RESEARCH ARTICLE

Reduction of Intestinal Polyp Formation in Min Mice Fed a High-Fat Diet with *Aloe Vera* Gel Extract

Takeshi Chihara[1], Kan Shimpo[1]*, Hidehiko Beppu[1], Akiko Tomatsu[1], Takaaki Kaneko[1], Miyuki Tanaka[2], Muneo Yamada[2], Fumiaki Abe[2], Shigeru Sonoda[1]

Abstract

   Aloe vera gel supercritical CO_2 extract (AVGE) has been shown to contain five phytosterols, reduce visceral fat accumulation, and influence the metabolism of glucose and lipids in animal model experiments. Recent epidemiologic studies have shown that obesity is an established risk factor for several cancers including colorectal cancer. Therefore, we examined the effects of AVGE on intestinal polyp formation in *Apc*-deficient Min mice fed a high-fat diet. Male Min mice were divided into normal diet (ND), high fat diet (HFD), low dose AVGE (HFD+LAVGE) and high dose AVGE (HFD+HAVGE) groups. The ND group received AIN-93G diet and the latter 3 groups were given modified high-fat AIN-93G diet (HFD) for 7 weeks. AVGE was suspended in 0.5% carboxymethyl cellulose (CMC) and administered orally to mice in HFD+LAVGE and HFD+HAVGE groups every day (except on Sunday) for 7 weeks at a dose of 3.75 and 12.5 mg/kg body weight, respectively. ND and HFD groups received 0.5% CMC alone. Between weeks 4 and 7, body weights in the HFD and HFD+LAVGE groups were reduced more than those in the ND group. However, body weights were not reduced in the HFD+HAVGE group. Mice were sacrificed at the end of the experiment and their intestines were scored for polyps. No significant differences were observed in either the incidence and multiplicity of intestinal polyps (≥0.5 mm in a diameter) among the three groups fed HFD. However, when intestinal polyps were categorized by their size into 0.5-1.4, 1.5-2.4, or ≥2.5 mm, the incidence and multiplicity of large polyps (≥2.5 mm) in the intestine in the HFD+HAVGE group were significantly lower than those in the HFD group. We measured plasma lipid (triglycerides and total cholesterol) and adipocytokine [interleukin-6 and high molecular weight (HMW) adiponectin] levels as possible indicators of mechanisms of inhibition. The results showed that HMW adiponectin levels in the HFD group were significantly lower than those in the ND group. However, the levels in the HFD+HAVGE group were significantly higher than those in the HFD group. These results indicate that HAVGE reduced large-sized intestinal polyps and ameliorated reduction in plasma HMW adiponectin levels in Min mice fed HFD.

Keywords: *Apc*-deficient Min mice - *Aloe vera* gel extract (AVGE) - intestinal polyp formation.

Introduction

   Aloe barbadensis Miller (*Aloe vera*) is a plant belonging to the family Liliaceae. *Aloe vera* gel, obtained from the inner thin-walled parenchyma cells, has been used as a health food and to treat burns and other wounds. Additionally, extracts from this gel showed preventive effects against insulin resistance in mice (Pérez et al., 2007), the anti-hyperglycemic effect in type 2 diabetic model mice (Tanaka et al., 2006), and lipid-lowering effect in rats (Rajasekaran et al., 2006). The gel contains about 98.5% water (Parks and Rowe, 1941) and its major components are polysaccharides, amino acids, lipids, sterols, tannins, and enzymes (Shelton, 1991; Vogler and Ernst, 1999). Tanaka et al. (2006) also reported that the anti-diabetic compounds were identified as five phytosterols (lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartanol, and 24-methylene-cycloartanol). Tanaka et al. (2012) recently produced an *Aloe vera* gel extract (AVGE) using a new procedure with supercritical carbon dioxide fluid. AVGE was also shown to contain phytosterols, which are a part of the active ingredient in terms of antihyperglycemia, antihyperlipidemia effects and the reduction in visceral fat accumulation in animal model experiments (Misawa et al., 2008). Furthermore, Nomaguchi et al. (2011) reported that lophenol and cycloartanol, two kinds of phytosterols, acted as ligands of peroxisome proliferator activated receptor (PPAR) α and γ, which regulate the metabolism of glucose and lipids in diet-induced obesity mice. In addition, Yamada et al. (2011) demonstrated that the ingestion of AVGE contributed to the reducing effects of serum alanine aminotransferase (ALT) and γ-glutamyl transpeptidase (GTP) levels in obese men with abnormal serum ALT

[1]*Fujita Memorial Nanakuri Institute, Fujita Health University,* [2]*Functional Food Research Department, Food Science and Technology Institute, Morinaga Milk Industry Co., Ltd, Japan *For correspondence: shimpo@fujita-hu.ac.jp*

values.

Recent epidemiologic studies (Bianchini et al., 2002; Larsson and Wolk, 2007) have shown that obesity is an established risk factor for several cancers including colorectal cancer. Adipose tissue produces and secretes adipocytokines, which include several bioactive substances (Berg et al., 2002). Adiponectin, which is one of these substances, produces diverse biological effects including anti-inflammatory properties (Okamoto et al., 2006). Additionally, serum levels of adiponectin were shown to be reduced in obesity (Arita et al., 1999). Moreover, it has been suggested that visceral fat accumulation and a low plasma adiponectin level may promote colorectal carcinogenesis (Otake et al., 2005; Takahashi et al., 2009). Meanwhile, Misawa et al. (2012a) recently reported that oral administration with lophenol or cycloartanol, which are included in AVGE, ameliorated serum adiponectin concentrations in Zucker diabetic fatty rats.

The *Apc*-deficient Min (multiple intestinal neoplasia) mouse is a model for human familial adenomatous polyposis (FAP) in which many tumors are characteristically found in the intestinal tract within three months (Moser et al., 1990). In this mouse, serum triglyceride levels are markedly higher (10-fold) than those in the wild-type mouse. Hyperlipidemia may be associated with intestinal polyp development (Niho et. al., 2003). Moreover, mRNA levels of lipoprotein lipase (LPL), which catalyzes the hydrolysis of triglycerides in lipoprotein particles into fatty acids and monoacylglycerol, were clearly lower in the liver and small intestine (Niho et al., 2003). Niho et al. (2005) also reported that the selective inducer LPL suppressed both hyperlipidemia and intestinal polyp formation in Min mice. Furthermore, Baltgalvis et al. (2009) reported that a high-fat diet increased the number of polyps observed in Min mice. High-fat diets may influence the immune system. Fenton et al. (2005) showed that leptin, which is one of the adipocytokines, induces colonic epithelial cell proliferation in Min mice. Additionally, they demonstrated that adiponectin blocked cell proliferation (Fenton et al., 2008).

In this study, we examined the effects of AVGE on intestinal polyp formation in Min mice fed a high-fat diet. Additionally, we measured plasma lipid and adipocytokine levels.

Materials and Methods

AVGE dissolved in 0.5% carboxymethyl cellulose (CMC) solution was provided by Morinaga Milk Industry Co. Ltd. (Kanagawa, Japan). Male C57BL/6J-*Apc*^Min/+ mice (Min mice) were originally purchased from Jackson Laboratories (Bar Harbor, ME, USA) and were bred with female C57BL/6J-*Apc*^+/+ mice purchased from Charles River Japan, Inc. (Tokyo, Japan). The presence of the mutant *Apc* allele was detected in DNA from the tail using an allele-specific PCR assay as described by Jacoby et al. (1996). These mice were maintained under the management of laboratory animals in Fujita Health University, Fujita Memorial Nanakuri Institute. They were kept in groups of one or two in plastic cages on woodchip bedding and were fed the normal diet AIN-

93G (Oriental Yeast Co. Ltd., Tokyo, Japan) in an animal facility controlled at a temperature of 23±5°C, 60±5% humidity, and with 12-h light/dark cycle. The care and use for the animals was according to the 'Guidelines for the Management of Laboratory Animals in Fujita Health University' and the experimental protocols were approved by the Institutional Animal Care and Use Committee of Fujita Health University.

Experimental design

Experiments were performed as follows. Five-week-old male Min mice were divided into normal diet (ND), high fat diet (HFD), low dose AVGE (HFD+LAVGE) and high dose AVGE (HFD+HAVGE) groups. ND group was given an AIN-93G diet and the latter 3 groups were given modified high-fat AIN-93G diets (HFD; Oriental Yeast Co. Ltd., Tokyo, Japan) *ad libitum* for 7 weeks. AVGE was suspended in 0.5% carboxymethyl cellulose (CMC) and administered orally to mice in HFD+LAVGE and HFD+HAVGE groups every day (except on Sunday) for 7 weeks at a dose of 3.75 and 12.5 mg/kg body weight, respectively. ND and HFD groups received 0.5% CMC alone at a dose of 25 ml/kg. Mice were observed and food intake was measured daily. At the end of the experiment, all mice were anesthetized with Nembutal, exsanguinated via the heart into heparin-coated syringes, and carefully autopsied. After sacrifice, the small intestine and large intestine were removed from each mouse.

Intestinal polyp count and size comparison

The number and size of polyps were determined according to the procedure described in Ushida et al. (1998). Briefly, the entire intestine was flushed with saline and cut longitudinally. It was then spread on filter paper with the lumen side up and fixed in 10% neutral buffered formalin. Thereafter, we scored the number and size of polyps and categorized them into 0.5-1.4, 1.5-2.4, or more than 2.5 mm in diameter. We evaluated these by dividing the intestine into 3 parts: the small intestine, large intestine, and total (small+large) intestine.

Determination of plasma lipid levels

Plasma levels of triglycerides (TG) and total cholesterol (T-Cho) were enzymatically measured with Triglyceride E-Test Wako and Cholesterol E-test Wako kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively.

Determination of plasma adipocytokine levels

Plasma interleukin (IL)-6 (eBioscience Inc., San Diego, CA, USA) and high molecular weight (HMW) adiponectin (Shibayagi Co., Ltd., Gunma, Japan) levels were determined by enzyme-linked immunosorbent assay (ELISA) kits, according to the manufacturer's protocol.

Statistical analysis

Values are expressed as the mean±SE. The unpaired *t*-test was used to compare the ND group with the HFD group. Statistical analyses of the total number of large polyps and plasma HMW adiponectin levels were performed by one-way analysis of variance (ANOVA)

followed by the Dunnett's multiple comparisons test. Changes in body weight was compared with the Kruskal-Wallis test (nonparametric ANOVA) followed by the Dunn's multiple comparisons test. The incidence of large polyps was analyzed Fischer's exact test. These procedures were performed using Instat version 3.0 for Windows (GraphPad Software, Inc., San Diego, CA, USA). Differences were considered to be significant at p<0.05.

Results

Changes in body weight and food consumption

Body weights in the HFD and HFD+LAVGE groups were reduced more than those in the ND group from 28 days after the start of the experiment (Figure 1). However, body weights were not reduced in the HFD+HAVGE group and were significantly higher than those in the HFD group on days 31 and 38. HAVGE inhibited the weight loss associated with increasing polyp size.

Food consumption was not different among the three groups fed HFD (data not shown).

Number and size of polyps

As shown in Figure 2, the total number of polyps (≥0.5 mm in a diameter) in the small intestine was significantly higher in the HFD group than that in the ND group. However, the total number of polyps in the HFD+LAVGE and HFD+HAVGE groups was not lower than that in the HFD group. No significant differences were observed in the large intestine among the four groups. The total number of polyps in the total intestine (small and large intestine) in the HFD group was significantly higher than that in the ND group, while that in the HFD+LAVGE and HFD+HAVGE groups was similar to the HFD group.

As shown in Table 1, the incidence of large polyps (≥2.5 mm in a diameter) in the small intestine in the HFD+HAVGE group was significantly lower than that in the HFD group. However, no significant differences were observed in the large and total intestine among the four groups. As shown in Figure 3, the total number of large polyps in the small intestine was significantly higher in the HFD group than that in the ND group. The total number of large polyps in the HFD+HAVGE group only was significantly lower than that in the HFD group, while no significant differences were observed in the large intestine among the four groups. In the total intestine, the total number of large polyps in the HFD group was not significantly higher than that in the ND group, while that in the HFD+HAVGE group was significantly lower than

Table 1. Incidence of Large Polyps (≥2.5 mm in diameter)

Organ Group	Small intestine		Large intestine		Toatal intestine	
ND	11/15	73.3%	13/15	86.7%	14/15	93.3%
HFD	15/16	93.8%	9/16	56.3%	15/16	93.8%
HFD+LAVGE	13/15	86.7%	10/15	66.7%	13/15	86.7%
HFD+HAVGE	8/16	50.0%*	9/16	56.3%	11/16	68.8%

*The HFD+HAVGE group was significantly different from the HFD group at p<0.01 (Fischer's exact test)

that in the ND group.

Plasma TG and T-Cho levels

Plasma TG levels in the HFD group were 365.2±68.9 mg/dL and those in the ND group were 158.3±24.4. The difference was statistically significant. However, those in the HFD+LAVGE group were 468.8±61.6 and those in the HFD+HAVGE group were 406.8±78.8, no significant differences were observed between the HFD+LAVGE group and the HFD+HAVGE group and the HFD group.

Plasma T-Cho levels in the ND group were 117.4±3.7 mg/dL and those in the HFD group were 112.9±5.9 and those in the HFD+LAVGE group were 113.6±4.9 and those in the HFD+HAVGE group were 117.2±4.3. Those levels were not changed among the four groups.

Plasma IL-6 and HMW adiponectin levels

Plasma IL-6 levels in the ND group were 4.07±0.72 pg/ml and those in the HFD group were 5.58±062 and those in the HFD+LAVGE group were 7.49±1.06 and those in the HFD+HAVGE group were 5.57±0.90. Those levels in the HFD group were slightly higher than those in the ND group, whereas those in the HFD+LAVGE and HFD+HAVGE groups were not lower than those in the HFD group.

As shown in Figure 4, HMW adiponectin levels in the HFD group were significantly lower than those in the ND group. On the other hand, the levels in the HFD+HAVGE group were significantly higher (p<0.05) and those in the HFD+LAVGE group were slightly, but not significantly, higher than those in the HFD group. Furthermore, we confirmed that there was a negative correlation between
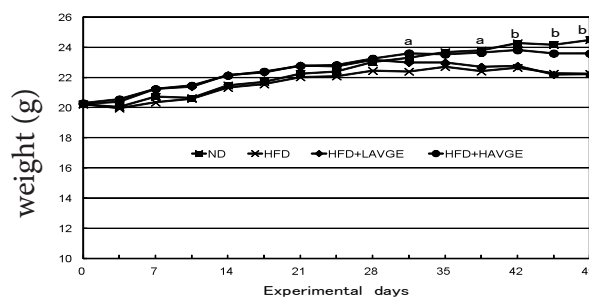
Figure 1. Changes in Body Weight. [a]The HFD+HAVGE group was significantly different from the HFD group at p<0.05 (Dunn's Multiple Comparisons test), [b]The ND group was significantly different from the HFD group at p<0.05 (unpaired t-test)

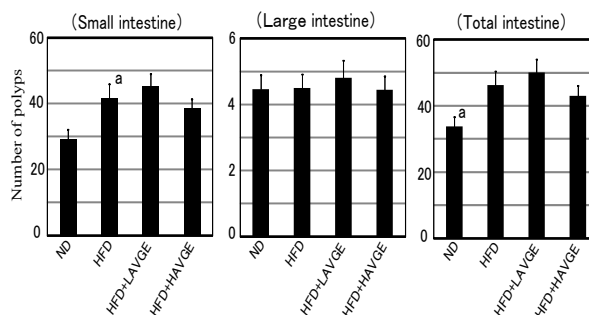**Figure 2. Multiplicity of Total Polyps (≥0.5 mm in diameter).** [a]The HFD group was significantly different from the ND group at p<0.05 (unpaired t-test)
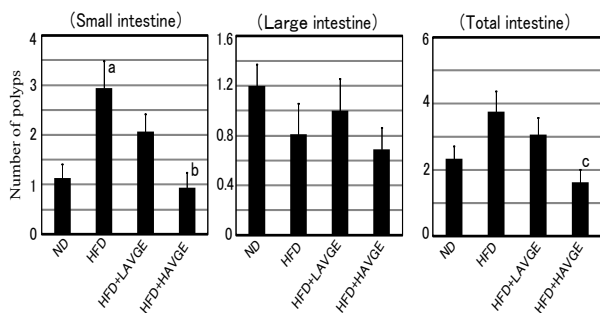
Figure 3. Multiplicity of Large Polyps (≥2.5 mm in diameter). [a]The HFD group was significantly different from the ND group at p<0.01 (unpaired *t*-test). [b]The HFD+HAVGE group was significantly different from the HFD group at p<0.01 (Dunnett's Multiple Comparisons test). [c]The HFD+HAVGE group was significantly different from the HFD group at p<0.05 (Dunnett's Multiple Comparisons test)
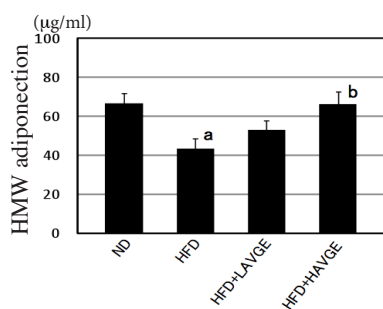

Figure 4. Plasma HMW Adiponectin Levels. [a]The HFD group was significantly different from the ND group at p<0.01 (unpaired t-test). [b]The HFD+HAVGE group was significantly different from the HFD group at p<0.05 (Dunnett's Multiple Comparisons test)

the number of total intestinal large polyps (≥2.5 mm in a diameter) and HMW adiponectin levels in the HFD group (r=-0.523, p=0.04). However, the measured value was higher than that of the reference value (Chung et. al., 2011), even though the cause is not known at this time. HMW adiponectin is thought to be involved in reducing large-sized intestinal polyps.

Discussion

Serum TG levels have been shown to be markedly increased, whereas LPL mRNA levels in the liver and small intestine were markedly decreased in Min mice (Niho et al., 2003). Therefore, the suppression of serum lipid levels by an increase in LPL activity may lead to a reduction in intestinal polyp formation (Niho et al., 2005). Niho et al. (2005) demonstrated that NO-1886 {4-[(4-bromo-2-cyanophenyl)carbamoyl]benzylphosphonate}, an LPL selective inducer, suppressed intestinal polyp formation in Min mice. In this study, we showed that the incidence and total number of large polyps (≥2.5 mm in a diameter) in the HFD+HAVGE group were significantly lower than those in the HFD group. However, plasma TG and T-Cho levels remained unchanged among the three groups (HFD, HFD+LAVGE, and HFD+HAVGE groups). Misawa et al. (2008; 2012a) and Nomaguchi et al. (2011) showed that the serum TG levels in obesity-induced and diabetic model animals fed HFD were reduced by the treatment

of lophenol and cycloartanol, which were contained in AVGE. Furthermore, Misawa et al. (2012b) reported that the TG levels were decreased by the administration of *Aloe vera* gel powder. However, in this experiment, AVGE did not improved the TG levels. LPL mRNA expression levels in the liver also remained unchanged (data not shown). We speculate that the differences in the TG levels depend mainly on the type of animal model.

IL-6, one of the adipocytokines, was reported to be associates with cell growth, differentiation, development, and survival through the activation of activators of transcription (STAT-3) (Battle and Frank, 2002). Fenton et al. (2006) showed that IL-6 was one such indirect mechanism of tumor growth. Steiner et al. (2003) and Brozek et al. (2005) also demonstrated that IL-6 was a potent growth factor in prostate cancer and colon tumor cells, respectively. However, no significant differences in IL-6 levels were observed in the three groups in this study.

Adiponectin, a 30-kDA protein, is secreted by adipose tissue and circulates at much higher concentrations in the blood than other hormones and cytokines (Whitehead et al., 2006). Adiponectin exists in the plasma in characteristic oligomeric complexes: a low-molecular weight (LMW) trimer, a middle-molecular weight (MMW) hexamer, and a high-molecular weight (HMW) multimer adiponectin (Pajvani et al., 2003). Biologically active adiponectin was shown to be HMW in several reports (Waki et al., 2003; Pajvani et al., 2004). Recent studies have shown that the absolute amount of HMW adiponectin rather than total adiponectin levels correlated better with weight reductions (Bobbert et al., 2005), the severity of vascular complications associated with obesity-associated disease (Hara et al., 2007), and metabolic syndrome (Seino et al., 2007). Therefore, we analyzed plasma HMW adiponectin levels. The results of the present study showed that these levels significantly inhibited the decrease by HAVGE. LAVGE also slightly inhibited that decrease. Misawa et al. (2012a) also reported that the serum HMW adiponectin concentration in Zucker diabetic fatty rats fed HFD were slightly increased by 44 consecutive days oral administration of lophenol and cycloartanol. Recent findings showed that azoxymethane-induced colorectal carcinogenesis under HFD conditions was enhanced in adiponectin-deficient mice (Fujisawa et al., 2008). They demonstrated that adiponectin suppressed colonic epithelial cell proliferation by inhibiting the mammalian target of the rapamycin pathway under that condition as a part of the mechanism. Mutoh et al. (2011) also showed that adiponectin-deficient Min mice had higher total numbers of polyps in the intestines. They demonstrated that hypoadiponectinemia promoted intestinal polyp development in Min mice, the phosphorylation of AMP-activated protein kinase was decreased in intestinal epithelial cells, and plasminogen activator inhibitor-1 (Pai-1) levels in the serum were increased in Min mice. Furthermore, Otani et al. (2010) reported that the exogenous administration of adiponectin suppressed the growth of intestinal adenomas in Min mice. By combining our results with the above findings, HMW adiponectin is thought to be involved in reducing large-sized intestinal polyps. Because of that, AVGE may

ameliorate plasma HMW adiponectin levels in Min mice fed HFD. Furthermore, the major components regulated by AVGE may be phytosterols.

In conclusion, HAVGE reduced large-sized intestinal polyps and ameliorated plasma HMW adiponectin levels in Min mice fed HFD. Recent findings showed that azoxymethane-induced colorectal carcinogenesis under HFD conditions was enhanced in adiponectin-deficient mice, and the exogenous administration of adiponectin suppressed the growth of intestinal adenomas in Min mice. Therefore, AVGE may regulate plasma HMW adiponectin levels in Min mice fed HFD. We are now continuing to clarify the precise mechanism of this suppression.

Acknowledgements

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