SUMMARY AND CONCLUSION

Release of reactive oxygen and nitrogen intermediates by alveolar macrophages (AMs) comprise an important aspect of their activation process. The present work was designed to evaluate the in vitro as well as in vivo effects of nimesulide, a specific COX-2 inhibitor on the activation of AMs in response to pro-inflammatory agents by estimating the superoxide and nitric oxide radical release. In spite of the extensive use of the drug nimesulide, no information is available on the effects of nimesulide intake on the functions of alveolar macrophages, hence was the aim of present work.

Initially, antiradical activity of nimesulide was estimated by using DPPH, a stable free radical. Nimesulide did not show any antiradical activity. Superoxide scavenging activity of nimesulide was studied in an in vitro model. Nimesulide was able to scavenge superoxide anions at concentrations of 250 μM or higher. It worked in synergism with superoxide dismutase (SOD) enzyme. Approximately 800 nmols of nimesulide showed $O_2^-$ scavenging activity equal to 1IU SOD. Similarly, antioxidant activity of the drug was estimated using lipid peroxidation assay by measuring the levels of malondialdehyde (MDA). Nimesulide did not affect the process of lipid peroxidation in AMs, rat liver homogenate and postmitochondrial supernatant, but it significantly inhibited the process of NADPH-induced lipid peroxidation in rat liver microsomes. The results altogether suggested that some of the metabolite(s) of nimesulide act as a scavenger(s) of OH$^-$ radicals, which initiate the process of lipid peroxidation in the presence of iron.

The study was then extended to the in vitro effects of nimesulide on the production of superoxide anions by intact alveolar macrophages in response to various stimulatory agents like PMA, LPS and TNF-α. PMA was able to stimulate AMs to produce maximum amounts of superoxide ions within 90 min. Nimesulide was found to scavenge superoxide ions produced in response to PMA effectively. LPS and TNF-α could not stimulate the generation of superoxide ions by AMs at any of the time periods studied, suggesting that these molecules take longer durations to activate AMs. But NADPH oxidase, an
Nitrile oxide radical production by stimulated as well as control AMs was estimated in the presence of nimesulide. AMs produced higher amounts of NO' radical in response to LPS and PMA. Nimesulide inhibited the release of NO' radical even in control cells in a somewhat dose-dependent manner. Nimesulide also inhibited the release of NO' in LPS-stimulated, but not in PMA-stimulated cells. To look into the mechanism for these differences, iNOS expression in these cells was studied. It was found that LPS induced the expression of iNOS enzyme, which was inhibited by nimesulide. On the other hand, PMA was unable to induce iNOS expression. TNF-α did not activate the cells to generate higher levels of NO'.

Further, male Wistar rats were fed with 9 mg/kg nimesulide twice a day for seven days, followed by intratracheal instillation with 2 μg LPS. LPS was chosen as a proinflammatory agent as humans and animals are commonly exposed to this agent through the respiratory tract. AMs and other tissues were isolated 18 hr after LPS instillations. Production of superoxide radical, nitric oxide radical and total oxidants by AMs from animals of different groups, was studied. Enhanced production of these oxidants in LPS-treated animals was suppressed to normal levels by nimesulide pretreatment. Even the enhanced expression of iNOS in LPS-treated animals was strongly inhibited by nimesulide pretreatment. Similarly, nimesulide pretreatment protected the AMs from enhanced lipid peroxidation in LPS-treated animals.

For a cell like alveolar macrophage, antioxidant defense system is quite important. Therefore, we studied the status of important antioxidant enzymes like SOD, catalase, glutathione peroxidase, glutathione reductase and levels of GSH, a non-enzymatic antioxidant in bronchoalveolar lavage fluid. LPS treatment enhanced the levels of SOD, which was suppressed to normal by nimesulide. Similarly, levels of GR decreased after LPS treatment which were restored to normal values by prior treatment with nimesulide. Enhanced GSH levels in BAL fluid as a result to LPS treatment were decreased to significant levels by nimesulide pretreatment. Nimesulide pretreatment could attenuate the
alterations in antioxidant defense system caused by LPS instillation. These results suggest that nimesulide pretreatment could control the extent of inflammation/oxidative stress caused by LPS instillation.

Antioxidant defense system and markers for oxidative stress were also studied in lung tissues of rats after various treatments. Alongwith lungs, remote organs like liver and kidneys were also analysed as liver is the major drug metabolizing organ and kidneys are the major route of excretion of drugs from the body. Oral feeding of nimesulide significantly inhibited the activity of superoxide dismutase in lungs, liver and kidneys. In addition, nimesulide feeding induced the levels of glutathione S-transferase in liver and glutathione reductase in kidneys, which might be the result of the action of different metabolites. Surprisingly, nimesulide feeding led to higher MDA formation in lungs, whereas in liver and kidneys this was not the case. The higher MDA levels in the lungs may be due to suppression of SOD enzyme by nimesulide, which might have led to accumulation of superoxide ions, leading to formation of peroxinitrite, a strong oxidizing agent. In case of liver and kidneys, this effect of suppressed SOD might have been attenuated by enhanced levels of GST and GR respectively.

To conclude, the drug could effectively control the production of pro-inflammatory oxidants and attenuate the markers of oxidative stress associated with them. The drug has also exhibited chemopreventive potential, being the inducer of phase-II detoxification system (GST). But the suppression in SOD enzyme in three major organs after nimesulide oral feeding can't be ignored as SOD is very crucial for life. Further long term studies are required to look into the adverse effects associated with this suppression of SOD activity. Besides this, results have raised a query (1): If PMA did not induce iNOS, then what was the source of NO release; (2) while AMs do have receptors for TNF-α, then why TNF-α did not act on AMs?