VITAMIN C FROM HISTORICAL PERSPECTIVE TO WONDER MOLECULE OF TWENTY FIRST CENTURY

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The discovery of wonder molecule vitamin C is closely related to the history of ancient disease scurvy. This disease was very common among the sailors, soldiers and some other persons who did not have the privilege of consuming fresh vegetables and citrus fruits in their diets. It was discovered that these foodstuffs contained an antiscurvy agent that was isolated in a crystalline form and named as vitamin C or ascorbic acid. Soon, various biochemical and medicinal properties of this newly discovered molecule were studied extensively. By virtue of excellent antioxidant properties associated with vitamin C, it is being investigated as a potential therapeutic agent for the treatment of many challenging ailments such as cancer, cardiovascular diseases, Alzheimer's disease, arthritis and metabolic syndrome. There is worldwide interest in elucidation of the molecular mechanism and biological effects of vitamin C.

Introduction

Vitamin C is also known as ascorbic acid or ascorbate (anion of ascorbic acid). It belongs to a group of organic compounds that are known as micronutrients, and are essential for human life. Although they are required in very small quantity in our diet, yet they play a significant role in various metabolic processes. Earlier, it was thought that all such vital compounds are amines, and therefore, they were named as *"Vitamines"* (meaning vital amines). Subsequently, it was discovered that all of the compounds of vital importance are not amines, but the name remained unchanged. Later on terminal "e" in the word *"Vitamine"* was dropped and it was modified as *"Vitamin"*. Vitamin C is one of the important water-soluble vitamins which is often referred to as ascorbic acid. The name ascorbic acid is based on "*a*" meaning "*no*", and "*scorbutus*" meaning "*scurvy*" (a disease caused due to deficiency of vitamin C)[1]. Vitamin C is synthesized in the liver of a large number of animals. However, humans, non-human primates and guinea pigs are unable to synthesize vitamin C because these species do not have the required enzyme, *Gulonolactone oxidase* in their bodies. Vitamin C requirement of human beings is fulfilled by providing it mainly through vegetables, fruits, fruit juices and tablets, etc. Vitamin C performs diverse biological functions in human life, and therefore, its presence is essential in human diet[2].

Importance of vitamin C was realized centuries ago, and originally it entered the realm of medical world as a panacea for the treatment of the catastrophic disease, scurvy. Advancements in medical science and biotechnology paved the way for the new researches on this old and structurally simple molecule. Vitamin C is endowed with excellent antioxidant properties, and consequently, it is being thoroughly investigated as a potent therapeutic agent for the treatment of many challenging ailments including cancer, rheumatoid arthritis, and metabolic syndrome. There is worldwide interest in elucidation of the molecular mechanism and biological effects of the vitamin C. The number of research publications in this area is increasing exponentially.

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Historical Persepective

History of vitamin C is intimately associated with the discovery of the ancient disease scurvy. Hippocrates (460-370 BC), an ancient Greek physician who is regarded as the father of medicine, had recommended the use of certain foodstuffs in human diet which during the present time are known as good sources of vitamin C and still required for the prevention of scurvy[2]. Ancient Egyptians also described a disease similar to scurvy[3]. In the middle ages of human civilization, long and adventurous sea voyages were quite common, and had an important place in human life. During these journeys sailors were deprived of fresh fruits and vegetables for long periods of time. Their diet mainly comprised nonperishable food items lacking in vital nutrients. Consequently, sailors were quite often afflicted by a "mysterious" disease claiming a heavy toll of their lives. This disease was named as scurvy, and after several years, deficiency of vitamin C in diet was discovered to be its cause. The symptoms of the scurvy include tiredness, swollen limbs, pain in joints and muscles, rashes on legs, bleeding gums and loss of teeth. It was observed that if the disease was left untreated, it ultimately led to death. According to Jonathan Lamb, when the famous Portuguese explorer Vasco da Gama sailed around the Cape of Good Hope, Africa's southernmost tip in 1479, one hundred of his 160-member crew had died from scurvy. In another devastating expedition led by Ferdinand Magellan during 1520-1522, 208 out of 270 sailors were killed by scurvy[4].

History is witness to the fact that besides the long sea voyages, wars had also made a miserable contribution to the scourge of scurvy. In many wars fought during the nineteenth century, in addition to weapons, scurvy also killed a vast number of the soldiers. It was reported that during American Civil War (1861-1865) twenty five percent of the captured soldiers who were kept in Andersonville Prison died of scurvy. In the Siege of Paris (1590) a huge number of soldiers became victims of scurvy and starvation. In the Crimean War (1854-1856), scurvy killed a large number of soldiers. During this war, the legendary Florence Nightingale saved the lives of many wounded and scurvy-afflicted soldiers by her dedicated services and kind heartedness. She was an English social reformer and founder of modern nursing. She was famous as "The *Lady with the Lamp*", as with a lamp in her hand, she used to take rounds throughout the nights to look after the wounded and sick soldiers. Her great service was described by the poet H.W. Long fellow:

Lo in that house of misery

A lady with the lamp I see

Pass through glimmering gloom,

And flit from room to room.

It is amazing that scurvy was also prevalent among the people of royal and aristocratic families of Europe during the 16th century. These people used to avoid vegetables in their diet because they thought that vegetables were grown in dirty soil and were befitting to the status of lower classes only. Their diet was heavy on meat and starchy food without vegetables and citrus fruits[2].

By the mid-18th century, the scourge of scurvy claimed the lives of innumerable sailors, soldiers and other human beings. The first step to eradicate scurvy came in 1747, when a British naval physician James Lind conducted a historic trial on sailors of British navy who were suffering from scurvy[4]. In his experiment, Lind provided two oranges and one lemon per day to sick sailors in addition to their normal diet. The result of this experiment conclusively proved that citrus fruits (oranges and lemons) are essential for the prevention of scurvy. This experiment of Lind is regarded as the first clinical trial in the history of medical science. But, even after this landmark discovery, for four decades British navy did not accept his finding, and countless sailors continued to die from scurvy. Ultimately, the significance of citrus fruits was accepted, and lemon juice was included in the ration of sailors as a measure for prevention of scurvy.

Isolation of Vitamin C From Natural Sources

In the year, 1907, Axel Holst and Alfred Frolhich recognized the existence of vitamin C in citrus fruits on the basis of its biological effects. Then, the next target was isolation of vitamin C from natural sources. Just after the World War I, Solomon Zilva *et al*. at the Lister Institute, London and Charles King at the University of Pittsburgh, USA started their efforts to isolate vitamin C from citrus fruits. But due to presence of various sugars in these fruits, it was difficult to isolate vitamin C in pure crystalline state. However, the isolated crude product exhibited powerful antiscorbutic property. Albert Szent-Gyorgyi, a Hungarian doctor and biochemist in 1928, while working at Cambridge University, London, isolated from adrenal gland of an ox a small quantity of sugar-like crystalline compound which was believed to be a new hormone[5]. He suggested the name *ignose* for this compound, the *ose* part being the ending as used in the names of sugars; and the *ig* part indicating his ignorance about its structure. Later on depicting his sense of humour, Szent-Gyorgyi renamed it as "*Godnose*" (God Knows) as he was still ignorant about the structure. Finally, at the suggestion of the editor of the Boichemical Journal

(where the work was published in 1928), the compound was named as hexuronic acid as it showed six carbon atoms in its formula, $C_6H_8O_6$. Subsequently, Szent-Gyorgyi also isolated the so called hexuronic acid from two plant sources: orange juice and cabbage. In 1931, Charles King and W.A. Waugh isolated the antiscorbutic factor as a crystalline solid from lemon juice and named it vitamin C. These workers arrived at the conclusion that vitamin C isolated by them and the hexuronic acid previously isolated by Szent-Gyorgyi were one and the same compound. Although citrus fruits were a good source of vitamin C, its isolation in pure form was extremely difficult due to the presence of sugars. Therefore, Szent-Gyorgyi started searching for other convenient sources of vitamin C.

In 1930, Szent-Gyorgyi returned to Hungary and joined the University of Szeged as a Professor of medical chemistry. Szeged was then and even today is a major centre for cultivation of paprika. As mentioned in his autobiography, one night his wife served him fresh red paprika as a side dish in dinner. Instead of eating red paprika, he thought that it could be tried as a source of vitamin C. He immediately left the dinner table and rushed to his laboratory with the served paprika. By the midnight, he isolated a "treasure *chest full of vitamin C*". After few weeks, Szent-Gyorgyi had successfully isolated three pounds of pure crystalline vitamin C. Hungarian paprika was found to be a particularly rich source of vitamin C, and due to the absence of sugars, the process of isolation was convenient.

Now, sufficient quantity of vitamin C was available to the sugar chemist Norman Haworth, Professor of Chemistry at the University of Birmingham who was a collaborator of Szent-Gyorgyi and working on chemical aspects of vitamin C. Szent-Gyorgyi and Haworth renamed the substance as "ascorbic" acid because it prevented scurvy. Soon the importance of this small molecule was recognized by the scientific community. In 1937, Szent-Gyorgyi was awarded Nobel Prize in medicine for his work on biological aspects of vitamin C, and Haworth, Nobel Prize in chemistry for determining the structure and synthesis of vitamin C[4]. Haworth shared the Nobel Prize in chemistry with Paul Karrer who was honored for his work on carotenoids, flavins, vitamin A and B_2.

It is amazing that some other chemists viz., Charles King and A Holst and their associates who made significant contribution in isolation and biochemical study of Vitamin C could not get due recognition and honour for their monumental achievements. It is rightly said, *"destiny is not kind to all"*.

Ascorbic acid is a chiral molecule and, therefore, it has two enantiomeric forms which are known as D-ascorbic acid and L-ascorbic acid. The name vitamin C always refers to L-ascorbic acid only which is widely distributed in nature. D-Ascorbic acid is devoid of vitamin activity or any other physiological activity and it does not occur in nature. However, it can be prepared by synthetic method.

Sources of Vitamin C[2,6] : Vitamin C is widely distributed in the vegetable kingdom. Fruits and vegetables are very good natural sources of this vitamin. The concentration of vitamin C (per 100 gram) in some commonly used fruits is as follows: guava (200-300 mg), blackcurrant (200 mg), kale (186 mg), orange (50 mg), lemon (50 mg), lime (25 mg), litchi (45 mg), strawberry (60 mg), Indian gooseberry '*amla'* (445 mg). In various vegetables, the concentration (per 100 gram) of vitamin C is: green pepper (128 mg), horseradish (120 mg), broccoli (113 mg), Brussels sprouts (87-109 mg), cauliflower (64-78 mg) spinach (51 mg), cabbage (46-47 mg), tomato (20-25 mg). In fruits and vegetables, the concentrations of vitamin C usually vary with various parameters such as source, soil condition, weather, etc. Hence, different amounts of vitamin C from the same source are reported. Milk also contains vitamin C. In human milk, concentration of vitamin C is 4 mg/ 100 gram while in the milk of cow and goat, its concentration is 2 mg /100 gram.

Requirement of Vitamin C[7] : All the species of plants including algae and fungi, and large number of animals such as amphibians, reptiles, fishes, birds, mammals (except human and guinea pigs, bats, passeriform birds, teleost fishes) possess the capacity of synthesizing vitamin C in their bodies. Being unable to synthesize vitamin C, human beings are entirely dependent on outer sources to meet their requirement. Presence of fruits and vegetables in human diet provides an adequate amount of vitamin C. This vitamin is also taken through fruit juices, tablets, powder, liquid and some other supplements.

In India[8], the recommended daily allowance (RDA) of vitamin C for adult men and women has been fixed at 40 mg per day by ICMR. In advanced countries like UK, USA, USSR and Japan, RDA's of vitamin C are 30 mg, 60 mg, 90 mg and 100 mg per day, respectively[2]. WHO had recommended 45 mg per day from the nutritional stand point. It has been observed that the requirement of vitamin C varies with age, sex, stress and physiological state of an individual. Alcohol addiction, smoking, post-surgery condition, pollution and pregnancy and lactation in women considerably enhance the requirement of vitamin C. As

vitamin C is non-toxic and water-soluble and can be excreted out of the body easily, there is no harm in exceeding the RDA. In 1970 and the following years, the great chemist Linus Pauling highlighted the importance mega doses of vitamin C (up to 10 gram in a single dose!) for the prevention and cure of colds, flu and cancer. No doubt Linus Pauling was a great chemist. He received Nobel Prize in Chemistry in 1954 for his work on the nature of chemical bond, and again in 1962 for his efforts on opposing the testing of nuclear weapons, he was awarded Nobel Prize for peace. But despite the eminence of Pauling as a scientist, his views could not get general acceptance from medical establishment.

On industrial scale vitamin C is manufactured from glucose. Both synthetic and natural vitamin C are absolutely identical in every respect. The richest natural source of vitamin C is the rose hips of the *Rosa macrophylla*, grown in pristine slopes of the lower Himalayas. The concentration of vitamin C in rose hips is 1.0-5.3 gm / 100 gram. Natural vitamin C is gently extracted from this source[4].

Biological Functions[9,10] : It has been clearly defined that vitamin C plays a significant role in modulating a number of useful enzymatic reactions in living beings though its function at cellular level is not very clear. It is involved in the synthesis and metabolism of hydroxyproline and hydroxylysine which are required in the synthesis of the protein collagen. Collagen represents one third of the total body protein. It constitutes the main protein of skin, bones, teeth, cartilage, tendons, blood vessels, heart valves, intervertibral discs, cornea and eye lens. Vitamin C is essential for the normal functioning of fibroblasts, osteoblasts, adrenal hormones and carnitine biosynthesis. Carnitine (β-hydrox-γ-butyrobetaine) is present in liver, heart and skeletal muscles and is required for the transport and transfer of fatty acids into mitochondria for production of energy. The enzymatic reaction involved in the conversion of the neurotransmitter dopamine to norepinephrine also requires vitamin C. Thus, it is essential for the synthesis of catecholamines. In addition, the enzymatic reaction responsible for the activity of hormones oxytocin, vasopressin, cholecystokinin and melanotripin also involves vitamin C as a co-factor.

The enzymatic conversion of cholesterol into bile acids is also assisted by vitamin C. Due to deficiency of vitamin C, this conversion is impaired and consequently, cholesterol is accumulated in blood and liver leading to health problems such as atherosclerotic changes in coronary arteries, and formation of cholesterol gallstones. Deficiency of vitamin C is also responsible for the elevation of triglyceride level in the blood of animals.

Therapeutic Applications[2,4,5,10] : The first medicinal use of the vitamins C was in the treatment of scurvy, an ancient hemorrhage disease that inflicted the mankind for centuries. Now, due to advancement in medical science, a large number of other therapeutic applications of this vitamin have been discovered, and it is being used in the treatment of vast spectrum of diseases ranging from common cold and flu to degenerative diseases. Some of these applications are of sound provenance while others are highly controversial, requiring further investigation. The medicinal properties of vitamin C constitutes a subject of recent research activity all over the globe. Its pivotal role in the treatment of a large number of complicated diseases including bursitis, gout, Crohn's disease, multiple sclerosis, gastric ulcers, obesity, osteoarthritis, herpes simplex infections, Parkinson's disease, anemia, coronary heart disease, autoimmune diseases, miscarriages, rheumatic fever, cataract, diabetes, alcoholism, infertility, schizophrenia, depression, Alzheimer's disease, and various forms of cancers is being investigated with great interest.

Old Age and Vitamin C[2,11] : With the advancement of age, there is considerable change in lifestyle which is associated with many physiological changes. As a consequence of these changes, production of free radicals within the body is increased. A free radical is highly reactive and highly unstable chemical entity (an atom or group of atoms or any chemical compound) containing one or more unpaired electrons. Free radicals are generally referred to as (i) reactive oxygen species (ROS), and (ii) reactive nitrogen species (RNS). They are produced in the living organism due to normal cellular metabolism. Besides the metabolic reactions, free radicals are also produced by some other factors, viz., pollutants, chemicals, irregular and insufficient diet, high salt intake, smoking and heavy drinking, wounds, etc. Production of free radicals in excess amount can cause serious oxidative damage to cell structures such as carbohydrates, proteins, lipids and nucleic acids. Due to this damage to these essential macromolecules, a hazardous situation is developed which is known as "*oxidative stress*". This undesirable situation produces a number of pathological conditions such as cancer, neurological disorder (Parkinson's disease, Alzheimer's disease, dementia, ageing), atherosclerosis, cardiovascular diseases, diabetes and various types of pulmonary diseases.

In order to protect the body from this devastating oxidation caused by free radicals, certain compounds, viz., β-carotene, vitamin E and vitamin C, etc. are used as antioxidants or scavengers. They retard or stop the process of oxidation by eliminating or scavenging the free radicals.

Vitamin C is regarded as an excellent antioxidant or free radical scavenger due to its favourable chemical structure and solubility in water. As advocated by Linus Pauling, some recent studies show the benefits of vitamin C in fighting the old age diseases and brand it as a "Youth In a Bottle"[2]. However, confirmation of such claims is still awaited. As a chiral synthon, vitamin C is capable of producing a large number of chemotherapeutic agents[12]. The central structural feature of vitamin C is the presence of a butenolide ring. Recently, a new group of compounds with a butenolide ring in their structures have been synthesized and found to exhibit antimicrobial activity[13,14].

Concluding Remarks

Vitamin C performs many important functions inside the human body. Many scientists believe that this little molecule is essential for good health and longevity. According to some research findings, it retards the process of ageing and acts miraculously in age-related diseases. They regard it as a fountain of good health. However, such reports are controversial and need to be resolved by further investigations. It is expected that new facts in chemistry and biochemistry of vitamin C would emerge in coming years. General awareness and interest in vitamin C are evident from the fact that during the international year of Chemistry, 2011, the Swiss Post issued a stamp bearing the depiction of a molecule of vitamin C. ❒

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