

RESEARCH COMMUNICATION

Effects of *Aloe arborescens* Ingestion on Azoxymethane-induced Intestinal Carcinogenesis and Hematological and Biochemical Parameters of Male F344 Rats

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Abstract

We examined the modifying effect of freeze-dried whole-leaf *Aloe arborescens* Miller var. *natalensis* Berger (Kidachi aloe in Japan; designated as 'ALOE') on azoxymethane (AOM)-induced intestinal carcinogenesis in rats. Male F344 rats (4 weeks old) were fed basal diet or experimental diet containing 0.2% or 1% ALOE for 28 weeks. Starting two weeks later, the animals received subcutaneous injections of AOM once weekly for 10 weeks. The incidence of colorectal adenocarcinomas in the 0.2% (but not 1%) ALOE group showed a strong tendency for decrease ($p = 0.056$) from the control group. Further, the adenocarcinoma incidence in the entire intestine (small and large intestines) in the 0.2% ALOE group was significantly ($p = 0.024$) decreased compared to the control value. However, there were no significant differences in tumor multiplicities of colorectal or entire intestines among the 3 groups. In addition, we also studied the safety of long-term ingestion of ALOE as a health food or natural thickening stabilizer. Rats were fed the basal diet or 1% ALOE diet for 35 weeks without AOM treatment. Feeding with 1% ALOE did not affect most hematological and serum biochemical parameters in the rats. These results indicate that a low level of ALOE ingestion might have a mild suppressive effect on intestinal tumor growth without harmful side effects.

Key Words: Intestinal tumors - azoxymethane - hematology - biochemistry - *Aloe arborescens* Miller var. *natalensis* Berger
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Introduction

The leaves of *Aloe arborescens* Miller var. *natalensis* Berger (*Liliaceae*) (Japanese name Kidachi aloe) are widely used in Japan, not only as a traditional remedy for gastrointestinal complaints, skin injury and burns, but also as an ingredient in health foods (Beppu et al., 2003a; Yagi, 2004). Aloe extract product from the leaves of *Aloe arborescens* is also used as a natural thickening stabilizer in Japan (Iso et al., 2005). *Aloe arborescens* and many other Aloe species contain barbaloin (= aloin) as one of the major components (Groom and Reynolds, 1987). Barbaloin, an anthrone C-glucoside, is known to have potent purgative action in humans but shows weak activity in experimental animals (Ishii et al., 1981; Hattori et al., 1988).

Chronic abuse of anthranoid-containing laxatives including aloe has been suggested to be a risk factor for the induction of colorectal tumors (Siegers, 1992; Siegers et al., 1993a; Van Gorkom et al., 1999). However, we previously demonstrated that dietary freeze-dried whole-leaf *Aloe arborescens* (designated as 'ALOE') inhibits the formation

of azoxymethane (AOM)-induced aberrant crypt foci (ACF; early lesions in colorectal carcinogenesis) in the rat colorectum (Shimpo et al., 2001a). Most recently, we also found that ALOE reduces DMH-induced colorectal proliferative lesions, especially adenomatous hyperplasia, in mice (Shimpo et al., 2003c).

This study was therefore designed to examine the modifying effect of ALOE on AOM-induced intestinal carcinogenesis in rats. Additionally, we also tested the safety of the long-term ingestion of ALOE, as a health food or natural thickening stabilizer, in rats treated without AOM.

Materials and Methods

Materials

Azoxymethane(A-9517; AOM) was purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan). All other chemicals were of the highest grade available and were obtained commercially. ALOE was kindly provided by Yurika Co. Ltd. (Tsu, Japan). The phenolic compounds of ALOE used in this experiment were analyzed by high-

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performance liquid chromatography as described previously (Kuzuya et al., 2001). The contents (mg/g dry weight) were: aloenin, 15.9; barbaloin (aloin A), 10.9; isobarbaloin (aloin B), 5.9; and aloin (aloin A + B), 16.8.

Animals and diets

Male F344 rats (3 weeks old) were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were kept in groups of two or three in plastic cages on woodchip bedding and fed a basal diet, Oriental MF diet (Oriental Yeast Co. Ltd., Tokyo, Japan), in an animal facility with a controlled temperature of 23±5°C, 60±5% humidity, and with a 12 h light/dark cycle. ALOE was finely pulverized with an Oster Power Blender (Osaka Chemical Co., Ltd., Osaka, Japan), added to the basal diet at 1 or 0.2%, and thoroughly mixed using a ball mill. All rats were fed ad libitum with the basal diet, 1 or 0.2% ALOE diet and tap drinking water. The care and use of the animals were according to the ‘Guidelines for the Care and Use of Laboratory Animals’ of Fujita Health University.

Experimental protocol

The experimental design of Experiments 1 and 2 is shown in Figure 1. Experiment 1 for AOM-induced intestinal carcinogenesis was performed as described by Ohkami et al (1995). After acclimatization for 1 week, rats in Groups 2 and 3 were fed for 28 weeks on diets containing 1% and 0.2% ALOE, respectively. Rats in group 1 were fed the basal diet as controls for 28 weeks. From 2 weeks after starting diet administration, animals were given subcutaneous injections of AOM (7.4 mg/kg) once weekly for 10 weeks. The rats were observed daily and weighed twice every week. At the end of week 28, all surviving animals were anesthetized with diethyl ether, exsanguinated through the heart into heparin-coated syringes, and completely autopsies were performed. Moribund and dead animals were also complete autopsied for histological examination. All organs, especially the intestines, were carefully inspected grossly. The intestinal tissues were fixed with 10% buffered formalin, processed for histological examinations and stained with hematoxylin and eosin. Each tumor was classified according to histological criteria described by Ward (1974).



Figure 1. Experimental protocol. AOM, 7.4 mg/kg body weight, subcutaneous injection

Experiment 2: In addition, we assessed long-term feeding effects of ALOE on hematological and biochemical parameters in rats. For 35 weeks, rats in groups 4 and 5 were fed the basal diet and 1% ALOE diet, respectively. The rats were observed daily and weighed twice every week. At the end of week 35, all animals were fasted overnight, anesthetized with diethyl ether, the abdominal cavity opened, and blood withdrawn from the postcava. The blood samples were collected into heparinized tubes to determine hematological parameters and non-heparinized centrifuge tubes for serum preparation. Serum was prepared by centrifugation of the blood at 3000 r.p.m. for 15 minutes at 4 °C, and the separated serum was analyzed to evaluate the biochemical parameters. After drawing blood, complete autopsies were performed. The main organs were weighed, carefully inspected grossly, fixed with 10% buffered formalin, processed for histological examination and stained with hematoxylin and eosin.

Hematological and biochemical analysis

The following hematological parameters were measured using an automated hematology analyzer (Model K-4500; Sysmex Corp., Kobe, Japan): white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PTL). Biochemical profiles were measured on a Keysys clinical chemistry analyzer (Roche Diagnostics K.K., Tokyo, Japan) for the following parameters: serum concentrations of total protein, albumin, lactate dehydrogenase, creatine phosphokinase, amylase, uric acid, leucine aminopeptidase, creatinine, urea nitrogen, glucose, triglycerides, total cholesterol, total bilirubin, direct bilirubin, high density lipoprotein, C-reactive protein, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, g-glutamyltransferase and calcium. Serum sodium, potassium and chloride levels were measured with a STAX-2 electrolyte analyzer (Techno Medica Co., Ltd., Yokohama, Japan).

Statistical analysis

Data on the final body weights and the multiplicity of intestinal tumors in Experiment 1 were analyzed statistically by ANOVA followed by Dunnett’s multiple comparisons test. The incidences of tumors were analyzed by Fisher’s exact test. The data on the final body weights, relative organ weights, and hematological and biochemical parameters in Experiment 2 were analyzed statistically using the Student’s t test or Welch’s t test with p<0.05 as the cutoff. These statistical procedures were performed with InStat 3.0 for Windows (GraphPad Software, Inc., San Diego, CA, USA).

Results

Experiment 1: Effects of ALOE on AOM-induced intestinal carcinogenesis in rats

Final body weights, food consumption and aloin

Table 1 . Body Weights, Food Consumption and Intakes of ALOE and Aloina in Experiments 1 and 2

Group/Treatment	No. of rats ^a	Body weights (g)	Food consumption (g/kg/day)	ALOE intake (g/kg/day)	Aloin ^b intake (mg/kg/day)
Experiment 1					
1/AOM + basal diet	27	400.5 ± 4.66	56.7 ± 4.04	-	-
2/AOM + 1% ALOE diet	27	400.5 ± 3.43	62.9 ± 4.00	0.63 ± 0.04	10.5 ± 0.67
3/AOM + 0.2% ALOE diet	26	409.7 ± 5.72	59.7 ± 4.07	0.12 ± 0.01	2.0 ± 0.17
Experiment 2					
4/Basal diet	12	422.3 ± 5.10	55.2 ± 3.57	-	-
5/1% ALOE diet	12	416.9 ± 7.31	57.9 ± 3.59	0.58 ± 0.04	9.7 ± 0.67

Values are means ± SE. ^aRats that survived for more than 24 weeks. ^bAloins A (barbaloin) + B (isobarbaloin).

(barbaloin + isobarbaloin) intake are shown in Table 1. The final body weights in Groups 1 (AOM + basal diet), 2 (AOM + 1% ALOE) and 3 (AOM + 0.2% ALOE) were not significantly different among the 3 groups. No rats given the 1% or 0.2% ALOE diet developed diarrhea during the experimental period. The daily food consumption was similar between the control and the two ALOE-treated groups.

Table 2 indicates the tumor incidence (% animals with tumors). The incidence of colorectal adenocarcinomas in group 3, but not in group 2, tended to be lower (p = 0.056) than that in group 1. The incidence of adenocarcinomas in the entire intestine (small intestine and large intestine) in group 3 was significantly (p = 0.024) decreased compared to in group 1. However, the incidence of adenomas and total tumors in the entire intestine of groups 2 and 3 was not significantly lower than in group 1. There were no significant differences in tumor multiplicities (number of tumors/animal) of small, large or entire intestines among groups 1, 2 and 3 (data not shown).

Experiment 2: 35-week feeding of ALOE on hematological and biochemical parameters in rats

All animals survived until the end of the experiment. No rats given the 1% ALOE diet developed diarrhea during the experimental period. As shown in Table 1, the final body weights and daily food consumption were not significantly different between groups 4 (basal diet) and 5 (1% ALOE diet). There were also no significant differences of the relative organ weights between the two groups at the termination of the experiment. In addition, neither macroscopic nor microscopic observations revealed tissue changes in these organs.

There were no significant changes of hematological parameters except mean corpuscular hemoglobin concentration (MCHC) between the two groups. MCHC in

group 5 was significantly (p<0.05) higher than that in group 4, but the difference was slight (only 0.9%)(data not shown). There were also no significant differences between groups 4 and 5 in most of the biochemical parameters analyzed. Interestingly, serum concentration of C-reactive protein (CRP) was significantly (p<0.05) lower in group 5 compared to group 4 (0.05±0.01 as compared to 0.07±0.004) suggesting anti-inflammatory activity of ALOE.

Discussion

We previously demonstrated that 5-week administration of 5% and 1% ALOE diet significantly inhibited AOM-induced ACF in the rat colorectum (Shimpo et al., 2001a). However, rats given the 5% ALOE diet had mud-like or soft feces and significantly decreased body weight gains, whereas rats given the 1% ALOE diet had soft or normal feces with no change in the body weight gain (Shimpo et al., 2001a). In addition, we also found that ALOE (especially the 1% diet) reduced the incidence and multiplicity of DMH-induced colorectal proliferative lesions, especially adenomatous hyperplasia, in mice. In this study, we therefore examined the modifying effects of 1% and 0.2% ALOE diets on long-term AOM-induced intestinal carcinogenesis in rats. As a result, we found that 28-week administration of 0.2% ALOE diet significantly or almost significantly reduced the incidences of AOM-induced intestinal adenocarcinomas in male F344 rats.

Twenty years ago, some anthranoids were suggested to be associated with an increased risk of colon cancer (Siegers, 1992; Siegers et al., 1993a; Van Gorkom et al., 1999). In particular, synthetic 1,8-dihydroxyanthraquinone (chryszin, danthron) was found to induce tumors in the large intestine and liver of rodents (Mori et al., 1985, 1986), and also to promote DMH-induced colonic tumorigenesis in mice (Sugie et al., 1994). However, Siegers et al. (1993b) reported

Table 2. Incidences of AOM-induced Intestinal Tumors in Each Group

Group/Treatment	No. of rats	Small intestine			Large intestine			Entire intestine		
		AD	ADC	Total	AD	ADC	Total	AD	ADC	Total
1/AOM + basal diet	27	0	6 (22)	6 (22)	2 (7)	18 (67)	18 (67)	2 (7)	21 (78)	21 (78)
2/AOM + 1% ALOE	27	0	5 (19)	5 (19)	0 (0)	15 (56)	15 (56)	0 (0)	17 (63)	17 (63)
3/AOM + 0.2% ALOE	26	0	6 (23)	6 (23)	5 (19)	10 (38)	14 (54)	5 (19)	12 (46) ¹	16 (62)

Abbreviations: AD, adenoma; ADC, adenocarcinoma. Numbers in parentheses are percentages of animals affected. ¹Significantly different from group 1 by Fisher's exact test (p = 0.024).

that in a model of DMH-induced colorectal tumors in male mice, aloin (the main anthranoid glycoside in aloe) or sennoside (the main anthranoid glycoside in senna)-containing diets (0.03%) did not change the incidence or growth of adenomas or carcinomas after 20 weeks. Recently, Mascolo et al. (1999) showed that a low dose (10 mg/kg) of senna pod extract (SE) for 13 - 28 weeks did not change the appearance of ACF or tumors in the rat colon, whereas administration at a higher dose (100 mg/kg) for 13 weeks increased the appearance of ACF and tumors. Furthermore, they most recently reported that the development of ACF and tumors in rats treated with AOM was significantly reduced by SE (30 and 60 mg/kg) for 110 weeks (Borrelli et al., 2005). Thus, anthranoid-containing herbal drugs or health foods, such as ALOE or SE may have a chemopreventive effect against intestinal tumorigenesis.

The mechanisms by which ALOE reduces AOM-induced tumorigenesis in the rat intestine have not been clearly elucidated. However, it is possible that ALOE inhibits activation enzymes (phase 1 enzymes), activates detoxifying enzymes (phase 2 enzymes), or reduces DNA adduct formation. In a previous study, we found that oral feeding with ALOE or crude aloin (Sigma A0451) for 5 weeks significantly increased the activity of quinone reductase (QR), a phase 2 enzyme, in the rat liver with or without AOM treatment (Shimpo et al., 2001a, 2001b). We also demonstrated that dietary ALOE significantly decreased cytochrome P4502E1 (CYP2E1; a phase 1 enzyme)-dependent *p*-nitrophenol hydroxylase activity in the rat liver and the formation of DNA adducts by AOM in the rat colorectum (Shimpo et al., 2003). AOM is metabolized mainly by CYP2E1 (Sohn et al., 1991). ALOE was also found to inhibit the initiation stage of hepatocarcinogenesis induced by 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) (Tsuda et al., 1993). ALOE reduces CYP1A2 protein levels, which may be directly linked to the reduction of IQ-DNA adduct formation (Uehara et al., 1996). Recently, Furukawa et al. (2002) also demonstrated that ALOE proved markedly efficacious in inhibiting pancreatic tumorigenesis at the initiation phase in hamsters treated with *N*-nitrosobis-(2-oxopropyl)amine, in good agreement with the reduction of DNA adduct formation. Therefore, dietary ALOE may suppress the activation of AOM and thus reduce DNA adduct formation in the initiation stage of colorectal tumorigenesis.

It has been suggested that ALOE has anti-tumor-promoting effects at the post-initiation stage. Previously, we found that post-initiation-stage feeding with ALOE inhibited the development of *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine-induced duodenal tumors in mice (Chihara et al., 2000). In addition, free radical scavenging action (Shimpo et al., 2003b, Beppu et al., 2003b) and antiinflammatory activity (Obata et al., 1993, Teradaira et al., 1993, Beppu et al., 2006) in *Aloe arborescens* are considered to be other possible mechanisms of reduction of AOM-induced intestinal tumors in rats. Yagi et al. (2002) reported the antioxidant- and free radical-scavenging effect of aloesin derivatives in *Aloe vera*. Further, feruloylaloesin and *p*-coumaroylaloesin were found

to inhibit thromboxane A2 synthase activity in vitro. We confirmed that these aloesin derivatives were phenolic compounds in *Aloe arborescens* and had the most effective radical-scavenging activities (Shimpo et al., 2003). Interestingly, Sakai et al (2006) recently found that thromboxane synthase is overexpressed in human colorectal carcinoma and thromboxane A2 is involved in colon cancer cell proliferation. Therefore, thromboxane A2 synthase inhibition by aloesin derivatives may be one possible mechanism of decreased tumor incidences in the rat intestine; however, further examinations are required to clarify these points.

We also found in this study that 35-week administration of 1% ALOE diet (without AOM treatment) did not affect most hematological and serum biochemical parameters in rats. As shown in Table 1, the average daily intake of ALOE per kg body weight in Experiment 2 was 0.58 g. Extrapolating the value of 0.58 g to an average human, it represents about 38 g. However, the intake of *Aloe arborescens* powder has been defined as 1 to 2 grams per day by the standard of *Aloe arborescens* processed food which the Japan Health Food and Nutrition Food Association (JHNFA) established (JHNFA, 2003). Therefore, the daily intake of ALOE (1% of the diet) corresponded to about 20 to 40 times of that in the JHNFA standard. Previously, Kamiya (2002) reported the 90-day repeated-dose toxicity study of Aloe extract (a natural thickening stabilizer; from *Aloe arborescens*) in F344 rats with dietary administration at concentrations of 0, 0.06, 0.25, 1, or 4% (w/w). According to his report (Kamiya, 2002), the administration of Aloe extract did not result in significant treatment-related adverse effects at dietary dose levels of 0.06, 0.25 and 1%, although mild leukocytosis and decreased body weights were observed only in the highest dose (4% in diet) group. Therefore, he concluded that the toxicity of Aloe extract was very low.

Recently, Imaida (2004) reported the intermediate results of a one-year repeated-dose toxicity study with dietary administration at concentrations of 0, 0.16, 0.8, or 4% and a 2-year carcinogenicity study with dietary administration at concentrations of 0, 0.8, or 4% of Aloe extract (from *Aloe arborescens*) in Wistar-Hanover rats. The report showed that rats in the highest dose (4% of diet) Aloe extract-fed group induced diarrhea or laxation and reduced body weight, whereas rats fed the lower dose (0.16, or 0.8% of diet) did not. Further, Aloe extract-fed rats including the highest dose-fed group remained in good health throughout those experiments. These results reported by Kamiya (2002) and Imaida (2004), especially the results of 0.8% or 1% Aloe extract diet-fed rats, are similar to our results of 1% ALOE diet-fed rats. Therefore, feeding with about 0.8 - 1% ALOE or Aloe extract in rats may be the maximum dose inducing no diarrhea, no weight loss and no change of most hematological and serum biochemical parameters, although we look forward to the final report from Imaida.

Taking our previous and present results together, ALOE feeding (1% or less in the diet for 28-35 weeks) appears to

have a mild preventive effect against intestinal tumor growth and very low toxicity on blood parameters in rodents.

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