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The effects of tranexamic acid on the progression of post-traumatic osteoarthritis in mice

Dissertation

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1. Introduction

1.1 Osteoarthritis

Osteoarthritis (OA) is among the most prevalent musculoskeletal diseases and represents a degenerative joint disease, which is a major cause of chronic disability and decreased quality of life in elderly[1-4]. It is a progressive disease occurring in the articulating joints and most commonly affects the knee joints[5]. The hallmarks of OA include progressive degeneration of articular cartilage, together with chronic joint inflammation and abnormalities in subchondral bone remodelling[6]. The multiple anatomic and physiological alterations contribute to a variety of clinical manifestations in affected joints such as pain, joint stiffness and swelling, muscle weakness and, finally, loss of joint function[7, 8].

1.1.1 Epidemiology

At present, OA poses a significant public health challenge. The most recent study from the Global Burden of Diseases, Injuries and Risk Factors Study (GBD) 2017 reported that OA affected over 300 million people globally. The age-standardized prevalence and incidence rates were increased by 9.3% and 8.2% from 1990 to 2017, respectively. Also, the age-standardized years lived with disability rate of OA was increased by 9.6% from 1990 to 2017[3]. OA has become a severe disease due to high disability rates, increased morbidity and mortality, and causes a heavy psychological and physical burden on affected individuals and overall health care costs[3]. The burden caused by OA is increasing globally and the trend is expected to continue with the aging of today's population.

OA is usually classified into two types: primary OA and secondary OA[9]. Although most of the cases in clinic are classified as primary OA, the underlying cause is not known. It is currently accepted that primary OA results from a combination of risk factors, such as aging, gender, obesity, endocrine and genetics[10]. Firstly, chief among these is aging, which may be associated with accumulation of other risk factors and impaired tissue repair and regenerative capacity[11]. The global prevalence estimate increased with age, and was higher in women compared with men[3, 12]. Secondly, increased body mass index is strongly associated with an increased risk for OA. Studies showed that overweight contributed to increased susceptibility to radiographic and symptomatic

OA[13], and obesity was associated with a 3-fold increased risk of OA[14, 15]. Thirdly, the relationship between hormones and OA has been extensively studied. For example, some studies showed that low vitamin D levels were associated with the development of OA[16-18], and vitamin D supplementation could improve pain and function in patients with OA[19, 20]. Furthermore, 21 independent susceptibility loci for OA have been identified by genetic association studies, and the genetic factors contribute to 30-65% of the risk for OA[7, 21].

Different from primary OA, secondary OA is the result of an existing cause such as joint trauma, infection or joint dysplasia. Post-traumatic OA (PTOA) develops after joint injury[22], which can be intra-articular fractures, cartilage damage, cruciate and collateral ligament injuries, and meniscal tears[22, 23]. PTOA accounts for nearly 12% of the overall prevalence of symptomatic OA, and approximately 10% of all cases of knee OA[24]. It mostly occurs in younger people than other forms of OA. As reported, OA patients with a history of joint trauma were more than 10 years younger than those without[25]. The knees which sustain an isolated anterior cruciate ligament (ACL) injury were 4.2 times more likely to develop OA than those without injury[26]. However, surgical ACL reconstruction could not well protect those injured joints from developing PTOA[27, 28]. Despite ACL surgery is recommended for restoring normal joint biomechanics with overall favorable outcomes, PTOA still develops in a high percentage of patients as a long-term consequence[29].

1.1.2 Pathogenesis

OA was once regarded as a disease that only involved mechanical cartilage degradation. It is now well established that OA affects the whole joint and is not restricted to articular cartilage[30-32]. The pathogenesis of OA involves the crosstalk between cartilage and surrounding joint tissues[33]. As described below, alterations in cartilage, subchondral bone, and synovial tissues have been demonstrated to play important roles in OA pathogenesis.

1.1.2.1 Cartilage

Articular cartilage is hyaline cartilage comprised of sparse distribution of chondrocytes and dense extracellular matrix (ECM)[34]. The ECM consists mainly of proteoglycans, type II collagen (COL2) and water[35, 36]. The COL2 provides cartilage with a meshwork and tensile strength, and the proteoglycans are embedded and draw water into this meshwork, which yields compressive resistance[11]. The articular cartilage is

anatomically and functionally divided into four spatially distinct zones: the superficial (tangential), middle (transitional), deep, and calcified zones[37-39]. And the composition and structure of ECM varies among the different zones of cartilage[40]. The superficial zone accounts for 10-20% of cartilage thickness, containing primarily collagen and flattened chondrocytes. Collagen fibers in this layer are densely packed and run parallel to the articular surface, which protects the cartilage from sheer, tensile, and compressive stresses. The middle zone comprises 40-60% of cartilage thickness that contains proteoglycans, spherical and larger chondrocytes and collagen. Collagen fibers in this layer are organized obliquely and randomly, providing the resistance to compressive forces. The deep zone represents about 30% of cartilage thickness. This zone possesses the lowest water and highest proteoglycan content compared with the other zones. In this layer, the collagen fibers with bigger diameter are parallel to the chondrocytes with columnar arrangement and perpendicular to the articular surface, therefore offering the maximum resistance to compressive forces[34, 37, 40]. The calcified layer is separated from the deep zone by a basophilic line called the tidemark, which is considered as a metabolically active front of calcification[41, 42]. This layer is composed of partially mineralized ECM and sparse hypertrophic chondrocytes. The collagen fibers of the deep layer extending across the calcified layer anchor the cartilage to subchondral bone.

In joint tissue, the main function of cartilage is the absorption and removal of mechanical load, which is necessary for maintaining cartilage homeostasis[43]. If the homeostatic or repair mechanisms in cartilage are not able to adequately compensate structural injuries, cartilage degeneration occurs and OA may develop[44]. Cartilage degeneration is a key hallmark of OA[45], which usually starts with the loss of proteoglycans from the superficial layer followed by degradation of the collagen network in deeper layers. Chondrocytes are the only cell type residing in cartilage matrix[46], regulating cartilage anabolism and catabolism in response to the surrounding mechanical and biochemical perturbations. This includes intrinsic and extrinsic growth and release of inflammatory mediators and other cytokines[47]. At the initial stage of OA, chondrocytes in the middle zone try to compensate the loss of ECM by enhancing their metabolic activity such as increased synthesis of fibronectin, COL2 and proteoglycans. As disease progresses, the catabolic activity is increased, and cartilage degeneration becomes irreversible and inevitably decompensates[37, 40]. By this time, several proteins indicative of the catabolic state are increased, including various matrix

metalloproteases (MMPs), as well as some inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α)[40, 48]. One should notice that the activation of chondrocytes is induced not only by ECM changes due to cartilage degradation, but also the catabolic and/or inflammatory signaling pathways originating from the surrounding joint tissues like synovium and subchondral bone[11]. In this regard, early intervention on some reversible lesions has proven more effective than approaches at later stages[49].

1.1.2.2 Subchondral bone

Subchondral bone refers to the bone tissue beneath the calcified cartilage and cement line, which is divided into subchondral bone plate (SBP) and underlying subchondral trabecular bone[50, 51]. The SBP is a thin cortical lamella providing mechanical strength for tissue support, which has a marked porosity and channels linking cartilage to subchondral trabecular bone[42]. The vessels and nerves from subchondral bone traverse through these channels to reach the calcified cartilage and allow crossover communications[52, 53]. Therefore, the SBP plays important roles in substance interchange across the osteochondral interface[50, 54-56]. In contrast to the SBP, the underlying subchondral trabecular bone is more metabolically active and contains bone marrow in its cavities[57]. The unique structural network of richly vascularized subchondral trabecular bone serves a variety of critical functions, including shock absorption and tissue support in normal joints, as well as nutrient supply and metabolic regulation of cartilage tissue[58].

The changes in subchondral bone during OA can be segregated into distinct patterns according to the disease stage and anatomic location. These changes usually include alterations in SBP thickness, in the architecture of subchondral trabecular bone, and the formation of osteophytes, all of which are caused by abnormal bone remodelling[48]. In OA, subchondral bone undergoes an uncoupling of bone remodelling[59], which is characterized by enhanced bone resorption and bone formation manifesting in different stages[60, 61]. Therefore, the rate of bone remodelling in subchondral bone dynamically changes across the course of OA[60, 61], resulting in distinct microstructural alterations at different stages[59]. Early OA presents as subchondral bone loss including reduced bone mass and thickness of both the SBP and trabecular bone[60], which is mainly associated with the excessive bone resorption due to the increased osteoclast numbers and activity in subchondral bone[60-62]. In contrast, subchondral bone sclerosis

with decreased bone resorption and relatively increased bone formation occur in late OA[60, 63]. Subchondral bone sclerosis has several structural features such as increased thickness of the SBP and trabecular bone, an increase in osteoid volume, and a decreased trabecular separation. Even though the bone volume and density are increased, a decreased material stiffness of subchondral bone in OA results from abnormal mineralization[64]. Alterations in osteoblast parameters in OA are still not clear. Osteoblasts from OA subchondral bone were shown to express higher levels of osteogenic differentiation markers such as alkaline phosphatase, osteopontin and osteocalcin than those from healthy joints. Further, Lin et al. reported that the number of pre-osteoblasts and osteoblasts in subchondral bone of OA mice is increased in the early stage[65, 66], while their mineralization capacity might be potentially impaired due to abnormal production of type I collagen (COL1)[51, 67, 68]. Though the mechanisms underlying the changes of bone remodelling remain insufficiently characterized, other studies showed that early inhibition of abnormal bone remodelling and improvement of subchondral bone loss was able to slow down cartilage degeneration in PTOA[63, 69-73].

1.1.2.3 Synovium

The synovium is a specialized soft connective tissue that presents in synovial joints. It covers all inner surfaces of the joint capsule except articular cartilage[74, 75]. Fibroblast-like synoviocytes (FLS) are the major sources of synovial fluid, which is rich in hyaluronic acid and lubricin and plays important roles in lubricating and protecting the surface of articular cartilage[76]. As articular cartilage is avascular[77], chondrocytes are dependent on synovial fluid perfusion and, as mentioned above, subchondral bone for substance exchange and nutritional support[78]. The synovium can be divided into an intimal lining layer which is directly exposed to the synovial space, and a synovial sublining layer[79]. Type-A macrophage-like synoviocytes and type-B FLS are the dominant cell populations in the lining layer[80, 81]. The sublining layer comprises connective tissues containing fibroblasts, adipocytes, vessels, lymphatics, nerve fibers and resident immune cells such as macrophages[82]. Studies indicated that macrophages in both lining and sublining layer are differentiated from circulating bone marrow-derived monocytes, even though they show differing phenotypes[83].

Synovial inflammation appears early in OA and persists throughout the course of the disease[84], which usually manifests as synovial membrane thickening, pannus formation and fluid effusion[85]. Immune cell infiltration is common in OA synovium,

which mainly includes macrophages and T cells, along with a few mast cells and B cells[86-88]. The factors driving immune cell recruitment are very complex and may be associated with trauma, aging or systemic low-grade inflammation as observed in obesity and the metabolic syndrome[89]. Tissue debris from injured cartilage or meniscus, and factors released from degraded cartilage and subchondral bone are capable to activate synoviocytes and promote their proliferation. Activated synoviocytes secrete cytokines and chemokines to attract immune cells[90], which subsequently release inflammatory mediators and proteolytic enzymes (MMPs and aggrecanases). Together, this contributes to the further propagation of inflammation, promotes angiogenesis and cartilage degradation, and drives erosions of adjacent bone[91-93]. As a result, hyperplasia of lining layer, an important histological feature of the synovium in OA, is observed, and fibrosis and stromal vascularization with massive immune infiltration into the sublining layer occurs[94].

In this regard, synovial fluid from OA patients was shown to activate the expression of pro-inflammatory cytokines in human chondrocytes[95]. Likewise, the concentrations of proinflammatory cytokines were reported to correlate with the radiographic and clinical grades of knee OA[96]. Several clinical studies showed that synovitis in knees without radiographic OA and cartilage damage favors subsequent cartilage loss and development of OA[97, 98], suggesting that synovitis is an independent cause promoting disease progression in OA. Synovitis and cartilage degeneration thus promote each other, leading to progressive joint degeneration during OA development[93]. Even though there is still no clinical evidence supporting that anti-inflammatory drugs protect from joint degeneration, synovitis is certainly a potential candidate target for OA treatment.

1.1.3 Treatment

Currently, there is no cure for OA and only few effective treatment options are available[99]. Several guidelines for OA management have been developed by professional organizations such as the Osteoarthritis Research Society International (OARSI)[100], the American College of Rheumatology (ACR)[101], and the American Academy of Orthopedic Surgeons (AAOS)[102]. The treatment can be broadly classified into non-pharmacological interventions, pharmacotherapy and surgical treatments.

1.1.3.1 Non-pharmacological interventions

Patient education is strongly recommended by all existing guidelines as an integral part of the management of OA[103]. An important goal of non-pharmacological interventions for OA is the reduction of modifiable risk factors, such as obesity[99]. Weight loss was demonstrated to be associated with symptom improvement and delayed cartilage degeneration in OA populations[104, 105]. A number of clinical trials have shown that weight loss in overweight and obese adults reduces the risk of symptomatic OA[106] and markedly improves clinical and functional scores of knee OA patients[107-110]. In this regard, intensive diet and/or exercise induced weight loss is usually recommended[109]. Besides, OA patients without obesity also appear to benefit from exercise. Studies found that guided exercise could decrease pain and improve physical function in individuals with OA[111-115]. Additionally, other interventions such as acupuncture, transcutaneous electrical nerve stimulation, bracing and electromagnetic field therapy have been described, however compelling evidence supporting their effectiveness is still insufficient.

1.1.3.2 Pharmacotherapy

To date, no medication exits that has clearly been confirmed to have a therapeutic effect on cartilage degeneration. The principal goal of pharmacological therapy for OA patients is thus to alleviate the symptoms and improve joint function[116]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are generally considered as the first-line therapy in the treatment of OA[103, 117]. They are widely used in the clinic and effective for pain relief. However, gastrointestinal and renal adverse effects of NSAIDs should be noticed, especially when they are applied to the patients with history of gastrointestinal diseases like bleeding peptic ulcer[118]. For patients with NSAID intolerance, acetaminophen and selective cyclooxygenase-2 inhibitors like celecoxib are suitable alternatives. Other oral drugs including glucosamine sulfate and chondroitin sulfate were also described to be used in the clinic, with however uncertain efficacy.

Apart from oral medication, several drugs were developed for intra-articular (IA) injection in OA. IA injection of corticosteroids for knee OA is strongly recommended by the ACR guidelines because of its proven short-term efficacy[101]. IA corticosteroids were shown to relieve pain and increase joint mobility in knee OA for up to several weeks[119], which was associated with potent immunosuppressive and anti-inflammatory effects[120]. However, there are some concerns regarding the potential long-term adverse effects of IA corticosteroids, such as a deleterious impact on

articular cartilage[121, 122]. Therefore, recommendations range from optional to inconclusive within the different guidelines[100, 102]. Another adjunct therapy to oral NSAIDs is IA hyaluronic acid. Hyaluronic acid exists in synovial fluid and plays an important role in the lubrication of cartilage[123], and its concentration is decreased in OA synovial fluid. Some studies showed that IA hyaluronic acid as a visco-supplementation was effective for joint pain relief in knee OA patients[124-126]. However, solid evidence for clinical efficacy is still lacking and therefore it is still not recommended or conditionally recommended in various guidelines[100-102], similar to novel approaches including IA platelet-rich plasma or mesenchymal stem cells.

1.1.3.3 Surgery

The principle aim of surgery for OA patients is to improve quality of life through minimizing pain and disability that fails to respond to non-operative means. In some cases, surgeries are performed to delay the development of OA by removal of predisposing factors. For example, ACL reconstruction may delay onset or reduce risk of PTOA in the patients with ACL injuries[127, 128]. Periarticular osteotomies are suitable for patients with hip dysplasia. Likewise, varus or valgus deformities of the lower extremity with early and unicompartmental knee OA may be treated by distal femoral or high tibial osteotomies to correct the mechanical axis, delay the progression of OA and improve overall symptoms and outcomes[129-131]. However, evidence to support the effectiveness of these surgeries remains rather limited.

Joint arthroplasty is the only definite treatment option in patients with end-stage OA, who suffer persistent pain and functional loss[132]. In 25% of patients, knee OA is limited to a single joint compartment[133]. Several studies reported that unicompartmental knee arthroplasty (UKA) provided lower early complication rates, faster recovery and better long-term outcomes than total knee arthroplasty (TKA)[133-136]. Even though similar long-term outcomes were observed in some other studies, UKA is more recommended for the patients with late-stage unicompartmental OA[135, 137-139]. In case of TKA, it is now clear that the procedure achieves greater pain relief, improvement in function, and quality of life compared to conservative treatment[140]. However, it is also important to note that TKA may result in severe complications including deep vein thrombosis and infection[141], and bares the risk for revision surgery due to prosthesis loosening, fracture, wear, dislocation or infection[142]. As revision rates of TKA are lower than 10% over 20 years, older patients who receive TKA are likely not to require revision

surgery[140]. However, joint arthroplasty is not an ideal solution for young individuals due to the restricted mobility and limited life span of prostheses. Therefore, effective early interventions slowing the progression of OA is essential for younger patients to prevent or delay joint arthroplasty.

1.2 Tranexamic acid

Tranexamic acid (TXA) is a synthetic analog of the amino acid lysine and well known as the most widely employed antifibrinolytic drugs used to control excessive bleeding[143]. It was approved by the Food and Drug Administration (FDA) and listed among the most essential medications by the World Health Organization (WHO)[144]. Recently, the effects of TXA beyond antifibrinolysis have been noticed, while the underlying mechanisms are still unclear. Nonetheless, there has been a revival of interest in its potential new applications.

1.2.1 Pharmacotherapeutic properties

The coagulation and fibrinolytic systems maintain a dynamic balance to regulate fibrin deposition and degradation[145]. Fibrinolysis is driven by the activation of plasminogen into plasmin[146], which is activated along with coagulation cascade to counterbalance fibrin formation[147]. The kringle domains of plasminogen contain several lysine binding sites that dock onto lysine residues on the surface of fibrin[148]. Afterwards, the plasminogen activators (PAs) cleave plasminogen to generate plasmin that drives fibrin degradation[149]. As a synthetic analog of lysine, TXA has a high affinity for the lysine binding sites in plasminogen[150]. TXA is thus able to competitively inhibit plasminogen binding to the lysine residues on fibrin. At a high concentration (> 10 mM), TXA has a direct weak inhibition on the activity of plasmin[151], suggesting that TXA itself may function as a weak plasmin inhibitor. Overall, TXA exerts anti-hemorrhagic properties by inhibiting the fibrinolytic activities of plasminogen/plasmin. On the other hand, TXA also theoretically increase the risk of blood clot formation due to the inhibition of fibrinolysis[152]. To date, no evidence exists that TXA increased thrombotic events in bleeding patients[153]. However, TXA still needs to be used with caution in particular patients with hypercoagulable states or a history of prothrombotic conditions.

TXA associated seizures are a rare but well-recognized side effects, which were mostly reported in patients receiving high-dose TXA when undergoing cardiac surgery such as cardiopulmonary bypass[154-159]. In the clinical trials of obstetrics and trauma,

conventional-dose TXA did not increase the risk of seizures[160-164]. The incidence of TXA associated seizures in cardiac surgery was about 0.9%[156]. Currently, it is assumed that high systemic TXA levels may promote seizures as TXA can cross the blood-brain barrier and accumulate in cerebrospinal fluid[165, 166]. Several mechanisms have been proposed to explain TXA associated seizures. TXA is also a structural analog of glycine (Gly) which is a major inhibitory neurotransmitter in the central nervous system (CNS). Studies indicated that TXA may act as a competitive antagonist of Gly receptors[167, 168] and N-methyl-D-aspartate receptor[169] in the CNS, lowering the threshold for seizure activity and thus exhibiting a proconvulsant effect. Besides, some other receptors such as γ-aminobutyric acid type A receptors in the CNS were also reported to be involved[168, 170, 171]. Thus, plasminogen does not represent the only target of TXA, especially when it is applied with unconventional dosing and routes of administration.

1.2.2 Clinical use of TXA in trauma and orthopedics

The FDA-approved indications for TXA are reduction and prevention of heavy menstrual bleeding and short-term bleeding during tooth extraction in patients with hemophilia. Apart from that, off-label use of TXA is practiced in a broader clinical setting. To date, TXA has been used for decades to reduce bleeding and transfusion requirements in various clinical domains, including polytrauma and major orthopedic surgery[172].

Trauma is a serious global public health problem that causes over 6 million fatalities each year[173]. Severe hemorrhage accounts for about 40% of early deaths after trauma[174]. Trauma triggers the release of tPAs, leading to activation of fibrinolysis[175, 176]. Hyperfibrinolysis is one of the key features of acute traumatic coagulopathy that occurs in a considerable proportion of patients with severe trauma[177] and exacerbates bleeding[178]. Therefore, hopes were placed on the use of antifibrinolytic drugs to reduce bleeding and improve outcomes. This was supported by a number of studies demonstrating that TXA reduces mortality in traumatic hemorrhage, depending on the timing of TXA intervention. In this regard, the CRASH-2 trial was a large randomized controlled trial (RCT), which first showed that TXA treatment, given within the first 3 hours after trauma, significantly reduced mortality due to bleeding[179]. Following the CRASH-2 trial, a trial study called MATTERs was conducted to characterize the effects of TXA on wartime injury[180]. The findings from MATTERs study showed that the use

of TXA after combat injury resulted in improved survival. And recently, the CRASH-3 trial on patients with acute traumatic brain injury demonstrated that TXA treatment within 3 hours of the trauma event reduced head injury-related death[160]. Even though some studies do not support the beneficial effects of TXA in trauma patients[181, 182], the favorable safety profile and low cost of TXA led to its wide-spread use in trauma practice. Moreover, TXA treatment is now implemented in most major trauma guidelines[183, 184].

Major orthopedic surgeries such as total joint arthroplasty are associated with significant blood loss, often requiring perioperative blood transfusions and resulting in postoperative anemia[185]. A systemic review reported that the perioperative blood loss in total joint arthroplasty was 1600 ml on average, and about 30% of patients received blood transfusions[186]. Except for the well-known adverse effects such as transfusion-transmissible infections and allergic reactions[187], transfusions also increase the incidence of complications like thromboembolic events and infections after joint arthroplasty[188-190]. Over the past decades, numerous RCTs and meta-analysis have demonstrated that TXA could effectively reduce requirements for blood transfusion and blood loss in patients undergoing major orthopedic surgeries including spinal surgery[191, 192], joint arthroplasty[186, 193-196] and hip fracture surgery[197]. Besides, several studies reported TXA administration lowered the incidence of periprosthetic joint[198-202] and wound infections[203] after joint arthroplasty. However, strong evidence from sufficiently robust RCTs is still lacking. Despite some concerns regarding increased risks of thrombosis, there is no evidence supporting an elevated incidence of thromboembolic events due to TXA. As such, the routine use of TXA in total joint arthroplasty is recommended in the guidelines developed by multiple American professional societies[204].

1.2.3 Potential therapeutic effects in OA

A series of investigations showed that plasminogen is a key regulator of numerous physiologic and pathophysiologic processes[205, 206]. As a plasminogen inhibitor, TXA has thus been postulated to mediate several effects beyond its anti-fibrinolytic effect. In this regard, previous evidence suggested that TXA exerts anti-inflammatory and immunomodulatory actions. For instance, clinical studies showed that TXA reduced serum inflammatory markers and immunosuppression after cardiac surgery[207] or TKA[208]. TXA also modulated the levels of various cytokines and cellular immune

markers in healthy volunteers[207]. In murine burn models, the release of damage-associated molecular patterns and lung macrophage infiltration were reduced by TXA treatment[209]. The inflammatory nature of OA has been well established, including chronic systemic and local inflammatory aspects. Macrophages are the most predominant immune cells in OA synovium, which contribute to inflammatory activity and cartilage degradation[93]. In this regard, our previous study showed that TXA stimulation in vitro reduced expression of inflammatory cytokines in murine bone marrow-derived macrophages activated with LPS[210]. Furthermore, alterations in subchondral bone play a critical role in OA development, rendering it a suitable potential target for novel OA treatment approaches[51, 63, 211, 212]. In this respect, we also found that TXA stimulation in vitro increased cell proliferation and osteogenic differentiation of bone marrow-derived osteoblasts, while it inhibited the formation of osteoclasts[210]. Based on its immunomodulatory effects in macrophages and its impact on osteoblast and osteoclast differentiation, TXA might thus represent a suitable drug to the treatment and prevention of OA.

1.3 Aims of the work

OA continues to pose a significant clinical challenge today and there is an urgent need for novel therapies to alleviate or, ideally, reverse the progression of OA, especially in the early stage. Current evidence suggests that TXA can potentially be used to treat OA, in which inflammatory responses and bone metabolism are disturbed. In this study, the 4-week anterior cruciate ligament transection (ACLT) model was applied to pre-clinically study the impact of TXA on the early stage of OA in the murine knee.

2. Material and Methods

2.1 Material

2.1.1 Equipment

Name	Model	Manufacture
Analytical balance, 220 g / 0.01 mg	BP221S	Sartorius AG (Goettingen, DE)
Anesthesia machine	UniVet Porta	Groppler Medizintechnik (Deggendorf, DE)
Autoclave	Evo 130/ Evo75	MediTech Service GmbH (Henstedt-Ulzburg, DE)
Cold light source	KL 1500 LCD	Schott AG (Mainz, DE)
Digital Scale, 500 g / 0.01 g	DIPSE TP-500	SSR Produkt GmbH & Co. KG (Oldenburg, DE)
Digital Scale, 600 g / 0.01 g	SCOUT™ PRO SPU602	OHAUS Corporation (Parsippany, US)
Dumont forcep	11231-30	Fine Science Tools GmbH (Heidelberg, DE)
Fine scissor-ToughCut®	14058-11	Fine Science Tools GmbH (Heidelberg, DE)
Fridge	CBNes 6256 PremiumPlus BioFresh NoFrost	Liebherr-Hausgeräte GmbH (Ochsenhausen, DE)
Gas evacuation apparatus	R546W	RWD Life Science Co.,LTD (Guangdong, CN)
Gas filter canister	R510-31	RWD Life Science Co.,LTD (Guangdong, CN)
Graefe forcep	11054-10	Fine Science Tools GmbH (Heidelberg, DE)
Hair clipper	Hatteker RFC-690	Yiwu Kehan Electrical Appliances Co., Ltd. (Yiwu, CN)

Halsey needle holder	12001-13	Fine Science Tools GmbH (Heidelberg, DE)
High-speed centrifuge	5430R	Eppendorf AG (Hamburg, DE)
Histomaster Autotechnicon	2065-2-Z-Di	Rowi Elektronik (Steffenberg, DE)
Incubator	VT 6025	Heraeus Holding GmbH (Hanau, DE)
Induction chamber	UV17006-S	Groppler Medizintechnik (Deggendorf, DE)
Magnetic stirrer	RCT D S000	IKA®-Werke GmbH & Co. KG (Staufen, DE)
Micropipette, 0.1-2.5 µl	Eppendorf Research® plus	Eppendorf AG (Hamburg, DE)
Micropipette, 100-1000 µl	Eppendorf Research® plus	Eppendorf AG (Hamburg, DE)
Micropipette, 20-200 µl	Eppendorf Research® plus	Eppendorf AG (Hamburg, DE)
Micropipette, 2-20 µl	Eppendorf Research® plus	Eppendorf AG (Hamburg, DE)
Microscope	BX50	Olympus optical co., LTD. (Tokyo, JP)
Microscope camera	DP72	Olympus optical co., LTD. (Tokyo, JP)
Paraffin embedding workstation	HistoStar	Thermo Fisher Scientific Inc. (Waltham, US)
pH Meter	FiveEasy Plus FP20	Mettler-Toledo GmbH (Greifensee, CH)
Pipette controller	PIPETBOY	INTEGRA Biosciences AG (Zizers, CH)
Roller mixer	RS-TR05	Phoenix Instrument GmbH (Garbsen, DE)

Rotary microtome	Microm HM 355S	Thermo Fisher Scientific (Shanghai) Instruments Co., Ltd. (Shanghai, CN)
Safety cabinet	630165/170/2	asecos GmbH (Gründau, DE)
ThermoLux thermal pad	464265	Witte + Sutor GmbH (Murrhardt, DE)
Ultrapure water system	PF2XXXXM1	ELGA LabWater (High Wycombe, UK)
Waterbath	1000	pfm Medical AG (Köln, DE)
µCT scanner	vivaCT 80	Scanco Medical AG (Brüttisellen, CH)

2.1.2 Consumables

Name	Manufacturer
Biosphere® plus, filter tips, 0.1-20 µl	SARSTEDT AG & Co. KG (Nümbrecht, DE)
Biosphere® plus, filter tips, 1000 µl	SARSTEDT AG & Co. KG (Nümbrecht, DE)
Biosphere® plus, filter tips, 200 µl	SARSTEDT AG & Co. KG (Nümbrecht, DE)
Cover glasses (24×60 mm)	DWK Life Sciences GmbH (Mainz, DE)
Cutfix® disposable scalpels, # 11	Aesculap AG (Tuttlingen, DE)
DERMAGRIP® nitrile examination gloves	REMESCO Handelsges.m.b.H (Vienna, AT)
Disposable bags	SARSTEDT AG & Co. KG (Nümbrecht, DE)
Embedding cassettes	Engelbrecht Medizin- und Labortechnik GmbH (Edermünde, DE)
Foliodrape® surgical drapes	PAUL HARTMANN AG (Heidenheim, DE)

Injekt®-F syringes, 1 ml	B. Braun Melsungen AG (Melsungen, DE)
MERSILENE™ suture, 4-0	Ethicon Inc. (Raritan, US)
Microscope slides (76×26×1 mm)	Th. Geyer GmbH & Co. KG (Renningen, DE)
Microscope slides (76×26×1 mm)	Paul Marienfeld GmbH & Co. KG (Lauda-Königshofen, DE)
Microtome blades, A35	FEATHER Safety Razor Co., Ltd. (Osaka, JP)
Safe-Lock tubes, 1.5 ml	Eppendorf AG (Hamburg, DE)
SafeSeal reaction tubes, 0.5 ml	SARSTEDT AG & Co. KG (Nümbrecht, DE)
Serological pipettes, 10 ml	SARSTEDT AG & Co. KG (Nümbrecht, DE)
Serological pipettes, 25 ml	SARSTEDT AG & Co. KG (Nümbrecht, DE)
Serological pipettes, 5 ml	SARSTEDT AG & Co. KG (Nümbrecht, DE)
Sterican® needles, 26 G	B. Braun Melsungen AG (Melsungen, DE)
Sterican® needles, 27 G	B. Braun Melsungen AG (Melsungen, DE)
Sterile gauze swabs	Fink & Walter GmbH (Merchweiler, DE)
Sterile surgical blades	Bayha GmbH (Tuttlingen, DE)
Transfer pipettes, 3.5 ml	SARSTEDT AG & Co. KG (Nümbrecht, DE)

2.1.3 Chemicals and reagents

Name	Art.-Nr /PZN	Manufacturer
Acetic acid	A6283	Sigma-Aldrich Corp. (St. Louis, US)
Bepanthen® Eye and nose ointment	01578681	Bayer Vital GmbH (Leverkusen, DE)

Betaisodona® solution, 100 mg/ml	01970433	Mundipharma GmbH (Frankfurt am Main, DE)
Buprenovet® multidose, 0.3 mg/ml	14439053	Richter Pharma AG (Wels, AT)
Clindamycin, 150 mg/ml	04468504	Hikma Pharma GmbH (Martinsried, DE)
DPX new, non-aqueous mounting medium	1.00579	Sigma-Aldrich Corp. (St. Louis, US)
Eosin Y-solution 0.5% alcoholic	1.02439	Sigma-Aldrich Corp. (St. Louis, US)
Ethanol, 70%	2202.5000	Th. Geyer GmbH & Co. KG (Renningen, DE)
Ethanol, 80%	2203.5000	Th. Geyer GmbH & Co. KG (Renningen, DE)
Ethanol, 96%	2209.5000	Th. Geyer GmbH & Co. KG (Renningen, DE)
Ethanol, 100%	2212.5000	Th. Geyer GmbH & Co. KG (Renningen, DE)
Ethylenediaminetetraacetate (EDTA), Titriplex® III	1.08418	Merck KGaA (Darmstadt, DE)
Fast green FCF (C.I. 42053)	1.04022	Sigma-Aldrich Corp. (St. Louis, US)
Fast Red Violet LB Salt	F3381	Sigma-Aldrich Corp. (St. Louis, US)
Formafix 3.5% buffered	70002-3,5-5	Grimm med. Logisitk GmbH (Torgelow, DE)
Fuchsin acid (C.I. 42685)	1.05231	Sigma-Aldrich Corp. (St. Louis, US)
Glycerol	3783.1	Carl Roth Gmbh & Co. KG (Karlsruhe, DE)
Hematoxylin solution A acc. to Weigert	X906.1	Carl Roth Gmbh & Co. KG (Karlsruhe, DE)
Hematoxylin solution B acc. to Weigert	X907.1	Carl Roth Gmbh & Co. KG (Karlsruhe, DE)

Hydrochloric acid (HCl), 25%	1.00312	Merck KGaA (Darmstadt, DE)
Hydrochloric acid (HCl), 37%	1.00314	Merck KGaA (Darmstadt, DE)
Isoflurane	HDG9623	Baxter Deutschland GmbH (Unterschleißheim, DE)
Kaiser′s glycerol gelatine, phenol-free mounting medium	1.08635	Sigma-Aldrich Corp. (St. Louis, US)
Korsolex® extra instrument disinfectant	973802	Paul Hartmann AG (Heidenheim, DE)
Mayer's hemalum solution	1.09249	Sigma-Aldrich Corp. (St. Louis, US)
N,N-Dimethylformamide	D158550	Sigma-Aldrich Corp. (St. Louis, US)
Naphthol AS-MX phosphate disodium salt	N5000	Sigma-Aldrich Corp. (St. Louis, US)
Nitric acid (HNO_3), ≥ 65%	X943.1	Carl Roth Gmbh & Co. KG (Karlsruhe, DE)
Novaminsulfon-ratiopharm®, 500 mg/ml	03530402	Ratiopharm GmbH (Ulm, DE)
Paraffin pellets, M.P. 56-58 °C	40-0021-00	MEDITE Medical GmbH (Burgdorf, DE)
Picric acid solution, saturated	P6744	Sigma-Aldrich Corp. (St. Louis, US)
Safranine O (C.I. 50240)	1.15948	Sigma-Aldrich Corp. (St. Louis, US)
Sodium acetate	1.06268	Merck KGaA (Darmstadt, DE)
Sodium hydroxide (NaOH) pallets	0402	Avantor Performance Materials Poland SA (Gliwice, PL)
Sodium L-tartrate dibasic dihydrate	228729	Sigma-Aldrich Corp. (St. Louis, US)

Sterile saline (NaCl 0.9%)	06063042	B. Braun Melsungen AG (Melsungen, DE)
Sterile water (Aqua)	00088992	B. Braun Melsungen AG (Melsungen, DE)
Sweetener	4009418150907	Goldhand Vertriebsgesellschaft mbH (Düsseldorf, DE)
Tranexamic acid, 100 mg/ml	16533804	Carinopharm GmbH (Elze, DE)
Xylene	360.5000	Th. Geyer GmbH & Co. KG (Renningen, DE)

2.1.4 Buffers and solutions

Name	Composition	
Acetic acid solution, 1%	1% (v/v)	Acetic acid
		ad. H_2O
EDTA solution	0.5 M	Titriplex III
	0.45-0.5 M	NaOH pellets
		ad. H_2O
		pH 7.4
Fast green (FCF) solution, 0.01%	0.01% (w/v)	Fast green FCF
		ad. H_2O
HCl-ethanol solution	5 ml	25% HCl
	95 ml	96% Ethanol
Safranin O solution, 0.1%	0.1% (w/v)	Safranin O
		ad. H_2O
TRAP buffer	0.1 M	Sodium acetate
	0.6% (v/v)	Acetic acid
	50 mM	Sodium L-tartrate dibasic dihydrate
		ad. H_2O
		pH 5

TRAP substrate solution	20 mg	Naphthol AS-MX phosphate disodium salt
	2 ml	*N,N*-Dimethylformamide
	120 mg	Fast Red Violet LB Salt
	200 ml	TRAP buffer
Van Gieson's solution	4.27 mM	Fuchsin acid
	10% (v/v)	Glycerol
	0.5% (v/v)	65% Nitric acid
		ad. saturated Picric acid solution
Weigert's Iron hematoxylin solution	50% (v/v)	Hematoxylin solution A acc. to Weigert
	50% (v/v)	Hematoxylin solution B acc. to Weigert

2.1.5 Software

Name	Version	Manufacturer
Adobe illustrator CC	22.0.0	Adobe Inc. (San Jose, US)
CellSens Entry	1.6	Olympus Corporation (Tokyo, JP)
EndNote	X9	Clarivate Analytics (Philadelphia, US)
GraphPad Prism	9.1.1	GraphPad Software Inc. (La Jolla, US)
Microsoft Office 2019	16.22	Microsoft Corporation (Redmond, US)
OsteoMeasure 7	4.2.0.1	Osteometrics Inc. (Decatur, US)
µCT Evaluation Program	6.6	Scanco Medical AG (Brüttisellen, CH)
µCT Ray	4.0-4	Scanco Medical AG (Brüttisellen, CH)

2.2 Methods

2.2.1 Mice

All animal experiments were approved by the Ethics Committee of the University Medical Center Hamburg-Eppendorf and by the “Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz”, and were performed adherent to the policies and principles established by the animal Welfare Act (Federal Law Gazett I, p.1094) and the national institutes of health guide for care and use of laboratory animals. Twenty-five female C57BL/6J wildtype mice (12-14 weeks old; 20-25 g) were used in this study. The mice were kept in a specific pathogen-free animal facility with standard conditions: 12 h light-dark cycle, room temperature 22-24 °C and relative humidity 40-60%. During the whole experiment, all mice received food and water ad libitum and moved freely. Mice were allowed 14 days to acclimate to the environment before surgeries were conducted.

2.2.2 ACLT-induced knee OA

All mice received the ACLT surgery on the right knee joint to induce OA as described previously[213]. In detail, mice were anesthetized by inhaling isoflurane vaporized with 5% oxygen, using 4% isoflurane for induction and 1-2% isoflurane for maintenance. Before the operation, mice were injected intraperitoneally with a mixture of 150 mg/kg body weight clindamycin for preventing infection and 0.1 mg/kg body weight buprenorphine for analgesia. Eye ointment was applied to protect the eyes from drying. After shaving around the surgical site, the skin was sterilized with Betaisodona solution. For the ACLT surgery, a 5 mm long medial parapatellar incision was performed in the right hindlimb, followed by lateral dislocation of the patella to expose the knee joint. Subsequently, the knee was flexed to find and cut off the ACL with a scalpel. The complete transection of ACL was confirmed by a positive anterior drawer test. After that, the patella was relocated, and the joint capsule, muscle and skin were sutured layer by layer. For postoperative care, 0.3 ml saline was injected subcutaneously to prevent postoperative dehydration. Thereafter, the mice were placed in recovery racks with a temperature of 28 °C overnight and given drinking water substituted with 1 mg/ml metamizole (Novaminsulfon-ratiopharm®) for 3 days. All the mice had healthy contralateral knees without surgical interventions.

2.2.3 Study design

Mice were randomized into one of the four groups: systemic vehicle (n=6), systemic TXA (n=7), topical vehicle (n=6) and topical TXA (n=6). The mice in the systemic TXA group were treated with 100 mg/kg body weight TXA by daily intraperitoneal (IP) injection for 4 weeks, starting at the day of surgery. The mice in the topical TXA group were treated with 5 µl of 20 mg/ml TXA by once-weekly intra-articular (IA) injection into the operated knees for 4 weeks, which also started at the day of surgery. The equivalent volume of vehicle (0.9% saline) was administered in an identical manner to their corresponding vehicle groups. Importantly, even though some concerns were raised about the toxicity of high-concentration TXA against chondrocytes, tenocytes, synoviocytes and osteoblasts[214-216], TXA concentrations of less than 20 mg/ml are expected to be safe[216-219]. Therefore, the doses of TXA applied in this study were within the safe concentration range. The mice were sacrificed 4 weeks after surgery under deep anesthesia with isoflurane inhalation, followed by sampling of the operated and the contralateral healthy knee joints. The knee joints were fixed in 10% formalin (FormaFix) for 24 hours without internal or external rotation, and then processed for micro-computed tomography (µCT) and histological analysis. The experimental strategy is present in Figure 1.

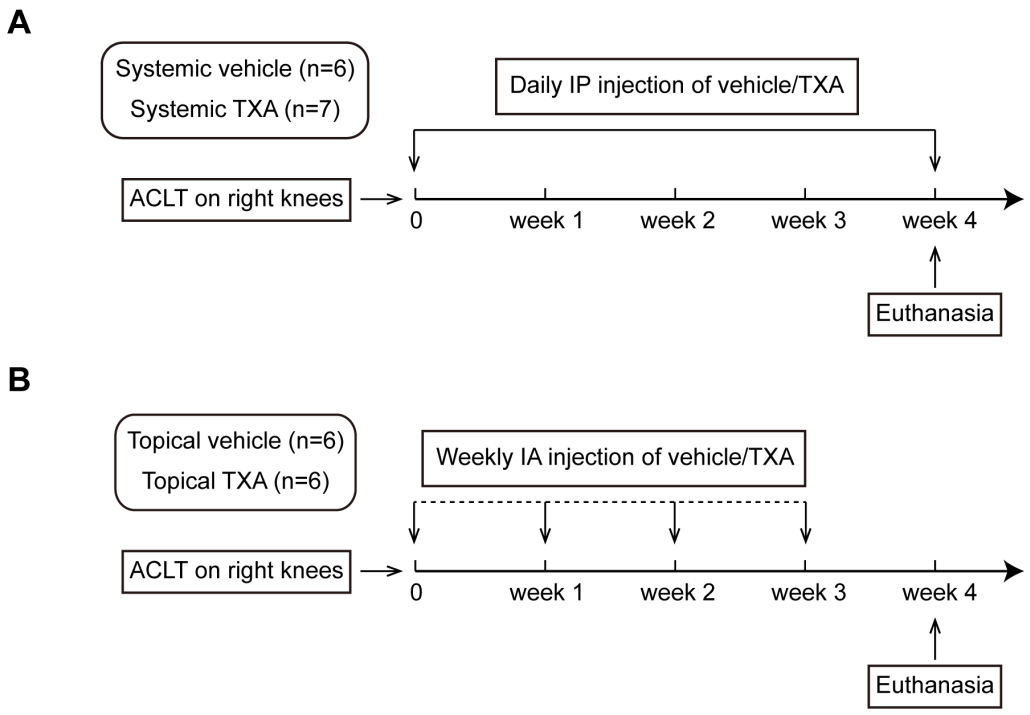

Figure 1. Schematic of the experimental protocol. (**A**) The experimental procedure of systemic treatment groups. (**B**) The experimental procedure of topical treatment groups. ACLT, anterior cruciate ligament transection; TXA, tranexamic acid; IP, intraperitoneal; IA, intra-articular.

2.2.4 μCT analysis

After fixation, the knee samples were scanned by μCT at 70 kVp, 113 μA and a 400 ms integration time with a voxel size of 15.6 μm. The three-dimensional (3D) models were reconstructed, and representative 3D pictures were generated by the program μCT Ray V4.0-4. The microarchitectural parameters of osteophyte and tibial subchondral bone were measured and calculated using μCT Evaluation Program V6.6. The assessed parameters included osteophyte volume (mm^3), bone volume fraction (BV/TV; %), trabecular number (Tb.N; mm^{-1}), trabecular thickness (Tb.Th; mm), and trabecular separation (Tb.Sp; mm).

2.2.5 Histological analysis

2.2.5.1 Paraffin embedding

After μCT scanning, the knee samples were processed for histological analysis. In detail, the knee samples were decalcified in 0.5 M EDTA (pH 7.4) solution for 4 days at 4 °C in a roller mixture, with a solution change at the 2^{nd} day. The decalcified knees were dehydrated in an Autotechnicon with sequential concentrations of ethanol and xylene baths. Subsequently, samples were embedded into paraffin blocks and sliced into 4 μm coronal sections. The program of the Autotechnicon is detailed in Table 1.

Table 1. Program of Autotechnicon

Reagent	Time (hour)	Times
70% ethanol	1	2
80% ethanol	1	1
96% ethanol	1	1
100% ethanol	1	2
Xylene	1	2
Paraffin (58-60 °C)	1	3

2.2.5.2 Hematoxylin and eosin (H&E) staining

In the H&E staining, hematoxylin stains nuclei blue and eosin stains the cytoplasm and extracellular matrix pink. The paraffin slices were stained with a basic protocol including dewaxing, rehydration, hematoxylin staining, differentiating, eosin staining,

dehydration, clearing and mounting. The detailed procedures are presented in Table 2.

Table 2. Steps of the H&E staining

Reagent/ Equipment	Time (min)	Times/Endpoint
60 °C Incubator	15-30	1
Xylene	5	3
100% Ethanol	2	2
96% Ethanol	2	1
80% Ethanol	2	1
70% Ethanol	2	1
Distilled water	2	1
Mayer's hemalum solution	6	1
Tap water	2	1
HCl-ethanol solution	Dipping	2-3
Running tap water	10	Blueing
Eosin Y-solution 0.5% alcoholic	3	1
80% Ethanol	Differentiation/Clearing	/
96% Ethanol	1	1
100% Ethanol	2	2
Xylene	2	3
DPX new mounting medium	Mounting	/

2.2.5.3 Tartrate-resistant acid phosphatase (TRAP) staining

TRAP is enriched in osteoclasts, making it suitable to identify these cells[220]. In the TRAP staining, osteoclasts are stained red with multiple nuclei (n > 3). The paraffin slices were stained with a basic protocol including dewaxing, rehydration, TRAP staining, hematoxylin staining, clearing and mounting. The detailed steps were performed as described in Table 3.

Table 3. Steps of the TRAP staining

Reagent/ Equipment	Time (min)	Times/Endpoint
60 °C Incubator	15-30	1
Xylene	5	3
100% Ethanol	2	2
96% Ethanol	2	1
80% Ethanol	2	1
70% Ethanol	2	1
Distilled water	2	1
TRAP substrate solution at 37 °C with shaking	30-60	1
Distilled water	Dipping	1
Mayer's hemalum solution, 1:10 diluted	1	1
Running tap water	10	Blueing
Kaiser′s glycerol gelatine mounting medium	Mounting	/

2.2.5.4 Bone-Inflammation-Cartilage (BIC) staining

The BIC staining was used to better identify the structures within the knee joints, such as bone, cartilage and synovium. In the BIC staining, bone is stained purple, cartilage is stained red, and synovium is stained blue to purple. The paraffin slices were stained as previously described[221]. The steps are described in detail in Table 4.

Table 4. Steps of the BIC staining

Reagent/ Equipment	Time (min)	Times/Endpoint
62 °C Incubator	60	1
Xylene	5	3
100% Ethanol	2	2
96% Ethanol	2	2
Weigert's Iron hematoxylin	10	1
Running tap water	30	Blueing
Van Gieson's Solution	15	1
Distilled water	Gently rinsing	Removing excess stain
0.01% Fast Green	3	1
1% Acetic Acid	10-15 sec	1
0.1% Safranin O	5	1
96% Ethanol	2	2
100% Ethanol	2	2
Xylene	2	3
DPX new mounting medium	Mounting	/

2.2.5.5 Histomorphometry

The histomorphometric analysis for tibial subchondral bone was performed using an OsteoMeasure system connected to a BX50 microscope equipped with a DP72 camera. For the trabecular area in tibial subchondral bone, the static indices of osteoblasts were quantified in H&E-stained sections and the indices of osteoclasts were measured in TRAP-stained sections. The assessed parameters included osteoblast surface per bone surface (Ob.S/BS; %), number of osteoblasts per bone perimeter (Ob.N/B.Pm; mm^{-1}), osteoclast surface per bone surface (Oc.S/BS; %) and number of osteoclasts per bone perimeter (Oc.N/B.Pm; mm^{-1}).

For the measurement of cartilage and SBP, a 400 μm × 300 μm region of interest was defined in the center of medial tibial plateau in H&E-stained sections[222]. The boundaries of hyaline cartilage (HC), calcified cartilage (CC) and SBP were marked

manually, and the thickness was calculated by the OsteoMeasure system automatically. The calculated parameters included the ratio of HC thickness to CC thickness (HC.Th/CC.Th), percentage of HC area per total cartilage area (HC area/total cartilage area; %), and SBP thickness (SBP.Th; μm).

2.2.5.6 Histopathological scoring

To evaluate the pathological changes in knee joints, the semi-quantitative histopathological scoring was performed with the BIC-stained sections. For each sample, four joint quadrants were scored, including the medial tibial plateau (MTP), the medial femoral condyle (MFC), the lateral tibial plateau (LTP), and the lateral femoral condyle (LFC).

2.2.5.6.1 OARSI scoring

The OARSI scoring system was used to grade cartilage degeneration (range 0-6)[223, 224]. The scoring details are given in Table 5.

Table 5. Description of the OARSI score

Grade	Changes
0	Normal
0.5	Loss of Safranin-O without structural changes
1	Small fibrillations without loss of cartilage
2	Vertical clefts down to the layer immediately below the superficial layer and some loss of surface lamina
3	Vertical clefts/erosion to the calcified cartilage extending to < 25% of the articular surface
4	Vertical clefts/erosion to the calcified cartilage extending to 25-50% of the articular surface
5	Vertical clefts/erosion to the calcified cartilage extending to 50-75% of the articular surface
6	Vertical clefts/erosion to the calcified cartilage extending > 75% of the articular surface

2.2.5.6.2 Synovitis scoring

The synovitis score was used to evaluate the synovial lining thickness and cellular density in the synovial stroma (range 0-6)[225, 226]. The scoring was done as described in Table 6.

Table 6. Description of the synovitis score

Grade	Changes	
	Enlargement of synovial lining cell layer	**Density of cells in synovial stroma**
0	1-2 cell layers	Normal
1	3-4 cell layers	Slightly increased
2	5-9 cell layers	Moderately increased
3	≥ 10 cell layers	Greatly increased

2.2.5.6.3 Bone erosion scoring

The bone erosion score was used to access the cortical bone erosion caused by pannus (range 0-3)[227]. The details of the score points are as presented in Table 7.

Table 7. Description of the bone erosion score

Grade	Changes
0	None
1	Partial thickness loss of cortical bone.
2	Focal complete loss of cortical bone - communication with marrow cavity at one small “vascular” communication site.
3	Widespread complete loss of cortical bone - communication with marrow cavity at multiple sites or broad area loss of cortical bone.

2.2.5.6.4 Osteophyte scoring

The osteophyte score was used to evaluate osteophyte formation, including osteophyte size and maturity (range 0-6)[228]. The scoring method is described in Table 8.

Table 8. Description of the osteophyte score

Grade	Changes	
	Size	**Maturity**
0	None	None
1	< 1 × the thickness as the adjacent cartilage	Predominantly cartilaginous
2	1-3 × the thickness as the adjacent cartilage	Mixed cartilage and bone with active vascular invasion and endochondral ossification
3	> 3 × the thickness as the adjacent cartilage	Predominantly bone

2.2.6 Statistical analysis

Data analysis and figure plotting were performed using GraphPad Prism. Mean and standard deviation (SD) are plotted with individual data points in each graph. In this study, inconsistent sample size between parameters is possible, due to loss or destruction of samples during processing. For multiple-group comparisons, data were analyzed by one-way or two-way ANOVA test as indicated, followed by Tukey's post-hoc tests. The significance level was set at $P < 0.05$.

3. Results

3.1 Both systemic and topical TXA treatment ameliorate ACLT-induced cartilage degeneration

First, we examined whether TXA may protect the articular cartilage from degeneration in ACLT knees of wildtype mice. In systemic and topical vehicle groups, the articular cartilage in OA knees showed obviously damaged integrity and loss of proteoglycans at 4 weeks after surgery, which was attenuated in both systemic and topical TXA groups (Figure 2A). The total OARSI scores of the operated knees from systemic TXA and topical TXA treated mice were significantly decreased when compared with the corresponding vehicle treated mice (Figure 2B). Moreover, the increased OARSI scores of the MTP was completely reversed by both systemic and topical TXA treatment (Figure 2B). In case of the other quadrants, the OA knees from mice treated with topical TXA showed significantly lower scores of the MFC and the LFC than those from mice treated with topical vehicle. No difference in the scores of the MFC, the LTP and the LFC between systemic vehicle and TXA groups was observed.

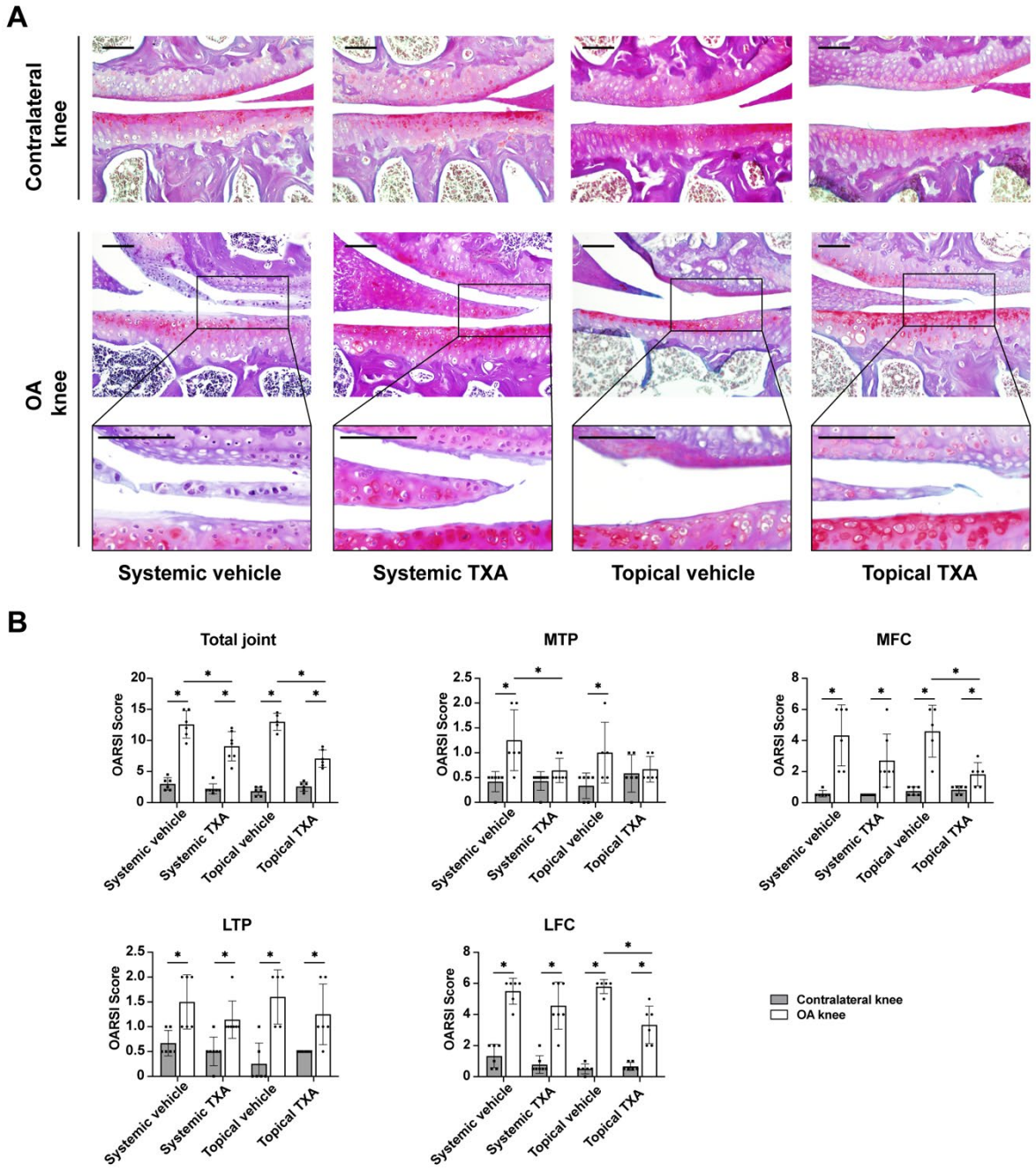

Figure 2. Systemic and topical TXA treatment attenuate articular cartilage degradation after ACLT. (**A**) Representative images of BIC staining of the medial knee joint 4 weeks after ACLT (scale bar = 100 μm). (**B**) OARSI scores of articular cartilage in total joint, MTP, MFC, LTP, and LFC. OA, osteoarthritis; TXA, tranexamic acid; ACLT, anterior cruciate ligament transection; BIC, Bone-Inflammation-Cartilage; OARSI, Osteoarthritis Research Society International; MTP, medial tibial plateau; MFC, medial femoral condyle; LTP, lateral tibial plateau; LFC, lateral femoral condyle. The data are expressed as the means ± SD, n = 5-7 per group as indicated (two-way ANOVA followed by Tukey's post-hoc test). *P < 0.05 compared as denoted by bar.

Next, we further evaluated the changes in chondro-osseous junctional region[229] in the MTP through histomorphometric analysis. The articular cartilage is separated into the HC and the CC by the tidemark, which is considered as a metabolically active front of calcification[41, 42]. With OA progression, the CC thickens and the HC becomes thinner due to the tidemark duplication. At 4 weeks after surgery, the OA knees from vehicle treated mice presented significantly decreased HC/CC thickness ratio and HC/total cartilage area ratio, which was reversed by both systemic and topical TXA treatment (Figure 3A, B). The microarchitecture of the SBP was also assessed with H&E staining. During early OA, the porosity and thickness of SBP are altered, which play important roles in substance interchange across the osteochondral interface[50, 54-56]. As displayed in Figure 3C, the SBP thickness was significantly decreased in OA knees from vehicle groups, consistent with previous reports[71, 230]. Most importantly, this reduction was reversed by systemic TXA treatment but not topical treatment.

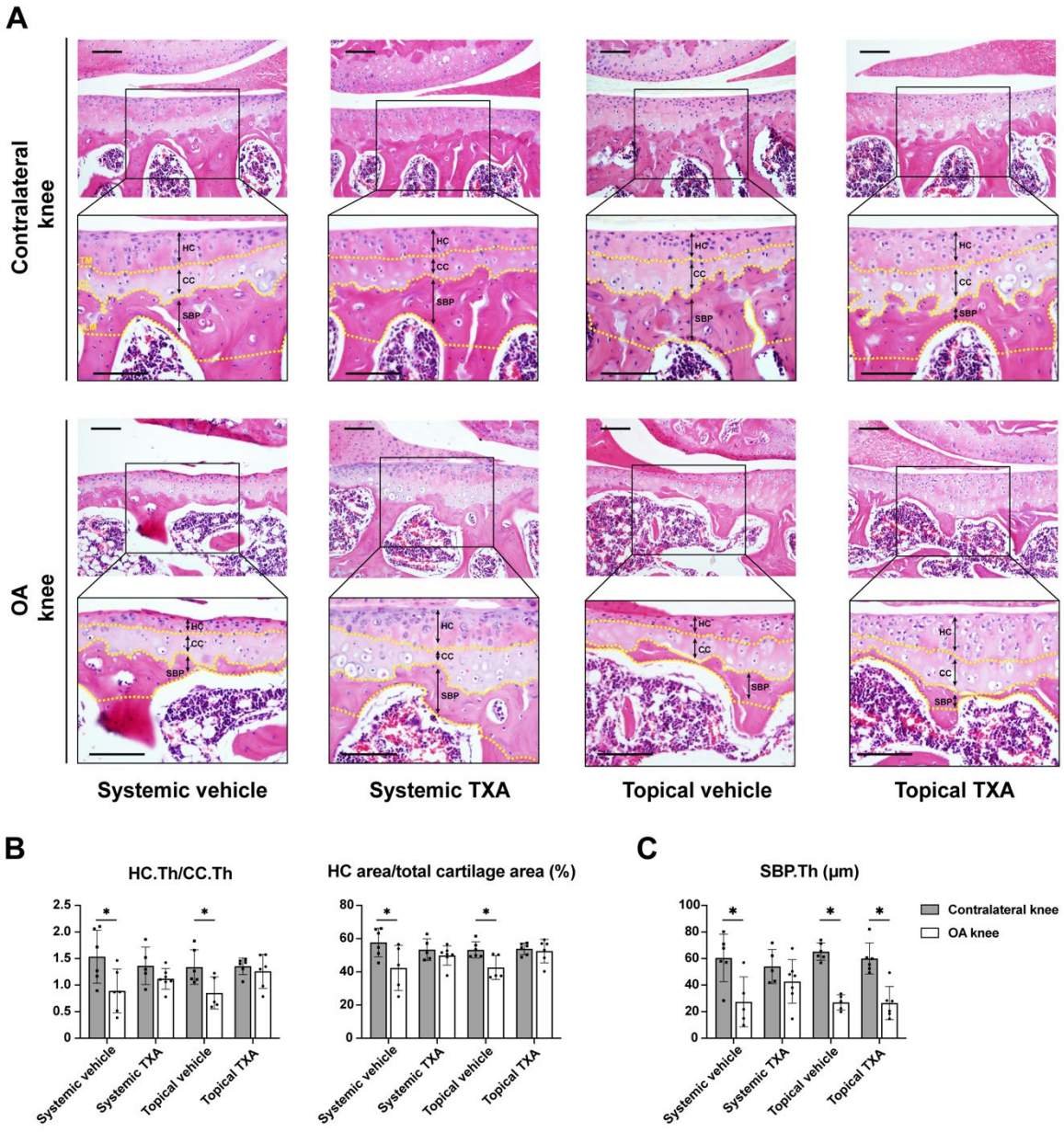

Figure 3. Systemic and topical TXA treatment preserve hyaline cartilage after ACLT. (**A**) The representative images of H&E staining on the medial tibial cartilage at 4 weeks after ACLT (scale bar = 100 μm). A box (400 μm × 300 μm) was centred on the medial tibial plateau. The TM, CL and LM of the SBP are marked by yellow dotted lines. The HC, CC and SBP are labelled with black double-headed arrows. (**B**) Quantitative analysis of the HC.Th/CC.Th and percentage of HC area out of total cartilage area. (**C**) Quantitative analysis of SBP.Th. OA, osteoarthritis; TXA, tranexamic acid; ACLT, anterior cruciate ligament transection; TM, tidemark; CL, cement line; LM, lower margin; SBP, subchondral bone plate; HC, hyaline cartilage; CC, calcified cartilage; HC.Th/CC.Th, ratio of HC thickness to CC thickness. All data are expressed as the means ± SD, n = 5-7 per group as indicated (two-way ANOVA followed by Tukey's post-hoc test). *P < 0.05 compared as denoted by bar.

3.2 Systemic TXA treatment reverses ACLT-induced subchondral bone loss whereas topical treatment does not

The effects of TXA on the microarchitecture of tibial subchondral trabecular bone were therafter analyzed by H&E staining and μCT (Figure 4A). In both vehicle groups, trabecular bone mass and trabecular numbers were significantly lower, whereas trabecular separation was higher in OA knees 4 weeks after surgery compared to the contralateral knees (Figure 4B). Moreover, the reduction in trabecular bone mass was reversed by systemic TXA treatment but not by topical treatment. Neither systemic nor topical TXA treatment significantly altered trabecular numbers or separation.

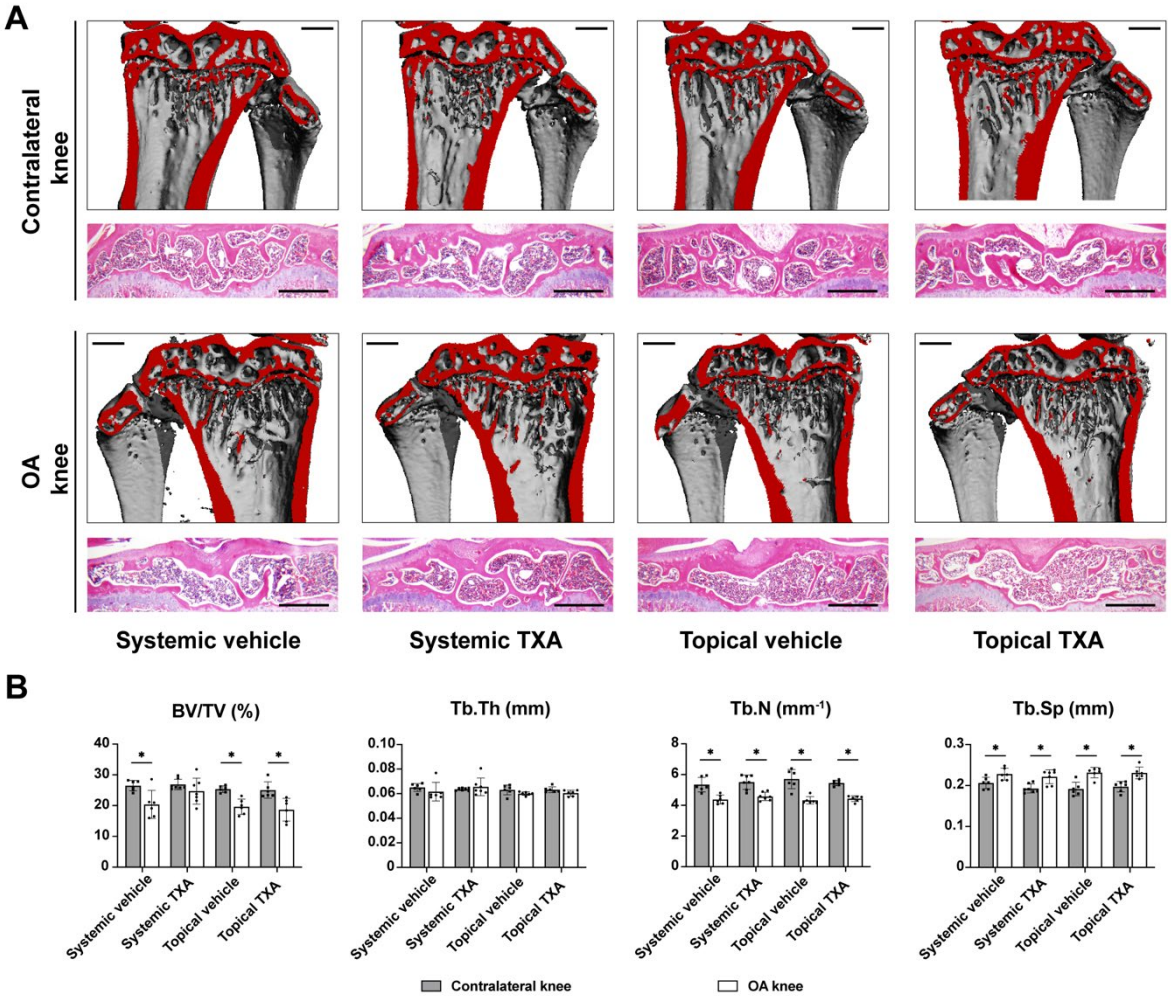

Figure 4. Systemic TXA treatment protects against subchondral bone loss after ACLT. (**A**) Representative coronal images of 3D-μCT reconstruction and H&E staining of tibial subchondral bone 4 weeks after ACLT (scale bar = 500 μm). (**B**) μCT quantitative analysis of tibial subchondral bone for BV/TV, Tb.N, Tb.Th, and Tb.Sp. OA, osteoarthritis; TXA, tranexamic acid; ACLT, anterior cruciate ligament transection; H&E, hematoxylin and eosin; BV/TV, bone volume fraction; Tb.N, trabecular number; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation. The data are expressed as the

means ± SD, n = 6-7 per group as indicated (two-way ANOVA followed by Tukey's post-hoc test). *P < 0.05 compared as denoted by bar.

3.3 Systemic TXA treatment inhibits ACLT-induced abnormal bone remodelling in subchondral bone whereas topical treatment does not

In the healthy organism, bone microstructure and quality are maintained by the well-balanced activities of bone-forming osteoblasts and bone-resorbing osteoclasts, the two key cell types involved in bone remodelling. Therefore, cellular bone histomorphometry of osteoclast and osteoblast parameters were evaluated to further account for the changes we found in the SBP and subchondral trabecular bone in OA. Increased bone resorption during early and progressive OA was reported in both mice and patients in previous studies[71, 230, 231]. In agreement, TRAP-activity staining indicated elevated osteoclast numbers and surface in tibial subchondral bone of vehicle OA knees compared to those in healthy contralateral knees (Figure 5A, B). Furthermore, systemic TXA treatment resulted in a normalization of osteoclast parameters in subchondral bone of OA knees, while topical TXA treatment had no effect (Figure 5A, B). In contrast, bone formation parameters including osteoblast numbers and surface in tibial subchondral bone were unaltered in vehicle OA groups (Figure 5C). Likewise, neither topical nor systemic TXA treatment significantly affected osteoblast parameters.

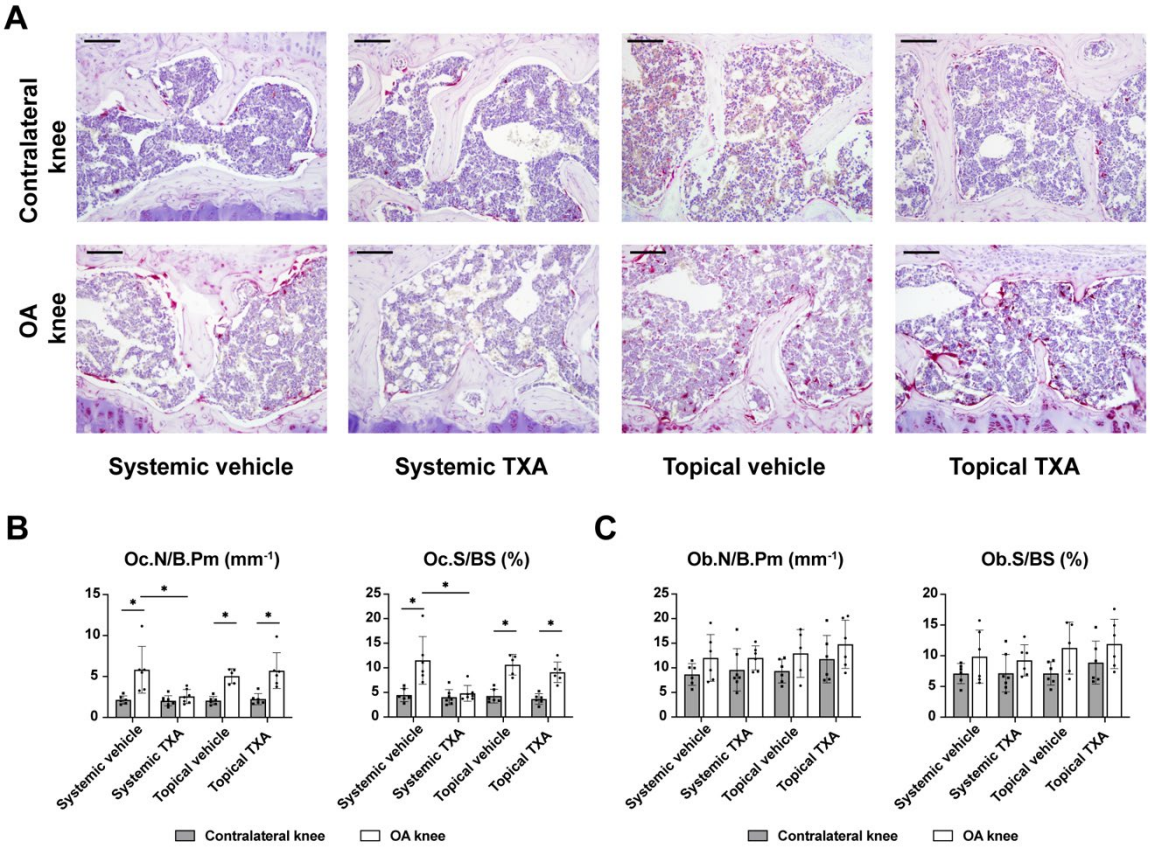

Figure 5. Systemic TXA treatment inhibits ACLT-induced bone resorption in subchondral bone. (**A**) Representative TRAP-stained histological sections of tibial subchondral bone at 4 weeks after ACLT. Scale bar = 100 μm. (**B**) Quantitative analysis of Oc.S/BS and Oc.N/B.Pm. (**C**) Quantitative analysis of Ob.S/BS and Ob.N/B.Pm. OA, osteoarthritis; TXA, tranexamic acid; ACLT, anterior cruciate ligament transection; TRAP, tartrate-resistant acid phosphatase; Oc.S/BS, osteoclast surface per bone surface; Oc.N/B.Pm, number of osteoclasts per bone perimeter; Ob.S/BS, osteoblast surface per bone surface; Ob.N/B.Pm, number of osteoblasts per bone perimeter. The data are expressed as the means ± SD, n = 5-7 per group as indicated (two-way ANOVA followed by Tukey's post-hoc test). **P* < 0.05 compared as denoted by bar.

3.4 Both systemic and topical TXA treatment alleviate synovitis in experimental OA

It is well-established that synovitis is a hallmark manifestation and promoting factor of OA. Therefore, we investigated the effects of TXA on synovial inflammation. In OA knees from vehicle groups, BIC-staining demonstrated significant inflammatory changes in the synovial tissues, such as synovial hyperplasia, pannus formation and pathological bone erosion (Figure 6A). In addition, the presence of TRAP-positive

osteoclasts in the hypertrophic synovium in close vicinity to eroded cortical bone was also observed in OA knees from vehicle groups. These pathological changes were alleviated in OA knees of both systemic and topical TXA groups (Figure 6A). Next, analysis of the synovial hyperplasia and bone erosion were performed by using semi-quantitative synovitis and bone erosion scores, respectively (Table 6,7). First, the total synovitis scores of OA knees were significantly lower in systemic and topical TXA groups compared to the corresponding vehicle groups (Figure 6B). Similar results were obtained when analyzing the femoral and tibial synovitis scores separately. And second, both systemic and topical TXA treatment were found to significantly decreased the total bone erosion scores compared to vehicle treatment (Figure 6C). However, while systemic TXA only reduced tibial but not femoral bone erosion, the opposite phenomenon was observed in the topical TXA group and respective control.

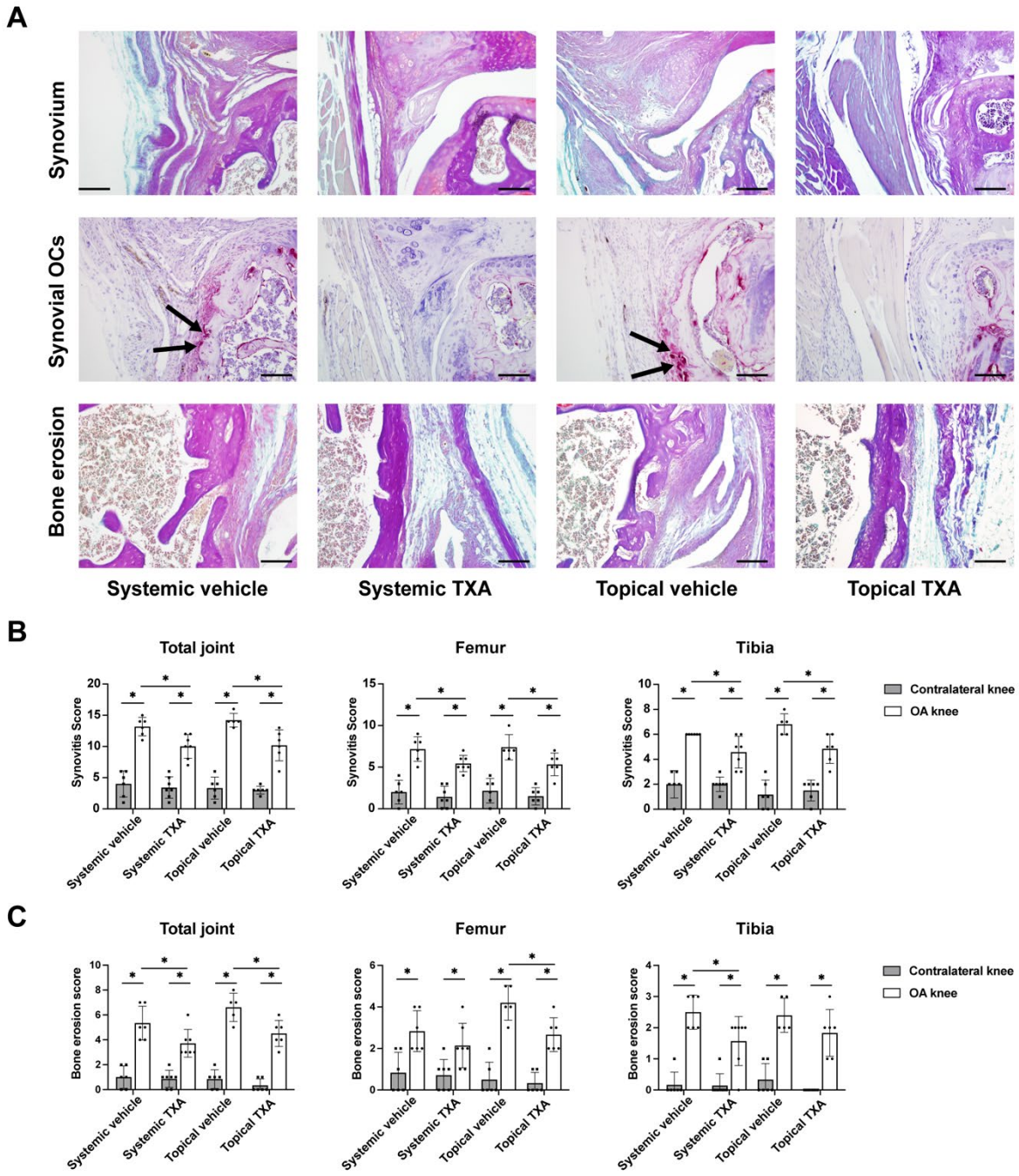

Figure 6. Systemic and topical TXA treatment alleviate synovitis after ACLT. (**A**) Representative BIC and TRAP staining images of the tibial knee joint for synovium, bone erosion and synovial osteoclasts (scale bar = 100 μm). Black arrows indicate TRAP-positive osteoclasts. (**B**) Synovitis scoring in total joint, femoral side and tibial side. (**C**) Bone erosion scoring in total joint, femoral side and tibial side. OA, osteoarthritis; OCs, osteoclasts; TXA, tranexamic acid; ACLT, anterior cruciate ligament transection; BIC, Bone-Inflammation-Cartilage; TRAP, tartrate-resistant acid phosphatase. The data are expressed as the means ± SD, n = 5-7 per group as indicated (two-way ANOVA followed by Tukey's post-hoc test). *$P < 0.05$ compared as denoted by bar.

3.5 Systemic TXA treatment reduces tibial osteophyte formation in ACLT models whereas topical treatment does not

Osteophyte formation leads to pain and limitation of free movement in affected joints. Osteophytes may form already at an early stage of OA and serve as a typical radiographic and histological features of OA[232]. Therefore, we applied µCT scanning and semi-quantitative osteophyte scoring to radiologically measure the osteophyte volume and histologically evaluate the degree of osteophyte formation, respectively (Figure 7A; Table 8). As evidenced by µCT analysis, the tibial osteophyte volume was significantly decreased in the systemic TXA group compared to the systemic vehicle group, while no significant difference was found in the femoral compartment between these two groups (Figure 7B). In contrast, neither femoral nor tibial osteophyte volume was altered by topical TXA treatment. The subsequent histological evaluation supported the µCT results, demonstrating a reduction in tibial osteophyte scores only in the systemic but not the topical TXA group (Figure 7C).

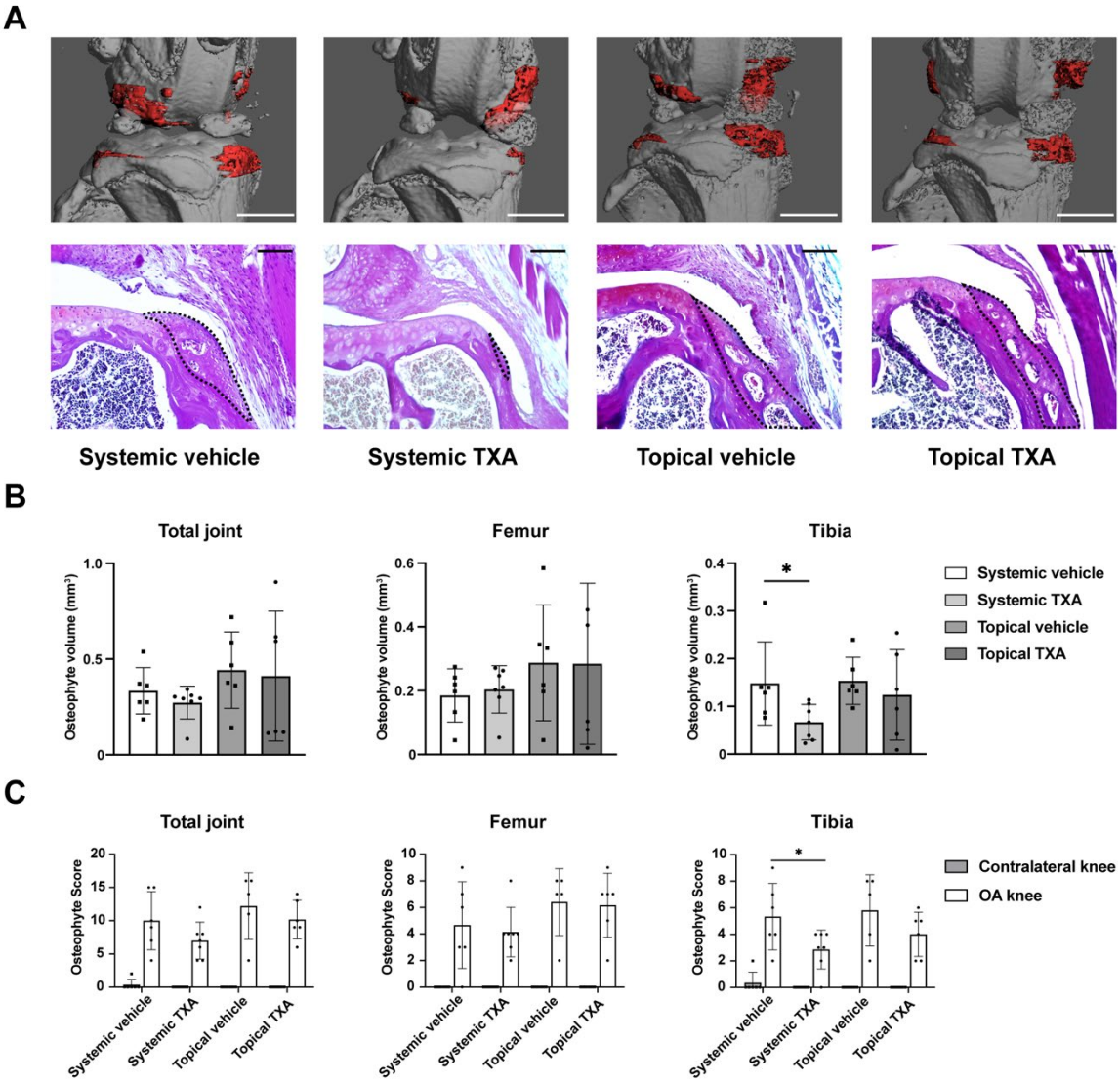

Figure 7. Systemic TXA treatment reduces tibial osteophyte formation after ACLT. (**A**) Representative images of 3D-μCT reconstruction and BIC staining for osteophytes at 4 weeks after ACLT. For upper panel, osteophytes are shown in red (scale bar = 1 mm); for lower panel, osteophytes are marked by black dotted line (scale bar = 100 μm). (**B**) μCT quantitative analysis of osteophyte volume in total joint, femoral compartment and tibial compartment. n = 6-7 per group as indicated (one-way ANOVA followed by Tukey's post hoc test). (**C**) Osteophyte scoring of total joint, femoral compartment and tibial compartment. n = 5-7 per group as indicated (two-way ANOVA followed by Tukey's post-hoc test). OA, osteoarthritis; TXA, tranexamic acid; ACLT, anterior cruciate ligament transection; BIC, Bone-Inflammation-Cartilage; The data are expressed as the means ± SD, *P < 0.05 compared as denoted by bar.

4. Discussion

The current clinical treatment options for OA are mainly symptomatic, i.e., targeting pain with anti-inflammatory and analgesic drugs, eventually requiring joint replacement if the disease progresses[233]. There is thus an urgent need for novel therapies to alleviate or, ideally, reverse the progression of OA, especially in the early stage. In this study, we demonstrate a beneficial and therapeutic effect of TXA in the early stage of experimental, post-traumatic OA. Both systemic and topical TXA treatment alleviated articular cartilage degeneration and lowered synovial inflammation induced by ACLT in mice. Moreover, systemic but not topical administration of TXA normalized subchondral bone remodelling and protected from osteophyte formation in OA knees.

TXA functions as a reversible plasminogen-inhibitor and, based on its high efficacy and safety, represents the most widely employed antifibrinolytic drug. It is routinely used for controlling excessive bleeding in clinical practice with only minimum risk of vascular adverse effects[143]. There is no consensus regarding the optimal doses and administration routes of TXA in various off-label indications such as trauma and orthopedic surgery. For example, TXA can be administrated systemically or topically into the operated sites during joint replacement and ACL reconstruction surgery[234-236]. For this reason, TXA was applied through systemic and topical routes in this pre-clinical study, in order to investigate the effects of TXA in preventing and/or treating post-traumatic joint degeneration in mice.

An essential finding of the current study is that ACLT-induced cartilage degeneration was significantly alleviated in the total joint by both systemic and topical TXA treatment and even reversed in the MTP. Although this clearly indicates that TXA may be beneficial to modulate the course of OA progression, the question remains through which exact mechanism TXA exerts these effects. In this regard, cartilage homeostasis during OA is affected by primary alterations of chondrocyte function, in addition to secondary effects induced by adjacent tissues including the synovium and the subchondral bone[237]. Previous in vitro experiments showed that TXA was cytotoxic to chondrocytes at concentrations higher than 20 mg/ml, inhibiting their viability, decreasing their expression of chondrogenic marker genes and leading to cell death[216-218, 238, 239]. In turn, TXA at concentrations lower than 20 mg/ml was reported to exert no measurable effect on chondrocytes[217, 239]. Therefore, both the systemic and topical TXA doses applied in this study were within the safe concentration range,

yet TXA may not directly affect chondrocytes homeostasis. Even though such a direct effect of TXA on cartilage cannot be excluded in vivo, TXA is more likely to exert indirect chondroprotective effects via both plasminogen-dependent and/or -independent pathways, for instance by modulating inflammatory responses and direct effects on bone remodelling[240, 241].

This notion is strengthened by our further data. We found that both systemic and topical TXA significantly alleviated synovial hyperplasia and bone erosion, indicating that TXA controlled the inflammatory response in OA. In particular, synovial inflammation appears early in OA and persists throughout the course of the disease[84]. It was reported that the concentrations of pro-inflammatory cytokines in synovial fluid correlate with the degree of knee OA[96]. Moreover, synovitis in knees increases the risk for cartilage loss and development of OA[97, 98], suggesting that synovitis is an independent cause promoting OA progression. Mechanistically, activated synoviocytes were shown to attract immune cells and release inflammatory mediators in addition to proteolytic enzymes[90], contributing to cartilage breakdown and driving the erosion of adjacent bone[91-93]. Accordingly, TXA may preserve cartilage integrity by reducing synovitis.

A growing body of evidence suggests that TXA exerts anti-inflammatory and immunomodulatory effects. Previous clinical studies showed that TXA could reduce serum inflammatory markers and immunosuppression after cardiac surgery[207] or TKA[208]. TXA also modulated the levels of various cytokines and cellular immune markers in healthy volunteers[207]. In murine burn models, the release of damage-associated molecular patterns and lung macrophage infiltration were reduced by TXA treatment[209]. The macrophages are the most abundant immune cell populations infiltrating OA synovium[242] and contribute to the production of most inflammatory cytokines[87]. In this regard, our previous study showed that TXA reduced the expression of inflammatory markers such as IL-1α and IL-1β in murine bone marrow-derived macrophages in vitro[210]. Furthermore, TXA reduced the expression of CD14, which plays important roles in the functions of innate immunity and inflammatory activity in macrophages[243]. It was reported that CD14 deficiency protects against cartilage degradation in destabilized medial meniscus (DMM) mouse models, likely through regulating macrophage polarization[244]. Thus, this could provide a possible mechanism through which TXA exerts its anti-inflammatory effect on the inflamed

synovium. On the other hand, CD14 is also essential for osteoclastogenesis[245], and CD14 deficiency influences the ability of bone marrow precursors to differentiate into osteoclasts[246]. Even though the degree of inflammation in OA is lower than that in rheumatoid arthritis, the pannus invading adjacent bone tissue in OA joints is characterized by the presence of TRAP-positive osteoclasts[212]. In this regard, we found less TRAP-positive osteoclasts present in the contact area between inflamed synovium and eroded cortical bone in the TXA groups compared to the vehicle groups. Previous studies demonstrated that the infiltrating osteoclasts in the inflammatory synovium were primarily derived from $CD14^+$ monocytes/macrophages[247, 248]. Monocytes cultured with OA synovial fluid showed increased osteoclastogenesis and bone resorption due to the presence of cytokines such as receptor activator of nuclear factor κ-B ligand (RANKL) and IL-1[249]. Thus, a possible mechanism by which TXA might reduce synovitis induced bone erosion could be through inhibiting the differentiation of synovial macrophages into osteoclasts.

In line with other studies, we additionally found thinning of the SBP and loss of subchondral trabecular bone in the early stage of OA[60]. The subchondral bone serves a variety of critical functions including mechanical support, nutrient supply and metabolic regulation of cartilage[58]. Increasing evidence is accumulating to support the fact that alterations in subchondral bone potently affect the behavior of the overlying articular cartilage[51]. Previous studies showed that restoring the alterations of subchondral bone is able to slow down cartilage degeneration during OA[63, 69-73]. In the present study, subchondral bone loss in OA knees was significantly improved by systemic TXA treatment. However, the microarchitecture of subchondral bone was not altered by topical TXA treatment.

Bone microstructural integrity is maintained by coupling bone formation to resorption, and an uncoupling of bone remodelling in subchondral bone is observed during OA[59]. Previous studies showed that subchondral bone loss in the early stage of OA was caused by increased bone remodelling, with enhanced osteoclast activity and increased net bone resorption[61, 62]. In agreement, we also found in this study that the ACLT surgery significantly increased subchondral osteoclast number and surface in operated knees compared to contralateral knees, while osteoblast parameters were not affected. Following systemic TXA treatment, the excessive osteoclast parameters in OA knees were normalized, while no alterations in osteoblast parameters were

observed. This is in part inconsistent with our previous results that TXA not only inhibited osteoclastogenesis, but also promoted extracellular matrix mineralization in bone marrow-derived osteoblasts in vitro[210]. This indicates that during experimental OA, the anti-resorptive effect of TXA is indeed more relevant to disease progression than its anabolic function in vivo. Consistent with the absence of microstructural alterations, topical TXA treatment did neither affect osteoclast and osteoblast parameters in subchondral bone nor the formation of osteophytes. The latter observation is most likely explained by the fact that only systemic TXA affected bone remodelling. Here, osteophytes usually form secondary to abnormal bone turnover, and early inhibition of bone remodelling was shown to reduce osteophyte formation in several studies[69, 250-253]. Together, our results indicate that systemic but not topical TXA restores abnormal subchondral bone remodelling through inhibiting excessive osteoclast activity while not affecting osteoblast function and bone formation.

Intra-articular injections are widely used in clinical practice to treat degenerative joint diseases. Even though intra-articular injections are associated with the risk of infections[254], there are several advantages when compared with systemic administration. For instance, topical administrations result in high initial pharmaceutical concentrations within the injected joint, which can be rapidly cleared[255]. Therefore, lower dosing of respective drugs is possible, reducing the risk for potential systemic adverse effects. Nevertheless, this may also account for our observation that systemic TXA normalized excessive bone remodelling in the subchondral bone whereas topical TXA did not. Following intra-articular injections, TXA may not achieve a sufficiently effective concentration to regulate bone remodelling in the subchondral compartment due to rapid drug clearance in the joint cavity and/or low systemic concentration after absorption into the circulation. Together, the bone-protective effects of TXA appear to be concentration-dependent and rely on the blood supply to subchondral bone, thus requiring a systemic route of administration.

Despite the promising results of TXA effects on OA, there are several limitations in this study that merit consideration. First, the mouse model we employed cannot be fully translated to human OA. Thus, the significance of our results for clinical application remains to be determined. Second, only female mice were employed in this study and it remains uncertain whether these observations can also be generalized to male mice. Third, as with any treatment, the benefits of TXA should always be considered in

relation to the potential side effects. Anticipated side effects include an increased risk of thromboembolism resulting from higher and repetitive systemic dosing or joint infections caused by topical injections. Although the safety of TXA in routine use has been demonstrated clinically, there needs to be caution in employing long-term and repeated administration of comparably high doses of TXA. And finally, we mainly used radiographic, histological and histomorphometric methods in this pre-clinical study to perform an in-depth morphologic analysis during OA progression. Even though several possible explanations have been put forth to account for our findings, the relevant detailed molecular mechanisms require further investigation.

Taken together, TXA was found to exert beneficial effects on the course of post-traumatic OA in the present work. Both systemic and topical TXA administration ameliorated cartilage degeneration and synovitis induced by ACLT in mice. Systemic TXA treatment restored subchondral bone microstructure and inhibited osteophyte formation through normalizing the excessive bone remodelling in OA. This work suggests that TXA may indeed benefit patients with post-traumatic OA.

5. Summary

5.1 Summary in English

OA is associated with high disability rates, morbidity and increased mortality, which exerts a severe psychological and physical burden on affected patients and causes tremendous health care costs. TXA is the most widely employed antifibrinolytic drug and commonly used in orthopedic trauma surgery. Previous studies indicated that TXA also modulates inflammatory responses and bone cell function, which are both disturbed during OA disease progression. The present study was therefore designed to investigate the effects of systemic and topical TXA treatment on the progression of knee OA in a pre-clinical mouse model.

In this study, murine OA was induced by ACLT operation of the right knee. Thereafter, mice received daily systemic or weekly topical injections of TXA or vehicle, starting at the day of surgery. Morphologic alternations were analyzed by histological, histomorphometric and radiological measurements: First, histopathological changes of cartilage degeneration, synovitis, bone erosion and osteophyte formation were scored histologically. Second, cartilage thickness and osteoblast/osteoclast parameters were measured histomorphometrically. And finally, µCT evaluation was performed to evaluate the subchondral bone microstructure and osteophyte volume.

We found that both systemic and topical TXA treatment significantly lowered the scores of cartilage degeneration, synovitis, and bone erosion, and increased the ratio of hyaline to calcified cartilage thickness in OA knees. Systemic TXA prevented subchondral bone loss and inhibited osteophyte formation induced by ACLT, while topical TXA did not. The number and surface of osteoclasts in subchondral bone from OA knees were reduced by systemic TXA, whereas those of osteoblasts were not altered. Neither osteoclasts nor osteoblasts parameters in subchondral bone were affected by topical TXA.

Based on our results, we conclude that both systemic and topical TXA protect against the progression of ACLT-induced OA in mice. While topical TXA is only effective to prevent cartilage degeneration, systemic TXA additionally prevents abnormal subchondral bone remodelling and osteophyte formation. Together, our findings provide new opportunities for drug repurposing of TXA in the prevention or treatment of OA.

5.2 Summary in German

Die OA ist mit einer hohen Morbidität und einer erhöhten Mortalität verbunden, was für die betroffenen Patienten eine schwere psychische und physische Belastung darstellt und enorme Kosten im Gesundheitswesen verursacht. TXA ist das am häufigsten eingesetzte Antifibrinolytikum, das bei einer Vielzahl von unfallchirurgischen und orthopädischen Operationen eingesetzt wird. Frühere Studien wiesen darauf hin, dass TXA auch Entzündungsreaktionen und die Funktion von Knochenzellen moduliert, die allesamt auch im Krankheitsverlauf einer OA verändert sind. In der vorliegenden Studie sollten daher die Auswirkungen einer systemischen und der topischen TXA-Behandlung auf das Fortschreiten der OA des Kniegelenkes in einem präklinischen Mausmodell untersucht werden.

In dieser Studie wurde die OA bei den Tieren mittels der operativen Durchtrennung des vorderen Kreuzbandes (ACLT) am rechten Knie ausgelöst. Danach erhielten die Mäuse täglich eine systemische oder wöchentlich eine topische Injektion von TXA oder Vehikel, beginnend mit dem Tag der Operation. Die morphologischen Veränderungen wurden durch histologische, histomorphometrische und radiologische Messungen analysiert: Zunächst wurden die pathologischen Veränderungen von Knorpeldegeneration, Synovitis, Knochenerosion und die Osteophytenbildung histologisch untersucht. Danach wurden die Knorpeldicke sowie die Osteoblasten- und Osteoklasten-Parameter histomorphometrisch gemessen. Abschließend erfolgte eine µCT-Auswertung, um die Mikrostruktur des subchondralen Knochens und das Osteophytenvolumen zu beurteilen.

Wir stellten fest, dass sowohl die systemische als auch die topische TXA-Behandlung das Ausmaß der Knorpeldegeneration, Synovitis und Knochenerosion signifikant verringerte und das Verhältnis von hyaliner zu kalzifizierter Knorpeldicke in den operierten Knien erhöhte. Die systemische TXA-Behandlung reduzierte zudem den subchondralen Knochenverlust und konnte die ACLT-induzierte Osteophytenbildung hemmen, während die topische TXA-Behandlung diesen Effekt nicht hatte. Die Anzahl der Osteoklasten und die von ihnen besetzte Knochenoberfläche im subchondralen Knochen in den Knien mit einer OA wurde durch die systemische TXA-Behandlung reduziert, während die Osteoblasten-Parameter nicht verändert wurden. Weder die Osteoklasten- noch die Osteoblasten-Parameter im subchondralen Knochen wurden durch topisch appliziertes TXA beeinflusst.

Unsere Ergebnisse lassen den Schluss zu, dass sowohl systemisches als auch topisches TXA vor dem Fortschreiten der ACLT-induzierten OA bei Mäusen schützt. Während die topische TXA-Behandlung nur die Knorpeldegeneration verhindert, beugt die systemische TXA-Behandlung zusätzlich dem gestörten subchondralen Knochenumbau und der Osteophytenbildung vor. Zusammengenommen bieten unsere Ergebnisse neue Möglichkeiten für den Einsatz von TXA in der Prävention oder Behandlung einer OA.

6. List of abbreviations

3D	Three-dimensional
AAOS	American Academy of Orthopedic Surgeons
ACL	Anterior cruciate ligament
ACLT	Anterior cruciate ligament transection
ACR	American College of Rheumatology
BIC	Bone-Inflammation-Cartilage
BV/TV	Bone volume fraction
CC	Calcified cartilage
CNS	Central nervous system
COL1	Type I collagen
COL2	Type II collagen
DMM	Destabilized medial meniscus
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetate
FDA	Food and Drug Administration
$FeCl_3$	Iron(III) chloride
FLS	Fibroblast-like synoviocytes
Gly	Glycine
H&E	Hematoxylin and eosin
HC	Hyaline cartilage
HC.Th/CC.Th	Ratio of hyaline cartilage to calcified cartilage thickness
HCl	Hydrochloric acid
HNO_3	Nitric acid
IA	Intra-articular
IL	Interleukin
IP	Intraperitoneal
LFC	Lateral femoral condyle
LTP	Lateral tibial plateau
MFC	Medial femoral condyle
MMPs	Matrix metalloproteases

MTP	Medial tibial plateau
NaOH	Sodium hydroxide
NSAIDs	Nonsteroidal anti-inflammatory drugs
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
Ob.N/B.Pm	Number of osteoblasts per bone perimeter
Ob.S/BS	Osteoblast surface per bone surface
Oc.N/B.Pm	Number of osteoclasts per bone perimeter
Oc.S/BS	Osteoclast surface per bone surface
PAs	Plasminogen activators
PTOA	Post-traumatic osteoarthritis
RANKL	Receptor activator of nuclear factor κ-B ligand
RCT	Randomized controlled trial
SBP	Subchondral bone plate
SBP.Th	Subchondral bone plate thickness
SD	Standard deviation
Tb.N	Trabecular number
Tb.Sp	Trabecular separation
Tb.Th	Trabecular thickness
TKA	Total knee arthroplasty
TNF-α	Tumor necrosis factor-α
TRAP	Tartrate-resistant acid phosphatase
TXA	Tranexamic acid
UKA	Unicompartmental knee arthroplasty
WHO	World Health Organization
μCT	Micro-computed tomography

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9. Curriculum Vitae

Lebenslauf aus datenschutzrechtlichen Gründen nicht enthalten.

10. Eidesstattliche Versicherung

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