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Inhibition of COX1/2 alters the host response and reduces ECM scaffold mediated constructive tissue remodeling in a rodent model of skeletal muscle injury

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A B S T R A C T

Extracellular matrix (ECM) has been used as a biologic scaffold material to both reinforce the surgical repair of soft tissue and serve as an inductive template to promote a constructive tissue remodeling response. Success of such an approach is dependent on macrophage-mediated degradation and remodeling of the biologic scaffold. Macrophage phenotype during these processes is a predictive factor of the eventual remodeling outcome. ECM scaffolds have been shown to promote an anti-inflammatory or M2-like macrophage phenotype *in vitro* that includes secretion of downstream products of cycolooxygenases 1 and 2 (COX1/2). The present study investigated the effect of a common COX1/2 inhibitor (Aspirin) on macrophage phenotype and tissue remodeling in a rodent model of ECM scaffold treated skeletal muscle injury. Inhibition of COX1/2 reduced the constructive remodeling response by hindering myogenesis and collagen deposition in the defect area. The inhibited response was correlated with a reduction in M2-like macrophages in the defect area. The effects of Aspirin on macrophage phenotype were corroborated using an established *in vitro* macrophage model which showed a reduction in both ECM induced prostaglandin secretion and expression of a marker of M2-like macrophages (CD206). These results raise questions regarding the common peri-surgical administration of COX1/2 inhibitors when biologic scaffold materials are used to facilitate muscle repair/regeneration.

Statement of significance

COX1/2 inhibitors such as nonsteroidal anti-inflammatory drugs (NSAIDs) are routinely administered post-surgically for analgesic purposes. While COX1/2 inhibitors are important in pain management, they have also been shown to delay or diminish the healing process, which calls to question their clinical use for treating musculotendinous injuries. The present study aimed to investigate the influence of a common NSAID, Aspirin, on the constructive remodeling response mediated by an ECM scaffold (UBM) in a rat skeletal muscle injury model.
The COX1/2 inhibitor, Aspirin, was found to mitigate the ECM scaffold-mediated constructive remodeling response both in an *in vitro* co-culture system and an *in vivo* rat model of skeletal muscle injury. The results presented herein provide data showing that NSAIDs may significantly alter tissue remodeling outcomes when a biomaterial is used in a regenerative medicine/tissue engineering application. Thus, the decision to prescribe NSAIDs to manage the symptoms of inflammation post-ECM scaffold implantation should be carefully considered.

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1. Introduction

Biologic scaffolds composed of ECM have been widely used to reinforce the surgical repair of soft tissue defects and to mediate an improved or constructive remodeling outcome [1–6]. While the clinical applications of ECM scaffolds are quite diverse and constantly expanding, skeletal muscle reinforcement (e.g. hernia repair and volumetric muscle loss) remains one of the most prevalent clinical applications for these materials [2,3]. When placed at the site of injury, ECM scaffolds orchestrate a complex host response that includes the recruitment of endogenous cells, such as immune cells and stem/progenitor cells [7–10]. Degradation of the scaffold by infiltrating host cells releases a variety of bioactive molecules that drive neovascularization, innervation, and site appropriate tissue formation [11–14].

One important feature of ECM scaffolds during the remodeling process is their ability to modulate macrophage phenotype. ECM scaffolds from a variety of source tissues promote an M2-like bias ($CD163^{high}$, $CD206^{high}$, $CD86^{low}$, $CCR7^{low}$) in the infiltrating macrophage population [9,15]. This bias has been shown to be a determinant factor in a favorable tissue remodeling outcome [9,10]. While a complete characterization of macrophage phenotype during tissue remodeling has yet to be completed, several studies have begun to describe this M2-like phenotype [16,17].

Recently, an enzymatically digested ECM scaffold derived from porcine urinary bladder (urinary bladder matrix, UBM) was found to up-regulate prostaglandin-E2 (PGE2) and prostaglandin-F2α (PGF2α) secretion in macrophages as part of a larger change in the overall macrophage phenotype [18]. Prostaglandin production requires the cyclooxygenase enzymes COX1 (constitutively expressed) and COX2 (inducibly expressed) [19]. Several studies have shown that COX2 knockout macrophages do not become fully M2 polarized and assume an M1-like phenotype [20,21]. Moreover, while prostaglandins can enhance the inflammatory response and pain states, these molecules are important mediators of tissue repair particularly in the context of skeletal muscle [22–24]. Collectively, these observations imply a potentially important role for COX1/2 in ECM-mediated macrophage polarization, and ultimately in constructive remodeling of ECM scaffolds.

COX1/2 inhibitors such as nonsteroidal anti-inflammatory drugs (NSAIDs) are typically available over-the-counter and taken for pain relief, and are routinely administered post-surgically, primarily for anti-inflammatory and analgesic purposes [25]. While COX1/2 inhibitors are important in pain management, they have also been shown to delay or diminish the healing process, including macrophage accumulation; leading some to question their clinical use in treating musculotendinous injuries [26–34]. The effect of administration of NSAIDs upon ECM scaffold remodeling is unknown. The purpose of the present study was to determine the effect of a common NSAID, Aspirin, on the constructive remodeling response mediated by an ECM scaffold (UBM) in a rat skeletal muscle injury model.

2. Materials and methods

2.1. Overview of experimental design

An established rodent skeletal muscle injury model was used to evaluate the effect of the COX1/2 inhibitor, Aspirin, on the ECM scaffold mediated constructive remodeling response [35,36]. Briefly, 3 days prior to the surgical procedure, animals were randomly assigned to either the Aspirin treated (3 mg/mL Aspirin in drinking water) or control (vehicle) group. Bilateral 1.5 cm $\times$ 1.5 cm partial thickness defects were created in the abdominal musculature. A size-matched pre-cast UBM hydrogel and an overlying 2 $\times$ 2 cm single layer sheet of UBM was then placed in the muscle defect area. The remodeling response was evaluated following 3, 7, 14, and 35 days by quantitative histomorphologic metrics [37,38], including characterization of macrophage phenotype and neo tissue deposition.

Established *in vitro* models were subsequently used to further interrogate the effect of Aspirin on ECM scaffold mediated macrophage function/polarization and myogenesis. *In vitro* macrophage function and polarization was characterized by quantification of secreted factor production and cell surface marker expression, respectively. *In vitro* myogenesis was characterized by an objective image analysis approach which quantified key events of myogenesis, such as the formation of multinucleated myotubes and myonuclear accretion.

2.2. Reagents

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO) unless otherwise specified. All cell culture supplies were purchased from Life Technologies (Carlsbad, CA) unless otherwise specified. All chemicals used in this study were molecular biology grade or cell culture grade where appropriate.

2.3. Urinary bladder matrix preparation

Porcine urinary bladders from market weight animals were acquired from Tissue Source, LLC. (Lafayette, Indiana). The ECM prepared from this tissue and referred to as UBM was prepared as previously described [39]. Briefly, the tunica serosa, tunica muscularis externa, tunica submucosa, and tunica muscularis mucosa were mechanically removed. The luminal urothelial cells of the tunica mucosa were dissociated by washing with sterile water. The remaining tissue consisting of basement membrane and subjacent tunica propria of the tunica mucosa was decellularized by agitation in 0.1% peracetic acid with 4% ethanol for 2 h at 300 rpm. The tissue was then extensively rinsed with phosphate-buffered saline (PBS) and sterile water. The UBM was then lyophilized into a dry sheet and used as such, where appropriate, or milled into particulates using a Wiley Mill with a #60 mesh screen [40].

2.4. Pepsin mediated ECM solubilization and hydrogel formation

UBM was enzymatically digested with pepsin as described [41]. Milled UBM particulates (10 mg/mL) and pepsin (1 mg/mL) were placed in 0.01 M HCl (pH 2.0, sterile filtered) and stirred at room temperature for 48 h. The thick slurry was then neutralized to a pH of 7.4 in sterile 1$\times$ PBS (137 mM NaCl, 2.7 mM KCl, 12 mM Phosphate, Fisher Scientific, Waltham, MA) to inactivate the pepsin. A solution of pepsin (1 mg/mL) in 0.01 M HCl, treated in the same fashion as the UBM sample, served as the control condition for all experiments. All steps were conducted under sterile conditions with sterile filtered solutions. To form hydrogels, the neutralized slurry was placed in a 1.4 $\times$ 1.4 $\times$ 0.5 cm plastic mold and incubated at 37 °C for 30 min. For cell culture experiments, the solid UBM hydrogel was broken into smaller pieces with vigorous agitation and pipetting. The subsequent slurry was then added directly to cells. For animal studies, the UBM hydrogel was removed from the mold and placed directly into the defect site.

2.5. In vivo study

Female Sprague Dawley rats (350–400 g at implantation) were purchased from Harlan Laboratories. Rats were housed on a 12 h light–dark cycle and fed standard laboratory chow and water ad libitum. All animal procedures were approved by The University of Pittsburgh Institutional Animal Care and Use Committee (IACUC).

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