

Effect of non steroidal anti-inflammatory drugs on inhibition of nitrite-induced methemoglobin formation

P. B. Miniyar, S. S. Toshniwal¹, R. S. Toshniwal¹, S. U. Deoda², S. J. Makhija³

Department of Pharm. Chemistry, Sinhgad Institute of Pharmacy, Narhe, Pune – 411 041, ¹Department of Pharm. Chemistry, Y.B. Chavan College of Pharmacy, Aurangabad - 431 001, ²Department of Biochemistry, SSUP College of Nursing, Aurangabad - 431 001, ³Department of Biochemistry, S. B. College of Science, Aurangabad - 431 001, India

Paracetamol, Diclofenac, Aceclofenac, Nimesulide, Ibuprofen and Rofecoxib belonging to the class of non-steroidal anti-inflammatory drugs (NSAID's) were tested for inhibitory role on nitrite-induced oxidation of hemoglobin *in vitro*. Result indicates a promising inhibitory role of aceclofenac and significant inhibitory role of ibuprofen in a concentration range of 0.25 mM each. Pro-oxidant activity was higher for remaining test compounds *viz.*, Diclofenac, Paracetamol, Rofecoxib and Nimesulide.

Key words: Free radical, methemoglobin, nitrite, NSAID

INTRODUCTION

Hemoglobin is subjected to severe oxidant stress. When hemoglobin binds to molecular oxygen, there is an accompanied risk of superoxide production along with the oxidation of hemoglobin to methemoglobin.^[1] There are several inherent antioxidant defense mechanisms that prevent methemoglobin formation. Superoxide dismutase, catalase, ascorbic acid and glutathione peroxidase constitute a few of these endogenous antioxidants.^[2] Oxidation of hemoglobin to methemoglobin occurs in response to a variety of chemical stimuli, which include drugs like primaquine, dapsone and environmental pollutants like nitrogen dioxide. The endogenous antioxidant defense system present in our body maintains the methemoglobin within one percent. Many antioxidants such as ascorbic acid, uric acid, 3-ribosyluric acid and glutathione are reported in literature.^[3] Curcumin, an established free-radical scavenger, protects hemoglobin against nitrite-induced-oxidation.^[4]

5-amino salicylic acid (5-ASA) and analogs have been already established as scavengers of several reactive oxygen species (ROS) such as superoxide dismutase.^[1] 5-ASA strongly inhibits nitrite-induced oxidation of hemoglobin in human blood-hemolysates.^[5,6] The objective of the present work is to study the effect of currently used NSAID's on inhibition of nitrite induced methemoglobin formation. Hence, paracetamol, diclofenac, aceclofenac, nimesulide, ibuprofen and rofecoxib these (NSAID's) were tested for inhibitory role

on nitrite-induced oxidation of hemoglobin *in vitro*.

MATERIALS AND METHODS

Blood samples were collected from the Pathology Dept. of SKN Hospital, Pune and were centrifuged (2000 rpm for 10 min) to remove the plasma and buffy coat of white cells. Erythrocytes thus obtained were suspended in phosphate buffer saline for 5 min and centrifuged again to remove the supernatant liquid containing white blood cells. The same procedure was repeated twice. The packed cell thus obtained above were suspended in 20 volumes of 0.7% sodium chloride solution for 30 min at room temperature and then centrifuged at 2000 rpm for 15 min to remove membrane and cell debris. The resulting solution (hemolysate, brilliant red color) was diluted with 0.7% sodium chloride solution to yield a final concentration of oxyhemoglobin suitable for spectrophotometric analysis. (Absorbance 0.5 at 577 nm and also scanned between 700 to 500 nm to get maximum absorbance and little or minimum peak at 631 nm)

Hemolysate prepared as shown above was incubated with different concentrations of test compounds (*viz.* 0.05 to 0.25 mM) for different time intervals (0 to 15 min). Curcumin was used at a concentration of 20 μ m. Compounds were added to the reaction mixture by dissolving them in ethanol. Controls contained an equivalent amount of ethanol. Hemoglobin exhibits maximum absorption at 577 nm and 560 nm while methemoglobin absorbs at 631 nm. Formation of

For correspondence: Dr. P. B. Miniyar, Department of Pharm. Chemistry, Sinhgad Institute of Pharmacy, Narhe road, Narhe, Pune – 411 041, M.S, India. E-mail: pankajmpharm@yahoo.co.in

Received: 28-05-2008; **Accepted:** 24-07-2008

Table 1: Absorbance at 631 nm of the NSAID's tested at 0.25 mM concentrations

	Acetoclofenac	Diclofenac	Paracetamol	Ibuprofen	Rofecoxib	Nimesulide
Blank	0.3456	0.3456	0.3456	0.3456	0.3456	0.3456
Conc. 0.25 mM	0.0989	0.4896	0.4123	0.2355	0.4123	0.4599

methemoglobin (induced by addition of sodium nitrite at a final concentration of 300 mM) was estimated by monitoring the absorbance at 631 nm using Jasco UV 430 spectrophotometer. Day to day variation was significant and results are representative samples drawn from trials.

RESULTS AND DISCUSSION

Preliminary investigations indicate that Aceclofenac inhibits nitrite-induced oxidation of hemoglobin. The highest concentrations of Aceclofenac were 0.25 mM, which gave good inhibition as compared to blank and other compounds tested [Table 1; Fig. 1]. The results are consistent at different time intervals tested *viz.* 0-15 min. However, curcumin showed a more significant activity even at lower concentration levels than Aceclofenac (The results are not included because the concentrations used were not comparative; curcumin concentration was kept low in the range of 20 μ m because of solubility problems). From various compounds tested, Ibuprofen also showed the inhibitory activity but less than that of Aceclofenac. On the other hand, Diclofenac, Paracetamol, Nimesulide and Rofecoxib enhanced the formation of methemoglobin at a concentration of 0.25 mM. The present investigation requires detailed studies under different experimental conditions especially *in vivo*. It is also important to confirm the results in purified hemoglobin. Since Aceclofenac, Ibuprofen and its structural analogues are prescribed drugs, results have therapeutic significance.

ACKNOWLEDGMENT

The authors are thankful to Prof. M. K. Unnikrishnan (College of Pharmaceutical sciences, Manipal) for continuous support and encouragement in setting up the experiment.

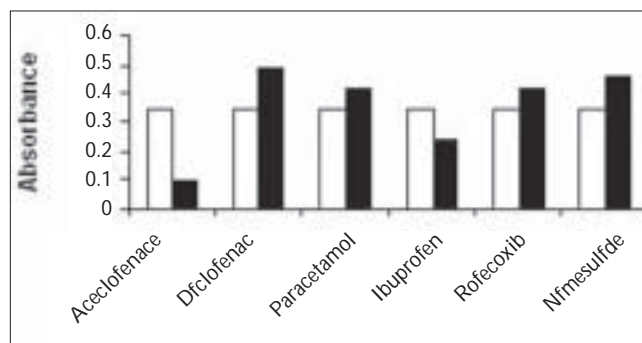


Figure 1: Inhibition of nitrite-induced methemoglobin. Results are expressed as absorbance at 631 nm (lambda max of methemoglobin) recorded at 15 min in hemolysate containing test compounds (0.25 mM) treated with nitrite. (Control bars [blank] are shown for comparison)

REFERENCES

1. Miles AM, Grisham, MB. Antioxidant properties of aminosaliclates. *Methods Enzymol* 1994;234:555-72.
2. Doyle MP, Pickering RA, Dykstra RL, Nelson CL, Boyer RF. Involvement of peroxide and superoxide in the oxidation of hemoglobin by nitrite. *Biochem Biophysics Res Commun* 1982;105:127-32.
3. Smith RC, Nunn V. Prevention by 3-N-ribosyluric acid of the oxidation of bovine hemoglobin by sodium nitrite. *Arch Biochem Biophys* 1984;232:348-53.
4. Unnikrishnan MK, Rao MN. Inhibition of nitrite-induced oxidation of hemoglobin by curcuminoids. *Pharmazie* 1995;50:490-2.
5. Unnikrishnan MK, Rao MN. Curcumin inhibits nitrogen dioxide induced oxidation of hemoglobin. *Mol Cell Biochem* 1995;146:35-7.
6. Miniyaar PB, Mahesh TG, Unnikrishnan MK. 5-Amino Salicylic Acid Inhibits Nitrite Induced Methemoglobin Formation, *I.J.P.S.*, Nov.-Dec., 2000;466-8.

Source of Support: Nil, **Conflict of Interest:** None declared.