Inhibition by *Chlorella* of *N*-Methylnitrosourea-induced Aberrant Crypt Foci in Rat Colon*

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Summary. An inhibitory effect of heterotrophically grown *Chlorella regularis* (green algae) against the formation of aberrant crypt foci (ACFs), putative precursors of colon cancer, was evaluated in rats. Six Sprague-Dawley rats in each group received three intrarectal doses of 2 mg *N*-methylnitrosourea in week 1 for inducing ACFs, and were killed at week 6 for quantifying ACFs. Treatment with a daily intragastric dose of 10 mg chlorella powder during weeks 1–5 and weeks 2–5, but not during week 1, resulted in a significant reduction of ACF formation, whereas treatments with a daily dose of 50 mg or 2 mg chlorella, or with 25 mg, 2.5 mg, or 0.25 mg sodium copper chlorophyllin during week 1–5, were less effective. The results support a protective effect for an optimum dose of chlorella against ACF formation and carcinogenesis in the colon, by the potent inhibition of the promotion phase rather than the initiation phase. The inhibition is presumably due to the combined effect of the large amounts of chlorophyll (2.4%) and carotenoids (0.6%) in the chlorella used.

Key Words: *Chlorella*—Chlorophyllin—Colon cancer—Aberrant crypt focus—Cancer chemoprevention

Introduction

It has been hypothesized that the ubiquitous pigment substances of vegetables and fruits, such as carotenoids and chlorophylls, have a protective role against carcinogenesis. Chlorophyll and its derivative chlorophyllin exert profound antimutagenic activities in studies in vitro and in vivo against a wide range of carcinogens [1–7]. The mechanisms which have been postulated include tight complex formation with carcinogen, scavenging of active oxygen species, and interference with metabolic activation of carcinogen. It has been demonstrated that colonic aberrant crypt foci (ACFs), which consist of one or more aberrant crypts (ACs) and can be quantitated on the surface of the unsectioned and whole-mounted normal-appearing colon mucosa under a light microscope, are putative precursors of colon cancer in animals and humans [8,9]. The formation of carcinogen-induced ACFs can be enhanced and inhibited by known tumor-promoting and -inhibiting substances, respectively, in animal models [10]. These findings have led to the proposed use of ACFs as a sensitive intermediate biomarker of colon cancer for short-term assay of carcinogenic and anticarcinogenic compounds.

The present study was designed to examine the protective effect of a powder of chlorella (heterotrophically grown green algae *Chlorella regularis*), rich in naturally occurring chlorophyll, against the induction of ACFs by the carcinogen *N*-methylnitrosourea (MNU) in the rat colon. Also, the effect of sodium copper chlorophyllin (Cu-CHL) was tested for comparison.

Materials and Methods

Female Sprague-Dawley rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan), seven weeks of age at the start of experiments, were used. They were housed in plastic cages with sterilized woodchip bedding in a specific-pathogen-free animal room under constant environmental conditions, with free access to standard laboratory chow CE-2 (CLEA, Tokyo, Japan) and drinking water. All rats in three separate experiments received three intrarectal instillations of 2 mg MNU dissolved in 0.5 ml of distilled water in week 1, using a metal feeding tube inserted into the colon lumen through the anal orifice. They were given a daily intragastric gavage of the indicated dose of chlorella (Nihon Chlorella Co., Tokyo, Japan) or Cu-CHL (Nakarai Tesque, Kyoto, Japan) suspended or dissolved in 0.2 ml of distilled water with a metal feeding tube during the indicated period. All six rats in each group were sacrificed under CO_2 euthanasia at week 6, when the colon was excised, cut open along its length, stretched and flattened on filter paper, and fixed in 4% neutral formalin solution. Thereafter, the colon was stained with 0.2% methylene blue solution for 10 min, and the number of ACFs and the total number of ACs were counted under a light microscope by two of the authors.

The presence of an AC was identified by an enlarged crypt opening, thicker surface epithelial cell lining, and widened

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