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Maiko Ochi, ND, James Hetherington, ND, and Davis W. Lamson, MS, ND

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The Concern about B-Vitamins Affecting the Oxidant Effect of Intravenous Ascorbate for Malignancy

Maiko Ochi, ND, James Hetherington, ND, and Davis W. Lamson, MS, ND

Abstract
The use of intravenous ascorbate has a long history in complementary medicine. Its efficacy against malignant cells via a pro-oxidant mechanism has been previously demonstrated. In some quarters, B-vitamins have been included with intravenous ascorbate therapy. Because of the antioxidant effect of some B-vitamins, the question arose as to whether their presence could decrease the anti-malignant effect of ascorbate. The data regarding the direct ability of several B-vitamins to decrease the concentration of the active agent providing the anti-malignant effect are summarized. The individual case of cobalamin in this regard is more complex than other B-vitamins, in that cobalamin and ascorbate generate hydrogen peroxide and kill tumor cells in vitro. The implications of this result certainly warrant in vivo studies. The overall conclusion is that data do exist demonstrating that some B-vitamins do have the capacity to decrease the concentration of the anti-malignant agent from ascorbate at the tissue of concern. The authors recommend that B-vitamins or other antioxidant materials not be included with intravenous ascorbate intended for anti-cancer purposes. (Altern Med Rev 2011;16(Supp):1S-5S)

Introduction
There is adequate in vitro demonstration that ascorbate, at concentrations attainable in humans, can have a cytotoxic effect on malignant cells.1,2 There is observation that intravenous ascorbate has benefited cancer patients.3-7

The proposed mechanism for the cytotoxic effect of ascorbate begins with the donation of an electron from ascorbate to molecular oxygen creating superoxide radical anion, followed by conversion to hydrogen peroxide by several mechanisms. It is postulated that the initial electron transfer proceeds first to iron (III), which reduces to iron (II), which then transfers an electron to oxygen (See Figure 1).2

Because of previous demonstrations of the antioxidant properties of several of the B-vitamins, the question arises whether concurrent administration of B-vitamins with intravenous ascorbate could be deleterious to the desired cytotoxic effect of ascorbate on malignant cells. Such concern was one factor leading to the Drisko-Khosh protocol for administration of intravenous ascorbate in the Program in Integrative Medicine at the University of Kansas Medical Center (ascorbate, magnesium chloride and sterile water). (See acknowledgement).
The following sections cite data found on the interactions of certain B-vitamins with superoxide or hydrogen peroxide, the intermediate agents believed chiefly responsible for the effect of ascorbate on malignant cells.

Thiamine – Vitamin B1
There are many publications on the antioxidant effects of thiamine under various conditions. The one most to the point at hand showed the scavenging ability of thiamine for superoxide (generated by the xanthine oxidase/hypoxanthine system) to be dose-dependent and able to be driven to completion under the in vitro experimental conditions. It was suggested that thiamine was sacrificed to some extent in the course of scavenging reactive oxygen species (ROS).8 A previous report demonstrated thiamine quenching of superoxide generated by pyrogallol autoxidation. It was also stated that no appreciable reaction of thiamine with hydrogen peroxide occurred, but no data was provided.9
Riboflavin – Vitamin B2
Direct and uncomplicated effects of riboflavin on hydrogen peroxide or superoxide in vitro were not able to be located.

Nicotinamide (Nicotinic Acid) – Vitamin B3
A 2000 publication referred to a 1969 report, which stated that nicotinamide had a high rate constant for reaction with superoxide (7 X 10^9).\(^{10,11}\) However, a 1999 publication determined that nicotinamide did not scavenge superoxide generated in vitro.\(^{12}\)

A 1995 report showed that superoxide generated by autoxidation of pyrogallol was quenched by nicotinic acid only at supraphysiologic concentrations. It was further stated that there was no appreciable reaction of nicotinic acid with hydrogen peroxide, but data was not provided.\(^{9}\)

Pantothenic Acid – Vitamin B5
Pantothene was stated to have no quenching effect on superoxide generated by pyrogallol autoxidation until a supraphysiologic concentration of 3.0 mM was reached. However, the table provided in the publication showed a small effect at 1.0 and 2.0 mM, with no entry for 3.0 mM. It was further stated that pantothene had no appreciable reaction with hydrogen peroxide.\(^{9}\)

Pyridoxine – Vitamin B6
In 1995, there was a report of both pyridoxal and pyridoxine (P) quenching superoxide, “rather poorly” according to the rate constant of 10^3 M\(^{-1}\)s\(^{-1}\).\(^{9}\) It was stated that no appreciable reaction with hydrogen peroxide was found. In a later 2001 study, high concentration glucose solutions were used for generation of superoxide and it was demonstrated that 1 mM P or pyridoxamine (PM) readily quenched superoxide to the range of 66-97%.\(^{13}\) (Note: P, PM, and pyridoxal-5-phosphate (PP) are inter-convertible in normal cells.)

Three publications after the 2001 report dispute the findings that P and PM inhibit superoxide radical generation. A 2006 report showed that in a system demonstrating reactions of superoxide, hydroxyl radical, and hydroperoxyl radical with organic molecules, superoxide radical did not react with P.\(^{14}\) A 2008 report claimed that PM did not react with superoxide radical or hydrogen peroxide generated by albumin-Amadori intermediates.\(^{15}\) A 2009 publication used an endothelial cell model to demonstrate whether pre-incubation with P, PM, or PP would inhibit superoxide generation from hydrogen peroxide. It was stated that only PP directly interacted with superoxide radical and that P and PM lowered superoxide by interaction with NADPH oxidase.\(^{16}\)

When U937 cells (a human leukemic monocytic lymphoma cell line) were exposed to hydrogen peroxide, superoxide was produced. When the cells were pre-incubated with P, PM, or PP, superoxide was quenched in a dose-dependent manner. While the concentrations of P, PM, and PP were above physiologic levels, the authors concluded that vitamin B6 compounds could reduce damage due to ROS.\(^{17}\)
Folic Acid – Vitamin B9

Folic acid was reported to have substantial scavenging ability against superoxide generated by the xanthine/xanthine oxidase system, to a degree comparable to that of the superoxide dismutase mimetic, Tempol.18

In 2000, it was shown that 5-methyltetrahydrofolate (5-MTHF), the main circulating folate in the body, directly scavenged superoxide generated by the xanthine/hypoxanthine system.19 However, in 2006, a report disputed the 5-MTHF findings and claimed that 1-10 µM concentrations of 5-MTHF did not scavenge superoxide generated by the xanthine/xanthine oxidase system. Concentrations of 100 µM led to only modest scavenging of superoxide.20

Incubation of porcine aortic endothelial cells with 1 mM homocysteine and 0.5 mM folic acid or 5-MTHF for 24 hours prevented the homocysteine-mediated generation of superoxide radicals. The authors thought it unlikely that this effect was due to a reaction between homocysteine and folic acid or 5-MTHF, as it takes weeks for homocysteine levels to fall after folic acid administration. It was thought more likely that folic acid and 5-MTHF directly scavenged superoxide or prevented its generation.21

Cobalamin – Vitamin B12

It was reported that cobalamin had potent superoxide scavenging ability. In a cell-free experiment, cob(II)alamin (Cbl(II), an intracellular form of vitamin B12) was reacted with superoxide (generated by the xanthine oxidase-acetaldehyde system). There was rapid oxidation of Cbl(II) with generation of hydrogen peroxide. The resulting Cbl(III) can be reduced to Cbl(II) intracellularly. Cbl(II) also disproportionates to Cbl(III) and Cbl(I), the latter of which reacts rapidly with oxygen to produce hydrogen peroxide. To elucidate the intracellular effect of a commonly used form of cobalamin, human aortic endothelial cells were pre-incubated with 100 nM cyanocobalamin for 24 hours. Superoxide generation by paraquat was prevented by the intracellular result of cyanocobalamin to the same degree as by superoxide dismutase.22

Hydrogen peroxide was rapidly produced by combination of 25 µM hydroxycobalamin and 500 µM ascorbic acid added to cell-free culture medium (containing nutrients including an iron salt). No peroxide was produced by hydroxycobalamin alone. Ascorbic acid alone produced about one-fifth as much peroxide.23

A similar accumulation of hydrogen peroxide occurred when hydroxycobalamin and ascorbic acid were added to human epidermoid larynx carcinoma cells (HEp-2). (Note: No hydrogen peroxide was produced by either of the two reactants alone.) Within 24 hours after addition of 25 µM hydroxycobalamin and 500 µM ascorbic acid, there was 90 to 95% cell death. Each of the agents alone produced no cell death.23

An earlier study looked at the combined effect of cobalamin with ascorbic acid on three ascites tumor cell types and found a synergistic effect decreasing mitotic counts and survival of tumor cells.24 For completeness, it should be mentioned that a 1991 publication25 claimed that the previous report by the same authors of growth inhibition and death of ascites tumor cells by cobalamin and ascorbic acid was due in fact to dehydroascorbic acid, not ascorbic acid. This concept was not discussed in the 2007 publication.23

Summary

Thiamine quenches superoxide, but not hydrogen peroxide. Data were not available to define these possibilities with respect to riboflavin. There are conflicting reports on the ability of nicotinamide to react with superoxide. Nicotinic acid at supraphysiologic concentrations is said to quench superoxide, with no appreciable effect on hydrogen peroxide. Pantothenate at supraphysiologic concentration also quenched superoxide, without effect on hydrogen peroxide.

In the group of vitamin B6 compounds, pyridoxal and P reacted poorly with superoxide according to one publication, with no reaction with hydrogen peroxide. In a later publication, 1 mM P or PM quenched superoxide extensively. Three even later publications disputed the reaction of P and PM with superoxide and reported a lack of reaction of P with hydrogen peroxide. It was stated that only PP reacted directly with superoxide.

Folic acid was claimed to react with superoxide comparably with superoxide dismutase. One study found that 5-MTHF also reacted with superoxide, but a later report disputed the finding and stated that 1-10 µm 5-MTHF did not scavenge superoxide, and that a concentration of 100 µm 5-MTHF gave only modest scavenging. One year later, 0.5 mM folic acid or 5-MTHF was reported to scavenge superoxide. Therefore, 5-MTHF’s ability to scavenge superoxide may be dependent on concentration.
Cobalamin, in a cell free experiment, was shown to rapidly quench superoxide and produce hydrogen peroxide. In the presence of oxygen, this became a cyclic reaction to convert all superoxide to hydrogen peroxide. In a cell culture experiment, 100 nM cyanocobalamin prevented detection of superoxide produced by a generation system. In cell-free culture medium, 25 µM hydroxycobalamin and 500 µM ascorbic acid rapidly produced hydrogen peroxide. In studies on tumor cell lines, the combination of cobalamin and ascorbic acid produced hydrogen peroxide and had a synergistic effect in decreasing mitotic counts and survival of tumor cells.

Conclusions

It was mentioned above that the anti-tumor effect of high concentration ascorbate performs its function in the interstitial fluid beyond the blood circulation. Ascorbate is converted to superoxide, which proceeds to the hydrogen peroxide believed to be the active agent. Most of the B-vitamins can quench superoxide under some conditions and would presumably lower the concentration of hydrogen peroxide available for anti-malignant action. Therefore a caution seems appropriate against the inclusion of B-vitamins with intravenous ascorbate aimed at tumor cell cytotoxicity. However, this general recommendation based on chemistry cited above needs the support of in vivo studies for certainty.

In the case of cobalamin specifically, the situation is more complex. As cobalamin and ascorbic acid can react in the intravenous reservoir bottle, it seems likely that less ascorbate would be delivered to the blood circulation when accompanied by cobalamin. It might appear a moot point as hydrogen peroxide results from the decreased ascorbic acid. However, it has been shown that almost all hydrogen peroxide in the systemic circulation is eliminated. This occurs chiefly because the erythrocyte membrane is extremely porous to hydrogen peroxide and allows peroxide disposal by erythrocyte catalase. Therefore, it does appear that a reduced amount of ascorbate would reach the interstitial fluid, with relatively little hydrogen peroxide.

However, cobalamin and the remaining ascorbate would both arrive in the interstitial fluid. The previously mentioned reaction of cobalamin and ascorbate would generate hydrogen peroxide. So the obvious question becomes, does the presence of cobalamin in the interstitial fluid contribute more than the detriment of less ascorbate being present. This becomes a question of chemical kinetics. Will the presence or the absence of cobalamin with ascorbate provide the most positive effect against malignant cells in vivo? Existing in vitro studies show that cobalamin and ascorbate cause more tumor cell death than ascorbate alone. But no matter what measurements or calculations might be acquired to predict the interstitial concentration of hydrogen peroxide, with and without cobalamin, the bottom line is that in vivo studies are required before this question can be answered.

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References


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