Effect of vitamin C and L-NMMA on the inotropic response to dobutamine in patients with heart failure

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Mak, Susanna, Christopher B. Overgaard, and Gary E. Newton. Effect of vitamin C and L-NMMA on the inotropic response to dobutamine in patients with heart failure. Am J Physiol Heart Circ Physiol 289: H2424–H2428, 2005. First published July 22, 2005; doi:10.1152/ajpheart.00453.2005.—The positive effect of vitamin C on left ventricular (LV) inotropic responses to dobutamine, observed in patients with preserved LV function, is lost in heart failure (HF). We tested the hypothesis that in HF, endogenous nitric oxide (NO) opposes the positive effect of vitamin C on adrenergically stimulated contractility by examining the effects of vitamin C on dobutamine responses during NO synthase inhibition. In 11 HF patients, a micromanometer-tipped catheter was inserted into the LV and an infusion catheter was positioned in the left main coronary artery. The peak positive rate of change of LV pressure (LV +dp/dt) was measured in response to intravenous dobutamine (Dob-1). After recontrol, intracoronary N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) was infused before reinfusion of dobutamine (L-NMMA + Dob-2). Finally, intracoronary vitamin C was infused in addition to intracoronary L-NMMA and dobutamine (L-NMMA + Dob-2 + vitamin C). Intracoronary L-NMMA alone had no effect on LV +dp/dt. After a stable inotropic response to intracoronary L-NMMA and dobutamine was established, the addition of intracoronary vitamin C resulted in a modest but significant increase in LV +dp/dt. The change in LV +dp/dt in response to dobutamine alone was 25 ± 5%, with intracoronary L-NMMA, 27 ± 6%, and with intracoronary L-NMMA plus vitamin C, 37 ± 5% (P < 0.05 vs. Dob-1 and L-NMMA + Dob-2). These findings demonstrate that an interaction between endogenous NO and redox environment exists and exerts some influence on stimulated contractility in HF.

THERE IS A DIVERGENT RESPONSE to an antioxidant intervention on stimulated contractility between humans with heart failure (HF) and those with preserved left ventricular (LV) function. We have demonstrated that vitamin C augments the LV inotropic response to dobutamine in patients with preserved LV function (18) but has no such effect in HF patients (20). We explored the possibility that in HF, an inotropic effect of vitamin C may have been supplanted by the concurrent antioxidant potentiation of endogenous nitric oxide (NO) generated in response to dobutamine stimulation. NO exerts a negative inotropic effect, likely mediated by cGMP (16, 21). NO generation stimulated by β-adrenergic agonists is augmented in the failing heart, as evidenced by demonstration that the inotropic effect of dobutamine is potentiated by NO synthase (NOS) inhibition in HF patients but not patients with preserved LV function (10, 11, 29). Furthermore, there is increasing evidence that the enzymatic generation, bioavail-

EXPERIMENTAL PROCEDURES

Study population. Eleven male HF patients referred for elective diagnostic heart catheterization participated in this study. All patients were being evaluated for stable New York Heart Association class II to III HF. Ten patients had a diagnosis of idiopathic dilated cardiomyopathy with angiographic confirmation of patent coronary arteries; one patient had coronary disease with occlusion of the right coronary artery only. Medical therapy included angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists (10 patients), β-blockers (10 patients), loop diuretics (9 patients), aldosterone antagonists (7 patients), and digoxin (9 patients). Other baseline characteristics are described in Table 1. The study was approved by the Mount Sinai Hospital Research Ethics Board, and all patients gave written informed consent.

Cardiac catheterization procedure and hemodynamic measurements. Patients were awake, nonsedated, and studied after a diagnostic right and left heart catheterization via the femoral approach was performed. All medications were withheld on the morning of the investigation. A 7-Fr micromanometer-tipped catheter (Millar, Houston, TX) was advanced via the right femoral artery into the LV. Femoral artery pressure was monitored via a 7-Fr sidearm sheath (Terumo Medical, Elkton, MD). A 6-Fr L4 Judkins catheter (Cordis Laboratories, Miami, FL) was advanced from the opposite femoral artery to the ostium of the left main coronary artery. When an active drug was not being infused, the catheter was continuously flushed with the vehicle (0.9% saline) at 2.7 ml/min with the use of a Harvard infusion pump. The ECG, femoral artery pressure, LV pressure, and peak positive rate of change of LV pressure (LV +dp/dt) were acquired with the use of methods previously described (17, 18, 20).

Study protocol. All patients received heparin (5,000 units iv) and a rest period after instrumentation. Study measurements were made sequentially under the following six conditions. 1) Intracoronary
infusion of vehicle was maintained at 2.7 ml/min (Control). 2) Dobutamine (Lilly, Indianapolis, IN) diluted in 5% dextrose in water was infused via a systemic vein at a rate of 2.5, 5.0, or 7.5 mg·kg⁻¹·min⁻¹, titrated to achieve at least a 25% rise in LV +dP/dt and until LV +dP/dt remained stable (+5%) for three consecutive measurements each separated by 1 min (Dob-1). 3) Dobutamine was stopped and measurements were repeated when LV +dP/dt returned to within 10% of the control value (Recontrol). 4) G⁻⁴-monomethyl-L-arginine (L-NMMA; 5 × 100-mg vials; Clinalfa) was infused by the intracoronary route at a rate of 1.1 ml/min (20 μmol/min) for 10 min (l-NMMA). During this condition, the intracoronary infusion of vehicle solution was decreased to 1.6 ml/min to maintain a total intracoronary infusion rate of 2.7 ml/min. 5) With the continued intracoronary infusion of L-NMMA, dobutamine was reinfused intravenously at the rate established during the first infusion (l-NMMA + Dob-2). In six patients, measurements were made at 10 min. In five patients, measurements were made at 10 and 15 min to ensure that the effect of L-NMMA on the inotropic response to dobutamine demonstrated by other investigators (10, 29) was fully developed. In all patients, LV +dP/dt had stabilized as defined in the second condition (Dob-1) by 10 min. Study measurements were reported from the final minute of this condition, whether 10 or 15 min. 6) With the continued intracoronary infusion of L-NMMA and the intravenous infusion of dobutamine, vitamin C (ascorbic acid injection, 10 mg/2 ml, pH adjusted with sodium hydroxide; Sabex, Boucherville, QC, Canada) was also infused by the intracoronary route at 1.2 ml/min (96 mg/min) (l-NMMA + Dob-2 + Vit C). During this condition, the intracoronary infusion of vehicle solution was stopped so that the total intracoronary infusion rate remained 2.7 ml/min.

Given the invasive nature of these experiments, we did not repeat a protocol to test the effect of vitamin C alone on the inotropic response to dobutamine (20).

**Statistical analysis.** All data are presented as means ± SE. A statistical software package was used for all analysis (Statview). Raw hemodynamic data were analyzed with a one-way ANOVA for repeated measures. Absolute and percent changes of LV +dP/dt in response to serial conditions were also analyzed with a one-way ANOVA for repeated measures. Post hoc testing was performed with the Student-Newman-Keuls test. A value of P < 0.05 was considered significant.

**RESULTS**

**Responses to l-NMMA.** The intracoronary infusion of l-NMMA alone had no effect on any hemodynamic parameter or on basal LV +dP/dt (Table 2).

**Effect of l-NMMA on dobutamine responses.** Intravenous dobutamine, infused at a mean rate of 5.5 ± 0.8 μg·kg⁻¹·min⁻¹, resulted in a mean increase in LV +dP/dt of 25 ± 5%. Dobutamine also resulted in an increase in LV systolic pressure.

The intracoronary infusion of dobutamine with the coinfusion of intracoronary l-NMMA resulted in a significantly greater rise in mean arterial pressure compared with dobutamine alone. There were also trends toward greater augmentation in both LV systolic pressure and LV +dP/dt, as has been demonstrated by other investigators. However, the LV systolic pressure and LV +dP/dt increases with combined dobutamine and l-NMMA, compared with dobutamine alone, did not reach statistical significance.

In five patients, the l-NMMA + Dob-2 condition was extended from 10 min up to 15 min (Fig. 1) to examine whether there was progressive augmentation of the inotropic response to dobutamine by l-NMMA. In these patients, we again observed a trend toward augmentation of the inotropic response to dobutamine at both 10 and 15 min. There was little change in the inotropic response between 10 and 15 min, suggesting that the LV +dP/dt response was stable.

**Effect of vitamin C on responses to dobutamine during l-NMMA infusion.** After a stable inotropic response to intracoronary l-NMMA and intravenous dobutamine was established, the addition of intracoronary vitamin C resulted in a significant increase in LV +dP/dt. The increase in LV +dP/dt in response to dobutamine alone was +193 ± 40 mmHg/s (25 ± 5%), with l-NMMA and dobutamine, +203 ± 46 mmHg/s (27 ± 6%), and with vitamin C, l-NMMA, and dobutamine, +284 ± 51 mmHg/s (37 ± 5%) (P < 0.05 vs. Dob-1 and l-NMMA + Dob-2) (Fig. 2).

**DISCUSSION**

We previously (18, 20) demonstrated that the positive effect of vitamin C on the inotropic response to dobutamine, observed in patients with normal LV function, is lost in HF patients. We hypothesized that this divergent response to an antioxidant was related to increased availability of NO in HF. The central result of the present study is that vitamin C had a modest positive inotropic effect on dobutamine-stimulated HF patients in the presence of a NOS inhibitor. This finding suggests that an interaction between endogenous NO and redox...
Fig. 1. Increase in left ventricular (LV) peak positive rate of pressure change ($\Delta$L V $\pm$ dP/dt) in response to infusion of dobutamine (Dob-1) tended to increase with added infusion of intracoronary NOS-monomethyl-L-arginine (L-NMMA), which was infused for 15 min in 5 patients. There was no difference in LV $\pm$ dP/dt response to dobutamine between 10 and 15 min of L-NMMA infusion (L-NMMA 10 min + Dob-2 and L-NMMA 15 min + Dob 2). Infusion of intracoronary vitamin C in addition to L-NMMA resulted in significant augmentation of LV $\pm$ dP/dt response to dobutamine. *P < 0.05 vs. Dob-1; † P < 0.05 vs. L-NMMA 10 min + Dob-2; ‡ P < 0.05 vs. L-NMMA 15 min + Dob-2.

environment exists and exerts some influence on stimulated contractility in HF.

At physiological concentrations, reactive oxygen species (ROS) participate in myocyte signal transduction pathways including virtually all elements of Ca$^{2+}$ handling and adrenergic receptor function (1, 8, 15, 27, 35). A large body of evidence suggests that ROS, particularly superoxide anion radical (O$_2^-$), can negatively modulate basal and adrenergically stimulated contractility (2, 24, 25, 33, 32). Our previous demonstration that vitamin C augmented inotropic responses to dobutamine in subjects with normal LV function (7, 18) provided functional evidence for redox regulation of stimulated contractility in humans.

The HF syndrome is characterized by oxidative stress. Myocardial sources of ROS production relevant to HF include mitochondria, uncoupled NOS, xanthine oxidase, and NADPH oxidase (19). There is also compelling evidence from animal studies that cardiac function is modulated by ROS in the settings of stunning and HF (1, 19) and that antioxidants can exert a positive inotropic effect (4, 5, 26, 32). Therefore, our subsequent observation that vitamin C did not augment inotropic responses to dobutamine in HF patients was surprising. We postulated that altering redox environment in HF by administration of vitamin C resulted in increased availability of NO, thereby potentiating the well-described role of NO as a negative modulator of adrenergically stimulated contractility in HF (10, 11, 29). We hypothesized that a positive effect of vitamin C on the inotropic response to dobutamine would be observed if a confounding negative influence of NO could be withdrawn by ROS blockade.

The effect of L-NMMA in our study suggests that endogenous NO limits a positive effect of vitamin C on the inotropic response to dobutamine. An antioxidant intervention such as vitamin C may increase NO availability by a variety of mechanisms including direct quenching O$_2^-$ (9, 14). Importantly, the dose of vitamin C in our studies (96 mg/min) was selected to achieve an intracoronary concentration of at least 1 mmol/l, which is sufficient in vitro to compete with O$_2^-$ and prevent its participation in a reaction with, and thereby consuming, NO (9, 14, 28). Despite the adequate concentration of vitamin C and the inhibition of NOS, the absolute quantitative augmentation of the inotropic response to dobutamine was modest, suggesting that redox environment, as least as modifiable by vitamin C, plays only a small role in the control of stimulated contractility in HF.

Our findings provide an example of the linkage between oxidative and nitrosative environments, an interaction that has been termed nitroso-redox balance (13). There is increasing evidence that nitroso-redox environment may be particularly relevant to the pathophysiology of HF. Our investigations in this field of study have dealt with dobutamine-stimulated contractility, where the effects of L-NMMA (10, 11, 29) and vitamin C administration (18, 20) have been documented experimentally in humans with and without HF employing consistent methodologies (10, 11, 29). Recently, the effect of altered nitroso-redox balance in HF on the more complex entity of cardiac efficiency has been investigated (26). NO negatively modulates myocardial O$_2$ consumption, preserving cardiac efficiency (31). There is evidence in a dog HF model that impaired cardiac efficiency observed in HF is related to the suppression or consumption of NO by oxidative stress as discussed above. The same investigators (5, 26) demonstrated that allopurinol augments basal cardiac efficiency in HF patients, potentially by its action to suppress superoxide generation. Whether this action of allopurinol was dependent on an intact NOS system in HF patients or whether allopurinol impacts cardiac efficiency in humans with normal LV function has not been tested.

Issues regarding the infusion protocol merit consideration. The current study demonstrated a modest trend toward augmentation of the dobutamine response by L-NMMA alone. In contrast, Hare et al. (11) found that dobutamine-stimulated LV $\pm$ dP/dt tended to rise at 5 min of L-NMMA and was significant.
cantly increased at 15 min, after intracoronary acetylcholine was infused between 5 and 10 min. Shinke et al. (29) also demonstrated a robust increase in LV +dP/dt when dobutamine was added to L-NMMA. The reasons for this discrepancy are unknown but may, in part, relate to differences in study populations, such as the degree of hemodynamic decompensation. NOS-2 activity contributes significantly to blunted adrenergic responsiveness in the failing human myocardium (6, 12, 36), and expression of this isoenzyme is directly related to hemodynamic decompensation (6). Our study group was on full contemporary therapy and was compensated [LV end-diastolic pressure (LVEDP) of 13 ± 2 mmHg], in contrast to the study of Hare et al. (11) (LVEDP of 25 ± 2 mmHg). Consideration must be given to the possibility that L-NMMA alone (and not the addition of vitamin C) caused the observed augmentation of the inotropic response to dobutamine. However, in the present study, there was little evidence of an ongoing increase in LV +dP/dt in response to L-NMMA, based on this condition being extended to 15 min in half of the patients. There were significant loading changes in response to the combination of L-NMMA and dobutamine. These changes would have tended to reduce any augmentation in LV +dP/dt by vitamin C.

This work builds on our previous studies (18, 20). Given the invasive nature of these experiments, we did not repeat an experiment to test the effect of vitamin C, without L-NMMA, on the inotropic response to dobutamine. In 10 HF patients, we have previously demonstrated that the change in LV +dP/dt from baseline in response to dobutamine is not significantly altered by the infusion of intracoronary vitamin C (20), in contrast to the effect of vitamin C on stimulated contractility in patients with normal LV function. The increase in LV +dP/dt of the failing ventricle when L-NMMA and vitamin C are added to dobutamine is quantitatively modest but, in the context of our previous observations, provides evidence that modulation of stimulated contractility depends on interrelated nitrosative and redox environments. Although nitroso-redox interaction may also be relevant in health, we did not repeat this experiment in subjects with normal LV function. Our previous observation that vitamin C alone augments the inotropic response to dobutamine suggests that the antioxidant intervention does not recruit NO-dependent negative modulation in this population. Consistent with this, endogenous NO does not appear to have a significant role in limiting adrenergically stimulated contractility in subjects with normal LV function (10). This is consistent with our view that the divergent effect of vitamin C in patients with and without HF is dependent on the divergent relevance of NO on stimulated contractility. Interestingly, blockade of endogenous NO appears to have divergent effects between HF patients and patients with normal LV function with respect to basal contractility (3, 34). Whether this finding is dependent on differing redox environments between these patient groups has not been tested.

Our findings demonstrate some level of interaction between the L-arginine/NOS system and redox environment in the setting of an acute hemodynamic mechanistic experiment in HF patients. The magnitude of this interaction was modest; however, it is consistent with other evidence that interdependence of oxidative and nitrosative environments occurs in HF.

**REFERENCES**


