Co- infusing Glutathione and Vitamin C during cancer treatment - a reply.

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Recently, a criticism has been raised by Chen et al to avoid the administration of intravenous Vitamin C and Glutathione at the same time[6]. We acknowledge that the authors understand the benefits of high dose intravenous Vitamin C and that from their studies they have deduced that in cancer treatment the "simultaneous administration" of glutathione (antioxidant effect by their definition) and Vitamin C (pro-oxidant by their definition) interferes with the cytotoxic effectiveness of the Vitamin C. Although from their results there is some suggestion of this interference by glutathione, at this stage we remain unconvinced that the common practice of administering low levels of glutathione (500mg to 1 gram) at the same time as high dose intravenous Vitamin C (30g to 100g) is not in the best interests of cancer patients who are usually under considerable systemic oxidative stress.

Of interest are the following observations concerning the Chen trial:

1. The amounts of Vitamin C and Glutathione used in vivo were distinctly *non-physiological* compared to the in vitro studies, where it was claimed in the paper (correctly) that the in vitro concentration of Glutathione was clinically relevant (640uM). 640uM is approx equivalent to the infusion of 1.5 gram of Glutathione into an adult human, presuming 5 liters of blood. Instead, they have used the equivalent of 48 grams of glutathione infused into a 60kg human. This was a massive overdose in terms of additional Glutathione but *still* there was some positive effect both on tumor reduction and mouse survival. It is hardly a relevant comparison with what is done in clinical practice and, therefore, potentially, not particularly meaningful in explaining the benefits or shortcomings of co-infusing Glutathione and Vitamin C.

2. The concentration of Vitamin C used in tissue culture was in fact quite low – let's say 2mM, which is approx 40mg%. However, the amount injected into the mice was 4000mg/kg – equivalence for an adult human body is 240,000mg/60kg, which could potentially result in levels in the blood stream at least as high as 400 to 600mg%, which is far in excess of what was used in vitro. No investigation was done into Vitamin C levels achieved in the blood stream of the athymic mice, so there is no way of evaluating if these high levels of Vitamin C were achieved and maintained in the mice. Unless the Vitamin C levels were/are measured in the mice (in the blood stream or preferably in situ at the cancer cell site), it is very difficult to make a deduction about the correlation of their tissue culture studies and the in vivo studies.

3. Virtually all cell lines had a 100% kill rate by ascorbate concentrations between 1mM and 2mM – this is quite remarkable because 100% kill rate of cancer cells at such low Vitamin C concentrations has not been reported across such a large range of cancer cell types.

However, research by the Riordan Institute[7] and by Mark Levine[8] have all reported 100% kill rates at ascorbate levels as high as 20mM, with a 50% kill rate reported at somewhat lower Vitamin C concentrations.

It is an interesting observation by Chen et al that virtually all cell lines had a very similar 100% kill rate by the same low concentration of Vitamin C (1 to 2mM); an unusual phenomenon.

4. Despite the claim that Glutathione totally stopped the effect of the Vitamin C in vitro, this is not what the accompanying graphs show. In virtually all cases in vitro there has been a decrease in cell viability when the combination of Vitamin C and Glutathione has been used. Who knows what the effect on cell survival may have been if they had used the same absolute concentrations of Vitamin C and Glutathione in vitro that they ended up using in vivo.

5. It is interesting that more mice survived up to 30 days in the Vitamin C + Glutathione-treated group than the Vitamin C group or the Glutathione group. Is successful treatment measured only by tumor shrinkage or by survival? (Preferably both, of course, but survival is imperative.)

6. It is also interesting that the same number of mice survived to 30 days in the Vitamin C group and the Glutathione group. Toxicity levels would appear to be the same.

7. Despite the claim that there was no reduction in tumor volume in the Glutathione group nor the Glutathione + Vitamin C group, in the graphs there *was* some reduction compared to the control and it was greater in the Glutathione + Vitamin C group compared to just the use of Glutathione. Who knows what would have ultimately happened if the experiment had been allowed to go to 'natural death', as the Glutathione + Vitamin C-treated mice were surviving in greater numbers.

8. Evidence is certainly accumulating that one of the mechanisms by which high-dose intravenous Vitamin C works is (*indirectly*) through the production of hydrogen peroxide. The addition of Glutathione in tissue culture in the absence of plasma, red cells and white cells, endothelial barriers, extracellular matrix barriers, systemic oxidative stress, liver uptake, brain uptake and metabolic catabolism, is hardly representative of an in vivo clinical situation of infusing a gram or two of Glutathione, especially when, because of pharmacokinetics, only a small amount of the infused Glutathione is likely to end up at the cancer cell site. So, it may well be that, in low concentration, Glutathione is a scavenger of reactive oxygen metabolites. However, the question could be and should reasonably be proposed that a small amount of co-infused Glutathione is far more likely to end up in multiple areas of the body – liver/brain/red cells/lungs – than suddenly concentrating in situ in a cancerous tissue. It is probable that these lower levels of Glutathione help protect the body from the systemic oxidative stress that exist in a person with cancer[11,12].

9. This paper unfortunately alludes to that old concept of *not* giving an antioxidant at the same time as an oxidizing agent. The medical literature now abounds with papers demonstrating quite the opposite: that the co-administration of an antioxidant along with a cytotoxic drug *decreases* side effects and *increases* the effectiveness of the cytotoxic drug in vivo[9, 10]. Once again we acknowledge that Chen et al have mentioned that glutathione is not uncommonly administered at the same time as platinum based cytotoxic drugs (pro-oxidant) where is has been demonstrated that the glutathione (anti-oxidant) does not interfere with their activity and decreases their side effects. The purpose of the Chen paper was to see if this pro-oxidant (Vitamin C) and anti-oxidant (Glutathione) combination was equally valid. We repeat that a massive amount of glutathione (48 grams equiv to a 60kg person) was given to the athymic mice to achieve the glutathione interference – other (not measured) physiological effects could well have occurred from such a massive amount unrelated to the "apparent" anti-oxidant interference of glutathione. Such levels of glutathione (48g) have never before been documented or infused by Doctors practicing nutritional medicine nor have they been used or documented in combination with platinum based cyto-toxic drugs.

10. The etiology of cancer, and almost certainly the propagation of metastases, involves oxidative stress. There is much evidence to support the use of antioxidants to slow cancer progress and to prevent its propagation[13,14,15,20]

11. Finally, like Vitamin C, if the right concentration of Glutathione can be found, it has been shown that even Glutathione can be cytotoxic and can produce hydrogen peroxide around cancer cells[16, 17]. Alpha lipoic acid has also been found to be very useful in increasing the cytotoxic effect of chemotherapeutics whilst decreasing the side effects[18]. Additionally, alpha lipoic acid has been found to dramatically increase the cytotoxic effect of high-dose Vitamin C[7].

Further observations of this paper include the use of athymic mice, presumably on the basis that immune stimulation by the high-dose Vitamin C was being excluded. For what reason? Surely in humans we are not going to remove the thymus gland before we administer high-dose intravenous Vitamin C and Glutathione. Probably, there is an attempt to continue the postulate that the only way high-dose Vitamin C works is by stimulation of hydrogen peroxide production and that Glutathione will inhibit this action; well, who knows what effect the Glutathione would have if there was still an intact thymus gland – the case in most humans. It is abundantly clear from the medical and scientific medical literature that high-dose intravenous Vitamin C works in multiple ways, some of which are described by Frei[2].

It is a misperception that megadose intravenous Vitamin C is acting as a pro-oxidant – it is quite the reverse. Vitamin C is a *reducing* agent – it donates electrons; the oxidized form of Vitamin C, dehydroascorbate (its redox pair), accepts electrons and, of course, is an *oxidizing* agent. In the claim that the 'pro-oxidant' Vitamin C produces hydrogen peroxide, ascorbate would be accepting electrons. This is contrary to the proposed mechanism of how megadose Vitamin C works in cancer treatment. Adding dehydroascorbate to tissue culture (the *oxidized* form of ascorbate) does not

result in the formation of hydrogen peroxide – the *reduced* form of ascorbate is required for hydrogen peroxide production.

Chen and Levine et al.[1] propose that ascorbate *donates* an electron (*reduction*) to a metal ion, the identity of which is still to be determined but may be cupric (Cu^{++}) or ferric (Fe^{+++}), to produce cuprous (Cu^{+}) or ferrous (Fe^{++}). It is the oxidizing molecule of cuprous or ferrous which then potentially interacts with oxygen to produce superoxide O_2^-, which in turn dismutates to produce hydrogen peroxide. Ascorbate does not directly produce hydrogen peroxide; in fact, ascorbate is a great scavenger of free radicals such as superoxide, so the concept of high doses of Vitamin C directly producing large amounts of superoxide free radical is contradictory. The addition of dehydroascorbate (the oxidized form of Vitamin C) by definition, according to the scheme outlined by Chen and Levine, would *not* result in the formation of hydrogen peroxide. So, if we follow Chen and Levine's discourse[1], Vitamin C acts as a *reducing* agent to indirectly produce hydrogen peroxide – this still suggests that in this instance Vitamin C is *not* directly pro-oxidant but is acting as a *reducing* agent albeit with an ultimate oxidative effect.

Further indirect confirmation of the requirement for Vitamin C to be in the reduced form has recently been published by Heaney et al [21]. They demonstrated that the in vitro addition of dehydroascorbate to myeloid leukemia and lymphoma cell lines *inhibited* the cytotoxic action of anti-cancer drugs rather than *increasing* their potency; a similar inhibitory effect on anti-cancer drugs occurred when dehydroascorbate was administered to mice with cancer xenografts. In general an *increased* cytotoxic action of anti-cancer drugs is observed when ascorbate (not dehydroascorbate) is used at the same time as anti-cancer drugs.[22,23,24,25,26.] These papers ad further confirmation to the importance of the *anti-oxidant* effect of megadose Vitamin C in cancer treatment adding a conundrum to the pivotal point of Chen's paper - that it is the *anti-oxidant* effect of glutathione which is interfering with the claimed pro-oxidant effect of the infused megadose Vitamin C.

Whatever the mechanism, high doses of the *reduced* form of Vitamin C appear to be involved with the production of significant amounts of hydrogen peroxide that have a pro-oxidant effect against cancer cells. Unfortunately, it has become axiomatic that this is how high-dose Vitamin C works in cancer treatment. There are undoubtedly *many* mechanisms by which high dose Vitamin C works[2].

Whatever the ultimate mechanism of action against cancer cells, in vitro and in vivo, it involves cancer cell death. In vivo there are probably mechanisms, such as generalized immune stimulation, inhibition of angiogenesis, hyaluronidase inhibition, collagen growth (to contain tumors), that are not easily quantified in vitro. Researchers in the Vitamin C and cancer field have succumbed to the temptation to apply the orthodox reductionist singularity approach of hydrogen peroxide production being the only mechanism by which high-dose Vitamin C works against cancer cells – most unfortunate, as so much excellent research is not being properly pursued and debated.

To date, cancer trials using high-dose Vitamin C have had mixed outcomes. Pauling and Cameron reported an increase in length and quality of life using just 10 grams of

intravenous Vitamin C in patients whose immune systems had *not* been compromised by long-term cytotoxic therapy[3]. Although there are occasional successful case history studies of the use of intravenous Vitamin C in cancer[19], more recently a Phase 1 clinical trial by Hoffer and Levine et al.[4] did not find remarkable results using doses of Vitamin C as high as 100g. However, on careful reading of the Hoffer and Levine paper, for 2 out of the 24 patients their cancers became stable. Additionally, cancer patients who received intravenous Vitamin C at levels $\geq$ 0.6g/kg maintained their quality of life compared with patients who received a lower dose. All of these cancer patients were phase 4 and had exhausted other treatment methods, including surgery, radiotherapy and chemotherapy (compared with the patients described above by Pauling and Cameron[3]). This illustrates that there are many complicating factors involved in high-dose Vitamin C therapy above and beyond the apparent measured in vitro cytotoxic effects. Not the least of these is the overall systemic oxidative stress that cancer patients are under, including widespread inflammation, SIRS (systemic inflammatory response syndrome) and the sepsis that often accompanies terminal cancer patients[5]. Cancer patients are often malnourished, cachectic and immune-overloaded/depleted, which can be caused by the cancer, associated disease or the treatment, or all of the aforementioned. Every one of these areas needs to be addressed.

Although it is tempting to use a singular approach in cancer treatment, this is fundamentally 'shortchanging' the patient in terms of their requirements to best deal with their cancer. Of course, the aim should be to remove any rapidly growing and/or life-threatening cancer tissue, to use appropriate and preferably targeted cytotoxic/radio therapies as required (which can include high dose intravenous Vitamin C and other appropriate nutraceuticals administered in combination or separately as indicated), maximize the patient's immune function, their nutritional status and their energy levels, diminish their toxic load (which may be contributing to their oxidative stress) and their depleted immune status, and to address their social and spiritual issues.

It has always been of interest that animals that are used to validate the effect of a known dose of Vitamin C manufacture their own Vitamin C – indeed mice were used that manufacture up to an equivalent of 17 grams per day based on a 60 kg human body weight. Of course, Glutathione is also manufactured by most mouse tissues, and tumor cells also manufacture abundant amounts of Glutathione. Without accounting for the Vitamin C and Glutathione levels in/around the mouse tumor before and after the administration of extra levels, it is not the easiest task to evaluate the real effect of loading extra Vitamin C and Glutathione. Also, how is this relevant to what happens in the human body, which starts off with *very* low levels of Vitamin C and unknown levels of glutathione in/around the tumor tissue?

In conclusion, the use of collateral antioxidants in the treatment of cancer has both clinical and scientific support. The Chen et al experiment needs to be repeated to give more clinically meaningful results: to include physiologically relevant levels of the intervention drugs and treatment continued until survival or natural death for all animals in the cohorts. Until there is conclusive evidence against the simultaneous use of Glutathione and Vitamin C, it would not seem imprudent to continue such a

protocol. It remains to be firmly established what is the best combination of antioxidants and other nutraceuticals (including Vitamin K3, Vitamin D3 and Selenium) for long-term survival and for use with orthodox cytotoxic therapy to increase its efficacy and decrease side effects. If at any stage it is clearly demonstrated that one or two grams of Glutathione and megadose Vitamin C (commonly 15g up to 100g) should not be administered at the same time, then naturally practitioners should cease this practice.

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Disclosure:
The authors are associated with a company which manufactures Vitamin C for injection.

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