

Microvascular function in patients undergoing chemotherapy

by

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B.S., University of Oklahoma, 2014

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Kinesiology
College of Human Ecology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2017

Approved by:

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Abstract

Adjuvant systemic chemotherapy for the treatment of certain cancers, particularly breast and lymphoma, adversely impacts cardiovascular health. However, the extent to which it impairs endothelial function is not well understood. Therefore, the purpose of this study was to determine if microvascular and macrovascular endothelial-dependent vasoreactivity is attenuated in breast cancer and lymphoma patients currently being treated with chemotherapy compared to healthy counterparts. With laser Doppler imaging, cutaneous microvascular function was evaluated via changes in cutaneous vascular conductance (CVC) in response to iontophoresis of acetylcholine (ACh). Endothelium-dependent flow-mediated dilation (FMD) was evaluated in the brachial artery via ultrasonography. CVC responses to iontophoresis of ACh in the cutaneous microcirculation was significantly lower in cancer patients than in control subjects (cancer (n=7): $959.9 \pm 187.3\%$; control (n=7): $1556.8 \pm 222.2\%$; $P = 0.03$). Furthermore, FMD was significantly lower in cancer patients than in control subjects (cancer: $2.2 \pm 0.6\%$; control: $6.6 \pm 1.4\%$; $P = 0.006$). These data provide evidence of microvascular and macrovascular dysfunction in breast cancer and lymphoma patients currently undergoing adjuvant chemotherapy, which may contribute to the increased long-term risk of cardiovascular disease morbidity and mortality in those treated for cancer.

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Dedication

For my dad, my most faithful supporter. You are missed but not forgotten. Because of you, I will continue to see the good in the world as I follow my path that you inspired me to take.

Chapter 1 - Introduction

Adjuvant systemic chemotherapy is used for the treatment of many cancers, including breast and lymphoma, and is associated with acute and late-occurring cardiovascular complications (2).

While effective in the treatment of cancer, anticancer chemotherapy increases the risk of developing multiple cardiovascular risk factors such as left ventricular dysfunction, hypertension, coronary artery calcification, ischemia, venous thromboembolism, QT prolongation, and bradycardia (50, 52). Furthermore, many cancer patients and survivors have an increased atherogenic profile compared to healthy control participants (5, 10, 50). Taken together, cancer patients receiving adjuvant systemic chemotherapy may be at an increased risk to develop both subclinical cardiovascular injury as well as overt cardiovascular disease (37, 50).

The development of chemotherapy-induced cardiotoxicity is predominantly characterized by progressive left ventricular dysfunction (52). However, other clinical manifestations, such as the development of endothelial vascular toxicity, may also occur (23, 45). In animal models, Doxorubicin chemotherapy has been shown to significantly impair endothelium-dependent vasodilation to acetylcholine (ACh) (11, 17). Similarly, conduit artery endothelium-dependent flow mediated dilation (FMD) is significantly decreased in adult cancer patients receiving cancer treatment with Doxorubicin (11) and Paclitaxel (47). While these initial studies provide some of the first evidence of endothelial dysfunction in cancer patients, there is still much that is not known about the progression and pathology of chemotherapy-induced endothelial dysfunction (34). This is concerning given that therapeutic interventions guided by evaluation of endothelial vascular function can provide a valuable method of detection, and therefore treatment, of those at risk for the development of overt cardiovascular disease (51).

To date the cutaneous microcirculation has been used to better understand the pathophysiological role of vascular dysfunction in heart failure, atherosclerosis, coronary artery diseases, peripheral vascular diseases, and type II diabetes (7, 14, 22, 39, 48, 49). Decreases in microvascular function occur early on in the progression of numerous cardiovascular and metabolic diseases (15, 36); therefore, evaluation of microvascular function in cancer patients may provide valuable insight into the pathological consequences of anticancer chemotherapy. When used in conjunction with iontophoresis of ACh, measurement of cutaneous microvascular red blood cell flux, via laser Doppler flowmetry, allows for non-invasive evaluation of endothelium-dependent microvascular reactivity. Therefore, the purpose of the present study was to determine whether breast cancer and lymphoma patients currently undergoing adjuvant systemic chemotherapy exhibit a reduced microvascular and macrovascular endothelium-dependent vascular function compared to healthy counterparts. Using laser Doppler flowmetry in response to ACh iontophoresis, we hypothesized that cutaneous microvascular function would be lower in chemotherapy-treated cancer patients than control subjects. In addition, using endothelium-dependent flow-mediated dilation of the brachial artery we hypothesized that macrovascular endothelial function would also be decreased in chemotherapy treated cancer patients, compared to control subjects.

Chapter 2 - Methods

Participants

The present study utilized a case-control cross-sectional study design (ClinicalTrials.gov ID:NCT03062878). Patients diagnosed with breast cancer (stage I-III n=3, stage IV n=2) or non-Hodgkin's lymphoma (stage I-III n=1, stage IV n=1) were recruited from Manhattan, Kansas and the surrounding communities. All patients were currently being treated with chemotherapy therapy at the time of testing (Table 2). Cancer diagnosis and chemotherapy regimen was confirmed by each patient's current oncologist. As all patients had already received anticancer treatment, healthy participants were recruited for comparison purposes. In a 1:1 ratio, cancer patients were matched with a healthy control based on sex, age, and body mass index (BMI). Upon enrollment in the study, health history and physical activity history questionnaires were completed. Both patients and controls were excluded from the study if they had known cardiovascular disease or two or more comorbidities (i.e. uncontrolled hypertension, diabetes, dyslipidemia, current tobacco use). Four participants (patients n=3, controls n=1) were currently taking hypertension medication (Angiotensin Converting Enzyme (ACE) inhibitors, Angiotensin II receptor blockers, Beta blockers, or diuretics). All procedures were approved by the Institutional Review Board of Kansas State University and conformed to the standards set by the *Declaration of Helsinki*. Written informed consent was obtained from all participants.

Experimental Procedures

Testing was conducted in a temperature-controlled room (21–23°C) after a > 4 hour fast. All experiments were performed while the participant remained in the supine position. Following a 10-min acclimation period, each participant was instrumented for continuous beat-by-beat blood pressure (systolic, diastolic, and mean) measurements via photoplethysmography (Finometer Pro, Finapres Medical Systems, Amsterdam, The Netherlands). Heart rate was monitored via three-lead ECG (S/5 Light Monitor). All blood pressure measurements were performed at heart level on the right side, unless contraindicated by lymph node dissection, in which case the left side was used, (n=2). To avoid the potential of trapped metabolites affecting forearm blood flow, testing order was not randomized with cutaneous microvascular function always preceding brachial artery FMD.

Cutaneous microvascular function. An iontophoresis drug delivery probe with an integrated laser Doppler probe and temperature regulator was placed on the left forearm 15 cm away from a conductive hydrogel drug dispersive electrode (PF 384, Perimed, Järfälla, Sweden). The integrated laser Doppler flowmeter (PeriFlux 5010 laser-Doppler perfusion monitor; Perimed, Jarfalla, Sweden) measured cutaneous red blood cell flux, which was used as an index of cutaneous blood flow. The temperature regulator maintained local skin temperature at 33°C around the perimeter of the probe. The drug delivery and drug dispersive probes formed a complete circuit in that they were both connected to a USB power supply (PF 751, Perimed, Järfälla, Sweden) that controlled the intensity, duration and interval of the current delivery. The drug delivery electrode contained 200 µL of a 2% ACh solution (Sigma-Aldrich, St. Louis, MO, USA) applied to the small sponge on the electrode. Following a 2-min baseline, a 100 µA anodal current was used to deliver the ACh in seven successive 20-s doses, with an interval of 60s.

Current delivery was managed and confirmed with the available software (PeriIont Software, Perimed, Järfälla, Sweden). Although current-induced vasodilation is a potential limitation of iontophoresis, previous work in clinical populations has implemented similar protocols (1, 38, 49) with minimal current-induced vasodilation elicited. Furthermore, this procedure follows recommended techniques (31), and pilot work from our lab confirmed that this procedure minimizes the current-induced vasodilation, thus demonstrating that the majority of dilation occurs via the actions of ACh.

Data acquisition software (DI-720, DATAQ Instruments, Akron, OH, USA) was used to record continuous measurements of cutaneous blood flow, reported as arbitrary perfusion units (PU); MAP, in mmHg; and heart rate (HR), in beats per minute during baseline, ACh iontophoresis, and for a minimum of 5 minutes following the completion of the last ACh delivery. Data were sampled at 100 Hz. Baseline averages of cutaneous blood flow, MAP and HR were calculated over a 2-min rest period. Data from the cutaneous blood flow response to ACh were binned to 10-s averages, from which the highest PU value was identified as the peak response. To normalize for MAP, cutaneous vascular conductance (CVC, PU/mmHg) was calculated as: $(PU/MAP) \times 100$. The relative change in CVC from baseline to peak was calculated as: $[(\text{peak} - \text{baseline CVC}) / \text{baseline CVC}] \times 100$. To reflect the cumulative effect of ACh iontophoresis, area under the curve (AUC) for arbitrary perfusion units and cutaneous vascular conductance was calculated for each patient and control subject.

Brachial artery FMD. In accordance to previously established guidelines (46), brachial artery endothelium-dependent FMD was performed on the left arm, unless contraindicated by lymph

node dissection, in which case it was performed on the right arm, (n=3). Instrumentation included a 6 cm tourniquet blood pressure cuff (Hokanson SC5, Bellevue, WA, USA) connected to an automated rapid cuff inflator (Hokanson E20, Bellevue, WA, USA), placed distal to the ultrasound transducer. A non-invasive 2D Doppler ultrasound equipped with a multi-frequency linear array transducer operating in duplex mode at a frequency of 10.0 MHz and 4.0 MHz, respectively (Logiq S8, GE Medical Systems, Milwaukee, WI, USA) was used to make simultaneous measurements of brachial artery diameter and blood velocity ~10 cm from the antecubital fossa (i.e. just proximal to the tourniquet cuff). The Doppler sample volume was set at the full width of the vessel, and the insonation angle was maintained at $< 60^\circ$. Following a 1-min baseline measurement, the tourniquet cuff was inflated to > 250 mmHg for 5 minutes. After the 5-min occlusion period, the tourniquet cuff was released (< 1 s), followed by a 2-min recovery period. Measurements of brachial artery diameter were made continuously during the 1-min baseline, the last 10 s of the occlusion period, and during the 2-min post-occlusion recovery period. A commercially available edge-detection and wall-tracking software package (Vascular Research Tools 6, Medical Imaging Applications, Coraville, Iowa, USA) was used to measure brachial artery diameters which were then averaged into 3-s bins. FMD was calculated, as both an absolute (mm Δ) and a relative (% Δ) value, as the peak post-occlusion diameter change from baseline.

Statistical Analysis. Statistical analyses were performed using a commercially available software package (SigmaPlot/SigmaStat 12.5, Systat Software, Point Richmond, CA, USA). Pair differences between the patient and control groups were determined by independent samples t-

tests. All data are presented as mean \pm SE, unless stated otherwise. Statistical significance was declared when $P < 0.05$.

Chapter 3 - Results

Demographic and clinical variables for chemotherapy patients and control subjects are presented in Table 1. There were no significant differences in age, height, weight, and BMI between chemotherapy patients and control subjects. Resting MAP was also not different between groups ($P = 0.5$). A description of cancer type and anticancer therapy parameters is presented in Table 2. Five (71.4%) patients were diagnosed with breast cancer. Two (28.6%) patients were diagnosed with Non-Hodgkin's lymphoma. All seven patients were receiving chemotherapy at the time of testing, and only one had received radiation.

Cutaneous microvascular function. Skin blood flow responses to ACh iontophoresis from representative subjects are illustrated in Figure 1. Values demonstrating cutaneous microvascular reactivity in response to ACh iontophoresis are presented in Table 3. In comparison to the control subjects, parameters of absolute cutaneous microvascular reactivity were significantly blunted in chemotherapy patients; peak perfusion units (PU) (Table 3; cancer: 73.3 ± 12.5 PU; control: 133.2 ± 29.2 PU; $P = 0.04$), peak cutaneous vascular conductance (CVC) (Table 3; cancer: 67.5 ± 11.4 PU/mmHg; control: 113.7 ± 22.8 PU/mmHg; $P = 0.048$). Similarly, relative changes in cutaneous microvascular reactivity were significantly reduced in chemotherapy patients; % change in PU (Table 3; cancer: $998.5 \pm 192.9\%$; control: $1652.8 \pm 215.2\%$; $P = 0.02$), % change in CVC (Table 3; Figure 2A; cancer: $959.9 \pm 187.3\%$; control: $1556.8 \pm 222.2\%$; $P = 0.03$). Additionally, as demonstrated by the area under the curve (AUC) values, the cumulative response to ACh iontophoresis was significantly lower in chemotherapy patients compared to the control group; AUC PU (Table 3; cancer: 34773.8 ± 7353.1 PU; control:

75925.0 ± 19190.3 PU; P = 0.03) and AUC CVC (Table 3; Figure 2B; cancer: 32915.4 ± 6901.0 PU/mmHg; control: 69403.6 ± 17352.1 PU/mmHg; P = 0.04).

Brachial artery FMD. Markers of endothelium-dependent brachial artery FMD are presented in Table 4. Resting brachial artery diameter (mm) prior to cuff inflation was not different between chemotherapy patients and control subjects (Table 4; cancer: 3.41 ± 0.24 mm; control: 3.21 ± 0.27 mm; P = 0.3). Following 5 min of arterial occlusion, the FMD response was significantly lower in chemotherapy patients when expressed in both absolute (Table 4; Figure 3A; cancer: 0.07 ± 0.02 Δ mm; control: 0.20 ± 0.03 Δ mm; P = 0.004) and relative terms (Table 4; Figure 3B; cancer: 2.18 ± 0.55 Δ %; control: 6.63 ± 1.41 Δ %; P = 0.006).

Table 1 - Subject Characteristics

	Chemotherapy Patients (n = 7)	Controls (n = 7)	P Value
Demographics			
Age (yr)	55 ± 13	54 ± 7	0.9
Females (%)	6 (85.7%)	6 (85.7%)	
Clinical Parameters			
Height (cm)	164 ± 6	162 ± 8	0.6
Weight (kg)	79.7 ± 28.7	71.1 ± 21.6	0.5
BMI (kg/m ²)	29.7 ± 11.5	26.9 ± 7.2	0.6
Resting MAP (mmHg)	106 ± 6	110 ± 14	0.5
HTN medication (%)	3 (42.9%)	1 (14.3%)	0.3

Values are means ± SD or n (%). BMI, body mass index; MAP, mean arterial pressure; HTN, hypertension.

Table 2 - Description of cancer and anti-cancer therapy

	Chemotherapy Patients (n = 7)
Breast Cancer	5 (71.4%)
<i>Stage</i>	
I-III	3 (60%)
IV	2 (40%)
<i>Pathobiology</i>	
IDC	4 (80%)
DCIS	1 (20%)
<i>Hormonal Status</i>	
ER +/-	4/1 (80%/20%)
PR +/-	2/3 (40%/60%)
HER2 +/-	3/2 (60%/40%)
Non-Hodgkin's Lymphoma	2 (28.6%)
<i>Stage</i>	
I-III	1 (50.0%)
IV	1 (50.0%)
<i>Pathobiology</i>	
Follicular, Nodular	1 (50.0%)
Nodal Marginal Zone B Cell	1 (50.0%)
Chemotherapy	7 (100%)
Alkylating Agent	4 (57.1%)
Anthracycline	2 (28.6%)
Antimicrotubule	2 (28.6%)
Aromatase Inhibitor	2 (28.6%)
Monoclonal Antibody	5 (71.4%)
Radiation	1 (14.3%)

Values are n (%). IDC, invasive ductal carcinoma; DCIS, ductal carcinoma in situ; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2 receptor.

Table 3 - Cutaneous microvascular reactivity

	Chemotherapy Patients (n=7)	Controls (n=7)	P Value
ACh			
Peak PU	73.3 ± 12.5	133.2 ± 29.2	0.04*
Peak CVC	67.5 ± 11.4.	113.7 ± 22.8	0.048*
% Change PU	998.5 ± 192.9	1652.8 ± 215.2	0.02*
% Change CVC	959.9 ± 187.3	1556.8 ± 222.2	0.03*
AUC PU	34773.8 ± 7353.1	75925.0 ± 19190.3	0.03*
AUC CVC	32915.4 ± 6901.0	69403.6 ± 17352.1	0.04*

Values are means ± SE. ACh, acetylcholine; PU, perfusion units; CVC, cutaneous vascular conductance; AUC, area under the curve; PORH, post-occlusive reactive hyperemia; FMD, flow-mediated dilation. *Statistically significant.

Table 4 - Flow-mediated dilation of the brachial artery

	Chemotherapy Patients (n=7)	Controls (n=7)	P Value
FMD			
Baseline (mm)	3.4 ± 0.2	3.2 ± 0.3	0.3
FMD (Δ mm)	0.1 ± 0.0	0.2 ± 0.0	0.004*
FMD (Δ %)	2.2 ± 0.6	6.6 ± 1.4	0.006*

Values are means ± SE. FMD, flow-mediated dilation. *Statistically significant.

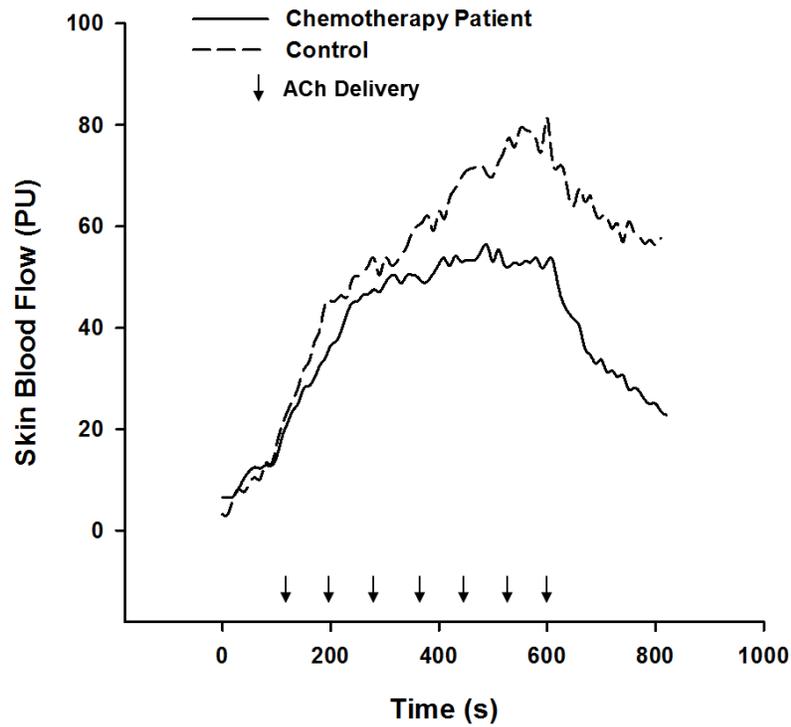


Figure 1 – Skin blood flow response, in arbitrary perfusion units (PU), to ACh iontophoresis in a representative chemotherapy patient (solid line) and their matched representative control subject (dashed line). Notice the overall pattern is very similar between the patient and the control; however, the magnitude is much greater in the control subject compared to the chemotherapy patient.

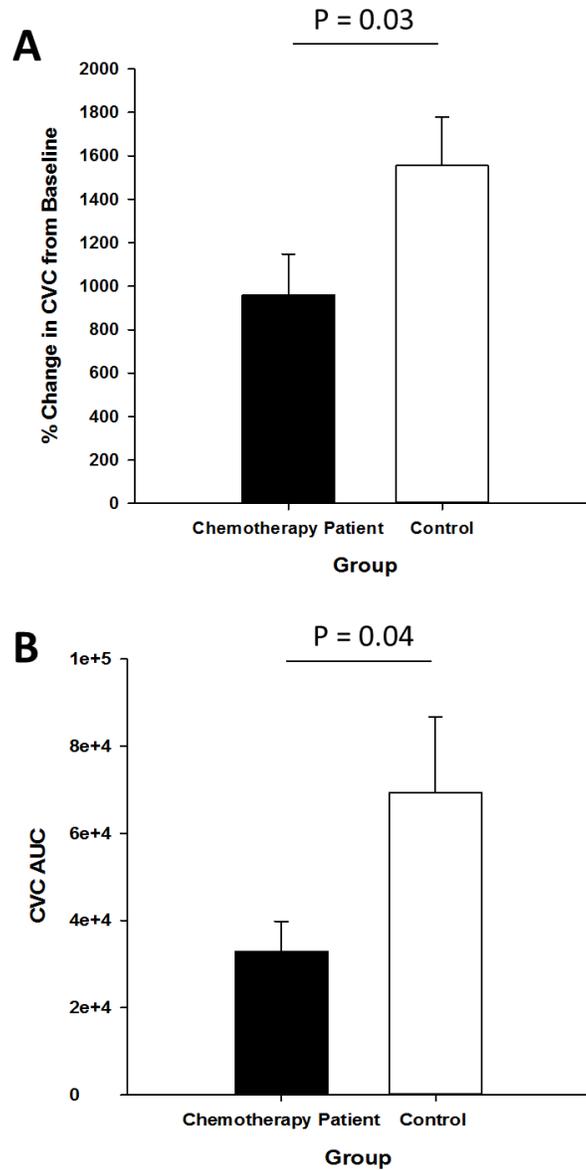


Figure 2 – (A) Relative increase in cutaneous vascular conductance (CVC) in response to ACh iontophoresis. CVC was significantly reduced in chemotherapy patients (filled bar), compared to controls (open bar) (P=0.03). (B) Cutaneous vascular conductance (CVC) area under the curve (AUC) in response to ACh iontophoresis. AUC, as an index of the overall response to ACh-mediated vasodilation, was significantly reduced in chemotherapy patients (filled bar), compared to controls (open bar) (P=0.04).

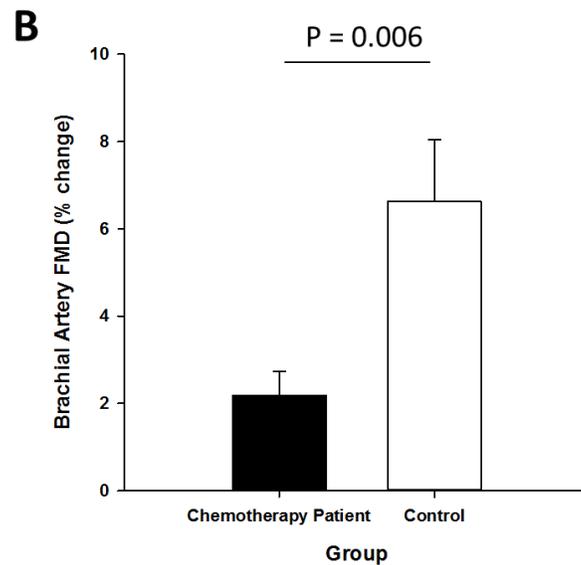
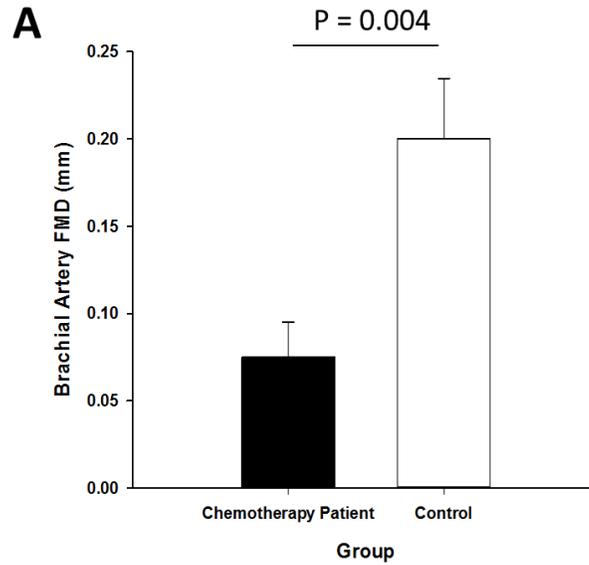


Figure 3 – (A) Absolute increase in brachial artery diameter (mm) in response to arterial occlusion. Flow-mediated dilation (FMD) was significantly reduced in chemotherapy patients (filled bar), compared to controls (open bar) (P=0.004). (B) Relative increase in brachial artery diameter (% change from baseline) in response to arterial occlusion. Flow-mediated dilation (FMD) was significantly reduced in chemotherapy patients (filled bar), compared to controls (open bar) (P=0.006).

Chapter 4 - Discussion

The major finding of this study is that microvascular and macrovascular endothelium-dependent vasoreactivity is attenuated in breast cancer and lymphoma patients currently being treated with chemotherapy compared to healthy counterparts. Specifically, significant decreases in ACh-mediated cutaneous vasodilation were observed. Additionally, our experiments demonstrated that these cancer patients also exhibit a significantly decreased endothelium-dependent FMD of the brachial artery. Taken together our findings suggest that the chemotherapy has a negative impact on endothelial health, which may contribute to the increased long-term risk of cardiovascular disease morbidity and mortality in cancer survivors (50, 52).

To the best of our knowledge, this is the first study investigating microvascular endothelial function in cancer patients currently being treated with chemotherapy, compared to healthy counterparts. We demonstrate that these patients treated with a combination of adjuvant systemic chemotherapies experience endothelial dysfunction at multiple locations. It is well established that impairments in endothelial signaling, leading to endothelial dysfunction, occur early in pathogenesis of cardiovascular disease (51). As such, vascular remodeling and decreases in endothelium-dependent vasodilation are clinically important, as they are likely the earliest pathological outcome associated with decreases in cardiovascular health (19, 51). Therefore, the decreased endothelial function within the intact circulation of cancer patients currently undergoing chemotherapy highlights that the chemotherapy-induced toxicity extends well beyond the myocardium and may be eliciting globalized systemic effects.

Cutaneous iontophoresis with ACh has previously been used to evaluate changes in microvascular function in a variety of clinical populations (33, 38, 49). The contribution of nitric oxide (NO) to ACh-mediated cutaneous vasodilation is well supported by several investigations (4, 16, 27, 32) but not all (24). In addition to NOS-dependent pathways, COX-dependent pathways and EDHF-dependent pathways are also thought to play a role in ACh-mediated cutaneous vasodilation (16, 24, 27, 32). However, while the exact mechanism(s) of ACh-induced cutaneous vasodilation are not fully understood, it does provide valuable insight into the adverse cardiovascular effects that may be occurring in cancer patients undergoing adjuvant chemotherapy. Since each of the ACh-mediated vasodilator pathways are endothelium-dependent, the findings suggest that cancer patients undergoing adjuvant chemotherapy experience some degree of endothelial dysfunction within the microcirculation.

Consistent with previous investigations we observed a significant decrease in macrovascular endothelium-dependent FMD of the brachial artery in our group of cancer patients. Duquaine et al. (2003) demonstrated significant reductions in endothelium-dependent FMD of the brachial artery following a single Doxorubicin infusion (11). Similarly, Vassilakopoulou et al. (2010) found that endothelial function was significantly impaired in cancer patients following treatment with the taxane-based chemotherapy drug Paclitaxel, as evidenced by significantly reduced brachial artery endothelium-dependent FMD (47). Furthermore, patients treated with both Paclitaxel and an anthracycline demonstrated the most severe reductions in endothelial function, possibly due to a negative synergistic effect of the drugs (47). Endothelium-dependent brachial artery FMD is largely NO dependent; suggesting that in our group of cancer patients treated with chemotherapy the observed decreases in FMD is due, in part, to decreases in NO bioavailability.

The potential mechanisms for the observed decreases in microvascular and macrovascular function in our cancer patients may be linked to the molecular actions of the prescribed chemotherapy. Of the chemotherapies in our experimental group, many are associated with factors known to affect endothelial function. For example, Cyclophosphamide is a cell-cycle non-specific alkylating agent that is commonly used to treat breast cancer and lymphoma (12, 42). It has been associated with left ventricular dysfunction, with both total dose and prior anthracycline exposure being risk factors of occurrence (20, 35). Although the precise mechanism of Cyclophosphamide-induced cardiotoxicity is unknown, it is speculated that it directly injures the endothelium, resulting in the extravasation of toxic metabolites, eventually damaging cardiomyocytes (21). While the consequences of this cascade in the peripheral circulation are largely unknown, based on the present study, we cannot rule out that a similar mechanism attenuates ACh-mediated cutaneous vasodilation and/or endothelium-dependent brachial artery FMD.

In addition to being a risk factor for cardiotoxicity in patients being treated with Paclitaxel, treatment with anthracyclines alone has been associated with cardiotoxicity (52). Anthracyclines, such as Doxorubicin and Epirubicin, are known to cause acute, subacute, and late cardiac damage (53), with the most widely accepted hypothesis pertaining to the generation of reactive oxygen species (ROS). Specifically, treatment with anthracyclines results in increased levels of ROS, which then leads to increases in apoptotic pathways, reduced myofilament synthesis, pathological myocardial hypertrophy, and altered cardiac metabolism (6). In addition to affecting cardiomyocytes, anthracyclines have also been implicated in the development of endothelial

dysfunction. Kotamraju et al. (2000) reported Doxorubicin induced bovine aortic endothelial cell apoptosis that was related to intracellular hydrogen peroxide formation (29). Furthermore, their findings demonstrated Doxorubicin-induced hydrogen peroxide generation and calcium release activated apoptosis (26). Taken together, it appears that anthracyclines cause endothelial dysfunction via generation of ROS (e.g. hydrogen peroxide), resulting in oxidative stress and apoptosis. Additionally, the endothelium itself, via eNOS uncoupling, may also be a contributing factor. Duquaine et al. (2003) demonstrated that endothelial denudation completely abolished ROS generation in rabbit aortic rings exposed to Doxorubicin, suggesting an endothelial source of free radical generation in the early stages of anthracycline exposure (11). The authors speculate this generation of ROS from eNOS is due to a change in its normal function, resulting in eNOS uncoupling. In health, eNOS is involved with NO production. However, when exposed to anthracyclines, it appears that eNOS begins to generate nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), resulting in ROS generation.

Perhaps in light of the well-characterized adverse effects on cardiac function, anthracyclines are no longer the most commonly used initial chemotherapy regimen (18). Indeed the use of other classes of chemotherapy, particularly taxane-based regimens (e.g., Paclitaxel and Docetaxel) appear to be implemented more frequently. Paclitaxel is a cell-cycle specific drug that inhibits cell division and replication, especially in rapidly dividing neoplastic cells (47), and is often used for treatment against solid tumors in patients with breast cancer (9). The effects of Paclitaxel on endothelial cells are still largely unknown; however, its function as an antimicrotubule agent may contribute to the observed endothelial dysfunction. Specifically, previous reports have highlighted the importance of the endothelial cytoskeleton in both maintaining structural

integrity, and regulating the production of endothelium-derived vasodilators (e.g. NO and prostaglandins) (8, 28). In addition to the adverse effects on the structural components of endothelial cells, Paclitaxel may also be eliciting inflammation. Cremophor EL, the formulation vehicle used for Paclitaxel, induces histamine release (40), which has been shown to disrupt vascular endothelial barrier function, resulting in inflammation (3). As such, it is possible that repeated and systemic exposure to Paclitaxel during treatment would be detrimental to the endothelium, both structurally and functionally.

In addition to the negative synergistic effects observed when Paclitaxel and anthracyclines are used concomitantly, similar outcomes have been reported when anthracyclines are used in conjunction with Herceptin. Indeed, patients treated with both anthracyclines and Herceptin are at a much greater risk of developing symptomatic heart failure than those treated with Herceptin alone (43). Herceptin, a monoclonal antibody, blocks the human epidermal growth receptor-2 (HER2) signaling pathway, which is overexpressed in HER2 + breast cancer patients (44), resulting in simultaneous ROS accumulation and decreased NO bioavailability, which have been proposed as mechanisms that impair endothelial function (41). Considering three (60%) of our breast cancer patients were HER2 +, these potential mechanisms should be further investigated.

Several experimental considerations should be recognized when interpreting the findings of this study. First, the sample size was modest, but very similar to previous investigations evaluating endothelial function in human cancer patients (11, 13) and the effect was large enough that significant differences in the cancer patients were still observed. Second, while documented, the type of treatment was not controlled for; however, we did obtain detailed treatment records from

each patient's treating oncologist. While the diversity in treatment limits our ability to identify treatment-specific mechanisms of endothelial dysfunction, it does make the results more generalizable to the cancer patient community. Third, the population measured was predominantly female, and is therefore not representative of cancer patients as a whole. Lastly, the use of cutaneous microcirculation does pose limitations to the extrapolation of our findings to other vascular beds, and therefore warrants additional discussion. Despite the cutaneous circulation being proposed as a model of generalized microvascular function (25), it is well-established that there are different mechanisms of regulation across vascular beds and along the arterial tree (30). Within the cutaneous circulation both vasoconstrictor and vasodilator pathways exist with multiple regulatory mechanisms (30). As such, extreme caution should be used when extrapolating the current findings in the cutaneous microcirculation to other vascular beds (e.g., coronary). Nevertheless, assessment of the cutaneous microcirculation does provide valuable insight into the changes in cardiovascular health that may be occurring during chemotherapy treatment. Decreases in cutaneous vasoreactivity are known to occur early on in the progression of atherosclerosis (48), and can be used to predict the risk of future cardiovascular events in patients with coronary artery disease (22). In the present study it was clear that the cutaneous microvascular response to ACh was abnormal in the cancer patients compared to healthy matched controls; thus, providing evidence that adjuvant systemic chemotherapy adversely affects vascular health.

In conclusion, the present study demonstrates that endothelium-dependent vasoreactivity within microvascular and macrovascular beds is attenuated in breast cancer and lymphoma patients currently undergoing chemotherapy. To date, various chemotherapy regimens, especially

anthracyclines, have been studied and characterized based on central cardiotoxicities affecting the heart. However, the present study indicates that not only is the peripheral circulation, at both the macro- and microvascular levels of the arterial tree negatively affected by chemotherapy, but also that other classes of chemotherapies (e.g. alkylating agent, taxanes and monoclonal antibodies) may be playing a role in the observed endothelial dysfunction. Indeed, the endothelium within these circulations appears to be affected, as evidenced by significantly reduced ACh-mediated cutaneous vasodilation and brachial artery FMD in breast cancer and lymphoma patients, compared to matched controls. Taken together these findings suggest patients undergoing adjuvant systemic chemotherapy experience endothelial dysfunction, which may contribute to the increased long-term risk of cardiovascular disease morbidity and mortality seen in cancer survivors.

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