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Pathogenesis and Prevention of Stomach Cancer

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In many Western developed countries, the incidence of stomach cancer has declined dramatically. This decrease was an extraordinary, "unplanned triumph", especially when compared to other cancers. Stomach cancer is still the most prevalent malignant tumor in Korea. Most Koreans carry Helicobacter pylori in their stomach. Thus, a new hypothesis, based on the relationship between the host and Helicobacter pylori, is presented as the carcinogenesis of human stomach cancer. The reasons for why the N-nitrosamide hypothesis should be dismissed as the etiology of stomach cancer, and why the contemporarily available principles and practice of intervention strategies to rapidly decrease the surprisingly high prevalence rate of Helicobacter pylori infection are impractical at this moment, are explained. In order to introduce an alternative provisional strategy of the "planned triumph" for the population vulnerable to stomach cancer, vitamin C is defined as an anti-inflammatory agent on the basis of the pathogenesis of Helicobacter pylori infection.

Key Words : *Helicobacter pylori, Stomach Cancer, Vitamin C, Gastritis, Carcinogenesis*

INTRODUCTION

The etiology of stomach cancer has been suggested in the following twelve epidemiological and pathological findings (Higginson, 1966 ; Mackay and Hislop, 1966 ; Stemmerman and Hayashi, 1968 ; Haenszel et al., 1972 ; Correa et al., 1973 ; Macdonald, 1974 ; Wynder, 1977 ; Cummings, 1978 ; Reddy et al., 1980 ; Correa et al., 1983 ; Weisburger and Horn, 1984 ; Montes et al., 1985 ; Howson et al., 1986).

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(1) The incidence of stomach cancer is higher in males than females.

(2) The incidence of stomach cancer increases proportionally to the 4th-5th power of age increment.

(3) The incidence of stomach cancer is higher in temperate zones than in tropical zones.

(4) Stomach cancer is found more frequently in mountainous areas with long winters.

(5) The incidence of stomach cancer is higher in rural areas than in urban areas. In rural areas, stomach cancer is found more frequently in inhabitants drinking underground well-water.

(6) In urban areas, stomach cancer is found more frequently in the lower socio-economic classes.

(7) The incidence of stomach cancer is correlated with an increased intake of salt and salted fish or vegetables, and inversely correlated with an increased intake of fresh vegetables, vitamin C, milk, and a diet with a high content of protein. Widespread use of

refrigeration for food storage has also decreased the incidence of stomach cancer.

(8) There are familial aggregations of stomach cancer.

(9) The incidence of stomach cancer is slightly increased in persons of blood group A.

(10) Susceptibility to stomach cancer is determined during childhood.

(11) The incidence of stomach cancer has declined rapidly in the USA and the United Kingdom in the past 50 years. The second and third generations of Japanese immigrants to the USA have experienced a rapid decline in the incidence of stomach cancer, and this phenomenon has also recently been observed in Japan.

(12) Chronic atrophic gastritis is the precursor disease of stomach cancer.

It is clear from the above observations that the incidence of stomach cancer changes, as temporal, spatial, and socioeconomic conditions change. Some factors increase the incidence, while others decrease the incidence, of stomach cancer. It is our proposal that the relationship between the human host and *Helicobacter pylori* in a state of deficiency of anti-oxidants which is dependent on the psychosociobiological conditions of human beings explains the twelve epidemiological and pathological suggestions for the etiology of stomach cancer (Rhee, 1988).

Before *Helicobacter pylori* was known as being the causative agent of type B antral gastritis, there were two hypotheses on gastric carcinogenesis. The first was Fibiger's hypothesis that an intestinal parasite might play a major role in the human gastric carcinogenesis. He based his theory on the analogy that a nematode, *Nippostrongylus neoplasticum*, causes gastric cancer in rats. This hypothesis earned him the Nobel prize in physiology and medicine in 1926, but further discussion is beyond the scope of this paper. The other hypothesis, that N-nitrosamide, a N-nitroso compound, might be a human gastric carcinogen, has been elaborated upon since the late 1960s by Takashi Sugimura (Sugimura and Fujimura, 1967 ; Sugimura et al., 1970 ; Sugimura and Sato, 1983) of the Japanese National Cancer Center Research Institute. A reevaluation of this hypothesis in regards to weighing and reconciling the juxtaposed or contradictory facts is essential for the purpose of arriving at the introduction of a new etiology of stomach cancer.

Reevaluation of the N-nitrosamide hypothesis on human gastric carcinogenesis

N-nitrosamine is one of the N-nitroso compounds. It is frequently confused with N-nitrosamide, even in the medical textbooks. However, N-nitrosamine is not relevant to the carcinogenesis of stomach cancer. Relevant properties of N-nitroso compounds must be understood in order to clarify the misleading interpretation of the N-nitrosamide hypothesis on the human gastric carcinogenesis.

N-nitrosamine is a very stable compound, synthesized from nitrite and secondary amines. Optimal pH for its synthesis is 2.5-3.5(Reed et al., 1981). Regardless of the route of administration, cancer develops only in a specific organ by a specific N-nitrosamine, because the specific cytochrome P450 enzyme of the specific organ can transform the specific N-nitrosamine (indirect carcinogen) into an active carcinogen (Mohla et al., 1988). Until now, no known N-nitrosamine compound has been able to induce stomach cancer in animal experiments(Mirvish,1977). N-nitrosamides are unstable compounds, synthesized from nitrite and compounds having the amide group at room temperatures. In contrast to N-nitrosamine, the required pH conditions for the synthesis of N-nitrosamides is more acidic. N-nitrosamides have direct carcinogenic effects on organs when administered without organ tropism(Hecht and Kozarich, 1973 ; Rustia and Shubik, 1974 ; Lee et al., 1977 ; Hecker et al., 1983 ; Yarosh, 1985 ; Barrows, 1986). When the pH is lowered, N-nitrosamides become more stable, and conditions for the synthesis become more optimal. However, when the pH is elevated, synthesis of N-nitrosamides stops ; N-nitrosamides become more unstable, with their half-life falling below a few seconds after being converted into an active mutagenic form.

While the N-nitrosamide hypothesis has been elaborated on by the experiment in which gastric adenocarcinoma very similar to the human gastric cancer were induced in rats, and simultaneously, by an attempt to accommodate the twelve epidemiological and pathological suggestions for the etiology of human stomach cancer already listed in the section of the Introduction, it is not clear to what extent the carcinogenicity of N-nitrosamides can be extrapolated to the human gastric adenocarcinoma, especially if we take the following eight inconsistencies arising from the paradoxical conditions for the synthesis of nitrite and N-nitrosamides into consideration :

(1) N-nitrosamides, which can be synthesized during food storage or cooking, are not relevant to the gastric adenocarcinoma of human beings, because they have very a short half-life in the pH range of edible foods.

Therefore, the central question in the N-nitrosamide hypothesis is whether the synthesis of N-nitrosamides in the stomach cavity of the precursor disease of human gastric adenocarcinoma, chronic atrophic gastritis, is possible or not.

(2) In the cases of chronic atrophic gastritis, the pH of the gastric juice is hypochlorhydric (pH 3-4). Even though a large amount of precursors, nitrite and amides, are present in the stomach cavity, the synthesis of N-nitrosamides is not favored within this elevated pH range of the condition of hypochlorhydria.

(3) The optimal conditions in which the nitrite, one of the precursor compounds of N-nitrosamides, can be formed in the stomach cavity, inherently conflict with the conditions for the synthesis of N-nitrosamides. Nitrite is unstable and reactive, with a short half-life. Furthermore, in the stomach cavity, it can be formed only by the physiological process of assimilatory or dissimilatory nitrate reduction of the normal bacterial flora of throat and intestine. The normal bacterial flora cannot survive in the usual pH range of gastric juice in a normal stomach, which is usually sterile. The pH condition is, however, good for the synthesis of N-nitrosamides. Although the nitrate is introduced into the stomach by the ingestion of natural salts, vegetables, and underground well-water, the nitrite can not be formed unless the normal bacterial flora survive in the hypochlorhydric stomach(Franklin and Skoryna, 1971 ; Stockbruegger et al., 1984 ; Borriello et al., 1985). Conversely, N-nitrosamides are synthesized only in a higher acidic concentration, in which normal bacterial flora cannot survive. Therefore, despite an abundance of nitrate in the acidic normal stomach, the nitrite is not formed in adequate amounts for the synthesis of N-nitrosamides, because the stomach cavity is too hostile for the normal bacterial flora to survive. The hypochlorhydria observed in chronic atrophic gastritis is not an optimal condition for the synthesis of N-nitrosamides. It is, however, an optimal condition for the synthesis of N-nitrosamines, irrelevant for gastric carcinogenesis.

(4) Skepticism may be raised about the real implications of the experiment on the induction of gastric adenocarcinoma in the rat by the oral administration of MMNG (N-methyl-N-nitroso-N'-nitro-guanidine) 1mg/day for 3-6 months. MMNG is one of the most potent carcinogenic N-nitrosamides. In these experiments, high doses of MMNG were used without consideration of the highest possible concentrations of N-nitrosamide precursors (nitrite and amides), or the MMNG precursor (methylurea) in the human stomach. This high concentration of N-nitrosamide is highly unlikely to be formed in the human stomach cavity, even though the concentration of N-nitrosamides in the human stomach cavity has never been measured experimentally because of their very short half-life.

(5) Even though a small amount of N-nitrosamide is formed in the human stomach cavity, it does not appear to be easy for this carcinogen to penetrate into the central portion of a gastric gland where stem cells of gastric epithelial cells are located. Except for a few chemicals such as ethanol and aspirin, the gastric mucus layer is impermeable to chemical substances. A good example is a simple H^+ ion in the normal stomach cavity.

(6) According to the N-nitrosamide hypothesis, N-nitrosamide was postulated as being the mutagen and NaCl as being the promoter. A two-mole NaCl solution was forcefully administered via a gastric tube during the experiment. The percentile concentration of normal physiological saline is 0.85% NaCl solution ; sea water, 3% ; and 2M NaCl is about 10%. It is difficult to imagine a human population drinking such highly concentrated salty water on the basis that humans do not drink even normal physiological saline because of the salty taste.

(7) Epidemiological data which demonstrates an inverse correlation between vitamin C intake and the incidence of stomach cancer, has been considered as a supportive evidence for the validity of N-nitrosamide hypothesis. The protective effects of vitamin C intake against stomach cancer have been explained by the synthesis of N-nitrosamides. The syntheis is inhibited by the removal of nitrite, the precursor of N-nitrosamide, by vitamin C. This explanation may appear compatible with the hypothesis that N-nitrosamide is the main cause of human gastric cancer. However, this explanation may be erroneous, or possibly partially true in a different sense. There may also be an alternative interpretation of the relationship between the vitamin C and stomach cancer. Nitrite is a strong oxidative agent of vitamin C. As a result of the reaction with vitamin C and nitrite, vitamin C is oxidized. The oxidized vitamin C loses its anti-oxidant activity and decays easily. Nitrite is formed through nitrate reduction by the normal bacterial flora in the hypochlorhydric stomach of patients with chronic atrophic gastritis. The vitamin C deficiency itself due to the consumption of vitamin C resulting from the increased nitrite production by the normal bacterial flora in the cavity of the hypochlorhydric stomach and additional vitamin C consumption factors resulting from the inflammatory condition of the precursor disease of

stomach cancer, which will be explained further in a later section, might play a more critical, straightforward role in the relationship between the human host and *Helicobacter pylori*.

(8) Finally, the relationship between *Helicobacter pylori* and human gastric mucosa is difficult to integrate into the N-nitrosamide hypothesis because *Helicobacter pylori* infection elevates the pH of the gastric juice. If there is no *Helicobacter pylori* infection, the nitrite can not be produced in the human stomach cavity. Therefore, formation of the nitrite for the synthesis of N-nitroso compound in the stomach is secondary to *Helicobacter pylori* infection, and pH conditions for the synthesis of nitrite and N-nitrosamides are mutually exclusive.

From these inconsistencies, it is clear that even though the N-nitrosamide hypothesis has proven that a chemical mutagen will induce a gastric tumor very similar to a human gastric tumor in the experimental animal, this hypothesis which is most frequently cited in medical textbooks has caused confusion about N-nitroso compounds. Therefore, it is not indispensable, and should be dismissed in the discussion of the human gastric carcinogenesis. Thus, the connection between vitamin C and the stomach cancer should be interpreted in a different perspective based on the pathogenesis of *Helicobacter pylori* infection.

Suggestions for a new etiology of stomach cancer

In the USA and in Australia, the prevalence rate of *Helicobacter pylori* infection has been reported as being less than 20% at 20 years of age (Marshall et al., 1984 ; Dooley et al., 1989). This rate increases with age, being up to approximately 50% at 50 years, and remains at that level after 50 years. In Korea, the prevalence rate is already 50% at 5 years of age, 80% at 8 years, and 90% at and above 20 years (Baik et al., 1990 ; Rhee et al., 1990 ; Youn et al., 1990). The prevalence rate in Korea is remarkably higher than those of developing countries whose socioeconomic status lags far behind (Megraud et al., 1989).

Most Koreans carry *Helicobacter pylori*, the causative agent of chronic gastritis, in their stomach for at least 40-45 years longer, or even for their entire life, compared to *Helicobacter pylori*-infected persons, or *Helicobacter pylori*-non-infected persons, in developed countries where the incidence of stomach cancer is lower (Rhee et al., 1988). The annual incidence of gastric cancer in Korea in 1986-1987 was estimated as 57.9 and 25.1/100,000 for males and females, respectively. This is one of the highest values in the world (Ahn et al., 1991). *Helicobacter pylori* causes type B antral gastritis. Chronic gastritis has been shown to be sequentially followed by chronic atrophic gastritis, intestinal metaplasia, dysplasia, and carcinoma in a susceptible population (Correa, 1992).

These impressive landmark observations on the prevalence rate of *Helicobacter pylori* infection in Korea has allowed us to put forth the hypothesis that stomach cancer is primarily determined by *Helicobacter pylori* infection, with the provision that the pathogenesis is modulated by anti-oxidants, in order to accommodate the twelve epidemiological and pathological suggestions for the etiology of the stomach cancer listed previously in the section of Introduction, and the fact that only less than 10% of *Helicobacter pylori* carriers develop stomach cancer (Rhee, 1988). Subsequently, the conclusion that infection with *Helicobacter pylori* is carcinogenic to humans (Group 1) has been announced, after evaluating the recently completed cohort and retrospective case-control studies on the seropositivity of *Helicobacter pylori* and the incidence and mortality of stomach cancer (IARC WHO monograph, 1994).

Some issues should be emphasized to eliminate confusion in the reported studies evaluating the presence of *Helicobacter pylori* infection in patients with stomach cancer, especially of the diffuse type of adenocarcinoma. *Helicobacter pylori* infection is a long-standing condition, and only rarely resolves spontaneously. However, *Helicobacter pylori* cannot be found in gastric cancer tissue because the gastric epithelial cells change so as to be unfavorable for *Helicobacter pylori* to live in ; these cells became atrophic, metaplastic, and cancerous. In countries with high prevalence rates of *Helicobacter pylori* infection from early childhood, like Korea, case-control studies to calculate the odds ratio could not be carried out. Carcinogens could not be detected in the cancer tissue (hit-and-run hypothesis in carcinogenesis). Therefore, the lack of *Helicobacter pylori* in the gastric epithelium of cancer patients is not incompatible with the hypothesis that *Helicobacter pylori* is the major determinant of stomach cancer. *Helicobacter pylori* infection in gastric mucosa is also reported to increase the risk of gastric lymphoma (MALToma) (Wotherspoon et al., 1993 ; Parsonnet et al., 1994).

This hypothesis is strengthened by the documented relationship between cancer and chronic inflammation in the literature, regardless of the etiology of inflammation:

(1) Chronic type A or fundal gastritis with pernicious

anemia and gastric cancer

(2) Chronic hepatitis by HBV or HCV and hepatocellular carcinoma

(3) Chronic cervicitis by human *Papillomavirus* 16 or 18 and cervical cancer

(4) Chronic Epstein-Barr virus infection and Burkitt's lymphoma or nasopharyngeal carcinoma

(5) Chronic HIV infection (AIDS) and Kaposi's sarcoma

(6) Chronic HTLV-1 infection and adult T-cell leukemia

(7) Hashimoto's thyroiditis and thyroid carcinoma

(8) Silicosis and lung cancer

(9) Asbestosis and mesothelioma

(10) Chronic clonorchiasis and cholangiocarcinoma

(11) Chronic schistosomiasis and squamous cell carcinoma of the bladder

(12) Ulcerative colitis and colon cancer

(13) Regional ileitis (Crohn's disease) and cancer of small intestine

(14) Barrett's esophagus and esophageal cancer

(15) Chronic pulmonary tuberculosis and lung cancer

The linking mechanism between *Helicobacter pylori* infection and stomach cancer must be understood on the basis of the pathogenesis of *Helicobacter pylori* infection.

Hypothetical carcinogenesis of *Helicobacter pylori* infection

The relationship between *Helicobacter pylori* infection and chronic gastritis is the central key to understanding the sequelae of *Helicobacter pylori* gastritis, such as peptic ulcer diseases and gastric cancer. Fragmentary and scattered observations of fundamental significance on the relationship between *Helicobactir pylori* and gastroduodenal diseases are assembled to build a bridge to a unified perspective on the pathogenesis of *Helicobacter pylori* infection.

Helicobacter pylori infection is an unusual bacterial disease. After infection, life-long gastric mucosal inflammation develops, along with a serological response (Rhee et al., 1990; Youn et al. 1990). In most other bacterial diseases, local tissue and immunological reactions eliminate bacteria. However, this is not the case with *Helicobacter pylori* infection. Nonetheless, from a microbiological point of view, a single bacterial species can cause a variety of diseases. A leading example is *Streptococcus pyogenes* which causes acute pharyngitis, scarlet fever, otitis media, meningitis, peritonitis, pneumonia, erysipelas, puerperal fever, cellulitis, impetigo, acute glomerulonephritis, and rheumatic fever. A variety of diseases may be manifested from a chronic infection by a single bacterial species. A prime example is *Treponema pallidum* which manifests primary, secondary, and tertiary syphilis, depending on the chronicity of infection. Therefore, *Helicobacter pylori* is not extraordinary in the sense that this organism causes diverse gastroduodenal diseases during the period of chronic infection.

Helicobacter pylori has usually been defined as a non-invasive organism, because it has not been found in the lamina propria using the various histopathological staining methods. Only a few inflammatory cells are observed in the lamina propria of gastric mucosa in normal, healthy *Helicobacter pylori*-free persons. However, in *Helicobacter pylori*-infected patients, there is a heavy infiltration of inflammatory cells, including polymorphonuclear leukocytes in the lamina propria of the stomach (Ko et al., 1996). The definition of *Helicobacter pylori* as being a non-invasive organism, and findings of heavy infiltration of inflammatory cells in the gastric mucosa, may appear contradictory. However, this condition may be understood as the following: First, even if *Helicobacter pylori* can invade the epithelial layers of the gastric mucosa, this organism cannot be observed intact because inflammatory cells phagocytoze the organism as soon as it penetrates the lamina propria. Until now, the possibility of *Helicobacter pylori* invasiveness has largely been ignored. Second, if *Helicobacter pylori* cannot penetrate the epithelial layers of the gastric mucosa, this organism must produce a large number of chemotactic factors into the mucus layer of gastric mucosa. They are absorbed into the lamina propria to induce the infiltration of inflammatory cells into the lamina propria.

From an immunological point of view, the host responds to invading microorganisms with both nonspecific and specific immune responses. Since the ecological niche of *Helicobacter pylori* is the mucus layer and intercellular junctions of the gastric epithelium, host inflammatory cells cannot eliminate the microorganisms. Enormous numbers of bacteria are found in the gastric mucosa, despite local and humoral immune responses. When the invading foreign body is not removed, despite full activation of defense mechanisms, adverse consequences of the immune responses on the host develop. This reaction is called the hypersensitivity reaction, and has been classified into types I, II, III, IV, and V by Coombs and Gel (Table 1).

Hypersensitivity reactions listed in Table 1 are likely

Table 1. Classification of hypersensitivity reactions

Type	Name	Example of Illness
I	Anaphylactic hypersensitivity	asthma, urticaria
II	Antibody dependent cytotoxic hypersensitivity	transfusion reaction, autoimmune hemolytic anemia
III	Immune complex mediated hypersensitivity	serum sickness, SLE, Arthus reaction
IV	Cell-mediated hypersensitivity	contact dermatitis, graft rejection
V	Stimulatory hypersensitivity	Graves disease

to occur in the gastric mucosa of patients infected with *Helicobacter pylori*. *Helicobacter pylori* and gastric epithelial cells share many immunologically cross-reactive antigens and thus the auto-immune mechanism may be a pathogenetic factor in *Helicobacter pylori* infection. Furthermore, the possibility of invasion of *Helicobacter pylori* into the lamina propria remains.

In this paper, only the phagocytic function of inflammatory cells will be reviewed in order to explain the mechanism of why vitamin C intake can interrupt the chains of pathogenesis of *Helicobacter pylori* infection. Phagocytes are the last scavengers involved in the termination of inflammatory reactions. The most remarkable physiological response of the phagocytes after uptake of a foreign body is the increased consumption of glucose and oxygen. This increase is for NADPH production via the pentose phosphate shunt to produce superoxide anion via NADPH oxidase. Hydrogen peroxides are formed from superoxide anions by the superoxide dismutase. Myeloperoxidase acts on these hydrogen peroxides in the presence of chloride or other halide anion generating hypochlorite, which is a bleaching agent. Hydroxyl radicals and singlet oxygens are generated from the reaction of superoxide anions and hydrogen peroxides(Weitzman and Gordon, 1990). The oxygen free-radicals not only digest foreign bodies by incineration of proteins and lipids, but also damage host cells, such as white blood cells, indiscriminately. This results in the formation of abscesses.

These oxygen free-radicals are also well-known endogenous mutagens which make single and double strand breaks in the DNA, and transform normal DNA bases to 8-hydroxydeoxyguanosine (Dizdaroglu, 1985), thymine glycol (Frenkel et al., 1981 ; Cathcart et al., 1984), and 5-hydroxymethyluracil(Teebor et al., 1982). The capacity of *Helicobacter pylori* to activate neutrophils to produce oxygen free-radicals has been found to be 10 times greater than that of *Escherichia coli* or *Staphylococcus aureus* (Han et al., 1995). Therefore, infection with *Helicobacter pylori* provides the life-long condition in which oxygen free-radicals are produced by the phagocytes which infiltrate the lamina propria of the human gastric mucosa.

In addition, one of the most constant properties of *Helicobacter pylori* is the production of a potent γ-glutamyltranspeptidase, which is 10 times more potent than those of *Escherichia coli* or *Staphylococcus aureus*. This γ-glutamyltranspeptidase is likely to deplete scavengers of oxygen free-radicals by the degradation of glutathione in the human gastric mucosa.

The oxygen free-radicals so produced, in a state of deficiency of free-radical scavengers, may override the human body's natural ability to repair the DNA damage of the rapidly proliferating epithelial stem cells of the gastric glands. If the major effect of oxygen free-radicals upon gastric epithelial stem cells is cell death, then atrophic gastritis would appear. Mutations of the DNA in stem cells induced by oxygen free-radicals could lead to intestinal metaplasia, dysplasia, and gastric carcinoma in the long term (Baik et al., 1996). Cell death and mitogenesis are also known to be promoters for mutagenesis as well as carcinogenesis(Ames and Gold, 1990).

Mice die showing Schwartzman reaction-like symptoms if 1mg of *Helicobacter pylori* sonicates is administered peritoneally. This lethal factor involved in this experiment may also be involved in the pathogenesis of *Helicobacter pylori*-induced diseases, if *Helicobacter pylori* invades into the lamina propria of the human gastric mucosa.

Some or all of the type I, II, III, IV, and V hypersensitivity reactions induced by *Helicobacter pylori* and auto-antigens may activate phagocytes in the lamina propria, to persistently produce oxygen free-radicals. This may, in turn, result in mucoepithelial injuries of the human gastric mucosa. When the disrupted mucoepithelial layer is exposed to attack by offensive acid-pepsin, secretion of which is up-regulated by the *Helicobacter pylori* infection itself and subsequent hypersensitivity reactions, peptic ulcer diseases may result. Thus, the mechanisms of pathogenesis of chronic gastritis, gastroduodenal ulcers, and gastric cancer

are basically unified into the imbalance of offence and defence systems of free radicals.

Limitations in the control of *Helicobacter pylori* infection

Helicobacter pylori infection is the major determinant of type B chronic gastritis, gastric ulcers, duodenal ulcers and gastric cancer. The problem of type B chronic gastritis, peptic ulcer disease and gastric cancer may be solved by the direct countermeasures targeting *Helicobacter pylori*. Unfortunately, however, strategies for the control of *Helicobacter pylori*-induced gastritis which are based on conventional principles and practice of microbiology are of limited utility, because of the following three reasons.

First, antimicrobial drugs are not effective in the eradication of *Helicobacter pylori*. In fact, *Helicobacter pylori* is sensitive to a wide range of antimicrobial agents *in vitro*(Rhee et al., 1989). Most of them, however, are unsuccessful *in vivo*(Marshall et al., 1987 ; McNulty, 1987 ; Goodwin et al., 1988 ; Axon, 1991 ; Park et al., 1994). To overcome this problem, combined chemotherapy of more than 2 weeks and up to 6 weeks has been tried. An eradication rate of 60-95% has been reported. Our experiments, however, on volunteers of asymptomatic *Helicobacter pylori* carriers, have revealed quite the contrary results, showing a possibility of over-estimation of the reported eradication rate. In order to understand the methodological limitations in the diagnosis of *Helicobacter pylori* infection from the gastric mucosal biopsy material, it should be noted that the type B gastritis shows a typical pattern in the distribution of the lesion, which is multi-focal or patchy in the human gastric mucosa. This contrasts with the diffuse nature of the lesion in type A fundal gastritis. This leads to a false interpretation of effectiveness of chemotherapy, which, while able to suppress infection, rarely eradicates infection(Park et al., 1994). Considering the expense and side effects of current antimicrobial chemotherapy, it does not seem practical to use chemotherapy to eradicate *Helicobacter pylori* from the stomach of patients with so-called asymptomatic gastritis in a country with a high prevalence rate of *Helicobacter pylori* infection.

Second, the development of a vaccine against *Helicobacter pylori* would be difficult. *Helicobacter pylori* infection is always accompanied by local and systemic immune responses. However, *Helicobacter pylori* survives in the human stomach for a lifetime. Thus, the immune responses does not seem effective for the eradication of *Helicobacter pylori* infection. The development of a vaccine is based on the phenomenon that a person who has suffered and recovered from a disease will never contract the same disease. There is no convincing evidence that this fundamental phenomenon exists with *Helicobacter pylori* infection. Therefore, unfortunately, it appears unlikely that an effective vaccine to prevent new infections may be developed. To make matters worse, *Helicobacter pylori* and epithelial cells of human gastric mucosa share a significant number of cross-reactive antigens, implying that a vaccine may cause auto-immune tissue damage.

Third, it seems difficult to block the transmission of *Helicobacter pylori* infection. In the USA and in Australia, the mean annual risk of *Helicobacter pylori* infection is estimated at 1% on average. However, in Korea, it is estimated at approximately 10%. The following epidemiology of *Helicobacter pylori* infection may suggest reasons why most Koreans are conditioned to be infected with *Helicobacter pylori* in early childhood.

(1) There are no differences in prevalence rates of *Helicobacter pylori* infection between men and women in both developed and developing countries(Megraud et al., 1989 ; Rhee et al., 1990).

(2) The prevalence rate of *Helicobacter pylori* infection is higher in developing countries than in developed countries(The Eurogast Study Group, 1993).

(3) In developed countries, the prevalence rate of *Helicobacter pylori* infection increases with age. The question arises of whether this increase represents the true increase of the prevalence rate, or reflects a cohort moving through the population. However, even in these countries, higher prevalence rates have been observed in blacks than in whites, and in persons of lower socio-economic class(Malaty et al., 1992).

(4) Even in developed countries, such as Australia, *Helicobacter pylori* infection is clustered among children within a family unit. Also, the prevalence rate of the closed population of mentally retarded children living in institutions is as high as that of Koreans, which suggests that the reservoir of *Helicobacter pylori* is human beings and its mode of transmission is via person-to-person. However, whether the route of transmission is fecal-to-oral or oral-to-oral is still open to conjecture (Berkowicz and Lee, 1987 ; Cho et al., 1995).

(5) According to Dubois in the USA(1995), the prevalence rate of *Helicobacter pylori* in Rhesus monkeys was low when they were first put into the cages; however, the prevalence rate increased after 1 year of being reared in the same cage.

The reason for the low prevalence rate of *Helicobacter pylori* infection in developed countries is suggested as being due to differences in living conditions between developed and developing countries, and being not due to the actual absence of *Helicobacter pylori* in developed countries. Even though upgrading living conditions and improving the socioeconomic status would help prevent the acquisition of *Helicobacter pylori,* the administration of such public health guidance will take time. These measures need concerted efforts for implementation and are more demanding economically.

These barriers, unfortunately, make it nearly impossible to rapidly decrease the prevalence rate of *Helicobacter pylori* infection by conventional measures in the near future(Lee et al., 1991 ; Park et al., 1994 ; Rhee et al., 1994). Nevertheless, a breakthrough for the control of *Helicobacter pylori*-induced diseases is needed urgently in a country with a high prevalence rate of *Helicobacter pylori* infection, like Korea. In addition to the gastroduodenal diseases induced by the *Helicobacter pylori* infection, hypochlorhydria or achlorhydria of chronic atrophic gastritis itself, weakens the nonspecific defence of gastric acidity. This results in an increased susceptibility to infections transmitted by an oral route. It is worthwhile to review the bibliographic documentation about the subscorbutic status of patients with chronic gastritis, gastroduodenal ulcers, and stomach cancer, before launching a strategy towards the development of new drugs with fascinating efficacy, in order to understand the reason why vitamin C protects againt the stomach cancer, and to develop an alternative approach, or an interim adjunct strategy of utility for the cessation of *Helicobacter pylori*-induced diseases.

Relationship between *Helicobacter pylori* infection and vitamin C

The vitamin C status of human beings is dependent not only on socio-psychological but also on biological conditions. Scurvy is closely associated with inflammation. It is necessary to reevaluate chronic inflammation in view of the fact that vitamin C is highly concentrated in white blood cells, up to 600-1,200 mg%, when the plasma concentration of vitamin C is 1 mg% in a vitamin C saturated person.

Vitamin C is the first line among the substances of defense against oxygen free-radical damage in the human body (Frei et al., 1988a ; Frei et al., 1988b). Therefore, increased oxygen free-radical production through prolonged hypersensitivity reactions will lead to increased consumption of vitamin C. In addition, as discussed previously, hypochlorhydria induced by *Helicobacter pylori* infection is likely to increase vitamin C consumption due to an increased nitrite production by the surviving normal bacterial flora in the human stomach cavity. To make matters worse, *Helicobacter pylori* produces a potent γ-glutamyltranspeptidase which degrades glutathione, resulting in the depletion of oxygen free-radical scavengers in the human gastric mucosa. Thus, a state of vitamin C deficiency is expected in the absence of supplementary intake of this vitamin, because unlike most other living creatures, humans are unable to synthesize vitamin C to meet the increased demands of the body. Taken together, *Helicobacter pylori* infection and vitamin C deficiency synergistically and uninhibitedly aggravate the situation in which oxygen free-radicals damage the human gastric mucosa.

Table 2 shows changes in the concentration of ascorbic acid, total vitamin C, and pH of gastric juice and total vitamin C concentration of plasma in a 30-year-old researcher, G.M. Sobala, before and after accidental *Helicobacter pylori* infection (Sobala et al., 1991).

A dramatic decrease in vitamin C concentration of gastric juice was found after the *Helicobacter pylori* infection. Before infection, fasting gastric ascorbic acid was 22 μmol/L. It rose rapidly to 125 μmol/L after an intravenous injection of 500 mg ascorbic acid. At 37 days after infection, ascorbic acid was scarcely detected in gastric juice. At 161 days, fasting gastric ascorbic acid was 2 μmol/L and rose to 30 μmol/L after injection. This case demonstrates that vitamin C concentration of gastric juice remains at a low level for a prolonged period of time once a person is infected with *Helicobacter pylori.*

Table 3 shows the relationship between *Helicobacter pylori* infection and vitamin C concentration of whole

Table 2. The concentrations of ascorbic acid, total vitamin C and pH of gastric juice and total vitamin C concentration of plasma before and after accidental *Helicobacter pylori* infection

Days to onset of infection	-170	+14	+37	+74	+161
Gastric juice pH	2.0	7.5	7.5	2.0	2.3
Gastric juice ascorbic acid	22	5	1	1	2
Gastric juice total vitamin C	37	5	2	9	8
Plasma total vitamin C (μmol/L)	35	-	30	-	53

Table 3. Median vitamin C levels of whole blood, plasma, and gastric juice, and median ratio of gastric juice/whole blood, and gastric juice/plasma vitamin C levels according to the degree of histological density of *Helicobacter pylori* infection

Degree of gastritis	No of patients	Vitamin C levels (mg/dl) of			Ratio of vitamin C	
		Whole blood	Plasma	Gastric juice	Gastric juice /whole blood	Gastric juice /plasma
Normal	221	1.74	1.36	5.58	3.44	4.50
Mild	45	1.65	1.27	2.19	1.34	1.86
Moderate	25	1.56	1.19	2.06	1.27	1.68
Severe	14	1.53	1.06	2.15	1.81	2.22

blood, plasma, and gastric juice of 148 male and 157 female children. They underwent gastroduodenoscopy at the Gyeongsang National University Hospital Department of Pediatrics, between February 1991 and June 1995.

As the grade of histological density of *Helicobacter pylori* infection increased, vitamin C levels of whole blood, plasma, gastric juice, gastric juice/whole blood ratio, and gastric juice/plasma ratio decreased. It is clear that childhood vitamin C levels of whole blood and plasma, as well as gastric juice, are interrelated closely with *Helicobacter pylori* infection.

The indophenol dye titration method, which has been used since the late 1920s, has allowed the measurement of vitamin C levels in tissue fluids in humans. These measurements have shown that subscorbutic levels of vitamin C in tissue fluids of human body have been found continuously and consistently in patients with chronic gastritis, gastric ulcers, duodenal ulcers, and gastric cancer for many years, before *Helicobacter pylori* is confirmed as the major determinant of these diseases, meeting the expectations from the pathogenesis of *Helicobacter pylori* infection.

1. Chronic gastritis and vitamin C

In 1922, before the discovery of vitamin C, Hess suspected that a lack of hydrochloric acid secretion in the stomach could cause a loss of antiscorbutic vitamin on the basis that antiscorbutic vitamin undergoes destruction when rendered even slightly alkaline. In 1939, Demole and Issler observed that low gastric acidity of hypochlorhydria was associated with low levels of ascorbic acid in gastric juice. In 1941, Ludden et al. reported that the plasma ascorbic acid levels of 28 patients with achlorhydria and chronic atrophic gastritis were among the lowest of admitted patients, and were prescorbutic. In 1946, Vilter et al. reported that hypochlorhydria or achlorhydria after histamine stimulation was found in all 19 adult patients with scurvy. Brown(1951) and Bronte-Stewart(1953) also reported that hypochlorhydira or achlorhydria was found in patients with scurvy.

Hypochlorhydria is invariably associated with chronic gastritis, which is accompanied by decreased levels of vitamin C in plasma and gastric juice.

2. Gastroduodenal ulcers and vitamin C

In 1928, Hutter observed that the peak incidence of peptic ulcers occurred during the late winter and early spring. These are the times of the year when intake of vitamin C is lowest and when body stores are at their minimum. In separate studies, Davidson in 1928, Wood in 1935, and Platt in 1936, observed scurvy in patients with peptic ulcers. They attributed the vitamin C deficiency to the usual ulcer diets of Sippy, Lenhartz, von Leube, and Alvarez, all of which lacked ascorbic acid. It was also attributed to the alkalis which these patients used extensively. In 1936, Payne found that 51 patients who had died of general or local peritonitis after partial gastrectomy at St. Bartholomew's Hospital in London had an almost complete absence of fibrous response along the suture line of the anastomosis. This was attributed to the effect of vitamin C deficiency. In 1936, Archer and Graham found that six of nine patients with duodenal ulcers were in a subscorbutic state, using the saturation test with ascorbic acid. In 1936, Harris et al. found that patients with gastric or duodenal ulcers were disproportionately predominant among hospital patients with the lowest urinary excretions of vitamin C. In 1937, Rivers and Carlson reported low levels of vitamin C in blood and urine, and a state of capillary fragility in patients at the Mayo Clinic who had suffered repeated gastrointestinal hemorrhages, with or without demonstrable ulceration. In 1937, Ingalls and Warren found that most patients with gastric or duodenal ulcers at the Peter Bent Brigham Hospital in Boston had subscorbutic plasma levels of vitamin C. In 1938, Portnoy and Wilkinson reported that the ascorbate status of 25

patients with peptic ulceration, and 31 patients with hematemesis was markedly deficient when compared to the 51 control subjects. In 1939, Warren et al. found that patients with duodenal ulcers utilized 20% more ascorbic acid per kilogram of body weight per day than did normal subjects. In 1944, Ebbesen and Rasmussen reported that 52 patients with gastric or duodenal ulcers showed a 75% drop in the serum level of ascorbic acid during the first 12 to 16 days of initial treatment with ulcer diet. In 1947, Crescenzo and Cayer reported that all patients with ulcers showed a low level of plasma vitamin C and a less steep saturation curve following the oral load test of vitamin C. In 1955, Weiss et al. reported excellent results after treating 19 patients who had bleeding duodenal and gastric ulcers, with the use of citrus bioflavonoids and ascorbic acid. In 1955, Drummond reported excellent results after treating 54 patients who had peptic ulcers with the combined use of high doses of ascorbic acid, corticotropin, and atropine. In 1960, Bodi and Weiss reported encouraging results after treating five patients with bleeding peptic ulcers with ascorbic acid and hesperidin. In the same year, Morris(1960) reported two cases of hemorrhagic gastritis which were successfully treated by the administration of large doses of vitamin C and citrus fruit juice.

From this bibliographic survey, it becomes apparent that there is a close link between gastroduodenal ulcers and a state of vitamin C deficiency. This status of prolonged vitamin C deficiency is likely to deplete vitamin C storage in the vitamin C-rich neuro-immuno-endocrine systems which are responsible for the communication between the human body and the environment. These deficiencies, in turn, jeopardize the homeostasis of internal milieu and the adaptive response of the normal human body to even a minor harmful stimulus.

3. Stomach cancer and vitamin C

In 1973, Bjelke found that vitamin C was the dietary variable discriminating most strongly between stomach cancer cases and control cases. The effect was significant for gastric carcinomas of diffuse and intestinal types. Correa et al.(1975) suggested that the pathogenesis of stomach cancer involves two stages. The first stage has a long-term effect, possibly initiated in childhood. It leads to severe atrophic gastritis and intestinal metaplasia in the gastric mucosa. The second stage of carcinogenesis occurs in a stomach that has undergone these mucosal changes. Fresh fruits and vegetables protect against these stages, possibly because of their high content of vitamin C. In 1985, Correa et al. found that fruits and dietary vitamin C were the protective factors against gastric cancer for both blacks and whites in Louisiana. Those in the lower quarter group of the vitamin C intake had twice the risk(whites) and three times the risk(blacks) compared with those in the upper quarter group of the vitamin C intake. In 1985, Risch et al. confirmed the protective effects of ascorbic acid against stomach cancer by studying 246 gastric cancer patients and control subjects. In 1987, Burr et al. conducted a survey in two British towns with high and low stomach cancer death-rates. Plasma concentrations of ascorbic acid and intake of fruits were lower in the high-risk area than in the low-risk area, and also in lower social classes. In 1987, La Vacchia et al. conducted a case-control study of 206 gastric cancer patients and control subjects in Italy. Those in the bottom one third of vitamin C intake had a 2.5 fold elevated risk than those in the upper one third group of vitamin C intake. In 1988, You et al. observed that the lowest quartile group of vitamin C intake had a two-fold increased risk of stomach cancer than the highest quartile group of vitamin C intake in a high-risk region for stomach cancer in China.

The incidence of stomach cancer is reported consistently as being inversely correlated with the vitamin C intake. Thus, a high intake of vitamin C has a protective effect against gastric cancer. If this message is indeed true, then we have to ask ourselves why we are not campaigning for the do-it-yourself practice of increased intake of vitamin C.

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