Inhibitory Effect of Long Term 1 α -Hydroxyvitamin D₃ Administration on *Helicobacter pylori* Infection

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Summary The effects of long-term supplementation with 1 α -hydroxyvitamin D₃ (1 α (OH) D₃) on serum pepsinogen (PG) I levels, and *Helicobacter pylori* (*H. pylori*) infection rate, were investigated in healthy elderly women, aged 70 to 99 years, in a nursing home. Of these 34 subjects, 15 have been administered one microgram of 1 α (OH) D₃ daily for twenty years, because of osteoporosis treatment, and 19 were free from the drug. Serum PG-I levels and *H. pylori* infection rate in subjects with 1 α (OH) D₃ were significantly lower than those without treatment ($p < 0.05$). These results suggested the inhibitory effect of long-term administration of 1 α (OH) D₃ on *H. pylori* infection.

Key Words: 1 α -hydroxyvitamin D₃, *Helicobacter pylori*, pepsinogen I, elderly women, nursing home

Introduction

Stomach cancer is one of the most prevalent malignancies in the world [1]. However, epidemiological studies have shown differences in its incidence in different countries and even different regions within a country [2]. Several causal factors have been suggested to explain the differences, including genetic, climatic, geographical, occupational factors, and dietary habits. For instance, with regard to diet, the risk of stomach cancer in Shandong, China, was found to decrease on dietary intake of carotene, vitamin C, and calcium [3].

Helicobacter pylori (*H. pylori*) is a microaerophilic, gram negative, urease positive, spiral bacterium that causes chronic infection of the human stomach [4]. *H. pylori* infection is thought to be a potential cause for gastric carcinoma

[5, 6]. Dietary vitamin C (ascorbic acid) intake may reduce gastric carcinoma risk by either reducing formation of N-nitroso compounds in the gastric juice [7] or by scavenging free radicals that may be enhanced by *H. pylori* in gastric mucosa [8]. Further, a high dose of vitamin C has been reported to inhibit the growth of *H. pylori* [9]. Serum pepsinogen (PG) I levels, which reflect the grade of atrophic gastritis, and a predisposition for gastric cancer, decreased after eradication of *H. pylori* [10]. In our previous study, 1 α -hydroxyvitamin D₃ (1 α (OH) D₃) had an inhibitory effect on N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced gastrointestinal carcinogenesis in Wistar rats [11]. Therefore, this study was carried out to investigate the relationship between 1 α (OH) D₃ supplementation and *H. pylori* infection in the elderly women at a nursing home.

Materials and Methods

Healthy elderly women aged 70 to 99 years, living in a nursing home, were recruited for this study (Table 1). All subjects had a similar dietary intake for longer than nine

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years. Of the 34 subjects, 15 have been receiving one microgram of 1α (OH) D_3 daily for twenty years as part of osteoporosis treatment, and 19 subjects were without treatment. Levels of serum anti-*H. pylori* IgG, PG-I, PG-II, gastrin, and other routine laboratory data were obtained by measurement through commercial diagnostics facilities (FALCO Biosystems, CO. Ltd., Kyoto). Fasting serum samples were taken for the determinations of PGI and PGII levels by chemiluminescent immunoassay (Architect Pepsinogen I, II; Dainabot, Tokyo, Japan), gastrin levels by radioimmunoassay (Gastrin · Riakit II; Dainabot, Tokyo,

Japan) and *H. pylori* Ig levels by enzyme immunoassay (Determiner · *H. pylori* antibody; Kyowa Medex, Tokyo, Japan). The χ^2 -test and Student's t-test were used for statistical analysis. Statistical significance was assigned for any p values less than 0.05.

Results

There was no significant difference between two groups (those on treatment and those without) in regard to age, height, weight, BMI, peripheral blood, and blood chemistry

Table 1. Clinical characteristics for two groups (mean $\pm$ SD)

	Group 1	Group 2
	Without 1α (OH) D_3	With 1α (OH) D_3
N	19	15
Age (yr)	85 $\pm$ 7.93	84.7 $\pm$ 6.87
Height (cm)	141.4 $\pm$ 6.82	143.6 $\pm$ 7.42
Body weight (kg)	39.3 $\pm$ 5.88	39.8 $\pm$ 6.72
Body mass index	19.8 $\pm$ 2.58	19.5 $\pm$ 3.66
White blood cell (per μ l)	5847 $\pm$ 2020	6225 $\pm$ 2851
Red blood cell ($\times 10^4$ /l)	379.6 $\pm$ 57.3	418.7 $\pm$ 62.5
Hemoglobin (g/dl)	11.5 $\pm$ 1.78	12.0 $\pm$ 1.48
Hematocrit (%)	37.0 $\pm$ 5.14	38.9 $\pm$ 4.72
AST (IU/l)	19.9 $\pm$ 5.62	24.2 $\pm$ 11.7
ALT (IU/l)	14.6 $\pm$ 7.98	15.3 $\pm$ 10.5
γ GTP (IU/l)	16.2 $\pm$ 6.01	24.5 $\pm$ 25.1
Total cholesterol (mg/dl)	202.5 $\pm$ 37.0	195.3 $\pm$ 33.9
High density lipoprotein (mg/dl)	52.2 $\pm$ 22.1	57.2 $\pm$ 16.2
Triglycerides (mg/dl)	124.1 $\pm$ 47.8	114.2 $\pm$ 48.7
Calcium (mg/dl)	9.05 $\pm$ 0.12	9.35 $\pm$ 0.14
Phosphate (mg/dl)	3.19 $\pm$ 0.13	3.03 $\pm$ 0.16
Uric acid (mg/dl)	4.98 $\pm$ 1.84	4.46 $\pm$ 1.34
Creatinine (mg/dl)	0.89 $\pm$ 0.37	0.75 $\pm$ 0.21
Blood sugar (mg/dl)	102.8 $\pm$ 29.1	116.4 $\pm$ 38.4

Table 2. Serum pepsinogen I and II levels, pepsinogen I/II ratio, *H. pylori* infection status, and gastrin levels with and without 1α (OH) D_3 administration

	Group 1	Group 2
	[Without 1α (OH) D_3]	[With 1α (OH) D_3]
N	19	15
pepsinogen I ^c (ng/ml)	86.93 $\pm$ 47.74	55.41 $\pm$ 28.64 ^a
pepsinogen II ^c (ng/ml)	26.74 $\pm$ 42.33	15.93 $\pm$ 9.960
pepsinogen I/II ratio ^c	5.52 $\pm$ 2.62	4.29 $\pm$ 1.94
<i>H. pylori</i> infection rate	13/19 (68.4%)	5/15(33.3%) ^b
gastrin ^c (pg/ml)	206.11 $\pm$ 216.94	283.00 $\pm$ 194.6

a) Significantly different from group 1 at $p < 0.05$ (two-sided) by Student's t-test.

b) Significantly different from group 1 at $p < 0.05$ by the χ^2 -test.

c) Mean $\pm$ SD

(Table 1). Serum PG-I and II levels, gastrin levels and *H. pylori* infection rate were compared in both the groups (Table 2). Serum PG-I levels and *H. pylori* infection rate in subjects with 1 α (OH) D₃ treatment were significantly lower ($p < 0.05$) than those without treatment. Similarly, PG-I/II and serum PG-II levels in the group being administered 1 α (OH) D₃ tended to be lower. Serum gastrin concentration in the second group also tended to be higher as compared to the first group.

Discussion

This is the first report suggesting that long-term administration of 1 α (OH) D₃ inhibits *H. pylori* infection. However, the exact mechanism for this observation is not elucidated. The results of earlier studies indicated that γ -glutamyl transpeptidase (GGT) is an important enzyme for the growth and survival of *H. pylori* *in vivo* [12]. Inhibition of GGT activity by a specific inhibitor (L-serine sodium borate complex, SBC) resulted in the growth retardation of *H. pylori* [13]. *H. pylori* possess strong urease activity, rapidly hydrolyzing urea, and producing large quantities of ammonia and carbon dioxide. These may in turn neutralize gastric acid, thus raising intra-gastric pH [14]. Consequently, *H. pylori* can survive in the stomach lumen for long periods of time. The inhibitory effect of vitamin C supplementation on *H. pylori* infection has been reported [9, 15–18]. Inhibition of urease production by *H. pylori* by vitamin C has also been reported [19]. The two mechanisms, i.e., inhibition of GGT and urease activities, may also be observed for vitamin D administration. The NADPH oxidase 1 (Nox 1) mRNA is induced by 1 α , 25-dihydroxyvitamin D₃ (1 α , 25 (OH)₂ D₃) [20]. Circulating phagocytes produce large amounts of reactive oxygen species (ROS) in response to infectious stimuli through activation of the NADPH oxidase complex [21]. The ROS are known to be microbicidal, and thus activation of NADPH oxidase leads to bacterial disinfection. *H. pylori* also might be phagocytosed by macrophages through the activation of Nox 1 induced by vitamin D. It has not been clarified that long-term supplementation of 1 α (OH) D₃ could prevent or eradicate *H. pylori* infection.

The observed decrease of serum PG-I levels may be due to inhibition of *H. pylori* infection by 1 α (OH) D₃. It has been reported that eradication of *H. pylori* decreased PG-I levels [10, 22]. Previously, chronic infection of *H. pylori* was shown to increase serum gastrin levels, and the gastrin levels in the patients decreased after eradicating *H. pylori* infection [10]. Our study showed that 1 α (OH) D₃ supplementation significantly decreased the *H. pylori* infection rate, but serum gastrin levels tended to be high. Administered 1 α (OH) D₃ is metabolized in the liver to form 1 α , 25 (OH)₂ D₃, which acts hormonally through its specific

receptors [23]. Stumpf *et al.* reported the presence of nuclear binding sites for 1 α , 25(OH)₂ D₃ in gastrin-producing cells in the mouse stomach [24], which might explain our results showing higher gastrin levels despite a decrease in *H. pylori* infection. These observations warrant further study on the role of vitamin D in *H. pylori* infection, and such studies may lead to developing a new approach to preventing gastric carcinoma.

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