NSAIDs have antioxidant properties and can be used effectively to alleviate pain in dogs and cats suffering from oral and dental pain.

The aim is to investigate the indicators of oxidative stress following the pre-operative administration of the opioid drug tramadol and the non-steroidal anti-inflammatory drug meloxicam in dental surgeries in dogs. These drugs are widely used in managing post-surgical pain in dental surgeries in small animals. Nevertheless, their effect on the indicators of oxidative stress is unclear.

Materials and Methods

Patient Selection

The study was performed on dogs referred to a referral pet hospital dental service for different dentistry procedures. Those without history of either surgical procedure or infection/ medication during the past 3 months were selected. The animal was excluded from the study if there is any physical signs of infection or diagnosed existing infection through either physical examination or complete blood test. Among them, 30 dogs entered the study. The criteria included dogs affected with stage 3 or 4 periodontal disease candidates of premolar and/or molar extractions. Dogs were assigned randomly into 2 groups.

Procedure

After admitting the patient, oral examination and dental probing were performed in either awake or anesthetized patients. The presence of advanced periodontal disease by observing furcation exposure of the premolar and/or molar teeth was confirmed. The procedure started after the written consent was received from the owners.

General anesthesia was performed using IV administration of a mixture of ketamine (Alfasan, Netherlands, 6.5 mg/kg) and diazepam (Chemidarou, Iran, 0.2 mg/kg) and continued inhalation of isoflurane (Piramal Healthcare Limited, India) in oxygen following intubation. The dogs in group A received meloxicam (Poyanmehrzarin, Iran, IV, 0.2 mg/kg), and in groups B, were injected tramadol (Caspian Tamin, Iran, IV, 3 mg/kg) at the time of induction of anesthesia. The analgesics were re-dosed for 24 hours postoperatively. Intra-oral X-ray confirmed extractions in the candidates. Simple dental extractions are performed in all of the patients by the same surgeon and using a similar procedure. The patients received intraoperative and postoperative antibiotic depending on the existed issue which were either long acting amoxicillin or ampicillin.

Blood Samples

Venous blood samples (1 ml each) were initially taken from all study subjects (T0). Then, 30 and 60 minutes and 3 hours after the induction of anesthesia blood samples (1 ml each) were drawn.

Laboratory Measurements

Serums were separated by centrifugation at 3,000 rpm for 10 min at 4 °C and frozen for subsequent assays by highly sensitive enzyme-linked immunosorbent assays (ELISA) kits to measure serum levels of lipid peroxidation product (MDA, mmol/l) antioxidant enzyme superoxide dismutase (SOD), and serum total antioxidant capacity (TAC, mmol/l). The samples were analyzed by an Immunoanalyser (Cobas® e 411 S/N 071227, Roche Diagnostics, Mannheim, Germany). The commercial kits were used to measure values of TAC (TAC test kit, Randox laboratories Ltd., GB), malondialdehyde (Thiobarbituric acid technique) and SOD (U/L) Human ELISA Kit (ab119694, Abcam, UK).

Statistical Analysis

Single point measurements, at any particular time, were analyzed using repeated measure ANOVA. The Dunnett's post hoc was used for analysis of measured variables using SAS. The Kruskal-Wallis test was implemented as a post hoc test using SAS for assessing nonparametric pain scores which the results were not illustrated in this study. Changes in parameters over time were analyzed using the LM procedure in SAS, including repeated measures in the model. Data were presented as Mean ± SD. A value of $p < 0.05$ was considered significant.

Results

All of the animals recovered from anesthesia uneventfully. The obtained results of MDA, SOD and TAC levels in groups A and B are illustrated in Table 1 and Figure 1. Results indicated that the mean serum levels of MDA in both groups increased at the different time points compared to T0 ($p < 0.05$). However, the increase was significant in group B at 30 min, 1, and 3 hours after the administration of tramadol. Also, we observed more increase in group B compared to group A at the different time points.

The mean ± SD of plasma SOD contents in groups A and B gradually decreased at different time points. However, the decrease was significant at 30 min, 1 hour, and 3 hours after the drug injection in group B ($p < 0.05$). While, at all times, serum levels of SOD significantly remained higher in group A compared to group B.

Results showed that the mean ± SD of TAC serum values at different time points decreased, however, it was not significant between the groups and within the groups of the study at 30 min and 1 hour and 3 hours after drug administration ($p > 0.05$).

Table 1. Mean ± SD of MDA, SOD and TAC in groups A and B before surgery, and at 30 min, 1 and 3 hours after dental extractions.

Measured variable	Group	T0	T30 min	T60 min	T3h
MDA (mmol /ml)	A	9.92 ± 4.9	12.12 ± 3.87	17.95 ± 2.24*	28.31 ± 4.46*
	B	8.93 ± 5.43	19.74 ± 2.11*	27.94 ± 3.24*	42.16 ± 7.51*
SOD (U/L)	A	292.25 ± 25.78	254.82 ± 28.87	239.02 ± 20.03	231.66 ± 18.09*
	B	284.24 ± 38.09	216.82 ± 14.21*	203.7 ± 13.22*	191.92 ± 21.27*
TAC (mmol /ml)	A	10.28 ± 0.31	10.01 ± 0.13	8.91 ± 0.41	6.88 ± 1.11
	B	10.40 ± 0.35	10.20 ± 0.26	9.22 ± 0.33	7.55 ± 0.61

Data with * superscripts showed significant differences within columns at the 0.05 significance level.

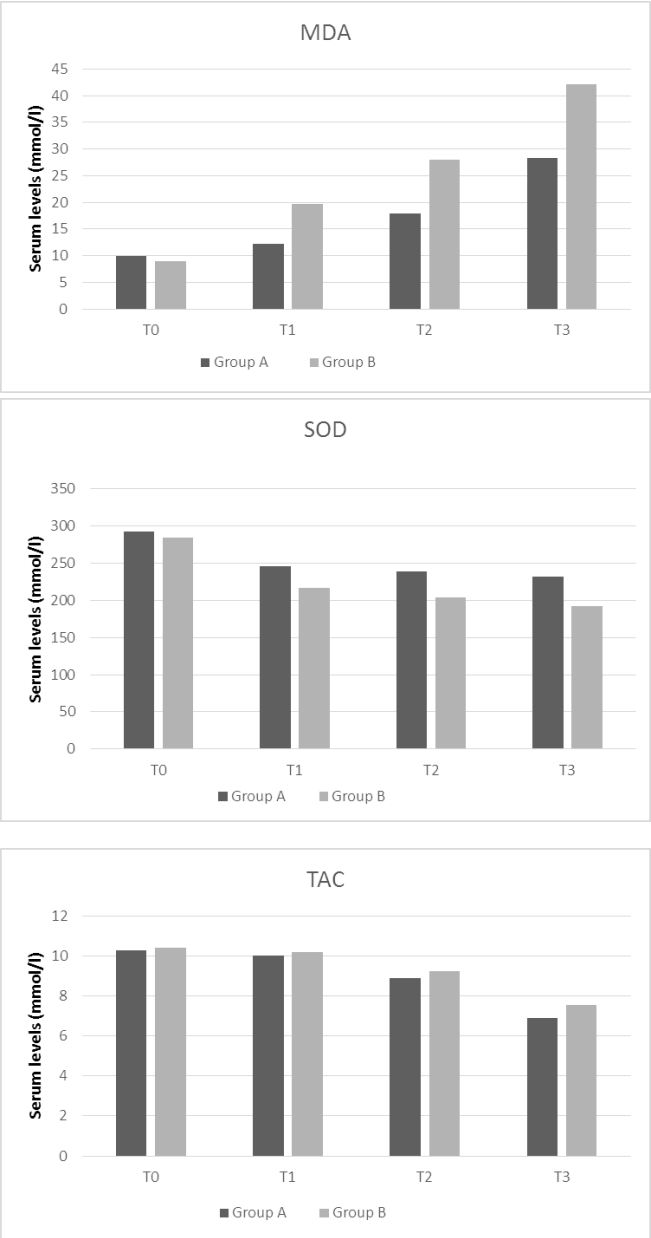

Figure 1. Mean levels of MDA, SOD, and TAC in groups A and B measured at different time intervals prior and after the extractions.

Discussion

Periodontitis, which causes destruction of periodontal tissues, is associated with oxidative stress.[2] The upregulation of ROS might play one of the most critical roles in the establishment and progression of periodontitis through the development of oxidative stress.[1,2] Meanwhile, some research has shown that tooth extraction procedures can cause severe oxidative stress.[2-4] At the same time, anesthesia leads to increased production of ROS. So, in such circumstances, several factors can potentiate the oxidative stress and therefore be tissues destruction.[11] Since several factors are involved in postoperative-induced oxidative stress, further research is needed to examine relevant findings aiming to reduce their destructive effects. Therefore, the purpose of the present study was to evaluate the impact of

meloxicam and tramadol, the two frequently prescribed pain medications on oxidative stress in a dog affected with advanced periodontitis that underwent dental extractions.

The body releases antioxidants to protect itself against the associated tissue destruction. Another related enzyme is SOD which is an antioxidant enzyme that acts against superoxide, an oxygen radical released in inflammatory pathways and causes connective tissue breakdown.[1,2,15-17] In addition, different drugs can cause oxidative stress. In this study, not only the periodontal disease -as a destructive inflammatory disease- but also administration of anesthetics which can potentiate the oxidative stress, were challenged.

Tramadol is a centrally acting analgesic drug that can induce a significant increase in MDA level, augmentation in tissue lipid peroxidation biomarker, disturbance in the antioxidant homeostasis, and a significant decrease in the levels of glutathione peroxidase and superoxide dismutase.[16-18] Also, meloxicam can decrease the activity of oxidative stress indicators including SOD and glutathione, and increase xanthine oxidase in the gastric mucosa.[13,19] It also has potential therapeutic effects by inhibiting lipid peroxidation, increasing antioxidant enzymes, and reducing acetylcholinesterase activity in the brain. It has been said that meloxicam led to the reduction of oxidative stress and inhibition of free radicals, and while administered, the AST and ALT enzymes were close to normal. Different indices are used to measure oxidative stress during anesthesia. Among them by considering available methods and kits in Iran, we found that measurement of MDA, SOD, and TAC are more efficient and applicable for us. TAC is decreased when more oxidative stress is predicted during a procedure. MDA amounts, the final product of lipid peroxidation, are expected to be increased after oxidative stress. While serum concentration of SOD has shown to be decreased following induced oxidative stress. Recent studies have discussed the increased levels of oxidative stress markers in periodontitis including MDA and SOD.

Levels of measured indicators of oxidative stress in this study including MDA, SOD, and, TAC confirmed that both groups of dogs during and after the dental extractions suffered from oxidative stress. This can be not only due to the presence if periodontitis itself but also might be due to the use of drugs that induced oxidative stress.

Several studies have demonstrated that oxidative stress is an essential factor in the pathogenesis of periodontitis. In one study, the position of MDA was advanced in the PICF (peri-implant crevicular fluid) of diseased compared to healthy peri-implant conditions, but this difference was not statistically significant. Several studies have anatomized the role of MDA in GCF (gingival crevicular fluid) of cases with periodontal complaints. They concluded that the levels of MDA in GCF of periodontally compromised cases were higher than in the periodontally healthy group. Akalin *et al.* reported no significant difference in the role of SOD in GCF of habitual periodontitis cases and the control group, and Tasi *et al.* suggested lower presence of SOD in GCF of periodontal cases. Another possibility may be that increased generation of ROS and oxidative damage may have suppressed SOD product in PICF.[15,20,21]

In other cases, a significant increase in MDA situations in diabetes groups compared to healthy controls was demonstrated and there was a positive correlation between the probing fund depth and MDA situations among periodontitis cases with diabetes. Increased oxidative stress is demonstrated in habitual periodontitis with and without type 2 diabetes indicating a common factor involved in towel damage. More severe destruction of periodontal tissue is associated with excessive ROS generation which is appreciatively identified in type 2 diabetic subjects.[22]

The surgery itself leads to increased ROS production and ischemia/reperfusion injury and increased perioperative oxidative stress has been identified with increased postoperative complications. Preoperative situations of oxidative stress cause increased threat of delayed recovery and complications after surgery.[7]

NSAIDs and opioids are generally used to manage post-operative dental and oral pain. NSAIDs inhibit the conflation of prostaglandin through inhibition of the cyclooxygenase enzymes hence producing their analgesic parcels. All evidence showed an increase after the dental surgery and the loftiest pain recorded was at 5 hours after surgery.[5] In the present study in group A we totally observed less oxidative stress, and previous studies confirmed the efficiency and superiority of meloxicam as an NSAIDS in reducing pain versus tramadol.[5]

As illustrated in the results, differences in MDA, SOD, and TAC levels in both groups have been reported. MDA levels, despite the similarity between both groups at T0, have shown a significant increase at T1, T2, and T3. At T0, groups A and B have shown similar MDA levels while group A has shown a slight increase compared to group B. However, at 30 min, 60 min, and 3 hours after the procedure, group B has shown a significant increase especially compared to group A which also increased in the mentioned time interval. Overall, group A has lower MDA levels which can demonstrate that meloxicam has a better effect on reducing oxidative stress. On the other hand, groups A and B have shown decreased SOD levels throughout the process. However, group A has shown much higher SOD levels compared to group B which indicates that meloxicam has a better effect on SOD levels which will decrease after oxidative stress.

While discussing TAC levels, there are no significant changes in either group but group B has shown slightly higher TAC levels throughout the process even though the difference between groups cannot be considered meaningful. TAC levels will decrease with oxidative stress so increased levels in group A can also indicate the better effect of meloxicam.

According to data illustrated in Table.1, we can conclude that group A, which received meloxicam, has shown better results compared to group B, administered with tramadol, in controlling the oxidative stress caused by periodontal procedures. Also, because the study was conducted on referral patients, that had been discharged after the surgical procedure and recovering from anesthesia, therefore, we could not follow the measured parameters for a longer duration. However, it is believed that oxidative stress is more evident during the early hours of anesthesia.[5] Group A had shown lower MDA levels and much higher SOD levels compared to group B, which can lead to meloxicam's effect on reducing oxidative stress.

Other study concluded that meloxicam possesses a better analgesic effect compared to piroxicam in dental procedures in which post-operative pain is a problem. Hence, patients benefit from receiving NSAIDs as they will get the optimal doses. These agents are effective, and safe and will reduce the need for opioids including tramadol.[23]

In conclusion, meloxicam provided less imbalances on intraoperative and during early hours post-operatively of oxidative stress and therefore, has beneficial effects in dental extractions due to advanced periodontitis in dogs, which is very probable done by regulating the inflammatory and oxidation factors.

Conflict of Interest

None to declare.

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