Elevated Glucose Inhibits Vasoconstriction but not Mannitol Following Balloon Angioplasty

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Abstract: Background: Balloon angioplasty (BA) reduces vasomotor relaxation by injuring the vascular wall. This experiment was to evaluate the effect of high glucose concentration on the vasomotor response following BA. Methods: 22 New Zealand White rabbits were used in this experiment. Both carotid arteries and both femoral arteries from each rabbit were isolated then perfused in a dual organ chamber with physiologic buffered solution (PBS) at 100 (G100, n = 20), 250 (G250, n = 17) or 500 (G500, n = 3) mg/dl glucose. To assess if the effects of glucose are due to higher osmolarity, 4 carotid arteries were perfused in the chamber with PBS at 100 mg/dl glucose plus 150 mg/dl mannitol (M150). Using norepinephrine (NE, $2x10^{-6}$ M) preconstriction, pharmacological challenge was performed with acetylcholine (Ach, $2x10^{-5}$ M) and sodium nitroprusside (SN, $2x10^{-5}$ M). BA was done with a 2.5x15 mm balloon catheter followed by re-challenge. Vessel diameter was measured using a computer planimetry. Vasomotor relaxation (%) was (Ach-NE)/NE x100 or (SN-NE)/NE x100 where NE, Ach and SN were the average diameter of the vessels perfused by PBS with NE, Ach or SN separately. The artery vasomotor function after BA was got from the ratio of vasomotor relaxation after BA to before BA, as it was assumed that there is no significant difference before BA. Results: Both 250 and 500 mg/dl glucose significantly reduced the artery vasomotor function after BA but 100 mg/dl glucose plus 150 mg/dl mannitol had no such effect, where $p < 0.05$ was considered significant. The after BA data of G100, M150, G250 and G500 were 0.43 ±0.35, 0.47 ± 0.12, 0.27 ± 0.14, 0.31 ± 0.12 for Ach and 0.49 ± 0.12, 0.49 ± 0.15, 0.30 ± 0.17, 0.31 ± 0.14 for SN, separately. Conclusion: Attenuation at high glucose levels may result in adverse outcomes after BA. The mechanism seems to be related to a non-osmotic effect of high glucose on injured vascular cells. [The Journal of American Science. 2006;2(4):40-44]

Keywords: artery; glucose; injury; vasomotor

Abbreviation: Ach: Acetylcholine; CABG: coronary artery bypass grafting; MI: Myocardial infarction; NE: Norepinephrine; NIDDM: Non-insulin-dependent diabetes mellitus; NZW: New Zealand White; PBS: Physiological buffered solution; PTCA: Percutaneous transluminal coronary angioplasty; SN: sodium nitroprusside

Introduction

Percutaneous transluminal coronary angioplasty (PTCA) is a very effective technology that allows, without surgery, successful mechanical revascularization of acutely or chronically of obstructed coronary arteries (Park, 1992). Diabetic patients with coronary artery disease have more adverse outcomes including increased mortality following balloon angioplasty when compared to coronary bypass surgery. According to Pandolfi's report (1996) local 24-hours hyperglycemia does not affect endothelium-dependent or -independent vasoreactivity in humans. It is not clear that the artery vasomotor influenced by glucose level under balloon injured condition. The effect of glycemic control on the incidence of restenosis after PTCA in non-insulin-dependent diabetes mellitus (NIDDM) has not been well analyzed (Baron, 1994). The cause for this observation has not been elucidated. In the placebo group, the diabetic patients suffered more recurrent coronary events such as nonfatal myocardial infarction (MI), coronary artery bypass grafting (CABG) and PTCA than did the non-diabetic patients (Goldberg, 1998). Complications including acute closure and thrombosis as well as restenosis have been attributed to vascular recoil (acutely) and remodeling of the artery as a

consequence of cellular infiltration. The purpose of this study was to help understand the effect of elevated glucose on vascular wall reactivity following vascular wall injury. In intact arteries, elevated glucose concentration has been reported to have little effect on normal vasoreactivity response (Morton, 2001). However, little has been evaluated in the setting of vascular injury.

Materials and Methods

Artery vasodilatation activity: Twenty-two New Zealand White (NZW) rabbits were sacrificed using an overdose of Euthanasia injected (contains sodium pentobarbital 390 mg/ml, 0.4 ml/kg rabbit, i.v., Delmarva Laboratories, Inc., Midlothian, VA). Forty-four carotid arteries and 30 femoral arteries from the 22 NZW rabbits were extracted by careful dissection. The arteries were mounted in a dual organic chamber and perfused using a physiologic buffered solution (PBS). Two arteries from one rabbit were perfused under same time for each experiment. The perfusion PBS contained either 100 or 250 mg/dl glucose, 100 or 500 mg/dl glucose, 100 mg/dl glucose or 100 mg/dl glucose plus 150 mg/dl mannitol, and 100 mg/dl glucose plus 150 mg/dl mannitol or 250 mg/dl glucose for each pair arteries from each rabbit. The perfusion condition was controlled under 60 mmHg pressure and 2.4 ml/min. Angioplasty balloon injury was processed by a 2.5×15 mm catheter (Cordis Corporation, Miami, FL) at 3 adjacent sites for 1 minute under 10 atm each in carotid arteries and 1 site for 0.5 minute under 2 atm each in femoral arteries; or crush injury on carotid arteries was performed using a hemostat clamp at three different adjacent sites for 1 minute each in carotid and 1 site for 0.5 minute each in femoral artery. After angioplasty, perfusion resumed using PBS (NaCl 119 mM, KCl 4.7 mM, $CaCl_2$ 2 mM, NaH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 22.6 mM and Na_2EDTA 0.03 mM) at responsible glucose concentration for 2 hours. Using norepinephrine (NE; 2×10^{-6} M, Sigma Chemical Co., St. Louis, MO) preconstriction, pharmacological challenge with acetylcholine (Ach; 2×10^{-5} M, Sigma) and sodium nitroprusside (SN; 2×10^{-5} M, Sigma) was conducted (Figure 1).

	Before Injury					After Injury				
Perfusion	Buffer	NE	Ach	SN	Buffer	Buffer	NE	Ach	SN	Buffer
Time (min)	10	10	10	10	10	10	10	10	10	10

Figure 1. Experimental Time Line of Perfusion

Lumen diameter change was reported as a percentage difference of PBS, Ach or SN from NE pre-constriction: Lumen diameter change (%)=(B–NE)/NE×100, where B was the diameter of artery perfused by PBS or PBS containing Ach or PBS containing SN and NE was the diameter of artery perfused by PBS containing NE.

Vascular diameter of the arteries was measured by a planimetry using digitized images obtained by a Sony camera connected to a computer system before and after balloon injury or crushing. Measurements were obtained at 10 seconds intervals from 8 sampled sites for each artery (Figure 2).

Nitrate/Nitrite: With a Nitrate/Nitrite Assay Kit (Cayman Chemical Company, Ann Arbor, Michigan, Catalogue Number 780001).

Glucose Concentration: Serum glucose concentration was measured with Sigma Diagnostic Kit (Sigma Chemical Co., St. Louis, MO, Catalogue Number 315). Glucose (Trinder) Reagent was mixed with sample and incubated at ambient temperature for 18 minutes, then read and record absorbance at 505 nm.

Results

Both balloon and crush injury of the artery reduced normal vasomotor activity in response to NE, Ach and SN. NE constriction of the artery was reduced after crush injury suggestive of trauma to the smooth muscle layers of the artery. NE constriction of the artery was reduced after balloon angioplasty showed that endothelium cells were damaged. Before injury, there was no significant

difference in vasomotor activity between the different perfusion that PBS contained corresponding glucose (mg/dl) (glucose 100 vs. 250, 100 vs. 500, 100 vs. 1000, glucose 100 vs. glucose 100 plus mannitol 150 and glucose 100 plus mannitol 150 mg/dl vs. glucose 250). These results showed that glucose concentration in perfusion PBS did not significantly influence normal artery vasomotor responding to NE, Ach and SN. There was no significant different between balloon injury and crush injury (Fig. 3).

The following results came from balloon injury experiments. For carotid artery dimension, before injury the artery diameter value obtained by (Buffer-NE)/NE×100, (Ach-NE)/NE×100 and (SN-NE)/NE×100 under different glucose content in the perfusion solution was around 40-60. There was no significant difference among the different glucose content in the perfusion solution (Table 1, Group 1). For femoral arteries, before injury the artery diameter value obtained by (Buffer-NE)/NE×100, (Ach-NE)/NE×100 and (SN-NE)/NE×100 was around 120-160 and there was no significant difference among the different glucose content in the perfusion solution (Table 1, Group 2). These results showed that glucose concentration under 500 mg/dl did not influence normal artery vasomotor under the experimental situation. The vasomotor of rabbit femoral artery was 2 to 3 times stronger than that of rabbit carotid artery (around 130±12 vs. 50±6; $p<0.03$, Table 1).

Both balloon and crush injury aroused the damage of both carotid arteries and femoral arteries and reduced artery vasomotor. For the carotid artery, balloon injury using a 2.5×15 mm catheter at 3 adjacent sites for 1 minute under 10 atmospheres (atm) each will reduce 80% of artery vasomotor function. For the femoral artery, balloon injury using a 2.5×4 mm catheter at one site for 0.5 minute under 2 atm will reduce 90% of artery vasomotor. Elevated glucose in perfusion solution increased the damage using both balloon and crush injury. For the carotid, the vasomotor function in 100 mg/dl glucose was around 1.5 times as that of 250 mg/dl glucose ($p < 0.03$) and the vasomotor function in 100 mg/dl glucose was around 1.8 times as that of 500 mg/dl glucose ($p < 0.03$) (Table 1). There was no significant difference between 250 and 500 mg/dl glucose. Crush injury on the artery in the presence of elevated glucose results was greater vasomotor activity blunting. This seems to be related to both endothelial as well as smooth muscle cell injury.

To make sure that the increased damage by elevated glucose concentration by the chemical property of glucose molecules or the osmotic property of glucose molecules, we made experiments using mannitol instead of glucose: 100 mg/dl glucose plus 150 mg/dl mannitol vs. 250 mg/dl glucose and 100 mg/dl glucose vs. 100 mg/dl glucose plus 150 mg/dl mannitol. There was no significant difference between each pair under the no injury condition. After injury, there was significant difference for vasomotor between 100 mg/dl glucose plus 150 mg/dl mannitol and 250 mg/dl glucose. For Ach result the ratio of 100 mg/dl glucose plus 150 mg/dl mannitol to 250 mg/dl glucose was 1.2±0.2 ($p<0.05$) for carotid arteries and 2.3±0.4 for femoral arteries, $p<0.05$), and it was not significant different for vasomotor between 100 mg/dl glucose and 100 mg/dl glucose plus 150 mg/dl mannitol (The ratio of 100 mg/dl glucose and 100 mg/dl glucose plus 150 mg/dl mannitol was 0.9 ± 0.3 for carotid arteries and 1.5 ± 0.4 for femoral arteries). These results showed that elevated glucose increasing the damage of artery wall was directly aroused from chemical property of glucose molecules rather than the osmotic property of glucose. Mannitol has the same molecular weight as glucose had but it did not have the characterization of damage increasing as the glucose did.

Discussions

Possible reasons of the increased damage of artery vasomotor by injury under elevated glucose concentration in perfusion buffer:

1. The half life of ATP in cells is only several seconds. In the beginning of glycolysis, it will consume 2 ATP for each glucose (Glucose + ATP→glucose 6-phosphate + ADP + H^+, Fructose 6-phosphate + ATP → fructose 1, 6-phosphate + ADP + H^+). In normal cells, the total concentration of ATP + ADP + AMP is around 2 - 10 mM. The normal concentration of 100 mg/dl glucose is 5.5 mM. When artery wall is damaged, the artery cell membrane will be damaged and the glucose entrance control will be destroyed. Then the glucose will enter cells and when glucose concentration reaches 250 mg/dl (13.75 mM) most of the cell ATP will be consumed by glucose with the initial of glycolysis as glucose concentration will be higher than total ATP + ADT + AMP in the cells. The later steps of glycolysis creating ATP will not process as soon as the consuming in the perfusion system. So that the conclusion is that over consuming ATP by the glucose over amount plus artery wall cell damage with initial of

glycolysis is one of the main reasons of that artery losses vasomotor with overdose glucose after artery wall damage. Under this condition the cell energy systems will be destroyed with the over-consuming of cell ATP.

2. Glucose transport process is coupled to the flow of sodium ion down its electrochemical gradient. In this process glucose is pumped into animal cells by the simultaneous entry of Na^+. In fact, Na^+ and glucose bind to a specific transport protein and enter together, as cotransport. Cotransporters use an existing gradient for a particular molecule to drive another molecule against its gradient. Na^+ gradient exists across most cell membranes, with more Na^+ outside than inside. As Na^+ moves down its gradient, it drags glucose with it. This is a property of a specialized protein called the Na^+/glucose cotransporter. Na^+ gradient is always maintained by some other means, namely, Na^+/K^+ ATPase. Thus Na^+/K^+ cotransporter does not use ATP directory, but it uses energy stored in the Na^+ gradient, which is maintained by ATP. The symport carries two species in the same direction. The rate and extent of glucose transport depend on the Na^+ gradient across the plasma membrane. Na^+ entering the cell in the company of glucose is pumped out again by the Na^+- K^+ ATPase. A Glucose transport through cell membrane combine with the cell ion-channels. At the molecular level, the Na^+/glucose transporter is a macromolecule that can bind Na^+ and sugar with a certain stoichiometry, in this case 2 Na^+ and 1 glucose molecule. When the cotransporter turns over, the Na^+ and glucose escape. As the cotransporter goes through its cycle facing out, binding 2 Na^+ and 1 glucose then facing in, it has the net movement of Na^+ and glucose into the cell. Na^+ is moving down its concentration gradient, dragging along glucose. This can be thought as a Na^+-sugar-selective pore because sugar cannot move without Na^+, and the Na^+ cannot move without sugar. This can be also thought as a way of moving Na^+ into a cell using sugar. Since Na^+ moves into the cell in a directed manner, it generates a Na^+ current. As 2 Na^+ ions move from outside to the inside, as did one sugar molecule, two negative charges on the transporter also translocated within the membrane (DeFelice, 1997). This Na^+/glucose moving generate current which important for cell electric characterization. For normal artery, cell will pump Na^+ outside the cells even over amount Na^+ will be transported into cell company with over content of glucose outside the cells. Under the damage condition of the arteries, cell membrane will be damaged and the ion transport system will be abnormal. In this condition the overdose of glucose in perfusion buffer will change the cell current and the artery vasoactivity is decreased. Myocardial glucose uptake is inhibited by cGMP (Depre, 1998). cGMP could be functioning in the glucose transport under this high glucose condition.

Crush and balloon injury to the normal arterial wall in this model suggested that in the presence of elevated glucose in the medium results in further aggravation of the normal vasomotor responses. This may help explain in part some of the adverse outcomes seen in diabetic patients who undergo PTCA through bypass surgery. Diabetic patients have been shown to have more adverse outcomes following PTCA compared to CABG. The cause for this observation has not been elucidated. Complications including acute closure and thrombosis as well as restenosis have been attributed to vascular recoil (acutely) and remodeling of the artery as a consequence of cellular infiltration. And, high glucose enhances growth factor-stimulated nitric oxide production (Trachtman, 1998). Nitric oxide could be a factor of the high glucose effect.

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