

Arginine/Asymmetric Dimethylarginine Ratio in Colorectal Surgery

Neli Ragina^{a, f}, Gabrielle Davis^b, Michael Doorly^c, Kyle Cologne^d,
Anthony J. Senagore^e

Abstract

Background: Asymmetric dimethylarginine (ADMA) is a naturally occurring amino acid that inhibits the effects of nitric oxide synthetases, producing considerable effects on the vascular system. Recent studies have suggested that increased ADMA is a major contributor to endothelial dysfunction, resulting in significant effects on cardiovascular and renal function.

Methods: The study sample consisted of five male and 11 female patients scheduled for elective laparoscopic colorectal surgery; patients were between 41 and 77 years of age. Venous whole blood (8 - 10 mL) was collected from patients prior to colorectal surgery, as well as on postoperative day 1 and 2. Arginine, citrulline, ADMA, and symmetric dimethylarginine levels were measured in collected blood using liquid chromatography coupled to mass spectrometry.

Results: Data from the amino-acid blood analysis demonstrated that the levels of L-citrulline and L-arginine decreased on postoperative days 1, coinciding with an increase of ADMA levels. The overall result of these changes was a decrease in the arginine to ADMA ratio by 21% from the preoperative period to postoperative day 2 (P = 0.02).

Conclusions: The data collected through this study demonstrate a significant increase in systemic ADMA levels following major laparoscopic colorectal surgery.

Keywords: ADMA; Citrulline; Nitric oxide; Surgery

Introduction

Asymmetric dimethylarginine (ADMA) is the most potent en-

dogenous inhibitor of all major isoforms of nitric oxide synthase (NOS). The elevation of ADMA has been implicated in disease pathology in a variety of conditions [1, 2]. Elevation of ADMA has been particularly identified as an independent risk factor for adverse cardiovascular events and death potentially due to its negative effects on endothelial function [3-6]. Nijveldt et al recently identified ADMA elevation as the strongest predictor of negative outcome in critically ill patients in the intensive care unit [7]. Elevation of ADMA is the result of impaired metabolism by its major degrading enzyme, dimethylarginine dimethylaminohydrolase (DDAH) and/or impaired renal or liver excretion [8]. A rise in ADMA may therefore indicate impending liver and/or kidney failure, or more ominously failure of the endothelial system at large, all of which are responsible for eliminating ADMA.

Maas et al were the first to demonstrate that an incremental increase in preoperative plasma ADMA concentrations were associated with a 1.33 odds ratio of postoperative surgical complications [9]. These findings suggest that ADMA may be a novel tool in identifying individuals at increased perioperative risk [10]. A limitation in the authors' data was the absence of simultaneous assessment of arginine (ARG) levels to assess the relative impact of the ARG/ADMA ratio. The ratio of ARG/ADMA is an indirect measurement of nitric oxide functionality. ADMA degradation by DDAH leads to citrulline (CIT) production. Citrulline has been demonstrated to increase organ perfusion and improve outcomes in sepsis and pathological conditions [11]. The purpose of this study, therefore, was to provide the first assessment of perioperative changes in ARG, CIT, and the ARG/ADMA ratio as a result of surgical stress in a laparoscopic colectomy population.

Methods

The study is a descriptive, observational study. It was approved by the Institutional Review Board at the University of Southern California. A consecutive series of patients (N = 16, five males and 11 females) between 41 and 77 years of age that were scheduled for elective laparoscopic colorectal surgical procedures were enrolled in the study after providing informed consent. All of the patients received an oral mechanical and antibiotic bowel preparation prior to surgery. Surgical care improvement project guidelines as defined by the Joint Commission during the study period were implemented in all study participants. Postoperative care was managed using our institutions' enhanced recovery protocol, which has been published

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^aCentral Michigan University, College of Medicine, Mt. Pleasant, MI, USA

^bStanford University School of Medicine, Palo Alto, CA, USA

^cTuality Healthcare, Hillsboro Surgical Associates, Hillsboro, OR, USA

^dKeck School of Medicine of the University of Southern California, Los Angeles, CA, USA

^eUniversity of Texas Medical Branch at Galveston, Galveston, TX, USA

^fCorresponding Author: Neli Ragina, Central Michigan University, College of Medicine, Mt. Pleasant, MI, USA. Email: raginane@gmail.com

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Table 1. Patients' Characteristics

Disease type	Age	Gender	Surgery
Recurrent rectal cancer	46	F	Laparoscopic proctectomy w/coloanal anastomosis and loop ileostomy
Cecal cancer	77	F	Laparoscopic right hemicolectomy
Diverticulitis	59	F	Laparoscopic sigmoid colectomy
Right colon cancer	71	M	Laparoscopic right hemicolectomy
Diverticulitis	54	M	Laparoscopic sigmoid colectomy with release of splenic flexure
Rectal/sigmoid cancer	62	F	Laparoscopic anterior resection converted to open w/loop ileostomy
Cecal mass	64	F	Laparoscopic right hemicolectomy
Rectal prolapse	41	F	Laparoscopic Wells procedure for rectal prolapse
Carcinoid	63	F	Laparoscopic small bowel resection
Diverticulitis	61	M	Laparoscopic sigmoid colectomy
Rectal cancer	66	F	Laparoscopic low anterior resection w/loop ileostomy
Colovaginal fistula	50	F	Laparoscopic low anterior resection w/primary anastomosis
Colon cancer	51	M	Laparoscopic left colectomy w/takedown of splenic flexure
Colon cancer	77	F	Laparoscopic extended right hemicolectomy
Colon cancer	70	M	Laparoscopic Hartmann procedure
Ulcerative colitis with malignant degeneration	50	F	Laparoscopic total proctocolectomy w/ileoanal Jpouch and loop ileostomy

previously, but includes early resumption of diet and activity and minimization of narcotic analgesia [12]. Blood was drawn preoperatively, on postoperative day 1 (POD1), and POD2. Our standard of care was discharge at either the afternoon of POD2 or morning POD3; therefore, we terminated the testing period to accommodate our standard discharge practice. Short-term complications were monitored and recorded up until the time of initial clinic follow-up.

Isolation of serum from whole blood

Eight to ten milliliters of venous whole blood was collected in red-topped tubes (Becton Dickinson (BD2)). The blood was allowed to clot by leaving it undisturbed at room temperature. The clot was removed by centrifuging at 1,000 - 2,000 × g for 10 min in a refrigerated centrifuge and the resulting supernatant (serum) was apportioned into 0.5 mL aliquots and stored at -80 °C.

CIT, ARG, ADMA, and SDMA analysis

Amino acids were measured by liquid chromatography coupled to mass spectrometry as previously described by Shin et al [13].

ARG, CIT, ADMA, and symmetrical dimethylarginine (SDMA) were purchased from Sigma. ARG, CIT, ADMA, and SDMA levels and ARG/ADMA ratio were assessed in serum at baseline (pre-operatively) and POD1 and POD2. Serum samples were diluted with water 100 times for ARG, CIT, ADMA and SDMA analysis. The analysis of ARG, CIT, ADMA and SDMA was performed by reverse-phase high performance liquid chromatography (HPLC) on-line to a TSQ Quantum Ac-

cess MAX triple stage quadrupole mass spectrometer. Quantification was performed via single reaction monitoring (SRM) using a fragment ion unique to ARG, CIT, ADMA and SDMA, respectively.

Statistical analysis

All data were presented as mean and standard deviation (SD) from the mean or ratios as defined by the data. Statistical analysis consisted of ANOVA and *t*-test with *P* < 0.05 considered as statistically significant.

Results

Table 1 lists the demographics of our patient population. All patients underwent laparoscopic colorectal procedures for cancer, complications of diverticulitis and rectal prolapse. Our data demonstrated a statistically significant increase of the ADMA levels on POD2 (22 ng/mL (SD: 18 - 26) vs. 28 ng/mL (SD: 22 - 34); *P* = 0.02) (Fig. 1). The net result of these changes was a decrease in the ARG/ADMA ratio by 21% from the preoperative period to POD2 (*P* = 0.02) (Fig. 1). The ARG/ADMA drop in our study was apparently related only to the surgical stress response as none of the patients developed clinical infections. No significant changes were noted in the CIT level by POD2.

Discussion

These are the first preliminary data to demonstrate a clear and

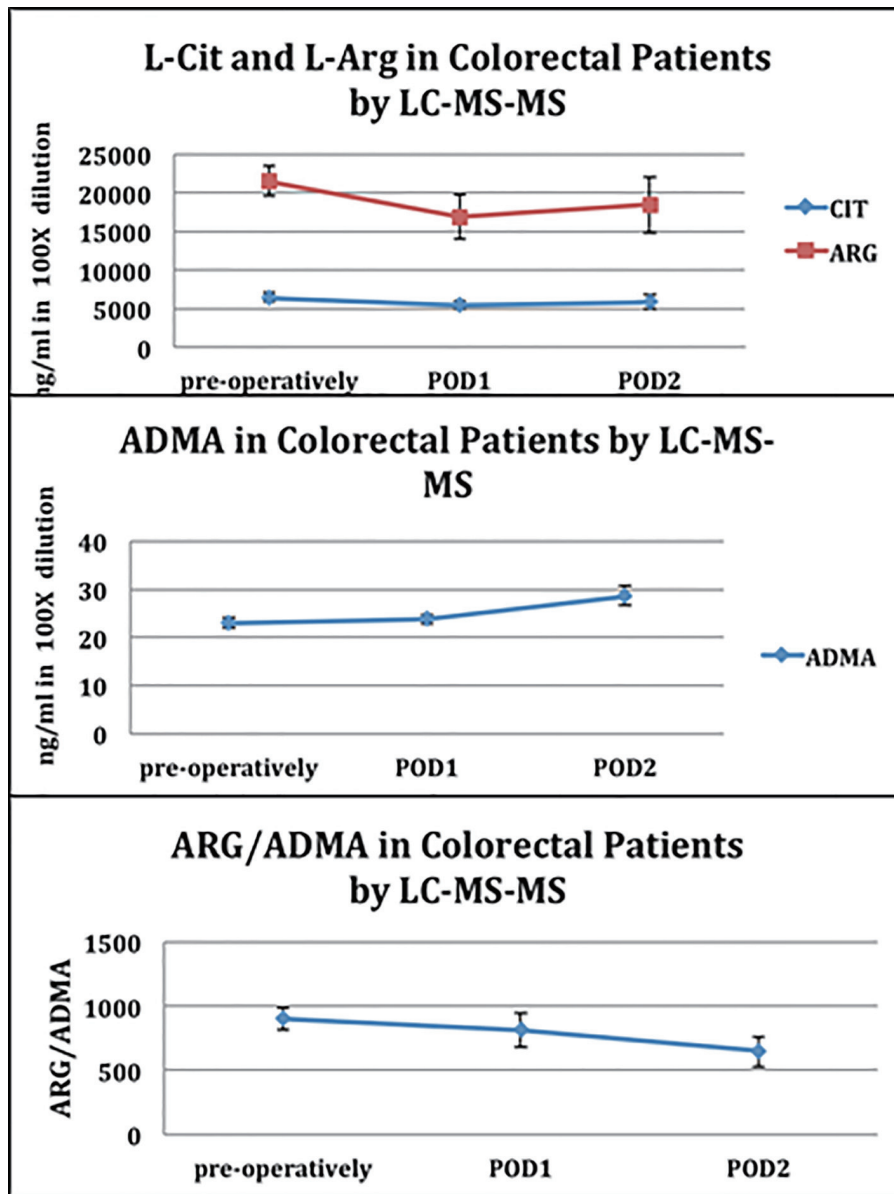


Figure 1. L-citrulline (L-Cit), L-arginine (L-Arg), ADMA and ARG/ADMA ratio in patients undergoing colon and rectal surgery.

significant rise in systemic ADMA levels after laparoscopic colorectal surgery, even in the absence of surgical complications. There was a subsequent reduction in the ARG/ADMA ratio that was statistically significant that indicates a decrease in nitric oxide functionality. The clinical significance of this remains uncertain; however, it does demonstrate that surgical stress leads to changes of these parameters.

Long-term inhibition of endothelial NOS (eNOS) by ADMA has been associated with disease progression and increased morbidity and mortality [14-16]. It has also been postulated that acute reduction in inducible NOS (iNOS) during episodes of acute inflammation such as in sepsis may be desirable, yet this theory is questionable [17-19]. The precise role of ADMA alone serves as either a physiologic brake to iNOS activity, or alternatively in combination with low L-arginine

plasma levels decreased the producing decreased systemic vascular resistance and impaired the central organ blood flow leading to multisystem organ failure [20-22]. There is a significant body of literature confirming that trauma results in a reduction in circulating ARG due to the activation of arginase. In addition, ARG reduction is directly correlated to injury severity [22, 23]. Although the liver is the predominant source of constitutively active arginase activity, which increases in response to surgical trauma, both the endothelium and neutrophil degranulation may be additional sources of circulating arginase in the trauma setting [24].

Interestingly, these data are the first to assess the interrelationship of ARG and ADMA in a major surgical population managed within an enhanced recovery program. This ratio has never been investigated in clinical trials of NOS inhibitors or

as part of immunonutrition studies. In fact, this relationship has been advocated as an important contributor to the “ARG paradox” describing the general failure of ARG supplementation in improving clinical outcomes in various treatment trials.

The data in this study will require confirmation in a larger patient population, and correlated with other clinic outcome parameters to determine if ADMA fluctuations could be used as a marker of surgical outcomes. It will also be important to determine if inhibiting ADMA by its inhibitor, DDAH, could be used to combat the deleterious effects of ADMA. In our data, the elevation in ADMA and the decrease in the ARG/ADMA ratio were still declining at 48 h post-surgery and therefore we cannot comment on the time required for resolution. These limited data make it difficult to draw any conclusions regarding the relative impact of a preoperative ARG or CIT supplementation. Ultimately, data from an extended perioperative time frame will be required to assess the impact of alterations in ADMA levels and the ARG/ADMA ratio on NO functionality during the various phases of the postoperative period: 1) the acute phase response; 2) during the development of complications; or 3) during the uncomplicated healing phase after surgery. However, the data are supportive of the potential role for the ARG/ADMA ratio as an important surrogate marker in perioperative responses to surgical stress and recovery. These data also suggest the feasibility of using L-citrulline as possible regulator of ADMA levels and ARG/ADMA ratio, via upregulation of L-arginine synthesis, in patients during the postoperative phase. A larger study with longer follow-up appears well warranted to more fully understand the variation in clinical responses of the ARG/ADMA ratio and clinical outcome.

Disclosures

This is a research involving human subjects and is performed under an approved IRB and signed informed consent by the participants.

Conflicts of Interest

The authors disclose that they do not have any potential conflicts of interest.

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