Long-term effects of a low dosage of grape seed proanthocyanidin extract on blood pressure in spontaneously hypertensive rats

Nico J. Allers[a*], Leon Hay[a], Paul J. Schutte[a], Christiaan M.L. Steinmann[b*], Sandra H. du Plooy[b] and Linde H. Böhmer[b]

Most studies on the antihypertensive effects of bioflavonoids have reported short-term effects (within 7 weeks) at high concentrations (40–100 mg kg^{-1} day^{-1}). The present study by contrast has investigated long-term effects of low concentrations of bioflavonoids on arterial blood pressure and left ventricular performance in spontaneously hypertensive rats (SHR). Spontaneously hypertensive rats were divided into a treated (n = 16) and a control (n = 16) group. The treated group received daily a grape seed proanthocyanidin extract (GSPE) at a concentration of 4 mg kg^{-1} day^{-1} over six months. Arterial blood pressure (ABP) was measured once monthly on six randomly selected rats from both groups using an indirect tail-cuff method. After three months, the remaining rats underwent catheterizations to measure left ventricular performance and aortic pressure. The possible role of nitric oxide (NO) in the effects of GSPE was investigated by blocking NO synthase with N-nitro-L-arginine methyl ester (L-NAME). Animals in the treated group had significantly lower arterial end-diastolic pressures (AEDP) after three months of treatment compared with control animals, and this trend continued until six months. In the treated group, left ventricular systolic pressures (LVSP) were reduced by 16.6% (P = 0.005), their dP/dt_{max} (left ventricular pressures) were reduced by 19.7% (P = 0.050), and cardiac work was reduced by 22.0% (P = 0.045) at the end of three months. Treatment with L-NAME suggested a contribution of NO to the effects of GSPE on blood pressure. A low concentration of GSPE administered over six months lowered AEDP significantly, and the L-NAME response suggested that NO is involved. The decreased AEDP had a lowering effect on left ventricular dynamics of hypertensive rats.

Introduction

In the case of the Dietary Approaches to Stop Hypertension (DASH) diet, it was found that the inclusion of increased bioflavonoids in the diet substantially reduced ABP in humans.[1] Most animal studies on the antihypertensive effects of bioflavonoids reflect short-term effects (1–7 weeks) at high concentrations (40–100 mg kg^{-1} day^{-1}).[2–7] Little information is available on long-lasting antihypertensive effects at bioflavonoid concentrations of 4–10 mg kg^{-1} day^{-1} in animals, a concentration which would correlate better with the bioflavonoid intake in humans following a diet rich in fruit and vegetables. Various human studies have provided evidence that bioflavonoids have a protective effect in lowering BP.[8–10] The DASH diet, rich in fruits, vegetables and low-fat dairy foods, reduced arterial peak systolic pressure (APSP) by 5.5 mm Hg or more, and arterial end diastolic pressure (AEDP) by at least 3.0 mm Hg.[11]

In an observational study based on a parametric model, it was suggested that a long-term drop in AEDP of between 5 and 6 mm Hg was associated with an approximated 35–40% reduction in the number of cerebral strokes and a 20–25% reduction in the number of CHD.[12,13] Another study suggested that a reduction in AEDP as small as 2 mm Hg could result in a 15% decline in the risk of strokes and a 6% decline in the risk of coronary heart disease (CHD).[14]

A cross-cultural correlation study[9] involving seven countries indicated that the mean average bioflavonoid intake varied from 2.6 mg day^{-1} in west Finland to 68.2 mg day^{-1} in Ushibuka, Japan, whereas the average bioflavonoid intake in the Zutphen study,[8] which examined the relation between bioflavonoids and mortality in elderly men, was 26 mg day^{-1}. When the previous results are taken into account, the 68.2 mg day^{-1}(rounded off as 70 mg day^{-1}) (or ~1 mg kg^{-1} day^{-1} for a 70-kg human) value can be considered as the highest natural bioflavonoid intake in humans. Since adult rats have a metabolic rate about four times faster than humans,[15] a matching GSPE dose would be 4 mg kg^{-1}day^{-1} for these animals.

The aim of this study was to assess long-term blood pressure-lowering effects of bioflavonoids in a hypertensive rat model consuming GSPE at a concentration which reflects a human diet rich in fruits and vegetables. In addition, the effects of GSPE on left ventricular variables were determined, and the question asked whether NO played a role in the BP-lowering effects of GSPE.

Materials and methods

See Appendix.

Results

Effects of GSPE on arterial blood pressure over a six-month period

Between three and six months, during which the AEDP of the control group had increased to a level above 100 mm Hg, a significant lowering and stabilizing effect of the AEDP was seen in the GSPE-treated group (Table 1).

Effects of GSPE on left ventricle parameters and aortic blood pressure at three months

Rats receiving the GSPE had significantly lower cardiac parameters (except LVEDP, HR and VBR, which did not change significantly) when compared with the control group (Table 2). Their LVPSP was less by 16.6%, their dP/dt_{max} by 19.7% and CW by 22.0%. The A_oPSP, A_oEDP, MA_oP, and A_oPP values recorded during this procedure were also significantly lower in the treated group than in the control group (Table 2).

Effects of L-NAME on GSPE-treated rats after three months

The results show a drop in A_oDBP of 12.5 ± 4.5 mm Hg for the control group and 28.7 ± 4.7 mm Hg for the treated group (P = 0.019) with the L-NAME intervention. L-NAME had a much greater effect on the A_oEDP of treated animals (130% higher) than on the non-treated control animals, while all the other parameters (A_oPSP, MA_oP, and HR) were not significantly affected by the L-NAME procedure (Table 3).

Discussion and conclusion

The study indicated that a low dosage of GSPE (4 mg kg^{-1} day^{-1}) given to SHR daily over a six-month period led to lower AEDP

[a]Department of Physiology, University of Limpopo, Medunsa Campus, P.O. Box 130, Medunsa 0204, South Africa.
[b]Department of Biomedical Sciences, Tshwane University of Technology, Pretoria, South Africa.
*Authors for correspondence. E-mail: nico@medunsa.ac.za or steinmannc@tut.ac.za

Table 1. Effect of GSPE (4 mg kg^{-1}day^{-1}) on arterial blood pressure (tail-cuff) over a six-month period (n = 6). Values are mean pressures in mm Hg ± s.e.m. Significance of difference between the control and treated group at each time interval was assessed by Student's t-test, $P \leq 0.05$ considered to be significant.

	1 month	2 month	3 month	4 month	5 month	6 month
APSP: Control	123 ± 4.3	122 ± 5.2	146 ± 6.8	148 ± 6.4	148 ± 3.9	152 ± 3.7
GSPE treated	118 ± 2.8	146 ± 2.7*	151 ± 6.2	148 ± 3.8	147 ± 3.7	143 ± 4.3
AEDP: Control	77 ± 3.9	89 ± 4.9	105± 3.5	103 ± 1.8	107 ± 3.1	111 ± 2.5
GSPE treated	75 ± 4.1	79 ± 5.8	94 ± 2.2*	91 ± 3.7*	92 ± 2.8*	91 ± 2.9*
MAP: Control	90 ± 5.2	100 ± 4.8	115 ± 4.6	118 ± 3.7	117 ± 3.2	124 ± 2.6
GSPE treated	88 ± 3.9	101 ± 4.6	113 ± 1.4	113 ± 2.8	109 ± 3.3	111 ± 2.3*

values than those of control rats (Table 1); this lowering effect became significant after three months compared with the control group. The higher indirect APSP for the treated group compared with that of control group at the end of month 3 cannot be explained; because arterial systolic blood pressure is predominantly a function of the heart's pressure development and not of arterial action, and as the direct effect of GSPE is mainly on arterial dynamics, systolic pressure is not the parameter of interest, however. The aortic blood pressures (Table 2), especially the A_oPSP, did not correspond with the blood pressures measured at the end of the third month with the tail-cuff method; different states of consciousness could have contributed to this. (It is well documented that blood pressures taken with the tail-cuff method are lower than intra-arterial measured aortic pressures.[17,18]) The decreased contractility as reflected by the decrease in dP/dt_{max}, as well as the decrease in LVPSP in the treated group, suggest the beneficial effect of a reduced afterload caused by GSPE (Table 2). The decreased contractility in this study should not be confused with decreased contractility during pathological fibrosis, since the results did not show any significant difference in the ventricular weight/body weight ratio (VBR) (Table 2), indicating the absence of myocardial hypertrophy during the first six months in the lifespan of the SHR. As the AEDP values were lowered in the treated group (Table 1), their left ventricles in turn generated less pressure to eject blood into the aortas as seen from the reduced LVPSP, reduced dP/dt_{max} and reduced CW (Table 2).

We surmised that the decrease in contractility in the treated group might be attributable to a NO-induced reduction in afterload. Administration of L-NAME, a NO synthase inhibitor, resulted in a larger increase in A_oEDBP in the treated group (Table 3). This suggests that higher concentrations of NO might have been present in the GSPE-treated group when compared with the control group, and points to a mechanism through which GSPE can produce blood pressure-lowering effects, because it is known that NO is a powerful vasodilator.

In conclusion, this study indicates that a low dosage of GSPE (4 mg kg^{-1} day^{-1}) administered over a six-month period significantly reduces the AEDP in SHR after three months (Table 1). The study further suggests that the reduction in afterload leads to a significantly beneficial reduction in left ventricular contractility as well as cardiac work in hypertensive rats (Table 2). The results support the concept that this *in vivo* antihypertensive effect of the extract might in part at least be due to the vasodilator actions of NO. Lastly, this study supports studies on humans which have indicated that a high consumption of fruit and vegetables which contain large amounts of bioflavonoids might provide protection against hypertension and other related cardiovascular diseases.[7,11]

We thank Harold Majane, Hosea Lephalala, Danila Correia and Annette de Frietas for technical assistance. This research was supported by a grant from Value Added Life (Pty) S.A. and we declare that there is no conflict of interest between any parties.

Table 2. Effects of GSPE (4 mg kg^{-1} day^{-1}) after three months of treatment.

	Control (n = 10)	GSPE treated (n = 10)	P-value (between groups)
A_oPSP (mm Hg)	197.2 ± 5.1	165.8 ± 5.5*	0.002
A_oEDP (mm Hg)	137.2 ± 4.5	118.6 ± 3.8*	0.013
MA_oP (mm Hg)	162.0 ± 5.2	143.7 ± 6.1*	0.045
A_oPP (mm Hg)	60.3 ± 1.8	42.3 ± 2.0**	<0.001
H_r (beats/min)	218 ± 8.3	215 ± 4.1	NS
LVPSP (mm Hg)	188.7 ± 5.2	157.3 ± 6.9*	0.005
LVEDP (mm Hg)	12.3 ± 2.2	15.2 ± 2.7	NS
dP/dt_{max} (mm Hg/s)	2949 ± 103	2369 ± 239*	0.050
Cardiac work (mm Hg/beat min^{-1})	41 143 ± 2161	32 084 ± 1049*	0.045
Ventricular weight/ body weight	0.387 ± 0.020	0.392 ± 0.011	NS

*$P \leq 0.05$ and **$P \leq 0.001$. Values are mean ± s.e.m. NS, not significant.

Table 3. Effect of L-NAME (10 mg kg^{-1}) on aortic blood pressure parameters of treated versus control SHR rats (values are expressed as mean ± s.e.m. for n = 10).

Parameter (n = 10)		Mean difference in L-NAME between control value and maximum response to L-NAME
HR (beats/min):	Control	–6.8 ± 3.9
	Treated	–11.0 ± 5.3
A_oPSP (mm Hg):	Control	20.5 ± 3.5
	Treated	30.4 ± 6.9
A_oDBP (mm Hg):	Control	12.5 ± 4.5*
	Treated	28.7 ± 4.7*
MA_oP (mm Hg):	Control	15.0 ± 10.5
	Treated	17.3 ± 17.4

*$P \geq 0.05$ considered to be significant.

Received 31 October 2008. Accepted 8 May 2008.

1. Most M. (2004). Estimated phytochemical content of the Dietary Approaches to Stop Hypertension (DASH) diet is higher than the control study diet. *J. Am. Diet Assoc.* **104**(11),1725–1727.
2. Bernatova I., Pechanova O., Babal P., Kysela S., Stvrtina S. and Andriantsitohaina R. (2002). Wine polyphenols improve cardiovascular remodeling and vascular function in NO-deficient hypertension. *Am. J. Physiol.* **282**, H942–H948.
3. Duarte J., Perez-Palencia R., Vargas F., Ocete M.A., Perez-Vizcaino F., Zarzuelo A. and Tamargo J. (2001). Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. *Br. J. Pharmacol.* **133**, 117–124.
4. Duarte J., Jimenez R., O'Valle F., Galisteo M., Perez-Palencia R., Vargas F., Perez-Vizcaino F., Zarzuelo A. and Tamargo J. (2002). Protective effects of the flavonoid quercetin in chronic nitric oxide deficient rats. *J. Hypertens.* **20**, 1843–1854.
5. Pataki T., Bak I., Kovacs P., Bagchi D., Das D.K. and Tosaki A. (2002). Grape seed proanthocyanidins improved cardiac recovery during reperfusion after ischemia in isolated rat hearts. *Am. J. Clin. Nutr.* **75**, 894–899.
6. Sato M., Bagchi D., Tosaki A. and Das D.K. (2001). Grape seed proanthocyanidin reduces cardiomyocyte apoptosis by inhibiting ischemia/reperfusion-induced activation of JNK-1 and C-JUN. *Free Radic. Biol. Med.* **31**, 729–737.
7. Soares D.M., Costa Viana F.S., Souza M.A., Kovary K., Guedes D.C., Oliveira E.P., Rubenich L.M., Carvalho L.C., Oliveira R.M. *et al.* (2002). Antihyper-

tensive, vasodilator and antioxidant effects of a vinifera grape skin extract. *J. Pharm. Pharmacol.* **54**, 1515–1520.
8. Hertog M.G., Feskens E.J., Hollman P.C., Katan M.B. and Kromhout D. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *Lancet* **342**, 1007–1011.
9. Hertog M.G., Kromhout D., Aravanis C., Blackburn H., Buzina R., Fidanza F., Giampaoli S., Jansen A., Menotti A. and Nedeljkovic S. (1995). Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. *Arch. Intern. Med.* **155**, 381–386.
10. Rimm E.B., Katan M.B., Ascherio A., Stampfer M.J. and Willett W.C. (1996). Relation between intake of flavonoids and risk for coronary heart disease in male health professionals. *Ann. Intern. Med.* **125**, 384–389.
11. Apple L.J., Moore T.J., Obarzanek E., Vollmer W.M., Svetky L.P., Sacks F.M., Bray G.A., Vogt T.M., Cutler J.A., Windhauser M.M., Lin P. and Karanja N. (1997). A clinical trial of the effects of dietary patterns on blood pressure. *N. Engl. J. Med.* **336**(16), 1117–1124.
12. Collins R., Peto R., MacMahon S., Hebert P., Fiebach N.H., Eberlein K.A., Godwin J., Qizilbash N., Taylor J.O. and Hennekens C.H. (1990). Blood pressure, stroke, and coronary heart disease. Part 2. Short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. *Lancet* **335**, 827–838.
13. Hebert P.R., Moser M., Mayer J., Glynn R.J. and Hennekens C.H. (1993). Recent evidence on drug therapy of mild to moderate hypertension and decreased risk of coronary heart disease. *Arch. Intern. Med.* **153**, 578–581.
14. Cook N.R., Cohen J., Hebert P.R., Taylor J.O. and Hennekens C.H. (1995). Implications of small reductions in diastolic blood pressure for primary prevention. *Arch. Intern. Med.* **155**, 701–709.
15. Preuss H.G., Montamarry S., Echard B., Scheckenbach R. and Bagchi D. (2001). Long-term effects of chromium, grape seed extract, and zinc on various metabolic parameters of rats. *Mol. Cell. Biochem.* **223**, 95–102.
16. Bunag R.D. (1973). Validation in awake rats of a tail-cuff method for measuring systolic pressure. *J. Appl. Physiol.* **34**, 279–282.
17. Bunag R.D. (1983). Facts and fallacies about measuring blood pressure in rats. *Clin. Exp. Hypertens. A* **5**(10), 1659–1681.
18. Kurtz T.W., Griffen K.A., Bidani A.K., Davisson R.L. and Hall J.E. (2005). Recommendations for blood pressure measurements in humans and experimental animals. Part 2. *J. Hypertens.* **45**, 299–321.
19. Hay L., Schutte P.J., du Plooy W.J., Kgwefane K. and Kahler C.P. (1995). The effects of left ventricular loading on three indices of isovolumic relaxation rate in primates. *Med. Sci. Res.* **23**, 285–286.

Appendix

Animals and experimental design. A six-month study was conducted on 32 spontaneously hypertensive female rats (SHR), obtained from the Animal Unit of the University of the Witwatersrand, Johannesburg, when they were eight weeks old. On arrival, the 32 rats were randomly divided into a control group ($n = 16$) and a treated group ($n = 16$). The rats were kept in separate cages in a temperature-controlled room, and had free access to water and nutritionally balanced rat cubes (Epol, South Africa). The dietary composition of the cubes was given by the manufacturer to be 18% crude protein, 57% carbohydrate, a minimum of 2.5% fat, a maximum of 6% fibre, 1.8% calcium, 0.7% phosphorus and 12% moisture. The study was approved (approval no. AEC08/O4) by the Research and Ethics Committee of the University of Limpopo (Medunsa Campus) and was conducted in accordance with internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC).

Experimental diet. The treated group received gelatine blocks enriched with GSPE (4 mg kg^{-1}) on a daily basis for six months, in addition to the rat cubes. The increase in the average mass of the rats from 146.8 ± 7.8 g to 221 ± 7 g during the experiment was taken into account when the dose was determined. There was no significant difference between the mass increase between the experimental and control group of rats (control = 148.2 ± 4.8 g to 224.3 ± 5.2 g). The rats were fond of the gelatine blocks and all these blocks were consumed by the rats within minutes after introduction into the cages. GSPE is a commercially available product (Value Added Life, South Africa) prepared from the seeds of *Vitis vinifera* (Vitaceae). Grape seed extract contains up to 78% of proanthocyanidin.[5] Proanthocyanidin belongs to the second-largest group of the bioflavonoids. The control group received non-enriched gelatine blocks over the same period.

Indirect arterial blood pressure measurements. APSP, AEDP, mean arterial pressure (MAP) and heart frequency (H_f) were measured in 12 of the 32 rats using tail-cuff plethysmography.[16] For this purpose, six rats were randomly selected from the treated group and six from the control group. The pressures and H_f values were measured on these selected animals at the end of each month for six months. Measurements were made with a tail-cuff placed around the base of the tail and pulses detected with a LE 5002 blood pressure recorder (Letica Scientific Instruments, Spain). This instrument is equipped with a microprocessor with a memory specifically designed not only to measure non-invasively APSP, MAP and H_f but also AEDP. The method includes the restraining and pre-warming of rats for 30 min in an environmental chamber at 32°C to cause dilatation of the caudal artery. Rats were sufficiently preconditioned to become used to the restraining and higher environmental temperature for two days before the actual procedure. Because of the controversy[17,18] around the use of tail-cuff plethysmography and to minimize errors, our laboratory has validated our method under uniform experimental conditions.

Direct blood pressure measurements and cardiodynamic parameters. Since the tail-cuff blood pressure measurements suggested significant differences between the AEDP of the two groups after three months, additional cardiac information was obtained to elucidate the mechanism of action via direct catheterization via the carotid artery of the aorta and the left ventricle using methods previously described.[19]

For this purpose, the remaining rats (10 in each group) were anaesthetized with an intramuscular injection of 0.5 ml of a mixture of ketamine (100 μg ml^{-1}) (Kyron Laboratories, South Africa) and xylaxine (2%) (Premier Pharmaceuticals, South Africa) at a ratio of 1:3. The anaesthesia was maintained throughout the procedure by 0.1-ml injections when needed. Anaesthetic depth was regularly assessed by means of testing for the tarsal pinch reflex. The procedure was performed in a sterile environment on a pre-warmed operating table. Rats were placed on their backs and the right carotid artery was exposed. The distal end of a catheter (Cordis 2.5 F) was inserted into the carotid artery and advanced into the left ventricle. Great care was taken to minimize blood loss to a minimum during these procedures. Finally, the proximal end of the catheter was connected to a fluid-filled Hewlett-Packard (HP) quartz transducer, which was connected to a HP 8-channel recording system (HP7758). The recorded data were stored on a personal computer for later analysis. During these procedures, pressure recordings were also monitored on an oscilloscope to confirm the position of the catheter in the left ventricle and to verify the quality of the pressure curves. After correct positioning of the catheter, enough time was allowed for the left ventricular blood pressure to stabilize (approximately 7 min) before baseline pressure curves were recorded and stored on the computer. Thereafter, the catheter was carefully pulled into the aorta and aorta peak systolic pressure (A_oPSP), aorta peak diastolic pressure (A_oEDP), and aorta mean pressure (A_oMP) recorded.

L-NAME intervention. Further experiments were then conducted using the NO synthase inhibitor, N-nitro-L-arginine methyl ester (L-NAME). During this procedure, L-NAME (10 mg kg^{-1}) was administered as a single bolus injection to both groups of rats in order to ascertain the possible contribution of NO to the blood pressure-lowering effects of GSPE. This injection was done after the baseline recordings in the ventricle and aorta. The post-baseline values were recorded in the aorta for 15 s, at time zero, and thereafter at one-minute intervals for a 15-min post-injection period. The maximum aortic blood pressure response recorded (between 7–10 min after the injection of L-NAME) was compared with the baseline readings obtained directly before the L-NAME administration. The following indices were obtained from the aortic and left ventricular pressure curves: H_f, left ventricular peak systolic pressure (LVPSP), left ventricular end-diastolic pressure (LVEDP), the maximum rate of rise in left ventricular pressure (dP/dt_{max}) and cardiac work (CW), calculated by multiplying HR and SBP. Aortic peak systolic pressure (A_oPSP), and aortic ventricular end-diastolic pressure (A_oLVEDP) were also recorded.

Statistical analysis. Results are expressed as means ± s.e.m. Student's *t*-test indicates significance of difference between control and treated group at each time interval, and between pre-L-NAME and post-L-NAME administration