The Aging/Precancerous Gastric Mucosa

A Pilot Nutraceutical Trial

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ABSTRACT: The aim of this study was to test the effect of antioxidant supplementation on enzymatic abnormalities and free radical–modified DNA adducts associated with premalignant changes in the gastric mucosa of elderly patients with HP-negative atrophic gastritis (CAG). Sixty patients with atrophic gastritis and intestinal metaplasia underwent a nutritional interview and a gastroscopy with multiple biopsy samples in the antrum that were processed for histology and for assaying: alpha-tocopherol, MDA, xanthine oxidase (XO), ornithine decarboxylase (ODC), and 8-OHdG. Patients were randomly allocated into three matched groups and supplemented for 6 months with (1) vitamin E, 300 mg/day; (2) multivitamin, two tablets t.i.d.; and (3) Immun-Age 6 g/day nocte (ORI, Gifu, Japan), a certified fermented papaya preparation with basic science-validated antioxidant/immunomodulant properties. Ten dyspeptic patients served as controls. Histology and biochemistry were blindly repeated at 3 and 6 months. CAG patients showed a significantly ($P < .05$) increased level of mucosal MDA and XO concentration that were reverted to normal by each supplementation ($P < .05$). All supplements caused a significant decrease of ODC ($P < .01$), but Immun-Age yielded the most effective ($P < 0.05$) and was the only one significantly decreasing 8-OhdG ($P < 0.05$). These data suggest that antioxidant supplementation, and, namely, Immun-Age, might be potential chemopreventive agents in HP-eradicated CAG patients and especially in the elderly population.

KEYWORDS: oxidative stress; atrophic gastritis; ODC activity; 8-OhdG; antioxidants

INTRODUCTION

Atrophic gastritis changes are commonly found to be increased with age and with an annual incidence of 1–3%. Although the prevalence of this condition often

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parallels the one of *Helicobacter pylori*, such association does not follow a strictly direct correlation as seen in some long-term studies.[1] Indeed, several other causative factors have been involved such as alcohol, nonsteroidal anti-inflammatory drugs, smoking, biliary reflux, and thermal injury. Moreover, genetic variables in the host may contribute to the susceptibility or resistance to the progression of chronic gastritis to gastric atrophy and, finally, to intestinal-type gastric cancer. Indeed, adenocarcinoma of the stomach is the second most common malignancy in the world, and the principal cause of mortality from cancer in the developing regions of Asia, Africa, and South America. There has been an increasing interest on abnormality in ornithine decarboxilase (ODC) activity in association with clinical premalignant and malignant lesions of the gastrointestinal tract. Evidence for involvement of active oxygen species in the promotion stage of carcinogenesis has been repeatedly reported.[2] Indeed, one of these free radical–modified DNA adducts, 8-hydroxydeoxyguanosine (8-OhdG), has recently received a great deal of attention as a potential biomarker.[3] Indeed, 8-hydroxyguanine is one of the major products of base damage when DNA is exposed to physiologically relevant systems producing OH and $^1 0_2$. The purpose of the current investigation was to test the effect of oral supplementation of a certified papaya-fermented product (Immune-Age FPP; Osato Research Institute, Gifu, Japan), which is endowed by an effective acid- and heat-resistant antioxidant property,[4] on enzymatic abnormalities and free radical–damaged DNA parameters associated with premalignant changes in the upper gastrointestinal mucosa.

MATERIALS AND METHODS

Sixty patients with known atrophic gastritis and intestinal metaplasia and with a recent negative result of urea breath test were selected as our population study group. Each of these patients was carefully interviewed for dietary habit with particular attention to an estimated vitamin intake, alcohol, smoking, and drugs or nutraceutical consumption. In particular, patients were instructed not to consume any vitamin or "health supplement" during the study period. All subjects underwent a routine upper gastrointestinal endoscopy during which multiple biopsy samples were taken in the antrum. Biopsy samples were processed for routine histology with particular attention to intestinal metaplasia and *H. pylori* presence. The other samples were processed to test alpha-tocopherol, malonyldialdehyde, xanthine oxidase, ODC activity, 8OHdG, and for mRNA expression for ODC, COX-2, and gastrin by reverse transcription polymerase chain reaction (RT-PCR).

An overall plasma antioxidant status also was assessed at entry. Patients were divided in three groups comparable for age, gender, drinking, and smoking habit. After overnight fasting, all patients underwent baseline blood chemical evaluation as described below. All subjects were randomly allocated into one of the following 6-month supplementation trial: (1) Immun-Age FPP 6 g/day nocte (FPP, obtained from biofermentation of carica papaya, pennisetum purpureum, and sechium edule; Osato Research Institute, Gifu, Japan); (2) vitamin E 300 IU/day nocte; (3) multivitamin preparation (Supradyn, Roche, Switzerland) two tablets t.i.d. All the above histological and biochemical parameters were repeated at 3 and 6 months. Histological assessment was conducted in a blind fashion and reviewed by one experienced investigator (R.B.).

RESULTS

There was no statistical difference among the groups for dietary composition during the study period. Routine blood chemistry were within the reference range at entry and did not change during the study period, irrespective of the treatment used.

Plasma Oxidant/Antioxidant Status

The baseline oxidant/antioxidant assessment at entry, as measured by the serum level of α-tocopherol, malonyldialdehyde, superoxide dismutase, hydroperoxide, and glutathione peroxidase, was within normal limits, and it did not change irrespective of any of the antioxidant treatments used.

Gastric Mucosal Oxidant/Antioxidant Assessment

As compared with controls, mucosal concentration of MDA and XO in patients with atrophic/metaplastic changes were significantly higher ($P<.05$). Each of the three antioxidant treatments used brought about a normalization of this parameter ($P<.05$) without any significant difference among them.

ODC Activity in Gastric Mucosa

As compared with control dyspeptic subjects, patients with CAG showed a significant increased concentration of ODC in gastric mucosa ($P<.05$). All three supplementations caused a significant improvement of this parameter. However, at 6-month observation, Immune-Age FPP yielded the most significant improvement ($P<.05$ vs. the two other supplements).

8-OhdG Concentration in Gastric Mucosa

Patients with CAG showed a significant increase of 8-0hdG ($P<.05$) which was unrelated to other tested parameters. Such abnormality was not affected either by vitamin E or by multivitamin supplement throughout the study period. However, Immune-Age FPP brought about a significant, although partial, decrease at the end of the study period ($P<.05$).

Gene Expression Study

The expression of COX-2 and ODC was significantly upregulated in the gastric mucosa of patients with atrophic gastritis and, in particular, in 18%, 26%, and 22% in groups treated with Immun-Age FPP, vitamin E, and multivitamin preparation, respectively. However, only the former treatment yielded a significantly reduced expression ($P<.05$ vs. baseline value).

CONCLUSION

Although the incidence of gastric cancer is declining, it remains a common cause of death from malignancy worldwide. Strong epidemiological evidence supports the correlation of age and markers of poverty with both *H. pylori* prevalence and

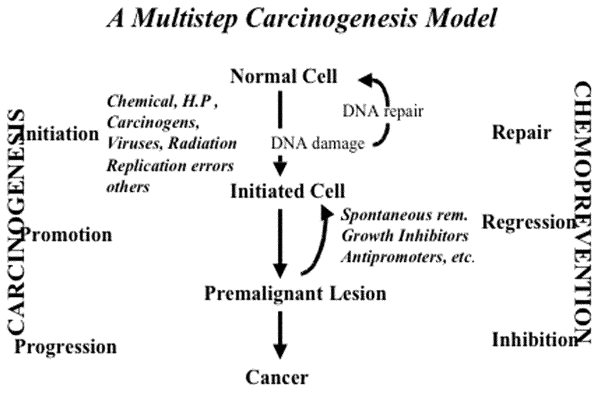

FIGURE 1. An overview on the multistep theory of carcinogenesis. H.P., *H. pylori*.

stomach cancer. There is substantial evidence to support a sequence of histological changes in the mucosa before the development of intestinal-type gastric carcinoma. The development of inflammatory gastritis progresses to gastric atrophy, to intestinal metaplasia, to dysplasia, and finally to intestinal type carcinoma. On the other hand, increased concentrations of lipid peroxidation products have been found in the serum of gastric cancer patients. Oxidative damage to DNA may result in base modification, sugar damage, stand break, and DNA–protein cross-links. Of these, modification of guanine by hydroxyl radicals at the C-8 site, frequently estimated as 8-OhdG, is the most commonly studied lesion. Albeit there have been scanty reports tackling the issue of vitamin/antioxidant supplementation in patients with premalignant gastrointestinal lesions in view of reducing ODC activity,[5] although a lack of a more comprehensive study of the issue still remains. From this study, it appears that patients with CAG, although maintaining a normal plasmatic redox status, show a significant impairment of it at a mucosal level. Virtually any antioxidant supplement[7] seems to improve such an abnormality to a certain extent. However, this is the first study showing that a nutritional intervention with a product endowed with immunomodulator and NO modulator[8] properties associated with lipid[4] and protein[9] antioxidant effect can significantly improve oncological biomarker in the gastric mucosa, something which poses some hopes, given the multistep characteristic of gastric carcinogenesis (FIG. 1). The positive preliminary data thus obtained are all the more encouraging when considering the rather inconclusive results coming from survey trials[6] which are likely to have been biased by the intrinsic limitations involved in any large-scale epidemiological study. This holds particular interest when considering that, although *H. pylori* is now recognized as one of the most widespread human pathogens in the world, it is difficult to eradicate by antibiotic therapy in 15–20% of individuals. Ongoing *in vitro* studies by a French group seem to suggest that a 500-dalton fraction of Immun-Age FPP has the best antioxidant/immunomodulator properties (unpublished data).

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