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Mast cell activation and degranulation in acute artery injury: a target for post-operative therapy

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Abstract

The increasing incidence of cardiovascular disease (CVD) has led to a significant ongoing need to address this surgically through coronary arterial bypass grafting (CABG) and percutaneous coronary interventions (PCI). From this, there continues to be a substantial burden of mortality and morbidity due to complications arising from endothelial damage, resulting in restenosis. Whilst mast cells (MC) have been shown to have a causative role in atherosclerosis and other vascular diseases, including restenosis due to vein engraftment, here we demonstrate their rapid response to arterial wire injury, recapitulating the endothelial damage seen in PCI procedures. Using wild-type mice, we demonstrate accumulation of MC in the femoral artery post-acute wire injury, with rapid activation and degranulation, resulting in neointimal hyperplasia, which was not observed in MC deficient $Kit^{W-sh/W-sh}$ mice. Furthermore, neutrophils, macrophages and T cells were abundant in the wild-type mice area of injury but reduced in the $Kit^{W-sh/W-sh}$ mice. Following bone marrow derived MC (BMMC) transplantation into $Kit^{W-sh/W-sh}$ mice, not only was the neointimal hyperplasia induced, but the neutrophil, macrophage and T cell populations were also present in these transplanted mice. To demonstrate the utility of MC as a target for therapy, we administered

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Author Contributions:
Harper, involved in analyzing and interpreting the data. Conceiving, drafting and revising the manuscript. *Fang*, designed, collected and analyzed the data. Yang, assisted in methodology and manuscript revision. *St Hilaire, Negro, Chen, Yu, Schwartzbeck, Walts, Kovacic* assisted in methodology and data acquisition. *San* performed animal surgery and ensured welfare of animals. *Dmitrieva* assisted in revision writing. Boehm conceived, designed the experiments, and revised the manuscript.

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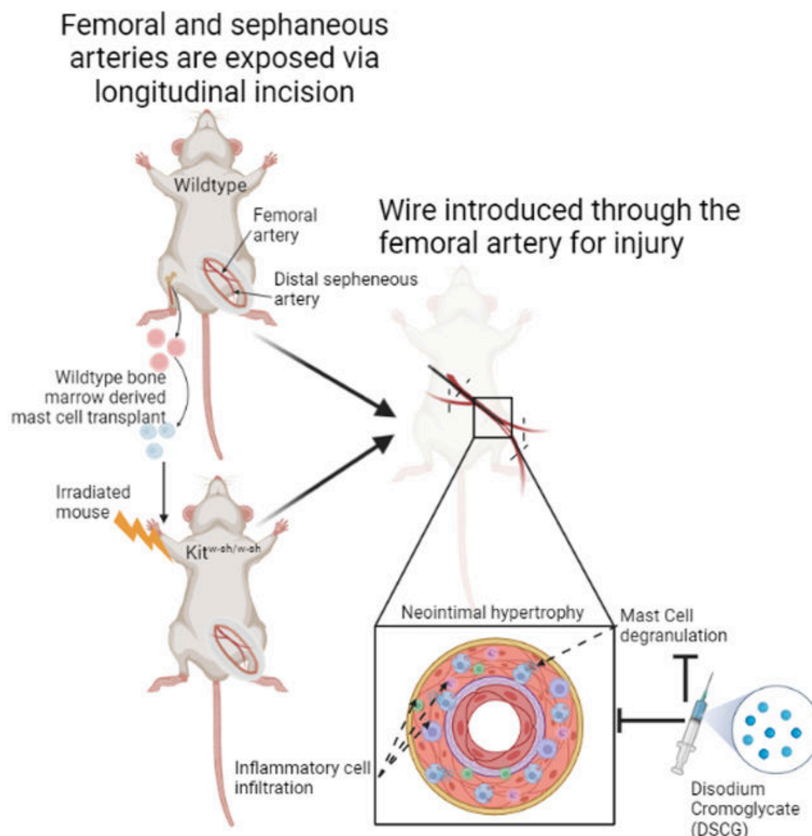
The authors declare no conflicts of interest.

the MC stabilizing drug, disodium cromoglycate (DSCG) immediately following arterial injury and were able to show a reduction in neointimal hyperplasia in wild-type mice. These studies suggest a critical role for MC in inducing the conditions and coordinating the detrimental inflammatory response seen post endothelial injury in arteries undergoing revascularization procedures, and by targeting the rapid MC degranulation immediately post-surgery with DSCG, this restenosis may become a preventable clinical complication.

Graphical Abstract



Acute arterial wire injury in mice triggers an inflammatory cascade mediated through mast cell degranulation resulting in inflammatory cell infiltration and ultimately, neointimal hypertrophy of the artery. Disodium cromoglycate (DSCG) inhibits mast cell degranulation, resulting in decreased inflammatory cell infiltration and reduced neointimal hypertrophy, demonstrating that DSCG may be a good candidate as a therapeutic following arterial surgical intervention.

Keywords

Restenosis; revascularization; blood vessel; mast cell; neointima hyperplasia; cardiovascular disease; acute arterial injury; coronary arterial disease; percutaneous coronary intervention

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Introduction:

As cardiovascular disease (CVD) incidence rises, so does the need for surgical intervention. Accounting for 32% of global deaths, 85% of these are due to stroke or heart attack, with over three quarters taking place in low or middle-income countries.[1] With the development of coronary arterial bypass grafting (CABG) and percutaneous coronary interventions (PCI), there has been a significant reduction in deaths related to coronary artery disease (CAD).[2] However, despite these advances in revascularization interventions, CAD is the leading cause of morbidity and mortality in high-income countries, with peripheral artery disease (PAD) as the third leading cause of disease morbidity.[3,4] Peripheral artery disease is present in at least 20% of people 50yrs and older, and is not only a global health burden in itself, it's becoming a serious risk factor for those receiving revascularization intervention.[5] Adding to this, half of those diagnosed with PAD are asymptomatic, undermining the accuracy of pre-operative assessment and when present, it has been shown to have a significantly poorer prognosis. [6,7] Limb ischemia has specifically been linked to adversely affecting vascular remodeling in regions of coronary and cerebral arteries and has been associated with endothelial dysfunction. [5,8–10]

Damage to the endothelium during revascularization interventions has been shown to trigger inflammatory processes that are linked to excessive smooth muscle cell (SMC) proliferation, specifically in the medial layer of the artery resulting in neointima hyperplasia and ultimately, restenosis of the vessel.[11] Restenosis is the result of increased smooth muscle cell proliferation, causing hyperplasia in the media layer of the arterial wall. It's defined as a reduction in the luminal diameter of a blood vessel, usually greater than 50%.[12] PCI, with or without stent implantation is the precipitating event that causes restenosis, leading to increased morbidity and mortality as a direct result of these procedures. Indeed, it has been shown that PCI has significantly increases cardiac and non-cardiac related adverse events compared to CABG and other cardiovascular interventions.[13] Restenosis follows a distinct timeline of progression post vascular intervention, starting with an early elastic recoil, followed by an acute onset of vascular remodeling resulting in neointima hyperplasia. Thus, by identifying the causative molecular agents in this process it is possible to develop a post-procedure therapy to prevent the onset. The success of this strategy has been seen in the wide use of post-operative therapies for other cardiac surgeries such as CABG, which have greatly reduced morbidity and mortality in this cohort.[5]

The importance of mast cells (MC) in cardiovascular disease progression was identified as early as 1954 when these cells were found in atherosclerotic plaques[14], and subsequent studies correlated the number of MC in these plaques with increased plaque rupture and disease progression.[15,16] Furthermore, there is also evidence of MC accumulation and degranulation in areas of heart ischemia and reperfusion.[17]Mast cells play a pivotal role in early/ acute inflammatory events, and are known to play a role in neointima hyperplasia.[18–20] Derived from progenitors, MC mature in tissue, generally residing near blood vessels, nerves, airways, glands, gastrointestinal tract or just below epithelium.[20] They are usually a heterogeneous population of cells, adapting to the microenvironment of where they develop and mature.[21] The strategic location of MC allows them to migrate, expand locally or secrete cytokines and chemokines to coordinate the immune system through

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early recruitment and activation of other immune cells, in a rapid response to invading pathogens or tissue injury. A wide range of stimuli can activate MC, and the type of mediators that are released is stimuli dependent.[22–24] The well-defined activation pathway for MC is the crosslinking of IgE, bound to the IgE receptor, FcεRI, whereas MC are also known to be activated by complement 3a and 5a via toll-like receptors (TLR) and other cytokines and chemokines.[21,25] In addition, Mas-related G protein-coupled receptor member X2 (MRGPRX2) was recently discovered as a key receptor mediating IgE-independent MC degranulation.[26] Mast cells regulate both the innate and adaptive immune responses and are important in maintaining many physiological functions as well pathophysiological manifestations such as autoimmune diseases, cancer and diabetes.[27,28] Specifically, MC are involved in angiogenesis, enhancing this process through secreting many pro-angiogenic factors such as VEGF, bFGF, TGF-β, TNF-α and IL-8, heparin and other proteases, as well as inducing vascular permeability through the release of histamine.[28,29] Activation of MC leads to degranulation, which is the process of exocytosis or release of granules into the surrounding tissue. The components of the secreted mediators and timing of release is dependent on what the activating stimuli is.[23] The effect of these mediators on the surrounding tissue and nearby cells has been the focus of many studies, however the role of MC in neointima hyperplasia following acute arterial injury is largely unknown. Herein, we have shown that MC activation and degranulation occurs immediately post arterial injury, controlling the resulting inflammatory milieu and smooth muscle cell hyperplasia in a mouse arterial wire injury model.

Advances in post-operative pharmaceutical treatments such as statins, angiotensin converting enzyme (ACE) inhibitors, antihypertensives and beta-blockers have significantly reduced deaths, however these therapies are mostly aimed at reducing symptoms arising from the physiological changes that can occur post revascularization surgery.[5] Through identifying MC are key mediators of post arterial injury restenosis, we assessed the utility of a MC stabilizer, disodium cromoglycate (DSGC) in preventing neointima hyperplasia post arterial wire injury in mice. We found that by targeting this process, we were successful in reducing restenosis, implicating that this may be an effective peri- or post-operative treatment.

Methods:

Mice

All animal work was performed in compliance with the guidelines and approved by the National Heart, Lung, and Blood Institute (NHLBI), Animal Care and Use Committee, National Institutes of Health (NIH). We used female C57BL/6 (Wild-type), GFP and MC-deficient ($kit^{W\text{-}sh/W\text{-}sh}$) mice, which were all originally obtained from Jackson Laboratories. All animals were anesthetized using ketamine HCI at 90–120 mg/kg + xylazine 5–10 mg/kg intraperitoneal (IP); Avertin at 0.015–0.017ml/g bodyweight of freshly prepared 2.5% solution (IP); 1–3% isoflurane administered through a nose cone; or Pentobarbital 80 mg/kg via IP when appropriate.

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Arterial wire Injury, vessel fixation, and vessel harvest (Adopted from Roque, M et al, 2000)[33]

Mice underwent femoral artery wire injury at 12–16 weeks of age, resulting in complete endothelial denudation as previously described.[34–36] Briefly, the femoral artery and saphenous artery of the animal were exposed via a longitudinal incision (2.5 cm) on the anterolateral surface of the left and right legs. The distal saphenous artery was tied off with a 6–0 silk suture. Using a 27 gauge needle the proximal artery was punctured, and a 0.010 inch flexible wire passed into the saphenous artery by introducing the wire through the femoral artery punctured to a depth of approximately 1.5 cm. The wire was pulled and pushed 3 times to denude the intima of the artery. After withdrawing the wire, the distal saphenous artery was tied off with 6–0 silk suture. Wounds were inspected for bleeding and arterial blood loss. When hemostasis was achieved, the incision was closed. *Vessel harvesting:* For vessels intended for immunofluorescence staining, following PBS infusion, 20 ml of 1.5% paraformaldehyde plus 0.1% glutaraldehyde in 1× PBS at 4°C was infused at the same rate. Vessels were then harvested and placed in 20% sucrose in 1× PBS at 4°C overnight. The next day, vessels were embedded in OCT compound, prepared as fresh frozen slides (10-μm–thick sections), and stored at −80°C pending analysis. For neointimal assessment, following PBS infusion, mice were perfused with 20 ml of 10% neutral buffered formalin at the same rate. Vessels were then harvested and placed in fresh 10% neutral buffered formalin overnight and transferred to 70% ethanol the following morning. Vessels were then embedded in paraffin, mounted on slides (5-μm–thick sections), and stained with H&E. Neointima was assessed as previously described.[36]

Bone marrow derived MC (BMMC) Isolation and Culture

Bone marrow harvesting: Donor animals were euthanized by CO_2 asphyxiation prior to removal of marrow. Skin was dissected from legs using sterile forceps and scissors, and femur and tibias were removed. Tissue was scraped away from the bones. Epiphyses (bone ends) were cut with scissors to expose the medullary cavity. Using a 5ml syringe with a 25-gauge needle, bone marrow was flushed out of each bone with PBS. The collected bone marrow was pooled from each mouse into one collection tube. This was centrifuged at 500xg for 5 min at room temperature (RT). Supernatant was removed and the pellet resuspend in culture medium consisting of modified Dulbecco's Modified Eagle's Medium (DMEM) with 4 mM L-glutamine adjusted to contain 4.5 g/L glucose and 1.5 g/L sodium bicarbonate and supplemented with recombinant murine 100ng/L SCL (Prepotech) and 100ng/L IL3 (Prepotech), 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 10% Rat T-STIM (Becton Dickenson) and 10% fetal bovine serum, and seeded into a flask. Following 2 days of culture, suspension cells (leaving debris and adherent cells) were transferred to a new flask with fresh culture medium. Complete differentiation of BMMC took 4–6 weeks, and the maturity of BMMC was assessed via flow cytometry (FACS).

Flow Cytometry

Cultured BMMC cells were lifted and washed with FACS buffer (PBS + 0.5% FBS) in a 5ml polystyrene round bottom tube. After centrifugation at 500g for 5 min at 4°C, the supernatant was aspirated, and cells were incubated for 5 mins in anti-mouse CD16/32 (BD

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Sciences) monoclonal antibodies at a dilution of 1/200 in FACS buffer to block nonspecific binding. Following blocking, cells were then stained in PE conjugated anti-mouse FcεRIα (BD Biosciences) 1/200 and FITC conjugated anti-mouse CD117 (BD Biosciences) 1/200 in FACS buffer. These were incubated for 15–30 min on ice, washed in FACS buffer and centrifuged at 500g, 5mins at 4°C. Cell pellets were resuspended in 200–400μl of diluted propidium iodide (PI) in FACS buffer (final concentration 1μg/ml). Data acquisition was performed on BD FACS CANTO (BD Biosciences) and the results were analyzed with FACS Diva Software (BD Biosciences).

Bone Marrow Transplantation

Mice underwent bone marrow (BM) transplantation at 20–24 weeks of age. Mice were prepared for transplant with four daily intraperitoneal (IP) injections of erythropoietin (300 Units/kg, diluted from stock solution of rHuEPO, Amgen) to promote erythropoiesis and prevent post-transplant anemia. Recipient female kit$^{W\text{-}sh/W\text{-}sh}$ mice (>2 months old) were lethally irradiated with 850–1000rads using a cesium irradiator to ablate the bone marrow. $2.5–5 \times 10^6$ donor marrow cells in PBS were injected into the tail vein of the recipient mice. Six weeks after the BMMC transplantation, recipient mice were subjected to femoral injury as described. Successful engraftment was confirmed via IHC of the toluidine blue staining of the femoral artery, spleen and ear.

Immunohistochemistry

Femoral arteries were serially sectioned in 10μm-thick sections. Mast cells were stained by toluidine blue (Sigma-Aldrich). Degranulated and non-degranulated MC were distinguished by bright-field assessment of cell morphology after toluidine blue staining. Degranulated MC show and release toluidine blue–positive remnants, whereas non-degranulated MC show intracellular and intravascular accumulation of toluidine blue. Mouse fetal liver was used as a positive control. For vessels intended for immunohistochemistry and immunofluorescence staining, following PBS infusion, 20 ml of 1.5% paraformaldehyde plus 0.1% glutaraldehyde in 1× PBS at 4°C was infused at a constant rate. Vessels were then harvested and placed in 20% sucrose in 1× PBS at 4°C overnight. The next day, vessels were embedded in OCT compound, prepared as fresh frozen slides (10μm–thick sections), and stored at −80°C pending analysis. Immunohistochemistry and immunofluorescence staining was performed on fresh frozen slides with primary antibodies against rabbit anit-CD117 (c-kit) (Cell Signaling) at 1:400 dilution, FceR1(eBioscience), at 1:50 dilution, as well as mouse anti-CD115 (R&D Systems) at 1:200, rat anti-CD15 and rabbit anti-CD3 (Abcam) at 1:100 dilution. Control slides were routinely stained in parallel by substituting the primary antibody for IgG or specific IgG isotypes from the same species and at the same final concentration as the primary antibody. Immunohistochemistry secondary antibodies were anti-rabbit, -mouse and -rat (Vector Laboratories) at 1:1000 and counterstained with hematoxylin using Pathcore Autostainer. For immunofluorescence, secondary antibodies were donkey anti-rat, -mouse or -rabbit Alexa Fluor 488 (Invitrogen) at 1:500 dilution or donkey anti-rat, -mouse or-rabbit 594 (Invitrogen) at 1:200 dilution. Mounting medium containing DAPI (Vector Laboratories) was then applied. Images were acquired using a Zeiss Axioskop *plus* light microscope with AxioVision V4.3 software, or a Zeiss LSM 510 UV laser scanning confocal microscope system (Carl Zeiss GmbH). All

immunofluorescence staining was performed at least 8 times and using sections obtained from differing vessels/mice. Cell counting was performed by Image J (1.37v) software.

Mast cell stabilization in vivo

After mice (C57BL/6, male, 10 weeks old) recovered from wire injury surgery (day 0), they received the first intraperitoneal (IP) injection of DSCG (Sigma-Aldrich, 25 mg/kg/d). Each animal received daily injections for the duration of the experiment thereafter (14 days). Wild-type (WT) or MC-deficient $Kit^{W-sh/W-sh}$ mice at 6 weeks of age received disodium cromoglycate (DSCG) intraperitoneally.

Statistics

Statistical analyses were done using GraphPad Prism 9 software. All data were tested for normality before the analysis. If the normality test was passed, comparisons were performed by 2-tailed unpaired Student’s t test or by 1-way ANOVA followed by Tukey’s multiple comparisons test. If not, the comparisons were performed by nonparametric Mann-Whitney U test or by Kruskal-Wallis test followed by Dunn’s multiple comparisons test. P values of less than 0.05 were considered significant.

Study approval

The study was approved by the Institutional Review Board of the NIH Clinical Center, NHLBI. Mouse studies were approved by the Animal Care and Use Committee of NHLBI. All mouse studies were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals (National Academies Press, 2011).

Results:

Mast cells degranulate in response to acute vascular injury.

To evaluate the effect of acute injury on the presence and degranulation of MC in a large artery, a femoral wire injury model was used in mice (Figure 1A). Following an acute femoral arterial injury in mice, MC were recruited to the adventitia of the femoral artery (Supplemental Figure 1A) and degranulate within the adventitia of the inured artery in wild-type mice (Figure 1B). Absence of degranulation staining in $Kit^{W\text{-}sh/W\text{-}sh}$ knockout mice confirmed absence of MC in this mouse model (Figure 1C). The observed degranulation seen in 82.8% ±14.3 of MC in the wild-type mice, and is a rapid response to the injury, peaking in the first 6–12 hours. However, this acute effect did not completely resolve until 7 days following injury. (Figure D). Interestingly, the number of MC does not change throughout this time, indicating that the acute injury induces activation and subsequent degranulation of tissue resident MC (Figure E). Comparison of the systemic inflammatory state between wild-type and the $Kit^{W\text{-}sh/W\text{-}sh}$ mice showed no significant differences (Supplemental Figure 1B-E).

Mast cell activation and degranulation cause neointimal hyperplasia.

To determine the effect of MC activation and subsequent degranulation on neointimal hyperplasia, the intima to media ratio was measured in wild-type and $Kit^{W\text{-}sh/W\text{-}sh}$ mice,

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as well as an assessment of other inflammatory cell recruitment into the adventitia of the injured femoral artery. There was a significant increase in neointimal hyperplasia in wild-type mice that is absent in the MC deficient mice following acute arterial injury (Figure 2A). This absence of neointimal hyperplasia in the $Kit^{W\text{-}sh/W\text{-}sh}$ mice is coupled with decreased inflammatory cell recruitment (Figure 2B-D, Supplemental Figure 2D-F). These results suggest MC activation and recruitment are not only responsible for the increased neointimal hyperplasia, but also for orchestrating or triggering further recruitment of other inflammatory cells into the site of injury. This phenomenon was observed only following acute injury, as baseline inflammatory cells were similar between wild-type and $Kit^{W\text{-}sh/W\text{-}sh}$ mice when uninjured (Figure 2A-C). (Representative images: Supplemental Figure 2A-C).

Bone marrow derived mast cells transplantation from wild-type to $Kit^{W\text{-}sh/W\text{-}sh}$ reestablishes acute vascular inflammation and neointima size.

To further show the critical role of MC in neointimal hyperplasia and global inflammatory response following acute arterial injury, bone marrow derived MC (BMMC) were transplanted into prepared recipient $Kit^{W\text{-}sh/W\text{-}sh}$ mice via tail vein injection (Figure 3A). Following culture and prior to transplantation, mature BMMC were confirmed via FACS for CD117 (cKit) and FcεRIα. Confirmation of transplanted BMMC is shown in the femoral artery and ear (Figure 3B-C) as well as an accumulation shown in the spleen (Supplemental Figure 3B).

Transplantation of BMMC into $Kit^{W\text{-}sh/W\text{-}sh}$ mice led to similar neointimal hyperplasia in the femoral artery post-acute injury as seen in the wild-type mice (Figure 3D). This phenomenon was coupled with increased recruitment of inflammatory cells in the arterial adventitia (Figure 3E-G). This further demonstrates the role of MC in orchestrating the detrimental effects of acute injuries in large arteries.

The mast cell stabilizer, disodium cromoglycate (DSCG), attenuates vascular remodeling after acute vascular injury.

To assess the potential therapeutic benefit of the MC stabilizer, disodium cromoglycate (DSCG) on vascular remodeling following acute arterial injury, we treated wild-type mice with intraperitoneal DSCG immediately following recovery from the arterial wire injury. Following 14 days of treatment, mice with DSCG treatment had a significant decrease in neointimal hyperplasia compared to mice treated with vehicle (Figure 4A). Decreased MC degranulation in the adventitia was also observed (Figure 4B), indicating that this may be an effective peri-operative treatment for preventing the formation of scar tissue and the occurrence of detrimental vascular remodeling that leads to poor outcomes. Additionally, inflammatory cell infiltration such as T cells (CD3) and macrophages (CD115) were seen to be significantly decreased at the site of injury (Figure 4C-D). Assessment of the presence of neutrophils was not assessed in this study as DSCG has been shown to inhibit both neutrophil infiltration and activation.[30–32]

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Discussion:

Here we show the role of MC in restenosis post-acute arterial wire injury in mice, and the pharmaceutical prevention of this process using the MC stabilizer, DSCG. This study demonstrates the presence and activation of MC resulting in degranulation not only results in neointima hyperplasia, but also plays a pivotal role in coordinating the activation and migration of other inflammatory cells to the site of injury. The clinical relevance of these results is demonstrated through the prevention of this process with the MC stabilizer, DSCG, resulting in decreased restenosis and providing proof-of-concept to the potential utility of this drug as a post-operative therapy for those undergoing revascularization surgery.

Mast cells are known to be early or acute responders following the detection of foreign antigens, tissue or cell damage. Best known for their role in allergic reactions, MC also control other immune cells to coordinate innate and adaptive immune responses.[27,28,37] There is a growing body of evidence implicating MC in cardiovascular diseases, such as atherosclerosis, aortic aneurysm, and restenosis. [27,38] With the known involvement of MC in vascular disease, we sought to investigate the role MC play following the surgical or procedural intervention in these blood vessels. Our MC knockout mouse studies show that when perivascular MC are not present, neointimal hyperplasia is significantly reduced following an acute arterial wire injury, modeling the endothelial damage seen in revascularization procedures. This is further evidenced when MC knockout mice who received MC transplantation, developed neointimal hyperplasia.

Our results are consistent with other studies that demonstrated accumulation and activation of MC at sites of vascular injury. Thus, mast cells have been investigated in the context of vein graft induced neointima hyperplasia, where they are known to accumulate in the tissue surrounding the grafts.[39] When transplanted at the site of the vein graft in MC deficient mice, neointimal hyperplasia was reestablished to a similar extent as wild-type mice.[39] Importantly, further studies showed reduced neointima hyperplasia when MC derived chymase was pharmacologically inhibited.[40] In a chronic kidney disease (CKD) mouse model, MC activation was shown to aggravate vein graft disease, and MC accumulation was seen in human CKD arteriovenous fistula veins.[41]

Degranulation of MC results in the release of both newly formed and pre-existing mediators, and what factors are predominantly released is dependent upon the type of stimuli. This release of heterogenous mediators is important in maintaining homeostasis through the effective clearance of foreign antigens and coordination of other immune cells. MC degranulation is also known to effect smooth muscle layers in airways as well as vascular integrity. In these settings, MC have been implicated in the pathogenesis of disease and hypersensitivity in humans.[42] In fact, MC derived factors have been used as markers for certain diseases, such as mastocytosis, kounis syndrome and asthma.[42] Our studies demonstrate that MC are critical in coordinating an immune response involving neutrophils, T cells and macrophages following an acute arterial wire injury. Inflammatory cell recruitment at the site of endothelial injury follows the deposition of platelets and fibrin.[43] Most studies have concentrated on the monocyte lineage and neutrophils when investigating restenosis following PCI,[33,44,45] and many studies have shown that the inhibition of the

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recruitment of these cells through various means leads to decreased neointima formation in animal models using vascular stents.[44] Furthermore, mice models blocking the recruitment of T cells and the Th1 response been shown to exhibit reduced vascular remodeling.[46,47] A humanized immunodeficient mouse model was developed by Moser *et al* to study the type of immune response and immune cell recruitment to varying severities of vascular injury.[48] The underlying mechanisms involved in immune cell recruitment and activation are complex and whilst many studies have attempted to address individual immune cell involvement, this study has shown the critical role for MC as the first responder cells in coordinating other immune cell recruitment either directly or indirectly through endothelial cell activation. It is natural then to target therapeutic intervention at the source of the whole inflammatory response.

Using DSCG, a MC stabilizing drug, we were able to prevent neointimal hyperplasia follow acute arterial wire injury. This study not only reinforces the critical role of MC in arterial restenosis following acute endothelial injury, but also provides a viable treatment for addressing a critical need for improvement of morbidity and mortality following surgical revascularization interventions. Disodium cromoglycate is already in clinical use for asthma, seasonal rhinitis, allergic or venereal conjunctivitis, mastocytosis and ulcerative colitis,[49] with the mechanism of action being to stabilize MC by preventing degranulation, resulting in the inhibition of mediators such as histamine, cytokines and chemokines from being released. Recently, Gan *et al* have looked at the utility of DSCG in a pre-clinical myeloperoxidase glomerulonephritis-ANCA associated vascularitis mouse model to study its effectiveness autoimmune disease.[50] They were able to show that DSCG blocked MC degranulation without affecting other immunoregulatory functions to maintain the tissue environment homeostasis. In addition, a renal fibrosis model using ureteric ligation to induce damage, demonstrated MC recruitment, activation and degranulation within 6 hrs of injury, associated with the resultant fibrosis, and attenuated with DSCG treatment.[51] Thus the utility of DSCG in minimizing the detrimental effects of MC activation and degranulation post-acute injury across multiple tissue settings indicates that this drug, which is already in clinical use, is a good candidate for therapy. Future studies may explore additional options for pharmacological inhibition of MC following surgical revascularization interventions. These options include using alternative MC stabilizers[52] or targeting ~~major~~ pathways of MC activation.

In summary, we have shown that the rapid recruitment, activation, and degranulation of MC following acute arterial wire injury is causal of arterial neointimal hyperplasia in this pre-clinical restenosis mouse model. Furthermore, we offer a viable, clinically approved therapeutic solution to prevent this phenomenon, aimed at patients with restenosis complications due to revascularization intervention.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability statement:

The data that support the findings of this study are available in the methods and/or supplementary material of this article.

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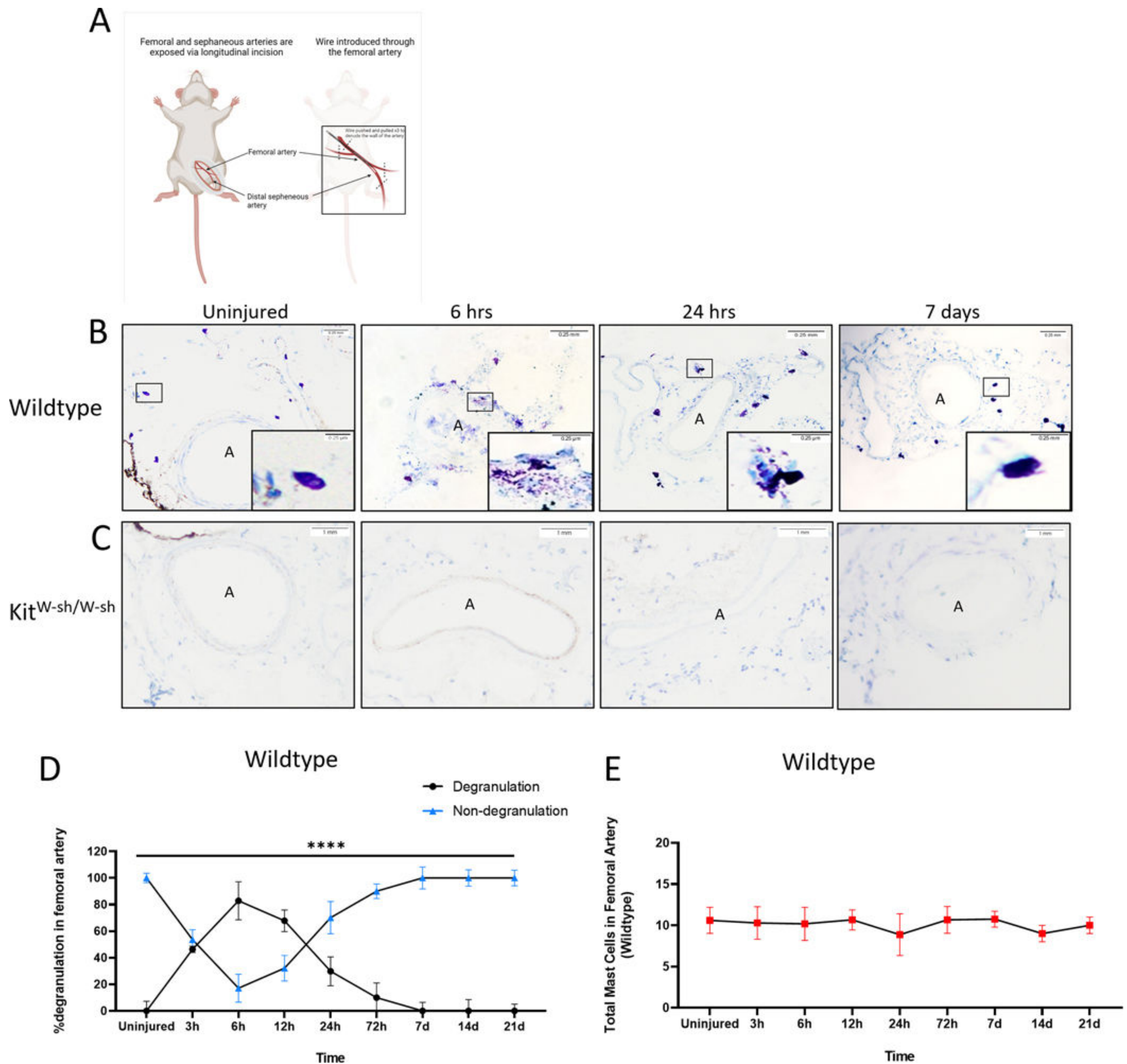

Figure 1: Mast cells degranulate in response to acute vascular injury.

Following an acute femoral artery injury in mice (A), MC are recruited and degranulate within the adventitia of the injured artery. (B) Toluidine blue staining of MC in wild-type mice showing degranulation at 6h and 24h post-acute arterial injury, with the presence of intact MC in uninjured vessels and at 7 days post injury. Absence of Toluidine blue staining in KitW-sh/W-sh mice confirms the staining specificity for mast cells and their absence in KitW-sh/W-sh mice (C). (D) Quantification of the percentage of MC degranulating in the wild-type mice demonstrates a rapid increase and peak by 6h, which steadily dissipates within 72h. (E) The total number of MC per field of view in wild-type mice does not change

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pre- and post-injury. Data are shown as a mean±SEM. ****p 0.0001. Data analyzed with ANOVA with repeated measures. N=3–6 mice per timepoint.

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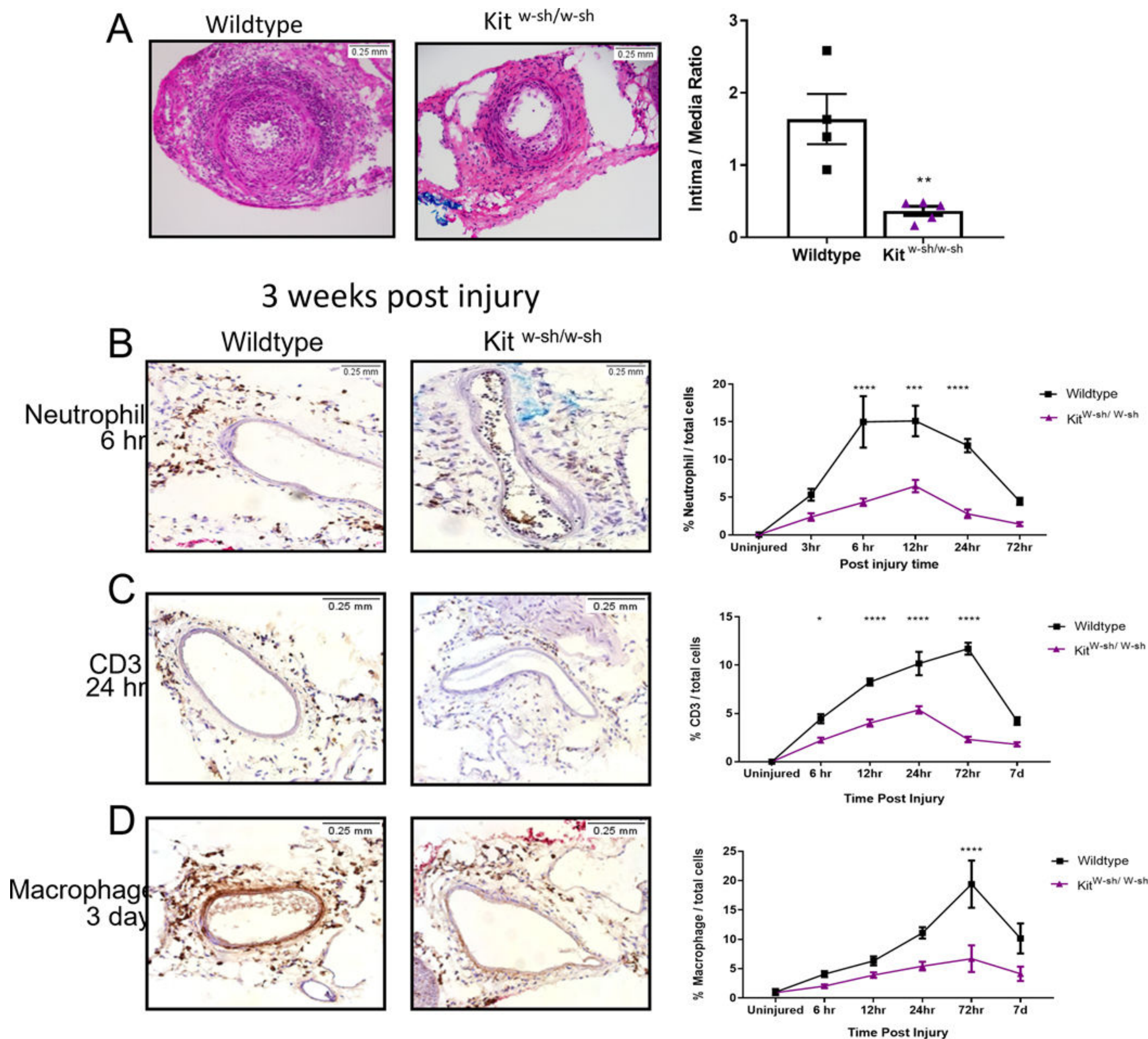

Figure 2: Mast cell activation and degranulation cause neointimal hyperplasia.

(A) H&E stain of large arteries showing increased neointima hyperplasia in the wildtype compared to the MC deficient mice. Decreased recruitment of neutrophils, macrophages and CD3+ T cells in mice with a deficiency in MC following an acute vascular injury. Decreased neutrophil (B) at 6hr, CD3 T cell at 24h (C) and macrophage (D) recruitment in MC deficient mice post arterial injury induction. Data are shown as a mean±SEM. ****p 0.0001, ***p 0.001, **p 0.01 & *p 0.05. Data analysis of intima/ media ratio with Students T-test between groups at each group. Data analyzed with ANOVA with repeated measures. N=5–7 mice per group/ per timepoint.

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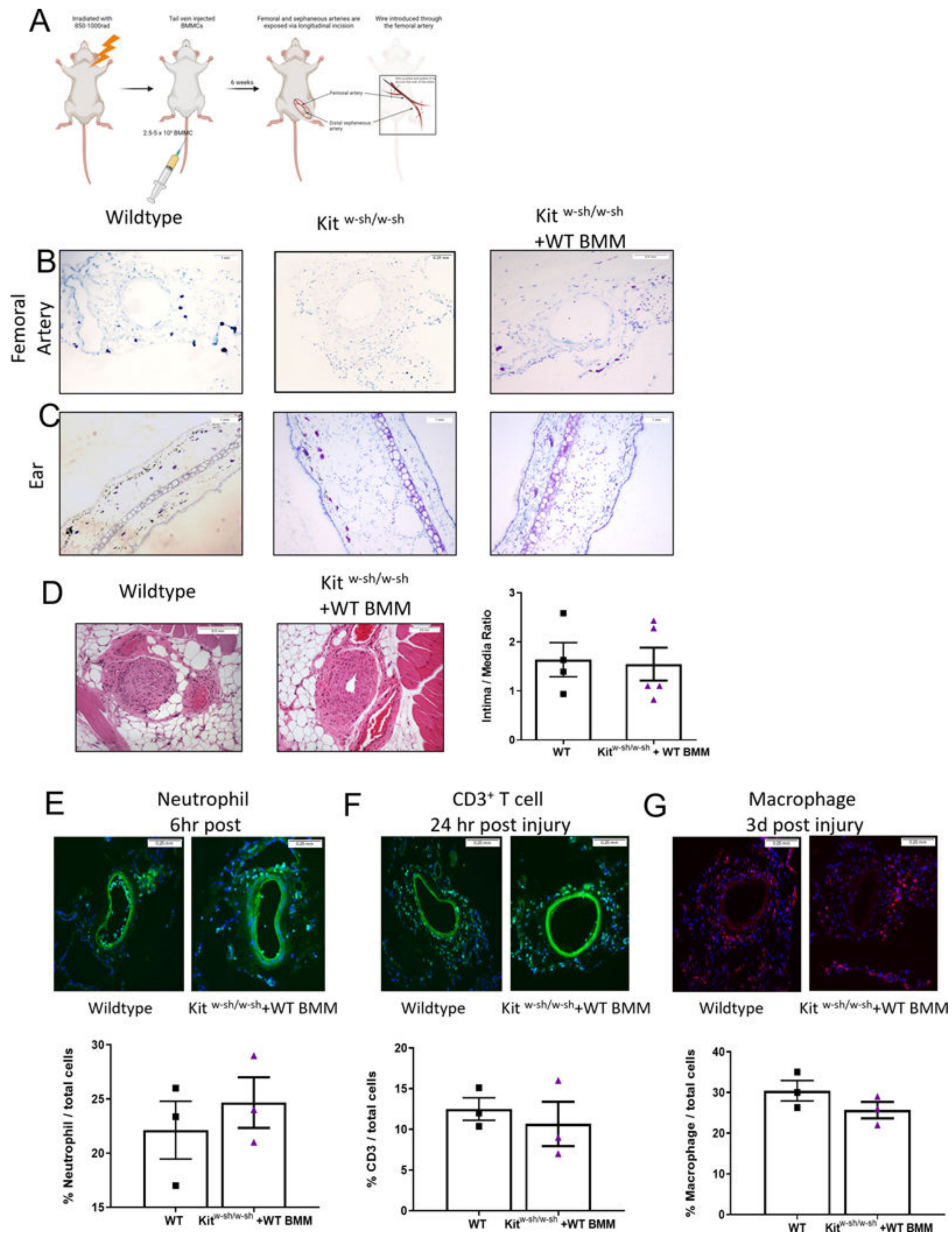

Figure 3: Bone marrow derived mast cell (BMMC) transplantation from wild-type to KitW-sh/W-sh reestablishes acute vascular inflammation and neointima size.

Reconstitution of mast cell deficient mice with wild-type bone marrow-derived MC (A) restored the acute inflammatory response and neointima size seen in wild-type mice. Presence of MC in WT, KitW-sh/W-sh and KitW-sh/W-sh + WT BMM in the femoral artery (B) and Ear (C) 6h post injury. (D) H&E stain of large arteries showing increased neointima hyperplasia in the wildtype and KitW-sh/W-sh + WT BMM. Neutrophil (E), CD3 T cells (F) and macrophage (G) recruitment was restored in the KitW-sh/W-sh + WT BMM. Data are

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shown as a mean±SEM. Data analysis with paired Students T-test between groups at each group. N=3–4 mice per group.

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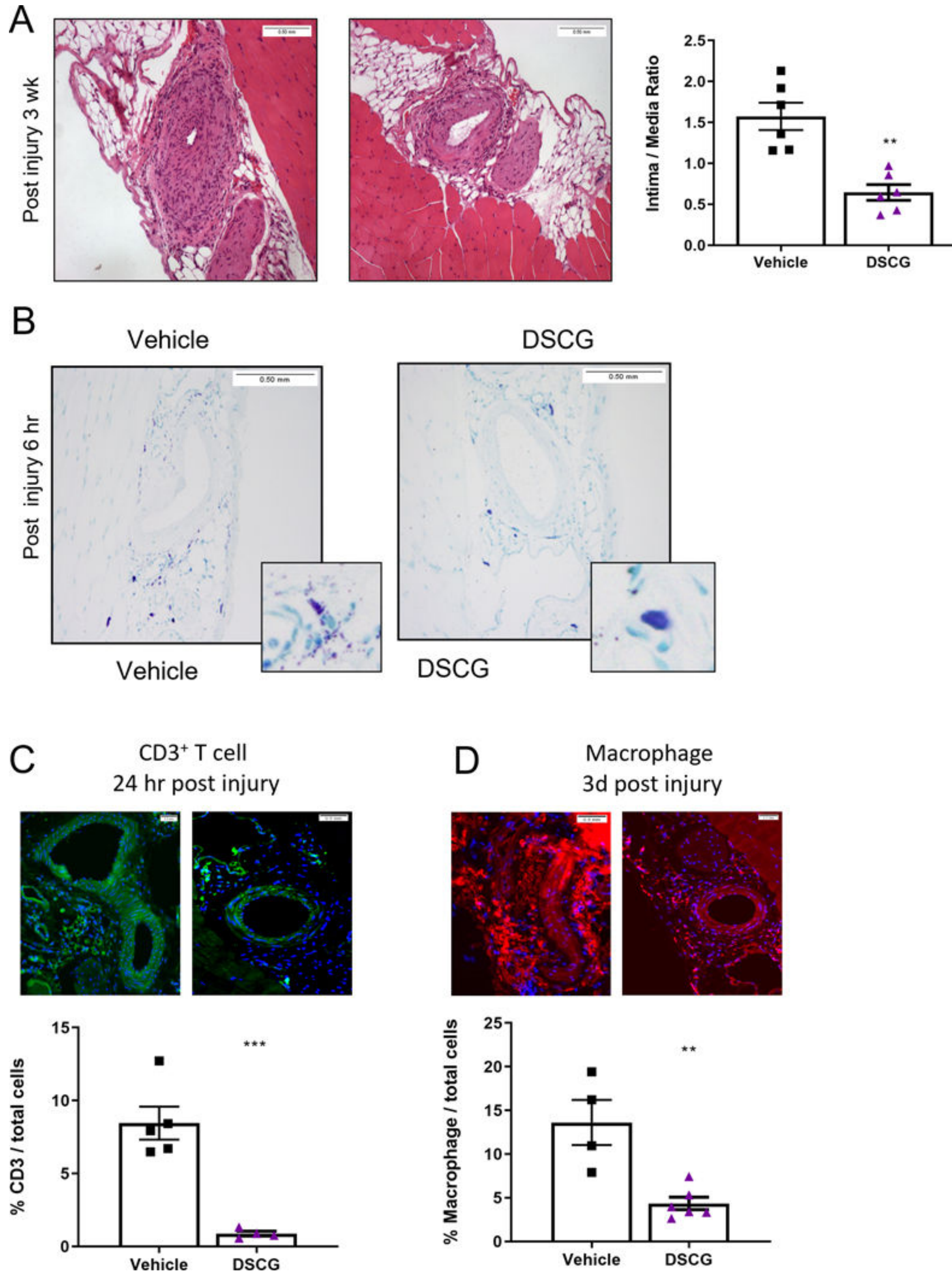

Figure 4: Vascular remodeling is attenuated following treatment with disodium cromoglycate (DSCG).

Mast cell activation and degranulation are prevented following treatment with DSCG (A). H&E stain of large arteries showing decreased neointima hyperplasia in the DSCG compared to vehicle. (B). Degranulation shown with toluidine blue staining of the femoral artery is reduced in DSCG treated mice. (C) The percentage of CD3 T cells is significantly decreased 24hr post injury following DSCG treatment. (D). The percentage of macrophages is significantly decreased 3days post injury following DSCG treatment. Data are shown as a

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mean±SEM. **p 0.01 and ***p p 0.001. Data analysis with paired Students T-test between groups at each group. N=4–7 mice per group.