Risk Assessment — Vitamin C

General information

Chemistry

Vitamin C is a six-carbon compound structurally related to glucose, consisting of two inter-convertible compounds: L-ascorbic acid, which is a strong reducing agent, and its oxidised derivative, L-dehydroascorbic acid.

Natural occurrence

Most animals are able to synthesise vitamin C from glucose and galactose. However, primates and guinea pigs are unable to do so.

Occurrence in foods, food supplements and medicines

Food of plant origin, particularly citrus and soft fruits and leafy green vegetables, are major sources of vitamin C. Kidney and liver are good animal-derived sources of vitamin C. Vitamin C is readily lost during cooking, due to oxidation. Ascorbic acid is a permitted anti-oxidant in food with no specified limits on the level of fortification. Vitamin C is present in numerous dietary supplements and in licensed medicines at doses of up to 3000 mg.

Recommended amounts

In 1991, COMA recommended a RNI for ascorbic acid of 40 mg/day for adults, with an increase in pregnancy to 50 mg/day, and during lactation to 70 mg/day (COMA, 1991).

Analysis of tissue levels and vitamin C status

Plasma and urinary vitamin C levels may be measured but reflect recent dietary intake rather than the level of vitamin C in body stores. Leucocytes contain higher concentrations of vitamin C than plasma, whole blood or serum but measurement of leucocyte vitamin C is technically more difficult than estimation of plasma or urinary levels. A leucocyte vitamin C level below 0.01 mg per 10^8 cells is generally regarded as deficient.

Brief overview of claimed non-nutritional beneficial effects

It has been claimed that vitamin C protects against the common cold. Beneficial effects on conditions such as cancer, vascular disease, cataracts, diabetes, asthma, arthritis, Parkinson's disease, autism and depression have also been suggested.

Function

Vitamin C is a strong reducing agent and as an antioxidant is involved in prevention of the damaging effects of free radicals. Vitamin C is involved in the synthesis of collagen, neurotransmitters and carnitine; it is an enzyme co-factor and also increases the gastrointestinal absorption of non-haem iron.

Deficiency

Vitamin C deficiency in humans leads to the clinical syndrome of scurvy. Early symptoms in adults include fatigue, weakness, aching joints and muscles. In later stages scurvy is characterised by anaemia, bleeding from the gums, petechial and sheet haemorrhages, and delayed wound healing.

Interactions

Absorption of metal ions may be altered by vitamin C.

Absorption and bioavailability

Gastrointestinal absorption of vitamin C is efficient and occurs in the small intestine via a saturable active transport mechanism. Absorption efficiency of low oral doses of vitamin C (4 – 64 mg) may be as high as 98%, but decreases with increasing doses of the vitamin.

Distribution and metabolism

Ascorbic acid is widely distributed in all tissues of the body, with higher levels found in the adrenal glands, pituitary and retina, and lower levels in kidney and muscle tissue.

Vitamin C is oxidised to dehydroascorbic acid, which is hydrolysed to diketogulonic acid and then oxidised to oxalic and threonic acid. Some oxidation to carbon dioxide occurs at high doses.

Excretion

Unmetabolised vitamin C and vitamin C metabolites, such as oxalate, are largely excreted in the urine. Approximately 3% of a 60 mg oral dose is excreted in the faeces. More of the vitamin is excreted unchanged at higher levels of vitamin C intake.

Toxicity

Human data

Gastrointestinal effects are the most common adverse clinical events associated with acute, high doses of vitamin C given over a short period of time.

Other suggested adverse effects include metabolic acidosis, changes in prothrombin activity and 'conditioned need' scurvy (where ingestion of an excessive amount of vitamin C during pregnancy may condition the offspring to require greater than the expected or recommended daily intakes of the vitamin).

Adverse effects related to the urinary route of excretion have been claimed, including renal stones, renal tubular disease and oxaluria. It has been suggested that vitamin C consumption may increase oxalate excretion and cause the formation of urinary stones, and subjects with a predisposition to the formation of kidney stones may be more sensitive to increases in urinary oxalate associated with vitamin C. However, studies in humans have not revealed a substantial increase in urinary oxalate after high intakes of vitamin C, and the moderate increases reported may be an experimental artefact (see later).

Vitamin C increases iron uptake from the gut and it is possible that this may be important in subjects with conditions such as haemochromatosis or in subjects heterozygous for this condition. However, when doses of vitamin C of 2000 mg/day were given to healthy volunteers for 20 months or more, no effects on iron status were found.

Supplementation trials

Vitamin C was administered (in combination with other vitamins and minerals) at doses of up to 1000 mg/day for up to 5 years in two supplementation trials with good compliance and no reported adverse effects. Reduced vitamin B_{12} levels in 3 (of 90) individuals consuming more than 1000 mg/day over a minimum of 3 years were reported in an earlier trial, although the relevance of these observations has been questioned.

Animal data

Vitamin C has low toxicity when large doses are given over a short period of time. High doses of vitamin C are associated with decreased growth rates in guinea pigs (50 mg/day), increased cholesterol levels in rats (150 mg/kg bw/day) and interference with trace element metabolism in chicks. No effects on reproductive or developmental parameters have been reported. A conditioned increase in vitamin C requirements has been reported in guinea pigs.

Carcinogenicity and genotoxicity

No data on carcinogenicity have been identified. Some positive *in vitro* mutagenicity tests have been reported, although results are generally mixed. However, the positive results tended to occur when vitamin C was tested in the presence of copper. Short-term vitamin C supplementation has been reported to cause an increase in modified DNA bases, and this has been attributed to pro-oxidant effects. *In vitro*, vitamin C caused an increase in DNA reactive metabolites formed from lipid hydroperoxides. There is no reliable evidence of mutagenicity *in vivo*.

Vulnerable groups

Individuals unable to regulate iron absorption due to haemochromatosis or thalassaemia may be vulnerable to any enhanced iron absorption caused by vitamin C. Although clear and substantial rises in urinary oxalate excretion have not been demonstrated during increased vitamin C intake, it remains possible that individuals with risk factors such as uricosuria will be more sensitive.

Mechanism of toxicity

Urinary stones may result from an increase in urinary oxalate excretion (see discussion below). Adverse effects attributed to vitamin C may be due to increased sensitivity to oxidant stress, because vitamin C can be pro-oxidant at very high concentrations.

Dose-response characterisation

The dose-response is unclear, as many studies have used only one dose level. However, where found, adverse effects are generally reported at levels in excess of 1000 mg vitamin C/day.

Genetic variations

Polymorphisms in haptoglobin and transferrin have been reported to be associated with altered metabolism of vitamin C. Certain conditions may increase sensitivity to the adverse effects associated with this compound e.g. haemochromatosis, thalassaemia or a pre-disposition to urinary or renal stones.

Studies of particular importance in the risk assessment

(For full review see http://www.food.gov.uk/science/ouradvisors/vitandmin/evmpapers or the enclosed CD).

Cameron and Campbell, 1974

In a stepped study, healthy human volunteers were given vitamin C with the dose increasing each week by 1000 mg. Abdominal distention, flatulence, diarrhoea and transient colic were described as 'fairly frequent' effects at supplemental dose levels of 3000-4000 mg daily. No other details were provided.

Cook et al., 1984

Seventeen adult volunteers were given 2000 mg vitamin C/day with meals for 16 weeks in a study examining the effect of dietary iron absorption and assimilation on body iron status. Nine subjects continued in the study, taking these doses of vitamin C for 24 months. No subjective side effects were reported. Although vitamin C enhances absorption of non-haem iron, no increase in serum ferritin levels was found, despite wide variation in initial iron status between the volunteers. The authors concluded that supplemental vitamin C had a negligible effect on iron stores. Vitamin C supplementation continued for a further 20 months in 4 iron-deficient and 4 iron-replete subjects. No effect of vitamin C on body iron reserves (as measured by serum ferritin levels) was apparent and no intestinal adaptation to the enhancing effect of the vitamin had occurred. The authors considered several possible explanations for their findings. No adverse effects were reported in this study of large supplemental doses of vitamin C but the authors noted that the study did not exclude possible adverse effects in individuals who are heterozygous for the haemochromatosis gene (approximately 10% of the population). The study involved small numbers of participants with variable iron status and was not blinded, though compliance was checked.

Mai et al., 1990

Twenty patients with multiple sclerosis were randomised to receive either supplements providing 2000 mg/day vitamin C, 6 mg/day sodium selenite and 480 mg/day vitamin E, or placebo for 5 weeks. The patients were interviewed about side effects after 2 weeks and 4 weeks of treatment. Out of the 10 patients receiving the active supplement, one reported slight facial erythema at week 2, which subsequently subsided during continued treatment, one reported passing urine with a peculiar smell. Three of the 10 patients receiving the placebo reported an increased number of headaches.

Urivetzky et al., 1992

Fifteen patients with calcium oxalate renal stones (presumed by the authors to be at greatest risk from the effects of ascorbic acid), were given 100, 500, 1000, or 2000 mg supplemental ascorbic acid on days 2 and 3 following operations to remove the stones. Intrarenal urine specimens were collected by nephrostomy catheter directly into EDTA and sodium thimerosol preservative to stabilise ascorbic acid and oxalate; oxalate was measured following the removal of ascorbic acid with sodium nitrite. The increase in urinary oxalate was statistically significant at doses of administered vitamin C of 500 mg or more. The authors estimated that there was a 6-13 mg/day increase in urinary oxalate excretion per 1000 mg/day administered ascorbic acid, and concluded that vitamin C supplementation was associated with an increased risk of calcium oxalate renal stones.

Wandzilak et al., 1994

Fifteen healthy volunteers were given 1000, 5000 and 10,000 mg vitamin C each for 5 days, separated by 5 days with no supplementation. Twenty-four-hour urine samples were preserved by adding hydrochloric acid to reduce the pH to 2 and frozen. Ascorbate appeared to be converted non-enzymatically into oxalate during analysis, confounding the analytical measurement. This may explain the previously published results in other studies, where oxalate excretion was affected by ascorbate consumption. A moderate, dose-dependent increase in urinary oxalate was found in the volunteers. However, a clear trend was not apparent when the non-enzymatic ascorbate-to-oxalate conversion was taken into account. Subjects were advised to take the vitamin C tablets at mealtimes to minimise potential adverse gastrointestinal effects. However, two out of 15 volunteers experienced diarrhoea when consuming 10,000 mg supplemental vitamin C/day and were unable to continue taking this level. The study was not blinded.

Levine et al., 1996; Levine et al., 1999

Steady state plasma ascorbic acid concentrations, urinary ascorbic acid and urinary oxalate concentrations (the latter determined by an enzymatic method reported to be free from interference by ascorbic acid) were measured in 7 young healthy non-smoking subjects. At vitamin C intakes of 1000 mg/day there were statistically significant increases in urinary oxalate excretion (though this was still within physiological limits) and uric acid excretion.

Auer et al., 1998

Ten healthy male volunteers (with no history of stone formation) ingested 4000 mg vitamin C daily (in 4 doses per day) for 5 days. The urine collection procedure allowed the analysis of oxalate in the presence and absence of an EDTA preservative and for the analysis of ascorbic acid. In the preserved samples there was no significant increase in oxalate excretion at any stage of the protocol. Excretion of ascorbic acid increased when vitamin C ingestion commenced but levels stabilised after 24 hours suggesting saturation of the metabolic pool. While transient and statistically significant changes

occurred in some of the biochemical risk factors, it was concluded that large doses of vitamin C did not affect the principal risk factors associated with calcium oxalate kidney stone formation.

Gokce et al., 1999

In a randomised, double-blind, placebo-controlled study, flow-mediated dilation of the brachial artery was measured in 21 patients with coronary artery disease 2 hours after a single supplementary dose of 2000 mg vitamin C and following a 30-day period during which 500 mg vitamin C was taken daily. A significant increase in flow-mediated dilation of the brachial artery was observed, compared to controls, following the single dose of 2000 mg vitamin C. This improvement was sustained after the 30-day supplementation period with 500 mg/day vitamin C. The report does not mention assessment of adverse effects.

Morton et al., 2001

A retrospective cohort study of 994 women, of whom 277 were regular users of vitamin C supplements, assessed the effects of vitamin C supplementation on bone mineral density (BMD). Amongst the users of vitamin C supplements, supplemental intake ranged from 100 mg/day to 5000 mg/day; the mean was calculated to be 745 mg/day. Average duration of vitamin C supplement use was 12.4 years. BMD levels were assessed at the hip, lumbar spine and ultradistal and midshaft radius of the arm. Vitamin C supplement users were reported to have BMD levels 3% higher than non-users at the midshaft radius, femoral neck and hip, after adjusting for age, body mass index and total calcium intake. A significant increase in BMD of the femoral neck and a small increase in BMD of the total hip were still apparent in vitamin C supplement users even after adjusting for age, body mass index, exercise, alcohol consumption, smoking, physician-diagnosed osteoarthritis and use of calcium supplements, multivitamin supplements, thiazides, oestrogen, thyroid hormones and oral corticosteroids. No parameters other than BMD were assessed, thus no side effects were reported.

Exposure assessment

Total exposure/intake:

Food   Mean: 64 mg/day
                              97.5th percentile: 160 mg/day (from 1986/87 NDNS)

Supplements   up to 3000 mg/day (Annex 4)

Estimated maximum
daily intake: 160 + 3000 = 3160 mg

Vegetarians are a potential high intake group.

Risk assessment

The available data suggest that vitamin C is not associated with significant adverse effects and there are no obvious specific key toxic endpoints for vitamin C dose given orally to healthy subjects. High oral doses of vitamin C are associated with gastrointestinal effects, generally at doses of several grams, but have also been reported at doses of 1000 mg (1 g). There are few controlled studies specifically investigating this adverse effect. Controlled studies do not support anecdotal reports of other possible adverse effects, such as infertility.

Earlier suspicions of potential adverse effects, such as destruction of vitamin B_{12}, have not been confirmed following subsequent developments in analytical techniques. Data on increased oxalate excretion attributable to vitamin C are conflicting. Some reported increases in urinary oxalate might be attributable to experimental artefact (Auer *et al.*, 1998) though increased oxalate was apparent at doses of more than 500 mg/day in subjects with calcium oxalate stones. In contrast, doses of 4000 mg/day vitamin C were not associated with increased urinary oxalate excretion in normal subjects.

Potential vulnerable groups include sufferers from disorders of iron metabolism or storage.

Vitamin C has been reported to produce a variety of pro-oxidant effects. The significance of this for the general population is uncertain.

Vitamin C has very low acute toxicity in animals and no effects on reproductive parameters have been reported. However high doses of vitamin C are associated with decreased growth rates in guinea pigs (50 mg/day) and increased cholesterol levels in rats (150 mg/kg bw/day).

ESTABLISHMENT OF GUIDANCE LEVEL

There are insufficient data to set a Safe Upper Level for vitamin C. The vitamin may be of low toxicity, though adverse effects, in particular on the gastrointestinal system, may occur in subjects consuming quantities of vitamin C greater than 1000 mg/day. The precise dose at which such effects occur is variable. These effects may be a serious problem for individuals with disordered gastrointestinal function. A limited study by Cameron and Campbell (1974) suggests a LOAEL for gastrointestinal effects in humans of 3000-4000 mg/day. For guidance purposes, based on a LOAEL of 3000 mg/day, and applying an UF of 3 for LOAEL to NOAEL extrapolation, a supplemental dose of 1000 mg/day supplement would not be expected to have any significant adverse effects. The dose is equivalent to 17 mg/kg bw/day in a 60 kg adult. A guidance level for total vitamin C intake has not been estimated since adverse effects appear to follow supplemental, bolus doses rather than intake of vitamin C in food. It should be noted that higher levels of vitamin C may be without adverse effects in many individuals.

A number of potentially vulnerable groups have been identified; these include individuals who are heterozygous for haemochromatosis, and thalassaemia or those with a pre-disposition to urinary or renal stones. Data on the possible adverse effects of vitamin C on these individuals are also conflicting, but, appear to occur at intakes above 1 g/day.

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