Effect of Vitamin A-ComP on Heme catabolism

Ramesh Chandra*, Mukta Dhawan and Rajni Malhotra
B.R.Ambedkar Center for Biomedical Research, University of Delhi, Delhi-110007, India.

The formation of Bilirubin from Biliverdin, a reductive process occurs so rapidly that no appreciable levels of biliverdin normally accumulate. Bilirubin is produced in the reticuloendothelial cells of the Spleen, Liver and Bone Marrow. Heme Oxygenase (HMOX) [6] is the regulatory enzyme of heme catabolism, catalyses the specific cleavage of heme at the alpha-methylene bridge, with the formation of stoichiometric amounts of biliverdin IXa, CO and iron. Recently, HMOX has been reported to be a major 32 kd stress protein, which shows change in its activity by a number of environmental pollutants, natural and synthetic hormones and dietary constituents like Proteins, Carbohydrates, Vitamins etc. One of the dietary constituent, Vitamin A has been shown to increase the splenic HMOX activity.

Male Male Wistar rats (10 days old) with a weight range of 25-30 g were fasted for 24 hrs., but were allowed free access to water. After 48 hrs. animals were divided into 3 groups with 8 animals in each group. Group I: 20 µl of groundnut oil was orally given to animals of this group. Group II: Animals in this group were orally given 10,000 I.U. of retinoic acid (RA) dissolved in groundnut oil (1 mg of RA is equal to 3333 I.U.). Group III: Animals in this group were given 10,000 I.U. of RA and 50 µmol/Kg b.w. of Cobalt Mesoporphyrin (CoMP), was simultaneously injected subcutaneously to these animals. After 48 hrs. animals were sacrificed and spleens were removed, cleaned with saline and homogenised in a Potter-Elvehjem type glass homogenizer in 3 volumes of 0.1 M potassium phosphate buffer (pH 7.4) having 0.25 M sucrose [1]. Homogenate was centrifuged at 10,000g for 10 min., its supernatant at 20,000g for 20 min. and again its supernatant obtained at 105,000g for 60 mins. at 4°C to get the microsomal fraction. Pellet was suspended in 0.1 M potassium phosphate buffer (pH 7.4) [2]. Microsomal pellet was used as the source of HMOX, which was assayed by the method of Frydman et al. [3].

Regulatory enzyme of heme catabolism is affected by various factor such as - Vitamin A which has a major role in visual cycle, and plays a significant role in maintaining the integrity of cell membrane and normal cell growth. It is also required for the mobilisation of iron from the liver. Therefore, we have studied the effect of dietary factor, Vitamin A on the Heme catabolism in the spleen. Vitamin A, which was orally given (10,000 I. U.) to the animals acts as a stress factor for HMOX which is shown by increase in the activity of this enzyme. Our results clearly indicate that the excess of vitamin A leads to induction of HMOX activity by ~ 2-fold. Induction of HMOX activity leads to depletion of cytochrome P-450, thus the drug metabolism of the body is suppressed due to hyperbilirubinemia.

Our results also indicate that heme analogue, CoMP in association with Vitamin A inhibit the HMOX activity, which was induced significantly by excess Vitamin A. Hence, it can be concluded that excess of dietary Vitamin A is toxic, further increases the bilirubin level and thereby further increasing neonatal Hyperbilirubinemia. Inhibition of heme degradation by agents as Tin Mesoporphyrin (SnMP) leads to the prompt excretion into bile of unmetabolised heme [4,5]; the same may be true in our study [6]. Since SnMP, administered can also potentially inhibit intestinal HMOX [7], the possibility is raised that the mechanisms of development of iron deficiency, anemia after prolonged CoMP Vitamin A administration reflects, at least in part, diminished absorption of heme and thus of heme iron from the intestinal lumen. Hemoglobin is an important source of dietary iron, which is released from the heme ring by the action of HMOX [8]. The effects in vivo of high doses of CoMP on P-450 mediated monoxygenases activities may be due in part to a decrease in HADPM cytochrome P-450 reductase activity. Our earlier studies had shown that at a dose that marginally affect total P-450 content, the activity of the reductase is appreciably decreased. This is in contrast to the conclusion of Speathe and Jollow [9] that CoPP is highly selective for cytochrome P-450. The P-450 can be almost obliterated by high doses of CoPP, but not so much by CoMP. Thus cytochrome may not be the limiting component in studies that use cobalt-substituted porphyrins to inhibit P-450 in vivo. This is especially likely if the systematic concentration of inhibitor is allowed to decrease [10]. These actions of heme oxygenase inhibitors could thus be of potential experimental and clinical significance in certain iron overload diseases.

Authors (MD & RM) thank the UGC, New Delhi for the award of Junior Research Fellowships. The author (RC) thanks the UGC, for Career Award and Research Scientist Award, and The Rockefeller Foundation, USA for Biotechnology Career Award.

References
10 Yanni, S.B. et al. (1993) Chemico-Biological Interactions 89, 73-87