Journal of Applied Pharmaceutical Science Vol. 3 (11), pp. 070-075, November, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.31112
ISSN 2231-3354

Possible involvement of leptin in a Mas-receptor agonist, AVE-0991-induced improvement in dyslipidemia and cardiomyopathy in STZ-induced diabetic rats

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ARTICLE INFO

Article history:
Received on: 21/08/2012
Revised on: 27/05/2013
Accepted on: 17/06/2013
Available online: 29/11/2013

Key words:
AVE-0991, Leptin, Mas-receptor, Diabetic Cardiomyopathy.

ABSTRACT

Present study was designed to investigate the possible involvement of leptin in the pharmacological activation of Mas–receptor in STZ-diabetic rats, with cardiomyopathy. A single administration of STZ (50 mg/kg, i.p.) produced diabetes which leads to cardiomyopathy after 8 weeks. Estimation of serum glucose has been used as a marker of hyperglycemia. Cardiomyopathy was assessed by measuring LV collagen content, absolute LV weight, LVW/BW ratio, LVDP, dp/dt_{max} and dp/dt_{min}. Furthermore, serum triglyceride, serum cholesterol and serum HDL levels were estimated as an index of dyslipidemia. Rat serum leptin was quantitatively estimated by using enzyme-linked immunosorbent assay (ELISA) kit. STZ-diabetic rats were associated with significant hyperglycemia, hypertriglyceridemia, decreased cardiac functions and decreased serum leptin level. Both the low and high dose AVE-0991 treatment, significantly decreased hyperglycemia and increased serum leptin level in diabetic rats. Whereas, AVE-0991 only at high dose significantly improved lipid profile and cardiac function. on the basis of above, it may be concluded that downstream activation of leptin may be responsible for the beneficial effect of AVE-0991, a Mas-receptor agonist in STZ-induced diabetic cardiomyopathy in rats.

INTRODUCTION

Diabetes related heart disease is mainly associated with premature development of atherosclerotic disease, microvascular structural and functional abnormalities and diabetic cardiomyopathy (DC) (Levy *et al.,* 2002; Bugger and Abel, 2009). The DC is characterized by left ventricular hypertrophy, cardiac dysfunction, alteration in coronary microcirculation, dyslipidemia and myocardial fibrosis (Finck *et al*., 2003; Wang *et al.,* 2006; Singh *et al.,* 2011). Like human DM, chronic diabetes induced by STZ in rats has been shown to be associated with heart dysfunction, including reduced heart rate, depressed peak vascular pressure, and depressed rate of contraction and relaxation in the left ventricle (Dhalla *et al.,* 1998). The pathophysiology of diabetes-induced cardiomyopathy is still not well understood. Various mechanisms have been proposed such as oxidative stress, excess generation of AGE, PARP over activation, activation of PKC, RAAS activation, increased lipid profile and altered Ca2+ handling (Bugger and Abel, 2009; Asghar *et al.,* 2009). Furthermore, alterations within RAAS are considered to be important contributor for the subcellular remodeling, heart dysfunction and other diabetic complications (Dhalla *et al.,* 1998). Moreover, Cumulative evidence suggests that upregulation of Ang-II/ AT_1 receptor axis and downregulation of ACE-2/Angiotensin (1-7)/Mas-receptor axis may be responsible for secondary complications of diabetes (Sajad *et al.,* 2004; Singh *et al.,* 2011). ACE-2/Angiotensin (1-7)/Mas-receptor axis is the survival axis of RAAS (Sim˜oes e Silva *et al.,* 2006; Chappell 2007). Angiotensin (1-7)/Mas-receptor axis activation produces NO dependent vasodilation, antiproliferative, anti-arrhythmic, antithrombotic, natriuretic, antiatherosclerosis effect (Ferrario, 2006; Ferreira *et al.,* 2006). Downregulation of Mas-receptor axis has also been implicated in cardiac dysfunction observed in diabetes (Ye *et al.,* 2004). In diabetic patients, dyslipidaemia and hypertension are major factors for the development of diabetic cardiomyopathy

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(NCEP Adult Treatment Panel III final report, 2002). Angiotensin (1-7) significantly attenuates diabetes-induced dyslipidemia and subsequently improves myocardial functions in diabetic rats (Singh *et al.,* 2011). AVE0991, a non-peptide Mas-receptor agonist, mimics the many pharmacological actions of Ang (1-7) in vasodialation and natriuretic effect (Weimer *et al.,* 2002). AVE0991 resembles cardioprotective effect of Ang (1-7). It has longer $t^{1/2}$ and is more stable than Ang (1-7) because of its non-peptide nature (Weimer *et al.,* 2002; Singh *et al.,* 2012). However, the mechanism of attenuation of dyslipidemia by Angiotensin (1-7) treatment is not known. It has also been reported that leptin protects from lipotoxicity and the relatively hypoxic milieu associated with diabetic cardiomyopathy. In addition, exogenous administration of leptin reversed both LV dysfunction and hypertrophy in leptin-deficient mice (McGaffin *et al.,* 2008). Above evidences suggests that leptin may be responsible for beneficial actions of AVE-0991 on lipid profile. The present study was designed to investigate the possible involvement of leptin in the Mas–receptor activation induced beneficial effect in diabetic rats with cardiomyopathy

MATERIAL AND METHODS

Age matched Wistar rats, of either sex, weighing about 180-260g, were employed. Rats were fed on standard chow diet and water was provided *ad libitum.* They were acclimatized in animal house and were exposed to normal day and light cycle. The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee (meeting-1/2011/protocol no. 11).

Induction of experimental diabetic cardiomyopathy

Diabetes mellitus was induced by single injection of streptozotocin (STZ) (50 mg/kg, i.p.) dissolved in freshly prepared ice cold citrate buffer (pH 4.5) (Singh *et al.,* 2011) and animals having random serum glucose more than 240 mg/dl were considered as diabetic. Persistent hyperglycemia after 8 weeks of STZ administration has been reported to induce DC as reported earlier (Hamblin *et al.,* 2007).

The serum glucose concentration was estimated spectrophotometrically by glucose oxidase-peroxidase (GOD-POD) method (Trinder, 1969) using commercially available kit (Coral clinical system, Goa, India).

Experimental protocol

In the present study five Groups were employed and each group had 6 wistar rats. The vehicle or test drug AVE-0991 treatment was initiated on 6th week post STZ and was continued for next 2 weeks administration. Group I: Normal Control rats; Group II: STZ Induced Diabetic Control (50 mg/kg, i.p, once) (Singh *et al.,* 2011); Group III: AVE-0991 Perse (576 µg/kg i.p daily) in Normal Rats; Group IV: AVE-0991 (288 µg/kg i.p daily) treated; Group V: AVE-0991 (576 µg/kg i.p daily) treated (Benter *et al.,* 2006).

Assessments of haemodynamic and morphological parameters

This animal model resembles human diabetes mellitus characteristics, heart dysfunction, reduced heart rate, depressed peak vascular pressure, and depressed rate of contraction and relaxation in the left ventricle (Dhalla *et al.,* 1998). After 8 weeks of study period, the heparinized (500 U/kg body weight) rats were sacrificed by cervical dislocation, thorax was opened and the heart was excised and placed into chilled, heparinized perfusate to arrest the beating of the heart. The heart was immediately mounted on digital Langendorff's apparatus (RADNOTI, Monrovia, CA, USA) (Langendorff, 1898) and perfused with Kreb's-Henseleit solution, gassed with 95% O_2-5% CO_2, pH 7.4, maintained at 37°C. For the measurement of cardiac functions, a double distilled water filled latex balloon was inserted through the mitral valve into the left ventricle, and left ventricular developed pressure (LVDP)(mmhg), rate of pressure development (dp/dt_{max}) and rate of pressure delay (dp/dt_{min}) were measured using pressure transducer (BIOPAC MP100 System, California, USA). The left ventricle including interventricular septum weight was weighed and expressed as milligram per gram of body weight.

Assessment of Serum Lipid Profile

Estimation of serum total cholesterol

The total cholesterol was estimated spectrophotometrically at 505 nm by cholesterol oxidase peroxidase CHOD-POD method (Allain *et al.,* 1974) using commercially available kit (Coral Clinical System. Goa, India).

Estimation of serum triglycerides

The serum triglyceride was estimated spectrophotometrically at 505 nm by glycerophosphate oxidase peroxidase GPO-PAP method (Werner *et al.,* 1981) using commercially available kit (Coral clinical system. Goa, India).

Estimation of high density lipoprotein (HDL)

The HDL was estimated spectrophotometrically at 505 nm by cholesterol oxidase peroxidase CHOD-POD method (Allain *et al.,* 1974) using commercially available kit (Coral Clinical system. Goa, India).

Estimation of Left Ventricle Collagen Content

LV collagen content was determined by measuring the hydroxyproline concentration spectrophotometrically at 558 nm. Then hydroxyproline values was converted to collagen content by multiplying by a factor of 6.94 (as hydroxyproline represents approximately 14.4% of the amino acid composition of collagen) and expressed further as mg collagen/gram of tissue (Gallop and Paz, 1975).

Assessment of serum leptin level

The serum leptin was estimated by using rat leptin Enzyme-Linked Immunosorbent Assay (ELISA) kit (RayBiotech, Inc.), according to manufacturer's instructions. The results were expressed as ρg/ml.

Table. 1: Effect of various pharmacological interventions on Serum Glucose and lipid profile.

Groups	Serum Glucose (mg/dl)	Serum Cholesterol (mg/dl)	Serum Triglycerides (mg/dl)	Serum HDL (mg/dl)
NC	110.6±8.998	49.05±5.72	82.43±13.87	35.33±6.24
DC	440.6±34.96 [a]	54.20±5.23	234.4±48.21 [a]	37.40±6.20
AVE-0991(576 µg/kg) perse	109.8±6.922	49.30±6.55	85.60±14.37	42.60±6.75
DC+AVE-0991(288 µg/kg)	348.8±38.08 [b]	55.60±5.88	197.0±11.07	49.80±6.48 [b]
DC+AVE-0991(576 µg/kg)	288.4±25.29 [b]	56.80±7.18	182.3±8.479 [b]	56.20±6.37 [b]

All the values are expressed as Mean ± S.D. a= p<0.05 versus normal control; b=p<0.05 versus diabetic control. Abbreviation: NC, normal control; DC, diabetic control.

Statistical Analysis

All values were expressed as mean ± S.D. Statistical analysis was performed using the Graph Pad Prism 5 Software. The data obtained from various groups were statistically analyzed by using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The p value < 0.05 was considered to be statistically significant.

Drugs and Chemicals

Streptozotocin was purchased from Sigma Aldrich Ltd., St. Loius, USA. AVE 0991 was obtained from Sanofi-Aventis, Germany as an Ex-gratia. All other chemicals used were of analytical grade.

RESULTS

Administration of STZ (50 mg/kg *i.p.* once) produced hyperglycemia, which was assessed in term of serum glucose 7 days after STZ administration and rats with serum glucose level ≥240 mg/dl were considered as diabetic and used for experimentation. The administration of AVE-0991 to normal rats did not produce any significant, per se, effect on various parameters assessed in the present study.

Effect of AVE-0991 on serum glucose level and lipid profile

A significant increase in serum glucose was noted in diabetic control rats, when compared with age matched normal rats. Both with high and low dose AVE-0991 treatment significantly decreased serum glucose levels. A significant increase in serum triglycerides was noted in diabetic control rats, when compared with age matched normal rats. There was no significant change in serum cholesterol and serum HDL level. In diabetic rats, treatment with high dose AVE-0991, but not with low dose AVE-0991, significant reduced triglycerides. Significantly increased HDL level was in both high and low dose treatment of AVE-0991 in diabetic rats as compared to diabetic control rats. (Table 1)

Effect of various pharmacological interventions on, absolute left ventricle weight; absolute left ventricle weight/body weight (LVW/BW) ratio; and absolute left ventricle collagen content

Absolute left ventricle weight was significantly reduced in diabetic rats as compared to normal control rats. Treatment with AVE-0991 didn't produce any change in absolute left ventricle weight as compare to diabetic control rats. (Figure 1A) A significant increase in LVW/BW ratio was noted in diabetic rats when compared with age matched normal rats. Both at high and low dose, AVE-0991 significantly decreased LVW/BW in diabetic rats. (Figure 1B) A significant increase in left ventricular collagen content was noted in diabetic control rats when compared with age matched normal rats. Treatment with AVE-0991 decreased left ventricular collagen content in diabetic rats, but significantly decrease was shown only with high dose treatment of AVE-0991 (Figure 2).

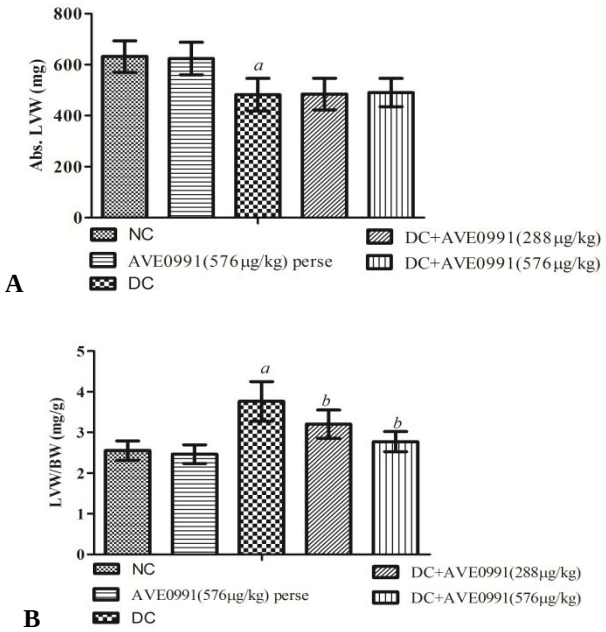

Fig. 1: Effect of various pharmacological interventions (A) on absolute left ventricle weight and (B) on absolute left ventricle weight/body weight (LVW/BW) ratio. All the values are expressed as Mean ±S.D. a= p<0.05 versus normal control; b=p<0.05 versus diabetic control. Abbreviation: NC, normal control; DC, diabetic control.

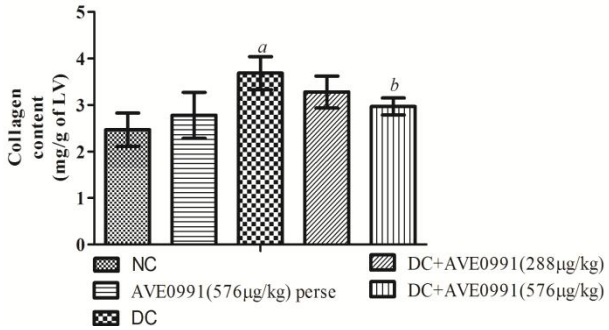

Fig. 2: Effect of various pharmacological interventions on absolute left ventricle collagen content. All the values are expressed as Mean ± S.D. a= p<0.05 versus normal control; b=p<0.05 versus diabetic control. Abbreviation: NC, normal control; DC, diabetic control.

Effect of various pharmacological interventions on haemodynamic parameters

A significant decrease in LVDP, dp/dt_{max} and dp/dt_{min} was noted in diabetic control rats, as compared with age matched normal rats. In diabetic rats, treatment with AVE-0991 increased LVDP, dp/dt_{max} and dp/dt_{min}, but significantly decrease was observed only with high dose treatment of AVE-0991. (Figure 3; 4 A and B)

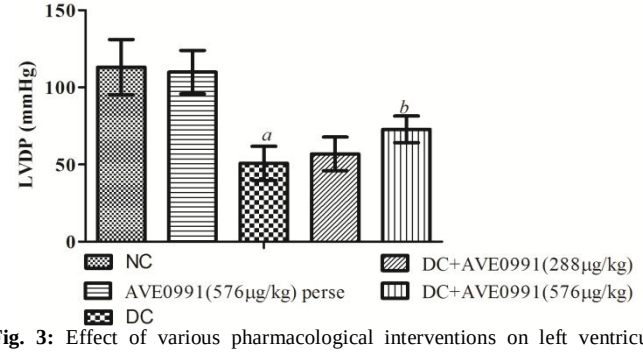

Fig. 3: Effect of various pharmacological interventions on left ventricular developed pressure (LVDP) (mmHg). All the values are expressed as Mean ±S.D. a= p<0.05 versus normal control; b=p<0.05 versus diabetic control. Abbreviation: NC, normal control; DC, diabetic control.

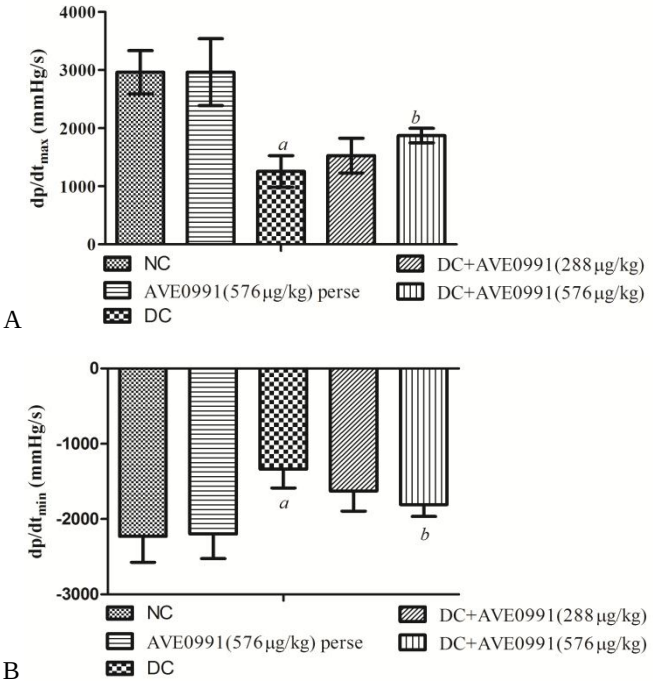

Fig . 4: Effect of various pharmacological interventions on dp/dt_{max} (mmHg/s) and dp/dt_{min} (mmHg/s). All the values are expressed as mean ± S.D. a= p<0.05 versus normal control; b=p<0.05 versus diabetic control. Abbreviation: NC, normal control; DC, diabetic control.

Effect of various pharmacological interventions on serum leptin levels

A significant increase in serum leptin level was observed in AVE-0991 per se in normal rats, when compared with normal control rats. Diabetic rats showed a significant decrease in serum leptin level, when compared with normal control rats. However, treatment with AVE-0991 produced significant increase in serum leptin as compare to diabetic control rats.

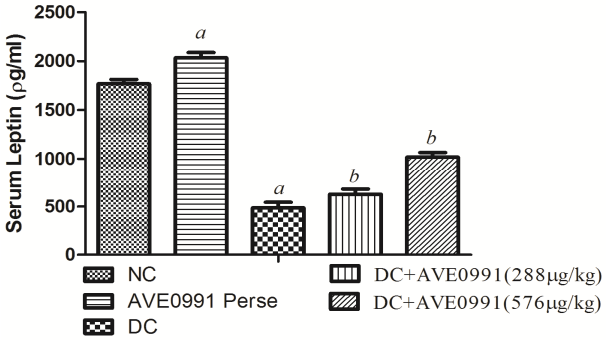

Fig. 5: Effect of various pharmacological interventions on serum leptin levels. All the values are expressed as Mean ± S.D. a= p<0.05 versus normal control; b=p<0.05 versus diabetic control. Abbreviation: NC, normal control; DC, diabetic control.

DISCUSSION

The principle findings of the study are that treatment with AVE-0991, a Mas-receptor agonist produced a beneficial effect on cardiomyopathy by reducing hyperglycemia, hypertriglyceridemia and increasing the level of leptin in STZ-induced diabetic rats.

Both hyperglycemia and dyslipidemia (i.e. increased in serum LDL cholesterol and decreased serum HDL cholesterol-levels) are reported to play a key role in the pathogenesis of diabetic cardiomyopathy (NCEP Adult Treatment Panel III final report, 2002). In the present study, it has been observed that that STZ-diabetic rats or associated with an elevated levels of triglyceride, but there is no significant change in serum cholesterol and HDL levels. This is consistent with our previous reported data (Singh *et al.*, 2011). Furthermore, in vehicle treated diabetic rats, there is significant increase in LV collagen content, decrease in absolute LVW and a significant increase in LVW/BW ratio, a significant decrease in LVDP, dp/dt_{max} and dp/dt_{min} in diabetic rats. These results are consistent with previously reported studies (Singh *et al.*, 2012).

The 2 week treatment with AVE-0991 has been shown to attenuate hyperglycemia. It also significantly increased LVDP and dp/dt_{max} and significantly decreased LVEDP in STZ-treated rats. On the other hand, 2 week treatment of AVE-0991 has shown to significantly attenuate serum triglyceride level only at high dose treatment group.

It has been reported that activation of Mas-receptor produced beneficial role in glucose metabolism (Walther *et al.*, 1998). Similarly, in present study, 2 week AVE-0991 treatment, both at low and high dose, produced significant anti-hyperglycemic effect. Exogenous administration of angiotensin (1-7) or AVE-0991 showed antifibrotic and antiproliferative effects in cardiac tissue (Ferreira and Santos, 2005; Iwata *et al.*, 2005). Similarly, in the present study AVE-0991 treatment has decreased in LV collagen content and LVW/BW ratio. It indicate that the antifibrotic and antiproliferative effect of Mas-receptor agonist.

Also, AVE-0991 treatment has significantly increase LVDP, dp/dt_{max} and dp/dt_{min}. It indicates the improvement of cardiac function in STZ-diabetic rat hearts.

STZ-induced diabetic rats were associated with a rapid and significant decrease in circulating leptin concentration, possibly via decreased glucose metabolism and subsequent decrease in m-RNA expression of leptin in adipocytes (Havel *et al.,* 1998; Lin *et al.,* 2002). An hypoinsulinemia associated with STZ-induced diabetes has shown to decrease the mRNA expression of leptin in adipocytes and subsequent development of hypoleptinemia (McGiffin *et al.,* 2008). Leptin has shown to play a crucial role into the glucose and lipid metabolism (Kershaw and Flier, 2004). Both clinical and experimental studies clearly evidencing that leptin deficiency is associated with insulin resistance and development of diabetes (Chan and Mantzoros *et al.,* 2005; Dardeno *et al.,* 2010).

Leptin replacement improves dyslipidemia, insulin sensitivity, and lipodystrophy (Chong *et al.,* 2010; Oral and Chan, 2010). In STZ induced diabetic rats, hypoglycemic effect of leptin is may be due to its insulin sensitizing effect (Denroche *et al.,* 2011). In present study, STZ induced diabetic rats are associated with a significant decrease in serum leptin levels as compare to normal rats. However, treatment with both high and low dose of AVE-0991 significantly increases the leptin levels, as compared to diabetic control rats. Activation of Mas-receptor significantly attenuates diabetes-induced hyperglycemia and dyslipidemia, the major culprits for Cardiac dysfunctions in diabetes (Singh *et al.,* 2011).

It suggests that leptin may be responsible for the observed anti-hyperglycemic and anti-triglyceridemic effect of AVE-0991 on diabetic cardiomyopathy. However, no data is available on the mechanism of the beneficial effect of Mas-receptor agonist in diabetic cardiomyopathy.

CONCLUSION

On the basis of above discussion, it may be concluded that the beneficial effect of AVE-0991, a Mas-receptor agonist, in diabetic cardiomyopathy, may be due to downstream activation of leptin pathway.

ACKNOWLEDGMENTS

We express our sincere thanks to the management and Chairman, Mr. Praveen Garg, ISF College of Pharmacy, Moga, Punjab, India for providing necessary facilities.

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How to cite this article:

Shiv Kumar Yadav, Bijjem V. Krishna Reddy and P.L. Sharma. Possible involvement of leptin in a Mas-receptor agonist, AVE-0991-induced improvement in dyslipidemia and cardiomyopathy in STZ-induced diabetic rats. J App Pharm Sci. 2013; 3 (11): 070-075.