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Suppression of Lipopolysaccharide and Galactosamine-Induced Hepatic Inflammation by Red Grape Pomace

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ABSTRACT: Grape pomace is generated in the production process of wine and grape juices and is an industrial waste. This study investigated whether an intake of grape pomace was able to suppress chronic inflammation induced by lipopolysaccharide (LPS) and galactosamine (GalN) in vivo. When Sprague−Dawley rats were orally given methanolic extracts from red and white grape pomace, the extracts inhibited the LPS/GalN-evoked activation of nuclear factor-κB (NF-κB) dose-dependently, and red grape pomace exerted a stronger effect than white grape one. Next, rats were fed an AIN93 M-based diet containing 5% red grape pomace for 7 days, followed by the intraperitoneal injection of LPS and GalN. The intake of the red grape pomace-supplemented diet was found to suppress the LPS/GalN-induced activation of NF-κB and expression of inducible nitric oxide synthase and cyclooxygenase-2 proteins. These results suggest that red grape pomace may contain an abundance of effective compound(s) for anti-inflammatory action.

KEYWORDS: *grape pomace, inflammation, liver, lipopolysaccharide, galactosamine, NF-κB*

INTRODUCTION

Wine is made from red and white grapes and is drunk regularly all over the world. However, the use of grapes as agricultural crops leads to large amounts of byproducts in agricultural areas. The Organización Internacional de la Viña y el Vino (OIV) estimated that the worldwide production amount of wine in 2009 was about 30 billion liters, and about 10% of the amount of the used grapes corresponded to grape pomace after pressing. Almost all grape pomace has become an industrial waste, although it has been slightly used as part of the feed for ruminants.

Grape pomace mainly consists of skins and seeds. Grape skins and seeds are abundant sources of polyphenols, mainly procyanidins, anthocyans, flavonols, phenolic acids, and stilbenes.[1] Actually, grape (*Vitis vinifera*) pomace is reported to contain anthocyans, flavonols, phenolic acids, and stilbenes,[2] and grape (Nero d'Avola) pomace also has a high content of quercetin in particular.[3] Polyphenols have multiple functions dependent on their antioxidative activity.[4] In a previous study, it was reported that methanolic extracts of grape (*V. vinifera*) pomace exerted an antioxidant activity in vitro and in vivo.[5] It was demonstrated that the extracts from grape pomace had antioxidative activity and exerted an anti-inflammatory effect in diet-induced obesity.[6] In addition, the grape pomace extracts selectively inhibited an intestinal α-glucosidase activity and suppressed postprandial hyperglycemia in diabetic mice.[7] Therefore, there is a high possibility that grape pomace possesses beneficial compounds and can exhibit a suppressive effect against various oxidative stresses.

Various phenolic compounds in grapes and wines have been demonstrated to exert various health-promoting effects, for example, inhibition of low-density lipoprotein oxidation,[8,9] and on the basis of these reports, the "French paradox" has been widely discussed. Almost all of the phenolic compounds in grapes and wine are present in grape skins and seeds. Because grape pomace mainly consists of skins and seeds and also the industrial waste, it is suggested that grape pomace can be reused as a functional food or material for extraction of effective compounds. Therefore, the aim of the present study is to find novel functionality of grape pomace, and the following experiments using a mouse model based on the lipopolysaccharide (LPS) and galactosamine (GalN)-evoked hepatic disorder accompanied by inflammation were carried out: First, we investigated whether methanolic extracts from red and white grape pomace can suppress chronic inflammatory responses induced by LPS and GalN in vivo (experiment I). We next examined whether the diet containing the red grape pomace can attenuate the LPS/GalN-induced chronic inflammatory responses in vivo (experiment II).

MATERIALS AND METHODS

Materials. The pomace of red and white grape (*V. vinifera*) was kindly provided from the Industry and Agriculture Promotion Bureau of Kobe City (Japan). Both grape pomaces were preliminarily dried in the shade for 3 days before drying at 60 °C for 6 h. For the electrophoretic mobility shift assay (EMSA), an oligonucleotide for the NF-κB responsive element probe containing the nuclear factor-κB (NF-κB)-binding site, 5′-AGT TGA GGG GAC TTT CCC AGG C-3′ (coding) and 5′-TCA ACT CCC CTG AAA GGG TCC G-3′

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(noncoding), was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). For the Western blot analysis, anti-inducible nitric oxide synthase (iNOS) and anti-cyclooxygenase-2 (COX-2) antibodies were obtained from Santa Cruz Biotechnology Inc. For the analysis of caspase-3 activity, Ac-peptidyl methylcoumarylamide (MCA) substrates, that is, Ac-DEVD-MCA, were purchased from Peptide Institute, Inc. (Osaka, Japan), and were dissolved at 10 mM in dimethyl sulfoxide.

Preparation of the Red and White Grape Pomace Extracts. The dried red and white grape pomaces were pulverized with liquid nitrogen and lyophilized. Lyophilized powders obtained were extracted with methanol containing 1% acetic acid overnight to obtain a variety of hydrophobic molecules such as polyphenols including anthocyans. The extract solutions were filtered before methanol and acetic acid were removed from the solutions using a centrifugal evaporator. The resultant extracts were dissolved by 5 g/L polyethyleneglycol (PEG) 6000, followed by the oral administration to rats (experiment I).

Animal Experiments. All animal treatments in this study were approved by the Institutional Animal Care and Use Committee and carried out according to the Kobe University Animal Experimentation Regulations. Male Sprague−Dawley rats (6 weeks old, 160−180 g, Japan SLC, Inc., Shizuoka, Japan) were housed in a temperature-controlled (23−25 °C) room at 60 ± 5% humidity under a 12 h light−dark cycle and acclimatized for 7 days with a commercial chow and distilled water. This study includes two kinds of the experiments as follows. In experiment I, the rats were divided at random into six groups of eight rats each, and the rats were orally given the red and white grape pomace extracts at concentrations of 100 and 500 mg/kg BW after food was withheld for 12 h. The other two groups were given 0.8 mL of 5 g/L PEG6000 solution alone as a vehicle control. The dosage concentrations of grape pomace extracts were decided by reference to previous papers about grape pomace.[6,7,10] After 1 h, half of the rats in each group (n = 4) were further given a single intraperitoneal (ip) injection of LPS at 10 μg/kg BW and GalN at 250 mg/kg BW, and the other half was injected with 400 μL of saline solution (0.85% NaCl) as a vehicle control. They were sacrificed under anesthetization 24 h after the LPS/GalN injection, and then the livers were excised and perfused with ice-cold phosphate-buffered saline (PBS). In experiment II, the experimental diet was based on a modified AIN-93 semipurified diet (AIN93 M diet). Rats were divided at random into two groups of six rats each and fed the AIN93 M diet containing 5% red grape pomace or the control diet. The body weight and the amount of food consumed were measured daily. After 7 days, half of the rats in each group (n = 3) were injected intraperitoneally with LPS at 10 μg/kg BW and with GalN at 250 mg/kg BW after food was withheld for 12 h, and the other half was injected with 400 μL of saline solution as a vehicle control. They were sacrificed under anesthetization 24 h after the LPS/GalN injection, and blood was collected from cardiac puncture. The plasma was separated from heparinized whole blood by centrifugation at 1600g for 10 min and used for measurement of alanine aminotransferase (ALT) activity. The livers were excised and perfused with ice-cold PBS. Preparation of the nuclear extract and the cell lysates from the livers was carried out according to the method described in our previous paper.[11]

EMSA. The DNA binding activity of NF-κB was evaluated by EMSA according to the method of our previous paper.[12] The specificity of the detected bands was confirmed by the competition experiment using a 100-fold excess of the corresponding non-radioactive cold probe (data not shown).

Western Blot Analysis. The cell lysate (50 μg of proteins) was separated by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) using a 7.5% (for iNOS) or 10% (for COX2) gel. After electrophoresis, the proteins were transferred onto a PVDF membrane (GE Healthcare Bio-Science Co., Piscataway, NJ, USA), and Western blotting using anti-iNOS (1:1000) or anti-COX2 (1:1000) antibody was performed. Specific immune complexes were detected with an ECL plus Western Blotting Detection System (GE Healthcare UK Ltd., Buckinghamshire, England).

Enzymatic Activity Assay. The plasma ALT activity was measured using the commercially available kit (Wako Pure Chemical Industries Ltd., Kobe, Japan) according to the manufacturer's instructions. Caspase-3-like protease activity was measured using fluorogenic peptide substrates as described previously,[13] and the activity was calculated as the amount of released 7-amino-4-methylcoumarin (AMC)/min/mg protein.

Instrumental Analysis. Anthocyans in the red grape pomace were measured as described previously.[14] Briefly, the dried red grape pomace was extracted with methanol containing 1% trifluoroacetic acid (TFA) overnight at 4 °C (12.5 mg/mL). The extracted solution was filtered and diluted with 0.5% TFA solution. Aliquots of the diluted solution (100 μL) were injected into a high-performance liquid chromatography (HPLC) system. HPLC was carried out on a Develosil ODS HG-5 (4.5 × 150 mm) at 40 °C using 25% methanol containing 0.5% TFA as the elution solvent at a flow rate of 1.5 mL/min, and the elution peaks were monitored at 520 nm.

Statistical Analysis. Data are expressed as the mean ± SE. Statistical significance was analyzed using Student's t test, and the 0.05 level of probability was used as the criterion of significance.

RESULTS AND DISCUSSION

Experiment I was performed to find novel functionality of grape pomace and investigate whether certain compounds in red or white grape pomace are effective for regulating inflammatory reactions in the liver. In this experiment, rats were orally given red and white grape pomace extracts (Figure 1). Both extracts

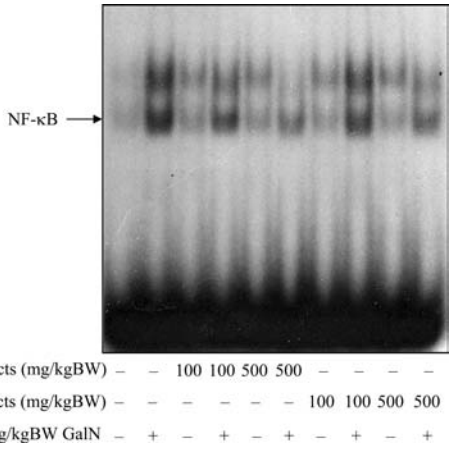


Figure 1. Effects of red and white grape pomace extracts on the DNA-binding activity of NF-κB in the liver. Rats were fasted for 12 h and orally given the red or white grape pomace extracts at 100 and 500 mg/kg BW, or 5 g/L PEG6000 solution alone as a vehicle control. After 1 h, they were intraperitoneally given LPS at 10 μg/kg BW and GalN at 250 mg/kg BW, or a physiological salt solution as a vehicle control. They were sacrificed 24 h after the LPS/GalN injection, and the DNA-binding activity of NF-κB in the liver was examined by EMSA as described under Materials and Methods. The arrow represents the specific band of NF-κB. Typical images are shown from one of four independent rats.

dose-dependently could suppress the LPS and GalN-induced DNA binding activity of NF-κB, which is a rate-controlling factor in inflammatory reactions, and the red grape pomace extract was more potent than the white grape pomace extract. Neither red nor white grape pomace extract alone caused the activation of NF-κB. LPS induces the release of tumor necrosis factor α (TNF-α) from a variety of the cells such as macrophages into the bloodstream, and TNF-α plays a critical role in the LPS and GalN-induced inflammation via the activation of NF-κB.[15] Therefore, the suppression of NF-κB activation by grape pomace extracts must lead to down-regulation of inflammatory and pro-inflammatory cytokines

including TNF-α, indicating that grape pomace, especially red grape pomace, contains an abundance of the anti-inflammatory compound(s).

The results from experiment I raise the possibility of novel functionality of red grape pomace. If an intake of the diet containing red grape pomace itself but not its extract can exert the beneficial effects, red grape pomace may widen the range of its reuse and there is a much greater chance for its reuse. In experiment II, we prepared the diet containing 5% red grape pomace and then investigated whether the red grape pomace-supplemented diet can suppress the LPS and GalN-caused inflammation. The intake of red grape pomace did not affect the body weight gain of rats and the amount of food intake (data not shown). Pro-inflammatory stimulus activates NF-κB through sequential steps of the activation of IκB kinase, phosphorylation of IκB, and release of IκB from the NF-κB–IκB complex.[16] The activated NF-κB translocates to the nucleus, acts as a transcription factor, and regulates the expressions of a battery of genes encoding iNOS, COX-2, interleukin-1β, cyclin D1, and so on.[17] Excessive accumulation of NO by iNOS is deeply involved in mutagenesis[18] and carcinogenesis.[19] In addition, NO attacks the variety of the cells as a free radical. COX2 produces pro-inflammatory mediators such as prostacyclins and prostaglandins, and prostaglandin E2 (PGE2) is one of the prostaglandins that are closely related to inflammation and carcinogenesis. Actually, a high level of PGE2 is found in breast cancer,[20] colon cancer,[21] and lung cancer.[22] Thus, hyperexpression of iNOS and COX2 proteins is associated with not only inflammatory diseases but also other diseases, and decreases in the expressions of iNOS and COX2 will lead to suppression of multiple diseases including inflammatory diseases. Therefore, we investigated whether a red grape pomace-supplemented diet can suppress the LPS and GalN-induced expressions of iNOS and COX2 proteins in the liver (Figure 2). As a result, the expressions of both proteins

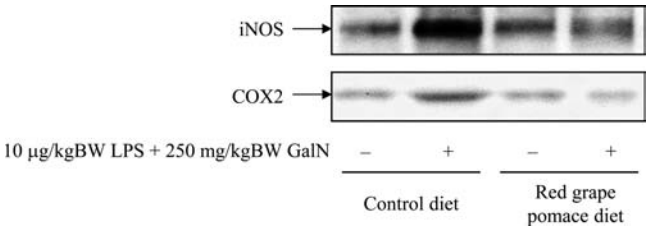


Figure 2. Effects of the red grape pomace-supplemented diet on the expression of iNOS and COX2 proteins in the liver. Rats were fed the AIN93 M diet containing 5% red grape pomace or the control diet for 7 days. They were intraperitoneally given LPS at 10 μg/kg BW and GalN at 250 mg/kg BW, or a physiological salt solution as a vehicle control. They were sacrificed 24 h after the LPS/GalN injection, and detection of iNOS and COX2 proteins in the liver was performed by Western blotting as described under Materials and Methods. Arrows represent the specific bands. Typical images are shown from one of three independent rats.

were perfectly suppressed via an intake of the red grape pomace-supplemented diet. In addition, the red grape pomace-supplemented diet could inhibit the LPS/GalN-enhanced DNA binding activity of NF-κB (Figure 3). These results exhibit that the red grape pomace-supplemented diet could suppress the expressions of iNOS and COX2 proteins through the inhibition of NF-κB activation, and the intake of red grape pomace may be effective for suppression of inflammatory diseases. The

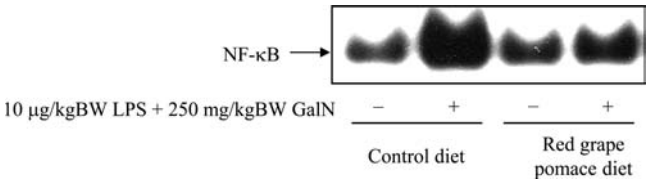
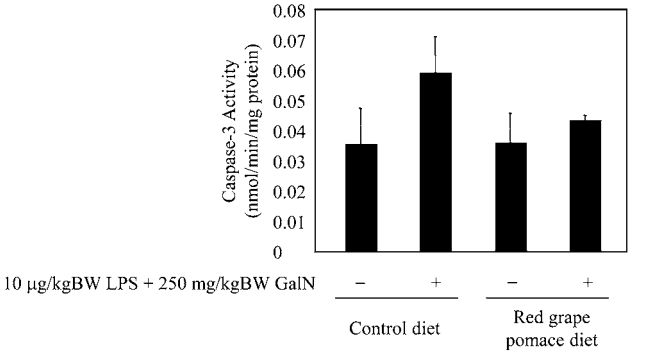


Figure 3. Effects of the red grape pomace-supplemented diet on the DNA-binding activity of NF-κB in the liver. Rats were treated as described for Figure 2. The DNA-binding activity of NF-κB in the livers was examined by EMSA as described under Materials and Methods. The arrow represents the specific band of NF-κB. A typical image is shown from one of three independent rats.

binding of TNF-α to TNF receptor stimulates not only the NF-κB signaling pathway but also the apoptosis signaling pathway.[15] Therefore, we investigated whether the red grape pomace-supplemented diet can protect rats from LPS and GalN-induced apoptosis in the liver (Figure 4). The grape

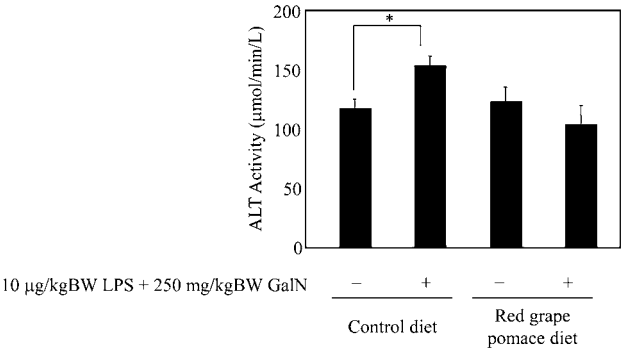


	p value
(Control + Control diet) vs (LPS&GalN + Control diet)	0.23
(Control + Control diet) vs (Control + red grape pomace diet)	0.98
(Control + Control diet) vs (LPS&GalN + red grape pomace diet)	0.55
(LPS&GalN + Control diet) vs (Control + red grape pomace diet)	0.22
(LPS&GalN + Control diet) vs (LPS&GalN + red grape pomace diet)	0.26
(Control + red grape pomace diet) vs (LPS&GalN + red grape pomace diet)	0.53

Figure 4. Effects of the red grape pomace-supplemented diet on caspase-3 activity in the liver. Rats were treated as described for Figure 2. The caspase-3 activity in the livers was measured as described under Materials and Methods. Data are shown as the mean ± SE (n = 3).

pomace-supplemented diet tended to reduce the elevated caspase-3-like activity (red grape pomace-supplemented diet + LPS and GalN vs control diet + LPS and GalN: p = 0.26), although the injection of LPS and GalN did not significantly elevate the caspase-3-like protease activity under this experimental condition (control diet vs control diet + LPS and GalN: p = 0.23). A simultaneous injection of LPS and GalN also causes liver injury, and liver injury leads to the leakage of ALT into the bloodstream, resulting in an increase in the plasma ALT activity.[23] Therefore, we examined the influence of the red grape pomace-supplemented diet on LPS and GalN-evoked liver injury. The red grape pomace-supplemented diet tended to cancel the increase in the plasma ALT activity (red grape pomace-supplemented diet + LPS and GalN vs control diet + LPS and GalN: p = 0.058) (Figure 5). These results suggest that red grape pomace suppress the LPS and GalN-caused hepatic inflammation leading to attenuation of liver injury.

In this study, red grape pomace could inhibit the activation of NF-κB and the downstream events, namely, expressions of



	p value
(Control + Control diet) vs (LPS&GalN + Control diet)	0.039*
(Control + Control diet) vs (Control + red grape pomace diet)	0.70
(Control + Control diet) vs (LPS&GalN + red grape pomace diet)	0.50
(LPS&GalN + Control diet) vs (Control + red grape pomace diet)	0.12
(LPS&GalN + Control diet) vs (LPS&GalN + red grape pomace diet)	0.058
(Control + red grape pomace diet) vs (LPS&GalN + red grape pomace diet)	0.40

Figure 5. Effects of the red grape pomace-supplemented diet on the plasma ALT activity. Rats were treated as described ifor Figure 2. The plasma ALT activity was measured as described under Materials and Methods. Data are shown as the mean ± SE (n = 3). (*) Significant differences from the corresponding control group (p < 0.05).

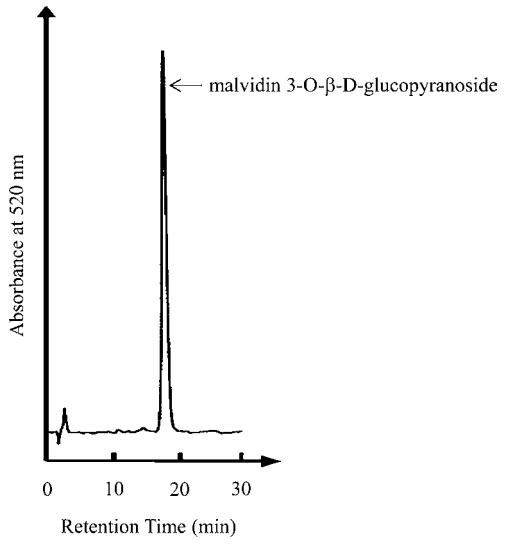


Figure 6. Detection of anthocyans in red grape pomace. Anthocyans in red grape pomace were detected by HPLC as described under Materials and Methods. A typical chromatogram at a wavelength of 520 nm is shown from one of three independent experiments. The peak for malvidin 3-*O*-*β*-D-glucopyranoside is indicated by an arrow; other anthocyans were not detected.

iNOS and COX2 proteins (Figures 2 and 3), whereas it could not suppress the increased caspase-3-like protease activity (Figure 4). Because injections of bith LPS and GalN cause the NF-κB activation and apoptosis via secretion of TNFα, the targeting point of effective compound(s) in red grape pomace may exist in the NF-κB-related pathway. Activation of NF-κB requires multiple steps, such as IκB kinase activation, IκB phosphorylation, and release of IκB from the NF-κB−IκB complex.[16] Until now, it has been demonstrated that a variety of dietary plant constituents could suppress these steps. For example, epigallocatechin-3-gallate and theaflavin inhibited the IκB kinase activity.[24,25] Epigallocatechin-3-gallate also suppressed the NF-κB activation via inhibition of the degradation of IκB.[26,27] Therefore, red grape pomace may contain active compound(s) with similar actions. Pomace from red grape (*V. vinifera*), which is the same breed as the grape used in this study, was reported to possess anthocyanins abundantly,[2] and anthocyanins could exert the anti-inflammatory effect in vivo.[28] Moreover, our results demonstrated pomace from red grape possessed stronger activity than that from white grape (Figure 1). Because red grape has a considerably abundant amount of anthocyans compared with white grape, we investigated which anthocyans exist in red grape pomace (Figure 6). As a result, among anthocyans, malvidin 3-*O*-*β*-D-glucopyranoside alone was identified in red grape pomace, and the amount was 0.714 nmol/mg red grape pomace. In a previous paper, it was revealed that malvidin could not inhibit the LPS-induced expression of COX2 protein in RAW 264 cells, whereas delphinidin and cyanidin suppressed it.[29] However, in the paper by Hou et al.,[29] the effect of malvidin 3-*O*-*β*-D-glucopyranoside on inflammatory responses was not evaluated, and so its anti-inflammatory activity has not been proven yet. Therefore, malvidin 3-*O*-*β*-D-glucopyranoside may have the potent anti-inflammatory effect. In this study, the rats ate 14.5 g of the diet containing red grape pomace/dayrat on average, and so the intake amount of red grape pomace itself was 690 mg/day/rat. On the basis of the amount of malvidin 3-*O*-*β*-D-glucopyranoside in red grape pomace, the rats ate malvidin 3-*O*-*β*-D-glucopyranoside at the concentration of 493 nmol (=243 μg)/day/rat. Compared with a previous report of the suppressive effect of the grape pomace extracts on obesity-related inflammation,[6] the intake amount of malvidin 3-*O*-*β*-D-glucopyranoside in this study seems to be relatively small. Therefore, malvidin 3-*O*-*β*-D-glucopyranoside may have strong anti-inflammatory activity, or several compounds in red grape pomace including malvidin 3-*O*-*β*-D-glucopyranoside may contribute to suppression of LPS and GalN-evoked hepatic inflammation. Resveratrol may be also one of the candidates for active compound in red grape pomace, although we did not measure the amount of resveratrol in red grape pomace. In the previous study, resveratrol was reported to exist in grape pomace abundantly.[2] Resveratrol could inhibit the phorbol ester-induced expression of COX2 protein and activation of NF-κB in an in vivo system using mouse skin.[30] In addition, it was reported that resveratrol could inhibit TNF-α-induced inflammation via Sirtuin1, which plays a role in the regulation of inflammation.[31] Resveratrol was also revealed to block the JAK/STAT-1 pathway that controls inflammatory responses in IFN-γ-activated macrophages.[32] Thus, the anti-inflammatory activity of resveratrol is well-known, and its molecular mechanisms have also started to be elucidated. Anthocyans and resveratrol may contribute to the suppression of inflammatory reactions by red grape pomace. Moreover, the presence of other effective compounds in red grape pomace cannot be denied, and further studies are required to find the active compound(s) for the detailed understanding and evaluation of grape pomace.

Grape pomace is the industrial waste generated in the production process of wine and grape juices, but the extract derived from red grape pomace could suppress NF-κB activation. Therefore, grape pomace may contain the active compound(s) for suppressing inflammatory responses and may be suitable to be used as material for the extraction and identification of anti-inflammatory agent candidates. In addition, the successive intake of a diet supplemented with

red grape pomace led to the LPS and GalN-evoked NF-κB activation and iNOS and COX2 expressions, and therefore grape pomace may be able to be used as an ingredient of supplements or functional foods in the future.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

LPS, lipopolysaccharide; GalN, galactosamine; NF-κB, nuclear factor-κB; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2; EMSA, electrophoretic mobility shift assay; MCA, methylcoumarylamide; PEG, polyethyleneglycol; ip, intraperitoneal; ALT, alanine aminotransferase; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; AMC, 7-amino-4-methylcoumarin; TFA, trifluoroacetic acid; HPLC, high-performance liquid chromatography; TNF-α, tumor necrosis factor α; PGE2, prostaglandin E2.

REFERENCES

(1) Pastrana-Bonilla, E.; Akoh, C. C.; Sellappan, S.; Krewer, G. Phenolic content and antioxidant capacity of muscadine grapes. *J. Agric. Food Chem.* **2003**, *51*, 5497−5403.

(2) Kammerer, D.; Claus, A.; Carle, R.; Schieber, A. Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *J. Agric. Food Chem.* **2004**, *52*, 4360−4367.

(3) Careri, M.; Corradini, C.; Elviri, L.; Nicoletti, I.; Zagnoni, I. Direct HPLC analysis of quercetin and *trans*-resveratrol in red wine, grape, and winemaking byproducts. *J. Agric. Food Chem.* **2003**, *51*, 5226−5231.

(4) Scalbert, A.; Manach, C.; Morand, C.; Remesy, C.; Jimenez, L. Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 287−306.

(5) Murthy, K. N.; Singh, R. P.; Jayaprakasha, G. K. Antioxidant activities of grape (*Vitis vinifera*) pomace extracts. *J. Agric. Food Chem.* **2002**, *50*, 5909−5914.

(6) Hogan, S.; Canning, C.; Sun, S.; Sun, X.; Zhou, K. Effects of grape pomace antioxidant extract on oxidative stress and inflammation in diet induced obese mice. *J. Agric. Food Chem.* **2010**, *58*, 11250−11256.

(7) Hogan, S.; Zhang, L.; Li, J.; Sun, S.; Canning, C.; Zhou, K. Antioxidant rich grape pomace extract suppresses postprandial hyperglycemia in diabetic mice by specifically inhibiting α-glucosidase. *Nutr. Metab. (London)* **2010**, *27*, 71.

(8) Miyagi, Y.; Miwa, K.; Inoue, H. Inhibition of low-density lipoprotein oxidation by flavonoids in red wine and grape juice. *Am. J. Cardiol.* **1997**, *80*, 1627−1631.

(9) Zern, T. L.; West, K. L.; Fernandez, M. L. Grape polyphenols decrease plasma triglycerides and cholesterol accumulation in the aorta of ovariectomized guinea pig. *J. Nutr.* **2003**, *133*, 2268−2272.

(10) Lizarraga, D.; Vinardell, M. P.; Noé, V.; van Delft, J. H.; Alcarraz-Vizán, G.; van Breda, S. G.; Staal, Y.; Günther, U. L.; Carrigan, J. B.; Reed, M. A.; Ciudad, C. J.; Torres, J. L.; Cascante, M. A lyophilized red grape pomace containing proanthocyanidin-rich dietary fiber induces genetic and metabolic alterations in colon mucosa of female C57BL/6J mice. *J. Nutr.* **2011**, *141*, 1597−1604.

(11) Nishiumi, S.; Yamamoto, N.; Kodoi, R.; Fukuda, I.; Yoshida, K.; Ashida, H. Antagonistic and agonistic effects of indigoids on the transformation of an aryl hydrocarbon receptor. *Arch. Biochem. Biophys.* **2008**, *470*, 187−199.

(12) Nishiumi, S.; Yoshida, M.; Azuma, T.; Yoshida, K.; Ashida, H. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin impairs an insulin signaling pathway through the induction of tumor necrosis factor α in adipocytes. *Toxicol. Sci.* **2010**, *115*, 482−491.

(13) Hashimoto, T.; Ashida, H.; Sano, T.; Furuyashiki, T.; Shiotani, B.; Kanazawa, K.; Danno, G. 3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) induces apoptosis in rat splenocytes and thymocytes by different mechanisms. *Mutat. Res.* **2000**, *457*, 57−67.

(14) Ichiyanagi, T.; Shida, Y.; Rahman, M. M.; Hatano, Y.; Matsumoto, H.; Hirayama, M.; Konishi, T. Metabolic pathway of cyanidin 3-*O*-β-D-glucopyranoside in rats. *J. Agric. Food Chem.* **2005**, *53*, 145−150.

(15) Silke, J. The regulation of TNF signalling: what a tangled web we weave. *Curr. Opin. Immunol.* **2011**, *23*, 620−626.

(16) Liu, F.; Xia, Y.; Parker, A. S.; Verma, I. M. IKK biology. *Immunol. Rev.* **2012**, *246*, 239−253.

(17) Yamamoto, Y.; Gaynor, R. B. Therapeutic potential of inhibition of NF-κB pathway in the treatment of inflammation and cancer. *J. Clin. Invest.* **2001**, *107*, 135−142.

(18) Grisham, M. B.; Jourd'heuil, D.; Wink, D. A. Review article: chronic inflammation and reactive oxygen and nitrogen metabolism–implications in DNA damage and mutagenesis. *Aliment. Pharmacol. Ther.* **2000**, *14*, 3−9.

(19) Yang, G. Y.; Taboada, S.; Liao, J. Induced nitric oxide synthase as a major player in the oncogenic transformation of inflamed tissue. *Methods Mol. Biol.* **2009**, *512*, 119−156.

(20) Bennett, A.; Charlier, E. M.; McDonald, A. M.; Simpson, J. S.; Stamford, I. F.; Zebro, T. Prostaglandins and breast cancer. *Lancet* **1977**, *2*, 624−626.

(21) Rigas, B.; Goldman, I. S.; Levine, L. Altered eicosanoid levels in human colon cancer. *J. Lab. Clin. Med.* **1993**, *122*, 518−523.

(22) Bennett, A.; Carroll, M. A.; Stamford, I. F.; Whimster, W. F.; Williams, F. Prostaglandins and human lung carcinomas. *Br. J. Cancer* **1982**, *46*, 888−893.

(23) He, P.; Noda, Y.; Sugiyama, K. Green tea suppresses lipopolysaccharide-induced liver injury in D-galactosamine-sensitized rats. *J. Nutr.* **2001**, *131*, 1560−1567.

(24) Yang, F.; Oz, H. S.; Barve, S.; de Villiers, W. J.; McClain, C. J.; Varilek, G. W. The green tea polyphenol (−)-epigallocatechin-3-gallate blocks nuclear factor-κB activation by inhibiting IκB kinase activity in the intestinal epithelial cell line IEC-6. *Mol. Pharmacol.* **2001**, *60*, 528−533.

(25) Aneja, R.; Odoms, K.; Denenberg, A. G.; Wong, H. R. Theaflavin, a black tea extract, is a novel anti-inflammatory compound. *Crit. Care Med.* **2004**, *32*, 2097−2103.

(26) Khan, N.; Afaq, F.; Saleem, M.; Ahmad, N.; Mukhtar, H. Targeting multiple signaling pathways by green tea polyphenol (−)-epigallocatechin-3-gallate. *Cancer Res.* **2006**, *66*, 2500−2505.

(27) Singh, R.; Ahmed, S.; Islam, N.; Goldberg, V. M.; Haqqi, T. M. Epigallocatechin-3-gallate inhibits interleukin-1β-induced expression of nitric oxide synthase and production of nitric oxide in human chondrocytes: suppression of nuclear factor κB activation by degradation of the inhibitor of nuclear factor κB. *Arthritis Rheum.* **2002**, *46*, 2079−2086.

(28) Yamaura, K.; Shimada, M.; Ueno, K. Anthocyanins from bilberry (*Vaccinium myrtillus* L.) alleviate pruritus in a mouse model of chronic allergic contact dermatitis. *Pharmacognosy Res.* **2011**, *3*, 173−177.

(29) Hou, D. X.; Yanagita, T.; Uto, T.; Masuzaki, S.; Fujii, M. Anthocyanidins inhibit cyclooxygenase-2 expression in LPS-evoked macrophages: structure-activity relationship and molecular mechanisms involved. *Biochem. Pharmacol.* **2005**, *70*, 417−425.

(30) Kundu, J. K.; Shin, Y. K.; Kim, S. H.; Surh, Y. J. Resveratrol inhibits phorbol ester-induced expression of COX-2 and activation of NF-κB in mouse skin by blocking IκB kinase activity. *Carcinogenesis* **2006**, *27*, 1465−1474.

(31) Zhu, X.; Liu, Q.; Wang, M.; Liang, M.; Yang, X.; Xu, X.; Zou, H.; Qiu, J. Activation of Sirt1 by resveratrol inhibits TNF-α induced inflammation in fibroblasts. *PLoS One* **2011**, *6*, e27081.

(32) Chung, E. Y.; Kim, B. H.; Hong, J. T.; Lee, C. K.; Ahn, B.; Nam, S. Y.; Han, S. B.; Kim, Y. Resveratrol down-regulates interferon-γ-

inducible inflammatory genes in macrophages: molecular mechanism via decreased STAT-1 activation. *J. Nutr. Biochem.* **2011**, *22*, 902−909.