Nimesulide affects antioxidant status during acute lung inflammation in rats

Kiranjit K Sohi and Krishan L Khanduja*
Department of Biophysics, Postgraduate Institute of Medical Education and Research, Chandigarh 160 012, India

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Antiradical activity of nimesulide, a commonly used COX-2 specific inhibitor, was estimated in vitro by 1, 1 diphenyl-2-picyrhydrazy (DPPH) assay, nitroblue tetrazolium reduction assay and lipid peroxidation assay, respectively. The biochemistry of antioxidant functions of nimesulide was also investigated under control and inflammatory conditions, caused by intratracheal instillation of lipopolysaccharide (LPS). Pro-inflammatory conditions generally end up in oxidative insults, which have been suggested to be the cause of multiple organ failure in inflammation. A primary defense, constituted of antioxidant enzymes, against this oxidative damage has evolved in the body. In this study, male Wistar rats were orally administered with nimesulide (9 mg/kg/twice daily for 1 week), followed by intratracheal instillation with 2 μg of LPS and after 18 hr, antioxidant defense system and lipid peroxidation were measured in liver, lungs and kidneys. Nimesulide pretreatment was found to protect the tissue from enhanced levels of lipid peroxidation, and also stimulated the levels of glutathione-S-transferase (GST) in liver and glutathione reductase in kidneys. Surprisingly, nimesulide oral feeding also significantly suppressed superoxide dismutase (SOD) activity in all the three organs. Although, in our study, nimesulide proved to be an inducer of GST (a marker for chemoprevention) and a scavenger of superoxide anions at higher concentrations (> 250 μM), but the relevance of suppression of SOD enzyme activity, which may contribute to the drug’s toxic effects cannot be ignored. The work suggests that further long term studies are needed to confirm nimesulide as a safe drug.

Key words: Nimesulide, antioxidant defense, superoxide anions, lipid peroxidation

Conventional non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in clinical medicine to treat pain and inflammation. However, a serious threat has been posed in their application by their adverse effects ranging from dyspepsia to life threatening perforation and bleeding in gastrointestinal ulcers. Therefore, use of COX-2 specific inhibitors has been recommended recently to reduce these adverse effects. Nimesulide, a NSAID belonging to sulfonamide class, is a preferential COX-2 inhibitor. The drug is already in clinical use for reducing pain, inflammation and fever associated with respiratory tract infections. It also exhibits anticar cinogenic activity in rodents. It could suppress the formation of superoxide anions (O₂⁻) and protect the DNA from oxidative damage in rats under colonic inflammation.

Superoxide anions are one of the potent reactive oxygen species (ROS), which are well characterized etiological factors for the pathogenesis of various diseases. Also, the oxidative damage may be the major cause of multiple organ failure resulting from inflammation. To counteract the devastating effects of ROS, network of antioxidant enzymes have evolved in the body as a primary defense. Certain factors present in the diet or some drugs may directly scavenge the free radicals and/or may enhance the endogenous antioxidant defense system in the body. But, reports on these aspects with respect to nimesulide in relation to inflammation are lacking. Hence, the present study was designed to explore the antioxidant functions of nimesulide in major tissues under control and inflammatory conditions, simulated by intratracheal instillation of lipopolysaccharide (LPS), as it is widely recognized that LPS exposure occurs via respiratory and systemic gram-negative bacterial infections. Elevated respiratory tract exposure to
LPS occurs in a variety of occupational environments. These include grain processing, waste treatment plants and poultry and swine industries. Furthermore, this endotoxin is implicated as a health hazard in indoor air quality. The sick building syndrome was found to be associated with endotoxin contamination. Asthma exacerbation in homes of children allergic to house dust mite depends on endotoxin levels rather than mite levels. Hence, intratracheal instillation of LPS was chosen as a route of endotoxin exposure, which has already been established as a model of lung inflammation. The interesting feature of this study is that nimesulide, though a weak antioxidant, suppressed the activity of SOD enzyme, an important component of antioxidant defense system, in liver, lungs and kidneys of male Wistar rats.

Materials and Methods

Chemicals

Nimesulide, lipopolysaccharide (LPS), nitroblue tetrazolium (NBT), 1,1 diphenyl-2-picrylhydrazyl (DPPH), glutathione reductase (GR) and superoxide dismutase (SOD) were procured from Sigma Chemicals, St. Louis, USA. All other chemicals of analytical grade were procured locally.

Radical scavenging assay

Antiradical activity of nimesulide was determined using DPPH assay as described. Briefly, different concentrations of nimesulide in DMSO were mixed with 50 mM Tris HCl buffer (pH 7.4), containing 0.5 μmoles of DPPH. Decrease in absorbance of DPPH at 517 nm, due to its scavenging, was followed spectrophotometrically up to 20 min. Antiradical activity of nimesulide was compared with trolox, a water soluble analogue of vitamin E, a well known antioxidant. In all in vitro assays, nimesulide was dissolved in DMSO, the final concentration of which was not exceeding 0.5%.

Superoxide anion (O₂⁻) scavenging assay

Various concentrations of nimesulide in 10 μl DMSO were used to estimate O₂⁻ scavenging activity. Superoxide anions were generated by the autoxidation of hydroxylamine hydrochloride as described. NBT reduction by O₂⁻ anions in the absence of nimesulide was taken as 100%. Assay was carried out with or without the presence of 1 IU of SOD enzyme.

Animals

Adult male Wistar rats (150-200 g) were housed in polypropylene cages (3 animals/cage) embedded with rice husk, kept in a temperature controlled room with 12 hr light-dark cycles. Water and standard laboratory chow were made accessible to the animals ad libitum.

Lipid peroxidation studies

Extent of lipid peroxidation was estimated in PMS. Levels of lipid peroxidation both spontaneous as well as induced with 250 μM FeSO₄: 500 μM t-butyl hydroperoxide (tBHP), were estimated in PMS of animals. For in vitro studies, liver PMS was incubated with 100 and 250 μM nimesulide.

NADPH-dependent lipid peroxidation in rat liver microsomes was estimated by the method as described. Microsomes (0.5 mg protein) were incubated with 100 and 250 μM nimesulide for spontaneous lipid peroxidation in a metabolic shaker at 37°C for 30 min. For NADPH-dependent lipid peroxidation, 0.25 μmols of NADPH and 3.0 nmols of FeSO₄ were used both in the absence and presence of nimesulide. Levels of thiobarbituric acid reactive substances (TBARs) were measured spectrophotometrically at 532 nm. Levels were expressed as nmols malondialdehyde (MDA)/mg protein. Protein concentrations were determined by the method of Lowry et al.

Treatments

Animals were divided into four groups: Group 1 (control); group 2 (LPS) received 1% (w/v) CM cellulose as a vehicle for nimesulide through oral administration, twice daily for one week; group 3 (nimesulide); and group 4 (nimesulide + LPS) received nimesulide (9 mg/kg, twice a day, for one week), finely suspended in 1% (w/v) CM cellulose to ensure uniform distribution. This dose was calculated according to human consumption [1.5 mg/kg × 6 (conversion factor)]. Nimesulide suspension was prepared fresh daily.

Intratracheal instillation of LPS

After one week of nimesulide/vehicle treatment, all animals were anaesthetised with a single i.p. injection of pentabarbital (40 mg/kg). Two hundred microlitres of sterile phosphate buffer saline (PBS) containing 2 μg LPS were instilled intratracheally to the animals of groups 2 and 4. Animals of groups 1 and 3 received 200 microlitres of sterile PBS intratracheally as a vehicle.
Tissue preparation

Animals were killed by cervical dislocation under light ether anesthesia, 18 hr after LPS instillation. To minimize diurnal variations, rats were routinely killed between 8.30 and 9.30 hours. Tissues were immediately rinsed, perfused with ice cold normal saline, trimmed and stored in liquid nitrogen till analysed. Tissues were thawed on ice before analysis. All subsequent processing procedures were carried out at 0-4°C. Tissue homogenates (25% w/v) were prepared in 50 mM Tris-HCl buffer (pH 7.4), using motor driven teflon homogeniser. Homogenates were centrifuged for 30 min (4°C, at 12,000 rpm) and the resultant post-mitochondrial supernatant (PMS) fractions were used for various assays.

Antioxidant enzyme assays

Catalase (EC 1.11.1.6) activity was measured as described25. Final reaction mixture contained 50 mM Tris-HCl (pH 7.0), 30 mM of H₂O₂ and 50-100 μg of tissue protein. Decrease in absorbance at 240 nm after the addition of sample was recorded spectrophotometrically for 4 min. Catalase activity was calculated by using extinction coefficient of 43.6 mM⁻¹ cm⁻¹.

Superoxide dismutase (EC 1.15.1.1) activity was measured21 using hydroxylamine hydrochloride-nitroblue tetrazolium system. The rate of NBT reduction by O₂⁻ anions, generated by autooxidation of hydroxylamine hydrochloride, was recorded spectrophotometrically at 560 nm. Activity was expressed as IU/mg protein, where 1 IU activity is defined as the amount of enzyme required to inhibit the rate of NBT reduction by 50%.

Glutathione peroxidase (EC 1.11.1.9) activity was assayed with a coupled enzyme system in which reduction of oxidized glutathione (GSSG) was coupled to NADPH oxidation by glutathione reductase (GR) as described26. Reaction mixture contained 50 mM potassium phosphate buffer (pH 7.4), 1 mM EDTA, 1 mM NaN₃, 0.2 mM NADPH, 1 unit of GR and 1 mM reduced glutathione. After incubation at 25°C for 5 min, the reaction was initiated by adding 50 μl of 12 mM H₂O₂ in final volume of 1 ml. The decrease in the absorbance at 340 nm was recorded spectrophotometrically up to 4 min.

Glutathione reductase (EC 1.6.4.1) assay was carried out by the method reported earlier27. Reaction mixture contained phosphate buffer (pH 6.6), NADPH and GSSG. Reaction was started by adding enzyme source and the decrease in absorbance due to the oxidation of NADPH was measured spectrophotometrically at 340 nm. Glutathione-S-transferase (EC 2.5.1.18) assay was carried out by the method as described28. Reaction mixture contained phosphate buffer, 0.1 ml of 1 mM GSH, 0.1 ml of 1 mM 1-chloro-2, 4 dinitrobenzene (CDNB). Reaction was started by adding enzyme source. Formation of CDNB conjugates was recorded at 340 nm spectrophotometrically for 4 min.

Statistical analysis

Data are represented as mean and the standard deviation from the mean. Statistical evaluation of the difference in mean separation was performed by one way analysis of variance (ANOVA) followed by Scheffe’s test. The level of significance at 95% confidence level was considered at p< 0.05.

Results

Antiradical activity of nimesulide

Antiradical activity of nimesulide was estimated by DPPH assay and compared to trolox. Trolox at 56 μM could scavenge half of DPPH (IC₅₀), when reacted for 20 min. IC₅₀ value for nimesulide could not be calculated, as it did not scavenge DPPH at all (Fig. 1).

O₂⁻ Anion scavenging assay

Ten microlitres of DMSO as a vehicle did not interfere in NBT reduction. Fig. 2 shows that lower concentrations of nimesulide did not affect NBT

![Fig. 1—DPPH scavenging activity of different concentrations of nimesulide (▲) and trolox (■), a water soluble analogue of vitamin E [IC₅₀ for trolox to scavenge DPPH to half of its original concentration was found to be 56 μM, whereas nimesulide did not scavenge DPPH]
reduction by $O_2^-$ anions generated by the chemical system. Nimesulide at concentrations of 250 $\mu M$, 500 $\mu M$ and 1 mM effectively scavenged $O_2^-$ anions by 34%, 56% and 66% respectively. Approx. 850 nmols of nimesulide showed activity equivalent to 1 IU of SOD i.e. the concentration which could inhibit NBT reduction by 50% (Fig. 2). Interestingly, nimesulide at concentrations higher than 250 $\mu M$ worked in synergism with SOD in scavenging $O_2^-$ anions.

**In vitro lipid peroxidation**

To study *in vitro* effects of nimesulide on spontaneous lipid peroxidation in PMS and microsomes, samples were incubated with 100 and 250 $\mu M$ nimesulide for 1.5 hr in case of PMS and for 30 min in case of microsomes. Nimesulide at neither concentration affected the extents of spontaneous lipid peroxidation in PMS (Fig. 3A). Non-enzymatic (tBHP+FeSO$_4$) induction of lipid peroxidation in PMS remained unchanged with nimesulide, but nimesulide could effectively ($p<0.05$) reduce the extent of induced levels of TBARs production when microsomes were incubated in the presence of 0.25 $\mu$mols of NADPH (Fig. 3B).

**Antioxidant defense system**

Table 1 shows the specific activities of various enzymes of the antioxidant defense system in liver, lungs and kidneys. In liver, activities of catalase (CAT), glutathione peroxidase (GpX) and glutathione reductase (GR) remained unchanged in all the four groups. However, the activity of glutathione-S-transferase (GST), an important antioxidant and a phase II drug metabolizing enzyme, was significantly enhanced by nimesulide. LPS treatment could also enhance activity of hepatic GST enzyme significantly. Oral feeding of nimesulide (9 mg/kg/twice daily for 1 week) suppressed the activity of SOD enzyme from 394 $\pm$ 104 IU/mg protein to 254 $\pm$ 17 IU/mg protein. LPS treatment also suppressed SOD activity significantly ($p<0.05$).

In lung tissue, neither LPS nor nimesulide affected the activities of CAT, GpX, GR or GST. However, SOD activity was suppressed significantly by LPS, nimesulide and nimesulide + LPS treatments. In kidneys, nimesulide treatment could significantly enhance the GR enzyme levels, whereas all other enzymes, except SOD remained unaltered by any of the treatments. Activity of SOD was significantly suppressed from 1.88$\pm$0.45 to 1.37$\pm$ 0.14 IU/mg protein by nimesulide treatment. LPS also suppressed the SOD activity to some extent, but it could not attain statistical significance (Table 1).

![Fig. 2—$O_2^-$ Anion scavenging activity of different concentrations of nimesulide with or without 1 IU SOD [NBT reduction in control was taken as 100%. Nimesulide at concentrations higher than 250 $\mu M$ inhibited NBT reduction effectively]

![Fig. 3—Levels of spontaneous and induced lipid peroxidation *in vitro* in the absence ($\oplus$), 100 $\mu M$ ($\odot$) and 250 $\mu M$ nimesulide (●) in PMS (A) and in liver microsomes (B) [Values are means $\pm$ SD of representative of triplicates assays; *Significant as compared to respective control; $p<0.05$, ANOVA followed by Scheffe’s test]
Formation of TBARs

In liver PMS, TBARs levels due to autooxidation at 37°C for 1.5 hr, were higher in LPS-treated animals (1.00 ± 0.102 nmols/mg protein) than in control (0.710 ± 0.11 nmols/mg protein). With nimesulide treatment, these levels, in average, were lesser than control. Nimesulide treatment prior to LPS instillation could arrest the induction of TBARs production by LPS to basal values (Fig. 4A).

In vitro induction of lipid peroxidation by t-BHP : FeSO₄ in animals treated with nimesulide was significantly less than in LPS-treated group. Nimesulide pretreatment prior to LPS instillation could also arrest this induction in lipid peroxidation significantly. Surprisingly, TBARs levels produced due to auto-oxidation of PMS of lungs were significantly higher in nimesulide treated animals, compared to control (Fig. 4B); whereas no difference was observed in induced levels. However, TBARs produced due to auto-oxidation as well as induced by t-BHP : FeSO₄, in PMS of kidneys were the same in all the four groups (data not shown).

Discussion

Oxygen free radicals are believed to contribute to the cellular and tissue injury associated with endotoxin-induced inflammation. Bacterial endotoxin (lipopolysaccharide) results in multiorgan NFκB activation in the pathogenesis of systemic

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Table 1—Antioxidant defense system of rat liver, lungs and kidneys in control and in animals treated with LPS (2 µg), Nimesulide (9 mg/kg twice daily for 7 days) and Nimesulide + LPS

<table>
<thead>
<tr>
<th></th>
<th>Catalase</th>
<th>SOD</th>
<th>GpX</th>
<th>GR</th>
<th>GST</th>
</tr>
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<tr>
<td></td>
<td>(µmols/mg protein)</td>
<td>(IU/mg protein)</td>
<td>(nmols/mg protein)</td>
<td>(nmols/mg protein)</td>
<td>(nmols/mg protein)</td>
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<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>0.218±0.074</td>
<td>394±104</td>
<td>10.8±1.74</td>
<td>39.0±6.50</td>
<td>59±5.4</td>
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<tr>
<td>LPS</td>
<td>0.237±0.055</td>
<td>284±42*</td>
<td>10.2±2.1</td>
<td>41.0±4.60</td>
<td>86.4±6.4*</td>
</tr>
<tr>
<td>Nimesulide</td>
<td>0.213±0.0462</td>
<td>254±17*</td>
<td>9.6±2.0</td>
<td>37.4±0.94</td>
<td>118±25*</td>
</tr>
<tr>
<td>Nimesulide+ LPS</td>
<td>0.192±0.083</td>
<td>254±28*</td>
<td>11.5±2.0</td>
<td>37.5±5.20</td>
<td>130±28*</td>
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<tr>
<td><strong>Lungs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>27.8±4.1</td>
<td>3.56±0.23</td>
<td>14.1±2.30</td>
<td>40.7±4.80</td>
<td>7.98±1.38</td>
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<td>LPS</td>
<td>39.7±7.24</td>
<td>2.88±0.38*</td>
<td>15.9±1.90</td>
<td>32.3±6.19</td>
<td>7.61±1.0</td>
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<td>Nimesulide</td>
<td>33.3±2.2</td>
<td>3.06±0.13*</td>
<td>13.8±1.20</td>
<td>41.6±8.30</td>
<td>7.38±0.89</td>
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<tr>
<td>Nimesulide+ LPS</td>
<td>34.2±6.3</td>
<td>2.25±0.10*</td>
<td>12.1±1.36</td>
<td>43.0±10.0</td>
<td>8.37±1.60</td>
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<td><strong>Kidneys</strong></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>0.39±0.079</td>
<td>1.88±0.4</td>
<td>49.3±6.2</td>
<td>84±18</td>
<td>4.86±0.99</td>
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<tr>
<td>LPS</td>
<td>0.326±0.167</td>
<td>1.45±0.15</td>
<td>50.6±4.0</td>
<td>90.6±16</td>
<td>4.65±1.20</td>
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<tr>
<td>Nimesulide</td>
<td>0.364±0.09</td>
<td>1.37±0.14*</td>
<td>50.2±6.8</td>
<td>130±7*</td>
<td>4.88±0.91</td>
</tr>
<tr>
<td>Nimesulide+ LPS</td>
<td>0.334±0.100</td>
<td>1.27±0.14*</td>
<td>47.1±3.5</td>
<td>118±9*</td>
<td>4.09±0.39</td>
</tr>
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</table>

*Significant as compared to control ; † significant as compared to LPS p<0.05, ANOVA followed by Scheffe’s test
inflammation involving liver, lungs, spleen in a dose-dependent manner\(^2\). Lancaster et al.\(^2\) suggested that a liver-lung interaction in the inflammatory response of lungs to systemic LPS treatment as NFkB inhibition in liver could protect lungs from neutrophilic alveolitis. However, in the present study, we looked for the impact of intratracheally administered LPS on lungs as well as on remote organs like liver and kidneys to explore the inter-tissue interaction under acute lung inflammation, and also to investigate the antioxidant effects of nimesulide, if any, as pro-inflammatory conditions generally end up in oxidative insults, which lead to inflammation.

Nimesulide, in vitro did not exhibit any radical scavenging activity with respect to antioxidant trolox, in DPPH scavenging assay, suggesting that it does not behave as antiradical like some other antioxidants such as vitamin E, polyphenols etc. In in vitro \(\text{O}_2^-\) scavenging assay, nimesulide proved to be a strong scavenger of \(\text{O}_2^-\) anions at high concentrations which is in well accordance with the earlier studies\(^8\). In vitro, it did not abrogate the effects of SOD on NBT reduction. Results of in vitro lipid peroxidation assay suggest that as nimesulide inhibits the process of lipid peroxidation in microsomes in the presence of NADPH. Microsomes are rich in drug metabolizing enzymes of Cyt P\(_{450}\) family. In the presence of NADPH, an electron donor, these enzymes might have led to metabolism of the drug to certain metabolites that might be responsible for these antioxidant functions. Earlier, it has been reported that 4\(^\prime\)-hydroxynimesulide, a major metabolite of nimesulide, is a strong inhibitor of NADPH-mediated lipid peroxidation\(^3\). However, our results on effects of nimesulide OH-mediated lipid peroxidation by Fe/tBHP system differ from their findings on OH-induced depolymerisation of hyaluronic acid. This difference may be due to different assay models.

To further check such antioxidant effects of the drug in vivo, male Wistar rats were made to consume nimesulide for one week as explained earlier. In our study, intratracheally instilled LPS could significantly affect the antioxidant enzymes and lipid peroxidation in remote organs, showing multiorgan involvement in acute local inflammation in lungs. It is generally accepted that LPS stimulates inflammatory cells like neutrophils, macrophages that release a collection of inflammation mediators including cytokines, ROS, proteolytic enzymes and metabolic products of lipid metabolism\(^32,33\). These mediators in addition to their pro-inflammatory effects also induce the synthesis of second wave of cytokines by other cell types\(^34\). These cytokines also diffuse away and influence structure and functions of neighboring cells and tissues\(^34\). Of these mediators, ROS have stimulated considerable interest in recent years as a major mechanism of LPS-induced tissue injury. After 18 hr of instillation, LPS enhanced the levels of hepatic GST, possibly to take care of oxidative stress in LPS-treated animals.

Nimesulide has come out to be an inducer of hepatic GST, as there was 2-fold increase in this enzyme activity. It is well known that GST family of enzymes catalyses the conjugation of a variety of structurally diverse compounds with reduced glutathione. In a way, GSTs inhibit reactive electrophiles from reaching cellular targets. GST levels have been found to be decreased in a number of cancerous conditions\(^35-37\) and induction of these enzymes by synthetic or natural agents is becoming a promising chemopreventive strategy. Nimesulide feeding to animals could protect both spontaneous and induced lipid peroxidation in liver of animals treated with LPS. This protection seems to be taken care by enhanced levels of GST in liver, by direct scavenging of ROS and lipid hydroperoxides.

When PMS of lungs from various groups of animals were incubated at 37°C for auto-oxidation, TBARs levels were significantly higher in nimesulide treated animals, whereas there was no effect on this process by any of the treatment in kidneys. When data was carefully examined, it was found that nimesulide treatment enhanced the levels of GR enzyme in kidneys, whereas it was not the case in lungs. Enhanced levels of GR, which maintain the cell glutathione homeostasis, seem to be responsible for keeping the TBARs levels under control in kidneys, whereas, increase in TBARs due to auto-oxidation in PMS of lungs might have been due to the suppression of SOD enzyme. Results obtained in in vitro experiment revealed that nimesulide is not a direct inhibitor of this enzyme, rather it seems to regulate its activity at transcriptional or translational levels.

SOD enzyme is essential for life as its total or partial inhibition leads to increased susceptibility to oxidative stress and severe dysfunctions of mitochondria resulting from elevation of ROS\(^38\). A decline in MnSOD has been reported in cancer, aging, asthma, progeria and transplant rejection. Also, inactivation of MnSOD leads to accumulation of...
superoxide anions and concomitant increase in peroxynitrite (ONOO⁻), which could lead to tyrosine nitration/oxidation of many key proteins, which ultimately leads to cell death. Besides its inherent toxicity, ONOO⁻ degrades into another reactive intermediate peroxynitrous acid (ONOOH) which finally may degrade to form hydroxyl radicals (OH), the most reactive oxygen species. Evidences implicate oxygen derived free radicals, especially O₂⁻, OH and ONOO⁻ as mediators of inflammation.

ROS can initiate a wide range of toxic oxidative reactions, such as initiation of respiratory peroxidation, direct inhibition of respiratory chain enzymes, inactivation of glyceraldehyde-3-phosphate dehydrogenase, inhibition of membrane Na/K ATPase, inactivation of membrane channels and other oxidative modifications of proteins, DNA and lipids etc. ROS including O₂⁻, OH and ONOO⁻ are capable in initiating DNA single strand breakage with subsequent activation of nuclear enzyme poly (ADP-ribose) synthetase, leading to eventual severe energy depletion of cells and then necrotic cell death. Role of SOD in oxygen derived pulmonary injury evaluated in SOD gene knock-out mice, demonstrates that O₂⁻ produced in mitochondria or extracellularly by infiltrating neutrophils and its derivatives like OH, ONOO⁻ etc. are important mediators of pathogenesis of pulmonary oxygen toxicity in SOD knock-out mice, which were taken care of by restoration of SOD gene. Overexpression of extracellular-SOD has been found to attenuate several markers of lung injury such as lung edema, lipid peroxidation, and myeloperoxidase activity. NfκB controls the transcription regulation of extracellular-SOD in alveolar type II cells. It may be possible that nimesulide inhibits NfκB activation which may lead to inhibition in transcription of SOD gene. But, the hypothesis can only be confirmed after studying NfκB activation by nimesulide oral feeding in different tissues in vivo.

Although in our study, nimesulide has come out to be an inducer of GST, scavenger of O₂⁻ anions at higher concentrations and has shown protective effects against LPS-induced oxidative stress, but inhibition of SOD enzyme in three major organs could not be ignored. This inhibition in SOD may contribute to toxic effects of the drug. However, further extensive studies, need to be carried out on the long term effects of nimesulide, before this COX-2 inhibitor could be confirmed as a completely safe drug.

References