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Research Article

Cardio protective hypolipidemic activity of amino guanidine against Streptozotocin induced diabetic mice heart (an In vivo) and high glucose induced lipid accumulation cardiac fibroblasts (an in vitro study)

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ABSTRACT

This study mainly will focus on cardio protective antilipidemic activity of Aminguanidine against streptozotocin induced diabetic heart and high glucose induced lipid synthesis in H9C2 cardiac myoblasts. Excess lipid accumulation is one of the most diabetes associated complications in diabetic patients. Excess lipid may be stored as triglycerides, but are also shunted into non-oxidative pathways that disrupt normal cellular signaling leading to organ dysfunction and in some cases apoptosis, a process termed lipotoxicity. Lipid accumulation in cardiac turns Obesity and insulin resistance are associated with ectopic lipid deposition in multiple tissues including heart. Single dose of Streptozotocin (100 mg/kg) were given tail vein for diabetic induction. Normal and diabetic mice receive Aminoguanidine orally (100mg/kg) throughout the study period of eight weeks. H9C2 cardiac myoblasts grown and exposed to high glucose and Aminoguanidine for 48 hrs to study whether Aminoguanidine can successfully reduces high glucose induced lipid accumulation. Aminguanidine significantly reduces the cholesterol, Triglycerides, Low density lipoproteins in Streptozotocin induced diabetic mice and reduces accumulated Triglycerides and Phospholipids in cell lysates of H9C2 exposed to high glucose and Aminoguanidine. Exact mechanisms responsible for the high glucose induced lipid accumulation and by which lipotoxicity alter cardiac structure and function are incompletely understood. This study mainly focuses on cardio protective antilipidemic activity of Aminguanidine against streptozotocin induced diabetic heart and high glucose induced lipid synthesis in H9C2 cardiac myoblasts.

Keywords: Aminoguanidine; Glucose; Streptozotocin; Triglycerides; Phospholipids.

INTRODUCTION

It has been suggested that the dramatic increase in the prevalence of obesity and cardiovascular disease worldwide, termed the metabolic syndrome pandemic (Wende & Abel) may result in a decline in the life expectancy of the current generation (Caterson et al., 2004; and Johnson et al., 2007). Due to changed food habitats there is a dramatic increase in the prevalence of obesity and cardiovascular disease worldwide. Obesity also increases the susceptibility to diabetes, which is not only increases atherosclerotic heart disease but also increases the risk of developing heart failure (Eckel, 1997). The metabolic syndrome which is caused by an imbalance between nutrient uptake and energy expenditure is associated with ectopic deposition of lipid in non-adipose tissue such as blood vessels, heart, liver and other organs. Although these organs can initially store some of this surplus as triglycerides, excess lipids are eventually shunted into non-oxidative pathways resulting in the accumulation of toxic lipid species which alter cellular signaling, promote mitochondrial dysfunction and increase apoptosis (Lehtonen et al; Maiorana et al 2007; Slawik & Vidal-Puig, 2006).

Hypertriglyceridemia and increased circulating free fatty acids (FFA) are correlated with lipotoxicity in many tissues such as the heart, liver and β-cell (Cusi et al 2007; Fatehi-Hassanabad & Chan, 2005). In addition to increased circulating lipids, co-existent hyperglycemia and increased inflammatory cytokines may accelerate progression of cellular dysfunction and death, leading to the concept of glucolipotoxicity (Rita Costa, Elisa Rodrigues, & Oliveira 2010).

Under patho physiological conditions like hypertrophy, hear failure and infarction, switches back to the fetal transcriptional program that is with reduced fatty acid metabolism gene expression and relative increase in gene products involved in glucose metabolism. (Montessuit, Papageorgiou, Tardy-Cantalupi, 2000). These changes results in the preferable utilization of glucose for energy production instead of fatty acids. Notably lipids accumulate in failing myocardium especially in diabetes and associated obesity with increased levels of toxic intermediates leading to lipotoxicity (Schulze, 2009). These lipotoxic intermediate products may further worsen cardiac function and metabolism with the

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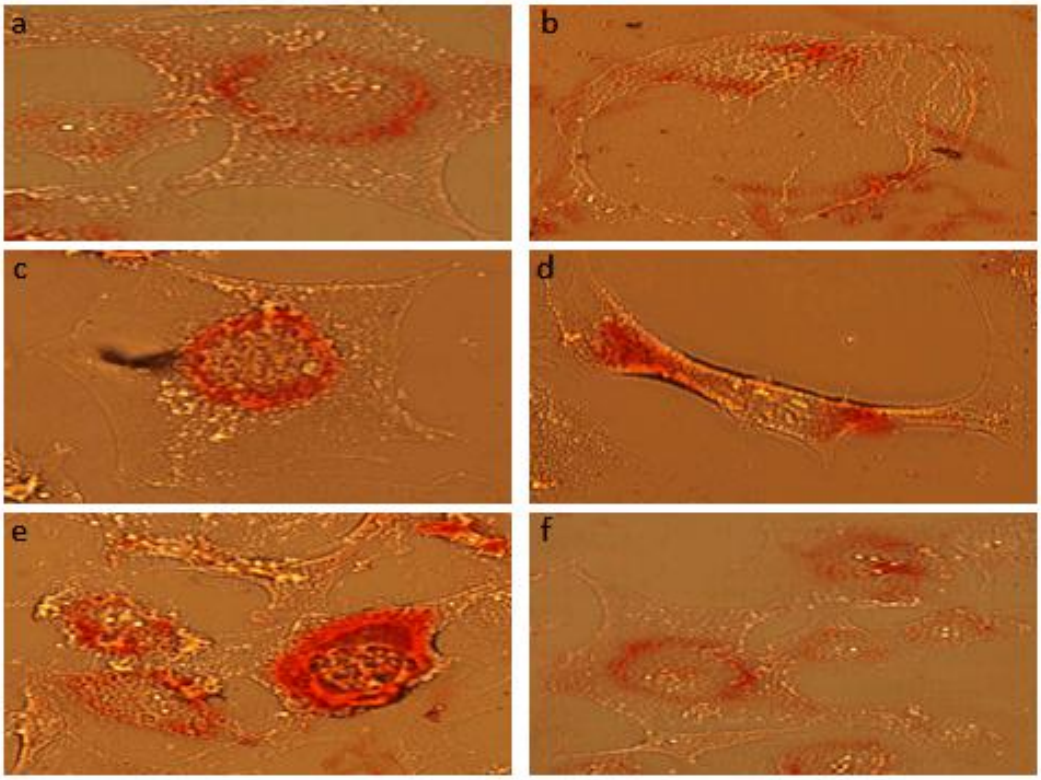

Figure 1: Oil red O staining of H9C2 cells treated with high glucose and AG for 48 hrs

(a-f) represents (a) Normal control cells (4.5 mM glucose). (b) Cells treated with AG (4.5 mM glucose + 10 mM AG). (c) Cells exposed to high glucose (20 mM). (d) Cells exposed to high glucse and treated with AG (20 mM +10 mM AG). (e) Cells exposed to high glucse (30 mM) and (f) Cell exposed to high glucose and treated with AG (30 mM) glucose + 10 mM AG.

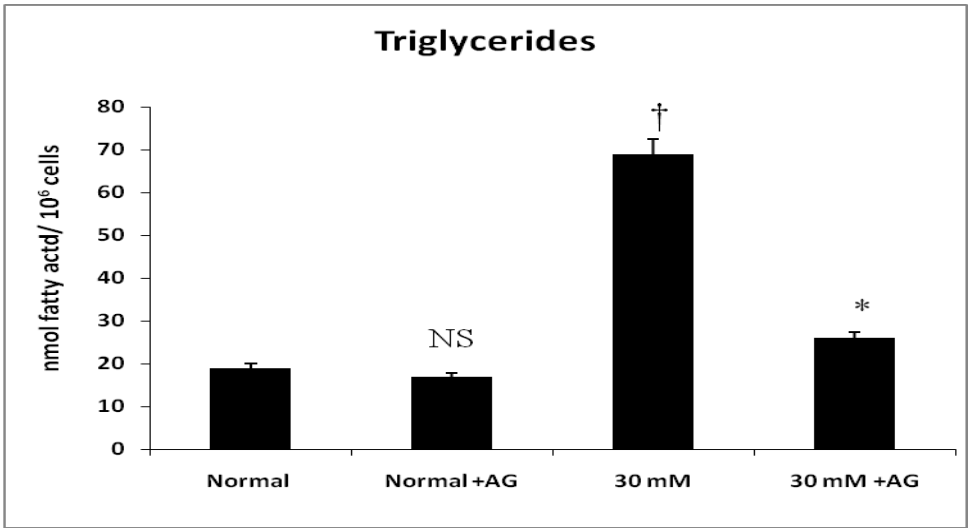

Figure 2: Effect of high glucose and Aminoguanidine on total triglyceride in H9C2 cells

Fig 2 shows the levels of total triglyceride content in H9C2 Cell lysates from cells exposed to normal glucose (Normal control-4.5 mM), Normal glucose treated with AG (10 mM), high glucose (30 mM), high glucose treated with AG. † *P*< 0.05 high glucose (30 mM) Vs Normal control (4.5 mM). Each value is expressed as mean ± S.D. Statistical significance at P < 0.05, as compared with †Group I, *Group II. NS non significant compared to group I.

development of progressive myocardial atrophy. Under normal physiological conditions, most triglycerides are stored in adipocytes with only minimal accumulation of lipids in other tissues such as liver and muscle. Increased cholesterol and triglycerides were detectable in myocardium of animals with obesity and diabetes (Tripathi & Srivastava, 2006). Findings were correlated in patients with diabetes and heart failure. Amount

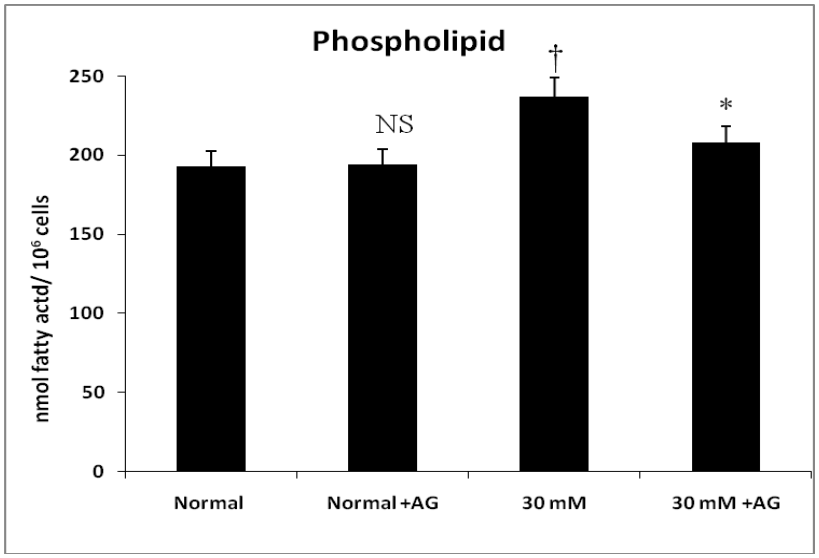

Figure 3: Effect of high glucose and Aminoguanidine on total Phospholipids in H9C2 cells

Fig 3 shows the levels of total Phospholipids in H9C2 Cell lysates from cells exposed to normal glucose (Normal control-4.5 mM), Normal glucose treated with AG (10 mM), high glucose (30 mM), high glucose treated with AG. † *P*< 0.05 high glucose (30 mM) Vs Normal control (4.5 mM). Each value is expressed as mean ± S.D. Statistical significance at P < 0.05, as compared with †Group I, *Group II. NS non significant compared to group I.

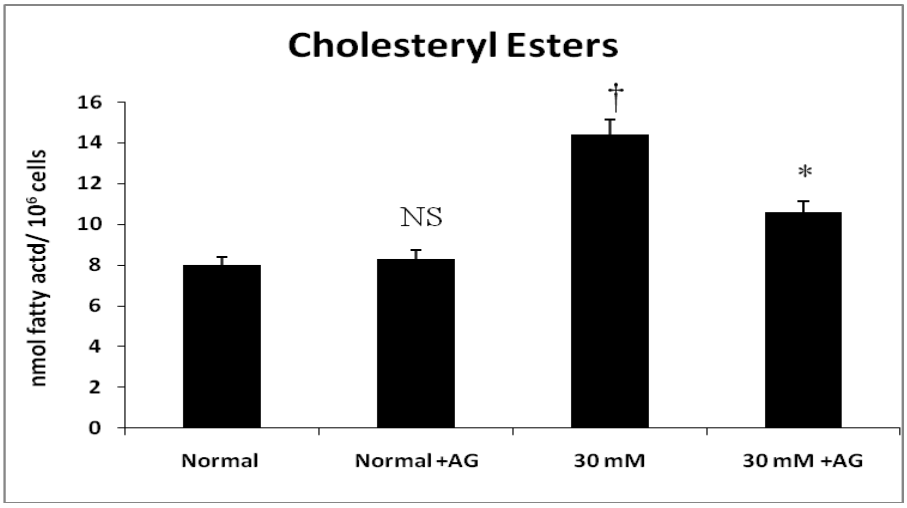

Figure 4: Effect of high glucose and Aminoguanidine on Cholesteryl Esters in H9C2 cells

Fig 4 shows the levels of Cholesteryl Esters in H9C2 Cell lysates from cells exposed to normal glucose (Normal control-4.5 mM), Normal glucose treated with AG (10 mM), high glucose (30 mM), high glucose treated with AG. † *P*< 0.05 high glucose (30 mM) Vs Normal control (4.5 mM). Each value is expressed as mean ± S.D. Statistical significance at P < 0.05, as compared with †Group I, *Group II. NS non significant compared to group I.

and composition of lipids in myocardium called lipotoxicity directly contributes to the development of cardiac dysfunction (Ruberg, 2007). The mechanism of increased lipid accumulation in myocardium was partially understood. Any compounds with and hypoglycemic and hypolipidemic activity had drawn attention in treatment of diabetes and associated obesity (Bedekar, Shah, & Koffas).

Aminoguanidine (AG) a nucleophilic hydrazine derivative of small molecular size, exhibiting antioxidant, hypoglycemic and hypolipidemic activity (Panagiotopoulos et al 1998). AG can interact with carbonyl groups of many biological constituents. Chemical interaction of AG with oxoaldehydes prevents the formation of highly reactive advanced glycation end products (AGEs) which helpful in treating diabetes associated complications (Prevot et al., 2007). AG had playing imp role in the AG has been proposed to delaying the pathogenesis of vascular and renal complications that are secondary to diabetes (Takeuchi et al 2010) and (Wang et al 2007). However detailed mechanism by which the AG protecting the diabetic heart is not known.

In the present study, we planned to answer these questions: (i) do Aminoguanidine inhibit excess lipid

Table 1: Effect of AG on serum Total Cholesterol, TGL, LDL and HDL in normal control, diabetic control and treated mice groups

	Total Cholesterol	Triglycerides	LDL	HDL
Group-I	84.40 ± 4.40	55.25 ± 5.11	32.80 ± 2.13	8.90 ± 2.28
Group-II	198.50 ± 10.20[a]	130.14 ± 9.52[a]	89.30 ± 3.73[a]	14.20 ± 1.29[a]
Group-III	129.34 ± 8.30[b]	94.20 ± 6.0[b]	56.25 ±3.15[b]	29.14 ± 2.16[b]
Group-IV	90.27 ± 6.11 [NS]	58.91 ± 8.24 [NS]	35.46 ± 2.3 [NS]	35.94 ± 1.95 [NS]

Each value is expressed as mean ±S.D. Statistical significance at P < 0.05, as compared with aGroup I, bGroup II. NS is not significant. Units are expressed as mg/dl for Cholesterol, Triglycerides, LDL and HDL.

Table 2: Effect of AG on cardiac Total lipids, Total Cholesterol, Triglycerides and Phospholipids in normal control, diabetic control and treated mice groups

	Total lipids	Total Cholesterol	Triglycerides	Phospholipids
Group-I	20 ± 1.03	2.11 ± 0.10	13.48 ± 0.87	14.52 ±0.66
Group-II	38 ± 5.6[a]	3.98 ± 0.23[a]	28.89 ± 1.73[a]	24.75 ± 1.29[a]
Group-III	26 ± 8.30[b]	2.80 ± 0.15[b]	20.56 ±1.35[b]	18.29 ± 1.08[b]
Group-IV	22 ± 6.11 [NS]	2.20 ± 8.24 [NS]	12.32 ± 2.3 [NS]	13.35 ± 0.98 [NS]

Each value is expressed as mean ±S.D. Statistical significance at P < 0.05, as compared with aGroup I, bGroup II. NS is not significant. Units are expressed as mg/dl Total lipids, Total Cholesterol, Triglycerides and Phospholipids.

accumulation in high glucose induced lipid synthesis in streptozotocin induced diabetic mice heart? (ii) Effect of Aminoguanidine against high glucose induced lipid accumulation in rat cardiac myoblasts (H9C2)?

MATERIALS AND METHODS

Chemicals and their Source

Streptozotocin and Aminoguanidine were purchased from Sigma Chemicals Co (St. Louis, MO, USA). All other chemical used in this study were of analytical grade and obtained from SRL Chemicals, Mumbai, India. Dulbecco modified Eagle medium (DMEM), Trypsin-EDTA, Fetal Bovine Serum, and Antibiotic solution from HIMEDIA (Mumbai, India); Whole cell lysis buffer was purchased from Thermo Scientific. All other chemicals used were of reagent grade (SRL-Mumbai).

EXPERIMENTAL PROTOCOL

Animal Model

Eight week old male Swiss albino mice were purchased from Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram, Chennai, India, and maintained in a controlled environmental condition of temperature and humidity on alternatively 12 h light/dark cycles. All animals were fed standard pellet diet (Hindustan Lever Ltd., Bangalore) and water *ad libitum.* All animal were maintained by procedures approved by the Institutional Animals Care and Ethical Committee (IACE-No: 01/069/09).

Drug induction, animal grouping and treatment

Total four groups were made and each group consists of six animals. Group I animals serving as vehicle controls. Group II consists of diabetic mice, received tail vein injection of a single dose of streptozotocin at 100 mg/kg body weight dissolved in freshly prepared sodium citrate buffer (pH 4.5). Group III consist of diabetic mice treated with Aminoguanidine (100 mg/kg). Group IV consist of normal mice treated with AG. Group III and Group IV animals receive Aminoguanidine dissolved 100 mg/kg orally. On day 5 after STZ treatment, whole blood glucose obtained from mouse tail-vein was detected using a complete blood glucose monitor (Bayer-contour plus). STZ-treated mice with whole blood glucose higher than 250 mg/dl were considered as diabetic. Mice serving as vehicle controls were given the same volume of sodium citrate. At the end of the experimental period the mice were fasted overnight, body weight were observed and then anesthetized followed by cervical decapitation. The blood samples were collected for further experimental purpose.

Cardiac myoblasts (H9C2) Cell culture and Treatment

Cultured rat cardiac myoblast (H9c2) cells were obtained from National Centre for Cell Science (NCCS, Pune). The cells were maintained in Dulbecco modified Eagle medium (DMEM) (HIMEDIA, Mumbai) supplemented with 10% fetal bovine serum, 90 U/ml penicillin, 90 µg/ml streptomycin and 5 µg/ml amphotericin B under atmospheric conditions of 95% air–5% CO2 at 37°C. Cells were seeded in 6-well plates until semi confluence and then the cells were maintained in 2 ml of DMEM without FBS for 20 h prior stimulation and during the stimulation. The conditioned cell media were collected 24 h after stimulation with glucose concentration ranging from 4.5 mM (normal) to 10, 15, 20, 25 and 30 mM (high). One set of cells with the same concentrations were exposed along with Aminoguanidine (10 mM) for 48 hr time period. At the end of the

experiments cells were scrapped and cell lysates were used for total lipid analysis.

Biochemical studies

Analysis of lipid Profiles in Serum, tissue and in cell lysates were carried out following by published papers. Serum separated for determination of parameters like triglycerides, Cholesterol, HDL using commercially available Accurex Diagnostic kit. (VLDL- cholesterol and LDL-cholesterol were calculated using the Friedewald's formula VLDL = Triglycerides / 5 and LDL = Total cholesterol – (HDL-CH + VLDL-CH). (Friedewald, Levy, & Fredrickson, 1972).

At the end of the stipulated period of eight weeks animals were sacrificed and heart was removed. Heart tissue lipids were extracted using the standard procedures of (Folch, Lees, & Sloane Stanley, 1957) extraction technique. Tissue cholesterol was estimated by the method of (Zlatkis et al., 1953), tissue triglycerides by the method of (Van Handel & Zilversmit, 1957). The method of (Bartlett, 1959) was used for the determination of tissue phospholipids phosphorous.

The cells were harvested, extracted and washed with 3 ml of ice cold phosphate buffered saline. Two ml of methanol: water (1:1, v/v) was added to the dish and the cells were removed, using a rubber policeman, into screw cap tubes. The suspension was vortexed twice and a 25 µl aliquot taken for the determination of protein. Lipids were extracted by a modified method of Folch et al. Total triglycerides, phospholipids and Cholestryl esters were estimated following the procedure of commercially available kits (biosystem assay).

Oil Red O Staining for Cultured Cells

Oil Red O Staining for Cultured Cells don by the method of (Pittenger et al., 1999) briefly culture and treated cultured cells in tissue culture plate as needed, once the treatment period over, take the culture plate out of incubator and remove the medium. Add ~2 ml of PBS to wash the cells and remove PBS completely. Add 2 ml of 10% formalin (RT) and incubate for 10 min at RT. Incubate for at least 1 hour. Remove formalin with a pipette and wash cells with ddH2O twice, and with 2 ml of 60% isopropanol for 5 min at RT. Let the cells dry completely at RT. Add 1 ml of Oil Red O working solution and incubate at RT for 10 min. Remove Oil Red O solution and immediately add double distilled water. Washing with ddH20 can repeat for 4 times. Acquire images under the light microscope for analysis.

Statistic Analysis

Statistical differences in the experimental animals were calculated by one way analysis of variance (ANOVA) followed by LSD test using SPSS/10 student version. All the results were expressed as the mean ± Standard deviation for six animals in each group. Differences were considered significant when $P<0.05$.

RESULTS

Table 1 Shows the on serum Cholesterol, Triglycerides, High density lipoproteins (HDL) and Low density lipoproteins (LDL) normal control, diabetic control and treated mice groups. Increase in total cholesterol, Triglycerides and LDL levels were noted in STZ induced mice group in compared to normal control, significant decrease in these levels were observed in rats given STZ and AG. Marked reduction of HDL levels was noticed in group II mice which receive STZ. HDL levels were come back to normal in AG treated group III mice group. No significant changes were observed group-IV.

Table 2 represents the on cardiac Total lipids, Cholesterol, Triglycerides and Phospholipids of experimental groups. Increase in total lipids, cholesterol, Triglycerides and Phospholipids were observed in STZ induced mice group in compared to normal control. These altered levels were bring back to normal were observed in rats given STZ and AG. No significant changes were observed group-IV.

Figure 1(a-f) illustrates the lipid accumulation revealed by Oil red O staining of H9C2 cells. Severe and gross changes were observed in cells exposed to high glucose. Dense staining of lipids seen in cells exposed to high glucose. AG treatment successfully reduced the lipid accumulation in H9C2 cardiac myoblasts.

Figure 2 depicts Effect of high glucose and AG on levels of total triglycerides of H9C2 cells exposed to high glucose and AG for 48 hrs. Significantly ($p < 0.05$) increased level of triglycerides were observed in cells exposed to high glucose (30 mM). Where as (Group III) shows low levels of total triglyceride upon AG co-treatment. AG alone treated cells are non significant when compared to normal control cells.

Figure 3 and 4 shows Effect of high glucose and AG on levels of total Phospholipids and Cholesteryl esters of H9C2 cells exposed to high glucose and AG for 48 hrs. Significantly ($p < 0.05$) elevated levels were seen in cells exposed to high glucose (30 mM). Whereas in Group III AG co-treatment reduced the levels of total Phospholipids and Cholesteryl esters when compare to cells treated high glucose. AG alone treated cells are non significant when compared to normal control cells.

DISCUSSION

Hyperglycemic and obesity affects cardiac structure and function in various ways. Hypertriglyceridemia and increased circulating free fatty acids (FFA) are correlated with lipotoxicity in many tissues such as the liver, and β-cell and heart (Lewis et al 2002). Short term lipid infusion enhances myocardial lipid accumulation and depresses cardiac function (Stanley, Recchia, & Lopaschuk, 2005). Cardiac triglyceride positively correlates with both body mass index and left ventricular (LV) mass in subjects with impaired glucose tolerance or obesity and inversely with systolic function. Hyperglycemia induced lipid accumulation has been linked to

structural and functional changes of the heart including LV hypertrophy (LVH), contractile dysfunction, apoptosis, fibrosis, lipid accumulation (Vaughan & Bell, 2006). Multiple molecular mediators have been proposed to promote these lipotoxic effects, such as high glucose induced reactive oxygen species (ROS) and nitric oxide (Y. Wang et al., 2009).

Diabetes associated obesity leads to cardiac dysfunction and heart failure due to accumulation of cholesterol especially low density lipoproteins (LDL) (Leichman et al., 2006). Intrinsic cardiac metabolism of the adult heart depends mainly on the utilization of the fatty acids for oxidative phosphorylation and generation of Adenosine tri phosphate (ATP) (Scheuer, 1967). Fetal heart preferably uses glucose for energy production, but in contrast after birth there is an increased expression of myocardial genes involved in the fatty acid metabolism. Mechanism of these increased gene expression is not understood yet.

Correlations between myocardial lipid accumulation and cardiac dysfunction have been noted in humans for nearly 150 years. However, only recently has there been renewed interest in the link between lipid accumulation and cardiac dysfunction. Indeed intra myocardial lipid accumulation in the failing heart shares many similarities with that seen in the lipotoxic rat heart. In addition to increased circulating lipids, coexistent hyperglycemia and increased inflammatory cytokines may accelerate progression of cellular dysfunction and death, leading to the concept of glucolipotoxicity. (van Herpen & Schrauwen-Hinderling, 2008) and (Balistreri, Caruso, & Candore, 2010).

Recent study conducted in cell culture, animal and human studies that link excess lipid delivery and accumulation to cellular apoptosis, contractile and metabolic dysfunction. Cell culture experiments have defined potential mechanisms for lipid-induced cell death (Turpin et al., 2009). Studies in animal models of obesity have demonstrated triglyceride accumulation in the heart and correlated these changes with potential mechanisms such as mitochondrial dysfunction and apoptosis (Trivedi & Barouch, 2008). Due to excess glucose the alternate mechanism of using surplus glucose in system is to synthesis of lipid in cell and stored mainly in adipocytes and some extent in tissues. Stored lipids mainly used in the providing energy in diabetic patients (Frayn et al 2003). When an excess amount of lipids accumulated in tissues leads to steatosis and turns toxic. Accumulation of low density lipoproteins in cardiac tissue and blood vessels leads to reduced function of heart and failure termed atherosclerosis (Fabbrini, Sullivan, & Klein).

To confirm the cardio protective, anti lipidemic activity if AG against high glucose induced diabetes, we used streptozotocin induced experimental diabetic mice and during the study period of 8 weeks, we observed the significant increase in total lipids in plasma and heart tissue. The cholesterol, triglycerides and phospholipids were drastically elevated in STZ induced heart. Ag treatment significantly reduced the elevated fractions of these lipids. The exact mechanism of the anti-lipidemic activity of AG not completely understood. This may be due the antioxidant and hypoglycemic activity of AG (Suthar, Rathore, & Pareek, 2009). The AG successfully reduced the diabetes associated complications (Oak, Youn, & Cai, 2009). To confirm the Anti-lipidemic activity we further used cardiac myoblast (H9C2) and exposed to normal and high glucose exposing to high glucose mimics the diabetic conditions *in vitro.* AG successfully reduced the high glucose induced cardiac lipids.

Oil red O stain extensively used for the lipid accumulation in adipocytes and other cell types. With the Oil red O stain it was clearly understood that the high glucose induces lipid accumulation in H9C2 cardiac myoblasts, and the AG treatment significantly reduced the accumulated cardiac lipids. Lipid extraction and estimation form cells further confirms the AG role against high glucose induced lipid accumulation. Total lipids, Triglycerides, and phospholipidawere neutralized upon AG treatment. Though each type of lipids have great significant in cell membrane synthesis and vast applications, but when it synthesized excess leads to lip toxic effect and further reduction of these necessary to balance the normal metabolism and prevention of diabetes and its assoiciated complications. The exact mechanism of lipid accumulation in diabetes is not understood yet.

CONCLUSION

Over all from the above study we are confirming the cardio protective effect of AG against high glucose induced lipid accumulation in H9C2 cardio myoblasts and in STZ induced experimental diabetic mice heart. Aminoguanidine can improve cardiac function in the diabetic and failing heart. Thus, we hypothesized that Aminoguanidine have a cardio protective effect against high glucose induced lipid accumulation and improve cardiac function in STZ induced diabetic heart.

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REFERENCES

Balistreri, C. R., Caruso, C., & Candore, G. The role of adipose tissue and adipokines in obesity-related inflammatory diseases. Mediators Inflamm, 2010, 802078.

Bartlett, G. R. (1959) Phosphorus assay in column chromatography. J Biol Chem, 234, 466-468.

Bedekar, A., Shah, K., & Koffas, M. Natural products for type II diabetes treatment. Adv Appl Microbiol, 71, 21-73.

Caterson, I. D., Hubbard, V., Bray, G. A., Grunstein, R., Hansen, B. C., Hong, Y., et al. (2004) Prevention Conference VII: Obesity, a worldwide epidemic related to heart disease and stroke: Group III: worldwide comorbidities of obesity. Circulation, 110, e476-483.

Cusi, K., Kashyap, S., Gastaldelli, A., Bajaj, M., & Cersosimo, E. (2007) Effects on insulin secretion and insulin action of a 48-h reduction of plasma free fatty acids with acipimox in nondiabetic subjects genetically predisposed to type 2 diabetes. Am J Physiol Endocrinol Metab, 292, E1775-1781.

Eckel, R. H. (1997) Obesity and heart disease: a statement for healthcare professionals from the Nutrition Committee, American Heart Association. Circulation, 96, 3248-3250.

Fabbrini, E., Sullivan, S., & Klein, S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology, 51, 679-689.

Fatehi-Hassanabad, Z., & Chan, C. B. (2005) Transcriptional regulation of lipid metabolism by fatty acids: a key determinant of pancreatic beta-cell function. Nutr Metab (Lond), 2, 1.

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem, 226, 497-509.

Frayn, K. N., Karpe, F., Fielding, B. A., Macdonald, I. A., & Coppack, S. W. (2003) Integrative physiology of human adipose tissue. Int J Obes Relat Metab Disord, 27, 875-888.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem, 18, 499-502.

Johnson, R. J., Segal, M. S., Sautin, Y., Nakagawa, T., Feig, D. I., Kang, D. H., et al. (2007)

Lehtonen, H. M., Jarvinen, R., Linderborg, K., Viitanen, M., Venojarvi, M., Alanko, H., et al. Postprandial hyperglycemia and insulin response are affected by sea buckthorn (Hippophae rhamnoides ssp. turkestanica) berry and its ethanol-soluble metabolites. Eur J Clin Nutr, 64, 1465-1471.

Leichman, J. G., Aguilar, D., King, T. M., Vlada, A., Reyes, M., & Taegtmeyer, H. (2006) Association of plasma free fatty acids and left ventricular diastolic function in patients with clinically severe obesity. Am J Clin Nutr, 84, 336-341.

Lewis, G. F., Carpentier, A., Adeli, K., & Giacca, A. (2002) Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr Rev, 23, 201-229.

Maiorana, A., Del Bianco, C., & Cianfarani, S. (2007) Adipose Tissue: A Metabolic Regulator. Potential Implications for the Metabolic Outcome of Subjects Born Small for Gestational Age (SGA). Rev Diabet Stud, 4, 134-146.

Montessuit, C., Papageorgiou, I., Tardy-Cantalupi, I., Rosenblatt-Velin, N., & Lerch, R. (2000) Postischemic recovery of heart metabolism and function: role of mitochondrial fatty acid transfer. J Appl Physiol, 89, 111-119.

Oak, J. H., Youn, J. Y., & Cai, H. (2009) Aminoguanidine inhibits aortic hydrogen peroxide production, VSMC NOX activity and hypercontractility in diabetic mice. Cardiovasc Diabetol, 8, 65.

Panagiotopoulos, S., O'Brien, R. C., Bucala, R., Cooper, M. E., & Jerums, G. (1998) Aminoguanidine has an anti-atherogenic effect in the cholesterol-fed rabbit. Atherosclerosis, 136, 125-131.

Pittenger, M. F., Mackay, A. M., Beck, S. C., Jaiswal, R. K., Douglas, R., Mosca, J. D., et al. (1999) Multilineage potential of adult human mesenchymal stem cells. Science, 284, 143-147.

Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. Am J Clin Nutr, 86, 899-906.

Prevot, D., Soltesz, Z., Abello, V., Wanecq, E., Valet, P., Unzeta, M., et al. (2007) Prolonged treatment with aminoguanidine strongly inhibits adipocyte semicarbazide-sensitive amine oxidase and slightly reduces fat deposition in obese Zucker rats. Pharmacol Res, 56, 70-79.

Rita Costa, A., Elisa Rodrigues, M., Henriques, M., Azeredo, J., & Oliveira, R. Guidelines to cell engineering for monoclonal antibody production. Eur J Pharm Biopharm, 74, 127-138.

Ruberg, F. L. (2007) Myocardial lipid accumulation in the diabetic heart. Circulation, 116, 1110-1112.

Scheuer, J. (1967) Myocardial metabolism in cardiac hypoxia. Am J Cardiol, 19, 385-392.

Schulze, P. C. (2009) Myocardial lipid accumulation and lipotoxicity in heart failure. J Lipid Res, 50, 2137-2138.

Slawik, M., & Vidal-Puig, A. J. (2006) Lipotoxicity, overnutrition and energy metabolism in aging. Ageing Res Rev, 5, 144-164.

Stanley, W. C., Recchia, F. A., & Lopaschuk, G. D. (2005) Myocardial substrate metabolism in the normal and failing heart. Physiol Rev, 85, 1093-1129.

Suthar, M., Rathore, G. S., & Pareek, A. (2009) Antioxidant and Antidiabetic Activity of Helicteres isora (L.) Fruits. Indian J Pharm Sci, 71, 695-699.

Takeuchi, M., Takino, J., & Yamagishi, S. Involvement of the toxic AGEs (TAGE)-RAGE system in the pathogenesis of diabetic vascular complications: a novel therapeutic strategy. Curr Drug Targets, 11, 1468-1482.

Tripathi, B. K., & Srivastava, A. K. (2006) Diabetes mellitus: complications and therapeutics. Med Sci Monit, 12, RA130-147.

Trivedi, P. S., & Barouch, L. A. (2008) Cardiomyocyte apoptosis in animal models of obesity. Curr Hypertens Rep, 10, 454-460.

Turpin, S. M., Ryall, J. G., Southgate, R., Darby, I., Hevener, A. L., Febbraio, M. A., et al. (2009) Examination of 'lipotoxicity' in skeletal muscle of high-fat fed and ob/ob mice. J Physiol, 587, 1593-1605.

Van Handel, E., & Zilversmit, D. B. (1957) Micromethod for the direct determination of serum triglycerides. J Lab Clin Med, 50, 152-157.

van Herpen, N. A., & Schrauwen-Hinderling, V. B. (2008) Lipid accumulation in non-adipose tissue and lipotoxicity. Physiol Behav, 94, 231-241.

Vaughan, T. B., & Bell, D. S. (2006) Diabetic cardiomyopathy. Heart Fail Clin, 2, 71-80.

Wang, X., Chang, T., Jiang, B., Desai, K., & Wu, L. (2007) Attenuation of hypertension development by aminoguanidine in spontaneously hypertensive rats: role of methylglyoxal. Am J Hypertens, 20, 629-636.

Wang, Y., Huang, Y., Lam, K. S., Li, Y., Wong, W. T., Ye, H., et al. (2009) Berberine prevents hyperglycemia-induced endothelial injury and enhances vasodilatation via adenosine monophosphate-activated protein kinase and endothelial nitric oxide synthase. Cardiovasc Res, 82, 484-492.

Wende, A. R., & Abel, E. D. Lipotoxicity in the heart. Biochim Biophys Acta, 1801, 311-319.

Zlatkis, A., Zak, B., & Boyle, A. J. (1953) A new method for the direct determination of serum cholesterol. J Lab Clin Med, 41, 486-492.