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Reduction in Aluminum Induced Oxidative Stress by Meloxicam in Rat Brain

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ABSTRACT

Background: Non-steroidal anti-inflammatory drugs (NSAID) have been associated with antioxidant property and have been shown to improve the circulating antioxidant status on daily dosing in different inflammatory conditions. The present study was conducted to investigate the antioxidant role of meloxicam in aluminum induced oxidative stress in rat brain. **Methods:** In the *in vivo* experiments, Sprague-Dawley rats where randomized into 4 groups receiving daily treatment for 4 weeks: 1) double distilled water i.p., 2) 4.2 mg/kg aluminum i.p. 3) meloxicam (5.0 mg/kg, i.m.) 4) 5.0 mg/kg, meloxicam i.m. + 4.2 mg/kg aluminum i.p. brain homogenates from the above animals were assayed for lipid peroxidation levels as well as superoxide dismutase activity. In the *in vitro* experiments, brain homogenates from Sprague-Dawley rats were treated with either, aluminum, meloxicam or their combinations and were then assayed as per the *in vivo* samples. **Results**: *In vivo* data showed elevated lipid peroxidation levels in brain homogenate in aluminium-treated group as compared to aluminium + meloxicam treatment which showed a significant decrease in the malonaldehyde levels. Similar results were observed in the *in vitro* experiments when brain homogenates were treated with either, aluminum, meloxicam or their combinations. Furthermore, no change was observed in superoxide dismutase activity in any treatment group as compared to the control in either experiment. **Conclusion:** These results indicate that NSAID can be used in Alzheimer management. *Iran. Biomed. J. 10 (3): 151-155, 2006*

Keywords: Superoxide dismutase (SOD), Lipid peroxidation (LPO), Meloxicam, Alzheimer's disease, Aluminium

INTRODUCTION

Dementia is an organic brain disease defined as a loss of intellectual ability of sufficient severity to interfere with either the occupational functioning or usual social activities. Prevalence of dementia increases exponentially with advancing age, ranging from 10% in the age group of 60-65 years to 38.6 in the age group of 90-95 years [1]. The dementing condition that has received the utmost attention is Alzheimer's disease. Unlike other dementias, Alzheimer's is a progressive neurodegenerative condition [1]. A number of environmental factors have been put forward as possible contributory cause of Alzheimer's disease in some people and aluminum is identified as one of these factors [2].

Aluminum has been implicated in human neurodegenerative diseases like Alzheimer's. Possible mechanisms of aluminum induced neurotoxicity have been related to cell damage via free radical production. Increased lipid peroxidation (LPO) is the major consequence associated with oxidative stress. Aluminum has been shown to be associated with both plaques and tangles in the Alzheimer. Various investigations have suggested that Alzheimer's disease is more common in areas where the aluminum content in water supplies is the highest [3, 4].

Several antioxidants have been studied for the reduction of oxidative stress occurring during Alzheimer's disease [5, 6]. Non-steroidal anti-inflammatory drugs (NSAID) are the group of drugs which work by interfering with the cyclooxygenase

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pathway. NSAID have been associated with antioxidant property and have been shown to improve the circulating antioxidant status on daily dosing in rheumatoid arthritis [7, 8].

Meloxicam is a NSAID that appears to have a greater selectively inhibitory activity against the inducible cyclooxygenase-2 (COX-2) isoform, than against the constitutive isoform cyclooxygenase-1 (COX-1). Inhibition of COX-1 is associated with gastrointestinal, renal, and platelet aggregation adverse effects. Therefore, meloxicam and other COX-2 selective inhibitors are promoted for their safer profile of side effects. Meloxicam also has been shown to possess significant antioxidant property [9]. The objective of the present study was to investigate the anti oxidant role of meloxicam in aluminum induced oxidative stress in rat brain.

MATERIALS AND METHODS

Reagents. Aluminum chloride ($AlCl_3$) was obtained from S.d Fine Chemicals Ltd, Mumbai, India and Meloxicam injections (5 mg/mL) were a gift from INTAS Pharmaceuticals, Ahmedabad, India.

Animals. Sprague Dawley rats (n = 30), weighing 184.64 ± 5.81 g, of either sex bred in the animal colony at the B.V. Patel PERD, Centre, Ahmedabad, from the original stock obtained from National Institute of Nutrition, Hyderabad, were used for the study.

Housing conditions. The animals were housed 3 per cage in polypropylene cages and were moved to the experimental room where they were allowed to acclimatize for a day before treatment. The environmental conditions of the animal room were as per a specific design. A 10% air exhaust in the air conditioning unit was maintained along with a relative humidity of 60 ± 5% and a temperature of 25 ± 3°C were stabilized. A 12 hour light/dark cycle was also regulated for the experimental animals. Amrut certified rodent diet (Maharashtra Chakan Oil Mills Ltd., India) and tap water (boiled water cooled to room temperature) were provided ad libitum to the experimental animals.

All experimental protocols were reviewed and accepted by the Institutional Animal House Ethics Committee prior to the initiation of the experiment. Animals were divided into the following treatment groups: 1. Control (double distilled water) 2. Aluminum Treatment 3. Meloxicam Treatment 4. Aluminum and Meloxicam treatment. All the experiments were conducted both *in vivo* and *in vitro*.

***In vivo* treatment.** Animals in all the groups were dosed for 28 days as per the above treatment (n = 6 per group). Double distilled water and aluminum (4.2 mg/kg body weight aluminium chloride) were dosed by i.p. injections [10] whereas meloxicam (5 mg/ kg body weight) was dosed by intra muscular route. Animals were sacrificed on the 29th day by euthanasia. Brain was removed and washed with saline to remove blood and divided into two halves. One half was transferred to HBSS (pH 7.4) and the second half chilled Tris buffer 50mM (pH 8.2), for the estimation of LPO [11] and assay of superoxide dismutase (SOD) activity [12], respectively.

In vitro treatment. Previously, untreated animals (n = 6) were sacrificed and the brains were excised, divided into two equal halves and transferred into the respective buffers for LPO and SOD estimations as mentioned above. The tissues were minced thoroughly and each half was further divided into four equal parts. First part was treated with double distilled water, second part was treated with aluminum chloride (4 µg/ml), the third part was treated with meloxicam (5 µg/ml) and the fourth part was treated with aluminum and meloxicam (4 µg/ml and 5 µg/ml, respectively). The treated minced tissues were incubated for 20 minutes and were then estimated for LPO and SOD activity.

Estimation of LPO. The brain tissue was taken in 2 ml HBSS and was homogenized at 3000 rpm using Polytron homogenizer (three cycles 30 seconds each). The homogenate was then centrifuged at 710 ×g for 10 minutes. The pellet was resuspended in 1 ml of HBSS that was then used for the estimation of LPO. LPO was measured in terms of malonyl-aldehyde (MDA): thiobarbituric acid (TBA) reaction by Okhawa *et al.* [11]. The reaction mixture contained 0.1 ml of tissue homogenate, 0.2 ml of sodium dodecyl sulfate, 1.5 ml of acetic acid and 1.5 ml of aqueous solution of TBA. The pH of 20% acetic acid was pre-adjusted with 1 M NaOH to 3.5. The mixture was made up to 4 ml with distilled water and heated at 95ºC for 1 hour, in a water bath. After cooling, 1 ml of distilled water and 5 ml of mixture of n-butanol and pyridine (15:1) were added and mixture was shaken vigorously on a vortex mixer. After centrifugation at 450 ×g for 10 min the amount of MDA formed was measured by absorbance of the upper organic layer at 532 nm.

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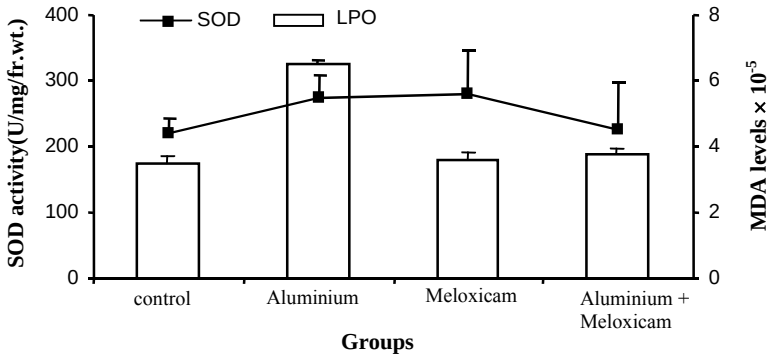

Fig. 1. *In vivo* changes in the LPO (expressed as MDA levels) and SOD activity in different groups of rat brain homogenate. A sharp increase in the MDA levels in the aluminum-treated group ($p<0.05$) and a significant decrease in the aluminum and meloxicam-treated group ($p<0.05$) was observed. However, no significant changes were observed in the SOD activity in any of the groups. SOD, superoxide dismutase; LPO, lipid peroxidation; MDA, malonylaldehyde.

Measurements were made by using Systronics ultra violet/visible spectrophotometer.

Assay of SOD activity. The brain tissue was washed with chilled saline to remove blood and was taken in 4 ml of chilled Tris buffer 50 mM (pH 8.2). It was then homogenized at 13000 rpm (3 cycles of 30 seconds each) using Polytron homogenizer. The homogenate was treated with 1 ml of % Triton X - 100 for 20 minutes at 4ºC. Homogenate was then centrifuged at a speed of 26895 ×g at 4ºC for 30 minutes using Sorvall high speed centrifuge. The supernatant was used for the assay of SOD activity by method of Marklund and Marklund [12]. All the calculations were made as per g fresh weight.

Circulating SOD levels. Animals were treated with aluminum and meloxicam for 28 days. On the 29th day, blood was collected by cardiac puncture in heparinized disposable syringes. Blood was then centrifuged and plasma was collected and stored at -70°C until assayed for SOD activity. Red Blood Cells were then subjected for extraction of SOD as described below.

Extraction of SOD from red blood cells. Heparinized blood was centrifuged at 710 ×g for 30 min at 4ºC and the plasma was carefully separated. To the erythrocyte pellet, saline was added to the original volume and was transferred to the 15 ml centrifuge tube. After the cells were washed thrice with saline, they were diluted with 4 ml double distill water to lyse the erythrocytes. Ethyl alcohol 1 ml and chloroform 0.6 ml were added to remove the hemoglobin. The tubes were shaken vigorously for 15 min. and centrifuged at 710 ×g at 4ºC for 10 min. Water ethanol layer was aspirated and was diluted with 0.7 ml of water. SOD activity was measured in plasma and ethanol water layer as described above.

RESULTS AND DISCUSSION

Figure 1 shows the changes in the superoxide anion radical and SOD activity *in vivo* in different groups of rat treated with aluminum, meloxicam and aluminum and meloxicam. A sharp increase was seen in the MDA levels in the aluminum-treated animal group when compared to the control group ($p<0.05$). A significant decrease in the MDA levels was observed in the group of animals treated with aluminum and meloxicam when compared to the only aluminum-treated group ($p<0.05$). However, no significant changes were observed in the SOD activity in any of the groups.

Figure 2 shows the changes in the superoxide anion radical and SOD activity in different groups *in vitro*. In this system, results are exactly similar to the *in vivo* study, a significant increase is in the MDA levels in aluminum-treated homogenate in comparison to the control group ($p<0.05$) and a decrease in MDA levels in the group of homogenate treated with aluminum and meloxicam as compared to the aluminum-treated group ($p<0.05$). SOD activity did not change in any groups and also the levels of circulating SOD activity in plasma and SOD extracted from red blood cells (results not presented).

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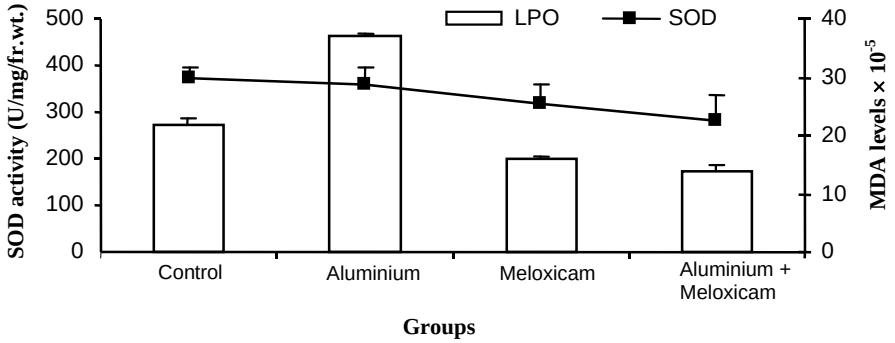

Fig. 2. *In vitro* changes in the LPO (expressed as MDA levels) and SOD activity in different groups of rat brain homogenate. A sharp increase in the MDA levels in the aluminum-treated group ($p<0.05$) and a significant decrease in the aluminum and meloxicam-treated group ($p<0.05$) was observed. However, no significant changes were observed in the SOD activity in any of the groups. SOD, superoxide dismutase; LPO, lipid peroxidation; MDA, malonylaldehyde.

Animals loaded with aluminum developed brain lesions, that is similar to those found in Alzheimer's disease [13, 14]. Aluminum uptake in the brain does not depend on the alterations to blood brain barrier (BBB) permeability. Transport of aluminum from brain interstitial fluid (BIF) to blood is believed to be mediated by a monocarboxylate transporter located in BBB. Several processes like endocytosis, facilitative diffusion, active transport are implicated in the uptake of aluminum and its relative contributions in each cell type in each brain region [13].

The proteolytic cleavage of the amyloid precursor protein and the production of amyloidogenic beta amyloid peptide are believed to be critical events in the etiology of Alzheimer's disease. Aluminum is bound by the beta amyloid and is found co-localized with beta amyloid in the Alzheimer's disease brain [15].

Amyloid fibrils formed in the presence of aluminum are slightly thicker, significantly longer and spirally wound around each other. Subsequent studies have been conducted to confirm that Amyloid beta sheets (Aβ) (1-40) will bind up to 4 aluminum atoms and that binding increased the β-sheet content of the peptide [16]. The neurotoxicity of Aβ in whatever form may involve the formation of reactive oxygen species. Aluminum is a pro-oxidant and is known to promote the oxidation activity of Aβ in the presence of iron. Aluminum has also been linked to Aβ production through the immune response. It is also linked to activate complement which in turn has been linked to the enhanced aggregation of Aβ. [15]

One group of brain cells in which aluminum is found are the glial cells. Their primary function in the brain is maintenance of the short term constancy of BIF. Glial cells respond to brain toxins by increased expression of glial fibrillary acidic protein. Disruption of glial cell function by aluminum could lead to accumulation of unwanted, possibly cytotoxic debris as well as modulation of synaptic transmission and neuron-glial signaling. Exposed glia does accumulate aluminum and show cellular changes indicative of oxidative stress [15]. Active oxygen is known to cause many lesions. Mechanism of the inhibition of active oxygen species appear to be important for the treatment of disease. Recently, many investigators have studied the ability of various drugs to eliminate active oxygen in particular.

The mechanism of aluminum neurotoxicity has been postulated as a possible contributing factor in several neurodegenerative diseases such as Alzheimer's disease [17]. In the current study, the group of animals treated with aluminum chloride expressed high MDA levels which suggest that aluminum induces superoxide anion radicals in the rat brain cells both *in vivo* and *in vitro*. NSAID have been shown to have anti-oxidant like properties [8]. Although, the exact mechanism of action is yet not known, many investigators have shown that these NSAIDs eliminate the active oxygen species, superoxide anion radical in particular [8]. In the current study, the role of meloxicam in reducing free radical load in the aluminum-treated animals was studied. Significant decrease in the MDA levels was seen in meloxicam- and aluminum-treated groups both *in vivo* and *in vitro*.

Meloxicam, a preferential COX-2 inhibitor showed reduction in MDA levels in the brain.

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Meloxicam has been reported to be able to cross the BBB [18] and has also been reported to reduce oxygen radical generation in rat gastric mucosa [19]. No inhibition was observed in SOD levels in brain and in the circulating levels with aluminum or meloxicam treatment. However, we had earlier shown that circulating SOD levels were improved with NSAID treatment in rheumatoid arthritis [7]. Decrease in the MDA levels with meloxicam in aluminum-treated animals is an important finding which can be studied further for Alzheimer management.

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