Increased cyclic guanosine monophosphate levels and continuous-flow left-ventricular assist devices: Implications for gastrointestinal bleeding

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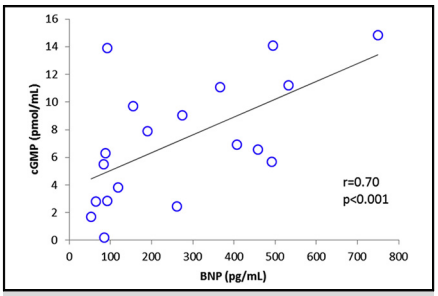
ABSTRACT

Objectives: We examine the hypothesis that cyclic guanosine monophosphate (cGMP) levels are elevated in recipients of continuous-flow left ventricular assist devices (CF-LVADs) and that elevated cGMP levels are associated with a risk of gastrointestinal (GI) bleeding events.

Methods: The levels of cGMP, nitric oxide, platelet activation markers, platelet-derived growth factors (PDGF) AB/BB and AA, and the inflammatory mediator C-reactive protein (CRP) were examined in 19 CF-LVAD recipients, 21 patients who had heart failure, and 19 healthy control-group participants.

Results: The median level of cGMP was significantly higher in CF-LVAD recipients, compared with healthy participants (6.6 vs 2.1 pmol/mL, $u = 62.5$; $P = .001$; $r = -0.55$). Median cGMP levels in the heart failure group (12.5 pmol/L) were higher, compared with both CF-LVAD recipients ($u = 75.0$; $P = .001$; $r = -0.53$) and healthy participants ($u = 4.0$; $P < .001$; $r = -0.83$). Compared with the healthy group, median CRP levels were significantly higher in CF-LVAD recipients (2.9 vs 8.0 mg/L; $u = 58.0$; $P < .001$; $r = -0.63$) and heart failure patients (2.9 vs 7.0 mg/L; $u = 59.0$; $P < .001$; $r = -0.65$). In the subgroup of patients supported with the HeartMate II (Thoratec Corporation, Pleasanton, Calif), pulsatility index was significantly negatively correlated with cGMP levels ($r = -0.73$; $P < .05$), indicating that low pulsatility index is associated with higher cGMP levels. High cGMP levels were significantly associated with GI bleeding events, but not with bleeding events in general.

Conclusions: The primary finding of this study is that GI bleeding in CF-LVAD recipients is associated with significantly elevated cGMP levels, despite high levels of CRP, which interfere with cGMP production. Further studies are required to determine whether elevated cGMP levels can be used as a clinical marker for increased risk of GI bleeding in CF-LVAD recipients. (J Thorac Cardiovasc Surg 2016;151:219-27)



In CF-LVAD recipients, cGMP levels were significantly correlated with the levels of BNP. While elevated cGMP levels predicted GI Bleeding events, the levels of BNP did not predict bleeding events in these patients.

Central Message

In recipients of CF-LVAD, GI bleeding events were associated with high cGMP levels.

Perspective

High cGMP levels may increase the likelihood of GI bleeding events in CF-LVAD recipients, via impaired platelet aggregation. Further studies are required to determine whether elevated cGMP levels can be used as a clinical marker for increased risk of GI bleeding in CF-LVAD recipients.

See Editorial Commentary page 228.

See Editorials page 10 and 13.

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Left ventricular assist devices have been developed as mechanical supports to improve or replace the functions of a failing heart. The newer continuous-flow left ventricular assist devices (CF-LVADs) have been shown to confer advantages, compared with the older pulsatile pumps, including greater durability, smaller size, fewer associated infections,[1,2] and most notably, increased survival rates.[3] However, general increases in bleeding, particularly gastrointestinal (GI) bleeding, are a major source of frequent adverse events in patients supported by CF-LVADs.[4,5] A recent report suggests that,

Abbreviations and Acronyms

ANCOVA = analysis of covariance
BNP = B-type natriuretic peptide
CF-LVAD = continuous-flow left-ventricular assist device
cGMP = cyclic guanosine monophosphate
CRP = C-reactive protein
GI = gastrointestinal
HeartWare = HeartWare Ventricular Assist System
NO = nitric oxide
PDGF = platelet-derived growth factor

compared with pulsatile pumps, rates of GI bleeding with CF-LVADs are significantly higher.[6] Given that GI bleeding can be a substantial complication of CF-LVAD therapy, the mechanisms of GI bleeding are important to understand.

In patients with end-stage renal disease, GI bleeding is a well known complication.[7-9] One study of such patients found that defective platelet aggregation is associated with exaggerated production, by the platelets, of nitric oxide (NO) and of cyclic guanosine monophosphate (cGMP).[10] In uremic animal models, blocking of NO-cGMP production decreased bleeding time.[11] This effect on bleeding time was completely reversed by the NO precursor L-arginine, suggesting that elevated cGMP levels, contribute to platelet dysfunction.

The mechanisms leading to GI bleeding in patients with CF-LVAD support may be similar to those responsible for uremic bleeding. Specifically, a higher incidence of GI bleeding in CF-LVAD recipients may be related to augmented NO-cGMP levels, because cGMP impairs platelet activation.[12] In addition, studies have shown that the contact of blood with an artificial surface activates platelets and causes the α-granules to release their contents, which include platelet-derived growth factor (PDGF).[13-15] In patients supported with CF-LVADs, blood is in contact with the surface of the surgically implanted mechanical pump.

Studies have shown that shortly after CF-LVAD implantation, levels of inflammatory markers are elevated.[16,17] C-reactive protein (CRP), an important marker of systemic inflammation, is known to decrease NO-cGMP production levels by interfering with endothelial NO synthase expression.[18] Despite this increase in CRP, which potentially interferes with NO-cGMP production, and the possible activation of platelets by contact of the blood with the surface of the CF-LVAD, bleeding is a common adverse event in this patient population.[5] Thus, high levels of NO and cGMP may impair platelet activation.

In this study, we examined the hypothesis that cGMP levels are elevated in CF-LVAD recipients and are associated with the risk of GI bleeding events. The specific aims were as follows: (1) to examine the levels of cGMP, NO, PDGF, and CRP in CF-LVAD recipients and compare them to those of heart failure patients, and with healthy controls; and (2) to assess whether high cGMP levels are associated with either a risk of bleeding in general or with GI bleeding in particular in CF-LVAD recipients.

METHODS

Clinical Data

The study protocol was reviewed and approved by the University Health Network Ethical Review Board. Informed consent was obtained from each participant before the study. The total of 59 participants was comprised of 21 heart failure patients, 19 CF-LVAD recipients, and 19 healthy controls, all aged 30-72 years. The CF-LVAD recipients, who were supported for at least 2 months, were compared with heart failure patients, who received standard care, and healthy participants in terms of cGMP (pmol/mL), NO (μmol/L), CRP (mg/L), and PDGF levels.

The mean CF-LVAD treatment time, from implantation, was 9.9 $\pm$ 1.4 months (range: 2-27.5 months). Eight CF-LVAD recipients were supported by the HeartWare Ventricular Assist System (HeartWare; HeartWare International, Inc, Framingham, Mass); 9 by the HeartMate II (Thoratec Corporation, Pleasanton, Calif); and 2 by the DuraHeart (Terumo Heart, Ann Arbor, Mich). Among the 19 CF-LVAD recipients, 6 (32%) had general bleeding events, and 4 (21%) had GI bleeding events.

Blood samples were obtained during hospital admission for either GI or general bleeding. In the cohort of patients who did not have evidence of bleeding, blood samples were collected during a routine clinic visit at an average of 9.8 months after CF-LVAD implantation.

A GI bleeding event was defined as: (1) bleeding identified by endoscopy; or (2) heme-positive stool and a decrease in hemoglobin >1 g/dL. The international normalized ratio, white blood cell count ($\times 10^9$/L), and lactate dehydrogenase (U/L) were recorded. GI bleeding was identified in the upper and lower GI track, requiring intervention, including the temporary cessation of anticoagulation. A general bleeding event was defined as any type of bleeding (with the exclusion of a GI bleeding event). Age and heart failure etiology, the levels of B-type natriuretic peptide (BNP), creatinine, estimated glomerular filtration rate, and medication usage were documented.

Biochemical Assays

Blood samples were obtained and collected in chilled tubes containing ethylendiaminetetraacetic acid (EDTA). The samples were centrifuged at 2056 g for 15 minutes at 4°C, and the plasma was stored at −80°C until analysis. Concentrations of PDGF AA and AB/BB were measured in duplicate, using Milliplex human cytokine kit (EMD Millipore, St. Charles, Mo), following manufacturer directions. The patient samples were loaded onto a 96-well plate, together with appropriate standards and controls, and were run in duplicate. Antibody-immobilized beads were added to the wells. The beads were conjugated to antibodies for the PDGF antigens in the panel. After an incubation period, the unbound beads were removed, and an antibody detection cocktail solution was added to the wells, along with streptavidin-phycoerythrin for visualization. The plate was run on a Luminex 200 machine (Luminex Corp, Austin, Tex), and the levels of PDGF were quantified. Plasma CRP was measured by latex-enhanced nephelometry