Current Topics in Pharmacology
Vol. 20, 2016

Original Communication

Suppressive effect of agarose and cellulose on hyperuricemia induced by dietary RNA in rats

Takashi Koguchi[1,*], Hisao Nakajima[2], Saburo Takano[3], Toru Ota[4], Masahiro Wada[5], Satoshi Innami[6] and Tadahiro Tadokoro[7]

[1]Department of Human Education, Kokugakuin Tochigi Junior College, 608 Hirai-cho, Tochigi-shi, Tochigi 328-8588; [2]Old affiliation: Department of Human Ecology, Toyoko Gakuen Women's Junior College, 8-9-18 Todoroki, Setagaya-ku, Tokyo 158-8586; [3]Old affiliation: Faculty of Education, Bunkyo University, 3337 Minami Ogishima, Koshigaya-shi, Saitama 343-8511; [4]Department of Nutritional Science, Faculty of Nutritional Science, Morioka University, 808 Sunakomi, Takizawa-shi, Iwate 020-0694; [5]Department of Clinical Dietetics and Human Nutrition, Faculty of Pharmaceutical Science, Josai University, 1-1 Keyakidai, Sakado-shi, Saitama 350-0295; [6]Institute of Food Behavior Science of Japan, "Chaku-kan" 905 Clio Higashi-Shinjuku Ichibankan 7-27-3 Shinjuku, Shinjuku-ku, Tokyo 160-0022; [7]Department of Administrative Nutrition, Faculty of Health and Nutrition, Tokyo Seiei College, 1-4-6 Nishishinkoiwa, Katsushika-ku, Tokyo 124-8530, Japan.

ABSTRACT

The increasing prevalence of hyperuricemia is most likely caused by the "Westernized" lifestyle and environment. Excessive intake of purines increases serum uric acid (SUA) concentration and is thought to be one factor causative of hyperuricemia. This study was performed to examine the effect of agarose on the elevation of SUA concentration (experimental hyperuricemia) induced by dietary ribonucleic acid (RNA). *In vitro*, agarose significantly decreased the digestion rate of RNA by ribonuclease A (RNase A) and uptake of ^{14}C-labeled adenosine and adenosine-5'-monophosphate (5'-AMP) in the rat jejunum, compared with the fiber-free control. In addition, *in vivo* experiments with 5-week-old male Wistar rats fed diets with a 3% (w/w) yeast RNA and without dietary fiber (DF) or with 5% (w/w) DF (cellulose or agarose) for five days revealed that the cellulose and agarose groups significantly lowered SUA and allantoin concentrations and urinary excretions of related compounds, and increased the amount of RNA excreted into feces, compared with the fiber-free control group, except for the amount of urinary allantoin excretion in the cellulose group. The agarose group had significantly decreased SUA and allantoin concentrations, and increased uric acid clearance and the amount of RNA excreted into the feces, compared with the cellulose group (positive control). The present results suggest that experimental hyperuricemia induced by dietary RNA in rats can be suppressed by agarose and cellulose, and that the suppressive effect of agarose is greater than that of cellulose.

KEYWORDS: agarose, allantoin, cellulose, dietary fiber, RNA, hyperuricemia, rats, uric acid, uric acid clearance

ABBREVIATIONS

5'-AMP	:	adenosine-5'-monophosphate
ANOVA	:	analysis of variance
DF	:	dietary fiber
RNase A	:	ribonuclease A
RNA	:	ribonucleic acid
SV	:	settling volume
SUA	:	serum uric acid
WHC	:	water-holding capacity

*Corresponding author: echo130@nifty.com

1. INTRODUCTION

Hyperuricemia has increased in Japan and the incidence of hyperuricemia in adult males (aged ≥ 30 years) has reached 30% [1]. The introduction of the Western lifestyle to Japanese people, such as a diet containing greater amounts of meat, has been associated with increase in serum uric acid (SUA) levels and the incidence of hyperuricemia [2]. During the past five decades, the eating habits of the Japanese have changed in the following specific ways: fish and shellfish intake tended to decrease (77.2 g/person/day in 1955; 70.0 g/person/day in 2012), and meat and poultry intake markedly increased (12.0 g/person/day in 1955; 88.9 g/person/day in 2012) [3]. Reports of recent epidemiological studies indicate that increased intake of the purine-rich foods, such as meat [4-7] or fish and shellfish [4, 7-9] has been associated with increased hyperuricemia risk. Excessive intake of purines increases the SUA concentration [10, 11] and is thought to be one factor causative of hyperuricemia [12]. Therefore, it is considered that suppressing the elevation of SUA concentration induced by the excessive intake of purines leads to the prevention and suppression of hyperuricemia. As a basic study to search for a dietary method to control hyperuricemia other than by avoidance of excess intake of purines, we [13-18] previously examined the effect of dietary fiber (DF) on the elevation of SUA concentration caused by dietary purines in rats. We [13-18] found that the elevation of SUA concentration in such experimental hyperuricemia induced by dietary purines (hereafter called hyperuricemia) was suppressed by the tested DF, including cellulose. In those reports, we [13] observed that burdock dietary fiber and konjac flour, which are derived from foods, suppress hyperuricemia induced by dietary RNA in rats. Considering the results of the previous studies on dietary RNA [13-15, 18], we surmised that the suppression of hyperuricemia induced by dietary RNA could possibly largely be associated with the inhibition of digestion and/or absorption of RNA by agarose, which is a DF component agar. To our knowledge, studies confirming that agarose has such a functionality have never been reported.

The present study was performed to examine whether agarose or cellulose would suppress hyperuricemia induced by dietary RNA in rats, and further to compare the effect of agarose with cellulose as the positive control.

2. MATERIALS AND METHODS

2.1. Reagents and samples

2.1.1. Reagents

Yeast RNA (purity 94.9%, type VI from Torula Yeast) and ribonuclease A (RNase A) (EC3.1.27.5) (type II-A from Bovine Pancreas) were purchased from Sigma Chemical Co. (St. Louis, MO). [8-^{14}C] adenosine (specific activity 1.84 GBq/mmol) and [8-^{14}C] adenosine 5'-monophosphate (5'-AMP) (specific activity 1.68 GBq/mmol) were obtained from Amersham International Plc (Buckinghamshire, UK).

2.1.2. Samples

Two kinds of DF were used in the present experiment. Cellulose was purchased from Oriental Yeast Co. (Tokyo, Japan). Agarose was purchased from Sigma Chemical Co. (St. Louis, MO).

2.2. Measurement of physical properties of agarose and cellulose

Measurement of the physical properties of agarose and cellulose was carried out by the method described in previous reports [14-16]. The settling volume (SV) and water-holding capacity (WHC) of agarose and cellulose were measured by the method of Takeda and Kiriyama [19]. The cation and anion exchangeabilities of agarose and cellulose were measured by the column method [20] and the method of McConnell *et al.* [21] using a radiometer autoburette (Towa Denpa Kogyo, Tokyo).

2.3. Effects of agarose and cellulose on hydrolysis of RNA by RNase A *in vitro*

Measurement of the digestion rate of RNA by RNase A was carried out by the method described in the previous reports [14, 15]. One Kunitz unit of RNase A (EC3.1.27.5) is defined as the enzyme activity hydrolyzing 0.05% (w/v) RNA per minute at 25 °C and pH 5.0 [22]. Briefly, the reaction mixture consisted of 0.1 M sodium acetate buffer solution (pH 5.0), 3.0 g RNA (type VI), 2.5 mL RNase A solution (125 Kunitz units) and 0 ~ 1.0 g agarose or cellulose in a final volume of 100 mL. A designated amount (0.1 g, 0.25 g, 0.5 g, 0.75 g and 1.0 g) of the agarose or cellulose was sufficiently swelled in

the buffer solution and the RNA was added. After pre-incubation at 37 °C for 2 hours, the enzyme solution was added and then the enzyme reaction was allowed to proceed for 30 minutes. At end of the incubation, RNase A was adsorbed with 11.1 mL of bentonite suspension (3 mg/mL) to stop the enzyme reaction. Then, the reaction mixture was centrifuged at 30,700 × g for 10 minutes and the supernatant was filtrated with an ultrafiltration disk membrane (NMWL:3,000; Sigma Chemical Co., St.Louis, MO). Each experimental reaction was performed in triplicate, and each was repeated six times. The nucleic acid concentration in the filtrate was measured by the Schmidt-Thannhauser-Schneider (STS) method [23] and the digestion rate of RNA by RNase A was calculated. For evaluating the effect of agarose and cellulose on the RNase A activity, the relative digestion rate of RNA by RNase A was also determined by comparing with the value in the fiber-free sample (control). The digestion rates of RNA by RNase A in the fiber-free, agarose, and cellulose samples were determined at the same time; the results for the fiber-free and cellulose samples were the same as those reported in the previous study [14].

2.4. Effects of agarose and cellulose on the uptake of ^{14}C-labeled adenosine and 5'-AMP by jejunal sacs of rats *in vitro*

The measurement of the uptake of ^{14}C-labeled adenosine and 5'-AMP by jejunal sacs of rats was performed by the method described in previous reports [14-16]. Male Wistar rats at 7 or 8 weeks old (200 g - 210 g) fed the American Institute of Nutrition (AIN)-76^{TM} diet [24] were anesthetized with pentobarbital sodium, and the intestine was excised from the duodenum to the distal ileum. After washing the inside of intestinal tract with physiological saline, the jejunum (the proximal one-fourth of the small intestine except the duodenum) was divided into 2 or 3 segments, each 8 cm to 10 cm long. The jejunum was everted to form sacs according to the method of Wilson and Wiseman [25]: briefly, 0.7 mL of modified Krebs bicarbonate Ringer (pH 7.4) buffer [26] was injected into the everted sac. The incubation medium (pH 7.4) consisted of 0.25 mM [8-^{14}C] adenosine (9.25 KBq) or 0.25 mM [8-^{14}C] 5'-AMP (9.25 KBq), modified Krebs bicarbonate Ringer (pH 7.4) buffer [26], and 0.06 g of agarose or cellulose sample in a final volume of 15 mL. The pH of the incubation medium was adjusted at pH 7.4 with 0.05 M HCl or 0.05 M NaOH solution. The incubation medium was aerated with a mixture of oxygen (95%) and carbon dioxide (5%) and was pre-incubated at 37 °C for 5 hours. Each everted sac was placed into the incubation medium, which was continuously aerated with the above gas mixture, and was incubated at 37 °C for 1 minute according to the method of Harms and Stirling [27]. After incubation, the everted sac was washed with a cold physiological saline for 5 minutes to remove extracellular isotopes, cut open, and the serosal fluid was collected. The radioactivity of the purines incorporated into the serosal fluid was measured with a liquid scintillation counter (ALOKA Co, Tokyo). The uptake of ^{14}C-labeled adenosine and 5'-AMP by jejunal sacs of rats in the fiber-free, agarose, and cellulose samples were conducted at the same time; the results for the fiber-free and cellulose samples were the same as those of the previous study [14].

2.5. Animal experiments

Male Wistar rats, 4 weeks old, each weighing 80 g - 85 g, were obtained from Tokyo Experimental Animals Co. (Tokyo), and housed individually in cages in a room kept at 23 ± 1 °C and relative humidity of 50%-60% under a 12-hour light/dark cycle (lights on from 08:00 to 20:00). The rats were first domesticated to take as much fiber-free basal diet as possible within 16-hour periods (16:00 to 08:00) for 7 days. The fiber-free basal diet consisted of 20% casein, 5.0% corn oil, 12% corn starch, 0.3% DL-methionine, 3.5% mineral mixture, 1.0% vitamin mixture, 0.2% choline chloride and sucrose to bring the mixture to 100% (AIN-76^{TM}) [24]. The rats were allowed free access to water. After the adaptation period, rats weighing 120 g - 125 g were divided into 3 groups consisting of 6 rats each. The rats were fed a diet containing yeast RNA and were subdivided into 3 groups: the fiber-free control group, the agarose group, and the cellulose group. The amount of yeast RNA added was 3%, and that of DF was 5%, which replaced sucrose in the diet for the control group. During the experimental period, all rats were fed the same amount (16 g/day) for 5 days. Body weights were measured, and urine and feces collected every day. Fecal samples were weighed and stored at -80 °C until analysis of RNA content. At the end of each experimental period, the rats were anesthetized with pentobarbital sodium and sacrificed by exsanguination by cardiac puncture.

The collected blood was left to stand for 30 minutes, then centrifuged at 2,270 × g for 15 minutes to obtain serum. The animal experiments were approved by the Animal Research Committee of Toyoko Gakuen Women's Junior College (Tokyo, Japan). The animal research protocols were carried out according to the guidelines for breeding and safekeeping of laboratory animals [28].

2.6. Measurement of RNA and RNA metabolites

The SUA concentration and amount of urinary uric acid excretion were measured by the uricase peroxidase method (Quickauto-Neo UAs, SHINO-TEST Co., Tokyo) [29] in the Health and Science Laboratory (Yokohama City, Kanagawa Prefecture). The serum allantoin concentration and amount of urinary allantoin excretion were analyzed by the method of Borchers [30]. The uric acid clearance, an index for expressing the renal excretion efficiency of uric acid, was calculated by the following equation, as described in the previous report [16]:

$$\text{Cua} = \frac{\text{UUA x UV}}{\text{SUA}} \text{ x } \frac{1000}{\text{B.W.}} \text{ x } \frac{1}{1440}$$

where Cua is uric acid clearance (mL/minute/kg B.W.), UUA is urinary uric acid (mg/dL), UV is urine volume (mL), SUA is serum uric acid (mg/dL), B.W. is body weight (g), and 1440 is time (minutes).

The STS method [23] was applied for the extraction and measurement of RNA in the feces.

Urinary excretion of uric acid and allantoin and fecal RNA excretion are shown as the total amounts for the experimental period.

2.7. Statistical analysis

The data are expressed as the mean ± standard error of the mean (SEM). Before comparisons between the two groups (agarose and cellulose) and among the three groups (fiber-free, agarose, and cellulose) were performed, the normality test (Shapiro-Wilk test) was applied to assess the homogeneity of dispersion from the normal distribution and the equal variance test was evaluated. In cases where the normality test (Shapiro-Wilk test) and the equal variance test were passed, the statistical significance was determined by the t-test or the Tukey multiple comparison method after a one-way analysis of variance (ANOVA). In cases where the normality test (Shapiro-Wilk test) and/or the equal variance test failed, the statistical significance was determined by the Mann-Whitney rank sum test or the Dunn's multiple comparison procedures after a Kruskal-Wallis one-way ANOVA on ranks. The statistical significance for SV values was determined by the t-test. The statistical significance for WHC values was analyzed by the Mann-Whitney rank sum test. The statistical significance for the amount of urinary allantoin excretion was determined by the Dunn's multiple comparison procedures after a Kruskal-Wallis one-way ANOVA on ranks. The statistical significance for all the values obtained in the *in vitro* and *in vivo* studies except for the amount of urinary allantoin excretion were determined by Tukey multiple comparison method after a one-way ANOVA. A SigmaPlot 12.0 software program (version 12.0, Systat Software Inc, San Jose, CA) was used for statistical analysis. Differences were considered significant at $p < 0.05$.

3. RESULTS

3.1. *In vitro* studies

3.1.1. Physical properties of agarose and cellulose

The results are shown in table 1. The SV and WHC values of agarose were significantly higher than those of cellulose. Neither agarose nor cellulose showed the cation and anion exchange capacity.

3.1.2. Effects of agarose and cellulose on hydrolysis of RNA by RNase A *in vitro*

The results on the digestion rate and relative rate (in parentheses) of RNA by RNase A are shown in table 2. In comparison to the fiber-free (control), digestion rates were significantly suppressed in the presence of any level of agarose or cellulose added

Table 1. Water-holding capacity and settling volume of agarose and cellulose.[1]

Dietary fiber	WHC[2] g/g fiber	SV[3] mL water/g fiber
Cellulose	3.75 ± 0.05^{b}	3.92 ± 0.06^{b}
Agarose	12.20 ± 1.33^{a}	15.75 ± 0.02^{a}

[1]Each value shows the mean ± SEM of 6 trials in agarose or cellulose. Values in a column not sharing the same superscript letter are significantly different at $p < 0.05$. [2]Water-holding capacity. [3]Settling volume. The statistical significances for SV values were determined by the t-test and those for WHC values were analyzed by the Mann-Whitney rank sum test.

Table 2. Effect of agarose and cellulose on digestion rate of RNA by RNase A *in vitro*.[1]

Test substance	g/100mL of reaction mixture				
	0.10	0.25	0.50	0.75	1.00
	digestion rate (%)				
Fiber-free	$93.5 \pm 0.2^{a,*}$ (100)[2]	93.7 ± 0.2^{a} (100)	93.6 ± 0.1^{a} (100)	93.6 ± 0.1^{a} (100)	93.8 ± 0.1^{a} (100)
Cellulose	$83.4 \pm 0.2^{b,A}$ (89.2)	$75.9 \pm 0.5^{b,B}$ (81.0)	$69.8 \pm 0.6^{b,C}$ (74.5)	$66.7 \pm 0.6^{b,D}$ (71.2)	$64.9 \pm 0.6^{b,D}$ (69.3)
Agarose	$48.7 \pm 0.3^{c,A}$ (52.0)	$39.7 \pm 0.5^{c,B}$ (42.4)	$35.0 \pm 0.5^{c,C}$ (37.4)	$31.4 \pm 0.7^{c,D}$ (33.5)	$27.8 \pm 0.9^{c,E}$ (29.6)

[1]Each value shows the mean ± SEM of 6 trials in agarose or cellulose. [2]Values in parentheses indicate the relative digestion rate (%). *Values in a column not sharing the same superscript small letter are significantly different at $p < 0.05$. Values in a row not sharing the same superscript capital letter are significantly different at $p < 0.05$. The statistical significances for all values obtained in this *in vitro* study were determined by the Tukey multiple comparison test after a one-way ANOVA. Values obtained from fiber-free and cellulose are adapted from Koguchi, T., Nakajima, H., Wada, M., Yamamoto, Y., Innami, S., Maekawa, A. and Tadokoro, T. 2002, J. Nutr. Sci. Vitaminol., 48, 184 with permission from Center for Academic Publications, Japan.

to the reaction mixture. The suppressive effect of agarose was significantly greater than that of cellulose. The results also revealed that as the agarose or cellulose level increased, the digestion rates of RNA by RNase A significantly decreased, except at the 1.0% level of cellulose.

3.1.3. Effects of agarose and cellulose on the uptake of ^{14}C-labeled adenosine and 5'-AMP by jejunal sacs of rats *in vitro*

The results are shown in table 3. The uptake of ^{14}C-labeled adenosine into the jejunum of rats was significantly lowered in each incubation medium containing agarose and cellulose compared with that in fiber-free (control). The uptake of ^{14}C-labeled adenosine into rat jejunum in the presence of agarose was not significantly different from that with added cellulose. However, the uptake of ^{14}C-5'-AMP was significantly reduced in the agarose sample compared with that in fiber-free and cellulose samples.

3.2. *In vivo* studies

3.2.1. Body weight gain and weight of organs

The results are shown in table 4. When comparing the test groups, no differences were detected in body weight gain, or the weight of the small intestine and the cecal contents. The weights of the cecum and the large intestine (colon and rectum) of the agarose group were significantly higher than those of the fiber-free group, but did not differ significantly when comparing the agarose and the cellulose groups.

Table 3. Effect of agarose and cellulose on the uptake of ^{14}C-adenosine and ^{14}C-5'-AMP by jejunal sacs of rats *in vitro*.[1]

Test substance[2]	Adenosine[3]	5'-AMP[4]
	nmol/mg wet tissue/min	
Fiber-free	39.2 ± 3.19^{a} (100)[5]	26.2 ± 0.68^{a} (100)
Cellulose	23.8 ± 0.87^{b} (60.7)	24.6 ± 0.82^{a} (93.9)
Agarose	17.2 ± 2.92^{b} (44.0)	17.9 ± 0.41^{b} (68.4)

[1]Each value shows the mean ± SEM of 6 rats in agarose or cellulose. [2]0.4% (w/v) agarose or cellulose. [3]0.25 mM [8-^{14}C] adenosine (specific activity 1.84 GBq/mmol). [4]0.25 mM [8-^{14}C] 5'-AMP (specific activity 1.68 GBq/mmol). Values in a column not sharing the same superscript letter are significantly different at $p < 0.05$. [5]Values in parentheses indicate the relative uptake rate (%). The statistical significances for all values obtained in this *in vitro* study were determined by the Tukey multiple comparison test after a one-way ANOVA. Values obtained from fiber-free and cellulose are adapted from Koguchi, T., Nakajima, H., Wada, M., Yamamoto, Y., Innami, S., Maekawa, A. and Tadokoro, T. 2002, J. Nutr. Sci. Vitaminol., 48, 184 with permission from Center for Academic Publications, Japan.

3.2.2. Serum uric acid and allantoin concentrations

The results are shown in table 5. The SUA and allantoin concentrations in the agarose and cellulose groups were significantly decreased compared with those in the fiber-free group, and both concentrations

Table 4. Body weight gain and weight of organs of rats fed diets with RNA.[1]

Test substance	Body weight gain g/5d	Organ weight			
		Small intestine	Cecum	Contents in cecum	Colon and Rectum
		g			
Fiber-free	36.3 ± 0.96	5.01 ± 0.20	2.16 ± 0.16^{b}	1.74 ± 0.12	0.67 ± 0.05^{b}
Cellulose	35.5 ± 1.02	4.57 ± 0.18	2.36 ± 0.16^{ab}	2.06 ± 0.16	0.73 ± 0.02^{ab}
Agarose	35.4 ± 0.46	4.71 ± 0.12	2.72 ± 0.08^{a}	2.02 ± 0.10	0.88 ± 0.05^{a}

[1]Each value shows the mean ± SEM of 6 rats in each group. Values in a column not sharing the same superscript letter are significantly different at $p < 0.05$. The statistical significances for all values obtained for body weight gain and organ weight were determined by the Tukey multiple comparison test after a one-way ANOVA.

were significantly lower in the agarose group than the cellulose group.

3.2.3. The amounts of uric acid and allantoin excreted in urine

The results are shown in table 6. The amounts of uric acid and allantoin excreted into the urine in the agarose group and cellulose group were significantly reduced compared with those in the fiber-free group, except for the amount of allantoin excreted into the urine in the cellulose group. The amounts of uric acid and allantoin excreted into the urine in the agarose group were not significantly different from those in the cellulose group.

3.2.4. The uric acid clearance

The results are shown in figure 1. The uric acid clearance in the agarose group was significantly increased compared with that in the fiber-free group or cellulose group, and that in the agarose group was the highest among the test groups. The uric acid clearance in the cellulose group was not significantly different from that in the fiber-free group.

3.2.5. The amount of RNA excreted into the feces

The results are shown in table 7. The wet weight of the feces in the agarose and cellulose groups was significantly heavier than that in the fiber-free group, and the amount of RNA excreted into the feces in both the agarose and the cellulose groups was significantly higher than that in the fiber-free group. The amount of RNA excreted into the feces in the agarose group was significantly increased compared with that in the cellulose group, and was the highest among the three test groups.

Table 5. Effect of agarose and cellulose on serum uric acid and allantoin concentrations of rats fed diets with RNA.[1]

Test substance	Uric acid µmol/L	Allantoin mmol/L
Fiber-free	121.6 ± 2.23^{a}	12.63 ± 0.17^{a}
Cellulose	106.6 ± 1.82^{b}	6.23 ± 0.10^{b}
Agarose	76.3 ± 2.09^{c}	3.75 ± 0.07^{c}

[1]Each value shows the mean ± SEM of 6 rats in each group. Values in a column not sharing the same superscript small letter are significantly different at $p < 0.05$. The statistical significances for all values obtained for serum uric acid and allantoin concentrations were determined by the Tukey multiple comparison test after a one-way ANOVA.

Table 6. Effect of agarose and cellulose on amounts of uric acid and allantoin excreted in urine of rats fed diets with RNA.[1]

Test substance	Uric acid µmol/5d	Allantoin mmol/5d
Fiber-free	69.88 ± 2.26^{a}	6.61 ± 0.17^{a}
Cellulose	58.36 ± 1.77^{b}	3.97 ± 0.40^{ab}
Agarose	57.11 ± 1.75^{b}	2.43 ± 0.04^{b}

[1]Each value shows the mean ± SEM of 6 rats in each group. Values in a column not sharing the same superscript letter are significantly different at $p < 0.05$. The statistical significances for values obtained for the amount of uric acid excreted in urine were determined by the Tukey multiple comparison test after a one-way ANOVA and those for values obtained for the amount of allantoin excreted in urine were analyzed by the Dunn's multiple comparison procedures after the Kruskal-Wallis one-way ANOVA on ranks.

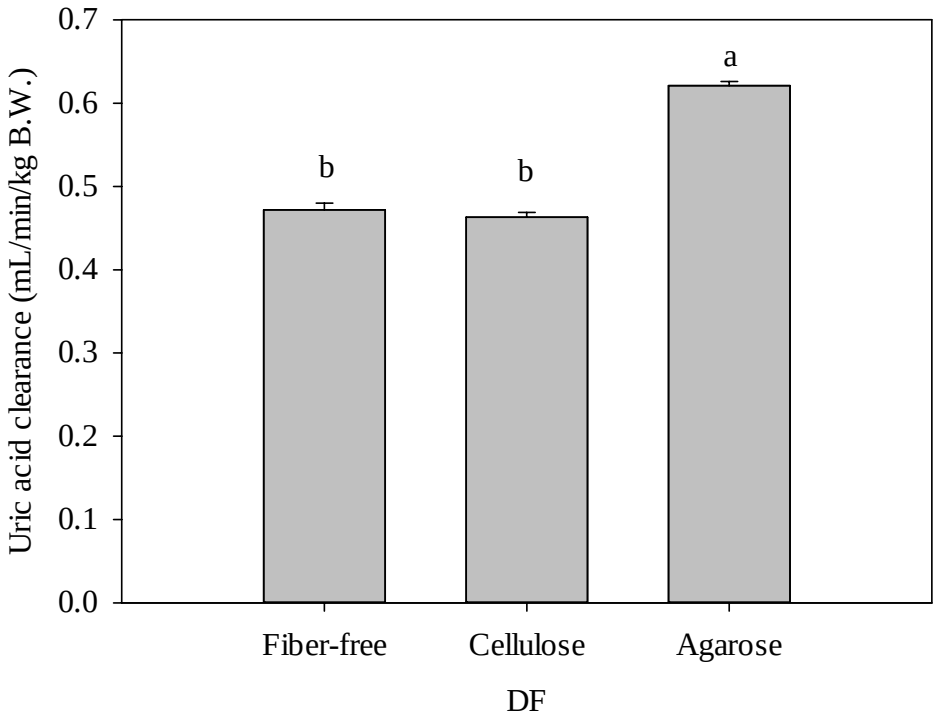

Figure 1. Effect of agarose and cellulose on uric acid clearance of rats fed diets with RNA. Each bar is the mean value for 6 rats, and vertical bar represent SEM. Different letters presented above the vertical bar indicate a significant difference at $p < 0.05$. The statistical significances for all values obtained in uric acid clearance were determined by the Tukey multiple comparison test after a one-way ANOVA.

Table 7. Effect of agarose and cellulose on fecal wet weight and amount of RNA excreted in feces of rats fed diets with RNA.[1]

Test substance	Fecal wet weight g/5d	Fecal RNA excretion mg/5d
Fiber-free	1.60 ± 0.14^{c}	39.23 ± 2.22^{c}
Cellulose	5.97 ± 0.29^{b}	77.26 ± 2.29^{b}
Agarose	8.73 ± 0.33^{a}	175.2 ± 3.87^{a}

[1]Each value shows the mean ± SEM of 6 rats in each group. Values in a column not sharing the same superscript letter are significantly different at $p < 0.05$. The statistical significances for all values obtained for fecal wet weight and the amount of fecal RNA excretion were determined by the Tukey multiple comparison test after a one-way ANOVA.

4. DISCUSSION

First, we examined the effect of agarose on hydrolysis of RNA by RNase A *in vitro* and on the *in vitro* uptake of ^{14}C-labeled adenosine and 5'-AMP by jejunal sacs of rats. Agarose significantly suppressed RNA digestion by RNase A and decreased the uptake of ^{14}C-labeled adenosine and 5'-AMP in rat jejunum. The suppressive effect of agarose on RNA digestion by RNase A was greater than that of cellulose (positive control). Compared with

cellulose, agarose significantly lowered the uptake of ^{14}C-labeled 5'-AMP into the jejunum of rats, and also tended to decrease the uptake of ^{14}C-labeled adenosine. This is presumed to be associated with the SV and WHC of agarose, which are significantly higher than those of cellulose. In the previous reports, we [14, 15] speculated that the suppressive *in vitro* effects of DF on RNA digestion by RNase A and on the uptake of ^{14}C-labeled adenosine and 5'-AMP by jejunal sacs of rats are associated with the physical properties of DF.

We [13, 14, 18] previously confirmed that dietary RNA is a substance that causes overproduction-type hyperuricemia (SUA concentration: purine-free, 75-90 μmol/L; RNA, 122-128 μmol/L). Johnson *et al.* [31-33] have reported that administration of oxonic acid, which is an inhibitor of uricase, or oral administration of a 60% of fructose diet causes hyperuricemia in rats (SUA concentration: control, 30.3-82.7 μmol/L; oxonic acid, 109.4 μmol/L; 60% fructose diet, 70.8-142.8 μmol/L). Results of the present experiment suggest that addition of RNA to the fiber-free diet causes hyperuricemia in rats. We reconfirmed the previous result [14, 18], demonstrating that cellulose suppressed the elevation of SUA concentration and the increase in the amount of urinary uric acid excretion induced by dietary RNA. A potential mechanism of cellulose in lowering the SUA concentration could be that cellulose suppresses digestion and/or absorption of dietary RNA in the digestive system. The suppressive effect of agarose on hyperuricemia was greater than that of cellulose. This may be explained by the data showing that SUA concentration in the agarose group was significantly lower, and the uric acid clearance significantly higher, than that in the cellulose group. Therefore, the suppressive mechanism of agarose on hyperuricemia induced by dietary RNA is presumed to be due to suppression of digestion and/or absorption of RNA and an increase in the renal excretion efficiency of uric acid.

We [13, 14, 18, 34] previously demonstrated that water-insoluble DF increases fecal RNA excretion in rats fed a diet with or without dietary RNA, although the degree of the response is different. This tendency was also observed in the present study. The effect of agarose on the amount of fecal RNA excretion was higher than that of cellulose. Fecal RNA can be divided according to its origin as either endogenous or exogenous. It is thought that the former is derived from exfoliated epithelial cells of the gastrointestinal tract and from enteric bacteria, while the latter is undigested and unabsorbed RNA originating in the diet. It is thought that approximately 30% of the dried feces is enteric bacteria [35]. Reports and speculation about the degradability of agarose and cellulose by enteric bacteria are as follows: (1) Oku *et al.* [36] have speculated that the decomposition rate of agar is 10% or less in animals and humans, suggesting that agarose, a major constituent of agar, is degraded with difficulty by enteric bacteria; (2) The digestion rate or degradation rate of cellulose by enteric bacteria *in vitro* is 6-7% [37]. In the previous studies, most of the cellulose administered to humans or rats has been recovered from the feces [37]. From the above reports, the degradability of agarose and cellulose by enteric bacteria is presumed to affect the extent of fecal RNA excretion. That is to say, agarose and cellulose are degraded in the large intestine to some extent, and are utilized as the carbon source for the proliferation of intestinal bacteria. Different effects of test DF on the amounts of endogenous and/or exogenous RNA were shown in our previous studies [13-15, 18, 34], and are similar to the observations seen in the present experiment. Therefore, as a possible cause of the increased fecal RNA excretion in the case of agarose and cellulose, we must consider not only the amount of exogenous RNA but also the effect of agarose and cellulose on the amount of endogenous RNA including the degradability of agarose and cellulose by enteric bacteria.

5. CONCLUSION

The present study showed that agarose and cellulose suppressed hyperuricemia induced by dietary RNA in rats. It seems that the effect of agarose and cellulose is associated with their physical properties. The suppressive effect of agarose on hyperuricemia was greater than that of cellulose. The *in vivo* and *in vitro* studies suggest that agarose may alter metabolic processes that contribute to hyperuricemia by lowering the SUA concentration, reducing the digestion and/or absorption of RNA, and increasing the renal excretion efficiency of uric acid and RNA excretion in the feces. It is very interesting that the suppressive mechanism of agarose on

hyperuricemia induced by dietary RNA is somewhat different from that of cellulose. Further study is warranted to investigate whether agarose suppresses hyperuricemia in rats fed a diet with dietary RNA for a longer rearing period. Moreover, further research is necessary to investigate how agarose affects concentrative nucleoside transporter 2 (CNT2), a transporter which exists the brush-border membrane of epithelial cells of the small intestine and kidney, Urate transporter 1 (URAT1/SLC22A12) and Glucose transporter 9 (GLUT9/SLC2A9), which are key renal urate anion transporters promoting reabsorption by the proximal renal tubule epithelial cell, and ATP-binding cassette transporter G2 (ABCG2), which is the proximal tubule renal urate anion secretory transporter in both non-hyperuricemic rats and hyperuricemic rats.

Recently, clinical studies have revealed that intake of DF lowers SUA concentrations in healthy subjects [38, 39] and in individuals with hyperuricemia [38, 40], and in gout patients [SUA concentration ≥ 9.0 mg/dL (535.3 μmol/L) in men and ≥ 6.5 mg/dL (386.6 μmol/L) in women] [41]. Epidemiological studies have shown that increased DF intake is associated with decreased SUA concentration [42-44] and hyperuricemia risk [6, 44]. It seems that the basic research results of our previous studies concerning the suppressive effect of DF on hyperuricemia stimulated subsequent epidemiological studies and clinical research on this matter. During the past six decades, consumption of DF by the Japanese population has decreased (27.0 g/person/day in 1952; 14.2 g/person/day in 2012) [3, 45]. We can surmise that consumption of more DF in daily life is required to prevent hyperuricemia in the general population of Japan. Hyperuricemic subjects [SUA concentration ≥ 7.0 mg/dL (416.4 μmol/L) in men and ≥ 6.0 mg/dL (356.9 μmol/L) in women] had a lower intake of DF compared to the non-hyperuricemic individuals [46]. After considering all these results together, we wish to emphasize the importance of recognizing agar intake as a potential method to prevent or suppress hyperuricemia in the general population.

ACKNOWLEDGEMENTS

The authors thank Journal of Nutritional Science and Vitaminology for the permission to re-use the *in vitro* study results of fibre-free and cellulose samples, and Dr. Akio Maekawa for his kind support and direction in this study, and Eiko Ota, Yuko Itabashi, Tamae Yanagita, Nao Fujiwara, and Yumi Kuwabara for furnishing references.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

REFERENCES

1. Yamanaka, H. 2010, Nucleosides Nucleotides Nucleic Acids, 30, 1018.
2. Kagan, A., Harris, B. R., Winkelstein, W. Jr., Johnson, K. G., Kato, H., Syme, S. L., Rhoads, G. G., Gay, M. L., Nichaman, M. Z., Hamilton, H. B. and Tillotson, J. 1974, J. Chronic. Dis., 27, 345.
3. National Institute of Health and Nutrition. 2016, The National Health and Nutrition Survey Japan, 2012, Daiichi-shuppan, Tokyo (in Japanese).
4. Choi, H. K., Liu, S. and Curhan, G. 2005, Arthritis Rheum., 52, 283.
5. Choi, H. K. 2010, Curr. Opin. Rheumatol., 22, 165.
6. Yu, K-H., See, L-C., Huang, Y-C., Yang, C-H. and Sun, J-H. 2008, Semin. Arthritis Rheum., 37, 243.
7. Guasch-Ferré, M., Bulló, M., Babio, N., Martínez-González, M. A., Estruch, R., Covas, M-I., Wärnberg, J., Arós, F., Lapetra, J., Serra-Majem, L., Basora, J. and Salas-Salvadó, J. 2013, J. Gerontol. A. Biol. Sci. Med. Sci., 68, 1263.
8. Villegas, R., Xiang, Y-B., Elasy, T., Xu, W. H., Cai, H., Cai, Q., Linton, M. F., Fazio, S., Zheng, W. and Shu, X-O. 2012, Nutr. Metab. Cardiovasc. Dis., 22, 409.
9. Chang, W. C. 2011, Aging Male, 14, 195.
10. Bowering, J., Calloway, D. H., Margen, S. and Kaufmann, N. A. 1970, J. Nutr., 100, 249.
11. Clifford, A. J., Riumallo, J. A., Young, V. R. and Scrimshaw, N. S. 1976, J. Nutr., 106, 428.
12. Zöllner, N. and Griebsch, A. 1974, Adv. Exp. Med. Biol., 41B, 435.
13. Koguchi, T., Wada, M., Ota, T., Nakajima, H., Tadokoro, T. and Maekawa, A. 1997, J. Agric. Sci. Tokyo Nogyo Daigaku, 42, 163.
14. Koguchi, T., Nakajima, H., Wada, M., Yamamoto, Y., Innami, S., Maekawa, A. and Tadokoro, T. 2002, J. Nutr. Sci. Vitaminol., 48, 184.

15. Koguchi, T., Nakajima, H., Koguchi, H., Wada, M., Yamamoto, Y., Innami, S., Maekawa, A. and Tadokoro, T. 2003, Int. J. Vitam. Nutr. Res., 73, 369.
16. Koguchi, T., Koguchi, H., Nakajima, H., Takano, S., Yamamoto, Y., Innami, S., Maekawa, A. and Tadokoro, T. 2004, Int. J. Vitam. Nutr. Res., 74, 253.
17. Koguchi, T., Koguchi, H., Nakajima, H., Takano, S., Yamamoto, Y., Innami, S., Maekawa, A. and Tadokoro, T. 2004, J. Jpn. Assoc. Dietary Fiber Res., 8, 83.
18. Koguchi, T., Koguchi, H., Nakajima, H., Takano, S., Kobayashi, K-I., Yamamoto, Y., Wada, M., Innami, S., Maekawa, A. and Tadokoro, T. 2008, J. Jpn. Assoc. Dietary Fiber Res., 12, 67.
19. Takeda, H. and Kiriyama, S. 1979, J. Nutr., 109, 388.
20. Fujimoto, M. and Murase, T. 1955, Ion exchange resin, M. Honda, H. Kakihana and Y. Yoshino (Eds.), Hirokawa Publishing, Tokyo, 21 (in Japanese).
21. McConnell, A. A., Eastwood, M. A. and Mitchell, W. D. 1974, J. Sci. Fd Agric., 25, 1457.
22. Kunitz, M. 1946, J. Biol. Chem., 164, 563.
23. Fleck, A. and Munro, H. N. 1962, Biochim. Biophys. Acta, 55, 571.
24. American Institute of Nutrition. 1977, J. Nutr., 107, 1340.
25. Wilson, T. H. and Wiseman, G. 1954, J. Physiol., 123, 116.
26. Bronk, J. R. and Parsons, D. S. 1965, Biochim. Biophys. Acta, 107, 397.
27. Harms, V. and Stirling, C. E. 1977, Am. J. Physiol., 233, E47.
28. Japanese Society of Nutrition and Food Science. 2000, J. Jpn. Soc. Nutr. Food Sci., 53, 56 (in Japanese).
29. Kabasakalian, P., Kalliney, S. and Westcott, A. 1973, Clin. Chem., 19, 522.
30. Borchers, R. 1977, Anal. Biochem., 79, 612.
31. Mazzali, M., Kanellis, J., Han, L., Feng, L., Xia, Y-Y., Chen, Q., Kang, D-H., Gordon, K. L., Watanabe, S., Nakagawa, T., Lan, H. Y. and Johnson, R. J. 2002, Am. J. Physiol. Renal Physiol., 282, F991.
32. Nakagawa, T., Hu, H., Zharikov, S., Tuttle, K. R., Short, R. A., Glushakova, O., Ouyana, X., Feig, D. I., Block, E. R., Herrera-Acosta, J., Patel, J. M. and Johnson, R. J. 2006, Am. J. Physiol. Renal Physiol., 290, F625.
33. Sánchez-Lozada, L. G., Tapia, E., Jiménez, A., Bautista, P., Crisóbal, M., Nepomuceno, T., Soto, V., Ávila-Casado, C., Nakagawa, T., Johnson, R. J., Herrera-Acosta, J. and Franco, M. 2007, Am. J. Physiol. Renal Physiol., 292, F423.
34. Koguchi, T. and Tadokoro, T. 2017, Int. J. Vitam. Nutr. Res., (in press).
35. Oku, T. 1995, Dietary fiber, S. Innami and S. Kiriyama (Eds.), Daiichi-shuppan, Tokyo, 80 (in Japanese).
36. Oku, T., Yamada, K. and Kanaya, K. 2002, J. Jpn. Assoc. Dietary Fiber. Res., 6, 81 (in Japanese).
37. Barry, J. L., Hoebler, C., Macfarlane, G. T., Macfarlane, S., Mathers, J. C., Reed, K. A., Mortensen, P. B., Nordgaard, I., Rowland, I. R. and Rumney, C. J. 1995, Br. J. Nutr., 74, 303.
38. Shirogane, H., Inoki, A., Yamaguchi, Y. and Tamesada, M. 2004, Journal of Nutritional Food, 7, 35 (in Japanese).
39. Carabin, I. G., Lyon, M. R., Wood, S., Pelletier, X., Donazzolo, Y. and Burdock, G. A. 2009, Nutr. J., 8, 9.
40. Yamaguchi, Y., Ando, C., Tsukamoto, S., Nagao, J., Ueda, T., Yamaguchi, H. and Akaoka, I. 2007, J. Jpn. Soc. Clin. Nutr., 29, 104 (in Japanese).
41. Osman, M. E., Abu Zeid, I. M. and Adam, F. A. 2011, Gum Arabic, J. F. Kennedy, G. O. Phillips and P. A. Williams (Eds.), Royal Society of Chemistry, Cambridge, UK, 301.
42. Zykova, S. N., Storhaug, H. M., Toft, I., Chadban, S. J., Jenssen, T. G. and White, S. L. 2015, Nutr. J., 14, 49.
43. Shatat, I. F., Abdallah, R. T., Sas, D. J. and Hailpern, S. M. 2012, Pediatric Research, 72, 95.
44. Sun, S. Z., Flickinger, B. D., Williamson-Hughes, P. S. and Empie, M. W. 2010, Nutr. Metab., 7, 16.
45. Tsuji, K. 1990, Gendai iryo, 23, 33 (in Japanese).
46. Ryu, K. A., Kang, H. H., Kim, S. Y., Yoo, M. K., Kim, J. S., Lee, C. H. and Wie, G. A. 2014, Clin. Nutr. Res., 3, 56.