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Original article

Major surgery diminishes systemic arginine availability and suppresses nitric oxide response to feeding in patients with early stage breast cancer

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SUMMARY

Background & aims: Plasma arginine (ARG) levels are reduced in breast cancer, suggesting diminished systemic ARG availability. ARG and its product nitric oxide (NO) are important in early postoperative recovery due to its roles in immune function and wound healing. It remains unclear whether major surgery further diminishes systemic ARG availability due to enhanced ARG catabolism and/or insufficient endogenous ARG synthesis negatively affecting NO synthesis in patients with early stage breast cancer.
Methods: In 9 women with early stage breast malignancy and 9 healthy women with genetic predisposition to breast cancer, whole body ARG and citrulline (CIT) rates of appearances were measured to determine their production rates prior to and within 24 h after major breast surgery by stable isotope methodology in the postabsorptive and postprandial state. The conversions of CIT > ARG, ARG > CIT, and ARG > Urea (markers of *de novo* ARG and NO synthesis, arginase activity, respectively), and ARG clearance (reflecting ARG disposal capacity) were calculated.
Results: Prior to surgery, plasma ARG, CIT and glutamine concentrations were lower in cancer ($P < 0.05$) but no differences were found in the rate of appearances of ARG, CIT and their conversions. Surgery increased ARG clearance and reduced CIT rate of appearance, conversion of CIT > ARG ($P < 0.001$), and plasma ARG, CIT, ornithine concentrations ($P < 0.001$). Furthermore, postprandial increase in ARG > CIT conversion ($P < 0.05$), plasma ARG ($P < 0.001$) and CIT ($P = 0.06$) concentrations were lower after surgery. The cancer group had lower values for postprandial increase in ARG > CIT conversion, plasma CIT ($P < 0.05$) and glutamine concentrations ($P = 0.08$).
Conclusions: Major surgery in early stage breast cancer further reduces systemic ARG availability in the early phase of recovery due to a combined process of increased ARG catabolism and impaired endogenous ARG synthesis. The suppressed postprandial NO increase in early stage cancer suggests that specific nutritional approaches are advised to increase ARG availability after major surgery although the effects on postoperative recovery remain unclear.
This trial was registered at clinicaltrials.gov as NCT00497380.

1. Introduction

A large proportion (91%) of breast cancer patients is characterized by a reduced plasma arginine level (ARG) [1,2]. ARG plays an important role in the immune function and in defense against tumor cells, and is used for whole body protein and urea synthesis [3]. Reduced plasma ARG levels in cancer patients are irrespective of tumor stage and type, and nutritional status [1], suggesting that a

Abbreviations: ARG, arginine; BMI, body mass index; CIT, citrulline; COPD, Chronic Obstructive Pulmonary Disease; CRP, c-reactive protein; FFMI, fat-free mass index; GLN, glutamine; HOMA, Homeostasis Model Assessment; iNOS, inducible nitric oxide synthase; MDSC, myeloid derived suppressor cells; NO, nitric oxide; ORN, ornithine.

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diminished systemic ARG availability can be found in weight stable breast cancer patients early in their disease trajectory.

Circulating myeloid derived suppressor cells (MDSCs) are elevated in patients with breast cancer [4,5], and even in early stage breast malignancy [6]. MDSC are potent suppressors of tumor immunity, and are able to deplete ARG levels and alter nitric oxide (NO) production by producing the ARG catabolic enzymes arginase and inducible nitric oxide synthase (iNOS). Elevated arginase activities in serum and human breast tissue samples have been found in breast cancer [7,8], suggesting that increased systemic and local (tumor) ARG catabolism play a role in the reduced ARG availability in these patients. Recently, we observed in advanced non-small cell lung cancer (NSCLC) patients that the reduced systemic ARG availability is related to a compromised endogenous ARG production from its precursor citrulline (CIT) [9] despite an elevated protein breakdown [10], and was associated with reduced whole body NO synthesis [9]. It remains unclear whether the reduced plasma ARG level in breast cancer patients [1,2] is the consequence of insufficient endogenous ARG production from CIT to balance the increased ARG catabolism.

ARG also plays an important role in postoperative recovery in cancer as it is required for adequate NO synthesis necessary for immune function, cell regeneration, tissue perfusion, and wound healing [11]. Low plasma ARG levels are present in patients with infected wounds, and impaired wound healing is related to altered arginine usage [12]. We previously observed that a tumor prohibits the normal postoperative increase in whole body ARG production [13] and reduces endogenous (*de novo*) ARG production from citrulline [14] after surgery, in mice indicating a compromised postoperative ARG metabolism. Major surgery in early stage breast cancer resulted in a preserved upregulation of protein breakdown [15], suggesting that their ARG production from protein breakdown is not attenuated by surgery. We therefore hypothesize that systemic ARG deficiency in patients with early stage breast cancer is enhanced by major surgery and mainly due to reduced endogenous ARG synthesis, negatively affecting NO synthesis.

The aim of the present study was to investigate whether the reduced systemic arginine availability in early stage breast cancer is due to enhanced ARG catabolism and/or reduced endogenous ARG synthesis, and to examine the effects of major breast surgery. Combined bilateral mastectomy and reconstruction surgery was chosen as it is a common and comparable major surgical procedure in women with breast cancer and in healthy women with a genetic predisposition to breast cancer. Arginine, citrulline and NO kinetics were measured in both groups prior to and within 24 h post-surgery using stable isotope techniques in the overnight fasted state and after intake of a conventional medical food supplement. Insight in the independent and combined effects of surgery and cancer on whole body arginine metabolism and NO synthesis may initiate novel nutritional approaches for cancer patients undergoing major surgery to enhance postoperative recovery.

2. Subjects and methods

2.1. Subjects

Women with Stage II breast cancer and healthy control women with a genetic predisposition to breast cancer scheduled for major breast surgery participated in the study [15]. All women were recruited during visits at the Women's Oncology Clinic Winthrop Rockefeller Cancer Institute at the University Arkansas for Medical Sciences and studied between November 2010 and March 2012. Exclusion criteria were neoadjuvant therapy and any surgery less than 4 weeks before the study, pre-existent cardiovascular disease or untreated metabolic disease, including liver or renal disease. Written informed consent was obtained from all subjects and the study was approved by the Institutional Review Board, and the Protocol Review and Monitoring Committee of the Arkansas Cancer Research Center, University Arkansas for Medical Sciences, Little Rock, AR.

2.2. Breast surgery procedures

Patients with invasive breast cancer and healthy control subjects scheduled for bilateral mastectomy – with or without lymphadenectomy – which was immediately followed by reconstruction surgery were invited to participate [15]. All patients underwent total skin sparing mastectomy. Initial reconstruction consisted of placement of pectoralis submuscular tissue expanders or implants. The bilateral mastectomy procedures were performed by the same breast cancer surgeon whereas the reconstruction surgeries were performed by two plastic surgeons using identical techniques. General anesthesia included opioid analgesics, and antibiotics, antiemetics, laxatives were provided to all patients and healthy subjects during surgery and/or post-surgery. Disease and treatment history, and surgery details were obtained from the subject's medical chart.

2.3. Study protocol

All breast cancer patients and healthy control subjects were studied the day prior to surgery at the UAMS Clinical Center of the Translational Research Institute, and the day after surgery at the Short Stay Unit of the UAMS Medical Center. The metabolic part of the two study days was identical and lasted 5.5 h [15]. In the early morning after an overnight fast, body weight, height and body composition by Dual-energy X-ray Absorptiometry (Hologic QDR 4500/Version 12.7.3.1 (Bedford, MA)) were measured. The anthropometric and body composition data were standardized for height [16]. Subsequently, for infusion of the stable isotopes, a peripheral line was placed in an antecubital vein of the arm. To measure ARG, CIT and NO kinetics, a primed, constant and continuous infusion was performed of the stable isotopes L-[guanidine-$^{15}N_2$]ARG (prime: 3.75 μmol/kg bw, infusion rate: 3.75 μmol/kg bw * h) and L-[ureido-^{13}C-$^{2}H_2$] or L-[5-^{13}C-$^{2}H_4$]CIT (prime: 0.88 μmol/kg bw, infusion rate: 0.30 μmol/kg bw * h), 1-^{13}C-Urea (prime: 17.5 μmol/kg bw, infusion rate: 5.0 μmol/kg bw * h) (Cambridge Isotopic Laboratories (Woburn, MA, USA)). Before intravenous administration of the priming dose of stable isotopes, a venous blood sample was collected to measure background enrichments. A second catheter for arterialized venous blood sampling was placed in a superficial dorsal vein of the hand or lower arm of the contralateral arm. The hand was placed in a thermostatically controlled hot box, a technique to mimic direct arterial sampling [17]. After 1.5 h of intravenous infusion, each subject ingested within 5 min a commercial medical food (Boost-HP drink (8 fl oz, 15 g protein, 240 kcal, 30% protein/11% carbohydrates/9% fat)). Blood samples were taken throughout the study and until 3.5 h after intake of the supplement.

2.4. Biochemical analysis

Blood was processed, stored at −80 °C, and analyzed batch-wise. Stable isotope enrichments and plasma amino acid concentrations were analyzed by LC-MS/MS [18].

2.5. Calculations for arginine and citrulline kinetics, and nitric oxide synthesis

Whole body ARG and CIT rates of appearances, CIT > ARG conversion (indirect measure of *de novo* ARG synthesis), ARG > CIT

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