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Original Article

Exercise pre-conditioning prevents vascular toxicity caused by infusion of 5-fluorouracil in male rats

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Abstract: 5-fluorouracil (5-FU) is one of the most common chemotherapies used in cancer treatment yet is often associated with acute cardiotoxicity (e.g., angina, vasospasm). To date, countermeasures to prevent 5-FU cardiotoxicity are lacking. Therefore, we tested the hypothesis that short-term, moderate-intensity exercise completed before 5-FU administration would prevent 5-FU-induced alterations in vascular and cardiac function. Male Sprague-Dawley rats were randomized to sedentary control (SEDCON, n = 9), sedentary 5-FU (SED5FU, n = 10), exercise control (EXCON, n = 8) or exercise 5-FU (EX5FU, n = 8) groups. Rats remained sedentary or completed 4-days of treadmill running (20-25 min, 20 m/min, 5% grade) with the final bout ending ~90-min before treatment with a clinically relevant dose of 5-FU (50 mg/kg bolus + 265 mg/kg 2-hr infusion) or volume matched saline. Echocardiographic indices of left ventricular function and Doppler measurements of aortic pulse wave velocity (PWV) were completed at baseline (BL) and after the 2-hr (2-hr) infusion. 5-FU did not induce changes in left ventricular function. PWV increased from BL to 2-hr in SED5FU (BL: 396 ± 39 cm/s; 2-hr: 452 ± 54 cm/s; P = 0.002), but not SEDCON (BL: 417 ± 55 cm/s; 2-hr: 392 ± 64 cm/s; P = 0.35), EXCON (BL: 408 ± 35 cm/s; 2-hr: 410 ± 46 cm/s; P > 0.99), or EX5FU (BL: 398 ± 13 cm/s; 2-hr: 417± 23 cm/s; P = 0.67). Additionally, PWV at the 2-hr time point was significantly higher in SED5FU compared to SEDCON (P = 0.002). These findings suggest that moderate-intensity exercise preconditioning may protect against 5-FU-induced alterations in arterial PWV—potentially mitigating early signs of cardiotoxicity. Future studies are warranted to identify the mechanisms of 5-FU-induced cardiotoxicity and the feasibility and efficacy of pre-treatment exercise regimens in human patients.

Keywords: Cardiotoxicity, cardio-oncology, exercise oncology, chemotherapy, pulse wave velocity, arterial stiffness

Introduction

5-fluorouracil (5-FU) chemotherapy has remained a cornerstone of numerous gastrointestinal, breast, and head/neck cancer treatment regimens since its inception in 1957 [1]. While this longevity speaks to its effectiveness as an anticancer agent, 5-FU has gained notoriety for its unfortunate association with treatment-induced cardiotoxicity in up to 19% of treated patients [2-7]. Reported side effects often arise during or within 72 hours of completing treatment [5], and include angina (i.e., chest pain), acute coronary syndromes at rest or with exertion, ECG alterations indicative of myocardial ischemia, and in severe instances, heart failure and sudden cardiac death [2, 4, 5, 8-11].

Evidence of coronary spasm [12, 13], peripheral vascular constriction/dysfunction [14-16], and increased arterial stiffness [17] in 5-FU treated patients have led to the predominant theory that 5-FU cardiotoxicity primarily arises via a direct effect on the vasculature [5]. However, data from preclinical studies suggest both myocardial [18-21] and vascular [22-28] etiology—with findings potentially influenced by

how closely experimental protocols replicate clinical infusion regimens and incorporate clinically relevant measures of cardiac and vascular injury. Preclinical studies have historically administered 5-FU via intraperitoneal or bolus intravenous (IV) injections alone [19-21, 29]. This differs from clinical practice where 5-FU is often delivered via IV 5-FU bolus (400 mg/m^2) followed by a continuous IV infusion of 5-FU (2,400 mg/m^2) for 46-72 hours [30-32]. Considering that rates of 5-FU cardiotoxicity are higher following continuous infusion compared to bolus alone [33, 34], preclinical models which employ continuous infusion protocols similar to those used clinically may elucidate whether 5-FU primarily mediates its cardiotoxic effects via the vasculature, the heart, or both.

Given its importance as a first-line cancer treatment, strategies to alleviate or prevent 5-FU cardiotoxicity have been of recent interest. Pharmacological therapy with nitrates or calcium channel blockers have yielded mixed findings [16, 35-37], and these treatments are typically only administered after a patient experiences adverse treatment-related side effects. As such, therapies administered prior to or during treatment with the potential to prevent cardiotoxicity are greatly needed. One such strategy is the implementation of an exercise preconditioning regimen, as aerobic exercise has established cardioprotective effects on both the heart and vasculature [38]. Yet, while long-term exercise preconditioning (7-12 wks.) can effectively prevent chemotherapy-induced cardiotoxicity in rodents [39-42], such programs may not be clinically feasible in the timeframe between initial diagnosis and treatment onset—especially if the patient is recovering from surgical procedures. Conversely, short-term exercise preconditioning programs can also offer cardioprotective benefits [43, 44] and could conceivably be implemented in the week preceding treatment. Studies in rodents have demonstrated 1-5 days of exercise preconditioning is sufficient to prevent cardiotoxicity caused by anthracycline chemotherapy [45-48]; however, the efficacy of exercise preconditioning as a means of preventing 5-FU cardiotoxicity remains untested. To address this gap, we developed a clinically relevant rat model of continuous 5-FU infusion and tested the hypothesis that acute aerobic exercise preconditioning would prevent signs of cardiac and vascular toxicity. We specifically evaluated the impact of 5-FU on left ventricular function and arterial stiffness to determine whether exercise may attenuate early manifestations of 5-FU-induced cardiotoxicity. Given recent evidence that cancer may independently contribute to cardiovascular maladaptation [49-52], the present studies were conducted in tumor naïve rats to eliminate potential confounding effects of cancer and determine the direct impact of 5-FU on cardiovascular well-being.

Materials and methods

Animals

All procedures performed herein were approved by the Kansas State University Institutional Animal Care and Use Committee and conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague Dawley rats (n = 38, 6-8 mo. old, ~670 g; Charles River; Wilmington, MA) were used for this study. All rodents were housed in a temperature-controlled facility (23 ± 2°C) on a 12:12-h light-dark cycle with standard rat chow and water provided *ab libitum*.

Study design

The week before the experimental protocol, all rats were habituated to treadmill exercise on a custom-built motor-driven rodent treadmill (< 5 min/day at 15 m/min, 0% incline for three days). Each rat was then randomized to either exercise or sedentary groups and to receive treatment with 5-FU or volume-matched saline. This resulted in a total of four experimental groups: sedentary + saline control (SEDCON, n = 10), sedentary + 5-FU (SED5FU, n = 10), exercise + saline control (EXCON, n = 9), and exercise + 5-FU (EX5FU, n = 9). Three days before 5-FU or saline treatment, exercise animals (EXCON and EX5FU) began a 4-day acute exercise preconditioning program (**Figure 1**). Briefly, rats ran on a motorized treadmill at 20 m/min (5% grade) for 20-25 min/day, with the final exercise session commencing ~90-min before 5-FU or saline treatment (see 5-FU or Saline Administration). Based on the prior work of others, we estimate this exercise intensity to be ~65% VO_2max [53].

Surgical procedures

Approximately 30-min following completion of the final exercise bout, rats were first anesthe-

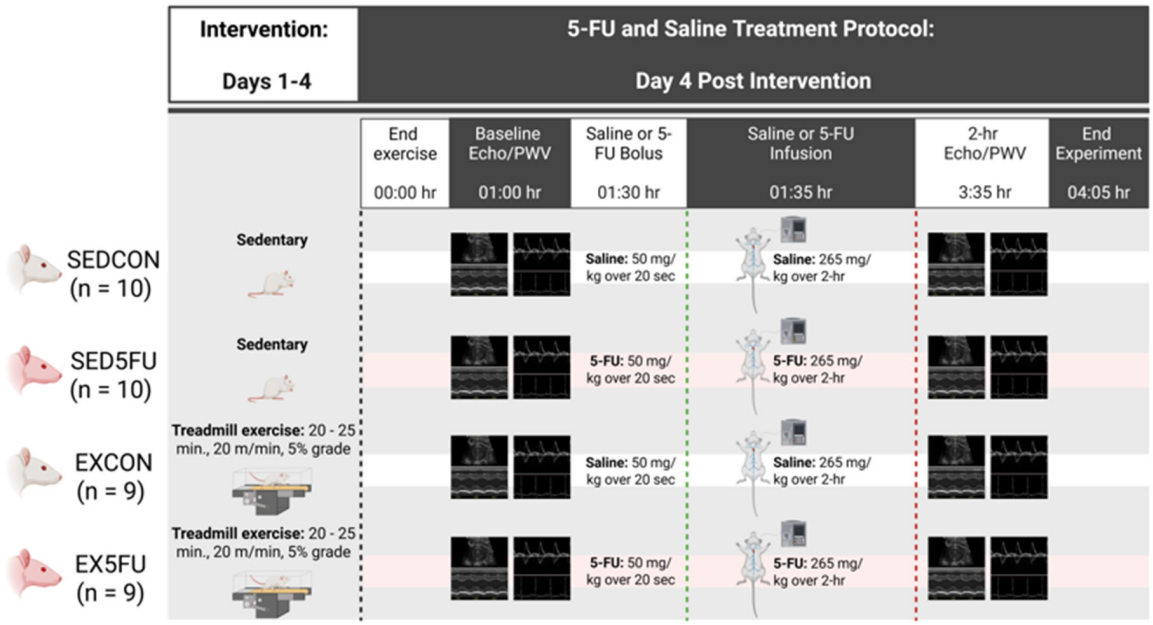

Figure 1. Study design. A timeline of events for the acute exercise preconditioning and 5-FU chemotherapy infusion protocol. Animals were randomized to sedentary or acute exercise preconditioning groups. Exercised animals completed four, once daily bouts of moderate intensity exercise on a motorized treadmill (20 m/min, 5% grade). Exercise preconditioning commenced three days prior to planned 5-FU/saline infusion with the fourth and final bout ending ~90 minutes prior to the start of the treatment. *In vivo* assessment of left ventricular function and aortic pulse wave velocity (PWV) were conducted immediately prior to and following completion of the 5-FU/saline infusion to assess the effect of 5-FU on cardiovascular function. Figure designed using Biorender.com.

tized with a 5% isoflurane-O_2 mixture (isoflurane vaporizer; Harvard Apparatus; Cambridge, MA) and subsequently maintained on 2.5% isofluorane-O_2 on a heated surgical station (Rodent Surgical Monitor, Indus Instruments; Houston, TX) at 37 ± 1°C. An incision was made at the left ventral neck to expose the left jugular vein for catheterization (PE-50 Intra-Medic polyethylene tubing, Becton Dickson, Sparks, MD) to administer 5-FU or saline. A second catheter (PE-10 connected to PE-50) was placed in the caudal artery for continuous blood pressure monitoring. Following instrumentation, rats were transitioned to pentobarbital sodium anesthesia (20 mg/kg body wt) administered via the caudal artery (total volume per injection ~0.2 ml) while concentrations of isoflurane were decreased and subsequently discontinued over ~30 min. The level of anesthesia was regularly monitored via toe pinch and palpebral reflex, with pentobarbital anesthesia supplemented (~16 mg/kg/hr) as necessary for the remainder of the experiment. After completing the 2-hr infusion and associated cardiovascular measurements (see below), all animals were euthanized via cardiac excision under deep plane anesthesia. A graphical depiction of the experimental timeline is presented in **Figure 1**.

5-FU and saline administration

To align our study with common clinical drug delivery methods, we designed a translational rat model of 5-FU-induced cardiotoxicity using allometric scaling [54]. Following the completion of surgical procedures and collection of baseline cardiovascular measurements (see below), a bolus dose of 5-FU (50 mg/kg) or volume-matched saline was delivered over ~20 seconds via a jugular vein catheter using a 10 mL syringe. The jugular vein catheter was then attached to a programmable infusion syringe pump (Pump 11 Elite; Harvard Apparatus; Holliston, MA) to initiate a 2-hour continuous infusion of 5-FU (265 mg/kg) or volume-matched saline. These 5-FU doses were determined using the human bolus (400 mg/m^2) and continuous infusion (2400 mg/m^2) doses commonly used in 5-FU based regimens (e.g., FOLFOX, FOLFIRI) [30, 32].

Hemodynamic variables

Heart rate (HR) and mean arterial blood pressure (MAP) were continuously monitored over the duration of the protocol using a commercially available blood pressure monitor (DigiMed BPA; Micro-Med; Louisville, KY) with analog output to a laboratory computer via a multifunction data acquisition device (NI USB 6211, National Instruments, Austin, TX). Data were sampled at 1,000 Hz and recorded for offline analysis. Measurements of HR and MAP were averaged over 8-10 minutes immediately before administration of the 5-FU/saline treatment (baseline, BL) and at the end of the 2-hour infusion (2-hr).

Echocardiographic evaluation of left ventricular function

Transthoracic echocardiography measures of left ventricular function were performed over three continuous cardiac cycles at BL and following the 2-hr continuous infusion (Logiq e; GE Medical Systems; Milwaukee, WI) as previously demonstrated by our group [49, 55-58]. Following removal of hair from the chest (Nair, Johnson & Johnson, New Brunswick, NJ), two-dimensional guided M-mode images were collected at the level of the mitral leaflets in the parasternal short axis view using a 22-MHz linear transducer (GE L10-22-RS, GE Medical Systems). Images were stored on an offline storage device for future measurement of left ventricular dimensions and posterior wall thickness, which were subsequently used to estimate left ventricular end-diastolic (EDV) and end-systolic (ESV) volumes using the Teichhholz formula and to calculate fractional shortening (FS), stroke volume (SV), and cardiac output (Q) [59].

Doppler pulse wave velocity

Measurements of aortic pulse wave velocity (PWV)—an established marker of arterial stiffness [60]—were made at BL and following the 2-hour continuous infusion using a commercially available Doppler flow velocity system (Indus Instruments; Houston, TX). Rats were positioned supine, with external needle electrodes placed on each paw for ECG collection. Two Doppler probes connected to a pulsed Doppler system transceiver were used to obtain simultaneous Doppler spectrograms from the descending and abdominal aorta. One probe was placed along the abdominal midline to capture abdominal aorta flow velocity and held in place by a micro-positioner. The second probe was placed to the right of the sternum near the base of the upper left limb to capture descending aorta velocity. Images were captured simultaneously, and time aligned with ECG for offline analyses using manufacturer software. The distance between probes (mm) was measured immediately following the obtainment of the image. PWV was calculated as follows: PWV = distance between probe tips (mm)/(time from R wave to the abdominal aorta - time from R wave to aortic arch) (ms) and reported in cm/s.

Data analysis

All statistical analyses were completed using a commercially available software package (GraphPad Prism 9.5.0; San Diego, Ca, USA). Echocardiographic and hemodynamic variables, as well as aortic pulse wave velocity, were assessed via two-way repeated measures analyses (mixed-model) with main effects for group (SEDCON, SED5FU, EXCON, EX5FU) and time (BL, 2-hours). Multiple comparisons were completed using Šídák's multiple comparisons test when significant interactions or main effects were present. Group differences in body mass were assessed using one-way ANOVA. All data are presented as mean ± standard deviation unless otherwise stated. Statistical significance was set at $P < 0.05$.

Results

A total of 38 rats were used in this study. Three rats died during the surgical procedures, and thus findings from a total sample of 35 rats (SEDCON: n = 9; SED5FU: n = 10, EXCON: n = 8; EX5FU: n = 8) are reported herein. There were no differences in body mass between the groups (SEDCON: 642 ± 41 g; SED5FU: 637 ± 87 g; EXCON: 728 ± 119 g; EX5FU: 685 ± 87 g; P = 0.13).

Hemodynamic variables

Mean values for hemodynamic variables are presented in **Table 1**. There was no significant group × time interaction for MAP (P = 0.99), HR (P = 0.78), or Q (P = 0.44). HR did not differ between the groups (P = 0.70), nor did it change significantly from BL to 2 hours (P > 0.99).

Table 1. Mean data for echocardiographic and hemodynamic variables

	Sedentary Control (SEDCON) n = 9		Sedentary 5-FU (SED5FU) n = 10		Exercise Control (EXCON) n = 8		Exercise 5-FU (EX5FU) n = 8	
	BL	2-hr	BL	2-hr	BL	2-hr	BL	2-hr
ESV (mL)	0.07 ± 0.04	0.14 ± 0.08**	0.09 ± 0.04	0.16 ± 0.10**	0.10 ± 0.02	0.13 ± 0.05	0.10 ± 0.06	0.18 ± 0.05**
EDV (mL)	0.82 ± 0.12	0.88 ± 0.21	0.82 ± 0.20	0.96 ± 0.26	1.02 ± 0.15	0.97 ± 0.15	0.92 ± 0.19	1.03 ± 0.13
SV (mL/beat)	0.75 ± 0.11	0.75 ± 0.16	0.73 ± 0.17	0.80 ± 0.17	0.91 ± 0.13	0.83 ± 0.11	0.82 ± 0.15	0.84 ± 0.08
FS (%)	59.8 ± 8.5	50.8 ± 9.7†	55.5 ± 6.1	50.0 ± 8.1*	55.8 ± 2.8	50.9 ± 3.5	57.3 ± 8.9	45.9 ± 2.8†
MAP (mmHg)	78 ± 12	86 ± 21	81 ± 9	87 ± 10	76 ± 15	85 ± 24	76 ± 10	84 ± 23
HR (BPM)	323 ± 26	326 ± 28	321 ± 31	314 ± 29	316 ± 28	308 ± 33	319 ± 57	329 ± 14
Q (mL/min)	243.0 ± 49.0	245.6 ± 68.3	234.9 ± 61.0	250.7 ± 52.7	286.3 ± 33.3	256.7 ± 46.2	264.6 ± 70.9	274.6 ± 25.3

Abbreviations: BL, Baseline; ESV, End systolic volume; EDV, End diastolic volume; SV, Stroke volume; FS, Fractional shortening; MAP, Mean arterial pressure; HR, Heart rate; Q, Cardiac output. Data are presented as mean ± standard deviation. *P < 0.05 baseline to 2-hr; **P < 0.01 baseline to 2-hr; †P < 0.001 baseline to 2-hr.

Similarly, Q was not different between the groups (P = 0.36) and did not change over the course of the infusion (P = 0.98). MAP did not differ between groups (P = 0.90) but increased significantly over the course of the 2-hour infusion (P = 0.01). However, multiple comparisons did not reveal a significant increase in MAP for any of the individual groups (SEDCON: P = 0.17; SED5FU: P = 0.26; EXCON: P = 0.19; EX5FU: P = 0.19).

Echocardiographic measurements

Echocardiographic measurements were conducted to assess how 5-FU may directly impact myocardial function and the potential cardioprotective role of exercise preconditioning. Group means for individual echocardiographic variables are reported in **Table 1**. There were no significant group × time interactions for any of the left ventricular volumes or parameters (all P > 0.05) (**Figure 2A-D**). Similarly, no significant main effects for group were detected (all P > 0.05). There was no significant main effect for time on EDV (P = 0.06) (**Figure 2B**) or SV (P = 0.96) (**Figure 2C**); however, ESV (P < 0.001) (**Figure 2A**) and FS (P < 0.001) (**Figure 2D**) did change significantly over the course of the 2-hour infusion. Post hoc analyses revealed a significant increase in ESV for SEDCON (P = 0.003), SED5FU (P = 0.002), and EX5FU (P = 0.002), but not for EXCON (P = 0.15), over the course of the 2-hour infusion (**Figure 2A**). Similarly, FS was reduced from BL to 2 hours in SEDCON (P < 0.001), SED5FU (P = 0.01), and EX5FU (P < 0.001), but did not reach statistical significance in EXCON (P = 0.05) (**Figure 2D**). However, it is important to note that the changes in FS remained within the normal physiological range for healthy rats [55-58, 61], and the absence of a treatment-dependent pattern suggests these effects may reflect time-dependent alterations related to prolonged anesthesia rather than true myocardial depression.

Aortic pulse wave velocity (PWV)

PWV was assessed to gain insight into the potential effects 5-FU may have on the vasculature. A significant group × time interaction was observed for PWV (P = 0.004). There were no significant differences in PWV between any of the groups at BL (all P > 0.05), however, SED5FU had a significantly greater PWV than SEDCON at the 2-hr time point (SED5FU: 452 ± 54 cm/s; SEDCON: 392 ± 64 cm/s; P = 0.03), indicating acute vascular dysfunction with 5-FU. No other group comparisons at the 2-hr timepoint reached statistical significance (all > 0.05). Additionally, PWV increased significantly from BL to 2-hr in SED5FU (BL: 396 ± 39 cm/s; 2-hr: 452 ± 54 cm/s; P < 0.001) but not in SEDCON (BL: 417 ± 55 cm/s; 2-hr: 392 ± 64 cm/s; P = 0.10). Rats that completed four days of moderate-intensity treadmill exercise before 5-FU or saline infusion showed no significant change in PWV (EXCON: BL: 408 ± 35 cm/s; 2-hr: 410 ± 46 cm/s; P = 0.86; EX5FU: BL: 398 ± 13 cm/s; 2-hr: 417 ± 23 cm/s; P = 0.24) over the same time period suggesting a protective effect of exercise preconditioning on 5-FU-induced increases in PWV (**Figure 3A**). When PWV was compared as a percent change from BL, SED5FU had a significantly greater change than SEDCON (SED5FU: 14.5 ± 10.9%; SEDCON: -5.3 ± 15.7%; P = 0.002) while no difference was observed between SED5FU and EXCON (0.72 ± 10.9%; P = 0.06) or EX5FU (4.87 ± 6.7%; P = 0.27) (**Figure 3B**). Taken together, these findings support the hypothesis that pre-treatment exercise protects against early 5-FU-induced vascular toxicity.

Discussion

The primary objective of this study was to determine whether acute exercise preconditioning is sufficient to prevent signs of cardiotoxicity in rats exposed to a clinically relevant 5-FU chemotherapy dosing regimen (i.e., bolus + infusion). In agreement with others, our findings suggest that acute 5-FU administration predominantly results in alterations in vascular function. While 5-FU caused no apparent changes in echocardiographic indices of left ventricular function, aortic PWV increased over the 2-hr infusion in SED5FU while remaining unchanged in all other groups. This increase resulted in significantly higher PWV at the 2-hr time point in SED5FU compared to SEDCON despite the absence of differences in key hemodynamic variables that can influence PWV (e.g., MAP, SV, Q, etc.). Notably, this increase in PWV was not observed in rats treated with 5-FU following 4 consecutive days of moderate-intensity treadmill running. We interpret these findings to suggest that vascular complications in rodents can arise following a single clinically relevant cycle of 5-FU (bolus + continuous infu-

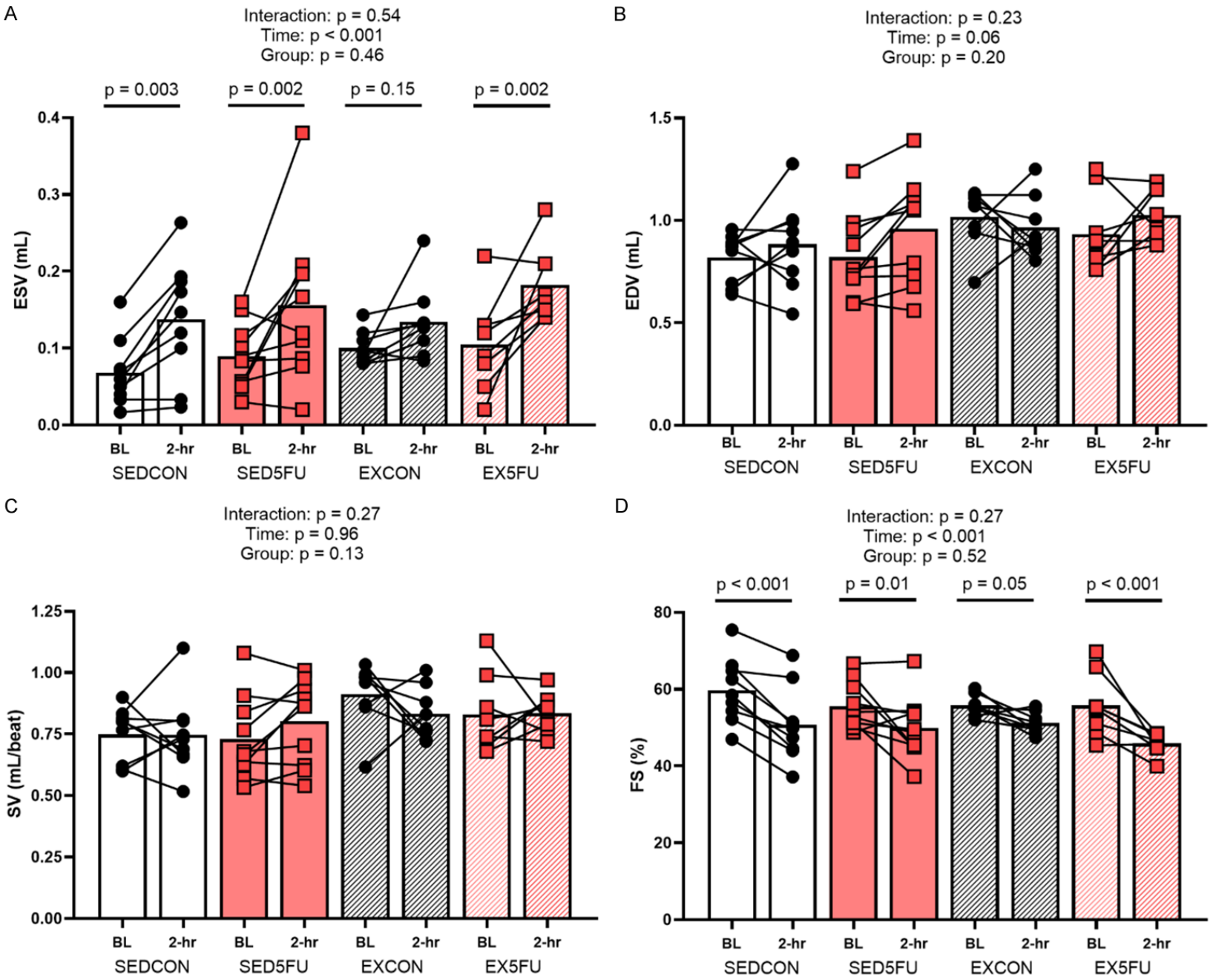
A
Interaction: p = 0.54
Time: p < 0.001
Group: p = 0.46
p = 0.003
p = 0.002
p = 0.15
p = 0.002
ESV (mL)
0.0
0.1
0.2
0.3
0.4
BL
2-hr
SEDCON
SED5FU
EXCON
EX5FU
B
Interaction: p = 0.23
Time: p = 0.06
Group: p = 0.20
EDV (mL)
0.0
0.5
1.0
1.5
C
Interaction: p = 0.27
Time: p = 0.96
Group: p = 0.13
SV (mL/beat)
0.00
0.25
0.50
0.75
1.00
1.25
D
Interaction: p = 0.27
Time: p < 0.001
Group: p = 0.52
p < 0.001
p = 0.01
p = 0.05
p < 0.001
FS (%)
0
20
40
60
80

Figure 2. Effect of 5-FU infusion on left ventricular variables. A. A significant main effect for time was present for ESV. There was a significant increase in ESV from BL to 2-hr in all groups aside from EXCON. B. There was no significant change over time or difference between the groups for EDV over the course of the infusion. C. There was no significant change over time or difference between the groups for SV over the course of the infusion. D. There was a significant effect of time on FS. FS was significantly reduced in all groups aside from EXCON over the course of the 2-hr infusion. SEDCON n = 9, SED5FU n = 10, EXCON n = 8, EX5FU = 8. Data analyzed using mixed effects model. Post hoc comparisons were completed using Šídák's multiple comparisons test when significant interactions or main effects were present. ESV = end systolic volume, EDV = end diastolic volume, SV = stroke volume, FS = fractional shortening.

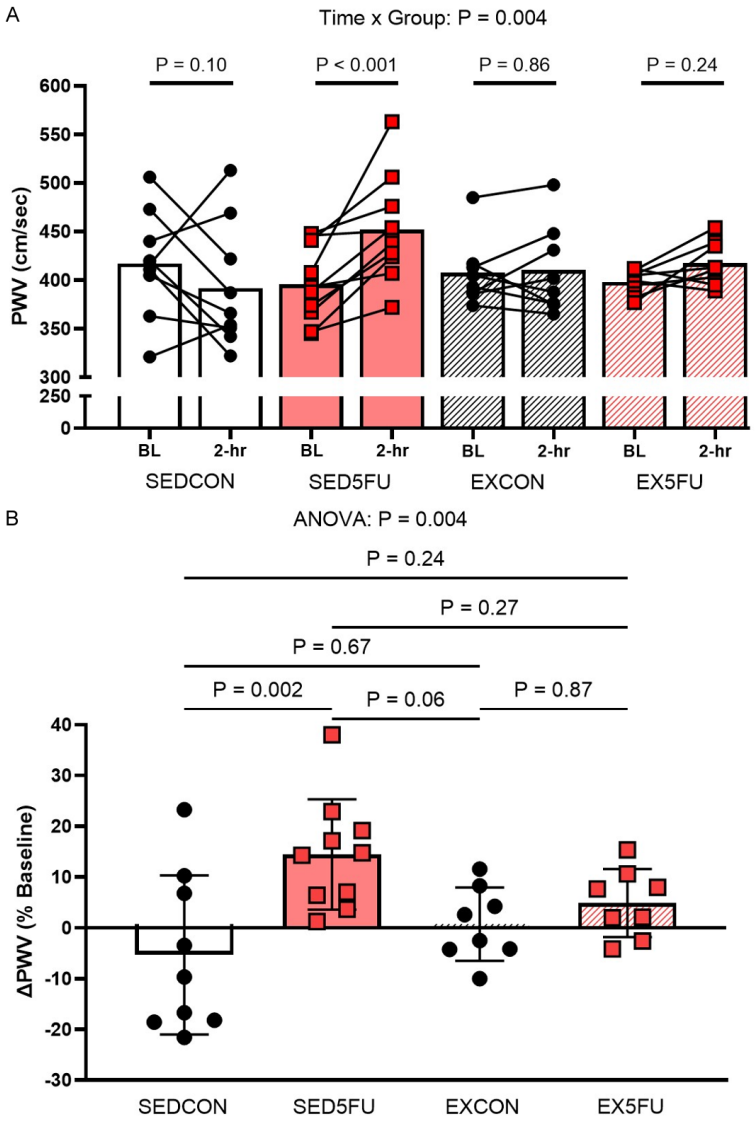

Figure 3. Effect of 5-FU infusion on aortic pulse wave velocity. A. PWV significantly increases from baseline (BL) to 2 hours (2-hr) in SED5FU but not in SEDCON, EXCON, or EX5FU, suggesting acute exercise may dampen the effect of 5FU on the vasculature. B. SED5FU had a significantly greater change in PWV from BL to the 2-hour time point than SEDCON. No other comparisons achieved statistical significance. Data were analyzed using a mixed effects model (post hoc comparisons made using Šídák's multiple comparisons test) (A) and one-way ANOVA (post hoc comparisons made using Tukey's multiple comparisons test) (B). SEDCON n = 9, SED5FU n = 10, EXCON n = 8, EX5FU = 8. PWV = pulse wave velocity.

sion) and that short-term aerobic preconditioning may be sufficient to prevent these adverse effects.

Despite continued advances in cancer care, 5-FU chemotherapy has remained a central component of numerous anticancer treatment regimens for over 60 years [62, 63]. However, a serious yet often underreported [64, 65] consequence of 5-FU is the onset of acute cardiotoxicities that arise during or shortly following cessation of treatment [5, 11, 66]. Heightened understanding of the mechanisms responsible for 5-FU cardiotoxicity coupled with identification of modalities to prevent its occurrence may help ensure patients can continue to receive 5-FU-based regimens without increased risk to cardiovascular health. While preclinical studies have played a significant role in growing the current understanding of how 5-FU impacts the cardiovascular system, prior works have exclusively employed IV bolus (20 sec. to 10 minutes) [20, 24, 26, 27, 67] or intraperitoneal [19, 21, 68, 69] dosing models. These models differ significantly from most common 5-FU-based regimens used in clinical practice where 5-FU is often delivered via IV bolus + continuous IV infusion over 46-72 hours [30-32, 70]. This prolonged exposure to 5-FU has been associated with greater treatment efficacy [71,

72] and heightened rates of cardiotoxicity compared to bolus injection alone [34]. Thus, we designed a preclinical dosing model incorporating a continuous infusion of 5-FU to align our study more closely with the delivery methods frequently used in clinical practice. While the present work was not specifically designed to assess the mechanisms of 5-FU cardiotoxicity, preclinical incorporation of a continuous infusion dosing model may provide a more translational approach to interrogate signaling pathways involved in the onset of 5-FU cardiotoxicity in the clinical setting.

In the present study, a single IV bolus + continuous infusion of 5-FU had no effect on left ventricular function compared to saline treatment. This is in agreement with the findings of Zhang and colleagues, who found no differences in FS between a control group and rats receiving 5 consecutive daily doses of either 25 or 50 mg/kg of 5-FU via intraperitoneal injection [21]. However, these authors did report changes in cardiac mitochondrial function following 5-FU treatment, suggesting 5-FU may be responsible for subclinical cardiac alterations that do not rise to the level of overt cardiac dysfunction. Reductions in left ventricular function following 5-FU treatment are seemingly rare in large-scale clinical studies but have been reported in several clinical case reports [8, 73, 74]. It is worth noting, however, that increases in ESV and reductions in FS were observed in each of our experimental groups over the course of the 2-hr infusion—with changes reaching statistical significance in 3 of 4 groups for both parameters. These alterations are seemingly independent of 5-FU and may be a consequence of prolonged use of pentobarbital anesthesia, as reductions in FS have been noted over the course of 3-hr of pentobarbital anesthesia in dogs [75]. Importantly, despite statistically significant reductions in FS, the values reported herein remain well above those seen in our rat model of heart failure (FS = ~20-26%) [55-58] and are consistent with previously published values in healthy rats [55-58, 61].

The most prevalent theory of 5-FU cardiotoxicity maintains that side effects (e.g., angina) are likely a consequence of a direct effect of 5-FU on the vasculature. In support of this notion, PWV was increased in SED5FU following the 2-hr infusion but remained unchanged in SEDCON, EXCON, and EX5FU. This change in PWV occurred without changes in hemodynamic variables known to influence PWV (e.g., MAP, Q, etc.). SED5FU also presented with significantly higher PWV at the 2-hr time point and as a change from BL compared to SEDCON. Notably, PWV increased from BL to the 2-hr time point in all 10 SED5FU animals (average increase of 14.5%). Our group has previously demonstrated a relationship between anticancer therapy and arterial stiffness [76, 77], as well as an association between an index of arterial stiffness and cardiovascular/cancer mortality in some cancer populations [78]. These data highlight the importance of PWV/arterial stiffness measurements throughout cancer survivorship. However, these measurements are typically made longitudinally (e.g., after completion of several chemotherapy cycles or in the years following treatment) rather than following a single chemotherapy cycle. To the best of our knowledge, others have yet to investigate changes in PWV following a single cycle of 5-FU. Visvikis and colleagues found a ~10% increase in the PWV of colorectal cancer patients after 6-12 cycles of 5-FU delivered via continuous IV infusion [17]. Interestingly, Groehs et al. found no significant change in PWV after 30 weekly bolus IV injections of 5-FU (Groehs et al. 2020) [79]. While the mechanisms responsible for 5-FU-induced vascular complications are not fully understood, they appear to involve a complex array of signaling events spanning the endothelial and smooth muscle layers. Our group and others have demonstrated impairments in endothelial function—an established contributor to increases in PWV and the onset of arterial stiffening [80]—in cancer patients following 5-FU treatment [14, 16]. In rodent models, acute inhibition of nitric oxide synthase is sufficient to increase PWV [81], suggesting that pathways involving nitric oxide signaling may contribute to the increased PWV seen in our study. Alternatively, preclinical studies have noted that 5-FU elicits a direct vasoconstrictor effect on aortic rings [28, 82] that seemingly involves endothelium-independent pathways involving protein kinase C [28]. Thus, future mechanistic studies aimed at illuminating the specific pathways in which 5-FU impairs vascular function remain warranted.

A critical novel finding of the present work was the protective effect of pre-treatment moder-

ate-intensity exercise on 5-FU-induced changes in PWV. Indeed, the significant increase in PWV—apparent in SED5FU over the 2-hr infusion—was not reciprocated in EX5FU. Similarly, the absolute PWV was not different between EX5FU and SEDCON at the 2-hr time point, nor were there differences between the groups when comparing the change in PWV from BL. We interpret these findings to suggest that 4 bouts of exercise preconditioning may induce stimuli capable of preventing the adverse vascular consequences of 5-FU treatment. Acute exercise preconditioning has previously been demonstrated to prevent cardiotoxicity induced by anthracycline chemotherapy in rodents [45, 46, 48] and human patients [83] with a specific focus on left ventricular function. To our knowledge, like studies have yet to be completed prior to 5-FU treatment. Using a long-term (4-8 weeks) exercise preconditioning model, Hayward and colleagues demonstrated that aortic rings isolated from exercise trained rats (20-25 m/min, 5 d/w, 8-wks) exhibit improved acetylcholine-mediated dilation following preconstriction with 5-FU [82]. The authors posit this improvement in endothelium-dependent vasodilation may be related to increased aortic content of endothelial NO synthase [82]. While the findings of Hayward and colleagues indicate the potential benefits of long-term exercise preconditioning prior to 5-FU exposure, implementation of such regimens (i.e., 4-8 weeks) may not be feasible in the time between diagnosis and treatment onset - especially considering that minimization of this timeframe is critical for cancer survival outcomes [84, 85]. In a non-cancer cohort of patients experiencing rest angina, Morikawa and colleagues found that three days of aerobic interval training improved endothelial function and reduced the incidence of coronary spastic angina [43]. Given that 5-FU cardiotoxicity most frequently presents as angina during or shortly following the first cycle of treatment [5, 34], these findings underscore the potential benefit of acute exercise for preventing the primary symptom of 5-FU cardiotoxicity. Though the risk for cardiovascular events in the months following 5-FU completion is seemingly low [86], this is not to say that additional exercise prescription of longer durations following treatment completion would not be beneficial. Courneya and colleagues recently found significant reductions in relative risk for disease recurrence, new primary cancer, and disease-free survival in colorectal cancer patients who participated in a three-year exercise training program initiated following completion of adjuvant chemotherapy containing 5-FU (or its oral prodrug Capecitabine) [87]. While cardiovascular outcomes were not reported, these findings signify the benefit post treatment exercise can have on overall cancer survival. Future work focused on implementation of individualized exercise prescriptions that prevent adverse cardiovascular effects of the specific treatment while simultaneously improving cancer survival outcomes are warranted and have the potential to greatly improve quality of life across the cancer survivorship continuum.

Experimental considerations

Several experimental considerations are pertinent to interpreting the present study's findings. First, a key component of our work included using a novel pre-clinical dosing regimen designed to reflect 5-FU delivery methods used in clinical practice. To perform this protocol, animals remained under pentobarbital anesthesia for approximately 150-180 minutes. Such prolonged periods of pentobarbital anesthesia can result in alterations in cardiac parameters (as discussed above) that should be considered in the interpretation of our findings. However, pentobarbital has been demonstrated to have less impact on cardiovascular function than other commonly used anesthetics (e.g., isoflurane, ketamine/xylazine) [88]. Additionally, anesthetic use was the same across all groups to minimize the potential effect of anesthesia on group comparisons. We cannot fully discount a potential interaction between pentobarbital sodium and 5-FU; however, since each animal was treated the same, and there were no differences in MAP or HR across groups (**Table 1**), the use of anesthesia likely had minimal impact on the PWV results herein. Future work could consider use of both implantable infusion pumps and/or telemetry devices that would allow for continuous infusion and measurement of cardiovascular parameters under minimal anesthesia. Second, only male animals were used in the present study. While large-scale studies have found no difference in the incidence of 5-FU cardiotoxicity between men and women [3, 7], the onset of

general cardiac and vascular pathology often differs between sexes. For example, women may have slightly higher angina incidence rates than men across the lifespan [89, 90] and menopausal transition can contribute to vasomotor changes and elevate cardiovascular risk [91]. Additionally, emerging evidence suggests potential differences in both molecular signaling and adaption to exercise in men and women [92]. This is an important and emerging topic of research that highlights the critical need to consider biological sex in future exercise oncology research. Future studies assessing the ability of exercise to mitigate 5-FU cardiotoxicity would benefit from the inclusion of both male and female rats to identify potential differences to either 5-FU treatment or the efficacy of exercise preconditioning. All rats used in the present study were free of cancer, as is common in most studies of chemotherapy-induced cardiotoxicity [19, 21, 24, 26, 27, 45-47]. While tumor naïve rodent models are key to isolate the effects of chemotherapy from those of the cancer itself, recent evidence suggests that the cancer alone [51] or in concert with various treatments [93] may increase the risk of future cardiovascular disease and mortality. Preclinical models also indicate that the tumor may contribute to cardiovascular pathology independent of cancer treatment [49, 50, 52]. Though it is possible that the underlying mechanisms of 5-FU cardiotoxicity may include complex interactions between 5-FU and the cancer itself, 5-FU has an established direct vasoconstrictor effect on isolated aortic rings [28, 82], and the acute nature of the toxicity, coupled with its disappearance upon cessation of 5-FU treatment suggest a direct effect of the drug independent of the tumor is likely. Nonetheless, inclusion of tumor-bearing animals in future studies may help illuminate a potential role of the tumor and further improve the translatability of preclinical models of 5-FU cardiotoxicity. Similarly, 5-FU is regularly delivered alongside several other chemotherapy drugs (e.g., leucovorin, oxaliplatin, bevacizumab, etc.) to improve cancer treatment efficacy. Some of these drugs have been suggested to increase the incidence of 5-FU-induced cardiotoxicity [33, 94]. While the focus of the current study was to understand the impact of 5-FU alone, future works may seek to include additional treatments to better understand the cardiovascular effects of specific 5-FU-based regimens (e.g., FOLFOX, FOLFIRINOX).

Conclusion

The present work sought to determine whether acute exercise preconditioning could prevent cardiotoxicity caused by a clinically relevant dose of 5-FU chemotherapy. Our findings demonstrate that a single cycle of 5-FU increases PWV (a clinical marker of aortic stiffness) but does not cause overt changes in left ventricular function in male rats. Importantly, exercise preconditioning was sufficient to prevent the 5-FU-induced increase in PWV. These findings indicate regimented moderate-intensity exercise in the days immediately before 5-FU treatment may mitigate the onset of 5-FU cardiotoxicity. Future studies designed to assess the mechanisms of 5-FU cardiotoxicity as well as the efficacy and feasibility of exercise preconditioning programs in human cancer patients remain critical to ensuring patients can continue to receive this effective treatment without detrimental effects to cardiovascular well-being.

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Disclosure of conflict of interest

None.

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