Clinical Trial to Find An Oral Dosage of Vitamin C That May Prevent Recurrence of Superficial Bladder Carcinoma

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Abstract In 1994, my group published in AGE the determination of an optimal dosage of vitamin C (AA), which produces the highest serum level before the kidneys remove it. That dosage is 500 mg BID, resulting in 0.1mM AA in the serum. This dosage may reduce free radicals and chronic disease, as proposed by AGE founder Denham Harman.

AA also kills cancer cells. The mechanism hypothesized is generation of extracellular hydrogen peroxide catalyzed at the cancer cell membrane. *In vitro*, this requires above 1mM AA. For treating most cancers *in vivo*, this can be achieved transiently through intravenous AA. This enhances the effectiveness of many chemotherapies.

For superficial bladder carcinoma (SBC), after surgical removal of the cancerous cells, chemotherapy is instillation of *Bacillus Calmette-Guerin* (*BCG*). The recurrence rate with this therapy is 85%. Recurrence routinely requires bladder replacement. But prior to our discovery that AA must be consumed twice daily to maintain high levels, high dosage vitamins including AA reduced recurrence to 40%.

It is unclear what effect high doses of vitamin C have on producing or preventing kidney stones. The major inconvenience of taking AA supplements found in 2010 is that 10% of those consuming them daily experience LUTS.

We investigated the oral dosage of AA that maintains the highest level of AA in the bladder. Varying the level and interval between dosages, we found AA above 2g BID was not absorbed. In a trial of 14 people, urinary [AA] varied from 2.7 to 27.8mM. With continuous exposure at that level in the bladder for 48 hrs, it is likely to be higher than needed to kill cancer cells. By consuming 2g BID two days each week, one may minimize the risk of LUTS while maintaining bladder [AA] likely to kill recurring cancer cells.

ABBREVIATIONS AA-ascorbic acid/vitamin C; BID-in divided does, twice a day; BCG-*Bacillus Calmette-Guerin*; IV-intravenously; SBC-superficial bladder carcinoma; LUTS-lower urinary tract sypmtoms; DCIP-dichlorophenol-indophenol; GRAS-generally recognized as safe

Introduction

Linus Pauling proposed that taking megadoses of vitamin C (ascorbic acid, AA) has many benefits. Among his results (Cameron 1978) are that AA significantly prolonged the fraction of survivors at times after date of onset of terminal stage (untreatable) cancer patients. Many patients had received an oral dose of 10 g daily.

AA enhances survival of cancer patients *in vivo* when properly administered IV rather than orally (Chen 2005). "Observational reports described ascorbate, given in pharmacologic doses of 10 g daily, as effective in treating some cancers and in improving patient well-being" (Cameron 19874, 1976, 1978). Subsequently, the same dose had no effect on patient well-being and survival in two double-blind placebo-controlled trials, and ascorbate was discarded as a treatment modality (Creagan 1979, Moertel 1985). Recent clinical evidence, however, indicates that the role of ascorbate in cancer treatment should be examined anew (Padayatty 2004). The originally reported observational studies used IV and oral ascorbate, but the subsequent double-blind placebo-controlled studies used only oral ascorbate. It was not recognized that the route of ascorbate administration might produce large differences in plasma concentrations. Recent pharmacokinetics studies in men and women show that 10 g of AA given IV are expected to produce plasma concentrations of nearly 6 mM, which are 75-fold higher than those concentrations from the same oral dose (Levine 2001, Padayatty 2004, Levine 1996). The maximum serum concentration achievable by oral AA is 80 mM.

It is estimated that 73,510 people will be diagnosed with and 14,880 will die of cancer of the urinary bladder in 2012 (National Cancer Institute 2012). Developed in 1976 by Morales (Morales 1980), normal chemotherapy for superficial bladder carcinoma (SBC) is instillation with intravesical *Bacillus Calmette-Guerin* (BCG). However, such cancers "commonly exhibit a very aggressive behavior and carry a grave prognosis ", and the 5-year recurrence rate is 91%. Lamm (1994) developed a megavitamin protocol that reduces recurrence to just 41%. However, many bladder oncologists do not use this modification.

When Lamm was publishing his megavitamin protocol, he was unaware of Ordman's discovery that AA must be taken twice a day (BID) to maintain elevated levels in the serum, a result we showed next for calcium (Cone 1996) and eventually assumed for all water-soluble nutrients. In 1994, we published in AGE (King 1994) that when people consume AA orally, AA intake above 500 mg is excreted in the urine over 12 hr, and that 500 mg must be consumed twice a day to guarantee continuous excretion of excess in the urine. Levine (1996) showed the relationship between oral AA dosage and serum levels, confirming Ordman's conclusion that 500 mg AA BID produces the highest statistically significant serum [AA] in people. Recent studies indicate 40% of Americans take AA supplements (Schleicher 2009).

In his megavitamin treatment, Lamm also did not note that the 2 g of AA was probably the active ingredient. Casciari (2001) demonstrated how 5mM AA kills cancer but not normal cells in tissue culture (Figure 4). Levine *et al* (2001) proposed the mechanism by which AA kills cancer cells without harming normal cells. Cancer cells catalyze the generation of hydrogen peroxide from AA, while normal cells do not. However, it is still accepted among many oncologists, e.g. at the Mayo Clinic web site, that high dose IV AA is ineffective (Moynihan 2012). Yet AA is used intravenously with cancer patients and increases efficacy in conjunction with a variety of chemotherapies, as reviewed by Levine at NIH (Levine 2011). A survey indicated more than 8800 patients were treated with IV AA in 2008 (Evers 2012).

Current SBC treatment with BCG does not include the megavitamin protocol. In reviewing Lamm's result, I noticed that his megavitamin included 2 g AA daily. In contrast to intravenous AA required to elevate serum concentrations to treat most cancers, oral AA is absorbed into the serum, and removed by the kidneys through the bladder in urine. Since AA kills cancer cells without harming healthy ones, we realized that an oral dose of AA could be found that would provide the maximum continuous [AA] in the bladder, and that this might significantly and safely reduce the recurrence of SBC.

Normal daily urine output is estimated 0.5 to 2 liters. Given that doses above 500 mg AA BID are excreted, we hypothesized that taking 2 g AA BID would produce a urine [AA] of approximately 14 mM, while transient exposure to 5 mM is what Casciari *et al* demonstrated to kill cancer cells.

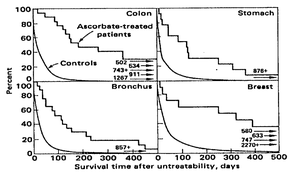

Figure 1 (Cameron 1978). Fraction of survivors at times after date of onset of terminal stage (untreatability) of AA-treated patients with primary cancer of colon, stomach, broncus, or breast, compared with that for matched controls (10 per ascorbate-treated patient). Many patients were given 10 g daily orally, but a variety of other AA treatments were used.

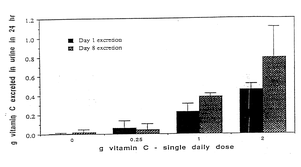

Figure 2 (King 1994). Relationship of dose of AA to total urinary excretion. Individuals took AA daily at 8am for 8 days. All urine was collected during the first and last day. Each bar represents the mean for 5 individiuals.

Method

This trial was approved by the IRB of Beloit College. Participants were 14 healthy members of the college community. Two g AA (four Spring Green 500 mg tablets) were taken at 9am and 9pm. Urine was collected 5-12 hours after the last vitamin intake and oxalic acid was immediately added to stabilize the AA. The samples were put on ice until they were assayed with dichlorophenol-indophenol (DCIP) (Omaye 1979) in the next few hours. This procedure is repeated twice with the same subjects.

Results

The results of the exploratory trial were used to determine 2 g AA BID dosage (Table 1) and BID dosing (Table 2) that would produce the highest [AA] in the bladder. The pilot trial showed that dosages above 500 mg BID were necessary to guarantee continuous excretion from the bladder, and that dosages above 2g BID could not be absorbed. The participants for this trial were from 18 to 22 yrs old and seemed to be of good health, with most being male (Table 3). Table 4 illustrates the results of the trial. The different [AA] within individuals in the two trials likely results from different volumes of urine production. Three of the 14 reported minor indigestion.

***Comparison of the Maximum AA achieved vs. the level required to kill cancer cells*:**
The AA levels ranged from 2.7 to 27.8mM AA which is much greater than the 1 mM level required to kill cancer cells *in vitro (Chen 2005)*.

Table 1. Effect of BID dose on the urinary [AA]

AA Dosage Taken Twice a Day	Urinary [AA], mM
1 g	1mM
2g	6mM
3g	7mM
6g	6mM

Table 2. Effect of frequency of dosing on urinary [AA]

Dosage	Frequency	Urinary [AA], mM
1g	Hourly	6mM
1g	Every 12 hr	1mM
2g	Every 12 hr	6mM

Table 3. Characteristics of participants

Person	Gender	Weight(kg)	Exercise (min/week)
1	M	102	900
2	M	93	840
3	F	79	0
4	M	77	1080
5	M	93	780
6	F	47	0
7	F	53	225
8	M	141	n.d.
9	M	90	1200
10	F	66	100
11	M	65	240
12	M	69	0
13	M	76	300
14	M	68	240

Table 4. Effect of BID dose on the urinary [AA] – Variation among participants

Person	Gender	mM AA first trial	second trial
1	M	2.66	
2	M	2.68	
3	F	3.85	
4	M	4.09	
5	M	5.64	6.4
6	F	6.37	
7	F	6.68	9.53
8	M	7.4	8.32
9	M	7.41	10.72
10	F	8.46	10.62
11	M	8.57	23.2
12	M	9.56	11.4
13	M	11.9	20.21
14	M	14.57	27.8
Ave females=		3.32	
Ave males=		11.975	

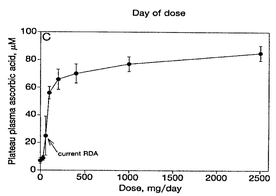

Figure 3 (Levine 1996). Plasma AA concentration (μM) as a function of total daily dose, delivered twice a day. For all plateau determiinations, concentration was defined as the mean of five or more samples drawn over at leastt 7 days.

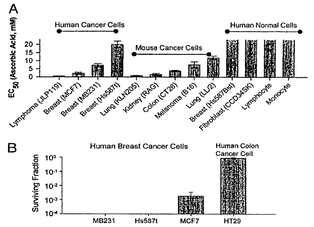

Figure 4 (Chen, Buettner, et al 2005). Effects of pharmacologic [AA] on cancer and normal cells. A. EC_{50} values of ascorbate in human and mouse cancer cells and normal humans cells. All cells were treated with AA for 1 hr, washed, and recultured without AA. EC_{50} values were determined 18-22 hr later. B. Colony formation of cancer cells in soft agar after 1 hr treatment with 5mM AA. Surviving fraction, expressed in log scale, indicates number of treated colonies compared with matched untreated control cells.

Table 5. Comparison of the maximum [AA] achieved vs. the level required to kill cancer cells

Condition	[AA]	dosage	Duration	Result
Human SBC in bladder	n.d.	2 g once daily (Lamm 1994)	6 hr	SBC Recurrence reduced from 91% to 41%
Human SBC in bladder	2.7 to 27.8mM	2 g BID	continuous	Clinical trial planned
Human cancer cells in Tissue culture	5 mM	*In vitro* 5 mM continuous	1 hr	No survival
Human normal cells in Tissue culture	20 mM	*In vitro* 20 mM continuous	1 hr	100% survival

Discussion

Taking 2g AA raises the concentration of AA filtered by the kidneys to a maximum of approximately 6 mM. Our previous work demonstrated that when AA is taken orally, the concentration in urine rises over 4 hr, remains elevated for the next 12 hr, and then falls rapidly, unless another dose of AA has been consumed. To maintain the 6 mM [AA] continuously, taking the dosage every 12 hr is necessary and sufficient.

AA is approved by the FDA as a GRAS (generally recognized as safe) substance. Two-time Nobel Prize winner Linus Pauling took 16 g daily for years without side effects. In the latest statement issued by the Food and Nutrition Board for the USDA, the chair of the panel on antioxidants, Maret Traber, set the upper limit for AA at 2 g daily (PDARC 2000). Using this level chronically may cause diarrhea in a few individuals. A review states clearly that high dosages of AA cause no apparent harm (Levine 2011). One hazardous side effect of AA is that wound healing takes longer, so it should not be consumed within a week of bleeding. Another side effect of high doses of supplemental AA that is documented in peer-reviewed literature was reported in 2011, when it was shown that taking AA supplements regularly causes LUTS in 21% of older men (Maserejian 2011). LUTS is lower urinary tract symptoms, specifically more difficulty with and frequency of urinating, similar to prostate hyperplasia. It remains unclear whether AA contributes to kidney stones. AA can be biochemically metabolized to oxalic acid, and oxalic acid forms kidney stones. For this reason many physicians believe that consuming AA increases the risk of kidney stones. However, a reduction in the risk for kidney stones was observed in those who take AA supplements (Naidu 2003). A 2013 study by Thomas *et al* (2013) reported a 0.15% increased risk in a Swedish population that takes single doses of 1g or more through supplements. Other documented effects of AA are beneficial. These include a minor reduction in blood pressure (Newberry 2012), a reduction in cortisol levels (Staff 2012), and a reduction in the risk of stroke damage (Polidori 2001, 2005, Zweifler 2003).

In order to reduce the chance of causing LUTS, and considering that cancer often develops tolerance for many forms of chemotherapy, the proposal to conduct a clinical trial on those with SBC will include 2g AA BID two consecutive days per week. Our pre-trial involved participants taking 2g of vitamin C twice a day (4g daily) to achieve a maximal concentration of AA in the bladder. Previously, it was thought that there was no benefit in taking more than 2g of vitamin C per day, but our research indicates that by taking a higher dose, 4g per day, will increase the concentration of AA in the bladder. We predict that the increased amount of AA in the bladder will help to prevent or treat SBC by killing any new cancer cells as soon as they develop in the bladder. We hope that this study may lead to a cure for SBC.

Conclusion

Research in urinary excretion of AA indicates that the AA level in the bladder can be elevated simply by oral consumption. The maximum [AA] achieved was 6mM, by consuming 2g AA BID. For SBC, this method provides a level of AA in the bladder that can kill cancer cells, not harm normal tissue, and is likely to significantly reduce the rate of recurrence from 91% to at least less than 41%, and potentially even less.

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