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Four-week Administration of Nimesulide, a Cyclooxygenase-2 Inhibitor, Improves Endothelial Dysfunction in the Hindlimb Vasculature of Streptozotocin-induced Diabetic Rats

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The aim of this study was to examine the effect of chronic administration of nimesulide, a cyclooxygenase-2 inhibitor, on endothelial dysfunction in streptozotocin-induced diabetic rats. Three groups of Sprague-Dawley rats (300-350 g, n = 6) were used. The first group served as normoglycemic control and the second and third groups were rendered diabetic by an intraperitoneal injection of streptozotocin (60 mg/kg). The third group received the selective COX-2 inhibitor, nimesulide (20 mg/kg/day), orally by gavage for 4 weeks while the second group received only drinking water and served as diabetic control. At the end of the treatment period, the rats were anesthetized with urethane (1.2 g/kg) and mean arterial pressure, heart rate and hindlimb blood flow were monitored. This was followed by the injection of acetylcholine (endothelium-dependent vasodilator, 0.1-0.8 μg/kg) and sodium nitroprusside (endothelium-independent vasodilator 1-4 μg/kg). Mean arterial pressure was significantly reduced and hindlimb vascular conductance was not significantly affected in the control diabetic group when compared to the normoglycemic control group. Nimesulide treatment did not cause any significant change in any of the measured hemodynamic parameters. Acetylcholine and sodium nitroprusside induced dose-dependent increases in hindlimb vascular conductance in control normoglycemic rats which were attenuated in diabetic control rats. Nimesulide reversed the attenuation of acetylcholine-induced increase in hindlimb vascular conductance. In conclusion, chronic administration of the selective COX-2 inhibitor, nimesulide improved endothelial dysfunction in the hindlimb vasculature of streptozotocin induced diabetic rats. This suggests that COX-2 products might be involved in the pathogenesis of endothelial dysfunction in streptozotocin-induced diabetic rats.

Key words: COX-2 inhibitors, Nimesulide, Blood pressure, Endothelium-dependent vasodilatation, Streptozotocin-induced diabetic rats

INTRODUCTION

The cyclooxygenase (COX) enzyme converts arachidonic acid to various prostaglandins and thromboxanes. This enzyme is found into 2 isoforms; the constitutive isoform, COX-1 and the inducible isoform, COX-2 (FitzGerald and Patrono, 2001). COX-2 inhibitors were introduced in 1999 as anti-inflammatory agents with lower incidence of gastrointestinal side effects by sparing COX-1 activity (Cosentino et al., 2003). Diabetic patients have an increased morbidity and mortality due to vascular complications (Calver et al., 1992). Endothelial dysfunction has been documented in streptozotocin-induced diabetic rats (Kamata et al., 1989; Angulo et al., 1998; Bardal et al., 2006) and in patients with type 1 and type 2 diabetes (Calles-escandon and Cipolla, 2001) and has been implicated in the pathogenesis of diabetic vascular disease (Calver et al., 1992). The role of superoxide anions in inducing diabetic endothelial dysfunction is clearly established as superoxide dismutase (SOD) was able to recover endothelium-dependent relaxations (Hattori et al., 1991; Angulo et al., 1998). This may be due to inactivation of nitric oxide (NO) by reactive oxygen species mainly the superoxide anion (Bardal et al., 2006). Previous studies showed that COX-2 inhibitors were shown to improve

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(Chenevard et al., 2003; Widlansky et al., 2003), worsen (Bulut et al., 2003) or had no effect on endothelial function (Title et al., 2003) in patients with coronary artery disease or hypertension. The mechanism by which COX-2 inhibitors can improve endothelial dysfunction is not clear. Treatment with a COX-2 inhibitor might reduce local production of superoxide anion and reactive nitrogen species and increases the bioavailability of endothelium-derived NO (Widlansky et al., 2003).

The aim of the present study was to examine if nimesulide, a selective COX-2 inhibitor, can improve endothelial dysfunction in an early stage of streptozotocin-induced diabetic rats. The selective COX-2 inhibitor, nimesulide was used in this study as it was shown to inhibit COX-2 with high selectivity (Llinas et al., 2000; Famaey 1997).

MATERIALS AND METHODS

Experimental animals

Male Sprague Dawley rats (300-350 g) were obtained from the animal house, Sultan Qaboos University. The rats were maintained under 12:12 h light dark cycle and supplied with standard laboratory chow diet and water ad libitum. The protocols were approved by the Animal Ethical Committee of the College of Medicine and Health Sciences, Sultan Qaboos University. Three groups of rats were used (n = 6). The first group served as normoglycemic control and received normal saline. The second and third groups were rendered diabetic by an intraperitoneal injection of streptozotocin (60 mg/kg) dissolved in normal saline. 72 h after streptozoticn injection, a drop of blood was taken from the tail vein and random blood glucose level was measured by using a blood glucose monitoring system "One Touch® Horizon™" (Life Scan, Inc., Milpitas, CA, USA) and diabetic rats were confirmed to have blood glucose level > 20 mmol/L. Once diabetes was confirmed, the third group received the selective COX-2 inhibitor, nimesulide (20 mg/kg/day), orally, once daily by gavage for 4 weeks while the second group received only drinking water and served as diabetic control. The dose of nimesulide was selected to be in the range of previous doses in the literature (Wu et al., 2005; Jain et al., 2005).

Experimental protocol

A drop of blood was taken from the tail vein to measure random blood glucose level at 18-24 h after the administration of the last dose of nimesulide. The rats were then anaesthetized with urethane (1.2 g/kg, i.p.). PE_{50} cannulae, filled with heparinized normal saline (25 IU/mL in 0.9% NaCl), were inserted into the right carotid artery for the measurement of mean arterial pressure by a pressure transducer (TSD104A, Biopac Systems, Santa Barbara, California, USA), and into the right jugular vein for the administration of drugs. Heart rate was derived electronically from the upstroke of the arterial pulse pressure. An ultrasonic probe (1.5RB, Hughes Sacks Electronik-Harvard Apparatus, March-Hugstetten, Germany) connected to a flow meter (Hughes Sacks Electronik-Harvard Apparatus, March-Hugstetten, Germany) was placed around the descending aorta to measure hindlimb blood flow.

After a 30-45 minutes stabilization period, baseline blood pressure, heart rate and hindlimb blood flow were monitored on a data acquisition system (MP 150, Biopac Systems, Santa Barbara, California, USA). Conductance of the hindlimb vasculature was calculated from blood flow /blood pressure.

Acetylcholine (0.1, 0.2, 0.4, and 0.8 μg/kg), an endothelium-dependent vasodilator, was injected at 3 min interval. After a 5 minutes interval, sodium nitroprusside (1, 2, and 4 μg/kg), an endothelium-independent vasodilator, was injected at 3 min interval. The magnitudes of the depressor response were expressed as % change in mean arterial pressure.

Statistical analysis

All values are presented as mean ± SEM. The data were analyzed by one way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparison Test or unpaired t-test, whichever appropriate. $P < 0.05$ was selected as the criterion for statistical significance.

RESULTS

Differences between streptozotocin-induced diabetic rats and normoglycemic rats

Table I shows that mean arterial pressure and body weight were significantly decreased while random blood glucose was significantly increased in streptozotocin-induced diabetic rats when compared to control normoglycemic rats. There were no significant changes in heart rate and hindlimb vascular conductance between streptozotocin-induced diabetic rats and normoglycemic rats.

Effect of nimesulide on mean arterial pressure, heart rate and regional hemodynamics and body weight

In streptozotocin-induced diabetic rats, nimesulide treatment did not cause any significant changes in mean arterial pressure, heart rate, hindlimb vascular conductance and body weight (Table I).

Effect of nimesulide on endothelium-dependent and endothelium-independent vasodilatation

Acetylcholine and sodium nitroprusside induced a dose dependent decrease in mean arterial pressure and increase in hindlimb vascular conductance. There were no significant changes between the decrease in blood pressure

Table I. Physiological parameters of non-diabetic and streptozotocin-induced diabetic rats divided into three groups; normoglycemic control (control), diabetic control (Control-D) and nimesulide (20 mg/kg/day) treated diabetic rats (Treated-D)

Parameter	Control	Control-D	Treated-D	P value
Number	6	6	6	
Body weight (grams)	374 ± 6	71 ± 21[a]	260 ± 9[a]	0.0061
Blood glucose levels (mmol/L)	5.7 ± 0.7	27.9 ± 1[a]	31.1 ± 1[a]	<0.0001
Mean arterial pressure (mmHg)	110 ± 3	95 ± 4[a]	97 ± 3[a]	0.0266
Heart rate (beats/min)	300 ± 23	272 ± 29	234 ± 11	0.1474
Hindlimb vascular conductance/100 gm body weight (mL/min.mmHg)	0.034 ± 0.03	0.035 ± 0.005	0.039 ± 0.05	0.6705

Results are means ± SEM. P values are for ANOVA comparing the three groups.
[a]Significantly different from normoglycemic control group.
When the blood glucose level was showing "HI", 33.3 mmol/L value was used for calculation.

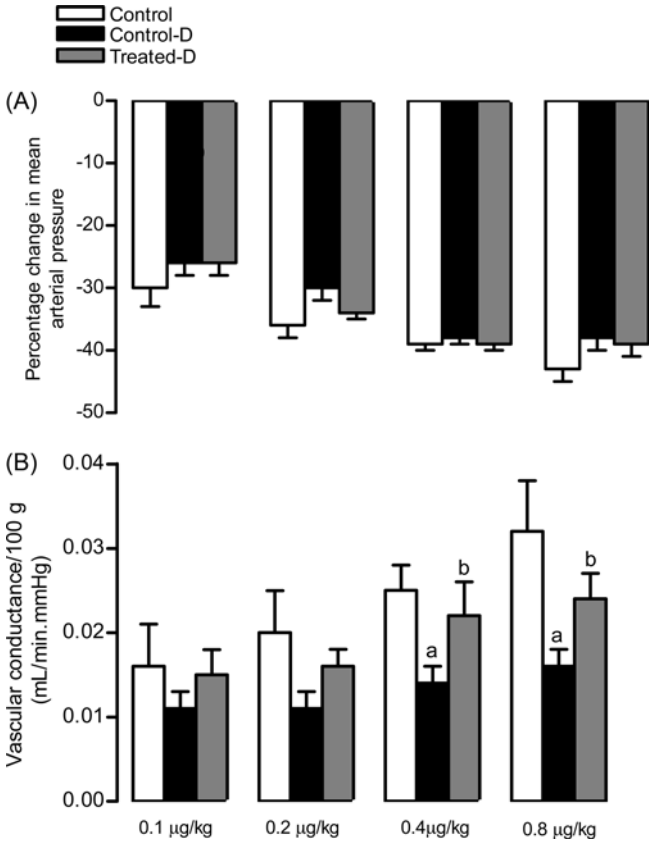

Fig. 1. Effect of nimesulide (20 mg/kg/day) on acetylcholine induced changes in A) mean arterial pressure and B) vascular conductance in urethane anaesthetized rats. Data are mean ± SEM of n = 6. Normoglycemic control (control), diabetic control (control-D) and nimesulide (20 mg/kg/day) treated diabetic rats (Treated-D). [a]Significantly different from normoglycemic control group, $P < 0.05$. [b]Significantly different from diabetic control group, $P < 0.05$.

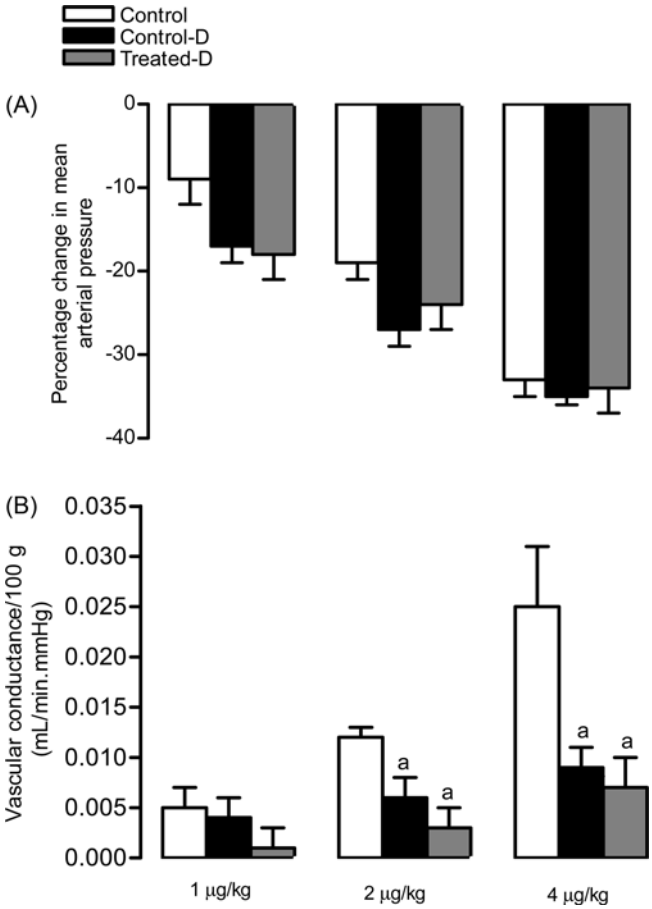

Fig. 2. Effect of nimesulide (20 mg/kg/day) on sodium nitroprusside induced changes in A) mean arterial pressure and B) vascular conductance in urethane anaesthetized rats. Data are mean ± SEM of n = 6. Normoglycemic control (control), diabetic control (control-D) and nimesulide (20 mg/kg/day) treated diabetic rats (Treated-D). [a]Significantly different from normoglycemic control, $P < 0.05$.

induced by acetylcholine and sodium nitroprusside in streptozotocin-induced diabetic rats and normoglycemic controls (Fig. 1a, 2a). In streptozotocin-induced diabetic rats, the increases in hindlimb vascular conductance induced by both acetylcholine and sodium nitroprusside were significantly attenuated when compared to normoglycemic control (Fig. 1b, 2b).

In streptozotocin-induced diabetic rats, nimesulide treat-

ment did not significantly affect the decrease in mean arterial pressure induced by acetylcholine or sodium nitroprusside. In streptozotocin-induced diabetic rats, nimesulide treatment reversed the attenuation in acetylcholine induced increase in vascular conductance but did not affect that of sodium nitroprusside (Fig. 1, 2).

DISCUSSION

The main finding of the present study is that administration of nimesulide, for four weeks, improved endothelial dysfunction in the hindlimb vasculature that was present in an early stage of streptozotocin-induced diabetic rats. Mean arterial pressure was significantly reduced in streptozotocin-induced diabetic rats relative to normoglycemic rats. There were no significant changes in heart rate and hindlimb vascular conductance. The decrease in mean arterial pressure in streptozotocin-induced diabetic rats is in accordance with previous reports (Tatchum-Talom et al., 2000; Rebolledo et al., 2001; Bardal et al., 2006) and may be related to vascular dilatation, hypovolemia secondary to osmotic diuresis or myocardial dysfunction (Bardal et al,. 2006). On the contrary, other studies showed that mean arterial pressure was not changed in streptozotocin-induced diabetic rats (Kiff et al., 1991; Angulo et al., 1998). In our study, the decrease in mean arterial blood pressure was associated with no change in hindlimb vascular conductance which suggests that other vascular beds may be responsible for the decrease in blood pressure. Previous studies showed renal and mesenteric vasodilatations and hindquarters vasoconstriction in conscious streptozotocin-induced diabetic rats relative to control rats (Kiff et al., 1991). Furthermore, the hindlimb perfusion pressure was increased in anesthetized autoperfused streptozotocin-induced diabetic rats (Angulo et al., 1998).

There was a significant decrease in both acetylcholine (endothelium-dependent) and sodium nitroprusside (endothelium-independent) induced increase in hindlimb vascular conductance in streptozotocin-induced diabetic rats when compared to normoglycemic rats. Impaired endothelium-dependent vasodilatation was previously reported in streptozotocin-induced diabetic rats (Kamata et al., 1989; Angulo et al., 1998; Bardal et al., 2006) and in patients with insulin-dependent diabetes mellitus (Johnstone et al., 1993). In our study we found that sodium nitroprusside (endothelium-independent) induced vasodilatation was also reduced in streptozotocin-induced diabetic rats. This is in accordance with previous studies which showed diminished relaxant responses evoked by sodium nitroprusside in anaesthetized autoperfused streptozotocin-induced diabetic rats (Angulo et al., 1998) and in diabetic patients (Calver et al., 1992). This might be due to an abnormality in vascular smooth muscle sensitivity to NO (Calver et al., 1992). In streptozotocin-induced diabetic rats, increased oxidative stress activates the production of COX-derived vasoconstrictor prostanoids causing hypersensitivity of vascular smooth muscle (Shi and Vanhoutte, 2008).

In this study, nimesulide did not affect mean arterial pressure in streptozotocin- induced diabetic rats. There are conflicting results in the literature on the effect of COX-2 inhibitors on blood pressure. In normtoensive rats, COX-2 inhibitors increased (Kiff et al., 1991), or had no effect on blood pressure (Harding et al., 2000).

In this study, nimesulide reversed the attenuated acetylcholine (endothelium-dependent vasodilatation) but not sodium nitroprusside (endothelium-independent vasodilatation) induced increase in hindlimb vascular conductance in streptozotocin-induced diabetic rats. This shows that nimesulide improved only the impaired endothelium-dependent vasodilatation in streptozotocin-induced diabetic rats. This suggests that COX-2 products have a role in the pathogenesis of endothelial dysfunction in the hindlimb vasculature of streptozotocin-induced diabetic rats. The exact mechanism of the improvement of endothelial dysfunction by nimesulide is not clear. However, it was suggested that treatment with a COX-2 inhibitor might reduce local production of superoxide anion and reactive nitrogen species and increases the bioavailability of endothelium-derived NO (Widlansky et al., 2003). Previous reports in the literature on the effects of COX-2 inhibitors on endothelial dysfunction are controversial. Celecoxb but not rofecoxib improved aortic endothelial dysfunction in Dahl salt sensitive rats (Herman et al., 2003). The COX-2 inhibitor, MF-tricyclic, did not influence acetylcholine induced aortic vascular relaxation in transgenic rats harboring mouse renin-2 gene (Cheng et al., 2002). In humans, there are studies that showed that COX-2 inhibitors improved endothelium-dependent vasodilatation in patients with coronary artery disease (Chenevard et al., 2003) and patients with hypertension (Widlansky et al., 2003). Other studies showed that COX-2 inhibitors impaired endothelium-dependent vasodilatation in patients with essential hypertension (Bulut et al., 2003). Furthermore, some studies showed that COX-2 did not appear to have any favorable or adverse effects on endothelium-dependent vasodilatation in patients with coronary artery disease (Title et al., 2003).

In conclusion, chronic administration of the selective COX-2 inhibitor, nimesulide, reversed the endothelial dysfunction in the hindlimb vasculature associated with the early stage of streptozotocin-induced diabetic rats. This suggests that COX-2 products have a role in the pathogenesis of endothelial dysfunction in the hindlimb vasculature of streptozotocin-induced diabetic rats. This

is mainly a hemodynamic study and additional studies are needed to explain the mechanism of COX-2 products that are in involved in the pathogenesis of endothelial dysfunction in diabetes.

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REFERENCES

Angulo, J., Rodriguez-Manas, L., Peiro, C., Neira, M., Marin, J., and Sanchez-Ferrer, C. F, Impairment of nitric oxide-mediated relaxations in anaesthetized autoperfused streptozotocin-induced diabetic rats. *Naunyn Schmiedbergs' Arch. Pharmacol.,* 358, 529-537 (1988).

Bardal, S., Misurski, D., Qiu, X., Desai, K., and McNeill, J. R., Chronic treatment with vascular endothelial growth factor preserves agonist-evoked vascular responses in the streptozotocin-induced diabetic rat. *Diabetologia,* 49, 811-818 (2006).

Bulut, D., Liaghat, S., Hanefeld, C., Koll, R., Miebach, T., and Mugge, A., Selective cyclooxygenase-2 inhibition with parecoxib acutely impairs endothelium-dependent vasodilatation in patients with essential hypertension. *J. Hypertens.,* 21, 1663-1667 (2003).

Calles-Escandon, J. and Cipolla, M., Diabetes and endothelial dysfunction: A clinical perspective. *Endocr. Rev.*, 22, 36-52 (2001).

Calver, A., Collier, J., and Vallance, P., Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. *J. Clin. Invest.*, 90, 2548-2554 (1992).

Chenevard, R., Hurlimann, D., Bechir, M., Enseleit, F., Spieker, L., Hermann, M., Riesen, W., Gay, S., Gay, R. E., Neidhart, M., Michel, B., Luscher, T. F., Noll, G., and Ruschitzka, F., Selective COX-2 inhibition improves endothelial function in coronary artery disease. *Circulation,* 107, 405-409 (2003).

Cheng, Z. J., Tikkanen, I., Vapaatalo, H., and Mervaala, E. M., vascular effects of COX inhibition and AT_1 receptor blockade in transgenic rats harboring mouse renin-2 gene. *J. Physiol. Pharmacol.,* 53, 597-613 (2002).

Consentino, F., Francia, P., and Volpe, M., Do COX-2 inhibitors really prevent hypertension and proteinuria? Another brick in the wall for the COX-2 inhibition and cardiovascular controversy. *J. Hypertens.,* 21, 501-503 (2003).

Famaey, J. P., In vitro and in vivo pharmacological evidence of selective cyclooxygenase-2 inhibition by nimesulide: an overview. *Inflamm. Res.,* 46, 437-446 (1997).

FitzGerald, G. A. and Patrono, C., The coxibs, selective inhibitors of cyclooxygenase-2. *N. Engl. J. Med.,* 345, 433-442 (2001).

Harding, P., Carretero, O. A., and Beierwaltes, W. H., Chronic cyclooxygenase-2 inhibition blunts low sodium-stimulated renin without changing renal haemodynamics. *J. Hypertens.,* 18, 1107-1113 (2000).

Hattori, Y., Kawasaki, H., Abe, K., and Kamo, M., Superoxide dismutase recovers altered endothelium-dependent relaxation in diabetic rat aorta. *Am. J. Physiol.*, 261, H1086-H1094 (1991).

Hermann, M., Camici, G., Fratton, A., Hurlimann, D., Tanner, F. C., Hellermann, J. P., Fiedler, M., Thiery, J., Neidhart, M., Gay, R. E., Gay, S., Lusher, T. F., and Ruschitzka, F., Differential effects of selective cyclooxygenase-2 inhibitors on endothelial function in salt induced hypertension. *Circulation,* 108, 2308-2311 (2003).

Jain, S., Gupta, M., Malhorta, S., and Pandhi, P., Effect of rofecoxib on antihypertensive effects of candesartan in experimental models of hypertension. *Method. Find. Exp. Clin.,* 27, 11-16 (2005).

Johnstone, M. T., Creager, S. J., Scales, K. M., Cusco, J. A., Lee, B. K., and Creager, M. A., Impaired endothelium-dependent vasodilation in patients with insulin-dependent diabetes mellitus. *Circulation*, 88, 2510-2516 (1993).

Kamata, K., Miyata, N., and Kasuya, Y., Impairment of endothelium-dependent relaxation and changes in levels of cyclic GMP in aorta from streptozotocin-induced diabetic rats. *Br. J. Pharmacol.,* 97, 614-618 (1989).

Kiff, R. J., Gardiner, S. M., Compton, A. M., and Bennett, T., Selective impairment of hindquarters vasodilator responses to bradykinin in conscious Wistar rats with streptozotocin-induced diabetes mellitus. *Br. J. Pharmacol.,* 103, 1357-1362 (1991).

Llinas, M. T., Rodriguez, F., Moreno, C., and Salazar, F. J., Role of cyclooxygenase-2 derived metabolites and nitric oxide in regulating renal function. *Am. J. Physiol.,* 279, R1641-R1646 (2000).

Rebolledo, A., Ayala-Paredes, F., Milesi, V., Grassi, A. O., and Rinaldi, G. J. Short-term streptozotocin-induced diabetes induces blood pressure decrease associated with reduced aortic (45)Ca(2+) uptake and selective depression of the sustained noradrenergic contraction. *Diabetes Metab.,* 27, 40-48 (2001).

Shi, Y. and Vanhoutte, P. M., Oxidative stress and COX cause hyper-responsiveness in vascular smooth muscle of the femoral artery from diabetic rats. *Br. J. Pharmacol.,* 154, 639-651 (2008).

Tatchum-Talom, R., Gopalakrishnan, V., and McNeill J. R., Radiotelemetric monitoring of blood pressure and mesenteric arterial bed responsiveness in rats with streptozotocin-induced diabetes. *Can. J. Physiol. Pharm.*, 78, 721-728 (2000).

Title, L. M., Giddens, K., McInerney, M. M., McQueen, M. J.,

and Nassar, B. A., Effect of cyclooxygenase-2 inhibition with rofecoxib on endothelial dysfunction and inflammatory markers in patients with coronary artery disease. *J. Am. Coll. Cardiol.,* 42, 1747-1753 (2003).

Widlansky, M. E., Price, D. T., Gokce, N., Eberhardt, R. T., Duffy, S. J., Holbrook, M., Maxwell, C., Palmisano, J., Keaney, J. F. Jr., Morrow, J. D., and Vita, J. A., Short and long-term COX-2 inhibition reverses endothelial dysfunction in patients with hypertension. *Hypertension,* 42, 310-315 (2003).

Wu, R., Laplante, M-A., and de Champlain, J., Cyclooxygenase-2 inhibitors attenuate angiotensin II-induced oxidative stress, hypertension and cardiac hypertrophy in rats. *Hypertension,* 45, 1139-1144 (2005).