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Original Research

Dietary psyllium fiber increases intestinal heat shock protein 25 expression in mice

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ABSTRACT

Heat shock proteins (HSPs) protect intestinal epithelial cell function, integrity and viability against many forms of stress. We hypothesized that dietary fibers (DFs) in the diet may increase HSP expression, since DFs are known to exhibit beneficial effects on intestinal health. The present study investigated the regulation of intestinal HSP expression by DFs, particularly psyllium fiber. Feeding psyllium fiber for 5 d increased HSP25 expression, but not HSP32 and HSP70 expression in the jejunum, ileum, and colon of mice at both the protein and mRNA levels. The increases in HSP25 expression did not correlate with cecal organic acid production by microbial fermentation. The water-insoluble fraction of psyllium fiber largely contributed to the induction of HSP25 expression, but feeding with other water-insoluble DFs from beet, wheat, and oats failed to induce intestinal HSP25 expression. Although the water-holding capacity of psyllium fiber was much higher than those of the other water-insoluble DFs examined, the increase in HSP25 expression induced by feeding polycarbophil, which possesses a high water-holding capacity similar to that of psyllium fiber, was much lower than that induced by psyllium fiber. Finally, induction of malondialdehyde production by hydrogen peroxide, an oxidant, in the colon of mice fed psyllium fiber was lower than that in mice fed with the control diets. Taken together, feeding psyllium fiber, especially the water-insoluble fraction, increases intestinal HSP25 expression and suppresses oxidant-induced malondialdehyde production.

1. Introduction

Intestinal epithelial cells line the luminal surface of the intestinal mucosa and have several functions essential for the maintenance of intestinal homeostasis. Among these functions, the intestinal barrier restricts the permeation of noxious substances including pathogens, toxins, and allergens present in the lumen [1]. Thus, the protection and maintenance of the intestinal barrier are essential for good health. Impairment of the barrier resulting in the permeation of noxious substances induces chronic and robust activation of intestinal immune cells and is involved in the pathogenesis of different intestinal disorders, such as inflammatory bowel diseases and irritable bowel syndrome. The intestinal barrier is organized by different barrier components, and the expression of heat shock proteins (HSPs) represents a major mechanism for the protection of intestinal epithelial cell function, integrity, and viability against many forms of stress [2].

Abbreviations: AU, arbitrary units; DF, dietary fiber; GGH, guar gum hydrolysate; HSF, heat shock factor; HSP, heat shock protein; IDF, water-insoluble dietary fiber; SDF, water-soluble dietary fiber; TLR, toll-like receptor; WHC, water-holding capacity.

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HSPs are involved in a wide range of physiological cellular processes and are particularly known for their ability to help cells survive under stress [3]. The mechanisms underlying their actions appear to be diverse, and are associated with the stabilization of critical cellular components and processes including cytoskeletal function, antioxidative activity, mitochondrial function, and apoptosis inhibition. Interestingly, stress response may be regulated in an organ- and cell-specific manner. For example, in intestinal epithelial cells HSP25 (the mouse homologue of human HSP27), 32, and 70 reportedly confer mucosal protection against a number of injurious agents and processes among the different HSPs [4-6]. Thus, much attention has been given to food factors and nutrients that enhance intestinal HSPs, thereby resulting in increased resistance to injurious stresses. Furthermore, recent studies have demonstrated that intestinal microbiota components such as lipopolysaccharides and metabolites such as short-chain fatty acids can regulate intestinal HSP25 and 70 expression [5,7]. Therefore, we hypothesized that supplemental dietary fibers (DFs), which are known to affect intestinal microbiota as well as intestinal functions, may effectively enhance intestinal HSP expression.

DFs exhibit different physiological functions in intestinal epithelial cells depending on their physicochemical properties, and are often divided into water-soluble and water-insoluble fibers (SDFs and IDFs, respectively) [8]. The viscosity of SDFs is determined by the degree of polymerization and molecular weight; SDFs with a higher molecular weight provide a more highly viscous solution. Many SDFs are readily fermented by intestinal microbes to produce organic acids, including short chain fatty acids (SCFAs). One of the major physiochemical properties of IDFs is water-holding capacity (WHC), and IDFs with a highly branched structure appear to hold more water molecules within their bodies [9]. This study focused on several DFs with different physiochemical properties, particularly on a fiber isolated from the psyllium seed husk. Psyllium fiber contains arabinoxylan, and consists of both SDF and IDF [10]. This fiber is known to be mildly fermented by intestinal microbes and it shows high WHC. Previous studies have demonstrated that it provides some physiological effects, including hypocholesterolemic action and an anti-constipation effect [11,12].

Thus, food factors such as DFs that have a potential to regulate intestinal HSP expression could be developed into novel functional foods. The present study investigated the regulation of intestinal HSP expression by the supplemental feeding of DFs in mice. In our first experiment (Expt 1), we examined the effects of 4 DFs with different physiochemical properties on intestinal HSP (HSP25, 32 and 70) expression to determine which DFs had the potential to induce intestinal HSP. Since we found that feeding psyllium fiber increased intestinal HSP25 expression, we then examined the effective dietary levels of psyllium fiber (Expt 2). Based on the complex components in psyllium fiber, we examined which fraction in psyllium fiber was responsible for the increased HSP25 expression, water-soluble or -insoluble fractions (Expt 3). Since the results of Expt 3 showed that the water-insoluble fraction of psyllium fiber contributes to the regulation of intestinal HSP25 expression, we then compared the effects of 4 IDFs, including psyllium fiber, on intestinal HSP25 expression (Expt 4). Psyllium fiber has a high WHC, and the WHC of IDFs is often responsible for their physiological effects. Thus, we examined the contribution of WHC to the psyllium-mediated HSP25 induction using polycarbophil calcium, a water-absorbing polymer (Expt 5). Finally, we hypothesized that feeding psyllium fiber protects the intestines from oxidative stress, since HSP25 is known to exhibit anti-oxidative effects in cells. We examined the hydrogen peroxide-induced production of malondialdehyde, an end product of fatty acid peroxidation, in intestinal segments from mice fed with psyllium fiber (Expt 6).

2. Methods and materials

2.1. Chemicals

Psyllium fiber was kindly provided by Bizen Chemical Co, Ltd (Akaiwa, Okayama, Japan). Guar gum and guar gum hydrolysate (GGH, Sunfiber®) fiber were kindly provided by Taiyo Kagaku (Yokkaichi, Mie, Japan). GGH was developed by controlled partial enzymatic hydrolysis of GG and presents lower molecular weight and viscosity. Cellulose fiber (Just Fiber®) was purchased from International Fiber (North Tonawanda, NY, USA). Beet fiber was kindly provided by Nippon Beet Sugar Manufacturing Co, Ltd (Obihiro, Hokkaido, Japan). Oat and wheat fibers (Vitacel® HF600 and WF600, respectively) were kindly provided by Fi Nutrition Ltd (Chiyoda-ku, Tokyo, Japan). Rabbit anti-HSP25, HSP32, and HSP70 were purchased from Enzo Life Sciences (Farmingdale, NY, USA). Mouse anti-β-actin and HRP-conjugated anti-mouse and -rabbit IgG were purchased from Sigma (St. Louis, MO, USA). All other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Animals and experimental design

All animal studies were preapproved by the Hiroshima University Animal Committee, and the mice were maintained in accordance with the Hiroshima University guidelines for the care and use of laboratory animals.

Male ICR mice (7-week-old, Japan SLC, Inc. Hamamatsu, Shizuoka, Japan) were housed in cages in a room with controlled temperature (22 ± 2°C), relative humidity (40%-60%), and lighting (light 08:00 to 20:00 h) throughout the study. The mice were allowed to acclimate to the laboratory environment with free access to a fiber-free AIN-93G formula diet (control diet, Table 1) [13] and distilled water for 1 week. In all experiments, mice were euthanized by exsanguination under isoflurane anesthesia for the sample collections.

Expt 1 examined the effects of 4 DFs with different physiochemical properties on intestinal HSP expression in mice. Mice (n = 30) were randomly divided into the following 5 groups: fiber-free, cellulose, guar gum, GGH, and psyllium groups (n = 6 per each group). The fiber-free group was fed the control diet (Table 1). The other 4 groups were fed diets containing 10% cellulose, guar gum, GGH, and psyllium fibers by weight, respectively. The fiber was added to the control diet by substitution for an equal amount of starch. Five days after the start of feeding, the jejunum (region after the ligament of Treitz, 10 mm in length), ileum (region before ileocecal