Neurosci Bull February 1, 2010, 26(1): 17-27. http://www.neurosci.cn
DOI: 10.1007/s12264-010-0713-x

·Original Article·

Protective effects of selective and non-selective cyclooxygenase inhibitors in an animal model of chronic stress

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Abstract: Objective Cyclooxygenase isoenzyme is known to be expressed in different regions of brain, and is mainly used for the treatment of pain and inflammation. Recently, it is proposed that cyclooxygenase isoenzyme may also play a key role in the pathophysiology of various brain-related disorders. The present study was aimed to explore the protective effect of cyclooxygenase inhibitors on stress by using an animal model of chronic stress. **Methods** The animals were forced to swim individually for a period of 6 min every day for 15 d. Then, the behavior (locomotor activity, anxiety and memory) and biochemical (lipid peroxidation, nitrite level, reduced glutathione, and catalase) alterations were assessed. **Results** Forced swimming for 15 d caused impaired locomotor activity, anxiety-like behavior and decreased percentage of memory retention, as compared to naïve mice (without chronic fatigue treatment). Biochemical analysis revealed significant increases in lipid peroxidation and nitrite level, while levels of reduced glutathione and catalase activity were both decreased. Chronic treatment with naproxen (14 mg/kg, i.p.), rofecoxib (5 mg/kg, i.p.), meloxicam (5 mg/kg, i.p.), nimesulide (5 mg/kg, i.p.) and valdecoxib (10 mg/kg, i.p.) significantly attenuated these behavioral and biochemical (oxidative damage) alterations in chronic-stressed mice. **Conclusion** The cyclooxygenase inhibitors could be used in the management of chronic fatigue-like conditions.

Keywords: chronic fatigue syndrome; naproxen; valdecoxib; rofecoxib; nimesulide; meloxicam

1 Introduction

Stress is triggered by many unexpected environmental, social or pathological stimuli during the lifetime of an individual, and determines the changes in all of his system. Although acute stress is necessary for survival, chronic and long-lasting stress can be detrimental[1]. Chronic stress is characterized by persistent fatigue, infection, and rheumatological and neuropsychiatric symptoms[2], and it has affected thousands of people. Chronic stress is a clinical state with defined symptoms, but undefined causes. The etiology of chronic stress remains still unclear, although evidence suggests that biological, psychological, and social factors contribute to its pathogenesis[3]. Recently, several hypotheses have been proposed concerning the underlying pathophysiological mechanism of chronic fatigue syndrome (CFS)[4]. Chronic stress has been reported to produce influences in various facets, such as cognitive deficits, anxiety, depression, neurochemical alterations and oxidative stress[5,6]. Currently, CFS treatment is based on the symptoms, being symptomatic rather than a cure. Therapies such as cognitive behavior therapy, graded exercise therapy, pharmacological interventions (e.g. antidepressants and corticosteroids), and nutritional supplements (antioxidants), have been tried, but with

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Article ID: 1673-7067(2010)01-0017-11
Received date: 2009-07-13; Accepted date: 2009-11-23

limited success. Although these drugs could attenuate the stress in patients[7], the exact mechanism of how these therapies ameliorate fatigue and other related problems have not yet been understood. There is, however, some evidence indicating that CFS is accompanied with signs of increased oxidative stress and inflammation in the peripheral blood, suggesting the involvement of inducible enzymes cyclooxygenase (COX) and inducible NO synthase (iNOS), inflammation, and oxidative and nitrosative stress in the pathogenesis of CFS. CFS further causes an intracellular inflammation, with increased productions of nuclear factor kappa beta (NF-kappa beta), COX-2 and iNOS. Thus, membrane fatty acids and functional proteins are damaged, due to the inflammation, and oxidative and nitrosative stress. A number of factors can trigger the inflammatory, oxidative and nitrosative stress pathways, including psychological stress, strenuous exercise, viral infections, *etc.* The 'psychosomatic' symptoms of CFS are caused by intracellular inflammation (ache and pain, muscular tension, fatigue, irritability, sadness, and the subjective feeling of infection), damage caused by inflammatory and oxidative and nitrosative stress (ache and pain, muscular tension and fatigue), and gut-derived inflammation (complaints of irritable bowel). Inflammatory pathways (monocytic activation) are also detected in somatizing disorder. Recent studies demonstrate that oxidative stress is also involved in the pathophysiology of CFS and significantly contributes to the clinical symptoms[8]. The oxidative stress mechanisms need to be understood in order to design therapeutic strategies. Chronic stress impairs the biosynthesis of n-3 and n-6 long-chain polyunsaturated fatty acids by inhibiting the delta-6 desaturation of the precursor essential fatty acids alpha-linolenic acid and linoleic acid. In turn, this influences the proper functions of cell membrane, including cell signaling, and has an adverse effect on the biosynthesis of eicosanoids from the long-chain polyunsaturated fatty acids dihomo-α-linolenic acid, arachidonic acid and eicosapentaenoic acid. Most of these changes are associated with the increases of inflammatory response and generation of free radicals[9,10].

Recent investigations in our laboratory have shown the protective effects of various COX inhibitors in epilepsy, drug addiction, sub acute stress and related disorders[11], suggesting the potential roles of prostaglandin in various neurodegenerative and neuropsychiatric disorders[12]. COX is an enzyme that catalyzes the convertion from arachidonic acid (a derivative from membrane phospholipids) into prostaglandins. COX has 3 isoforms, namely COX-1, COX-2 and COX-3. COX-1 is a housekeeping enzyme constitutively expressed in nearly all the tissues, mediating physiological responses. COX-2 is often referred to as the inducible isoform and is expressed during inflammation by macrophages, monocytes, and synoviocytes. It is responsible for the synthesis of prostanoids involved in cellular processes. COX-3 has been recently discovered as an RNA splicing variant of COX-1. COX-2 is one of the most important components of inflammatory response, and its reaction products are cytotoxic. Although COX-2 is expressed constitutively in brain, its expression is up-regulated in several neurological diseases, such as stroke, Alzheimer's dementia and seizures. The mechanisms by which COX-2 induces cell damage include the biosynthesis of prostaglandins, which then generate free oxygen radicals, resulting in cellular injury. On the other hand, prostaglandin itself is also responsible for cellular damage by inducing glutamate release from astrocytes or by apoptosis. The linkage between COX-2 and neurological damage has been demonstrated by numerous studies, which demonstrate that inhibition of COX-2 activity or deletion of its gene results in neuroprotective effect. Also, it is found that COX-2 mRNA level is significantly increased due to forced swim stress in mice[1].

Naproxen and meloxicam are non-selective COX (COX-1 and COX-2) inhibitors. Nimesulide is preferentially a COX-2 inhibitor. Rofecoxib and valdecoxib are selective COX-2 inhibitors. Naproxen and rofecoxib have been reported to have neuroprotective effect in mice against immobilization stress[11]. Recently, increases in COX-2 expression and activity have been observed in rat cortex after a short-time exposure (4-6 h) to restraint stress. Moreover, specific pharmacological tools that inhibit COX-2 activity (NS-398) could prevent the increase in membrane lipid peroxidation mediators and restore the antioxidant (glutathione) system, suggesting that COX isoform is involved in the accumulation of

oxidative damage during stress[1]. Studies have also indicated the neuroprotective effects of COX-2 inhibitors in various central nervous system (CNS)-related disorders[13-16].

Thus, the aim of the present study was to study the effects of various selective or nonselective COX inhibitors in chronic fatigue stress-induced behavioral and biochemical alterations.

2 Material and methods

2.1 Animals Male albino mice (Laca strain; weighing 20–30 g) were bred in Central Animal House (CAH) facility of the Panjab University, Chandigarh. The animals were housed under standard laboratory conditions under a 12-h light/dark cycle, with food and water *ad libitum*. The animals were acclimatized to the laboratory conditions 1 h prior to the experiment and thereafter they were kept in the lab every day in a similar way till the end of the experiment. All the experiments were carried out between 10:00 to17:00 in order to minimize the influences of circadian changes and inter-group variations. The experimental protocol was approved by the Institutional Animal Ethics Committee and was conducted according to the Indian Science Academy Guidelines for the use and care of experimental animals.

2.2 Drugs and treatment schedule The drugs were all purchased from Sigma Reagent Laboratories, including naproxen (14 mg/kg, i.p.), rofecoxib (5 mg/kg, i.p.), meloxicam (5 mg/kg, i.p.), nimesulide (5 mg/kg, i.p.) and valdecoxib (10 mg/kg, i.p.). Other standard chemicals were from LOBA and Himedia. The drug doses were selected according to the ED50 of the drugs and previous studies in our laboratory[11,28]. The animals were randomized into 7 groups (n=10 in each group), including the naïve group, in which the mice only received vehicle for 15 d without forced swimming session; the control (chronically stressed) group, in which mice received vehicle 30 min before the forced swimming session (6 min) for 15 d; the naproxen (14 mg/kg) group; the valdecoxib (10 mg/kg) group; the rofecoxib (5 mg/kg) group; the meloxicam (5 mg/kg) group; and the nimesulide (5 mg/kg) group. Drugs were suspended in 0.25% carboxymethylcellulose (CMC) and administered intraperitoneally, 30 min before the forced swimming session for 15 consecutive days.

After 15 d, various behavioral assessments followed by biochemical estimation were conducted, on the subsequent day 16. Besides, there was a sufficient time gap (at least 1 h) between the behavioral tests. Locomotor activity was assessed first, followed by elevated plus maze and mirror chamber test.

2.3 Measurement of immobility period The animals were forced to swim individually in a glass jar (25×12×25 cm^3) at (25±2) ºC for 6 min every day for 15 consecutive days. The water was 15 cm in height. After an initial period of vigorous activity, each animal attained a typical immobile posture. The duration of immobility was measured. The animal was considered to be immobile when it ceased to struggle and the limbs seldom moved to keep the head above the water[6]. This test session was repeated for 15 d.

2.4 Behavioral assessments Various behavioral parameters were assessed in mice on day 16, 24 h after the last forced swim challenge.

2.4.1 Measurement of locomotor activity The locomotor activity was recorded for 5 min using photoactometer. Each animal was observed in a closed square (30×30 cm^2) arena equipped with infrared light sensitive photocells using digital photoactometer. Locomotor activity was expressed in terms of total photobeam counts for 5 min per animal[17]. The apparatus was placed in a darkened, light and sound attenuated, and ventilated testing room.

2.4.2 Measurement of anxiety (mirror chamber test) The mirror chamber consisted of a wooden chamber with a mirror cube open on one side and the mirror cube was placed into a square plexiglass box. The box (40 × 40 × 30.5 cm^3) had a white floor and opaque black walls. The mirrored cube (30 × 30 × 30 cm^3) was made up of 5 pieces of mirrored glass with one mirrored side and an opposite side painted dark brown. In the standard configuration, the mirrored surfaces (3 side panes, a top pane and the floor pane) face the interior of the cube. Placement of the mirrored cube into the centre of the container forms a 5 cm corridor completely surrounding the mirrored chamber. The animal was placed individually at the distal corner of the mirror chamber at the beginning of the test. During the 5-min test, the following parameters were evaluated, including the latency to enter the mirror chamber,

the number of entries in mirror chamber, and the total time spent in mirror chamber. An anxiogenic response was identified when the number of entries and time spent in the mirror chamber were decreased[18].

2.4.3 Measurement of memory (plus maze test) Elevated plus maze was used to evaluate the spatial long-term memory[17,19]. Briefly, the apparatus consisted of 2 open arms (16 × 5 cm^2) and 2 enclosed arms (16 × 5 ×12 cm^3). The arm extended from a central platform (5 × 5 cm^2). The mice were placed individually at the end of one of the open arms facing away from the central platform. The time that the mice spent moving from the open arm to either of the enclosed arms was recorded. Transfer latency (TL) indicated the elapse between the placement of the animal on the open arm and its fully entry (all the 4 paws) in the enclosed arm. On the first day, the mice were allowed to explore the plus maze for 20 s after the measurement of TL. The mice returned to their home cages after the first trial. Retention was examined 24 h later. Each animal was again placed into the maze and TL was recorded. Percentage of the retention of memory was calculated as follows:

Memory retention = (Final transfer latency–Initial transfer latency)/Initial transfer latency×100%.

2.5 Biochemical parameters

2.5.1 Dissection and homogenization On day 16, animals were sacrificed by decapitation. The whole brain was removed and homogenized (10%, *w/v*) in 0.1 mol/L phosphate buffer (pH 7.4). Homogenates were centrifuged for 20 min at 15 000 g. The supernatant was collected for the estimation of lipid peroxidation and reduced glutathione levels. The post nuclear fractions for the catalase assay were obtained by centrifugation of the homogenate at 1 000 g for 20 min at 4 ºC. For other enzyme assays, the homogenate was centrifuged at 12 000 g for 60 min at 4 ºC.

2.5.2 Lipid peroxidation assay The quantitative measurement of lipid peroxidation in the whole brain was performed as described by Wills[20]. Malondialdehyde (MDA) is a second product of lipid peroxidation, and has been widely used as an indicator of lipid peroxidation. The level of MDA was determined by thiobarbituric acid method, and absorbance was read at 532 nm with a Shimadzu spectrophotometer (Kyoto, Japan). The results were expressed as nanomole of MDA per milligram protein using the molar extinction coefficient of chromophore [1.56×10^5 L/(mol.cm)].

2.5.3 Estimation of reduced glutathione Level of reduced glutathione in the brain was evaluated according to the method described by Ellman[21]. Supernatant (1 mL) was precipitated with 1 mL 4% sulfosalicylic acid and digested at 4 ºC for 1 h. The sample was centrifugated at 1 200 g for 15 min at 4 ºC. Supernatant was collected, and 2.7 mL phosphate buffer (0.1 mol/L, pH 8) and 0.2 mL 5, 5' dithiobis (2-nitrobenzoic acid) (DTNB) were added into 1mL supernatant. The yellow color developed and was read immediately at 412 nm using a Shimadzu spectrophotometer. Results were calculated using the molar extinction coefficient of the chromophore [1.36×10^4 L/(mol.cm)] and were expressed as nanomole GSH per milligram protein.

2.5.4 Catalase estimation Catalase activity was assessed according to the method of Luck H[22]. The breakdown of hydrogen peroxide (H_2O_2) is measured at 240 nm. Briefly, 3 mL H_2O_2 phosphate buffer and 0.05 mL supernatant of tissue homogenate were mixed. The change in absorbance was recorded at 240 nm using a Shimadzu spectrophotometer. Enzyme activity was calculated using the millimolar extinction Coefficient of H_2O_2. The result was expressed as micromoles of H_2O_2 decomposed per min/mg protein.

2.5.5 Nitrite estimation Nitrite is an indicator of the production of nitric oxide (NO). The accumulation of nitrite in the supernatant was determined by colorimetric assay using Greiss reagent [0.1% N- (1-napthyl) ethylenediamine dihyrochloride, 1% sulfanilamide and 2.5% phosphoric acid] as described by Green[23]. Equal volumes of supernatant and Greiss reagent were mixed, and incubated for 10 min at room temperature. Absorbance was read at 540 nm using Shimadzu Spectrophotometer. The concentration of nitrite in the supernatant was determined from a standard curve and expressed as the percentage of the control.

2.5.6 Protein estimation The protein content was measured according to the method of Lowry[24] using bovine serum albumin as the standard reagent.

2.6 Statistical analysis Data were expressed as mean±SEM, and analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test.

3 Results

3.1 Effects of naproxen, meloxicam, nimesulide, rofecoxib or valdecoxib on immobility period As shown in Fig. 1, the 15-d forced swimming (6 min each day) significantly increased immobility period as compared to the naïve group ($P < 0.05$). However, pretreatment with naproxen (14 mg/kg), valdecoxib (10 mg/kg), meloxicam (5 mg/kg), nimesulide (5 mg/kg) and rofecoxib (5 mg/kg) could all attenuate this increase.

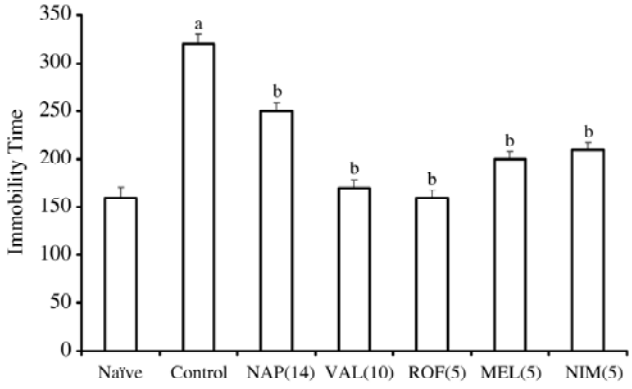

Fig. 1 Effects of COX-inhibitors (naproxen, meloxicam, nimesulide, rofecoxib and valdecoxib) on immobility period. $^aP < 0.05$ *vs* naïve group, $^bP < 0.05$ *vs* control (chornic fatigue) mice. n=10 in each group.

3.2 Effects of naproxen, meloxicam, nimesulide, rofecoxib or valdecoxib on locomotor activity As shown in Fig. 2, locomotor activity was significantly decreased in chronic stressed mice, as compared to that in naïve group (without chronically forced swimming). However, pretreatment with naproxen (14 mg/kg), valdecoxib (10 mg/kg), meloxicam (5 mg/kg), nimesulide (5 mg/kg) and rofecoxib (5 mg/kg) could all significantly reverse the locomotor activity in chronic stressed mice.

3.3 Effects of naproxen, meloxicam, nimesulide, rofecoxib and valdecoxib on anxiety After 15-d forced swimming test session, the mice showed a significant delay in the latency to enter the mirror chamber. Meanwhile, the number of entries and time spent in the mirror chamber were both decreased, as

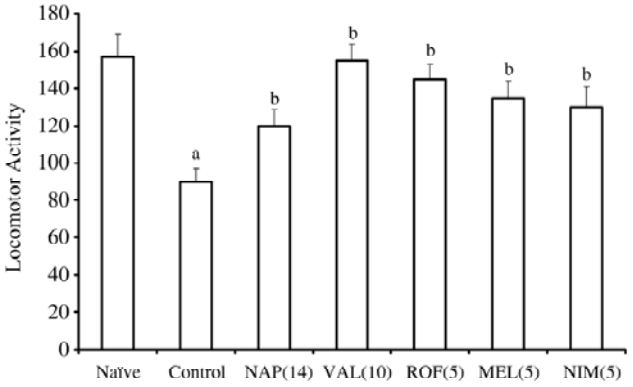

Fig. 2 Effects of COX-inhibitors (naproxen, meloxicam, nimesulide, rofecoxib and valdecoxib) on locomotor activity. $^aP < 0.05$ *vs* naïve group, $^bP < 0.05$ *vs* control group (chornic fatigue mice). n=10 in each group.

compared to those in naïve group. Pretreatment with naproxen (14 mg/kg), valdecoxib (10 mg/kg), meloxicam (5 mg/kg), nimesulide (5 mg/kg) or rofecoxib (5 mg/kg) could all significantly shorten the latency to enter the mirror chamber, and increase the number of entries and time spent in the mirror chamber, as compared with the control group (Table 1).

Table 1. Antianxiety effects of naproxen, meloxicam, nimesulide, rofecoxib and valdecoxib in mirror chamber test in chronically fatigue mice (Mean±SEM)

Groups	Latency to enter mirror chamber (s)	Number of Entries in mirror chamber	Time spent in mirror chamber (s)
Naïve	44.6 ± 2.18	6.2 ± 0.66	45.2 ± 2.9
Control	151.2 ± 11.19[a]	2.0 ± 0.33 [a]	21.0 ± 1.76 [a]
Nap (14)	100.6 ± 6.02 [b]	4.0 ± 0.87 [b]	30.4 ± 3.47 [b]
Val (10)	59.8 ± 4.97 [b]	6.0 ± 0.33[b]	40.8 ± 5.23 [b]
Rof (5)	65.6 ± 6.41 [b,c]	6.0 ± 0.28 [b]	42.66 ± 3.15 [c,e]
Mel (5)	110.3 ± 4.28 [a,b,d]	4.0 ± 0.32 [b]	35.33 ± 6.15 [b,d]
Nim (5)	90.0 ± 6.82 [b]	3.0 ± 0.37 [b]	33.66 ± 3.07 [c,e,f]

[a]$P < 0.05$ *vs* naïve group, [b]$P < 0.05$ *vs* control group, [c]$P < 0.05$ *vs* Val (10) group, [d]$P < 0.05$ *vs* Rof (5) group, [e]$P < 0.05$ *vs* Nap (14) group, [f]$P < 0.05$ *vs* Mel (5) group. n=10 in each group.

3.4 Effects of naproxen, meloxicam, nimesulide, rofecoxib and valdecoxib on memory (plus maze performance task) As shown in Fig. 3, animals with chronic fatigue showed a significant loss of memory, as indicated by a significant decrease in the percentage of retention of memory, as compared to that in naive group ($P < 0.05$). However, chronic administration of valdecoxib (10 mg/kg), meloxicam (5 mg/kg), nimesulide (5 mg/kg) or rofecoxib (5 mg/kg) could all significantly improve the memory retention, as compared to that in control group.

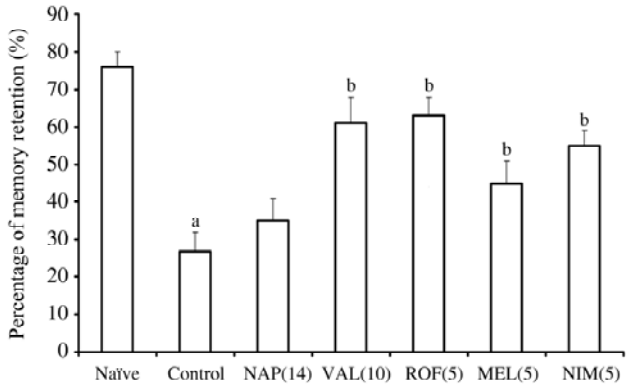

Fig.3 Effects of COX-inhibitors (naproxen, meloxicam, nimesulide, rofecoxib or valdecoxib) on memory, revealed by plus maze performance task. $^aP < 0.05$ *vs* naïve group, $^bP < 0.05$ *vs* control group. n=10 in each group.

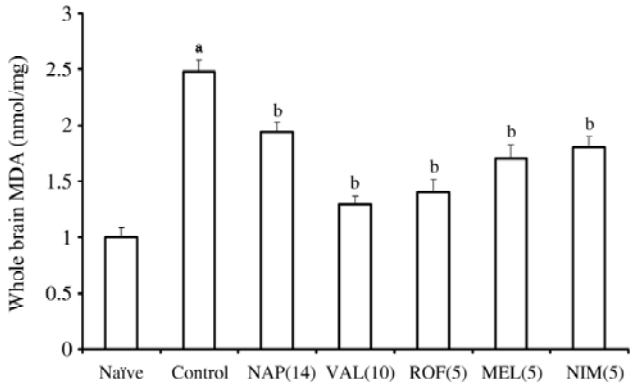

Fig.4 Effects of COX-inhibitors (naproxen, meloxicam, nimesulide, rofecoxib and valdecoxib) on brain lipid peroxidation. $^aP < 0.05$ *vs* naïve group, $^bP < 0.05$ *vs* control group.

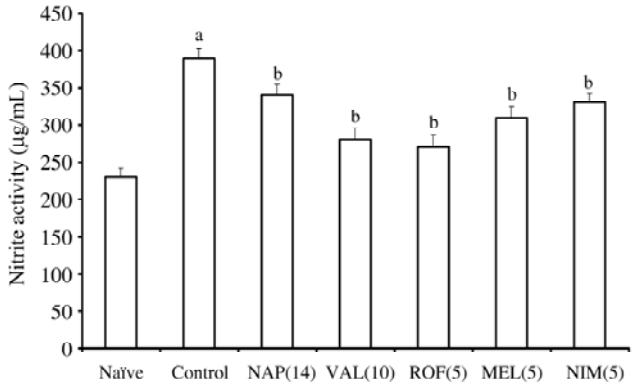

Fig. 5 Effects of COX-inhibitors (naproxen, meloxicam, nimesulide, rofecoxib and valdecoxib) on nitrite activity. $^aP < 0.05$ *vs* naïve group, $^bP < 0.05$ *vs* control group.

3.5 Effects of naproxen, meloxicam, nimesulide, rofecoxib or valdecoxib on the levels of brain lipid peroxidation, nitrite, reduced glutathione and catalase enzyme Chronic forced swimming for 15 d induced oxidative stress as shown by significant increases in the levels of whole brain MDA (Fig. 4) and nitrite (Fig. 5), and reduced the levels of GSH (Fig. 6) and catalase activity (Fig. 7). Chronic administration of naproxen (14 mg/kg), valdecoxib (10 mg/kg), meloxicam (5 mg/kg), nimesulide (5 mg/kg) or rofecoxib (5 mg/kg) significantly attenuated the increases in lipid peroxidation and nitrite level, and further restored the glutathione and catalase enzyme activities, as compared to the control level ($P < 0.05$).

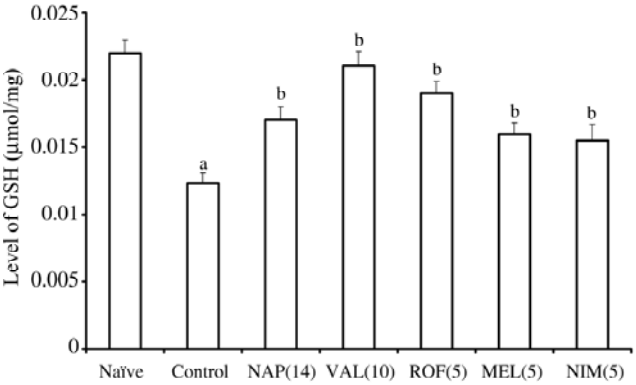

Fig. 6 Effects of COX-inhibitors (naproxen, meloxicam, nimesulide, rofecoxib and valdecoxib) on reduced glutathione levels. $^aP < 0.05$ *vs* naïve group, $^bP < 0.05$ *vs* control group.

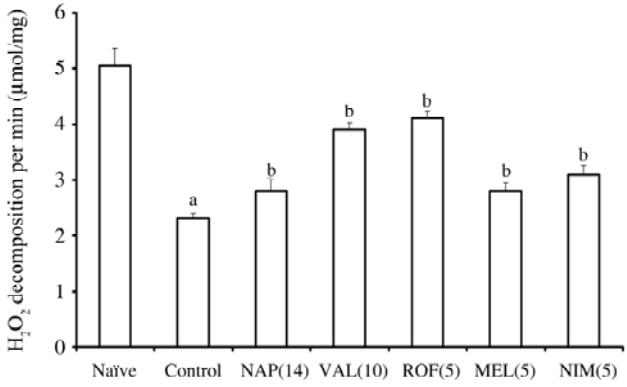

Fig. 7 Effects of COX-inhibitors (naproxen, meloxicam, nimesulide, rofecoxib and valdecoxib) on catalase activity. $^aP < 0.05$ *vs* naïve group, $^bP < 0.05$ *vs* control group.

4 Discussion

Chronic stress is characterized by various symptoms, including feverishness, recurrent sore throat, pain, myalgia, arthralgia, headache and post exercise weakness[25]. Besides, various neuropsychiatry and neuroimmunological disturbances such as difficulty to concentrate, memory deficit, and depression, are also present under chronic stress conditions[26,27]. However, the etiology of chronic stress remains still unclear. Studies in patients with CFS have revealed alterations of the hypothalamic-pituitary-adrenal (HPA) axis[1,27]. Here we investigated the protective effects of COX inhibitors (nimesulide, naproxen, meloxicam, valdecoxib, and rofecoxib) on chronic fatigue-induced stress.

Consistent with other published observations[28,29], 15-d forced swimming could induce CFS-like symptoms, as revealed by increases in despair (increased immobility period) and anxiety-like behaviors, and a reduction in locomotor activity[30]. Here repetition of 6-min session for 15 d caused significant chronic fatigue-like conditions in animals. It is also reported that chronic stress induces depressive behavior (increase in immobility time)[31] and significantly influences exercise and physical activity[32]. However, the underlying mechanisms are not well understood. These behavioral changes might be due to the accumulation of stress, since chronic stress has been well documented to cause anxiety-like behaviors, reduce locomotor activity and lead to stress-induced depression[28]. The impairment of motor activity could be due to the stress-induced depression[33]. It is clear that oxidative stress plays a role in the pathogenesis of motor activity. Hypoactivity of CNS has also been strongly implicated in the pathophysiology of anxiety. Besides, immobilization stress has been reported to induce a 2–3-fold elevation of plasma cortisol level[34], and an increase in cortisol level is correlated with anxiety-like behavior and painful response in human[35]. Moreover, acute stress has been reported to influence the behavioral activities, such as motor activity, anxiety-like activity and depression[36,37]. In the present study, administration of naproxen, rofecoxib and valdecoxib at clinically relevant doses significantly reversed the impairment in locomotor activity, and produced a remarkable neuroprotective effect against chronic stress in mice. Jain *et al.*[38] also report that COX inhibitor could reverse lipopolysaccharide-induced immobility period[38]. In other neurodegenerative disorders, the beneficial roles of COX inhibitors have also been suggested[39]. Here, chronic administration of nimesulide, naproxen, meloxicam, valdecoxib, or rofecoxib attenuated stress-induced decrease in locomotor activity, suggesting that prostaglandin may be involved in chronic stress-induced hypolocomotion.

Consistent with the previous report[40], here we also find that chronic stress could cause anxiety-like behavior and cognitive dysfunctions. When exposed to the mirror chamber, the chronic stressed animals exhibited anxiety, as revealed by decreases in the number of entries in mirror chamber and time spent in mirror chamber. This indicates that chronic stress could significantly induce anxiety-like behavior in animals. Studies in human and animals have shown that stress hormones glucorticoids influence cognition[41,42]. Glucocorticoids have 2 types of receptors, both of which are abundantly expressed in the hippocampus, a region involved in learning and memory and being one of the most vulnerable regions to stress in the brain[42]. In the hippocampus, chronic stress causes atrophy in the CA3 region. It has also been reported that exposure to chronic stress causes subsequent impairment of hippocampus-dependent memory in both human and animals[5]. Furthermore, the hippocampus also regulates the stress response and inhibits the response of the HPA-axis to stress. Chronic stress causes the atrophy of

dendrites of pyramidal neurons in the CA3 region of the hippocampus through a mechanism involving both glucocorticoids and excitatory amino acid neurotransmitters released during and after stress[43]. In the present study, acute immobilization stress caused poor retention of memory, which could be ameliorated by pretreatment of both non-selective and selective cyclooxygenase (COX-2) inhibitors. COX-2 has been reported to play a role in the selective loss of neural connections but not in their formation[44]. Recent study has indicated that COX-2 potentiates brain parenchymal amyloid plague formation, which leads to Alzheimer's disease, suggesting a therapeutic role of NSAIDs in the treatment of neurological diseases[45]. It has been well documented that high levels of stress and fear commonly cause memory loss and cognition disturbance[46].

Stress also stimulates numerous pathways leading to an increased production of free radicals. COX-2 then leads to the release of inflammatory prostaglandins such as PGE_2, which account for the accumulation of oxidative mediators in brain, mitochondrial inhibition[47], increased excitatory amino acid release[48], activation of second messenger systems[49] and decreased efficiency of antioxidant defense mechanisms[50]. CFS causes increased productions of NF-kappa beta, COX-2 and iNOS, and induces damage to membrane fatty acids and functional proteins through ROS and RNS[51]. Clinical reports also document that CFS patients show significantly lower levels of transforming growth factor-beta1 (TGF-beta1) and natural killer (NK) cell production, as compared to the normal control[52,53]. These factors are implicated in the increase of lipid peroxidation level in stress. In addition, forced swimming for 15 d significantly raised lipid peroxidation level and nitrite concentration, and inhibited the levels of reduced glutathione and catalase enzyme activity, thereby inducing oxidative damage. The roles of oxidative stress and related free radical generation in the pathogenesis of CFS have been well documented[4,54]. ROS generated by a severe stressor significantly compromise the *in vivo* antioxidant defense capability when animals are subjected to CFS[55]. Accordingly, chronic oxidative damage alters mitochondrial function, calcium homeostasis, energy pathways, neuronal precursors, neurogenesis, and cell death[56]. In the present study, pretreatment with COX inhibitors could reduce the levels of lipid peroxidation and nitrite, and restore the reduced glutathione level and catalase activity, suggesting their neuroprotective roles against chronic stress. However, there are few adequate reports exploring the protective effects of COX inhibitors in restraint stress and related conditions. Moreover, the discovery of selective COX-2 inhibitors has improved our understanding on COX and its biological properties. Therefore, the exact cellular events in their neuroprotection still need to be explored. An initial formation of large amounts of oxygen and nitrogen reactive species during stress may also initiate lipid peroxidation[57], as has been demonstrated in brain, liver and heart[58]. A positive correlation between nitrite and thiobarbituric acid reactive substance (TBARS) levels has been demonstrated by many reports, revealing that nitric oxide acts as a free radical in stress, and stress results in the expressions of iNOS and neuronal nitric oxide synthase (nNOS)[59,60]. There is a close relationship between NOS and COX pathways because an increased expression of both COX and nNOS have been found during the inflammatory processes. Moreover, nitric oxide has been shown to stimulate COX isoenzyme activity. However, some evidence indicates that CFS is accompanied by increased oxidative stress and that NO pathways are involved in its pathogenesis[61,62]. Moreover, free radical damage by reactive oxygen species (ROS) has been suggested to play a critical role in the pathophysiology of CFS and stress-induced depression[63, 64].

In conclusion, COX-2 selective inhibitors are comparatively more effective. However, variations among the effects of COX-2 selective inhibitors have also been noticed. Moreover, both selective and non-selective COX inhibitors could ameliorate chronic fatigue-induced various behavioral and biochemical alterations in mice, showing protective effects against chronic fatigue-induced stress.

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选择性和非选择性环氧合酶抑制剂对慢性压力小鼠模型具有保护作用

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摘要：目的 环氧合酶是广泛表达于大脑各区域的一类同功酶，主要用于治疗疼痛与炎症。最近研究还发现环氧合酶在大脑相关疾病的病理生理过程中扮演关键角色。本文运用慢性压力动物模型，对环氧合酶抑制剂的保护作用做一探讨。**方法** 每只小鼠每天被迫游泳 6 min，共持续 15 天。结束后进行行为学（包括活动能力、焦虑以及记忆能力）和生化指标（包括脂质过氧化、亚硝酸盐水平、还原性型谷胱甘肽和过氧化氢酶水平）的检测。**结果** 持续 15 天的强迫性游泳会损伤小鼠活动能力，引起焦虑样行为的产生，并削弱记忆力。在生化指标方面，脂质过氧化和亚硝酸盐水平均显著提高，还原型谷胱甘肽和过氧化氢酶活力则显著降低。此外，环氧合酶抑制剂，包括甲氧萘丙酸、罗非考昔、美洛昔康、尼美舒利和伐地考昔，都能显著减缓这些损伤。**结论** 环氧合酶抑制剂可被用来治疗慢性疲劳综合症。

关键词：慢性疲劳综合症；甲氧萘丙酸；伐地考昔；罗非考昔；尼美舒利；美洛昔康