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# Cranberry extract suppresses interleukin-8 secretion from stomach cells stimulated by *Helicobacter pylori* in every clinically separated strain but inhibits growth in part of the strains

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ABSTRACT

It is known that cranberry inhibits the growth of *Helicobacter pylori* (HP). In human stomach, HP basically induces chronic inflammation by stimulating stomach cells to secrete interleukin (IL)-8 and other inflammatory cytokines, and causes stomach cancer, etc. The aim of this study was to investigate the inhibiting effects of cranberry on HP growth and IL-8 secretion from stomach cells induced by HP, using clinically separated HP strains. HP growth in liquid culture and on-plate culture was evaluated by titration after 2-day incubation and by agar dilution technique, respectively. For IL-8 experiments, MKN-45, a stomach cancer cell line, was incubated with HP for 24 h and IL-8 in the medium was assayed by ELISA. Cranberry suppressed growth of the bacteria only in six of the 27 strains. Meanwhile, it suppressed IL-8 secretion in all the strains. The results may suggest a possible role of cranberry in prevention of stomach cancer by reducing gastric inflammation.



## 1. Introduction

Stomach cancer has been decreasing, but it still is one of the major causes of cancer death in the world and is the most popular cancer in Japan ("GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10," 2010; Malfertheiner et al., 2012). HP infection is recognized to induce chronic inflammation of stomach mucosa and causes various HP-related gastric and non-gastric disorders, such as peptic ulcer, gastric MALToma, idiopathic thrombocytopenic purpura (ITP) and stomach cancer (Cover & Blaser, 2009; Kim, Ruiz, Carroll, & Moss, 2011). For treatment and prevention of such diseases, HP eradication and suppression of chronic inflammation of stomach mucosa is a theoretically ideal solution and has been shown to be effective for treating peptic ulcer diseases (Asaka et al., 2003), early stages of gastric MALToma (Wotherspoon et al., 1993), and about half of chronic adult ITP in Japan (Suzuki et al., 2005) and other countries (Stasi et al., 2009). As for stomach cancer, its relationship with HP is clear (Uemura et al., 2001) but the cancer grows by itself even after eradication of HP; therefore, the role of HP eradication has focused on prevention.

According to the recently published Maastricht IV consensus (Malfertheiner et al., 2012), HP eradication is strongly

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recommended for reducing the risk of stomach cancer development. There was, however, another opinion that HP eradication was recommended for not all the HP infected individuals (Fuccio, Zagari, Minardi, & Bazzoli, 2007). In the patients of endoscopically treated early stomach cancer, who belong to a very high risk group of stomach cancer, the preventive effects of HP eradication on metachronal stomach cancer were clearly shown in a randomized controlled study in Japan (Fukase et al., 2008). However, such attempts against the infectants with no cancer histories did not yield decisive results (Wong et al., 2004), which is also a reference paper to the comment in the consensus. In spite of the consensus, HP eradication of all the infected people is actually not very common depending on the countries, which might be, at least partially, due to rather vague results of the paper (Wong et al., 2004).

The second is antibiotics resistance acquired by HP. Recently, clarithromycin resistant HP has been reported to be increasing and eradication failure of the first line regimen (proton-pump inhibitor, amoxicilin and clarithromycin) has also increased in Japan (Kobayashi et al., 2007), as well as in other countries (Megraud, 2004).

The third is health economics and the health insurance system in Japan. Although HP eradication in the communities with high risk for gastric cancer was cost-effective as described in the Maastricht IV consensus, the costs for the national HP eradication projects were much at least temporary. A common trend among developed countries, population of older people has increased and medical expenses have increased accordingly, making the nation economically worse off. HP eradication of all the infectants is a heavy medical expense, which might be difficult in such a situation, especially after the greater East-Japan Earthquake disaster. Furthermore, the Japanese medical insurance system currently limits coverage of HP eradications to several disorders excluding chronic gastritis, and abolishment of the limitation in the near future seems unlikely.

One possible solution for the problems is anti-HP natural food products such as broccoli sprouts (Galan, Kishan, & Silverman, 2004), green tea (Stoicov, Saffari, & Houghton, 2009), yogurt containing *Lactobacillus* (Sakamoto et al., 2001; Ushiyama et al., 2003) and cranberry (*Vaccinium macrocarpon*) (Matsushima et al., 2008; Xiao & Shi, 2003; Zhang et al., 2005), and studies on such functional food products might be clues for new drugs against HP. We have already reported growth-inhibiting action of cranberry on HP standard strains (Matsushima et al., 2008), but we could not determine whether the anti-HP growth effects of cranberry generally holds in differed HP strains. In this study, we tested the effects using 27 clinically separated HP strains.

HP infection essentially causes chronic inflammation in the stomach mucosa, which is believed to cause several gastric diseases including peptic ulcer and stomach cancer (Kim et al., 2011). Not only growth inhibition of HP but also suppression of gastric inflammation is, therefore, another possible target for prevention or treatment of HP-related disorders. The inflammation is mediated by cytokines secreted from stomach cells and IL-8 is one of the most important mediators (Andersen, 2007). We also tested the inhibiting effects of cranberry on IL-8 secretion from stomach cells using the clinically separated strains in the study.

## 2. Materials and methods

### 2.1. Materials

Cranberry extract powder (Kikkoman Corporation, Tokyo, Japan) was manufactured by mixing cranberry fruit with hot water. The cranberry extract (Lot No. CRE3006) used in the study contained 5.4% total polyphenols and 11.2% total organic acids and was kindly supplied by the manufacturer. Polyphenols in the extract were analyzed as aglycon forms by BML Inc. (Tokyo, Japan), essentially according to the method reported by Sakakibara, Honda, Nakagawa, Ashida, and Kanazawa (2003) and the results are shown in Table 1. Cranberry juice concentrate was supplied by Nippon Del Monte Corporation (Tokyo, Japan).

### 2.2. H. pylori culture

As standard strains, NCTC 11637 and 11638 were obtained from American Type Culture Collections (Manassas, VA,

**Table 1 – Polyphenol analysis of the cranberry extract.**

| Polyphenols | Amount (mg/kg sample) |
|---|---|
| Protocatechuic acid | 286.6 |
| Vanillic acid | 111.0 |
| Caffeic acid | 324.2 |
| Epicatechin | 361.9 |
| Ferulic acid | 126.5 |
| *p*-Coumaric acid | 848.2 |
| Quercetin | 2179.8 |
| Myricetin | 1072.5 |

Detection limit, 0.1 mg/kg sample.

Polyphenols under the detection limits were as follows; Gallic acid; Naringenin; Hesperetin; Apigenin; Eriodictyol; Galangin; Chrysin; Luteolin; Genistein; Daidzein; Glycitein; Fisetin; Kaempferol; *o*-Coumaric acid; Chlorogenic acid; Scopoletin; Catechin; Gallocatechin; Epigallocatechin; Catechin gallate; Epicatechin gallate; Gallocatechin gallate; Epigallocatechingallate; Ellagic acid; Salicylic acid; Syringic acid; Sesamol; Vanillin; Syringaldehyde; Eugenol; 7-Hydroxy coumarin; *p*-Hydroxybenzoic acid; $\alpha$-Resorcylic acid; $\beta$-Resorcylic acid; $\gamma$-Resorcylic acid; Gentisic acid; 2,3-Dihydroxybenzoic acid; *o*-Aminobenzoic acid; *m*-Aminobenzoic acid; *p*-Aminobenzoic acid; Theaflavin-3-gallate; Theaflavin-3′-gallate; Theaflavin-3,3′-gallate.