Measuring in vivo Effects of Chemotherapy Treatment on Cardiac Capillary Permeability


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Abstract — Background: Cardiotoxicity is a life-threatening side effect of chemotherapy that is multi-factorial in nature. Increased capillary permeability due to endothelial damage after anthracycline administration could be an important contributor to cardiac dysfunction. Methods: We investigated the cardiotoxic effects of the anthracycline doxorubicin (DOX) in rats using intraperitoneal injections of saline (n=10) or DOX (n=10) over 12 days for a cumulative dose of 18 mg/kg. We studied cardiotoxicity by serial echocardiography and with an isolated heart setup. We monitored perfusion and left ventricle pressures and measured permeability using a fluorescent indicator dilution method developed by our group. Results: There were significant differences (p=0.029) in permeability surface area product between control (0.047±0.009 cm$^3$/s) and DOX animals (0.068±0.025 cm$^3$/s). This is consistent with our hypothesis that chemotherapy-induced changes in the coronary capillary endothelium lead to increased permeability. We also observed changes in cardiac function consistent with chemotherapy-induced cardiotoxicity. Contractility (+dP/dt) and LVDP were significantly reduced (p = 0.030) in the DOX group. Average +dP/dt was 2465±183 mm Hg/s for controls vs. 1817±177 mm Hg/s for DOX-treated rats, and average LVDP was 92.4 mm Hg for controls vs. 78.7 mm Hg for DOX-treated rats. Fractional shortening (FS) echocardiographic measurements decreased significantly over the course of treatment for the DOX group (p = 0.028, end FS = 32.8%, n=5), but not for the control group (p = 0.209, end FS = 50.7%, n=5). Conclusion: Changes in permeability after chemotherapy treatment can be detected using a fluorescent indicator dilution method. These changes are consistent with other measures of cardiac function observed in the chemotherapy group. Efforts toward the development of chemotherapy drugs with reduced cardiotoxicity should consider the effect on the endothelial layer, and our method to measure permeability in an isolated heart setup could be useful during testing of new drug alternatives.

Keywords — Permeability, chemotherapy, isolated heart, fluorescent indicator dilution, cardiotoxicity.

I. BACKGROUND

Cancer is a major public health problem in the United States and it is the second cause of death after heart disease. In 2009 the American Cancer Society predicted that almost 1.5 million people would be diagnosed with cancer [1]. The 5-year relative survival rate for people diagnosed with cancer, based on data collected between 1996 and 2004, is 66% [1]. The fact that current diagnostics and therapeutics allow higher survival rates highlights the importance of developing chemotherapy approaches with reduced side effects. Anthracyclines are common agents used in cancer treatment. This family of drugs is very effective in causing tumor regression, but their long-term use is seriously limited by their systemic toxicity [2,3]. Cardiac toxic effects are particularly worrisome because they can have a very significant long-term impact on functional level and quality of life. Research efforts geared towards anthracycline derivatives or modified formulations with reduced cardiotoxic effects could have a major impact on the prognosis and quality of life of patients diagnosed with cancer.

Anthracyclines such as doxorubicin (DOX) damage myocardial tissue through the production of reactive radical species [4,5]. The damaging action results in fibrosis, degeneration of myocardial cells, cardiac dilatation, reduced contractile function and interstitial edema [6,7]. Although most studies of the effect of chemotherapy on the heart focus on cardiac function measures, Wolf et al have determined that DOX also causes endothelial dysfunction in vitro [8], and that capillary permeability changes could be a contributing factor to the development of myocardial edema and dysfunction after chemotherapy. To our knowledge, there are no in vivo studies of the effect of chemotherapy on cardiac capillary permeability.

The objective of this project was to study changes in cardiac capillary permeability in a short-term rat model of cardiotoxicity, along with more traditional measurements of cardiac function that are well established in the literature. This would allow us to determine whether cardiac capillary permeability is a variable of interest for in vivo testing of drug cardiotoxicity, and whether significant changes in permeability can be measured using a fluorescent indicator dilution method developed by our group. One of the classical ways to measure permeability of an organ or tissue is the multiple indicator dilution protocol. It typically uses radioactive tracers, but the fluorescent version is based on the same principles and avoids the use of radioactive materials. Two tracers are simultaneously introduced into the circulation. One of the tracers remains intravascular after injection and the second tracer can cross the capillary wall and will diffuse out of the capillary [9]. The output curves versus
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The researcher can then calculate, among other parameters, the permeability-surface area product of the tissue using the Crone-Renkin equation. This expression relates the maximum extraction of the diffusible tracer ($E_{\text{max}}$) and the perfusate flow rate ($F$) to the permeability-surface area product (PSP) as shown in (2):

$$PSP = -F \star \ln(1 - E_{\text{max}}) \quad (2)$$

II. METHODS

In order to study the cardiotoxic effects of anthracyclines in a rat model we randomly assigned animals to receive intraperitoneal injections of either saline or doxorubicin solution over a period of 12 days. Each rat received a 3 mg/kg dose of their assigned treatment on days 1, 3, 5, 7, 9, and 11, for a cumulative dose of 18 mg/kg. Cardiotoxicity was studied by (1) serial echocardiography at baseline and on day 11, (2) pressure measurements in a Langendorff isolated perfused heart setup on day 12, and (3) cardiac capillary permeability measurements with fluorescent indicator dilution technique in a perfused heart setup on day 12.

A. Langendorff Isolated Rat Heart Setup

The Langendorff isolated perfused rat heart preparation was used to examine cardiac function and capillary permeability after in vivo treatment ($n=10$ per group). The project was approved by the FIU IACUC. On day 12 of treatment, each rat was anesthetized with a 50 mg/kg i.p. injection of pentobarbital. The heart was surgically excised, placed in ice cold Krebs-Henseleit (KH) buffer, and quickly cannulated to a Langendorff setup. The heart was attached to a cannula by the aortic root and perfused retrogradely according to the Langendorff technique, with KH equilibrated to 95% O$_2$ and 5% CO$_2$ at 37°C, and artificially paced at approximately 300 bpm using a Harvard Apparatus Stimulator P.

The flow was adjusted to a perfusion pressure between 50 and 75 mmHg, measured with a fluid-filled catheter connected to a Statham strain gauge pressure transducer. A water-filled latex balloon was inserted through the mitral valve into the left ventricle, and connected through a fluid filled catheter to a Statham strain gauge pressure transducer to monitor left ventricular cardiac function (left ventricular pressure waveforms). All waveforms (aortic pressure and left ventricular pressure) were recorded by computer. The balloon was inflated to a left ventricular end diastolic pressure of approximately 5 mmHg.

The heart was allowed to stabilize for about 30 minutes with the perfusion flow rate adjusted to maintain the perfusion pressure between 50 and 75 mm Hg. This stabilization period ensures the acclimation of the heart to the setup, as well as the clearance of any anesthesia residue from the tissue. After the stabilization period, we started recording pressure waveforms, and the capillary membrane permeability was measured using the fluorescent indicator dilution technique.

We performed permeability measurements in three replicates separated by 15 minutes. The vascular tracer, which does not diffuse through the capillary wall, was Texas-Red conjugated dextran (TR, 70,000 MW; 1.56 mg/mL). The diffusible tracer was sodium fluorescein (NaFL, 376 MW; 1.56 μg/mL). For each replicate, we injected 25 μL of an equivolume dye mixture above the aortic cannula (12.5 μL each of TR and NaFL), and collected output samples for 45 seconds. The fluorescent intensities of the samples were measured using a Fluorolog-3 spectrofluorometer (Horiba Jobin Yvon) with characteristic excitation/emission wavelengths of 485/515 nm for NaFL, and 590/630 nm for TR.

To analyze permeability and pressure data, measurements were imported into Matlab and processed with custom-made algorithms created by our group [10]. Calculated variables included (1) dP/dt curves, dP/dt max, dP/dt min, heart rate, and left ventricular developed pressure (LVDP) for the pressure data; and (2) h(t) curves, extraction curves, and PSP values for the permeability data.

B. Serial Echocardiography

Serial echocardiography images were obtained on day 1 and day 11 of treatment ($n=5$ per group) using a Hewlett-Packard Sonos 2500® echocardiography machine equipped with a pediatric 5.5/7.5 MHz transducer selecting the 7.5 MHz mode. Although a higher frequency such as 12 MHz would be desirable to improve axial and lateral resolution given the small size of the rodent heart, the literature [11] and our results show that the 7.5 MHz transducer provides reasonably accurate M-mode images that can be used to monitor changes in heart function over time when the measuring technique is kept consistent. The animal was anesthetized using isoflurane through an inhalation cone with concurrent oxygen flow. Once anesthetized, the rat was placed in the left lateral decubitus position, the chest was shaved and cleaned with isopropyl alcohol, and we applied ultrasound gel for transducer/skin coupling.

Long-axis B-mode images were obtained from the left parasternal windows. From these B-mode images, we acquired M-mode images by taking a B-mode image section...