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5-Amino Salicylic Acid Inhibits Nitrite-Induced Methemoglobin Formation

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5 Amino salicylic acid (5-ASA) and several structurally-related analogs were tested for inhibitory role on nitrite-induced oxidation of hemoglobin in human blood-hemolysates. Results indicate a strong inhibitory role for 5-ASA in a concentration range of 0.2 to 0.8 mM. Although the inhibitory activity of 5-ASA was higher than other structural analogs tested, the activity of curcumin was much higher at equimolar levels. PABA also showed some activity at similar concentrations although it was lower than that of 5-ASA. Benzoic acid showed marginal activity at lower concentrations. On the other hand, acetyl salicylic acid and salicylic acid increased the methemoglobin levels in hemolysate. The pro-oxidant activity was higher at higher concentrations for these compounds. Higher level of activity of 5-ASA is consistent with previous findings. As acetyl salicylic acid and 5-ASA are currently being prescribed, it is worth investigating their *in vivo* activity in order to establish novel and hitherto unexplored clinical applications.

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 methemoglobin¹. There are several inherent antioxidant defense mechanisms which prevent methemoglobin formation. Superoxide dismutase, catalase, uric acid, ascorbic acid and glutathione peroxidase constitute a few of these endogenous antioxidants². In spite of this, oxidation of hemoglobin to methemoglobin occurs in response to a variety of chemical stimuli which include drugs like primaquine and dapsone and environmental pollutants such as nitrogen

dioxide. The endogenous antioxidant defense system present in our body maintains the level of methemoglobin within one percent. Many antioxidants such as ascorbic acid, uric acid, 3-ribosyluric acid and glutathione have been found to protect hemoglobin from nitrite-induced oxidation³. Nitrite oxidizes hemoglobin in two stages, viz. a slow stage followed by a rapid autocatalytic stage involving superoxide anion, hydrogen peroxide and nitrogen dioxide. Curcumin, an established free radical scavenger, protects hemoglobin against nitrite-induced oxidation^{4,5}.

There is a great deal of evidence to show that the antioxidant activity of 5 amino salicylic acid (5-ASA) is

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important in its mechanisms of action against ulcerative colitis which has a free radical pathophysiology. 5-ASA and a close analog, sulphasalazine are currently prescribed extensively in inflammatory diseases. 5-ASA scavenges hydroxyl radicals and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and also inhibits the lowering of reduced glutathione levels¹. 5-ASA and analogs have been already established as scavengers of several reactive oxygen species such as superoxide dismutase¹. The chief objective of this work was to identify new uses for 5-ASA, a drug currently in use. Finding novel therapeutic applications for existing drugs is an ideal strategy for poor countries.

5-ASA and several structurally-related analogs were tested for inhibitory role on nitrite-induced oxidation of hemoglobin in human blood-hemolysates. Blood samples were centrifuged (2500 g, 20 min) to remove the plasma and the buffy coat of white cells. Erythrocytes thus obtained were washed three times with phosphated buffered saline and were hemolysed by suspending them in 20 volumes of 20 mM phosphate buffer (pH 7.4). The hemolysate was then centrifuged at 2500 g for 1 h. to remove the membranes and cell debris. The resulting solution was diluted with phosphate buffer (pH 7.4) to yield a final concentration of oxyhemoglobin suitable for spectrophotometric analysis.

Hemolysate prepared as shown above was incubated with different concentrations of test compounds (viz, 5-ASA, 5-ASA, para amino benzoic acid, acetyl salicylic acid, salicylic acid and benzoic acid) 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 volume of ethanol. Hemoglobin exhibits maximum absorption at 577 nm and 560 nm while methemoglobin absorbs at 631 nm. Formation of methemoglobin (induced by sodium nitrite at a final concentration of 300 μ M) was estimated by monitoring the absorbance at 631 nm using a Shimadzu Graphicord UV 240 spectrophotometer⁴. Day to day variations were significant and results are representative samples drawn from a number of trials.

Preliminary investigations indicate that 5-ASA inhibits nitrite-induced oxidation of hemoglobin. Figure 1 shows a dose-dependent decrease in absorbance at 631 nm in the hemolysate treated with 5-ASA. The highest concentration of 5-ASA, was 0.89 mM which gave a significant

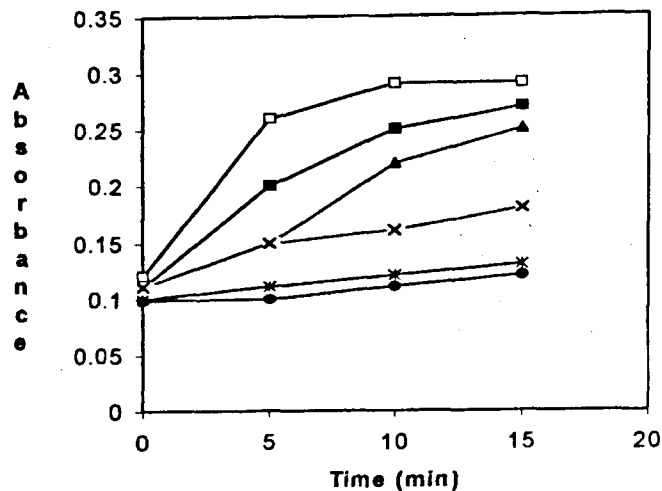


Fig. 1 : Inhibition of nitrite-induced oxidation of hemoglobin by 5-ASA

Results are expressed as absorbance at 631 nm (absorption maximum of methemoglobin) recorded at different times in hemolysate containing, 0.0 mM (□), 0.05 mM (■), 0.1 mM (▲), 0.15 mM (×), 0.2 mM (*) and 0.25 mM (●) 5-ASA treated with nitrite

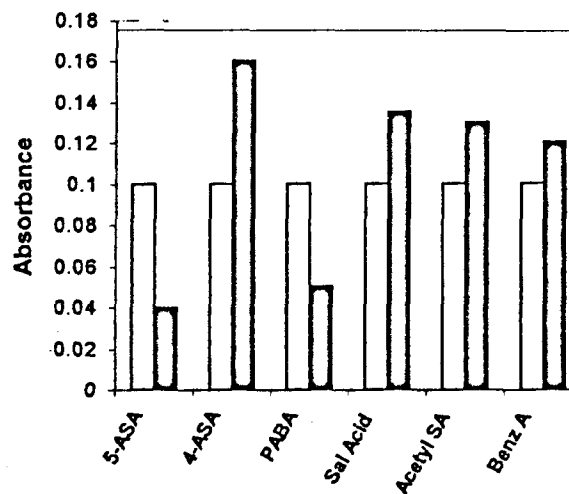


Fig. 2 : Inhibition of nitrite-induced methemoglobin

Results are expressed as absorbance at 631 nm (lambda max of methemoglobin), recorded at 10 minutes in hemolysate containing test compounds (0.89 mM) treated with nitrite. (Control bars [blank] are shown for comparison)

inhibition. The results are consistent at different time intervals tested, viz. 0 to 15 min. However, curcumin showed a more significant activity even at lower concentration levels than 5-ASA. (The results are not included

because the concentrations used were not comparative. Curcumin concentrations were kept low in the range of 20 μ M because of solubility problems). Of the other compounds tested, PABA showed maximum activity although it was lower in potency compared to 5-ASA. On the other hand, 4-ASA, acetyl salicylic acid, salicylic acid and benzoic acid may even enhance the formation of methemoglobin at a concentration of 0.89 mM (Fig. 2). The absorbance values at 631 nm are significantly above the control values ($P \leq 0.05$).

The results partly corroborate previous reports of antioxidant activity of salicylate¹. For example, inhibition of lipid peroxidation was much higher with 5-ASA than all the other tested derivatives, including sulphasalazine and sulfapyridine¹. 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 5-ASA and its structural analogs are prescribed drugs, results have therapeutic significance.

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Visible Spectrophotometric and HPLC Methods for the Estimation of Sertraline Hydrochloride from Tablet Formulations

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One visible spectrophotometric and one HPLC method has been developed for the estimation of sertraline hydrochloride from tablet formulations. Developed visible spectrophotometric method is based on the formation of chloroform extractable coloured complex of drug with nitrosonaphthol. The coloured complex shows absorbance maxima at 441.5 nm. Beer's law was obeyed in the concentration of 20-100 μ g/ml of sertraline hydrochloride. Developed HPLC method was a reversed phase chromatographic method using Intertsil C₁₈ column and methanol:acetate buffer (pH 2.8)::80:20 as mobile phase with detection at 220 nm. Caffeine was used as an internal standard and linearity was observed in the concentration range of 10-250 μ g/ml of sertraline hydrochloride for HPLC method. Results of analysis for both the methods were validated statistically and by recovery studies.

Sertraline hydrochloride, chemically (1S-cis)-4-(3,4-dichlorophenyl)-1,2,3,4 tetrahydro-N-methyl-1-naphthalenamine hydrochloride, is an antidepressant agent¹. For the estimation of sertraline hydrochloride from biological fluids, one GC² and four HPLC^{4,7} methods have

been reported. However, none of these methods report the estimation of the drug from formulations. An attempt has been made in the present study to develop a simple visible spectrophotometric and an HPLC method for the analysis of sertraline hydrochloride from tablets.

A Jasco UV/visible recording spectrophotometer with

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