BMC Pharmacology

BioMed Central

Research article

The enhancement of the hyperglycemic effect of S-nitrosoglutathione and S-nitroso-N-acetylpenicillamine by vitamin C in an animal model

Donovan McGrowder, Dalip Ragoobirsingh* and Tara Dasgupta

Address: Department of Basic Medical Sciences (Biochemistry Section) and Department of Chemistry, University of The West Indies, Mona, Kingston 7, Jamaica, West Indies

E-mail: Donovan McGrowder - dmcgrowd@yahoo.com; Dalip Ragoobirsingh* - dragoo@uwimona.edu.jm; Tara Dasgupta - Tara@uwimona.edu.jm

*Corresponding author

Published: 13 September 2002

BMC Pharmacology 2002, **2**:18

Received: 18 March 2002
Accepted: 13 September 2002

This article is available from: http://www.biomedcentral.com/1471-2210/2/18

Abstract

Background: S-nitrosoglutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP) are two of the most common sources of nitric oxide (NO) in the biomedical field. Vitamin C has been known to accelerate the decomposition of GSNO and SNAP increasing the release and availability of NO which is cytotoxic at non-physiological concentrations. The study investigates any potential detrimental effect of vitamin C and GSNO, vitamin C and SNAP on glucose metabolism in normotensive and normoglycemic dogs.

Results: The results showed that administration of vitamin C (50 mg/kg) and GSNO (35 mg/kg & 50 mg/kg), or vitamin C (50 mg/kg) and SNAP (10 mg/kg) to overnight fasted dogs resulted in significant elevation of the blood glucose levels, attaining maximum level at the 2.0 or 2.5 h time point postprandially. The elevated blood glucose levels were due to significant reduction in plasma insulin levels in the dogs treated with vitamin C and GSNO, or vitamin C and SNAP ($P < 0.05$). The decreased insulin response was associated with significant elevation of nitric oxide produced from GSNO and SNAP co-administered with vitamin C, as assessed by plasma nitrate/nitrite levels.

Conclusions: The results indicate that enhanced NO release by vitamin C affects postprandial blood glucose and plasma insulin levels and the reduced glucose tolerance is mainly due to impaired insulin release. The clinical relevance of the findings of this study suggest that hypertensive diabetic patients treated with GSNO or SNAP, who are on vitamin C supplements may be more predisposed to further decrease in their glycemic control.

Background

Vitamin C (ascorbic acid) is a naturally occurring antioxidant in human plasma and is capable of scavenging super oxide anion [1]. Vitamin C levels in plasma and tissues have been reported to be significantly lower than normal in diabetic animals and humans, and might contribute to the complications found at the late stages of diabetes [2]. Chronic vitamin C administration has beneficial effects upon glucose and lipid metabolism in aged non-insulin dependent (type 2) diabetic patients [3], and high-dose supplementation may have beneficial effect in type 1 diabetes [4]. However, research carried out by investigators

examining the adverse effects of vitamin C, discovered that elevated plasma ascorbic acid delays insulin response to a glucose challenge in normoglycemic adults, thereby prolonging the postprandial hyperglycemia [5]. On the other hand, other researchers have found that large doses of vitamin C administered intravenously did not alter glucose tolerance and fasting glucose levels, nor fasting serum levels in normal or in obese subjects [6].

Nitric oxide-donor drugs decompose in the body, by a variety of mechanisms, to generate NO. Initial clinical studies of s-nitrosothiols such as S-nitrosoglutathione (GSNO) and S-nitroso-N-acetlypenicillamine (SNAP) suggest that they may be of benefit in a variety of cardiovascular disorders [7]. In animals and human beings, GSNO has significant antiplatelet action at doses that cause little or no cardiovascular effect [8]. It is used therapeutically as an arterio-selective vasodilator [9] and a platelet-selective anti-thrombotic agent [10]. Reducing agents such as glutathione and vitamin C can stimulate decomposition of GSNO and SNAP by chemical reduction of transition metal ions such as Cu^{2+}[11]. Enhanced release of NO from GSNO has been shown to augment the hypotensive response to this agent [12]. The effects of vitamin C on the NO-like bioactivity of exogenous GSNO and SNAP as it relates to carbohydrate metabolism is unclear and serves the purpose of this study.

Experimental evidence from animals studies suggest that GSNO and SNAP had a beneficial effect of reducing blood pressure, however this was associated with decreased glucose tolerance [13,14]. Therefore, we sought to test the hypothesis that co-administration of vitamin C and GSNO, or vitamin C and SNAP in an animal model may possibly result in a potential detrimental effect that could include further deterioration of glycemic control.

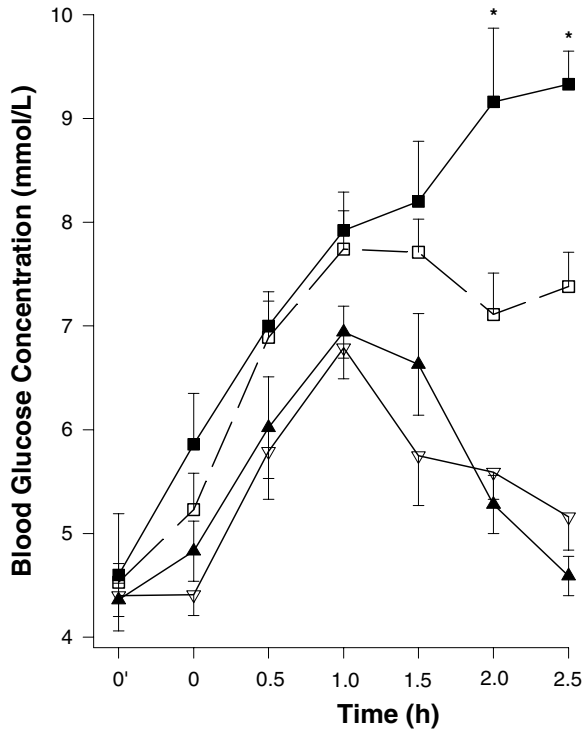

Figure 1
Line graphs showing the effect of 20 mg/kg captopril (▽), 35 mg/kg of S-nitrosoglutathione (□), 50 mg/kg of vitamin C and 35 mg/kg of S-nitrosoglutathione (■) on blood glucose levels. Water is used as the control solvent (▲). Statistical significant difference between GSNO-treated dogs and those treated with vitamin C and GSNO at different time points are indicated by $^{*}P < 0.05$.

Results

Effects of vitamin C and NO donors (GSNO & SNAP) on blood glucose levels

The glucose tolerance curve of dogs treated with 35 mg/kg of GSNO and 50 mg/kg of vitamin C was elevated above that of dogs treated with only 35 mg/kg of GSNO at the 2.0-h and 2.5-h time points (Figure 1). Statistical significant differences in mean postprandial blood glucose concentrations between the two groups were observed at the 2.0-h and 2.5-h time points with values of 9.16 ± 0.49 mmol/L and 9.33 ± 0.32 mmol/L in vitamin C and GSNO-treated dogs. These values were compared with 7.11 ± 0.40 mmol/L and 7.38 ± 0.33 mmol/L respectively in GSNO-treated dogs ($P < 0.05$). There were significant differences between the integrated area under the blood glucose concentration-time curve of dogs treated with vitamin C and GSNO, 1562.10 ± 101.40 mmol/L × 150 min compared with 1398.00 ± 69.00 mmol/L × 150 min in GSNO-treated dogs ($P < 0.05$). Further statistical analysis showed that areas under the blood glucose concentration-time curve differ significantly between vitamin C and GSNO-treated dogs, those treated with 20 mg/kg of captopril (1136.70 ± 69.00 mmol/L × 150 min; $P < 0.05$), and controls administered with water (1159.50 ± 72.00 mmol/L × 150 min; $P < 0.05$).

The elevated mean blood glucose concentrations of dogs treated with 50 mg/kg of GSNO and 50 mg/kg of vitamin C, and those treated with 50 mg/kg of GSNO were comparable from 0.5-h to 1.0-h postprandial time points (Figure 2). However, after the 1.0-h time point, the glucose tolerance curve for vitamin C and GSNO-treated dogs shifted upward. There were significant differences at the 1.5-h and 2.0-h time points with values of 10.22 ± 0.49 mmol/L and 10.23 ± 0.37 mmol/L respectively in the GSNO and

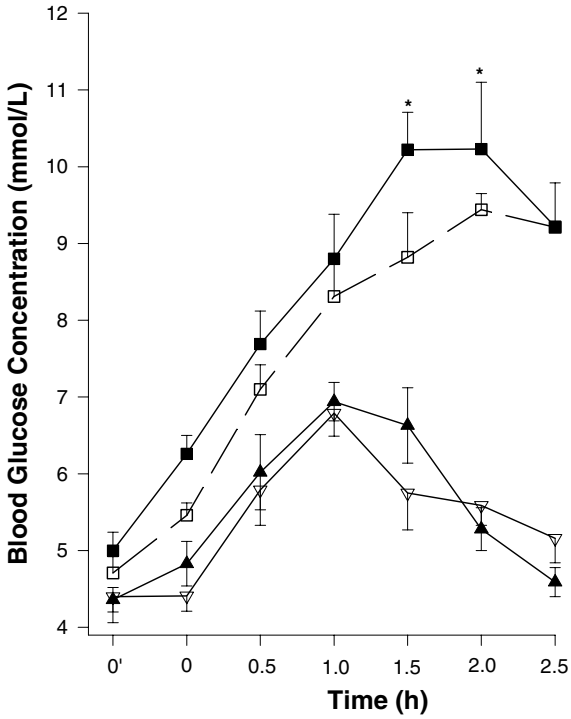

Figure 2
Line graphs showing the effect of 20 mg/kg captopril (▽), 50 mg/kg of S-nitrosoglutathione (□), 50 mg/kg of vitamin C and 50 mg/kg of S-nitrosoglutathione (■) on blood glucose levels. Water is used as the control solvent (▲). Statistical significant difference between GSNO-treated dogs and those treated with vitamin C and GSNO at different time points are indicated by $^{*}P < 0.05$.

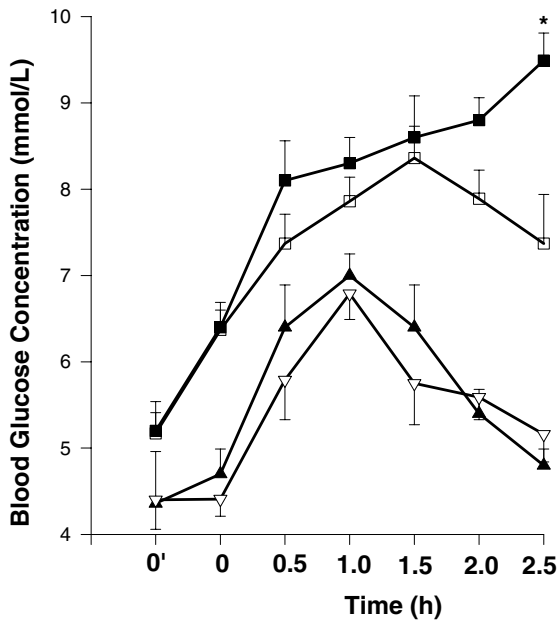

Figure 3
Line graphs showing the effect of 20 mg/kg captopril (▽), 10 mg/kg of S-nitroso-N-acetylpenicillamine (□), 50 mg/kg of vitamin C and 10 mg/kg of S-nitroso-N-acetylpenicillamine (■) on blood glucose levels. Water is used as the control solvent (▲). Statistical significant difference between SNAP-treated dogs and those treated with vitamin C and SNAP at different time points are indicated by $^{*}P < 0.05$.

vitamin C-treated dogs, compared with 8.82 ± 0.58 mmol/L and 8.44 ± 0.37 mmol/L respectively in the GSNO-treated dog ($P < 0.05$). There were significant differences between the integrated area under the blood glucose concentration-time curve of dogs treated with vitamin C and GSNO, 1722.60 ± 75.30 mmol/L × 150 min compared with 1591.50 ± 70.50 mmol/L × 150 min in GSNO-treated dogs ($P < 0.05$). Further statistical analysis showed that areas under the blood glucose concentration-time curve differ significantly between vitamin C and GSNO-treated dogs and those treated with 20 mg/kg of captopril ($P < 0.05$), and controls administered with water ($P < 0.05$).

The elevated mean plasma glucose concentrations in dogs treated with 10 mg/kg of SNAP and 50 mg/kg of vitamin C, and those treated with 10 mg/kg of SNAP were comparable from 0 to 1.5-h time points (Figure 3). However, at this point the glucose tolerance curve for vitamin C and SNAP-treated dogs shifted upward and there was significant difference at the 2.5-h time point with value of 9.49 ± 0.10 mmol/L in the SNAP and vitamin C-treated dogs. This was compared with 7.37 ± 0.57 mmol/L in the SNAP-treated dogs ($P < 0.05$). The integrated areas under the glucose concentration-time curve differ significantly between vitamin C and SNAP-treated dogs (1614.76 ± 71.40 mmol/L × 150 min) and those treated with SNAP (1511.70 ± 131.60 mmol/L × 150 min; $P < 0.05$), 20 mg/kg of captopril ($P < 0.05$), and controls administered with water ($P < 0.05$).

Effects of vitamin C and NO donors (GSNO & SNAP) on plasma insulin levels

In response to a glucose load, the mean plasma insulin concentration in dogs treated with GSNO increased to maximum of 15.60 ± 0.91 μIU/ml after 1.0-h postprandial and decreased steadily to 8.20 ± 0.91 μIU/ml after 2.5-

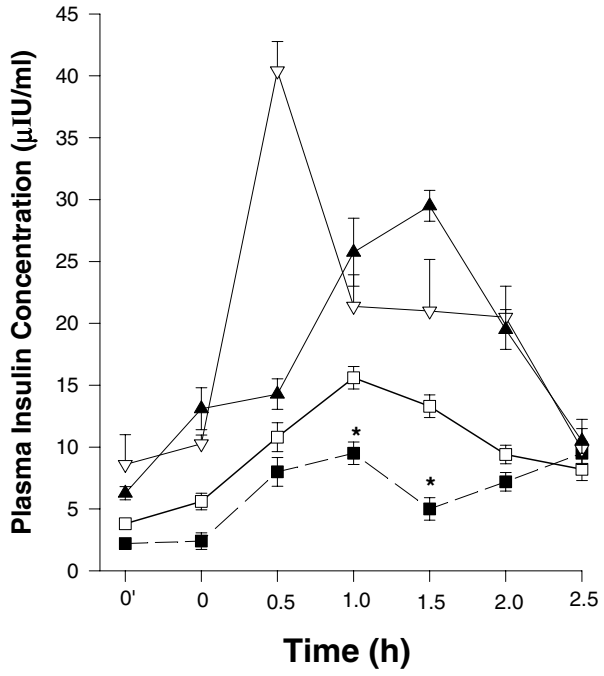

Figure 4
Line graphs showing the effect of 20 mg/kg captopril (▽), 50 mg/kg of S-nitrosoglutathione (□), 50 mg/kg of vitamin C and 50 mg/kg of S-nitrosoglutathione (■) on plasma insulin levels. Water is used as the control solvent (▲). Statistical significant difference between GSNO-treated dogs and those treated with vitamin C and GSNO at different time points are indicated by $^{*}P < 0.05$.

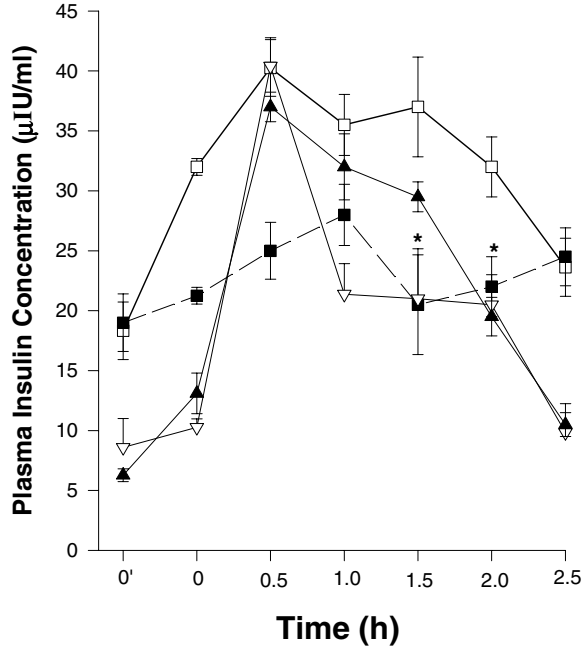

Figure 5
Line graphs showing the effect of 20 mg/kg captopril (▽), 10 mg/kg of S-nitroso-N-acetylpenicillamine (□), 50 mg/kg of vitamin C and 10 mg/kg of S-nitroso-N-acetylpenicillamine (■) on plasma insulin levels. Water is used as the control solvent (▲). Statistical significant difference between SNAP-treated dogs and those treated with vitamin C and SNAP at different time points are indicated by $^{*}P < 0.05$.

h postprandial (Figure 4). The mean plasma insulin concentration in dogs treated with 50 mg/kg of GSNO and vitamin C (50 mg/kg) increased gradually to 9.50 ± 0.70 μIU/ml at the 1.0 h time point, and then decreased to 7.20 ± 0.70 μIU/ml at the 2.0-h postprandial time point. Statistically significant differences between the mean plasma insulin levels of dogs treated with 50 mg/kg of GSNO, and those treated with 50 mg/kg GSNO and vitamin C (50 mg/kg) were observed at the 1.0-h and 1.5-h time points ($P < 0.05$). The mean plasma insulin concentrations in the dogs treated with 50 mg/kg of vitamin C and 50 mg/kg of GSNO, or 50 mg/kg of GSNO was significantly altered compared to those of controls or captopril-treated dogs ($P < 0.05$). There were significant differences between the integrated area under the plasma insulin concentration-time curve of dogs treated with vitamin C and GSNO, 1314.10 ± 125.80 mmol/L × 150 min. This is compared with 2001.00 ± 164.70 mmol/L × 150 min for GSNO-treated dogs ($P < 0.05$), 3959.40 ± 367.2 mmol/L × 150 min in captopril-treated dogs ($P < 0.05$), and 4448.10 ± 302.10 mmol/L × 150 min for controls administered with water ($P < 0.05$).

The decreased insulin response to the oral glucose challenge was depicted by mean plasma insulin concentration of 28.00 ± 1.50 μIU/ml at the 1.0-h postprandial time point in dogs treated with 50 mg/kg of vitamin C and 10 mg/kg of SNAP compared with 35.50 ± 0.50 μIU/ml in dogs treated with 10 mg/kg of SNAP ($P < 0.05$; Figure 5). Areas under the plasma insulin concentration-time curve did differ significantly between vitamin C and SNAP-treated dog (4814.7 ± 493.5 mmol/L × 150 min) compared with 6561.00 ± 500.40 mmol/L × 150 min for SNAP-treated dogs ($P < 0.05$). Further statistical analysis showed that areas under the plasma insulin concentration-time curve differ significantly between vitamin C and SNAP-treated dogs, those treated with 20 mg/kg of captopril ($P < 0.05$), and controls administered with water ($P < 0.05$).

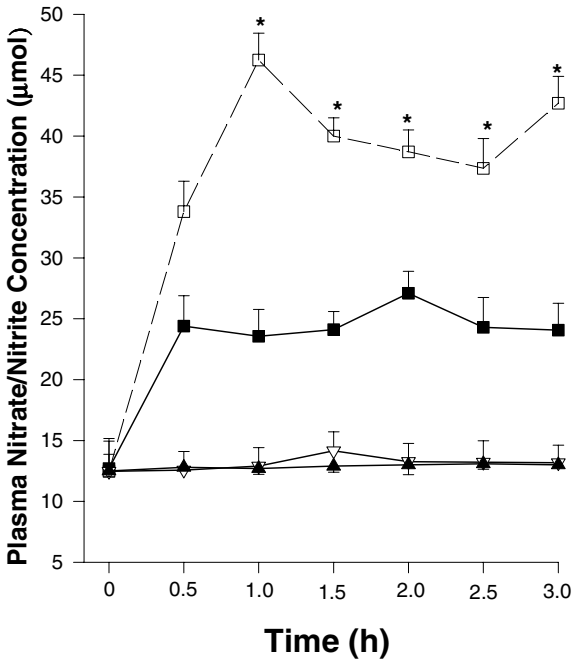

Figure 6
Line graphs showing the effect of 20 mg/kg captopril (▽), 50 mg/kg of S-nitrosoglutathione and 50 mg/kg of Vitamin C (□), 50 mg/kg of S-nitrosoglutathione (■) on plasma nitrate/nitrite concentrations. Water is used as the control solvent (▲). Statistical significant difference between GSNO-treated dogs and those treated with vitamin C and GSNO at different time points are indicated by $^{*}P < 0.05$

Determination of plasma nitrate/nitrite and vitamin C levels

The plasma nitrate/nitrite concentration increased two-fold over the basal value after 0.5 h, in dogs treated with of 50 mg/kg of GSNO (Figure 6). The plasma nitrate/nitrite concentration increased from 12.67 ± 0.47 μM to 24.53 ± 0.48 μM after 0.5 h, and then a steadily increased to a peak value of 27.13 ± 1.86 μM after 2.0 h. There was a slight decrease to 24.07 ± 0.23 μM after 3.0 h. On administration of 50 mg/kg of GSNO and 50 mg/kg of vitamin C, the plasma nitrate/nitrite concentration increased from 14.70 ± 0.47 μM to 40.00 ± 3.85 μM after 1.5 h, and then gradually decreased to 37.35 ± 1.10 μM after 2.5 h. The plasma nitrate/nitrite levels differ significantly between these two groups at all the time point ($P < 0.05$) throughout the experiment after drug administration, and also between vitamin C and GSNO-treated dogs, and captopril treated-treated dogs ($P < 0.05$). Plasma nitrate/nitrite concentration also differ significantly between vitamin C and GSNO-treated dogs, and controls administered with water ($P < 0.05$).

The plasma nitrate/nitrite concentration of dogs treated with 10 mg/kg of SNAP increased by 8 ± 0.5 % after 0.5 h, from 12.04 ± 0.30 μM to 13.00 ± 0.23 μM (Figure 7). This marginal rise in the plasma nitrate/nitrite concentration was followed by gradual increases over the 3.0 h period, with a maximum level of 19.12 ± 0.5 μM after 2.5 h. The plasma nitrate/nitrite concentration increased by 32 ± 2 % over the basal value after 0.5 h, in dogs treated with of 10 mg/kg of SNAP and 50 mg/kg of Vitamin C. The plasma nitrate/nitrite concentration increased from 12.15 ± 0.30 μM to 15.01 ± 0.48 μM after 0.5 h, and then a steadily increased to a peak value of 28.35 ± 1.50 μM after 3.0 h. Statistically significant differences between the plasma nitrate/nitrite concentration of SNAP (10 mg/kg) and vitamin C-treated dogs, and those treated with SNAP (10 mg/kg) were observed at 1.5-h to 3.0-h time points ($P < 0.05$). The mean plasma nitrate/nitrite concentration remained elevated in SNAP (10 mg/kg) and vitamin C-treated dogs, compared with those of controls administered with or captopril-treated dogs in which no increase in this parameter was observed ($P < 0.05$). Mean fasting plasma ascorbic concentration was in the normal range of 0.7 – 1.2 mg/100 ml. The increase in mean vitamin C concentration in plasma after administration of 50 mg/kg of vitamin C ranged from 1.03 ± 0.05 mg/100 ml – 2.79 ± 0.30 mg/100 ml that is comparable with literature values at similar vitamin C intake [15].

Discussion

We have showed previously that GSNO and SNAP had beneficial effects based on their reduction of blood pressure in normotensive dogs [13,14]. This was related to enhanced generation and release of NO. In addition, findings from the same study showed that the decreased glucose tolerance observed was associated with decreased insulin release [13,14]. In the present investigation, we tested the hypothesis that co-administration of vitamin C and GSNO, or vitamin C and SNAP in an animal model may possibly result in a potential detrimental effect that could include further deterioration of glycemic control. The salient finding of this study is that enhanced NO release from GSNO and SNAP by vitamin C, affects postprandial blood glucose and plasma insulin levels, and reduced glucose tolerance is mainly due to impaired insulin release. Therefore vitamin C deteriorates the decreased glucose tolerance by the NO donors. This observation strongly support the fact that elevated plasma ascorbic acid may delay or decrease insulin response to a glucose challenge in an animal model, thereby prolonging the postprandial hyperglycemia. The results from this study are in agreement with previous observations that indicated that elevated plasma vitamin C delays the insulin re-

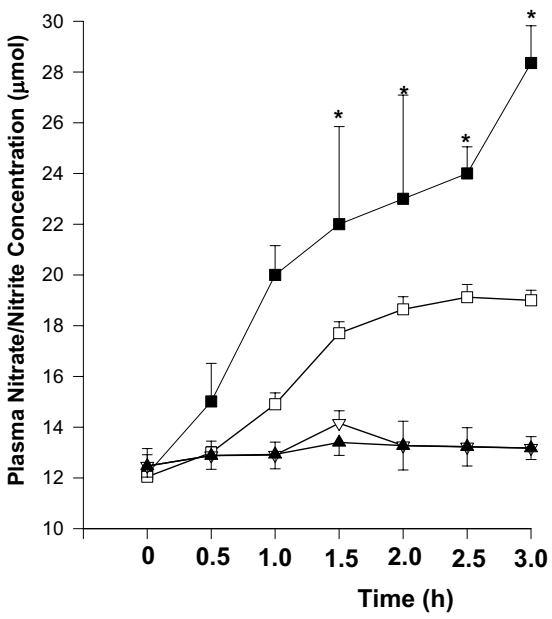

Figure 7
Line graphs showing the effect of 20 mg/kg captopril (▽), 10 mg/kg of S-nitroso-N-acetylpenicillamine (□), 10 mg/kg of S-nitroso-N-acetylpenicillamine and 50 mg/kg of vitamin C (■) on plasma nitrate/nitrite concentration. Water is used as the control solvent (▲). Statistical significant difference between SNAP-treated dogs and those treated with vitamin C and SNAP at different time points are indicated by $^{*}P < 0.05$.

sponse to a glucose challenge in normoglycemic adults, perhaps by competitively inhibiting glucose uptake by pancreatic beta-cells [5].

In the present study the insulin response to a glucose challenge in the dogs treated with vitamin C and SNAP or vitamin C and GSNO was notably delayed and significantly decreased compared with values in dogs treated with only SNAP or GSNO, captopril or controls administered with water. A likely explanation for the initial delayed in insulin release could be that as the blood glucose began to rise in the initial minutes after the glucose challenge, competition between glucose and vitamin C for transport into the islet cells may have slowed glucose entry, thereby impairing the glucose-sensing apparatus of the islet cells. Hence, less insulin was secreted 0.5-h postprandial in the vitamin C and GSNO or vitamin C and SNAP-treated dogs, than in dogs treated with GSNO, SNAP, captopril or controls administered with water. Decreased insulin secretion would also explain why mean blood glucose was significantly elevated in the vitamin C and GSNO, or vitamin C and SNAP-treated dogs at the 2.0 and 2.5-h time points compared with dogs treated with GSNO or SNAP, captopril or control administered with water thereby prolonging postprandial hyperglycemia. As evident by comparisons of the area circumscribed by the plasma insulin response curve, the quantity of insulin released by the pancreas was significantly affected by the co-administration of vitamin C with the NO-donors, suggesting that inadequate insulin was secreted to dispose of the glucose load in the vitamin C and GSNO, or vitamin C and SNAP-treated dogs. The blood glucose values for the vitamin C and GSNO or vitamin C and SNAP-treated dogs were well outside of the normal limits at the 2.0-h and 2.5-h time points, and significantly elevated above their counterparts treated with only GSNO or SNAP ($P < 0.05$) whose values were also outside the normal range, suggesting further deterioration of glucose tolerance.

Nitric oxide is toxic at high physiological concentration where it appears to function as a cellular effector molecule that mediates both cytostatic and cytotoxic effect [16]. Previous evidence have indicated that pancreatic islet cells exposed to the NO-donor streptozotocin caused lasting, damage to the beta-cells, characterized by a persistent impairment in glucose metabolism and a defective insulin response [17], and that the NO-donor sodium nitroprusside caused lysis of islet cells in a concentration- and time-dependent manner. The mechanism by which these drugs affect the pancreas could account for the hyperglycemic effect observed in the dogs treated with GSNO and SNAP. Vitamin C, an important determinant of the intracellular redox state, has been known to accelerate the decomposition of GSNO and SNAP increasing the release and availability of NO. The modulation of the bioactivity of GSNO by ascorbic acid is dependent on the presence of transition metal ions [18]. Reduced transition metal ions such as Cu^{+} catalyze the decomposition of GSNO and SNAP than do their oxidized forms eg, Cu^{2+}[18]. Elevated levels of plasma nitrate/nitrite levels, assessed as NO production, was observed in vitamin C and GSNO or vitamin C and SNAP-treated dogs indicating enhanced NO release. The prolonged and exacerbated hyperglycemic effect could be explained in terms of the fact that exposure of the beta-cells to increased NO levels resulting in further deterioration in beta-cell function characterized by impairment in glucose metabolism and defective insulin response [17]. The enhanced NO released caused marked reduction in the plasma insulin levels which are in agreement with previous observations which suggest that NO acts as a negative modulator of glucose stimulated insulin secretion, thus accounting for the pronounced hyperglycemic effect [19].

The solvent water was administered to the control dogs. Water was used because it can be considered as an "inactive treatment" with no capacity to enhance the generation of NO from its donors or possessing any antioxidant

properties. Captopril an angiotensin converting enzyme inhibitor was also used in the study. Reports on the metabolic effects of captopril on oral glucose tolerance and insulin action in the literature are conflicting. Studies have shown that captopril enhanced the effects of bradykinin, which via a stimulation of B_2 receptors caused the release of NO and of prostanoids, which resulted in increased blood glucose levels and reduced plasma insulin levels [20]. However the results from this study showed that captopril had no effect on oral glucose tolerance and insulin levels, and plasma nitrate/nitrite levels in captopril-treated dogs was comparable with age-matched controls administered with water. The results from our study is supported by similar findings by other investigators, where experiments carried out on spontaneously hypertensive rats showed that captopril reduced blood pressure and cardiac mass, but had no significant effect on oral glucose tolerance and insulin action [21]. In addition, analysis of results from the treatment of hypertensive patients with captopril showed that it had no effect on carbohydrate metabolism, which is advantageous for the treatment of hypertensive patients [22].

It is suggested that the lack of effect of captopril on glucose tolerance might be due to different tissue metabolisation and/or penetration of captopril, which could arise from (1) the redox status of the tissue, which limit captopril bioavailability by favouring the formation of disulfurs through captopril dimerzation or reaction with a different thiol compound; and (2) the existence of organ renninangiotensin systems with different levels of expressions and/or activity of angiotensin converting enzymes [23]. Captopril has been reported to possess hydroxyl and hypochlorous acid scavenging effects, which could contribute to its therapeutic activity in the clinical settings [24]. The sulfhydryl moiety (-SH) of captopril is responsible for its antioxidant properties [25] and it is suggested that captopril may protect tissues from oxidative damage by increasing enzymatic and non-enzymatic antioxidant deference [23] and inhibit microsomal lipid peroxidation [26].

Assessment of the clinical relevance of the present results should take into account the fact that ascorbic acid influences the decomposition of SNAP in rat striatal slices [27]. Islet cells were found to be very susceptible to the cytotoxic action of NO while hepatocytes and endothelial cells were relatively resistant [28]. Epidemiological studies have indicated that an association between increased intake of vitamin C and reduced risk of coronary disease [29]. This is the first documentation of the potential detrimental effect of vitamin C and NO donors on glucose tolerance in an animal model. The clinical relevance of the findings of this study allows us to suggest that hypertensive patients treated with GSNO or SNAP, who are on vitamin C supplements may be more predisposed to further decrease in their glycemic control. These findings should encourage further research on the effect of vitamin C supplementation on glucose homeostasis and insulin release in clinical settings involving hypertensive and diabetic patients is warranted. The elucidation of the underlying mechanism might help us to understand the reaction of antioxidants with NO donors and it is therefore suggested that vitamin C supplementation should be limited in patients treated with these drugs.

Conclusion

The findings of this study indicates that enhanced NO release affects postprandial blood glucose and plasma insulin levels and reduced glucose tolerance is mainly due to impaired insulin release. Vitamin C deteriorates the decreased glucose tolerance by the NO donors. This the first report of the detrimental effect of vitamin C on glucose metabolism in normoglycaemic dogs, treated with NO donors, and furthers current knowledge on the association of NO donors and antioxidants. An examination of the possible effect of vitamin C supplementation on glucose homeostasis and insulin release in clinical settings involving hypertensive and diabetic patients is warranted.

Methods

Animal care – experimental design

The protocol was conducted in accordance with the guidelines of the University of the West Indies Animal Committee. Mongrel dogs (15 males and 15 females) of 2–3 years, with average weight of 12.4 ± 0.4 kg were obtained from the Pre-Clinical Animal House of the Department of Basic Medical Sciences, University of the West Indies. The animals were maintained in the animal house under the supervision of attendants and a veterinary consultant. Dogs were fed on a diet of Purina Laboratory Chow (Purina, St. Louis MO. USA) and water administered *ad libitum*. The dogs were divided into five groups of six and each had normal glucose tolerance as indicated by an oral-glucose tolerance test (OGTT) performed at the beginning of the study. The first group of dogs was administered with water (controls), the second group was treated with 20 mg/kg of captopril, and the third group with 35 mg/kg of GSNO, then GSNO (35 mg/kg) and 50 mg/kg of vitamin C. The fourth group was treated with 50 mg/kg of GSNO, then GSNO (50 mg/kg) and 50 mg/kg of vitamin C and the fifth group was treated with 10 mg/kg of SNAP, then SNAP (10 mg/kg) and 50 mg/kg of vitamin C.

Oral glucose tolerance test

An oral glucose tolerance test was performed on each dog. Briefly, after an 18-h fast, dogs were anesthetized with sodium pentabarbital (30 mg/kg i.v.). Subsequently, the fasting blood sample [0'] was taken and in control experiments 20 mg/kg body weight of captopril (Sigma Chem-

icals Co. Ltd, St. Louis MO. U.S.A.) dissolved in water was administered intravenously. In test experiments, S-nitroso-N-acetylpenicillamine dissolved in dimethylsulphoxide (DMSO) was administered at a dose of 10 mg/kg body weight to the dogs at the 0' time point after a fasting blood sample was taken. S-nitrosoglutathione, dissolved in water (2 ml) was administered at doses of 35 or 50 mg/kg body weight to the dogs also at the 0' time point. Additional blood samples were collected 0.5, 1.0 1.5, 2.0, and 2.5-h time points after administration of a glucose load of 1.75 g/kg body weight. Each sample was collected in an EDTA tube and immediately placed on ice for subsequent biochemical analysis.

The plasma glucose levels were determined by the glucose oxidase method [30] and the absorbance measured at 420 nm using a Spectrophotometer (Spectronic Genesys). The plasma insulin concentrations were measured by radioimmunoassay with a commercial kit (Diagnostic Products Corporation, Los Angeles, California, USA), and radioactivity was determined using a gamma counter (Abbott Auto Logic Gamma Counter). The plasma ascorbic acid concentration was determined using the 2,4-nitrophenylhydrazine method of Omaye et al [31].

Nitric oxide formation was measured as plasma nitrate/nitrite concentration, using the Griess reaction [32]. Briefly, 50 μl of plasma was deproteinized by the addition of 100 μl of 35% sulfosalicylic acid. Treated samples were mixed by vortexing every 5 minutes and allowed to react for 30 minutes at room temperature. These were centrifuged at 10,000 g for 15 minutes. Two hundred microlitres (200 μl) of the supernatant was added to 4 ml of de-ionized water in a 1:20 dilution for analysis. The sample was passed through a copper-cadmium column of an autoanalyser (Autoanalyser, Technicon Instruments Corporation, Tarrytown, N.Y. U.S.A.), to reduce nitrate to nitrite. The resulting nitrite concentration was determined by the addition of Griess reagent [0.1% sulfanilamide in 5% concentrated phosphoric acid and 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride] to form a purple azo dye and the nitrite was quantified using $NaNO_2$. The data is presented as plasma nitrate/nitrite concentration.

Calculations and statistics

Results are reported as the mean ± S.E.M. Integrated area under the curve (iUAC) was calculated by subtracting the rectangle corresponding to the basal value from the total area under the curve [33]. Mean curved areas were compared by using the paired Student's t test for controls and all the groups in the test experiments. Analysis of variance (ANOVA) followed by the Bonferroni multiple comparison test [34] was used to examine differences in blood glucose, plasma insulin and plasma nitrate/nitrite concentrations at each time interval during the OGTT. Analysis of the data was done using the Sigma Plot and Sigma Statistics software packages (Jandel Scientific). A probability value of less than 0.05 was considered to indicate significance in all cases.

Authors' Contributions

DM carried out the oral glucose tolerance tests, radioimmunoassays, Greiss reaction and drafted the manuscript. DR conceived of the study, and participated in its design and coordination. TD participated in the design of the study. All authors read and approved the final manuscript.

Acknowledgements

The authors extend gratitude to Dr. Paul Brown for his assistance in the preparation of the manuscript. This work was supported by a grant from the Mona Campus Committee for Graduate Studies, University of the West Indies.

References

1. Frei B, Stocker R, Ames BN: **Antioxidant defenses and lipid peroxidation in human blood plasma.** *Proc Natl Acad Sci USA* 1988, **85:**9748-9752
2. Yew MS: **Effect of streptozotocin diabetes on tissue ascorbic acid and dehydroascorbic acid.** *Horm Metab Res* 1983, **15:**158
3. Paolisso G, D'Amore A, Balbi V, Volpe C, Galzerano D, Giugliano D, Sgambato S, Varricchio M, D'Onofrio F: **Plasma vitamin C affects glucose homeostasis in healthy subjects and in non-insulin-dependent diabetics.** *Am J Physiol* 1994, **266:**E261-E268
4. Eriksson J, Kohvakka A: **Magnesium and ascorbic acid supplementation in diabetes mellitus.** *Ann Nutr Metab* 1995, **39:**217-223
5. Johnson CS, Fan M: **Megadose of vitamin C delays insulin response to a glucose challenge in normoglycemic adults.** *Am J Nutr* 1994, **60:**735-738
6. Scarlett JA, Zeidler A, Hrochman , Rubenstein A: **Acute effects of ascorbic acid on carbohydrate tolerance.** *Am J Clin Nutr* 1976, **29:**1339-1342
7. Al-Sa'doni H, Ferro A: **S-nitrosothiols: a class of nitric oxide-donor drugs.** *Clin Sci (Lond)* 2000, **98:**507-520
8. De Belder AJ, MacAllister R, Radomski MW, Moncada S, Vallance PJT: **Effects of S-nitros-glutathione in the human forearm circulation: evidence for selective inhibition of platelet activation.** *Cardiovas Res* 1994, **28:**691-694
9. Macallister RJ, Calver AL, Riezebos J, Collier J, Vallance P: **Relative potency and arteriovenous selectivity of nitrovasodilators on human blood vessels: an insight into targeting of nitric oxide delivery.** *J Pharmacol Exp Ther* 1995, **273:**154-160
10. Langford EJ, Brown AS, Wainwright RJ, Belder AJ, Thomas MR, Smith REA, Radmski MW, Martin JF, Moncada S: **Inhibition of platelet activity by S-nitrosoglutathione during coronary angioplasty.** *Lancet* 1994, **344:**1458-1460
11. Singh RJ, Hogg N, Joseph J, Kalyanaraman B: **Mechanism of nitric oxide release from S-nitrosothiols.** *J Biol Chem* 1996, **271:**18596-18603
12. Sharfstein JF, Keaney JF, Slivka A, Welch GN, Vita JA, Stamler JS, Loscalzo J: **In vivo transfer of NO between a plasma protein-bound reservoir and low-molecular-weight thiols.** *J Clin Invest* 1994, **94:**1432-1439
13. McGrowder D, Ragoobirsingh D, Dasgupta T: **The hyperglycemic effect of S-nitrosoglutathione in the dog.** *Nitric Oxide: Biology and Chemistry* 1999, **3:**481-491
14. McGrowder D, Ragoobirsingh D, Dasgupta T: **Effects of S-nitroso-N-acetylpenicillamine administration on glucose tolerance and plasma levels of insulin and glucagon in the dog.** *Nitric Oxide: Biology and Chemistry* 2001, **5:**402-412
15. Berger L, Gerson CD, Yu TF: **The effects of ascorbic acid on uric acid excretion with a commentary on renal handling of ascorbic acid.** *Am J Med* 1977, **62:**71-79
16. Rabinovitch A: **Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM.** *Diabetes* 1994, **43:**613-621

17. Schmidt HHW, Warner TD, Ishii K, Sheng H, Merad F: **Insulin secretion from pancreatic β-cells caused by L-arginine-derived nitrogen oxides.** *Science* 1992, **255:**721-723
18. Dicks AP, Williams DL: **Generation of nitric oxide from S-nitrosothiols using protein-bound Cu^{2+} sources.** *Chem Biol* 1996, **3:**655-659
19. Mosen H, Salehi A, Lundquist I: **Nitric oxide, islet acid glucan-1,4-alpha-glucosidase activity and nutrient-stimulated insulin secretion.** *The Journal of Endocrinology* 2000, **165:**293-300
20. Demas J, Hallet C, Lefebre PJ: **Changes in blood glucose and plasma insulin levels induced by bradykinin in anesthetized rats.** *Br J Pharmacol* 2001, **134:**1312-1318
21. Swislocki AL, LaPier TL, Khuu DT, Fann KY, Tait M, Rodnick KJ: **Metabolic, hemodynamic, and cardiac effects of captopril in young, spontaneously hypertensive rats.** *Am J Hypertension* 1999, **12:**581-589
22. Krutikova EV, Slavina LS, Lobanova AM, Chilhladze NM: **Effect of captopril on insulin secretion and blood glucose in patients with arterial hypertension.** *Biull Vsesoiuznogo Kardiol Nauchn Tsentra AMN* 1988, **11:**69-71
23. de Cavanagh EM, Inserra F, Ferder L, Fraga CG: **Enalapril and captopril enhance glutathione-dependent antioxidant defenses in mouse tissues.** *Drug Metabolism and Disposition* 2000, **278(3):**R572-R577
24. de Cavanagh EM, Inserra F, Ferder L, Fraga CG: **Enalapril and captopril enhance antioxidant defenses in mouse tissues.** *Am J Physiol* 1977, **272:**R514-R518
25. Munzel T, Keaney JF Jr: **Are ACE inhibitors a "a magic bullet" against oxidative stress?** *Circulation* 2001, **104:**1571-1574
26. Chopra M, Beswick H, Clapperton M, Dargie HJ, Smith WE, Murray J: **Antioxidant effects of angiotensin-converting enzyme (ACE) inhibitors: free radical and oxidant scavenging are sulfhydryl dependent, but lipid peroxidation is inhibited by both sulfhydryl- and nonsulfhydryl-containing ACE inhibitors.** *J Cardiovasc Pharmacol* 1992, **19:**330-340
27. Reiser M, Schild L, Keilhoff G, Wolf G: **Interaction of nitric oxide donors and ascorbic acid on [^{3}H] aspartate efflux from rat striatal slices.** *Neurochem Res* 1999, **24:**61-67
28. Kroncke KD, Brenner HH, Rodriguez ML, Etzkorn K, Noack EA, Kolb H, Kolb-Bachofen V: **Pancreatic islet cells are highly susceptible towards the cytotoxic effects of chemically generated nitric oxide.** *Biochem Biophys Acta* 1993, **1182:**221-229
29. Riemersma RA, Wood DA, Macintyre CCH, Elton RA, Gey F, Oliver MF: **Low plasma vitamin E and C and increased risk of angina in Scottish men.** *Ann NY Acad Sci* 1989, **570:**291-295
30. St G Huggertt A, Nixon DA: **Use of glucose oxidase, peroxidase, and o-dianisidine in the determination of blood and urinary glucose.** *The Lancet* 1957, **314:**368-370
31. Omaye ST, Turnbull JD, Sauberlich HE: **Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids.** *Methods Enzymol* 1979, **62:**7-8
32. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR: **Analysis of nitrate, nitrite, and [^{15}N] nitrate in biological fluids.** *Anal Biochem* 1982, **126:**131-138
33. Godfrey K: **Statistics in practice: comparing the means of several groups.** *N Eng Med* **313:**1450-1456
34. LeFloch JP, Escuyer P, Baudin , Baudon D, Perlemuter L: **Blood glucose area under the curve: methodological aspects.** *Diabetes Care* 1990, **13:**172-75