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In Vitro Effects of Vitamins C and E on *Helicobacter pylori*

Vitamin C ve E'nin *Helicobacter pylori* Üzerindeki İn Vitro Etkileri

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Abstract

In this study, we aimed to evaluate the effects of vitamins C and E on *Helicobacter pylori* in vitro, in other words in the absence of the in vivo dynamics of the gastric mucosa. The study included 32 patients with *H. pylori*-positive nonulcer dyspepsia. Tissue samples from antrum and corpus, which were taken during upper gastrointestinal endoscopy, were Gram stained and seeded onto culture plates supplemented with 7% horse blood for *H. pylori* culture. Plates were incubated in a microaerophilic environment. Isolated *H. pylori* strains were identified on Gram-stained smears and with urease test. Different concentrations of vitamins C, E and E+C were added into the Columbia Blood Agar plates containing 7% defibrinated horse blood at appropriate temperature in order to produce vitamin added media. Equal amounts of *H. pylori* colonies (*equivalent to 1 McFarland turbidity standard unit*) were inoculated onto media plates. Media groups were as follows: 1. Control group, 2. Vitamin C added group, 3. Vitamin E added group, 4. Vitamins C and E added group. Growth of *H. pylori* was seen in all culture media. By counting each colony, the total number of colony forming units (CFUs) on each plate was determined as 10^3 CFU/ml in two groups (60 µl vitamin E group and 20 µl vitamin C+60 µl vitamin E group). The total number of CFUs in other groups (including the control group) were approximately 1-2x10^2 CFU/ml. Inhibition of growth was not observed in any groups and there was a 1 log increase in plates with high levels of vitamin E. The present study shows that vitamins C and E had no inhibitory effects on *H. pylori* in vitro.

Keywords: *Helicobacter pylori*; Vitamin C; Vitamin E; In vitro.

Özet

Bu çalışmada vitamin C ve E'nin *Helicobacter pylori* üzerine etkilerini in vitro ortamda, yani gastrik mukozanın in vivo etkilerinin olmadığı bir ortamda değerlendirmeyi amaçladık. Çalışmaya *H. pylori* pozitif ülser dışı dispepsisi olan 32 hasta dahil edildi. Üst gastrointestinal endoskopi sırasında antrum ve korpustan alınan

doku örnekleri Gram boyandı ve *H. pylori* kültürü için %7 at kanı ile desteklenmiş kültür plakalarına (besiyerlerine) ekildi. Örnekler mikroaerofilik bir ortamda inkübe edildi. İzole edilen *H. pylori* suşları, Gram boyanmış yaymalar ve üreaz testi ile tanımlandı. Vitamin katkılı besiyeri ortamı üretmek için uygun sıcaklıkta %7 defibrine at kanı içeren Columbia Blood Agar plakalarına farklı konsantrasyonlarda C, E ve E + C vitaminleri eklendi. Eşit miktarlarda *H. pylori* kolonileri (*1 McFarland türbidite standart birimine eşdeğer*) besiyeri plakalarına inoküle edildi. Besiyeri grupları şu şekildedir: 1. Kontrol grubu, 2. Vitamin C eklenen grup, 3. Vitamin E eklenen grup, 4. Vitamin C ve E eklenen grup. Tüm kültür ortamlarında *H. pylori* üremesi görüldü. Her bir koloni sayılarak her plakadaki toplam koloni oluşturan birim (CFU) sayısı hesaplandı ve iki grupta (60 µl vitamin E grubu ve 20 µl vitamin C + 60 µl vitamin E grubu) 10^3 CFU/ml olarak belirlendi. Diğer gruplardaki (kontrol grubu dahil) toplam CFU sayısı ise yaklaşık 1-2x10^2 CFU/ml idi. Hiçbir grupta büyüme inhibisyonu gözlenmedi ve yüksek E vitamini seviyelerine sahip plakalarda 1 log artış oldu. Bu çalışma, C ve E vitaminlerinin in vitro olarak *H. pylori* üzerinde herhangi bir etkisinin olmadığını göstermektedir.

Anahtar Kelimeler: *Helicobacter pylori*, Vitamin C, Vitamin E, İn vitro.

Introduction

Helicobacter pylori induces chronic gastritis and peptic ulcer when it colonizes the gastric mucosa. *H. pylori* also plays a role in the etiology of gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma [1-5]. This knowledge has a deep clinical impact with regard to the successful management of *H. pylori*. *H. pylori* eradication is very difficult in areas where antibiotic resistance is high due to uncontrolled use of antibiotics. In areas with low prevalence of resistance to antibiotics, 7-10 days lasting eradication treatment regimens seem to be sufficient [3-5]. However, in Turkey, 14-day treatment duration is recommended due to the high prevalence of antibiotic resistance [5-6]. Several combinations of antibiotics are prescribed for the eradication of *H. pylori*. Although standard triple therapy (clarithromycin 500 mg BID, amoxycillin 1 gr BID and a proton pump inhibitor BID) has been accepted as first-line therapy, alternative regimens are proposed because of higher eradication rates [5]. Recently, supplementation of prebiotics and probiotics [7], garlic [8] and vitamins to several treatment regimens have been examined in several research studies. Although it has been shown that supplementation of vitamins C and E increased the *H. pylori* eradication rate of some regimens in several studies, there is no consensus on the dose and duration of therapy and they were found to be ineffective in other studies [9]. In our previous studies, we showed that vitamins C and E had increased *H. pylori* eradication rates of several regimens. In some study groups, *H. pylori* eradication rates were higher than 80%; however, these were lower than ideal rates in patients with low antioxidant capacity [10-12]. We also showed that vitamins C and E decreased inflammation and colonization of *H. pylori* in the gastric mucosa in vivo [13]. In this study, we aimed to evaluate the effect of vitamins C and E on *H. pylori* in vitro, in other words in the absence of the in vivo dynamics of the gastric mucosa.

Material and Method

Ethics committee approval was received for this research from the ethics committee of the Haydarpaşa Numune Hospital, Istanbul. Thirty-four patients with *H. pylori*-positive nonulcer dyspepsia were included in the study. All subjects were informed about the study protocol, and written consent was obtained from all participants. Patients receiving antimicrobial, anticoagulant or proton pump inhibitors were excluded from the study. *H. pylori* positivity was detected with C-14 urea breath test. All patients underwent upper gastrointestinal (GI) endoscopy. Two tissue samples were taken from the corpus and antrum for histopathological examination during upper GI endoscopy. Tissue samples were transported to the laboratory in sterile isotonic NaCl solution within 5-10 minutes. Tissue samples were then Gram stained and seeded onto Christensen's Urea Agar and Columbia Blood Agar (Oxoid, UK) plates supplemented with 7% horse blood for *H. pylori* culture. Plates were incubated in a microaerophilic environment at 37°C for 5-10 days. A BD Gaspak Campy Container System was used to achieve a microaerophilic environment.

Isolated *H. pylori* strains were identified with typical morphology on Gram-stained smears and with urease test. Seventeen cultured tissue samples were positive for *H. pylori*. Different concentrations of vitamin C, vitamin E and vitamin E+C were added into the Columbia Blood Agar plates containing 7% defibrinated horse blood at appropriate temperature, in order to produce vitamin added media. Equal amounts of *H. pylori* colonies (*equivalent to 1 McFarland turbidity standard unit*) were inoculated onto media plates. Media groups were as follows: 1. Control group [Figure 1], 2. Vitamin C added group, 3. Vitamin E added group, 4. Vitamins C and E added group. Plates were incubated in microaerobic conditions and growth was monitored for 10-14 days. Added concentrations of vitamin C, vitamin E and Vitamin C+E in the medium are shown in Table 1.

Table 1. Added concentrations of vitamin C, vitamin E, Vitamin C+E in the medium.

		0 mg/dl	0.5 mg/dl	1 mg/dl	1.5 mg/dl	2mg/dl
Vitamin C	Drug	0	5 µl	10 µl	15 µl	20 µl
	Medium	100 ml	99.995 ml	99.99 ml	99.985 ml	99.98 ml
Vitamin E	Drug	0	15 µl	30 µl	45 µl	60 µl
	Medium	100 ml	99.985 ml	99.97 ml	99.955 ml	99.94 ml
Vitamin C+E	Drug	0	5+15 µl	10+30 µl	15+45 µl	20+60 µl
	Medium	100 ml	99.98 ml	99.96 ml	99.94 ml	99.92 ml

Reference values for serum levels of vitamin E and vitamin C in adults:
Vitamin E: 0.5- 1.8 mg/dl (12- 42 µmol/L)
Vitamin C: 0.5- 1.5 mg/dl (28- 85 µmol/L)
The concentrations of vitamin C and vitamin E were 500 mg/5 ml and 50 IU/ml (ampule-1 IU=0.67 mg), respectively.
µl: microliters, ml: milliliters, mg: milligram, µmol: micromole.

Results

Growth of *H. pylori* occurred in all culture media. At the beginning, dose escalation of the medium was carried out according to the table. Evaluation of dose escalation was done at the next inoculation of medium. By colony count, the total number of colony forming units [CFUs] on plates was determined to be 10^3 CFU/ml in two groups (60 µl vitamin E group and 20 µl vitamin C+60 µl vitamin E group).

The total number of CFUs in the other groups (including the control group) was approximately 1-2x10^2 CFU/ml. Inhibition of growth was not observed in any group. Particularly, a one log increase in plates with a high level of vitamin E was remarkable.

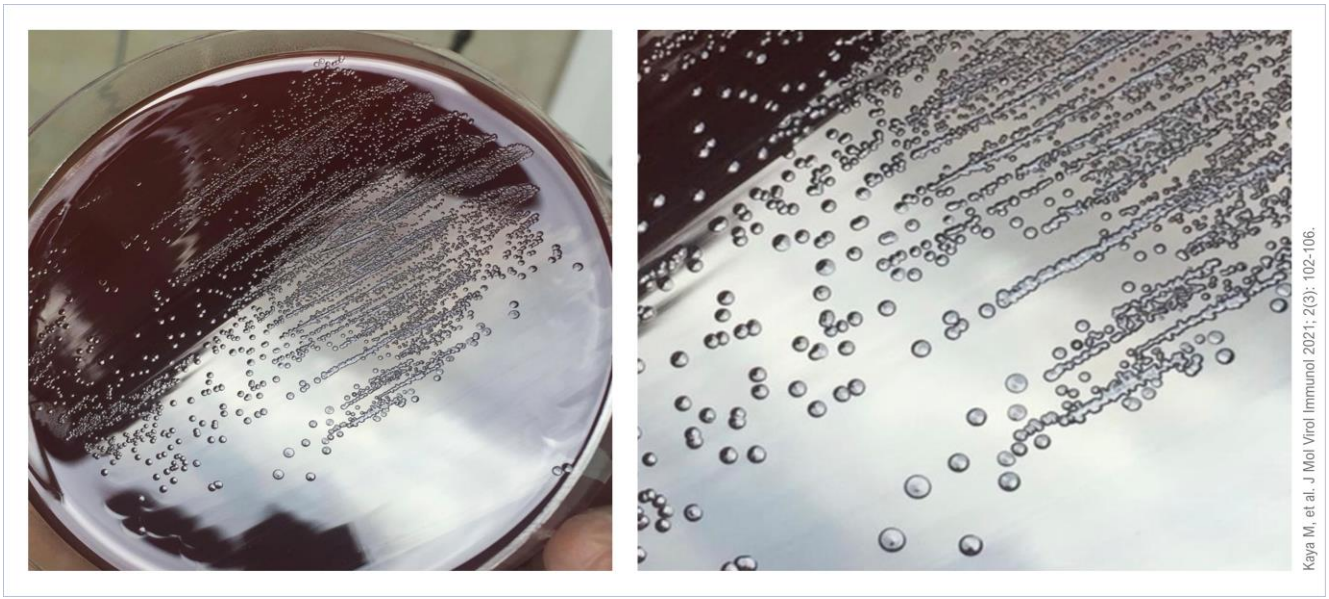

Figure 1. *H. pylori* colonization on Columbia Blood Agar plates.

Discussion

Since *H. pylori* culture is difficult to perform and expensive, it is rarely performed in daily clinical practice. The main difficulty is the requirement of an appropriate culture medium due to the microaerophilic characteristic of the bacteria. Culture of *H. pylori* gives the opportunity to determine antibiotic susceptibilities of infected patients. We usually use a rapid urease test and histopathologic examination to detect *H. pylori*, which are sufficient to prescribe eradication therapy. Culture is usually performed to determine antibiotic susceptibilities for patients who failed to respond to second line *H. pylori* eradication therapy [5]. Treatment-naïve patients were included in the present study.

We previously showed the efficacy of supplementation of vitamins C and E in various antibiotic regimens on *H. pylori* eradication in vivo [10-12]. Sun et al. showed that dietary supplementation of vitamins C and E had short-term beneficial effects on *H. pylori* gastritis in Mongolian gerbils; however, this benefit was not sustained when the infection persisted [14]. In another study, dietary supplementation of vitamin E had a protective effect on *H. pylori* related gastric mucosal injury in Mongolian gerbils. This benefit was attributed to inhibition of accumulation of activated neutrophils by vitamin E [15].

In an in vitro study, the effect of standardized extract of apple peel APPE (60% of total polyphenols; 58% of flavonoids; 30% of flavan-3-ols and procyanidins) on *H. pylori* gastritis was evaluated. This extract, which contains antioxidants, inhibited the accumulation of activated neutrophils, as in the previous study [16-17].

The efficacy of vitamin C, as an antioxidant and free radical scavenger, is well studied. Nonetheless, the mechanism of the direct inhibitory effect of vitamin C on *H. pylori* gastritis is yet to be defined. In 1997, Zhang et al. investigated the effect of vitamin C on *H. pylori* both in vitro and in vivo [18]. They showed that vitamin C had an inhibitory effect on *H. pylori* in vitro as well as in vivo. As we know, ascorbic acid is broken down by *H. pylori* [19]. In a previous study, concentrations of ascorbic acid in gastric juice in *H. pylori* positive and *H. pylori* negative patients were shown to be 2.8 μg/mL and 17.8 μg/mL, respectively [20]. Thus, by increasing the concentration of ascorbic acid in gastric juice, supplementation of vitamin C may have a toxic effect on *H. pylori*.

The aim of the present study was to evaluate the possible inhibitory effect of vitamins C and E on *H. pylori* in vitro. However, this beneficial effect of vitamins C and E was not achieved in vitro in the present study. Rather, *H. pylori* proliferation increased in the group in which vitamin E was added. This may be due to soybean oil, which was used as an additive in the vitamin E preparation, which might have increased *H. pylori* proliferation. We think that this result might not be due to the small number of patients. Probably due to the absence of in vivo dynamics between *H. pylori* and gastric mucosa, vitamins C and E had no bactericidal and/or bacteriostatic effects on *H. pylori* growth in defined medium. The combination of vitamin C, vitamin E and antibiotics provided higher eradication rates of *H. pylori* [10] and also dietary supplementation of vitamin C (without antibiotics) inhibited the colonization of *H. pylori* and the accumulation of activated neutrophils, but vitamins C and E had no inhibitory effects on *H. pylori* growth in defined medium. The limitation of this study is that the method for counting each *H. pylori* colony was close to ideal, but it was not mathematically sufficient. However, when we consider the results of the aforementioned studies and our research, it may be speculated that vitamins C and E have no inhibitory effects on *H. pylori* in vitro.

Conclusion

We previously showed that the oral intake of vitamins C and E had inhibitory effects on gastric mucosal colonization of *H. pylori* in vivo. However, in the present in vitro study, vitamins C and E had no inhibitory effects on *H. pylori*. This result leads us to think that substances that have inhibitory effects on *H. pylori* in vivo interact with each other in the microenvironment. Therefore, it does not seem logical to compare the in vivo dynamics of the host's stomach and the in vitro interactions of vitamins C and E with *H. pylori*.

Conflict of interest: The authors declare that there is no conflict of interest. The authors alone are responsible for the content and writing of the paper. **Financial disclosure:** There is no financial support to this study.

References

1. Kaplan M, Tanoglu A, Duzenli T, Tozun AN. Helicobacter pylori treatment in Turkey: Current status and rational treatment options. North Clin Istanb 2019; 7(1): 87-94. [Crossref]

2. Lee SW, Kim HJ, Kim JG. Treatment of Helicobacter pylori Infection in Korea: A Systematic Review and Meta-analysis. J Korean Med Sci 2015; 30(8): 1001-9. [Crossref]

3. Ishaq S, Nunn L. Helicobacter pylori and gastric cancer: a state of the art review. Gastroenterol Hepatol Bed Bench 2015; 8(Suppl 1): S6-S14.

4. Sezikli M, Sirin G, Cetinkaya Z, Tanoglu A, Guzelbulut F, Bunul F, et al. Comparison of the efficacy of six different helicobacter pylori eradication regimens: Greater than or equal to another. Biomed Res 2018; 29: 1143-8. [Crossref]

5. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, et al; European Helicobacter Study Group. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. Gut 2012; 61(5): 646-64. [Crossref]

6. Kadayifci A, Buyukhatipoglu H, Cemil Savas M, Simsek I. Eradication of Helicobacter pylori with triple therapy: an epidemiologic analysis of trends in Turkey over 10 years. Clin Ther 2006; 28(11): 1960-6. [Crossref]

7. Franceschi F, Cazzato A, Nista EC, Scarpellini E, Roccarina D, Gigante G, et al. Role of probiotics in patients with Helicobacter pylori infection. Helicobacter 2007; 12 Suppl 2: 59-63. [Crossref]

8. O'Gara EA, Maslin DJ, Nevill AM, Hill DJ. The effect of simulated gastric environments on the anti-Helicobacter activity of garlic oil. J Appl Microbiol 2008; 104(5): 1324-31. [Crossref]

9. Li G, Li L, Yu C, Chen L. Effect of vitamins C and E supplementation on Helicobacter pylori eradication: a meta-analysis. Br J Nutr 2011; 106(11): 1632-7. [Crossref]

10. Sezikli M, Cetinkaya ZA, Sezikli H, Güzelbulut F, Tiftikçi A, Ince AT, et al. Oxidative stress in Helicobacter pylori infection: does supplementation with vitamins C and E increase the eradication rate? Helicobacter 2009; 14(4): 280-5. [Crossref]

11. Sezikli M, Çetinkaya ZA, Güzelbulut F, Yeşil A, Coşgun S, Kurdaş OÖ. Supplementing vitamins C and E to standard triple therapy for the eradication of Helicobacter pylori. J Clin Pharm Ther 2012; 37(3): 282-5. [Crossref]

12. Sezikli M, Cetinkaya ZA, Güzelbulut F, Sezikli H, Özkara S, Coşgun S, et al. Efficacy of vitamins supplementation to therapy on Helicobacter pylori eradication in patients with low antioxidant capacity. Clin Res Hepatol Gastroenterol 2011; 35(11): 745-9. [Crossref]

13. Sezikli M, Çetinkaya ZA, Güzelbulut F, Çimen B, Özcan Ö, Özkara S, et al. Effects of alpha tocopherol and ascorbic acid on Helicobacter pylori colonization and the severity of gastric inflammation. Helicobacter 2012; 17(2): 127-32. [Crossref]

14. Sun YQ, Girgensone I, Leanderson P, Petersson F, Borch K. Effects of antioxidant vitamin supplements on Helicobacter pylori-induced gastritis in Mongolian gerbils. Helicobacter 2005; 10(1): 33-42. [Crossref]

15. Sugimoto N, Yoshida N, Nakamura Y, Ichikawa H, Naito Y, Okanoue T, et al. Influence of vitamin E on gastric mucosal injury induced by Helicobacter pylori infection. Biofactors 2006; 28(1): 9-19. [Crossref]

16. Pastene E, Speisky H, Troncoso M, Alarcón J, Figueroa G. In vitro inhibitory effect of apple peel extract on the growth of Helicobacter pylori and respiratory burst induced on human neutrophils. J Agric Food Chem 2009; 57(17): 7743-9. [Crossref]

17. Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr 2005; 81(1 Suppl): 317S-325S. [Crossref]

18. Zhang HM, Wakisaka N, Maeda O, Yamamoto T. Vitamin C inhibits the growth of a bacterial risk factor for gastric carcinoma: Helicobacter pylori. Cancer 1997; 80(10): 1897-903.

19. Odum L, Andersen LP. Investigation of Helicobacter pylori ascorbic acid oxidating activity. FEMS Immunol Med Microbiol 1995; 10(3-4): 289-94. [Crossref]

20. Banerjee S, Hawksby C, Miller S, Dahill S, Beattie AD, McColl KE. Effect of Helicobacter pylori and its eradication on gastric juice ascorbic acid. Gut 1994; 35(3): 317-22. [Crossref]