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Effects of a Polysaccharide Fraction from the Roots of *Bupleurum falcatum* L. on Experimental Gastric Ulcer Models in Rats and Mice

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Abstract—Effects of an acidic polysaccharide fraction, BR-2, from the roots of *Bupleurum falcatum* L., on HCl-ethanol, ethanol and water immersion stress-induced gastric lesions in mice and pylorus-ligated ulcers in rats have been studied. Oral administration of BR-2 at doses of 50 to 200 mg kg^{-1} inhibited the formation of the gastric lesions induced by necrotizing agents such as HCl-ethanol and ethanol, in a dose dependent manner. This protective effect was observed after oral, intraperitoneal, and subcutaneous administration of BR-2 (25-100 mg kg^{-1}). BR-2 also inhibited the formation of gastric ulcers which were induced by water immersion stress or pylorus-ligation. Prostaglandin E_2 in gastric juice from rats and in gastric mucosa from mice was not influenced by oral administration of BR-2. The protective action of BR-2 against HCl-ethanol-induced gastric lesions was not abolished by pretreatment with indomethacin (20 mg kg^{-1}, i.p.). The amount of alcian blue binding to mucosa also increased after administration of BR-2 (100 mg kg^{-1}, p.o.); however, the amount of hexosamine and *N*-acetylneuraminic acid in mucosa did not change significantly.

The roots of *Bupleurum falcatum* L. have been used clinically in Chinese and Japanese herbal medicine (Kampo medicine) for the treatment of chronic hepatitis, nephrotic syndrome and auto-immune diseases. The roots of *B. falcatum* contain several saponins (Aimi et al 1968), and their various pharmacological activities have been reported (Takagi & Shibata 1969; Abe et al 1982, 1986; Mizoguchi et al 1985). However, the clinical efficacy of the roots of *B. falcatum* cannot be explained by the action of saponin alone. Shibata et al (1973) reported that a crude saponin fraction showed weak anti-ulcerogenic activity in the pylorus-ligated ulcer model; however, no active principle was characterized (Shibata et al 1973).

Recently, we found a potent inhibitory activity against HCl-ethanol-induced ulcerogenesis in mice by the polysaccharide fraction of *B. falcatum* and the purified active polysaccharides, Bupleurans 2IIb and 2IIc (Yamada et al 1991b). These active polysaccharides were characterized as pectin-like polysaccharides which consist of a large polygalacturonan region and a small rhamnogalacturonan region with neutral side chains (Yamada et al 1989, 1991a). The activity was sensitive to periodate oxidation and endo-polygalacturonase digestion but resistant to pronase digestion (Yamada et al 1991b).

The present paper deals with the protective activity of a polysaccharide fraction from *B. falcatum* and its mechanism of anti-ulcer activity on several experimental gastric ulcer models in mice and rats.

Materials and Methods

Chemicals

16,16-Dimethyl prostaglandin E_2 (16-dmPGE$_2$) was purchased from Caymen Chemical Co., MI. ^{125}I-PGE$_2$ RIA KIT was obtained from New England Nuclear, MA. Indomethacin and L-tyrosine were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Alcian blue 8GX was purchased from Sigma, MO. Bovine serum albumin was purchased from Boehringer Mannheim (Germany).

Animals

Male ICR mice (SLC, Shizuoka, Japan) 8-10 weeks, 35-40 g, and male Wistar rats (SLC, Shizuoka, Japan) 8-10 weeks, 180–200 g, were housed and maintained at 24 ± 1°C and constant humidity (55%). After a period of fasting (24 h for mice, 48 h for rats), but with free access to water, the animals were used for the experiments.

General methods

Total carbohydrate, uronic acid, and protein contents were assayed by the phenol-sulphuric acid (Dubois et al 1956), *m*-hydroxybiphenyl (Blumenkrantz & Asboe-Hansen 1973), and Lowry (Lowry et al 1951) methods, respectively, using arabinose, galacturonic acid, and bovine serum albumin as the respective standards. Neutral and acidic components of sugars were analysed by GLC and their molar ratios calculated as described previously (Yamada et al 1987a). HPLC was performed on a Waters model ALG/GPC 244 equipped with a column of Asahi-pak GS-510+GS-320 (vinylalcohol co-polymer, Asahi Chemical Industry Co. Ltd, Japan) equilibrated with 0·2 M NaCl and the molecular weight was estimated by HPLC as described previously (Yamada et al 1987b).

Preparation of anti-ulcer polysaccharide (BR-2)

The crude polysaccharide fraction (BR-1) was prepared by hot-water extraction and precipitation with ethanol from the roots of *B. falcatum* L. as described previously (Yamada et al 1988). BR-1 was fractionated by treatment with cetyltrimethylammonium bromide (Cetavlon), and the acidic poly-

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saccharide fraction (BR-2) was obtained as the precipitate (Yamada et al 1989). BR-2 dissolved in water was administered at a volume of 10 mL kg^{-1} 50 min (for mouse) or 30 min (for rat) before the ulcer-inducing treatment of the animals. Control animals received water only.

Induction of ulcerogenesis in mice and rats

HCl-ethanol-induced gastric mucosal membrane lesions. The method is based on the modifications of Mizui & Doteuchi (1983). The mice were divided into groups of 8–10. After 24 h fasting, the mice were administered test drugs or water orally. Fifty min later, each mouse received 0·2 mL of 0·3 M HCl-60% ethanol orally. Each animal was killed by cervical vertebral dislocation 1 h after the administration of the necrotizing agent, and the stomach was excised and inflated by injection of saline (2 mL). The ulcerated stomachs were fixed in 5% formalin for 30 min. After opening along the greater curvature, gastric damage visible to the naked eye was found in the gastric mucosa as elongated black-red lines (1–10 mm long by 0·5–1·5 mm wide) parallel to the long axis of the stomach in mice, and were similar to HCl-ethanol induced lesions in rats. The lesion index was expressed as the sum of the length (mm) of all lesions in the fundic region.

To test the effect of indomethacin (20 mg kg^{-1}), the drug was suspended in saline with a trace amount of Tween 80, and administered to mice intraperitoneally 1 h before the dose of BR-2; 50 min after BR-2 administration, each mouse received HCl-ethanol orally.

Ethanol-induced gastric mucosal membrane lesions. The method is based on that of Robert et al (1979), and was carried out as above except that absolute ethanol was used as the necrotizing agent instead of HCl-ethanol.

Water immersion stress-induced gastric lesions. The method was carried out according to the modification of Yano & Harada (1973). The mice were given test samples orally and then immobilized in restraint cages. Fifty min later, animals were vertically immersed to the level of the xiphoid process in a water bath maintained at 19 ± 1°C. Seven h later, the mice were killed by cervical vertebral dislocation. The stomachs were immediately removed and inflated with saline solution (2 mL) and fixed in 5% formalin for 30 min. The lengths of haemorrhagic lesion were measured and the sum for each animal was taken as the ulcer index.

Pylorus-ligated gastric ulcers. The method was that of Shay et al (1945). The rats were fasted for 48 h, the abdomen was incised and the pylorus was ligated under ether anaesthesia 30 min after each test sample was administered orally. Twelve h after pyloric ligature, the stomach was excised, and the gastric juice was transferred to test tubes. The stomach was inflated by injecting saline (10 mL) and fixed in 5% formalin for 30 min. After incision along the greater curvature, degree of ulceration in the forestomach was estimated, using the following scoring system: no ulcer, grade zero; 1–5 ulcers 1 mm in diameter, grade one; 6–10, grade two; > 10, grade three; 1–5 streaked ulcers, grade four; 6–10, grade five; > 10 streaks or perforation, grade six. The ulcer grade was obtained from the sum of the number of ulcers multiplied by the corresponding score. The amount of haemoglobin in gastric juice was measured by employing a pseudoperoxidase reaction catalyzed by haemoglobin (Naumann 1964). The absorbance was measured at 405 nm and expressed as optical density.

Measurement of gastric secretions in rats and mice

Gastric acid and pepsin activity. The gastric juice from pylorus-ligated gastric ulcers was centrifuged at 3500 rev min^{-1} for 30 min and the volume of gastric juice from each rat was measured. Free and total acidities in the supernatants were determined by titration with 0·01 M NaOH according to the method of Reitman (1970) and expressed as mEq mL^{-1} gastric juice. Pepsin activity in the supernatants was determined using bovine serum albumin (0·5% (w/v) in 0·01 M HCl, pH 2) as substrate, and was expressed in terms of the amount of liberated tyrosine per 1 mL of gastric juice according to the methods of Prino et al (1971).

Amounts of mucus adherent to mucosal surface. Alcian blue binding to mucus adherent to mucosal surface was measured by the method of Bolton et al (1978). Each test sample or water was given orally to mice or rats fasted for 24 or 48 h, the abdomen was incised after 30 min, and the pylorus was ligated under ether anaesthesia. The animals were killed by cervical vertebral dislocation 4 h after ligation. The everted stomach was immersed immediately in either 10 mL (for mice) or 15 mL (for rats) 0·02% alcian blue-0·16 M sucrose in 0·05 M sodium acetate (pH 5·8), and incubated for 2 h. The alcian blue binding extract was centrifuged at 3000 rev min^{-1} for 10 min and the absorbance of supernatant was measured at 615 nm. Amount of mucus adherent to the mucosal suface was calculated from the amount of alcian blue binding to mucosa and expressed as mg alcian blue per g wet tissue.

Contents of N-*acetylneuraminic acid (NANA) and hexosamine in gastric mucosal layer.* The mucosal tissue layers of untreated mice which were treated with test drugs or water for 60, 120, and 240 min, were frozen by immersion in hexane in a dry ice-acetone bath as described by Kobayashi et al (1985). Each gastric mucosal tissue was weighed, homogenized in 2 mL of 90% ethanol and centrifuged at 3500 rev min^{-1} for 30 min. The precipitate was rehomogenized in water, and a part of this homogenate was hydrolysed with HCl. Hydrolysis conditions were as follows: 0·1 M HCl at 80°C for 60 min for NANA; 4 M HCl at 110°C for 16 h for hexosamine. NANA was measured by the fluorometric HPLC method of Hara et al (1989). HPLC was performed on a Shimadzu LC-6A system equipped with a column of TSK gel ODS-120T (5 μm, 4·6 × 150 mm, Tosoh Co.) and a Shimadzu RF-530 fluorescence spectrometer (Tokyo, Japan). A mixture of methanol-acetonitrile-water (3:1:10) was used as a mobile phase at a flow rate of 1·0 mL min^{-1}. Hexosamine was measured using a Hitachi model-835 amino acid analyser (Tokyo, Japan).

Extraction of PGE_2 from rat gastric juice. The method described by Powell (1980) was used with a modification for the final eluent for PGE_2. The gastric juice obtained in 4 h pylorus-ligated rats was centrifuged at 3500 rev min^{-1} for 30 min the supernatant solution was diluted with water and adjusted to pH 3·0 with 1 M HCl. The solution was loaded on

Table 1. Physicochemical properties of anti-ulcer polysaccharides.

	BR-2	Bupleuran 2IIb	Bupleuran 2IIc
Molecular weight (HPLC)	60000 ~ 30000	23000	63000
$[\alpha]_D$	n.d.	+120·0°	+199·7°
Neutral sugar	19·2%	23·4%	5·3%
Uronic acid	75·0%	75·5%	93·6%
Protein	5·8%	1·1%	1·1%
Methyl ester	n.d.	45·7%	3·6%
Acetyl ester	n.d.	n.d.	1·88 (w/w)%
Component sugar (mol. ratio)			
rhamnose	1·0	1·4	1·1
arabinose	1·9	2·1	1·1
xylose	0·1	trace	0·2
mannose	0·1	—	0·3
galactose	1·0	1·0	1·0
glucose	0·5	trace	0·4
galacturonic acid	11·6	13·0	61·5
glucuronic acid	—	—	—

Not determined, n.d.

a Sep-pak C18 cartridge (Waters Associates MA). The cartridge was washed with 20 mL of water and then with 20 mL of 15% ethanol in water, followed by 20 mL *n*-hexane to remove non-polar compounds. PGE_2 was eluted with 20 mL of a solvent mixture (ethylacetate : methanol, 9 : 1). The eluate was evaporated under reduced pressure at room temperature (21°C) and the residue was subjected to RIA for PGE_2. The recovery of PGE_2 from homogenate was 88·4% (the mean of two separate experiments).

Extraction of PGE_2 from mouse mucosa. The mucosal tissue layers of mice were frozen by immersion in hexane in a dry ice-acetone bath as described by Kobayashi et al (1985), homogenized in 2 mL of 90% ethanol containing 10 μM of indomethacin and centrifuged at 3500 rev min^{-1} for 30 min. The supernatant was diluted with water, adjusted to pH 3·0 and subjected to Sep-pak C18 cartridge fractionation as described above. The recovery of PGE_2 from gastric juice was 99·6%.

Statistical analyses of data

Data obtained from pharmacological experiments are expressed as mean ± s.e. Differences between the control and the treatment in these experiments were tested for statistical significance by Student's *t*-test.

Results and Discussion

The results of all the experiments are summarized in Tables 2–11.

Recently, we described the purification of two anti-ulcer acidic polysaccharides, Bupleurans 2IIb and 2IIc, which have potent inhibitory activity against the formation of HCl-ethanol-induced gastric ulcers in mice, from the roots of *Bupleurum falcatum* (Yamada et al 1991b). The strong acidic polysaccharide fraction, BR-2, showed most potent anti-ulcer activity among the polysaccharide fractions which were fractionated by cetavlone precipitation of crude polysaccharides (BR-1) (Yamada et al 1988, 1991b), and this activity was similar to the anti-ulcer agent, sucralfate at the same dose. Cytoprotective action of many drugs has been investigated using animal models of acute gastric injury induced by necrotizing agents such as ethanol, HCl and HCl-ethanol (Konturek et al 1983; Martin et al 1983; Shiratsuchi et al 1988). BR-2 significantly protected against a variety of gastric lesions, water immersion stress ulcer and pylorus-ligated ulcer in mice or rats (Tables 2, 3, 4, 5).

BR-2 was effective against HCl-ethanol induced gastric lesions by oral, intraperitoneal and subcutaneous administration (Table 2), indicating that BR-2 acted through a local action and a systemic action in the stomach. The cytoprotective action of some anti-ulcer drugs and the action of mild irritants are mediated by the action of endogenous prostaglandins known to play an important role in maintaining mucosal integrity (Miller 1983) and to protect the gastric mucosa against various damaging agents (Chaudhury &

Table 2. Effect of BR-2 on HCl-ethanol-induced gastric lesions in mice.

Treatment	Dose (mg kg^{-1})	Ulcer index	Inhibition (%)
Exp. 1 (p.o.)			
Control (vehicle)	—	24·4 ± 4·29	—
BR-2	200	3·9 ± 1·42**	84·0
BR-2	100	9·0 ± 1·88**	63·1
BR-2	50	12·5 ± 2·54*	48·8
BR-2	25	18·4 ± 2·84	24·6
Sucralfate	100	7·5 ± 2·07**	69·3
Exp. 2 (i.p.)			
Control (vehicle)	—	29·0 ± 4·95	—
BR-2	50	11·8 ± 3·25*	59·3
BR-2	25	17·1 ± 2·71*	41·0
BR-2	12·5	21·5 ± 4·30	25·9
16-dmPGE_2	0·005	5·8 ± 2·13**	80·0
Exp. 3 (s.c.)			
Control (vehicle)	—	17·6 ± 3·03	—
BR-2	100	8·9 ± 1·98*	49·4
BR-2	50	10·4 ± 2·07	40·9
BR-2	25	14·0 ± 2·26	20·5
BR-2	12·5	15·7 ± 2·64	10·8
16-dmPGE_2	0·005	3·9 ± 1·42**	77·8

Expressed as mean ± s.e. * $P < 0·05$, ** $P < 0·01$. n = 8 or 9.

Table 3. Effect of BR-2 on absolute ethanol induced gastric lesions in mice.

Treatment (p.o.)	Dose (mg kg^{-1})	n	Ulcer index	Inhibition (%)
Control	—	8	22·6 ± 3·5	—
BR-2	200	8	2·6 ± 1·0***	88·5
BR-2	100	8	7·7 ± 2·9**	65·5
BR-2	50	8	11·1 ± 2·8*	50·9
BR-2	25	8	18·7 ± 5·2	17·3
Sucralfate	100	8	5·9 ± 2·6**	73·8

Expressed as mean ± s.e. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 4. Effect of BR-2 on water immersion stress-induced gastric lesions in mice.

Treatment (p.o.)	Dose (mg kg^{-1})	n	Ulcer index	Inhibition (%)
Control	—	8	5·40 ± 0·73	—
BR-2	200	8	2·60 ± 0·79*	51·9
BR-2	100	8	3·13 ± 0·66*	42·0
BR-2	50	8	3·75 ± 0·72	30·6
16-dmPGE$_2$	0·025	8	1·12 ± 0·52***	79·3
Sucralfate	100	8	2·75 ± 0·75*	49·1

Expressed as mean ± s.e. * $P < 0.05$, *** $P < 0.001$.

Table 8. Effect of BR-2 on PGE_2 contents in gastric mucosa of untreated mice.

Treatment (p.o.)	Dose (mg kg^{-1})	n	Time after administration (min)	PGE_2 contents in mucosa (ng (g tissue)$^{-1}$)
Control	—	16	60	311 ± 25
BR-2	200	8	30	305 ± 25
BR-2	200	8	60	347 ± 50
BR-2	200	8	90	415 ± 52
Sucralfate	200	8	60	338 ± 42

Expressed as mean ± s.e.

Robert 1980; Robert et al 1983). Because the protective action of BR-2 was not abolished by pretreatment with indomethacin, an inhibitor of prostaglandin biosynthesis (Table 6), and the levels of PGE_2 in gastric juice and the mucosal layer were not affected by the oral administration of BR-2 (Tables 7, 8), it was suggested that endogenous prostaglandins did not mediate the protective action of BR-2. Gastric acid and pepsin are important factors for ulceration of pylorus-ligation ulcer in rats (Shay et al 1945). BR-2 markedly inhibited the formation of pylorus-ligated ulcer in the forestomach, decreased acidic concentration, inhibited pepsin activity, and reduced haemorrhage from the ulcer

Table 5. Effect of BR-2 on pyloric ligation-induced gastric lesions in rats.

Treatment (p.o.)	Dose (mg kg^{-1})	n	Grade of ulcer	Inhibition of ulcer formation %	Haemorrhage (OD_{405})
Control	—	9	3·75 ± 0·46	—	0·49 ± 0·07
BR-2	200	8	1·88 ± 0·40**	50·1	0·21 ± 0·07*
Sucralfate	100	8	1·63 ± 0·49**	56·5	0·24 ± 0·05*

Expressed as mean ± s.e. * $P < 0.05$, ** $P < 0.01$.

Table 6. Effect of pretreatment of indomethacin on the preventive effect of BR-2 against HCl ethanol-induced gastric lesion.

Treatment (p.o.)	Dose (mg kg^{-1})	Control (vehicle)		Indomethacin	
		Ulcer index	Inhibition (%)	Ulcer index	Inhibition (%)
Control	—	22·1 ± 3·5	—	36·9 ± 5·5	—
BR-2	200	7·9 ± 1·6**	64·3	13·1 ± 2·5*	64·5
16-dmPGE$_2$	0·025	2·3 ± 0·9***	89·6	4·4 ± 1·6**	88·1
Sucralfate	100	8·3 ± 1·8**	62·4	29·0 ± 5·3	21·4

Indomethacin (20 mg kg^{-1}) or vehicle (saline) was given intraperitoneally 60 min before oral administration of BR-2. Expressed as mean ± s.e. (n = 8) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 7. Effect of BR-2 on endogenous PGE_2 contents in gastric juice from pylorus-ligated rats.

Treatment (p.o.)	Dose (mg kg^{-1})	n	PGE_2 contents in gastric juice (μg/rat)
Control	—	12	1·26 ± 0·29
BR-2	200	8	0·89 ± 0·30
Sucralfate	100	8	2·40 ± 1·06*

Expressed as mean ± s.e. * $P < 0.05$.

(Tables 5, 9). These results suggested that BR-2 can suppress gastric damage induced by aggressive factors. Because BR-2 had no effects on pepsin activity and acidic concentration by the direct addition to gastric juice, it is suggested that the effect of BR-2 on the ulcerogenesis is caused by the indirect action on gastric secretion.

Certain anti-ulcer drugs increase the amount of gastric mucus secretion in gastric mucosa (Bolton et al 1976; Johansson & Kollberg 1979; Nezamis & Robert 1980; Murakami et al 1981; Robert et al 1984). This gastric mucus

Table 9. Effect of BR-2 on biochemical parameters in gastric juice from pylorus ligated rats.

Treatment (p.o.)	Dose (mg kg^{-1})	n	Volume of juice (mL)	Pepsin activity (L-tyrosine mg mL^{-1} h^{-1})	Total gastric acid (mEq mL^{-1})	Free gastric acid (mEq mL^{-1})
Control	—	9	6·92 ± 1·38	31·4 ± 5·5	0·320 ± 0·060	0·151 ± 0·019
BR-2	200	8	7·66 ± 2·31	16·1 ± 2·6***	0·213 ± 0·067*	0·126 ± 0·028**
Sucralfate	100	8	8·04 ± 2·87	16·9 ± 2·0***	0·116 ± 0·080***	0·067 ± 0·021***

Expressed as mean ± s.e. * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

consists of mucin-type glycoproteins which can be detected by the amounts of hexosamine and NANA, and alcian blue binding (Bolton et al 1978; Clamp et al 1978). BR-2 increased the alcian blue binding to mucosa (Table 10); however, the amount of hexosamine and NANA in mucosa did not change significantly (Table 11). Alcian blue dye is able to bind to negatively charged materials, and this increase in bound alcian blue suggests the presence of bound BR-2 on the surface of mucosa. These results suggest that the protective effect of orally administered BR-2 may be via the formation of a mucin-like gel layer of BR-2 on the surface of mucosa, or via the formation of protecting complexes between BR-2 and mucus as a barrier against several agents introduced into the stomach or via endogenously formed acid and pepsin in the stomach. In our earlier study, we observed a significant increase in hexosamine and NANA content as measured by colorimetric methods, in gastric juice from pylorus-ligated mice and rats and in gastric mucosa of untreated mice 90 min after oral administration of BR-2. However, the present results, obtained by HPLC and amino acid analysis, showed that BR-2 influenced the colorimetric methods for NANA and hexosamine, and HPLC and amino acid analysis should be more accurate for the measurement of NANA and hexosamine in gastric mucus.

Table 10. Effect of BR-2 on alcian blue binding to gastric mucosa from pylorus-ligated mice and rats.

Treatment (p.o.)	Dose (mg kg^{-1})	n	Bound alcian blue (mg (g wet tissue)$^{-1}$)
Exp. 1 (mice)			
Control	—	9	0·625 ± 0·066
BR-2	200	8	0·775 ± 0·094**
Sucralfate	100	8	0·705 ± 0·112
Exp. 2 (rats)			
Control	—	8	1·450 ± 0·122
BR-2	200	10	2·040 ± 0·160*
16-dmPGE$_2$	0·025	8	2·050 ± 0·187*
Sucralfate	100	10	1·960 ± 0·163*

Expressed as mean ± s.e. * $P < 0·05$, ** $P < 0·01$.

The present results also suggest that the major mechanism of gastric mucosal protection by orally administered BR-2 may be due to reinforcement of resistance of the mucosal barrier by a protective coating due to BR-2 in addition to its anti-secretory activity on acid and pepsin, as a possible explanation for the mode of action of orally administered BR-2 and a direct cytoprotective action can not be excluded.

In conclusion, BR-2, with both protective and anti-secretory activities, is consistently active in various experimental ulcer models and may be useful in the treatment of peptic ulcer in man. The mechanism of anti-ulcer activity by BR-2 is not proven from the present results and requires further investigation.

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Table 11. Effects of BR-2 on hexosamine and NANA contents in gastric mucosa of untreated mice.

Treatment (p.o.)	Dose (mg kg^{-1})	n	Time after administration (min)	Glucosamine (mg (g tissue)$^{-1}$)	Galactosamine (μg (g tissue)$^{-1}$)	NANA (μg (g tissue)$^{-1}$)
Control	—	10	60	2·22 ± 0·06	736 ± 24	229 ± 7
	—	10	120	2·21 ± 0·09	708 ± 40	228 ± 6
	—	10	240	2·35 ± 0·10	726 ± 30	235 ± 11
BR-2	100	10	60	2·24 ± 0·10	832 ± 50	220 ± 6
	100	10	120	2·41 ± 0·09	800 ± 35	251 ± 13
	100	10	240	2·23 ± 0·07	736 ± 22	230 ± 11

The contents were expressed as mg or μg per g wet tissue (mean ± s.e.).

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