CV247 is under investigation and its safety and efficacy have not yet been demonstrated

VITAMIN C

Chemistry

Vitamin C is a natural product contained in many fruits and vegetables. It is comprised essentially of 2 compounds, L-ascorbic acid (MW 176) and its oxidised derivative, L-dehydroascorbic acid. Although most vitamin C in the body is in its reduced form, both ascorbic acid and dehydroascorbic acid have biological activity and are interconvertible by an oxidation and reduction (redox) reaction, requiring the presence of the enzymes ascorbate oxidase and glutathione dehydrogenase. Ascorbic acid is a 6-carbon lactone which is synthesised in many animals from D-glucose or D-galactose via the glucuronic acid pathway. However several species including man and primates are unable to synthesise ascorbate because they lack hepatic gulonolactone oxidase, the terminal enzyme in the biosynthetic pathway. As a consequence such animals must ingest ascorbic acid to survive. Ascorbic acid is a strong reducing agent (an electron donor) and probably all of its biochemical function is accounted for by this property (Basu, 1996; Stipanuk, 2000).

Biochemical Role

Vitamin C is regarded as the most important water- soluble anti-oxidant in human plasma and mammalian cells, which have mechanisms to recycle and accumulate it against a concentration gradient, suggesting that the vitamin might have important intra-cellular and extra-cellular functions (Duarte, 2005).

Enzyme reactions

Ascorbic acid plays an important role in many reactions involving mono-oxygenases or di-oxygenases, reactions, which also require molecular oxygen and Fe2+ or Cu2+ as a cofactor. Essentially, ascorbic acid plays either of 2 roles, as a direct source of electrons for the reduction of oxygen, or as a protective agent for maintaining Fe and Cu in their reducing states.

A high concentration of vitamin C is found in the adrenal medulla of mammals. It is a cofactor, acting as a hydrogen donor, for dopamine β mono-oxygenase, which catalyses the synthesis of noradrenaline, in neurons, and the adrenal medulla. This mono-oxygenase system contains copper, which is an integral part of the functioning of vitamin C, and which accepts electrons from ascorbate as it is reduced to the cuprous ion. These electrons are subsequently transferred to oxygen and then to the substrate dopamine to yield the hydroxylated product noradrenaline. The protein bound copper is also re-oxidised to the cupric state during this reaction. Noradrenaline may subsequently be methylated to adrenaline. Adrenaline potentiates adenyl cyclase, which in turn activates the formation of cAMP from ATP. The breakdown of cAMP is reduced by ascorbic acid by inhibiting phosphodiesterase, and hence ascorbic acid increases tissue levels of cAMP. For many bioactive peptides to be active, a carboxy terminal α-amide must be added. α-amidation is mediated by peptidylglycine α-amidating monooxygenase, which is a

bifunctional enzyme containing 2 distinct domains. One, peptidylglycine α-hydroxylating mono-oxygenase, requires ascorbic acid, oxygen and copper to function.

A variety of reactions involve ascorbate dependant dioxygenases, many requiring α-ketoglutarate as a co-substrate, and all containing iron in its ferrous state. Such enzymes include prolyl 4-hydroxylase and lysyl hydroxylase which are responsible for the hydroxylation of prolyl and lysyl residues in procollagen, an essential reaction for the collagen molecule to aggregate into its triple helix configuration. Hence ascorbic acid deficiency leads to markedly abnormal collagen, affecting basement membrane in skin, connective tissues, blood capillaries and bone matrix.
Reduced carnitine levels may also be a sequel to ascorbic acid deprivation. L-carnitine is required as part of fatty acid metabolism for formation of acyl carnitine derivatives needed for transport of long chain fatty acids into mitochondria for subsequent oxidation and ATP formation. Carnitine can be synthesised de novo from lysine and methionine in a process which involves 2 hydroxylation reactions. Both enzymes, trimethyllysine hydroxylase and γ butyrobetaine hydroxylase, require a reducing agent, most probably ascorbic acid.
In total, vitamin C acts as an electron donor for 8 mammalian enzymes. Three participate in collagen hydroxylation, 2 in carnitine biosynthesis, and one each is required for noradrenaline biosynthesis, peptide hormone amidation, and tyrosine metabolism
In addition it is thought that vitamin C is one of the factors modifying the mixed function oxygenase system so important in the elimination process of lipid soluble environmental chemicals.

Other properties

Non-haem iron usually constitutes more than 90% of dietary iron, but it is less readily absorbed than haem iron. Ascorbic acid is a potent, but dose related enhancer of non-haem iron absorption, by maintaining iron in its reduced form in the gut and hence aiding absorption. Ascorbic acid also helps the transer of iron into the blood.

As a strong reducing agent, vitamin C has many biochemical functions. Ascorbic acid is oxidised to dehydroascorbate through a short-lived intermediate , ascorbate free radical or monodehydroascorbate. This intermediate forms a part of the body's antioxidant defences against reactive oxygen species (ROS) and free radicals. The antioxidant action of ascorbic acid is potentiated by the presence of other reducing agents, such as glutathione and NADH, which assist with the regeneration of ascorbic acid from its oxidation products. This protection against oxidative damage is relevant in tissue with high oxidant production and/or oxygen concentration such as neutrophils, monocytes/macrophages, lung and eye, Depending on the tissue and the radical generated, ascorbic acid could quench superoxide, hypochlorous acid, singlet oxygen or hydroxyl radicals (Sies, 1995). Ascorbic acid may also have a role in modulating extracellular oxidants, such as blocking oxidative modification of low density lipoprotein, and thus protecting against atherogenesis which maybe a consequence of LDL peroxidation (Jialal, 1995). Extracellular ascorbate may also quench oxidants that leak from activated neutrophils or macrophages.

Owing to its reducing properties, ascorbic acid appears to have potential importance as a nitrite scavenger. Nitrites are converted from nitrates ingested in various foods, and are subsequently may be amidated in the stomach to form potentially carcinogenic nitroso compounds. Ascorbic acid has the potential to block the formation of nitrosamines

because its nitrite scavenging activity is a more rapid process than the amine nitrite reaction. (Tannenbaum, 1991). Ascorbic acid, at doses up to 1g/day, has been shown in studies in healthy volunteers given fish meals as a source of amines and nitrosatable precursors, and nitrate containing drinking water, that the consequential increase in the levels of carcinogenic N-nitrosodimethylamine (NDMA) is significantly decreased (Vermeer, 1999).

Recommended daily allowance

Ascorbic acid is used extensively as a supplement in both animals and man.
Vitamin C status in man depends upon dietary intake, metabolic demands and renal clearance. The current RDA for vitamin C for healthy adult non smoking males and females is 60mg/day. This is based on the mean requirement of 46mg/day to prevent the deficiency disease, scurvy. The RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all healthy individuals, but in view of the benefits of increased intake to reduce the risk of chronic diseases such as cancer and heart disease, a daily intake of 90-100mg is required, and a new RDA of 120mg/day has been proposed for healthy individuals (Carr, 1999). Important functions such as the immune response, pulmonary function and iron absorption are related to vitamin C intakes, and at least 150-200mg per day may enhance these roles. In humans there is overwhelming evidence that reserves of vitamin C are depleted in both acute and chronic disease, such as cancer. In such circumstances where vitamin C levels are depleted, an even higher ascorbic acid intake is required to achieve normal concentrations. The literature documents that doses of 200mg vitamin C per day, and much higher, are safe (Weber, 1996). Indeed a comprehensive review of studies has confirmed the safety of higher than RDA intakes of vitamin C.. In a total of 8 placebo controlled, and 6 open studies, daily doses of up to 10g of vitamin C were consumed for up to 3 years (Bendich, 1995).
Ascorbic acid is available in a large number of preparations. Tablets typically contain from 50 to 1500mg of the vitamin.

Immune function

Cells involved in the immune response contain very high concentrations of vitamin C, some 40-60 times the levels found in plasma. Aging and chronic disorders, such as cancer, are associated with depression of immunity and are accompanied by low levels of both plasma and leucocyte vitamin C (Basu, 1996). Proposed mechanisms of action for ascorbic acid in the prevention and treatment of cancer include enhancement of the immune system (Head, 1998). Although there is little evidence that vitamin C is involved in the humoral side of the immune response (production of immunoglobulins), it may promote chemotaxis, reduce allergic reactions and raise interferon production (Anderson, 1981).

CLINICAL PHARMACOLOGY

Vitamin C is synthesised naturally in most animals including dogs.

Absorption, Distribution, Metabolism and Excretion

Absorption

Absorption in man occurs in the buccal cavity, stomach and small intestine. Buccal absorption is mediated by passive diffusion, whereas gastrointestinal absorption is via an active carrier mediated transport system, which is probably Na+ dependant. This system becomes saturated when the mucosal concentration is greater than 6mmol/L and may account for the fact that dietary vitamin C absorption decreases with increasing intake (Hornig, 1980). Bioavailability following oral administration is nearly 100% for single doses of up to 200mg but has been reported to decrease in man to 75% following a single 1g dose, to only 50% with 1250mg and just 20% following a single 5g dose (Levine, 1996). In a 3 month study, the serum response to oral administration of vitamin C at doses of 50 and 1000mg/day in 54 Japanese subjects in the age range 40-69 years, found that serum ascorbic acid was significantly higher following high dose administration, increasing by between 88 and 95% compared with control. Steady state was observed after 1 month and remained stable thereafter (Sasaki, 2000).
When 60mg vitamin C (the RDA) is consumed each day the concentration in plasma reaches about 0.8mg/dl (45microM) and the body store is approximately 1500mg. If intake is raised beyond 200mg/day, the body store tends to level off at about 2500mg with a plasma concentration of less than 2mg/dl.
The pharmacokinetics of vitamin C have been studied in 17 healthy subjects to determine whether plasma concentrations vary according to the route of administration. Bioavailability data was obtained following steady state at various doses from 0.015 to 1.25g. It was found that whatever dose of vitamin C was given intravenously (iv), mean peak plasma concentrations were significantly higher than when the same dose was administered by mouth, at the highest dose some 6.6 fold higher. Notably when given iv, Cmax increased with increasing dose, whereas following oral administration Cmax seemed to plateau with increasing dose. A 1.25 mg oral dose was found to result in a mean Cmax of 187 μmol/L. Using a 3 compartment vitamin C PK model, it was predicted that a single oral dose of 3g would only increase the Cmax to 206 μmol/L. Even if repeated at 3 hour intervals, a 3g oral dose was only predicted to produce a peak Cmax of 220 μmol/L. By contrast a 3g iv dose was predicted to result in a mean Cmax of 1760 μmol/L, whilst a 50g iv dose produced a Cmax of 13,350 μmol/L. At the 1,25g dose in the study, peak urine concentrations were 3.5 times higher after iv administration when compared with the same dose given orally. The data suggested that vitamin C plasma concentrations are tightly controlled when taken orally, whereas iv administration bypasses this control and results in very much higher plasma concentrations (Padayatty, 2004).

Distribution

Following absorption ascorbate is found in the plasma of man and animals. Ascorbate is transported by a carrier protein that is Na+ and energy dependant, whereas dehydroascorbic acid, transport of which is 10-20 fold faster than ascorbate is Na+ and energy independent (Welch, 1995) Because it is water soluble and not protein bound, ascorbate rapidly equilibrates in intra and extracellular compartments. The concentration of ascorbate in many tissues is 5 to 100 fold higher than that of plasma. Cells and tissues that accumulate ascorbate include the adrenal and pituitary glands, eye lens, brain, liver,

spleen, kidneys, heart muscle and leucocytes. Vitamin C exists mainly in the body in the reduced form as ascorbate, the oxidised form accounting for less than 10%. Ascorbate and dehydroascorbic acid transport depend upon substrate availability. Ascorbate is the predominant substrate under normal conditions under nonoxidising conditions and where ascorbate accumulation is mediated by the ascorbate transporter. Once oxidants are generated, however, dehydroascorbic acid is formed, transported into cells and reduced to ascorbate. The cycle of extracellular ascorbate oxidation and subsequent intracellular reduction is known as ascorbate recycling and is most likely to occur during the time that diffusible oxidants are present and increased intracellular ascorbate could be utilised for oxidant quenching. For example with neutrophils, the putative ascorbate transporter transports extracellular ascorbate and probably maintains microM concentrations within the cells. With activation, neutrophils secrete reactive oxygen species, which may oxidise ascorbate. The resultant dehydroascorbic acid is rapidly transported by glucose transporter isoenzymes into the cell where it is immediately reduced back to ascorbate, mainly by glutathione dependant, glutaredoxin. Ascorbate recycling thus allows cells to increase intracellular ascorbate concentrations rapidly and is therefore a potentially protective mechanism (Stipanuk, 2000).

The average half-life in adult humans is about 20 days with a turnover of 1mg/kg/day and a total body pool size of 1500mg. The daily utilisation of ascorbate is fairly constant, being around 0.2mg/kg fat free weight. Thus a man with a lean body mass of 70kg requires 14mg vitamin C daily to maintain the body pool (Basu, 1996).

Metabolism and Excretion

Healthy adults lose 3%-4% of their body store of vitamin C daily. Metabolism of vitamin C in man occurs by the irreversible hydrolysis of dehydroascorbic acid to diketogulonic acid, followed by oxidation to oxalic acid and threonic acid. These metabolites with some ascorbate-2-sulphate are excreted in the urine along with unmetabolised ascorbic acid.

As well as these metabolites, excretion of vitamin C in man can also occur in the forms of lyxonic acid, xylonic acid and xylose, which are the breakdown products of diketogulonic acid following decarboxylation. In addition a small portion of vitamin C (< 2%) is metabolised to CO2 and exhaled during respiration. In guinea pigs, on the other hand, 60%-70% of vitamin C is excreted as CO2. A number of factors, however, increase the rate of oxidation to CO2 in man, including the level of vitamin C intake, probably as a result of presystemic microbiological degradation of unabsorbed vitamin C in the gut. Iron toxicity is another factor thought to accelerate oxidation of vitamin C, and has been noted in patients with haemosiderosis.

The overall metabolism of vitamin C is affected by the level of its intake. At a physiological level, less than 10% is excreted as ascorbic acid and 90% as metabolites, whereas a reverse occurs when large doses (1-2g) are ingested. The ability of the renal tubules to re-absorb vitamin C is decreased when plasma levels rise to 0.75-1.0mg/dl, which explains why plasma levels rarely exceed 1.4mg/dl despite very large intakes of the vitamin (Basu, 1996).

CLINICAL EXPERIENCE

Vitamin C and Cancer

The fact that conventional therapies cause serious side affects, and some cancers have poor overall survival has resulted in an increased utilisation of alternatives treatments for cancer. Recent progress in molecular biology has increased the likelihood that cancer treatment will rely increasingly on interventions collectively termed chemoprevention, which is the use of agents to inhibit, delay or reverse carcinogenesis. Oxidative stress, whether from an increased production of oxidants or from a failure of physiological anti-oxidant systems, can cause cancer (Bjelakovic, 2004), and consequently the use of anti-oxidant supplements is widespread. Ascorbic acid, a deficiency of which is often seen in cancer patients (Head, 1998) and is a known potent water- soluble anti-oxidant, is the single nutrient supplement most commonly used by cancer patients (Block, 2003). Proposed mechanisms of action for ascorbic acid include:

a) enhancement of the immune system
b) stimulation of collagen formation necessary for walling off tumours,
c) inhibition of hyaluronidase which keeps the ground substance around the tumour intact and prevents metastases
d) prevention of oncogenic viruses
e) expedition of wound healing after cancer surgery
f) enhancement of the effect of certain chemotherapy agents
g) reduction of the toxicity of other chemotherapeutic agents, eg adriomycin
h) prevention of free radical damage
i) neutralisation of carcinogenic substances

Some Scottish and Japanese studies have also pointed to the potential benefit of high dose vitamin C for the treatment of terminal cancer (Head, 1998), and to increase survival (Block, 2003), though these findings are controversial, having been, on the whole, derived from uncontrolled trials or anecdotal reports. The controversy may, in part, be because of varying dosing regimens. Dose concentration studies in healthy subjects showed a sigmoidal relationship between oral dose and plasma and tissue vitamin C concentration, and hence optimal dosing is critical to intervention studies (Padayatty, 2003), so as to attain blood levels that are cytotoxic to malignant cells. Ascorbic acid when given in high enough doses to maintain plasma concentrations above levels that have been shown to be toxic to tumour cells in vitro, has the potential to selectively kill tumour cells in a manner similar to other cytotoxic agents. Unfortunately most studies of ascorbic acid and cancer have not utilised high enough doses to maintain a high plasma concentration (Riordan, 1995), though this is not always so. Nonetheless most people do not consume an optimal amount of vitamins by diet alone and low levels of the anti-oxidant vitamins including vitamin C may increase the risk of several chronic diseases (Fletcher, 2002), though observational studies have inconsistent results with respect to the relationship shown between specific dietary intake, or serum levels and risk of cancer (Ruffin, 2001). Several studies of serum and dietary anti-oxidants including vitamin C have suggested that higher levels, well above the current US RDA, are required to be protective, for example, in ovarian cancer (Fleischauer, 2001). The fact that diets high in fruit and hence vitamin C have been found to be associated with a lower risk of cancers of the oral cavity, oesophagus, stomach, colon and lung, but that some studies have been unable to find a strong relationship if the vitamin is added as a supplement, has led to one supposition that the protection may be derived from consumption of vitamin C in a complex mixture with other nutrients and bioactive compounds (Byers, 1995).
The effects of ascorbic acid may also be time dependant with different effects depending upon the species and the organ studied (Chan, 1998). One protocol for the use of vitamin

C in the treatment of cancer also emphasises the importance of continuous indefinite treatment with the emphasis on continuous and not intermittent (Cameron, 1991).

The use of Vitamin C to reduce cancer risk

A randomised, double blind trial in over 12,000 adults found that low dose anti-oxidant supplementation lowered the total cancer risk in men but not in women. In this study individuals received a daily diet supplement of different anti-oxidant vitamins and minerals including 120mg vitamin C and 30mg vitamin E, or placebo. The cut off limits for baseline serum concentrations were defined as 11.4 microM/L for vitamin C (0.3microM/L for beta-carotene, and 15 microM/L for vitamin E). It was found that after 7.5 years, the percentage of men who developed cancer was higher where the serum concentrations were under the cut off limits for the 3 vitamins. The risk of cancer was higher in men with baseline concentrations of serum vitamin C or vitamin E under the cut off limits (Galan, 2005). This finding of benefit to men is in part confirmed by a 10 year mortality study based on a cohort of over 11,000 US adults in the age range 25-74 years. The relation of the standardised mortality ratio (SMR) to increasing vitamin C intake for all cancers was lower in men (0.78) than in women (0.86). There was no clear relationship for individual cancer sites except possibly an inverse relationship for oesophageal and stomach cancers in men (Enstrom, 1992).

Numerous epidemiological studies have pointed to the importance of both dietary and supplementary ascorbic acid in the prevention of various types of cancer including bladder, breast, cervical, colorectal, oesophageal, lung, pancreatic, prostate, salivary gland, stomach. leukaemia and non-Hodgkins lymphoma (Head, 1998).

Other epidemiological and ecological studies have shown that L-ascorbic acid has a protective effect, in particular non-hormone dependant malignancies, such as oropharyngeal neoplasms (Chan, 1998). A review of the epidemiological literature from 1985 to 1993 relating only dietary intake of anti-oxidants and protection from cancer, found that for lung cancer there was only weak evidence of protection from vitamin C (2of 6 diet studies). For upper aerodigestive tract cancers (mouth, larynx and pharynx) and for cancer of the uterine cervix there was suggestive evidence for a protective effect (4 of 5 studies). But the available data did not support a protective effect of vitamin C in the diet for cancers of the colon , breast and prostate (Flagg, 1995).

Case control studies in Italy have suggested that a diet rich in fresh fruit and vegetables protects from the risk of most common epithelial cancers. Specific reference to the role of selected anti-oxidants, namely vitamins A,C and E showed a significant inverse relation with oral and pharyngeal, oesophageal and breast cancer risk, and for vitamins A, C and B6, colorectal cancer as well (La Vecchia, 2001). A population based prospective study of 59,000 women in Sweden found that, though there was no overall association between intake of ascorbic acid, retinol, beta-carotene and vitamin E, and the incidence of breast cancer, high intake of ascorbic acid was inversely related to breast cancer incidence among overweight women with a BMI>25 (hazard ratio 0.61), and for women with the highest consumption of linoleic acid (HR 0.72) (Michels, 2001).

However a review of placebo controlled studies that evaluated anti-oxidant supplementation (vitamins A, C and E with selenium, either in different combinations or separately) found that none of the supplements protected against oesophageal, gastric, colorectal or pancreatic cancers, and even increased overall mortality (Bjelakovic, 2004). Similarly the available data from prospective studies either only support a small reduction

in risk (Verhoeven, 1997), or do not support an association between vitamins C and E and risk of breast cancer (Zhang-Shumin, 2004). Another prospective study, in this case on over 34,000 postmenopausal women, was designed to assess the association between dietary anti-oxidant vitamin intake and the risk of breast cancer. It was found that women who consumed at least 500mg of supplemental vitamin C per day had a relative risk of breast cancer of 0.79 compared with women who did not take supplemental vitamin C. However the difference was not significant and provided little evidence that intake of vitamin C is associated with breast cancer risk (Kushi, 1996).

In a study that examined the association between stomach cancer mortality and regular (>15 times/month) use of vitamin C and other vitamin supplements among over one million US adults, it was found that the risk of stomach cancer mortality was reduced, though not substantially, with regular, albeit short (<10 years) vitamin C use at enrolment There was no benefit associated with vitamin E or multivitamins regardless of the duration of use (Jacobs, 2002).

The treatment of oesophageal squamous cell carcinoma and oesophageal adenocarcinoma was studies in a nationwide, population based case control study in Sweden. Subjects with a high parallel intake of vitamin C, beta-carotene and alpha tocopherol showed a 40-50% decreased risk of both types of cancer compared with subjects with a low parallel intake. Anti-oxidant intake was not associated with the risk of gastric cardia adenocarcinoma. Separately, vitamin C and beta-carotene reduced the risk of oesophageal carcinoma more than alpha-tocopherol. The data suggested that the inverse associations were even stronger in subjects with higher oxidative stress, such as that associated with smoking (Terry, 2000). Curiously another Swedish population based study, ascorbic acid was found to be inversely associated with all sub sets and sub types of gastric cancer in a significant dose response manner, with risk reductions between 40 and 60% (Ekstroem, 2000).

Some prospective epidemiological studies have suggested an inverse relationship between dietary vitamin C and the risk of lung cancer, but studies using pre-diagnostic plasma concentrations of ascorbic acid do not support the involvement of vitamin C in lung carcinogenesis (Virtamo, 1999).

Cancer of the salivary glands is rare, but a population based control study found that vitamin C intake of greater than 200mg/day compared with less than 100mg, was associated with a 60% decrease in salivary gland cancer risk, and that the inverse associations observed for carotene and vitamin E were diminished when adjusted for vitamin C intake (Horn-Ross, 1997).

Vitamin C and cancer treatment

Cancer patients are significantly depleted of ascorbic acid and indicates an increased requirement to potentiate the number of ascorbic acid associated mechanisms known to be involved in host resistance to malignant disease. The results of an open study in 100 terminal cancer patients given supplemental vitamin C found that the mean survival time was more than 4 times that for a group of 1000 untreated historical controls, Analysis of the survival time curves indicated that death occurred for 90% of the treated patients at about one third the rate for the controls, and that the remaining 10% had a much greater survival time averaging more than 20 times the untreated controls (Cameron, 1976).

A data bank recording the details of cancer patients attending 3 district general hospitals in Scotland over a 4.5 year period found that of 2804 patients with cancer, 1826 had reached an incurable stage. Of the latter, 294 had received supplemental ascorbate whereas 1532 had not. Analysis showed that the ascorbate supplemented patients had a median overall survival time of 343 days, almost double that of the non-supplemented group (Cameron, 1991). The use of vitamin C supplements has also been evaluated in terminally ill cancer patients at 2 hospitals in Japan. It was found in one hospital that the average survival time was 43 days for 44 low ascorbate patients, but 246 days, and with improved quality of life, for 55 patients who received high dose vitamin C supplementation. Results at a second hospital were similar (Murata, 1982).

A study at the Mayo Clinic on 150 patients with advanced cancer, who participated in a double blind study comparing high dose vitamin C (10g/day) and flavoured lactose placebo did not, however, support these findings. The 2 groups showed no appreciable difference in changes in symptoms, or performance status, and the median survival for all patients was about 7 weeks with the survival curves essentially overlapping (Creagan, 1979).

The positive results from the open label studies above and other similar trials have been criticised because they were not based on random, double blind principles, but if examined using broad inductive reasoning show that there is a strong possibility that vitamin C approximately doubled survival time as measured from the start of vitamin C treatment, regardless of whether this was after termination of conventional therapy or much earlier. It is suggested that Mayo Clinic study may be given an alternative interpretation, which supports this view (Jaffey, 1982).

It has been shown, both in vitro and in vivo, that combined vitamins C and K3 administration produced tumour growth inhibition and increased the life span of tumour bearing mice. The treatment also selectively potentiated tumour chemotherapy and radiotherapy, and caused inhibition of the development of cancer metastases (Buc, 2002).

Vitamin C and tumour cell death

Apoptosis may lower the risk of neoplasia by removing genetically damaged cells, and vitamin C may interfere with apoptosis by protecting such cells from reactive oxygen species dependant cell death. It has been found that in patients with adenomas, there was an inverse linear relationship between apoptosis and high vitamin C intake, leading the investigators to conclude that since a high rate of apoptosis has been linked to a reduced risk of colorectal adenomas and colorectal cancer, and vitamin C reduces that rate, that it should be avoided by patients with a history of adenomas (Connelly, 2003). However it has been reported that selective cancer cell death is caused by autoschizis, which is the primary form of vitamin induced tumour cell death (Taper, 2001), and which involves several mechanisms including the formation of hydrogen peroxide by vitamin redox cycling, oxidative stress, DNA fragmentation, nitric oxide caspase –3 activation and cell membrane injury with progressive loss of organelle free cytoplasm. Changes in the phosphorylation level of some critical proteins leading to inactivation of NF-kappaB appear as the main intracellular signal transduction pathways. NF kappaB factors are important in the transcription of many genes in both innate and adaptive immune responses.

It has been shown that vitamins C and K3 can induce autoschizis through activation of acid and alkaline DNAse's respectively (which are known to be deficient in all non-necrotic cancer cells in animals and man), at an early stage of cancer cell death (Verrax,

2003). Human prostate cancer cells (DU145) implanted into nude mice are deficient in DNAse activity. After administration of vitamin C and K3 in combination, both alkaline DNAse (DNAse 1) and acid DNAse (DNAse II) activities were detected in cryosections. Metyhyl green staining indicated that DNAse expression was accompanied by a decrease in the DNA content of the tumour cells leading to multiple forms of cell death, predominantly, autoschizis (Taper 2001).

The combination of hydroxycobalamin (vitamin B12) and vitamin C have also been observed to cause tumour cell death in vitro, specifically epidermoid human larynx carcinoma cells HEp-2, probably as a consequence of damage to the cells redox system (Akatov, 2000).

Vitamin C and the treatment of bladder cancer

A total of 65 patients with biopsy confirmed transitional carcinoma of the bladder enrolled in a randomised comparison of intravesical bacillus Calmette-Guerin (BCG) versus high dose multivitamin (vitamin A, 40,000 units, vitamin B6 100mg, vitamin C 2g, vitamin E 400 units and zinc, 90mg). It was found that after 10 months the addition of percutaneous BCG did not significantly lessen tumour recurrence, but recurrence in the patients receiving high dose vitamins was markedly reduced (Lamm, 1994). A study of the treatment of bladder cancer with vitamin C is also included in the following section

Vitamin C and the treatment of lung cancer

A systematic study of vitamin C blood levels in patients with cancer has been undertaken following long term, high dose (5g/day) ingestion of vitamin C. The results were evaluated in 24 patients of either sex with lung cancer and 35 patients with cancer of the bladder. There was no distinction made between patients who were operable and who were not. The study found that, at the start, the majority of patients had low (hypovitaminosis C) blood levels, particularly in the younger patients, which were rapidly increased after high dose vitamin C supplementation, frequently to a blood level above 1500 microg %, the upper end of the normal range, and which were maintained over time. It was observed that these high levels were of benefit, with regard to improved defence against cancer progresion (Greco, 1982).

Vitamin C and the treatment of patients with or pre-disposed to gastric cancer

High dietary intake of vitamin C has been observed to modify the relation between Helicobacter pylori and gastric cancer. In a case control study of 295 patients with histologically proven gastric cancer, it was found that in a stratified analysis after

adjusting for age sex and past history of gastritis, that H. pylori seropositivity was not found to be a significant risk factor when diets high in vitamin C were consumed (Kim, 2005). A population based double blind study which examined the effect of vitamin C supplementation on serum pepsinogen levels, H pylori infection and cytotoxin associated gene A in patients with chronic gastritis supported the beneficial effects of the vitamin. A total of 244 patients received vitamin supplementation at a dose of either 50 or 500mg/day for 5 years at the end of which, the difference in the change of pepsinogen I:II ratio was significant and dose related, drawing the conclusion that vitamin C supplementation may protect against progression of gastric mucosal atrophy (Sasazuki, 2003). Recent follow up studies on high- risk populations suggest that ascorbic acid protects against gastric cancer for which H pylori is a significant risk factor (Feiz, 2002). The role of vitamin C and E in the prevention of potentially pre-malignant modifications to DNA has also been investigated. In this study patients were selected, who, because of hypochlorhydria and possible depletion of gastric anti-oxidants, could be at increased risk of gastric cancer. A total of 100 patients undergoing surveillance for Barrett's oesophagus, who were on long term proton pump inhibitors, were randomised to receive vitamin C (500mg bd) and vitamin E (100mg bd) or placebo for 12 weeks. Even though gastric juice ascorbic acid and total vitamin C levels were raised in the supplemented group, there was no effect on mucosal levels and, possibly as a consequence, mucosal malondialdehyde and DNA damage levels were unaffected (White, 2002). A randomised controlled chemoprevention study has been undertaken in patients with confirmed histological diagnoses of multifocal non-metaplastic atrophy and/or intestinal metaplasia; both pre-cancerous lesions. Patients were assigned to receive anti H. pylori therapy, or dietary supplementation with ascorbic acid or beta-carotene, for 72 months. Increases in the rates of regression were observed in all 3 treatment arms in subjects with atrophy, and intestinal metaplasia. There was no additional benefit from combinations of the treatments (Correa, 2000). To assess whether long term ascorbic acid administration following H pylori eradication could affect intestinal metaplasia regression into the stomach, 65 patients were randomised to receive 500mg vitamin C per day or no treatment for 6 months. At the end of the period, the investigators found no evidence of metaplasia in 31% of patients compared with 3% in the untreated group. Moreover a further 20% of the vitamin C treated group with chronic inactive pangastritis with widespread intestinal metaplasia at entry, showed less extensive antritis with intestinal metaplasia after treatment (Zullo, 2000). The effect of oral vitamin C supplementation on intragastric ascorbate levels and gastric mucosal DNA damage as measured by the 32P post-labelling assay has been measured in 43 patients. In patients with normal gastric mucosa, vitamin C resulted in elevation of intragastric ascorbate levels, but in those with chronic atrophic gastritis, the effect was variable. Gastric mucosal DNA damage was decreased in 65% of patients after vitamin C supplementation which supports the epidemiological data that suggests vitamin C exerts a protective effect against the development of gastric cancer (Dyke, 1994).

Vitamin C and the treatment of breast cancer

The effect of long term, high dose (3mg/day) ascorbic acid supplementation has been investigated in 27 women with early breast cancer, and found that the prognosis of the disease did not seem to be affected. In addition 5 year survival was no different between women taking ascorbic acid and those who did not (Poulter, 1984).
In an historical cohort study in 90 women with non-metastatic breast cancer conducted in Canada, it was found that survival and disease free survival times were not improved

even following mega-dose multi anti-oxidant vitamin and mineral supplementation, that included vitamin C (Lesperance, 2002).
In vitro and animal studies do, however, suggest a benefit of vitamin C in breast cancer. Utilizing a microplate ATP bioluminescence assay on 2 human breast carcinoma cell lines (MCF 7 and MDA-MB-23), it was found that vitamin C exhibited cytotoxic activity at high concentrations (100 and 1000 microM) in vitro (Kurbacher, 1996).
In addition the effect of ascorbic acid on the growth of human mammary tumour xenografts was investigated using the 6 day subrenal capsule assay method. The results showed that ascorbic acid administered in the drinking water at a dose of 1 or 5g/L, significantly inhibited the growth of tumour fragments implanted beneath the renal capsule of immunocompetent mice. Administration in the mouse diet did not affect growth, however it was inhibited when the diet containing 50g vitamin C/kg was supplemented with copper sulphate at a dose of 18 or 90 mg/L of the drinking water (Tsao, 1988) suggesting an important additive or synergistic affect of copper with vitamin C.

Vitamin C and the treatment of colorectal adenomas and colorectal cancer

Colonic adenomas represent the natural precursor lesion of most colo-rectal cancers. A study was undertaken in 255 post polypectomy patients who were randomised to either lactulose, multivitamins (vitamin A 30,000 iu, vitamin C 1g and vitamin E 70mg/day) or no-treatment for an average of 18 months. Of the 209 evaluable subjects adenoma recurrence occurred in 5.7% of the patients on multivitamins, 14.7% of the patients on lactulose and 35.9% of the untreated patients, suggesting that treatment with anti-oxidant vitamins lowers the recurrence rate of adenomas of the large bowel (Roncucci, 1993). In another study, 20 patients with colorectal adenomas were given vitamins A,C and E for 6 months post polypectomy. A further 21 patients received a placebo. Biopsy samples were taken at intervals and 2 parameters of cell proliferation were evaluated, the ratio of the number of thymidine labelled cells to the total number of cells, and the the ratio of the number of labelled cells in the upper 40% of the crypt (Phih). The latter reflects abnormal expansion of the proliferative compartment, and is thought to be an intermediate biomarker of cancer risk. Both markers of cell proliferation decreased progressively from baseline values in the patients receiving vitamins with increasing significance after 3 months. There was no change in the placebo group (Paganelli, 1992). The control of large bowel adenomas has also been evaluated using vitamin C alone in a randomised double blind study in 49 patients with polyposis coli over a 2year period. Of 36 patients who were evaluable, 19 received ascorbic acid at a dose of 3g/day and 17 received placebo. The investigators found a reduction in polyp area in the ascorbate group, at the 9 month follow up and a trend toward reduction in both numbers and area of rectal polyps during the middle of the trial. A labelling study of the rectal epithelium with tritiated thymidine also suggested a treatment effect (Bussey, 1982).

However, high dose (10g/day) vitamin C has been investigated in the treatment of advanced colorectal cancer, and was found to be ineffective. 100 patients, who had not received any prior chemotherapy, were randomly assigned to receive either high dose vitamin C or placebo. It was found that vitamin C therapy showed no advantage over placebo with regard to either the interval between the beginning of treatment and disease progression or patient survival (Moertel, 1985).

Vitamin C as an adjunct to conventional cancer therapy

The majority of cancer patients combine some form of complementary medicine with conventional therapy (Drisko, 2003).
Concern has been raised that anti-oxidants might reduce oxidising free radicals created by radiotherapy and some forms of chemotherapy and thereby decrease the effectiveness of the therapy. However a review of the evidence has demonstrated that exogenous anti-oxidants alone produce beneficial effects in various cancers and both animal and human studies have not found any reduction in efficacy of either radio or chemo therapy when given with anti-oxidants. In fact the evidence suggests that the effectiveness of many cancer therapeutic agents is increased, as well as a decrease in adverse effects when given with anti-oxidants (Lamson, 1999).
In a study of high dose vitamin supplementation in patients with advanced non small cell lung cancer, 64 patients received 6.1g/day of vitamin C, 1.05g/day of vitamin E and 60mg/day of beta-carotene as an adjunct to chemotherapy. When compared to the cohort receiving chemotherapy alone it was found that the median survival times in the combination arm were slightly, but not significantly, higher, though 2 of the patients showed a complete response compared with none in the chemotherapy alone group. The high dose vitamin supplementation did not add to the toxicity profile due to chemotherapy (Pathak-Ashutosh, 2005).
Two patients with advanced epithelial ovarian cancer combined vitamin and mineral anti-oxidant therapy to conventional chemotherapy including the use of intravenous vitamin C at a dose of 60g administered twice weekly. Both patients responded well with normalisation of CA-125 even after 3 years. From these case histories it seemed that anti-oxidants when added adjunctively to first line chemotherapy improved the efficacy of that chemotherapy (Drisko, 2003).

Following anti-oxidant supplementation to patients being treated with cisplatin based chemotherapy, it was found in a placebo controlled study that those patients with the highest plasma concentrations of vitamin C, vitamin E and selenium, had significantly less loss of the high tone hearing often associated with cisplatin toxicity. In addition significant correlations were specifically found between the reduced/oxidised vitamin C ratio and malondialdehyde, markers of oxidative stress and cisplatin induced ototoxicity and nephrotoxicity (Weiji, 2004). Utilizing a microplate ATP bioluminescence assay, 2 human breast carcinoma cell lines (MCF 7 and MDA-MB-231) were tested against doxorubicin, cisplatin and paclitaxel alone and in combination with vitamin C. It was found that at both non-cytotoxic (1 microM) and moderately cyto-toxic concentrations (100 microM), vitamin C improved the cytotoxicity of all 3 chemotherapeutic drugs significantly (Kurbacher 1996).

It has been proposed that the combined vitamins C and K3 be used as adjuvant therapy, based upon the observation in studies in mice that the combination selectively potentiated both tumour chemo and radiotherapies (Buc, 2002).
Vitamin C also appears to increase the drug sensitivity of cervical carcinoma cells by stabilising P53. Cervical cancer is commonly associated with human papilloma virus (HPV) oncoproteins that target P53 protein for degradation and deregulation of the cell cycle. Vitamin C has been found to stabilise P53, which was associated with an increase in the target gene for P53 (bax) and a decrease in telomerase activity. Accumulation of P53 and bax then sensitised HeLa cells to cell cycle arrest and apoptosis induced by cisplatin and etoposide (Reddy, 2001).

An investigation into the treatment of acute myeloid leukaemia, found that ascorbic acid enhanced the apoptotic effect of arsenic trioxide, a drug which induces remission in a high proportion of patients (Bachleitner, 2001).
A study carried out in patients with advanced breast cancer evaluated the benefit of ascorbic acid as a chemosensitiser of polychemotherapy. It was found that in the group in which ascorbic acid was given as a supplement with 3 cycles of polychemotherapy, there was a significantly higher reduction in average breast lump size and tumour cell DNA content compared to the group receiving polychemotherapy only (Goel, 1999).

The influence of ascorbic acid and 6-chloro-6-deoxy ascorbic acid (6-Cl-AA) have been investigated on the growth of various human cell lines, lung fibroblasts, ovarian adenocarcinoma (HT-29), laryngeal carcinoma (HEp2), HEp2 cells resistant to vincristine, cervical carcinoma (HeLa), HeLa cells resistant to cisplatin (HeLaCIS), breast carcinoma (SK-BR-3), SK-BR-3 resistant to doxorubicin, mouse fibroblasts (L929), mouse melanoma (MelB16) and Chinese hamster fibroblasts (V79). Both drugs arrested the growth of HeLa, SK-BR-3 Dox, L929, and MelB16, but did not influence the growth of others. The results suggested that ascorbic acid and 6-Cl-AA could serve as potential anti-tumour agents especially tumour cells resistant to chemotherapy (Osmak,