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The Clinical Effects of Vitamin C Supplementation in Elderly Hospitalised Patients with Acute Respiratory Infections

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Summary: A randomised double-blind trial involving vitamin C/placebo supplementation was conducted on 57 elderly patients admitted to hospital with acute respiratory infections (bronchitis and bronchopneumonia).

Patients were assessed clinically and biochemically on admission and again at 2 and 4 weeks after admission having received either 200 mg vitamin C per day, or placebo.

This relatively modest oral dose led to a significant increase in plasma and white cell vitamin C concentration even in the presence of acute respiratory infection.

Using a clinical scoring system based on major symptoms of the respiratory condition, patients supplemented with the vitamin fared significantly better than those on placebo. This was particularly the case for those commencing the trial most severely ill, many of whom had very low plasma and white cell vitamin C concentrations on admission.

Various mechanisms by which vitamin C could assist this type of patient are discussed.

Introduction

There has been much controversy concerning the role of moderate to large doses of vitamin C in preventing or alleviating symptoms of the common cold. Studies have produced conflicting results and problems of interpretation exist with inadequate control, variable clinical assessment and large variations in the doses given. In general, the use of doses of the vitamin over and above normal dietary intakes have not been shown to have significant effects in preventing upper respiratory tract infections. Nevertheless a number of reviews including those of Chalmers (1975) [1], Anderson (1977) [2], Basu and Schorah (1982) [3], Briggs (1984) [4] and Hemila (1992) [5] have concluded that whilst no significant preventive effect can be demonstrated, there may be a modest effect of extra vitamin C in reducing the severity of cold symptoms. However most of the studies reviewed have used young or middle-aged adults with reasonable dietary intakes and tissue levels of the vitamin and relatively non-severe respiratory infection.

We have chosen to investigate the effect of pharmacological doses of vitamin C in elderly subjects with severe respiratory infections, many of whom had very low blood vitamin C levels. Vitamin C is normally found in high concentration in cells of the immune system and given that there tends to be a decline in immuno competence (especially cell-mediated immunity) with increasing age (Chandra, 1985 [6]; Chandra, 1989 [7]) and that animal studies have clearly demonstrated depressed immune function in vitamim C deficiency (Shilotri, 1977 [8]; Thomas and Holt, 1978 [9]; Anderson, 1981a [10]), it was felt that this category of patients

might be among the most likely to derive any benefit that vitamin C supplementation has to offer.

This study was a follow-up to one which had appeared to show a tendency for such patients to benefit from moderate vitamin C supplementation (Hunt, Chakravorty and Annan, 1984 [11]), but which had used a relatively crude assessment of clinical change.

Material and Methods

Clinical: The patients enrolled into this second phase study were suffering from acute bronchitis (often acute exacerbation of chronic bronchitis) or bronchopneumonia. Patients suspected or known to be suffering from lung cancer were excluded from the study, as were those who were judged by the clinician to be at high risk of death within a day or two of admission.

The patients were enrolled over a period of three years and were admitted mainly in the winter months. For consistency all clinical assessments were performed by the same Associate Specialist (GA). Three main diagnostic features of infective respiratory conditions, namely cough, breathlessness and radiographic evidence of chest infection were used. Each was scored by the clinician according to severity (Table I) on a scale that was thought to be as clinically sensitive as reasonably possible (details below). Then for each person, at each assessment interval, his or her three main diagnostic feature scores were added to give the "total respiratory score" ("TOTRESP"). By this procedure, the worst score that could be achieved by the most severely ill patient (whilst still alive) was 9, whilst those who were completely well with regard to the respiratory condition would score 3. A score of 10 was given for subjects who died during the trial. Details of the criteria used for clinical assessment are given in Table I.

Assessments were made and blood samples taken (see below) on admission (0 weeks) and at 2 and 4 weeks after admission. If patients were discharged from hospital as "well" before 4 weeks, therapy was discontinued and they were assumed to remain well for up to 4 weeks, for the purpose of clinical scoring (none of the patients discharged were readmitted during their 4 week assessment period). It had been decided from the phase 1 study, however, that excessive dropout through death or discharge would make it unworthwhile continuing patient follow up beyond 4 weeks.

Age and diagnoses other than "respiratory" were also recorded since other conditions, particularly heart disease could obviously influence clinical progress, although acute respiratory infection had, in all cases, been the primary reason for hospitalisation. Previous smoking habits of the patients were not recorded but none of them smoked whilst in hospital.

After the initial clinical assessment and blood sampling the patients commenced placebo or vitamin C therapy to which they were allocated on a randomised "double-blind" basis. This was in addition to their normal medication. 100 mg of vitamin C or placebo were taken twice each day at 8.30 am and 2.00 pm. The vitamin C and placebo tablets (Roche pharmaceuticals) were indistinguishable from each other by look or taste. (The placebo tablets were composed of lactose, corn starch, talc and magnesium stearate and the vitamin C tablets had ascorbic acid instead of corn starch, but otherwise the same ingredients).

Table I: Scoring system used in Phase 2 with regard to severity of respiratory illness

Severity of respiratory condition

(a)	Cough	very severe	4
		moderately severe	3
		less severe	2
		well	1
(b)	Radiological evidence of chest infection	present	2
		not present	1
(c)	Breathlessness	severe	3
		less severe	2
		well	1

(Scoring scale therefore ranges in 1 point steps from 3 for well to 9 for the severest possible condition).

Criteria used for clinical assessment:

Cough
- very severe – yellowish or greenish purulent expectoration
- moderately severe – a fairly large amount of mucoid whitish expectoration
- less severe – sputum is scanty, mucoid and tenacious
- well – no cough

Breathlessness
- severe – breathless at rest (much worse on exertion)
- less severe – breathless only on exertion
- well – no breathlessness

Biochemical Methodology: Blood samples were taken for the analysis of plasma, mononuclear cell, polymorphonuclear cell and platelet vitamin C levels. Estimation of vitamin C in the individual white cell fractions was preferred as fluctuations in the proportions of the individual cell types of the buffy layer during an acute phase response cause changes in the concentration of vitamin C measured in the buffy layer which do not reflect the levels in the individual cell types (Schorah *et al*, 1986 [12]).

Initial red blood cell sedimentation was achieved using Dextran-Isopaque solution which consisted of 2 volumes of Fisons Dextraven 150 solution (containing the glucose polymer dextran [mol wt approx 150,000] in sodium chloride injection BP) and 1 volume of 45% aqueous solution of Isopaque (440 mg I/ml).

The separation of the individual white cell fractions was achieved by differential centrifugation techniques based on the procedure of Boyum, 1968 [13], but modified slightly in order to not only isolate polymorphs and mononuclear cells but also platelets. Details were as follows:

1. Whole venous blood was collected into di K-EDTA anticoagulant and processed within half an hour. Two × 4 mls of whole blood were layered carefully onto the surface of dextran-isopaque and allowed to stand at 37 °C for 45–60 minutes. This sedimented the red cells to the bottom of the tubes leaving platelets and white cells in the supernatant.
2. The supernatant was diluted 1:1 with balanced salt solution (which consisted of 9 volumes of 0.14 Maq NaCl mixed with 1 volume of an aqueous solution containing D-glucose (0.1%); $CaCl_2$ $2H_20$ (5×10^{-5}M), $MgCl_2.6H_20$ (9.8×10^{-4}M), KCl (5.4×10^{-3}M) and tris hydroxymethyl methylamine (0.145 M), adjusted to pH 7.6 with 1MHC1). This diluted supernatant was then layered onto Ficollpaque. This was then centrifuged at 400 × g for 23 mins at 18–22 °C, which separated the cell fractions, with platelet-rich plasma as the top layer then a thin layer of mononuclear cells and at the bottom a 'button' of polymorph cells.
3. These three cell fractions were removed, washed by further centrifugation in balanced salt solution, and then (after cell counting) spun down and the vitamin C extracted in 0.6 ml of 5% trichloracetic acid solution.
4. Vitamin C in the extracts was estimated with 2, 4 dinitrophenyl hydrazine, (Denson and Bowers, 1961 [14]).
5. Plasma estimations were performed by the same colorimetric method on 1 ml of supernatant obtained after centrifuging a mixture of 1 ml plasma and 2 ml of 5% TCA.

Cross contamination of cell types in the different fractions were found to be less than 10% with the exception of platelet contamination of the mononuclear cells. In the latter, contamination ranged from 1–8 platelets per mononuclear cell. However, because platelets are so much smaller, the maximum error that could occur in the mononuclear vitamin C concentration was 9%. Duplicate samples from the same patient fractionated separately indicated a precision of 5% for plasma and platelet, and 10% for polymorph and mononuclear cell vitamin C levels.

Yields of the cell types were estimated by comparing counts obtained on both whole blood and the cell suspensions. Average yields were found to be 33.7% for mononuclear cells, 52.3% for polymorphs and 24.2% for platelets. These were higher than those of Evans *et al* (1982 [15]), particularly for polymorphs. Boyum (1968 [13]) obtained a similar yield of polymorphs (59%) but a much better yield of mononuclear cells (98%). This is because his technique was designed primarily to isolate the latter fraction in a one-step centrifugation whereas in this study the method was modified to allow extraction of platelets and a mononuclear cell fraction as free as possible of platelet contamination. This meant sacrificing some of the mononuclear yield during subsequent washing to reduce platelet contamination.

However, mononuclear vitamin C levels as measured here were compared with a method similar to Boyum's in which a higher yield of mononuclear cells was obtained. The vitamin C concentrations were very similar (within 5%) showing that differences in yield did not affect the values obtained.

Statistical Methods: The clinical score results were approximately normally distributed and therefore small-sample parametric statistics (student t-test) were used (and the same for biochemical data). However non-parametric statistics (Mann-Whitney U-test) are also presented for the clinical data in order to overcome the criticism that, whilst the clinical scoring scales were undoubtedly, at least ordinal (ie. meaningful on a "ranking" basis), they were not necessarily "interval" scales (ie. equidistant intervals between any one score and the next).

Furthermore by both methods a 1-tail rather than 2-tail test is used since the hypothesis of the study is that vitamin C patients fare better (not just differently) than the placebo patients (Hunt *et al*, 1984 [11]).

Results

Full clinical information was obtained on 57 patients at 0, 2 and 4 weeks. Four patients were excluded because of incomplete information. Table II shows the mean ages and numbers of patients in the vitamin C and placebo groups.

Table III shows blood vitamin C concentrations (plasma and white cell) on admission. It can be seen that 35% of the subjects commenced the trial with low plasma levels (less than 11.4μ mol/1 of plasma). Mean platelet and polymorphonuclear white cell concentrations were also very low compared with normal (Evans *et al*, 1982 [15]; Schorah *et al*, 1986 [12]). To the author's knowledge no-one has attempted to define lower threshold values for these in the same way as plasma, but the vitamin C levels quoted by Evans *et al* (1982 [15]) for healthy young adults (polymorphs, 0.527 ± 0.139μ mol/10^9 cells; platelets, 0.030 ± 0.013μ mol/10^9 cells: mean values ± SD) were considerably higher than the admission values found in this study (Table III). The concentrations remained low in the unsupplemented subjects at 2 and 4 weeks (Table III) suggesting either low vitamin C intake from the hospital diet or high utilisation rates or both. However, low plasma levels were almost completely eliminated and white cell levels significantly increased in the supplemented subjects by weeks 2 and 4, showing that 200 mg per day was sufficient to achieve this even in these acutely ill patients. Plasma platelet and polymorph levels on admission tended to be particularly low in those patients who were most severely ill (for whom TOTRESP∅ = 8 or 9), as shown in Table IV. Although the differences shown did not achieve statistical significance, the tendency was consistent for all four blood fractions.

Table II: Numbers and ages of patients recruited to study for whom full clinical information was obtained

	Vitamin C group				Placebo group			
		Age (years)				Age (years)		
	Number	Mean	SD	Range	Number	Mean	SD	Range
Males	13	81.4	7.1	66–93	14	80.4	5.7	74–94
Females	15	82.0	4.0	76–88	15	78.9	6.1	72–90
Total	28				29			

Table III: Mean (±SD) plasma and white cell vitamin C concentrations during the study; also % of subjects with low plasma levels (less than 11.4 μ mol/l)

		Weeks	Plasma (μ mol/l)			μ mol/10^9 cells					
						Platelets		Polymorphonuclear Cells		Mononuclear Cells ∅	
			N	Mean	% of Subjects <11.4	N	Mean	N	Mean	N	Mean
All patients combined (C+P)		0	57	23.3* (±22.7)	35.0	46	0.020 (±0.013)	44	0.293 (±0.196)	52	1.948 (±1.227)
All Patients		0	28	23.3 (±21.0)	31.0	23	0.018 (±0.009)	23	0.291 (±0.191)	27	1.755 (±0.784)
	C	2	19	85.8[a] (±43.8)	5.3	16	0.035[c] (±0.016)	15	0.677[e] (±0.298)	17	3.078 (±1.363)
		4	10	94.9[b] (±32.4)	0	11	0.033[d] (±0.018)	10	0.687[f] (±0.366)	11	3.096[g] (±1.948)
		0	29	23.9 (±25.0)	40.0	23	0.022 (±0.016)	21	0.296 (±0.204)	25	2.158 (±1.556)
	P	2	19	19.3[a] (±14.2)	36.8	13	0.019[c] (±0.009)	12	0.289[e] (±0.094)	16	2.380 (±1.636)
		4	12	24.4[b] (±19.9)	25.0	9	0.020[d] (±0.010)	9	0.426[f] (±0.256)	12	1.744[g] (±0.875)

NB. C = patients supplemented with vitamin C; P = unsupplemented (placebo) patients;
N = number of analyses; (*median 16.5); (∅ mainly lymphocytes)
Pairs of means with same superscript letter significantly different (t-test): a, b, c, e: $p<0.001$; d, f, g: $p<0.05$

Table IV: Mean (±SD) blood values of vitamin C on admission (week 0): Comparison of those most severely ill (TOTRESP∅ = 8 or 9) with the rest of the patients

	Patients with TOTRESP∅ = 8 or 9		All patients excluding those with TOTRESP∅ = 8 or 9	
	N	Mean	N	Mean
Plasma (μ mol/l)	28	19.9 (±6.2)	31	26.1 (±28.3)
Platelets (μ mol/10^9 cells)	20	0.019 (±0.010)	26	0.021 (±0.015)
Polymorphonuclear cells (μ mol/10^9)	20	0.249 (±0.119)	24	0.330 (±0.237)
Mononuclear cells (μ mol/10^9)	25	1.715 (±0.722)	27	2.164 (±1.534)

N = number of analyses

With regard to clinical progress, 6 patients died during the trial – 5 placebo and 1 vitamin C. This difference was not significant statistically but death was not related to age or underlying disease other than the respiratory condition.

Table V compares the vitamin C and placebo groups in terms of TOTRESP scores. Since different starting points in TOTRESP must be taken into account the change in TOTRESP scores from 0 to 4 weeks ("TOTCH") is also shown. The starting points for both groups were similar and so was the clinical progress towards well at 2 weeks. The improvement in scores from 0 to 4 weeks was highly significant in both groups

Table V: Means (± SD) of the total respiratory clinical scores ('TOTRESP') of the subjects at the assessment intervals and change in scores from 0 to 4 weeks (TOTCH)

		TOTRESP Weeks 0	2	4	Change in scores from 0 to 4 weeks (TOTCH)
Vitamin C group	Mean	7.18	4.46	3.75	3.43*
	SD	1.31	1.69	1.62	1.77
	N	28	28	28	28
Placebo group	Mean	7.45	4.86	5.14	2.31*
	SD	1.15	2.30	2.68	2.44
	N	29	29	29	29

* Comparing TOTCH score for vitamin C group with placebo group: Student t-test p = 0.026 (1-tail) (ie. vit C significantly better recovery progress than placebo) or Mann-Whitney U test p = 0.062 (1-tail), NS

Table VI: Mean clinical scores and TOTCH of patients who were most severely ill when admitted (ie. TOTRESP∅ = 8 or 9)

		TOTRESP Weeks 0	2	4	TOTCH
Vitamin C group	Mean	8.42	4.75	4.17	4.25*
	SD	0.51	1.91	2.29	2.14
	N	12	12	12	12
Placebo group	Mean	8.33	5.60	6.46	1.87*
	SD	0.49	2.61	1.48	3.00
	N	15	15	15	15

* p = 0.012 (t-test); p = 0.012 (Mann-Whitney U-test) (ie. TOTCH significantly greater for vit C group than placebo group)

($p < 0.001$, t-test). However, comparing groups, there was a divergence of scores by week 4, with the placebo group regressing whilst the initial improvement in the vitamin C group was continued.

In terms of TOTCH, the vitamin C group fared significantly better from 0 to 4 weeks than the placebo group (p = 0.026, t-test) but this difference did not quite reach conventional levels of significance by the Mann-Whitney test (p = 0.062). If the data is re-examined excluding the scores of those who died, then the difference in TOTCH between the two groups is reduced (vitamin C 3.59, placebo 3.17) and becomes non-significant by either statistical test.

Because plasma and cell levels of vitamin C tended to be lower in the most severely ill patients (Table IV) and because it is these who have the greatest potential for clinical improvement on supplementation, the clinical progress for this sub-group was similarly.

The results show (Table VI) that the supplemented patients fared better than the placebo group, and this difference was significant by both statistical methods. Furthermore, even when Table VI is recalculated excluding those who died, the better clinical progress of the vitamin C group remains close to significant (Table VII).

Table VII: Mean clinical scores and TOTCH of most severely ill patients (with TOTRESP∅ = 8 or 9) excluding those who died

		TOTRESP Weeks 0	2	4	TOTCH
Vitamin C group	Mean	8.36	4.27	3.63	4.73*
	SD	0.50	1.00	1.43	1.42
	N	11	11	11	11
Placebo group	Mean	8.40	4.50	4.70	3.70*
	SD	0.52	1.65	1.70	1.64
	N	10	10	10	10

* p = 0.072 (t-test); p = 0.061 (Mann-Whitney U-Test) (comparing TOTCH for vit C and placebo groups)

Discussion

On admission, both plasma and cell ascorbic acid levels were low in these patients. This could reflect the effect of disease or poor intake (Basu and Schorah, 1982 [3]). None of the patients smoked whilst in hospital, but if they had smoked prior to hospitalisation this could have contributed to these low levels. It is known that heavy smoking depresses plasma vitamin C levels by about 30% compared with non-smokers matched for intake, but this can be corrected by an additional intake of approximately 40 mg/day (Pelletier O., 1975 [16], Kallner *et al*, 1981 [17]) which was easily supplied in this trial in the supplemented subjects. Furthermore mean plasma levels of the vitamin C and placebo groups were almost identical on admission (Table III) which does not suggest significant differences between them in terms of prior smoking habits.

Although there have been some reports of difficulties in increasing blood (particularly white

cell) levels of vitamin C in acute illness (Shukla, 1969 [18]; Hume and Weyers, 1973 [19]; Schwartz *et al,* 1973 [20]), significant rises were achieved here in both plasma and cell levels (Table III) with a dose of 200 mg/day. Therefore relatively modest increases in intake boosted blood levels substantially even in the presence of disease. Encouraging increased intake of fruit juice could therefore be considered as an alternative to tablet supplementation.

Overall, the results of this study suggest that supplemented patients (particularly those who were most severely ill on admission) fared better in terms of clinical progress than unsupplemented ones. This effect therefore operated over and above those of normal medication (mainly antibiotics and cough medicines) to which all patients were exposed.

This is potentially of considerable importance and so we must look critically at the findings. There were no significant differences between the placebo and supplemented on admission in terms of gender, age, vitamin C status, severity and type of chest infection or associated disease (Tables II, III and VIII). The groups were therefore well matched at the start of the study.

Any study involving clinical judgement as opposed to relying on a more objective end point such as mortality is potentially open to criticism in terms of subjectivity of assessment. In terms of mortality, the difference between groups was not statistically significant (by adjusted chi-square) but the numbers were small. However, at the outset of planning the trial, the problem of relatively small numbers was anticipated and therefore more sensitive means of judgement of clinical progress than mortality was devised. The Associate Specialist performed all the clinical assessments and hence this gave internal consistency between and within patients in scoring the three major diagnostic features of the acute respiratory condition. The scoring was therefore valid at least on a ranking basis.

The next consideration is whether death led to bias in the results. It seemed unjustified to exclude death unless cause of death was unrelated to the primary condition (respiratory infection). Of the other conditions given for some individuals as secondary diagnoses (Table VIII), only ischaemic heart disease was likely to have affected mortality risk over and above the respiratory disease. However none of the subjects who died on the trial had any secondary diagnosis, including ischaemic heart disease, and death was attributed directly to respiratory infection in each case. There is, therefore, no valid reason for excluding the deaths from the scoring scheme.

Examining the results overall, the progression towards well by 4 weeks tended to be better in the supplemented than unsupplemented subjects (Table V). Taking those subjects who were most severely ill (TOTRESPØ = 8 or 9) on admission (Table VI) the difference in progress in favour of supplemented subjects becomes more obvious and is significant by both statistical methods. This category of patient might benefit most from treatment because there was a tendency for their blood vitamin C levels to be lower and, as the sickest, they can, on recovery, achieve the highest "TOTCH" scores.

There is now a considerable literature suggesting that vitamin C has an important role in the immune response to infection particularly cell mediated immunity. There is evidence of vitamin C's ability to stimulate chemotaxis in phagocytes (Anderson *et al,* 1980 [21]; Anderson, 1981b [22]; Oberitter *et al,* 1986 [23]) and to

Table VIII: Breakdown of primary and secondary diagnoses in the two groups

	Primary diagnosis	Number of patients with this diagnosis
Vitamin C group	Acute bronchitis	19
	Bronchopneumonia	9
		Total 28
Placebo group	Acute bronchitis	21
	Bronchopneumonia	8
		Total 29
	Secondary diagnosis	
Vitamin C group	Ischaemic heart disease	5
	Anaemia	1
	Anxiety	1
	Spondylosis	1
Placebo group	Ischaemic heart disease	4
	Anaemia	1
	Anxiety	1
	Diabetes	1
	Parkinson's	1
	Dementia	1

prevent deactivation of neutrophil chemotaxis which may otherwise occur during continual exposure to circulating chemotaxins (Patrone *et al,* 1980 [24]). Stimulation of lymphocyte transformation by vitamin C has been demonstrated (Anderson, 1981a [10] and 1981b [22]; Kennes, 1983 [25]) including during exposure to influenza virus (Manzella and Roberts, 1979 [26]). Improvements in various measures of cell mediated immunity have recently been reported in acutely and chronically sick elderly hospitalised patients given antioxidant supplements, including vitamin C (Penn *et al,* 1989 [27]).

In some of these situations, vitamin C may act by neutralising various oxidants produced by activated phagocytes. There is evidence that extracellular vitamin C helps to protect against this sort of damage during the inflammatory process by scavenging these compounds (Oberitter *et al,* 1986 [23]; Frei *et al,* 1989 [28], Theron *et al,* 1990 [29] and Hemila, 1992 [5]). Such protection may be especially important in acute respiratory infection.

There are thus a number of potential mechanisms by which vitamin C supplementation could improve the symptoms associated with the acute inflammatory response occurring in this type of patient.

Conclusion

Although this study was performed on a relatively small number of subjects the results suggest that moderate vitamin C supplementation could have clinical benefit to patients suffering from acute respiratory infection, particularly to those who are most severely ill on admission. In many of these patients, plasma and white cell vitamin C levels are likely to be low enough to be construed as representing at least marginal deficiency, but 200 mg per day boosts these considerably within 2 weeks. Conversely, in the unsupplemented patients', concentrations of the vitamin remain low. These results are therefore consistent with evidence that vitamin C assists immune function possibly by acting as an antioxidant.

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The study was approved by the Huddersfield Health Authority Medical Ethical Committee and informed consent was obtained from the patients taking part in the trial.

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