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THE EFFECTS OF FREE RADICALS ON COBALAMIN AND IRON

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The aim of this communication is to show the means by which free radicals could deleteriously alter the metabolism of cobalamin (vitamin B12) and iron in their attempt to protect the body against neoplasia or inflammation and in doing so, create the anaemia of chronic disease.

KEY WORDS: Cobalamin, iron, anaemia

COBALAMIN

It has been known for some time that the anaesthetic gas nitrous oxide administered with various concentrations of oxygen produces megaloblastic anaemia in man, especially when given over a long period of time as in the management of tetanus.[1,2] Subsequently it has been shown that the gas in aqueous solutions reacts with hydrated electrons to generate the hydroxyl radical.[3]

$$N_2O + e_{aq} \xrightarrow{H_2O} N_2 + OH^- + {}^{\cdot}OH$$

These radicals oxidize transitional-metal complexes such as the cobalt complex in the cobalamin derivatives and prevent the formation of the active form (methylcobalamin, MeCbl), hence the development of megaloblastic anaemia in their presence. When rats were exposed to various concentrations of nitrous oxide for 1–15 hours and their livers assayed immediately thereafter for the two cobalamin-dependent enzymes, the gas was found to have deleterious effect on the enzymes: methionine synthetase which is involved in methionine synthesis from homocysteine and methylmalonyl CoA-mutase responsible for isomerization of methylmalonyl CoA to succinyl CoA.[4]

In my laboratory, the deleterious effect of nitrous oxide *in vitro* on another cobalamin-related enzyme, thymidylate synthetase (TS), was studied.[5] This enzyme was chosen because previously we have shown that the enzyme was absent in phytohaemagglutinin (PHA)-stimulated lymphocytes of patients with cobalamin deficiency and that the activity of the enzyme returned following treatment with the vitamin.[6] Nitrous oxide by its production of free radicals as stated above could cause a state of cobalamin deficiency. In 14 experiments, normal human lymphocytes were cultured with PHA on day 0. The cells were exposed to nitrous oxide and oxygen (1:1 ratio) delivered from an anaesthetic machine on day 2 for ten minutes; methylcobalamin was delivered immediately thereafter to half of the cells. Untreated cells served as controls. On day 3 the cells were harvested, washed and lysed. After centrifugation, the extracts were assayed for TS in triplicate according to the method of Kammen.[7] Briefly stated, the enzyme activity is the result of the conversion of deoxyuridine

monophosphate (containing [5-^{3}H] dUMP) to thymidylic acid in the presence of 5,10-methylenetetrahydrofolic acid with the release of a tritium atom. The radioactivity of the latter, ^{3}H-fraction after separation from [5-^{3}H] dUMP, expresses the activity of the enzyme. The results of the 14 experiments were as follows: the geometric mean for the activity of TS in normal cells was 61 pmole per 10^6 cells (100% activity). It dropped to 19 pmole per 10^6 cells (31%, $p < 0.001$) when the cells were exposed to nitrous oxide. The cells exposed to the gas and methylcobalamin had a geometric mean activity of TS of 40 pmole per 10^6 cells (66%, compared to the above 31% $p < 0.001$). In none of the experiments was there a directional change of the results from one set of experiments to the next. There was no statistical significance in the thymidine or deoxyuridine incorporation by any of the three groups indicating that all the groups were growing well and that nucleoside incorporation was a poor index of TS activity.

IRON

Each person needs daily about 30 to 40 mg of iron in order to maintain a normal haemoglobin concentration in the blood. Food provides about 10 mg, 1–2 mg of which are absorbed and the rest is derived from the reticuloendothelial system (RES) following the digestion of effete red cells. Therefore this reutilization of iron is an important mechanism in the homeostatis of haemoglobin synthesis and accounts for at least 80 percent of the plasma iron turnover (30 to 40 mg).[8] The movement of iron in the RES (the macrophage) has proved elusive to investigate. A hypothesis that this author suggests to describe the traffic of iron in this system is based on indirect evidence. Under normal conditions iron is released from the effete red cell in the macrophage in a ferric state although its initial release from haeme by haeme oxygenase is in a ferrous state.[9] Ferric iron could react then with a transferrin species known to be produced by the cell[10] and form what is called the labile or pre-release iron pool. From the cell membrane it is carried by plasma transferrin to the bone marrow for hemoglobin synthesis where another cycle of reutilization is started. However, under abnormal conditions, such as inflammation or malignancy with generation of free radicals, the traffic of iron in the macrophage undergoes a detour. Generation of superoxide ($O_2^{\cdot -}$) reduces iron as it is released from the red cell thus allowing it to enter ferritin, one of two storage proteins of iron.[11] Alternatively the presence of other free radicals could oxidise iron to the ferryl state[12] which consequently is trapped by hemosiderin, the second storage protein of iron. The increase of haemosiderin iron may easily be visually appreciated by the Prussian blue stain of the bone marrow. This hypothesis can neatly explain the triad that is characteristic of anaemia of chronic disease where free radicals are produced. The triad consists of: increased haemosiderin iron, decreased serum iron (hypoferraemia) anad anaemia.[13]

In this respect, it is suggested that the body in the anaemia of chronic disease, protects itself against neoplastic cells or invading organisms by reducing the availability of the active forms of cobalamin and iron to these invaders through the production of free radicals.

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