Acta Pharmaceutica Sinica B 2024;14(7):2869–2884

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

REVIEW

The emerging role of tranexamic acid and its principal target, plasminogen, in skeletal health

Weixin Xie, Antonia Donat, Shan Jiang, Anke Baranowsky, Johannes Keller*

Department of Trauma and Orthopedic Surgery, University Medical Center Hamburg-Eppendorf, Hamburg 20246, Germany

Received 22 November 2023; received in revised form 28 February 2024; accepted 14 March 2024

KEY WORDS

Tranexamic acid;
Osteoporosis;
Osteoarthritis;
Fracture healing;
Bone metabolism;
Inflammation;
Plasminogen;
Fibrinolysis

Abstract The worldwide burden of skeletal diseases such as osteoporosis, degenerative joint disease and impaired fracture healing is steadily increasing. Tranexamic acid (TXA), a plasminogen inhibitor and anti-fibrinolytic agent, is used to reduce bleeding with high effectiveness and safety in major surgical procedures. With its widespread clinical application, the effects of TXA beyond anti-fibrinolysis have been noticed and prompted renewed interest in its use. Some clinical trials have characterized the effects of TXA on reducing postoperative infection rates and regulating immune responses in patients undergoing surgery. Also, several animal studies suggest potential therapeutic effects of TXA on skeletal diseases such as osteoporosis and fracture healing. Although a direct effect of TXA on the differentiation and function of bone cells *in vitro* was shown, few mechanisms of action have been reported. Here, we summarize recent findings of the effects of TXA on skeletal diseases and discuss the underlying plasminogen-dependent and -independent mechanisms related to bone metabolism and the immune response. We furthermore discuss potential novel indications for TXA application as a treatment strategy for skeletal diseases.

*Corresponding author.
E-mail address: j.keller@uke.de (Johannes Keller).

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2024.03.033

1. Introduction

Diseases of the skeletal system affect a significant number of patients of all ages worldwide. The Global Burden of Disease 2019 study shows that about 1.71 billion people are suffering from musculoskeletal diseases, which have been the highest contributor to the global need for rehabilitation[1]. The most common conditions, namely osteoporosis, osteoarthritis and bone fracture, are associated with significant disability and reduced quality of life. With the progressive aging of the general population, these diseases impose a heavy burden on individuals and public health. Exploring new treatment options for skeletal diseases is therefore of the utmost importance.

Bone remodeling is a precisely controlled dynamic process which is maintained by the coordinated action of bone-resorbing osteoclasts and bone-forming osteoblasts[2,3]. In most skeletal disorders, bone formation and/or bone resorption activity is affected, resulting in a disruption of balanced bone remodeling. Osteoporosis, the most prevalent bone disease worldwide, is characterized by low bone mass resulting from excessive bone resorption coupled with insufficient bone formation. Abnormal subchondral bone remodeling is also one of the hallmarks of osteoarthritis at different stages. Furthermore, bone remodeling is critical for fracture healing and essential to callus formation and maturation[4].

Apart from bone remodeling, many skeletal diseases are accompanied by a local or systemic inflammatory reaction[5]. Inflammation is a physiological response of the innate immune system to disrupted tissue homeostasis[6], which can be triggered by injury, infection as well as aging[7]. Bone injury usually elicits an acute inflammation that contributes to fracture healing, while the healing process can be delayed if the inflammatory response is dysregulated or becomes chronic due to the persistence of inflammatory stimuli[8]. Osteoporosis and osteoarthritis are two of the most prevalent degenerative skeletal diseases in the aging population. Despite differences in the underlying pathophysiological mechanisms, they also share common features, including impaired bone remodeling and activation of the immune system with pro-inflammatory responses. The “inflammaging”, as proposed by Franceschi et al.[9], describes the inflammatory state that occurs with increasing age and promotes the development of both osteoporosis[10] and osteoarthritis[7]. On the cellular level, macrophages are critical effectors of inflammation in osteoporosis and osteoarthritis. Experimental studies demonstrate that a reduced M1/M2 ratio exerts anti-osteoporotic[11] and -osteoarthritic[12,13] effects by inhibiting osteoclastogenesis and inflammation. Additionally, migration of macrophages to the fracture site is found during fracture healing. When macrophages are depleted, fractures will not heal effectively[14]. The interaction of inflammatory processes and bone metabolism plays therefore an important role in skeletal diseases.

As the development of osteoporosis increases the risk of bone fractures, the improvement of fracture healing and long-term treatment of osteoporosis go hand in hand. Currently, several agents have been approved for the treatment of osteoporosis, including bisphosphonates and denosumab, which mainly work by inhibiting bone resorption. However, there are some concerns about long-term application and their effects on fracture healing and osteoarthritis which are limited and controversial as well[15-17]. For osteoarthritis, there is currently no definite treatment available that significantly slows disease progression or cures joint degeneration[18]. The three lines of knee osteoarthritis treatment, supported by moderate to strong evidence from the American Academy of Orthopaedic Surgeons (AAOS) evidence-based guideline[19], include exercise, weight management and nonsteroidal anti-inflammatory drugs. However, these treatments provide only limited pain relief and do not reverse or delay disease progression. In this regard, joint replacement is now the most effective treatment for osteoarthritis, especially for patients with advanced osteoarthritis who have not responded to conservative treatment.

The increasing incidence of skeletal diseases and limitations in treatment options augment the urge to find novel therapeutic strategies. In this regard, the plasminogen (PLG) inhibitor tranexamic acid (TXA) is well known for its anti-fibrinolytic properties, however, specific effects unrelated to fibrinolysis and PLG have been discovered recently. *In vitro*, TXA was shown to directly promote osteogenesis of bone marrow-derived osteoblasts, inhibit osteoclastogenesis, and reduce the expression of inflammatory cytokines in macrophages[20]. Furthermore, several animal studies have reported that TXA treatment exerts beneficial effects on osteoporosis and osteoarthritis[21,22]. Thus, TXA may potentially alter bone and immune cell function in skeletal diseases *via* PLG-dependent or -independent manners, as discussed below. Herein, we review the potential therapeutic impact of TXA in skeletal diseases and discuss the underlying mechanisms.

2. Clinical usage and efficacy of TXA

TXA is a synthetic analogue of the amino acid lysine. It is included in the World Health Organization list of the most essential medications. The approved indications for TXA use vary in different countries and jurisdictions (Table 1). The US American Food and Drug Administration (FDA) approved the application of TXA for the treatment of menorrhagia and the prevention of short-term bleeding during tooth extraction in patients with hemophilia. Even though the European Medicines Agency and Pharmaceuticals and Medical Devices Agency have approved TXA for use in a broader range of indications (Table 1), off-label uses in emergency trauma medicine[23], orthopedic surgery[24-26] and dermatology (melasma treatment[27]) are common clinical practice[28].

The main purpose of TXA application is the reduction of bleeding and transfusion requirements in emergency trauma and surgery. The CRASH-2 trial, a large randomized controlled trial (RCT) investigating the usage of TXA in bleeding trauma patients, included 20,211 adult trauma patients in 274 hospitals in 40 countries. The study demonstrated TXA treatment reduced the risk of death due to bleeding when TXA was administered intravenously within the first hour after trauma (5.3% *vs.* 7.7%; RR = 0.68, 95% CI 0.57−0.82), and within 1 to 3 hours after trauma (4.8% *vs.* 6.1%; RR = 0.79, 95% CI 0.64−0.97)[23,33]. Following the findings from the CRASH-2 trial, TXA attracted considerable interest as a treatment option for trauma patients. The Military Application of Tranexamic Acid in Trauma Emergency Resuscitation (MATTERs) study was performed to characterize the effects of TXA in wartime injury. This retrospective study included 896 patients with combat injury, of which 293 received intravenous TXA. Here, in line with the findings of the CRASH-2 study, the usage of TXA following combat injury resulted in significantly lower in-hospital mortality (17.4% *vs.* 23.9%)[34]. Recently, results of the CRASH-3 trial, evaluating the effects of TXA in patients with acute traumatic brain injury, were published. This large RCT was performed in 175 hospitals in 29 countries and included 12,737 patients with traumatic brain injury

Table 1 Approved indications for tranexamic acid use in America, Europe, Australia and Japan.

Institutions	Indications
Food and Drug Administration, America[29]	1. Treatment of cyclic heavy menstrual bleeding 2. Patients with hemophilia for short-term use (two to eight days) to reduce or prevent hemorrhage and reduce the need for replacement therapy during and following tooth extraction
European Medicines Agency, Europe[30]	1. Haemorrhage caused by general or local fibrinolysis such as menorrhagia and metrorrhagia, gastrointestinal bleeding, and haemorrhagic urinary disorders 2. Ear, nose, and throat surgery 3. Gynaecological surgery or disorders of obstetric origin 4. Thoracic and abdominal surgery and other major surgical intervention such as cardiovascular surgery 5. Management of haemorrhage due to the administration of a fibrinolytic agent
Therapeutic Goods Administration, Australia[31]	1. For the reduction of peri- and post-operative blood loss and the need for blood transfusions in patients undergoing cardiac surgery or total knee arthroplasty or total hip arthroplasty
Pharmaceuticals and Medical Devices Agency, Japan[32]	1. Bleeding tendency probably induced by increased systemic fibrinolysis (leukaemia, aplastic anaemia, purpura, etc., and intra/postoperative abnormal haemorrhage) 2. Abnormal haemorrhage probably induced by increased local fibrinolysis (pulmonary haemorrhage, epistaxis, genital haemorrhage, renal haemorrhage, abnormal haemorrhage during and after prostatic operation) 3. Symptoms such as erythema, swelling, and itching in the following diseases: eczema and similar diseases, urticaria, drug eruption, toxicoderma 4. Symptoms such as pharynx pain, redness, hyperaemia, and swelling in the following diseases: tonsillitis, laryngopharyngitis 5. Oral pain and aphtha associated with stomatitis

(TBI). In this study, intravenous TXA treatment within 3 h of injury reduced the risk of head injury-related death in patients with mild-to-moderate TBI (RR = 0.78, 95% CI 0.64−0.95), but not in patients with severe TBI (RR = 0.99, 95% CI 0.91−1.07)[35]. While there are also some smaller RCTs showing that the use of TXA was not associated with reduced mortality or even resulted in increased mortality in trauma patients[36,37], further studies are needed to identify patients who will benefit from TXA administration.

TXA is also effective in reducing bleeding during various surgeries, including major orthopedic surgery. Several meta-analyses based on RCTs indicate that TXA can effectively and safely reduce blood transfusion and blood loss in patients undergoing spinal surgery[38,39], joint arthroplasty[24-26], and hip fracture surgery[40]. A summary of recent RCT studies regarding the applications of TXA in orthopedic trauma surgery is provided in Table 2.

Regarding the route of TXA application, there are also studies comparing the effects of intravenous (IV) injection to the topical application of TXA. A meta-analysis including 18 RCTs reported that there was no significant difference in blood loss and blood transfusion between IV and topical TXA in orthopedic surgery[56]. Topical administration provides initial high local drug concentrations followed by rapid clearance[57], and it was shown that topical TXA results in a 90% reduction of TXA plasma concentration when compared with IV TXA[56]. Therefore, the topical use of TXA may be a better choice for the treatment of local bleeding as it acts directly at the target site. This minimizes the systemic effects of TXA, which may be safer in patients at risk of venous thromboembolism, even though there is no evidence supporting an increased risk of thrombotic complications with IV TXA. However, systemic administration of TXA orally or intravenously is more appropriate in cases that require a high frequency of application. Systemic TXA also results in drug distribution *via* the circulatory system to multiple target organs or tissues, including subchondral bone, which is difficult to reach with topical TXA. Altogether, clinicians need to choose the way of administration according to the actual condition and needs of individuals.

Based on its anti-fibrinolytic activity, researchers have also investigated whether TXA treatment eliminates the need for surgical wound drainages. For example, Fenwick et al.[58] described in a retrospective cohort study that drainages would not be inserted when TXA was applied into the surgical site in their protocols of proximal femur fracture surgery. Chandran et al.[59] conducted a RCT with 210 hip fracture patients, comparing a group that received no drainages and was treated with TXA, a group that received drainages without TXA treatment, and a group that received neither drainages nor TXA treatment. The results showed that the average fall of hemoglobin

Table 2 Randomized controlled trials of TXA in orthopedic trauma surgery published from 2018 to 2023.

Study	Fracture type	TXA dose	Route	No. of patients; Age (TXA/Placebo)	Outcomes in TXA group (TXA/Placebo)
41	Intertrochanteric	1 + 1 g (10 min preop + 3 h later)	IV	61/61; 79.11 ± 11.91/ 76.07 ± 16.60	Decreased total blood loss by 391.44 mL; decreased transfusion rate (29.5%/60.7%); decreased transfusion units by 1 U; no difference in complications (90-day postoperative follow-up)
42	Intertrochanteric	1 g (15 min preop)	IV	50/50; 75.10 ± 8.27/ 77.82 ± 6.42	Decreased total blood loss by 255.88 mL; decreased transfusion rate (10%/54%); no difference in transfusion units; no difference in complications (1-month postoperative follow-up)
43	Trochanteric	15 + 15 + 15 mg/kg (10 min preop + during surgery + 3 h postop)	IV	88/88; 76.8 ± 7.0/ 77.4 ± 6.8	Decreased total blood loss by 205.5 mL; decreased transfusion rate (17%/35%); decreased transfusion units by 1 U; no difference in complications (6-month postoperative follow-up)
44	Intertrochanteric	10 + 10 mg/kg (10 min preop + 5 h postop)	IV	50/50; 77.74 ± 6.53/ 79.25 ± 6.55	Decreased total blood loss by 181.58 mL; decreased transfusion rate (48%/68%); decreased transfusion units by 0.55 U; no difference in complications (1-week postoperative follow-up)
45	Sub-capital femoral	1 g:10 mL (at the end of the surgery)	Topical	52/50; 84.08 ± 6.9/ 84.56 ± 10.4	Decreased total blood loss by 379.3 mL; no difference in transfusion rate; no difference in transfusion units; no difference in complications (1-year postoperative follow-up)
46	Intertrochanteric	1 g (at admission)	IV	63/62; 78.05 ± 7.62/ 78.66 ± 6.95	Decreased hidden blood loss by 234.58 mL; decreased pre-operative transfusion (11.11%/ 22.58%); no difference in complications (3-month postoperative follow-up)
47	Subcapital or intertrochanteric	15 mg/kg (5 min preop)	IV	77/88; 82.93 (61−96)/ 83.36 (48−96)	Decreased total blood loss by 323.9 mL; decreased transfusion

Table 2 (*continued*)

Study	Fracture type	TXA dose	Route	No. of patients; Age (TXA/Placebo)	Outcomes in TXA group (TXA/Placebo)
					rate (57%/73%); decreased transfusion units by 0.58 U
48	Acetabular	10 + 10 mg/kg (15 min preop + 3 h later)	IV	36/27; 49.05 ± 16.42/ 44.33 ± 17.39	No difference in total blood loss; no difference in transfusion rate; no difference in transfusion units; no difference in complications (12-week postoperative follow-up)
49	Intertrochanteric	15 + 15 mg/kg (15 min preop + after anesthesia)	IV	51/51; 76.0 ± 18.3/ 79.8 ± 10.5	Decreased total blood loss by 286.6 mL; decreased transfusion rate (8%/23.5%); decreased transfusion units by 8 U; no difference in complications (12-week postoperative follow-up)
50	Calcaneal	80 mL 0.5 g/L + 20 mL 0.5 g/L (washing wound at the end of the surgery + injecting into wound through the drainage tube)	Topical	20/20; 43.9 ± 11.3/ 40.2 ± 13.2	Decreased total blood loss by 136.5 mL; no difference in complications (during hospital stay)
51	Thoracolumbar burst fracture	1 g:100 mL (soaking wound during the surgery)	Topical	39/37; 38.85 ± 4.17/ 39.41 ± 6.51	Decreased total blood loss by 125.16 mL; decreased transfusion rate (2.56%/13.51%); decreased transfusion units by 4 U; no difference in complications (At least 1-month postoperative follow-up)
52	Proximal humeral	1 g (just preop)	IV	53/48; 68 (49−82)/68 (45−84)	Decreased total blood loss by 92 mL; no difference in complications (during hospital stay)
53	Mandibular	20 mg/kg (30 min preop)	IV	25/25; 29 ± 6.4/ 27.1 ± 5.2	Decreased total blood loss by 200.33 mL; no difference in complications (6-month postoperative follow-up)
54	Thoracolumbar fracture-dislocation	10 mg/kg + 1 mg/kg/ h (15 min preop + maintenance during the surgery)	IV	39/41; 41.2 ± 10.3/ 42.5 ± 9.5	Decreased total blood loss by 498 mL; no difference in complications (12-week postoperative follow-up); no difference in the levels of the prethrombosis-state molecular markers

(*continued on next page*)

Table 2 (*continued*)

Study	Fracture type	TXA dose	Route	No. of patients; Age (TXA/Placebo)	Outcomes in TXA group (TXA/Placebo)
55	Pelvic or acetabular	15 + 15 mg/kg (after anesthesia + 3 h later)	IV	54/54; 32.4 ± 10.9/ 37.9 ± 13.5	Decreased total blood loss by 207.2 mL; decreased transfusion units by 0.6 U

TXA, tranexamic acid; IV, intravenous.

levels in the group treated by TXA treatment without drainages was less compared to the other two groups (8.0%, 14.4% and 10.9%, respectively)[59]. Therefore, currently drainages appear to be not necessary when TXA is administered in selected orthopedic surgeries including proximal fractures of the femur[58,59] and knee arthroplasty[60,61]. However, as TXA in orthopedic and emergency trauma surgeries is still applied off-label in most cases, its use, particularly systemically, must for now be limited to indications where excessive bleeding is expected to lead to clinical complications, and contraindications such as an acquired or genetic increased risk for thromboembolic events must be taken into consideration carefully on a case-to-case basis.

Overall, current evidence supports positive effects of TXA on reducing blood loss and transfusion requirements in emergency trauma and orthopedic surgeries, without increasing the rates of complications such as thromboembolic events and adverse effects. This has resulted in TXA to evolve as one of the most used and widely studied anti-fibrinolytic drugs in orthopedic and emergency trauma surgery. Its extensive use also generated research interest to further investigate potential therapeutic effects beyond its role in hemostasis, including the immune system and inflammatory responses.

In this regard, inflammatory responses are key determinants of disease manifestations and outcomes in many skeletal diseases, including bone fractures, osteoarthritis and osteoporosis. Although not all directly related to the skeletal system, several recent clinical studies have shown that TXA regulates immune cell function and related inflammatory responses during major surgery. Draxler et al.[62] conducted a RCT including 41 cardiac surgery patients and 10 healthy volunteers to investigate the global effect of TXA on the immune profile. The results indicated that TXA reduced the level of pro-inflammatory interleukin (IL)-1β and surgery-induced immunosuppression in patients following cardiac surgery through upregulating immune-enhancing markers such as C−C chemokine receptor type 7 (CCR7) expression on natural killer cells, and downregulating indicators of immunosuppression such as latency-associated peptide on regulatory T lymphocytes. In healthy volunteers, TXA induced a temporary reduction of plasma levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and IL-6. Regarding these effects on clinical outcomes, TXA treatment significantly reduced the rate of surgical site infection after cardiac surgery (RR = 0.70, 95% CI 0.49−0.99), which was independent of the effect of TXA on reducing blood loss[62]. Similar findings were obtained in the few comparable studies in orthopedic surgery. A RCT involving 125 primary total knee arthroplasty (TKA) patients reported that intravenous TXA treatment following primary TKA decreased serum C-reactive protein and IL-6 levels by 30.4% and 32.7%, respectively, whereas increased complement C3 and C4 levels by 22.1% and 32.3%, respectively, were observed[63]. Additionally, TXA treatment was demonstrated to improve immunocompetence in TKA patients chronically exposed to dexamethasone. Here, several retrospective studies reported that administration of TXA reduced the rate of periprosthetic joint infection by approximately 50% after both primary and revised total joint arthroplasty[64-68], although relevant evidence from RCTs confirming these data is still lacking. With regard to wound healing, a meta-analysis involving 1608 patients from 25 RCTs showed that TXA had a very modest 2% reduction in wound complication rates after primary total hip arthroplasty[69]. However, the immunomodulatory effects of TXA were not observed in some other surgical procedures, such as spine surgery[39] and fixation of intertrochanteric fractures[42]. Thus, despite accumulating evidence for beneficial immunomodulatory functions, the effects of TXA on immune responses and infections in regard to clinical outcome parameters in orthopedic and emergency trauma surgery still need to be further delineated.

Collectively, the above RCTs demonstrated that TXA reduces blood loss and transfusion unit requirements without affecting postoperative complication rates. However, clinical trials investigating the effect of TXA on outcome parameters other than bleeding and net overall complication rates are scarce, highlighting the need for alternative research approaches to evaluate potential other indications for TXA that, if promising, could then be further tested in the clinical setting.

3. Preclinical evaluations of TXA in skeletal diseases

3.1. *Fracture healing*

Because it might prove difficult to clearly demonstrate therapeutic efficacy and mechanism of action in clinical trials in orthopedic surgery, preclinical animal models have been used as an appropriate tool to study the impact of TXA on skeletal diseases and the underlying mechanisms. Regarding the fracture healing process, three *in vivo* animal studies were successively published recently[70-72], all of which applied a rat fracture model with intramedullary Kirschner wire fixation. Karaduman et al.[70] conducted a rat experiment with a sample size of 6 and employed a closed femoral fracture model, and the results showed that a single dose of intravenous TXA (30 mg/kg) immediately following surgery accelerated early femoral fracture healing on Day 15 but not on Days 30 and 45. Çevik et al.[71] performed a rat experiment with a sample size of 8 and used an open femoral osteotomy model to compare the effects of topical and systemic TXA treatment on femoral fracture healing. In contrast to the former study[70], they found that an intravenous single dose of TXA (10 mg/kg) immediately after surgery negatively affected fracture healing on Days 14 and 28, whereas a topical dose (10 mg/kg) intraoperatively onto the fracture gap accelerated the healing

process. Unlike these two studies[70,71], Balkanlı et al.[72] used an open tibial fracture rat model with a sample size of 7. A TXA-embedded sponge (50 mg/kg) was locally applied over the fracture ends for the topical group, and an intravenous single dose of TXA (50 mg/kg) immediately following surgery was applied to the systemic group. The results showed that both topical and systemic applications of TXA did not alter tibial fracture healing on Days 14 and 21. However, the outcomes reported by these studies only include radiological and histological scores of fracture healing, and the scoring system was different among studies. The inconsistent findings from these studies may result from different dosages and application forms of TXA, the types of applied fracture models, and other methodological issues such as the chosen approaches to rate histological and radiological indices of fracture healing. Since important histological, morphological and biomechanical outcome parameters such as the quantification of callus formation and biomechanical strength were not reported, more comprehensive and standardized studies are needed to shed light on these discrepant results.

3.2. *Osteoporosis*

Concerning osteoporosis, a recent animal study with a sample size of 10 reported that oral TXA administration significantly improved bone mineral density (BMD) in age-induced, but not ovariectomy-induced, osteoporosis in mice[21]. However, the conclusions of the study are limited as a detailed morphological and histological assessment of the bone phenotype and bone remodeling parameters was not reported. *In vitro*, Wagenbrenner et al.[73] showed a decrease in proliferation rates of human mesenchymal stromal cells upon treatment with high concentrations of TXA (50 mg/mL), while there was no clear trend regarding the influence of different concentrations (0, 10, 20 and 50 mg/mL) of TXA on the gene expression of osteogenic markers. Another previous *in vitro* study investigated the effects of TXA on bone cells including murine osteoblasts, osteoclasts and macrophages[20]. The results showed that TXA treatment increased cell proliferation and matrix mineralization of bone marrow-derived osteoblasts in a dose-dependent manner (0, 0.01, 0.1 and 1 mg/mL), and long-term TXA stimulation (10 days) was associated with the increased expression of osteogenic marker genes. Similarly, TXA stimulation resulted in a potent inhibition of osteoclastogenesis and reduced the expression of inflammatory cytokines in bone marrow-derived macrophages activated with lipopolysaccharides[20]. The discrepancies between the results of TXA on osteoblast differentiation may arise from the cell sources, drug dosage and duration of treatment. Regardless, the obtained results suggest that TXA may directly be involved in the regulation of bone metabolism, independently of its anti-fibrinolytic action. It is therefore conceivable that TXA may yield potential benefits for the treatment of osteoporosis, however additional studies are warranted to delineate such effects. For example, a more comprehensive morphological and histological assessment of the bone phenotype at multiple skeletal sites should be performed in TXA-treated osteoporosis animals. Further studies using the *Plg*-knockout mice are also needed to investigate whether the respective effects of TXA are dependent or independent of the presence of PLG.

3.3. *Osteoarthritis*

Based on the modulatory effects on inflammatory responses and bone cell function[20], TXA may potentially attenuate key processes in the progression of osteoarthritis. Until now, only few studies have investigated the effects of TXA on chondrocytes and subchondral bone. Some *in vitro* experiments have demonstrated that TXA at concentrations higher than 20 mg/mL were cytotoxic to human[74], bovine, and murine chondrocytes[75]. In turn, TXA at concentrations lower than 20 mg/mL had no effect on chondrocytes[74,76]. Therefore, TXA may not directly affect chondrocyte homeostasis within the safe concentration range. A recent animal study with a sample size of 6 showed that both systemic and topical TXA treatment protected cartilage from degeneration and lowered inflammatory responses of the synovial tissue in mice with post-traumatic osteoarthritis after anterior cruciate ligament transection. Moreover, systemic TXA beneficially affected pathological subchondral bone remodeling and reduced osteophyte formation in wild-type mice that underwent anterior cruciate ligament transection[22], overall suggesting a potentially beneficial effect of TXA in patients with osteoarthritis. Thus, the results of a current phase II double-blinded RCT investigating the effects of systemic TXA on joint inflammation and cartilage health in anterior cruciate ligament reconstruction are eagerly awaited[77].

Collectively, the current evidence from animal experiments suggests beneficial effects of TXA on osteoporosis[21] and osteoarthritis[22], while the underlying mechanisms however, require further clarification. In terms of bone regeneration, the results from one preclinical study support a positive effect of TXA application on fracture healing[70], whereas the other two available studies suggest no influence[72] or even negative outcomes[71]. Further studies using standardized preclinical models with rigorous and comprehensive phenotypic evaluation and in-depth mechanistic exploration are therefore required.

4. Molecular mechanisms of TXA in skeletal diseases

4.1. *Anti-fibrinolytic mechanisms*

As described above, there is accumulating evidence that TXA might have beneficial effects on common skeletal disorders that are related or unrelated to fibrinolysis, and may thus also occur in either a PLG-dependent or a PLG-independent manner, respectively. Under physiological conditions, coagulation and fibrinolysis maintain a dynamic equilibrium. Upon activation of the coagulation system, prothrombin is converted to thrombin, which then cleaves circulating fibrinogen into fibrin (Fig. 1). Subsequently, fibrin polymerization and cross-linking occur and result in clot formation[78]. Along the coagulation cascade with fibrin deposition, fibrinolysis is activated, ultimately resulting in the decomposition and liquidation of fibrin clots. Various enzymes are essentially involved in the fibrinolysis system, including PLG and PLG activators, yet fibrinolysis can only take place efficiently when PLG is activated into plasmin. PLG contains several lysine binding sites that can bind to lysine residues on the surface of fibrin. When PLG is bound to fibrin, the tissue PLG activators (tPA) or urokinase PLG activators (uPA) convert PLG to plasmin. Subsequently, plasmin degrades fibrin into soluble fibrin degradation products (FDPs)[79].

Hyperfibrinolysis is one of the distinctive features of acute traumatic coagulopathy, which is present in a significant proportion of patients with severe trauma[80] and contributes to progressive hemorrhage and even death[81]. As a synthetic analogue of lysine, TXA exhibits high affinity for one of the lysine binding sites in PLG. When bound, TXA competitively prevents binding of PLG to the lysine residues on the surface of fibrin, which

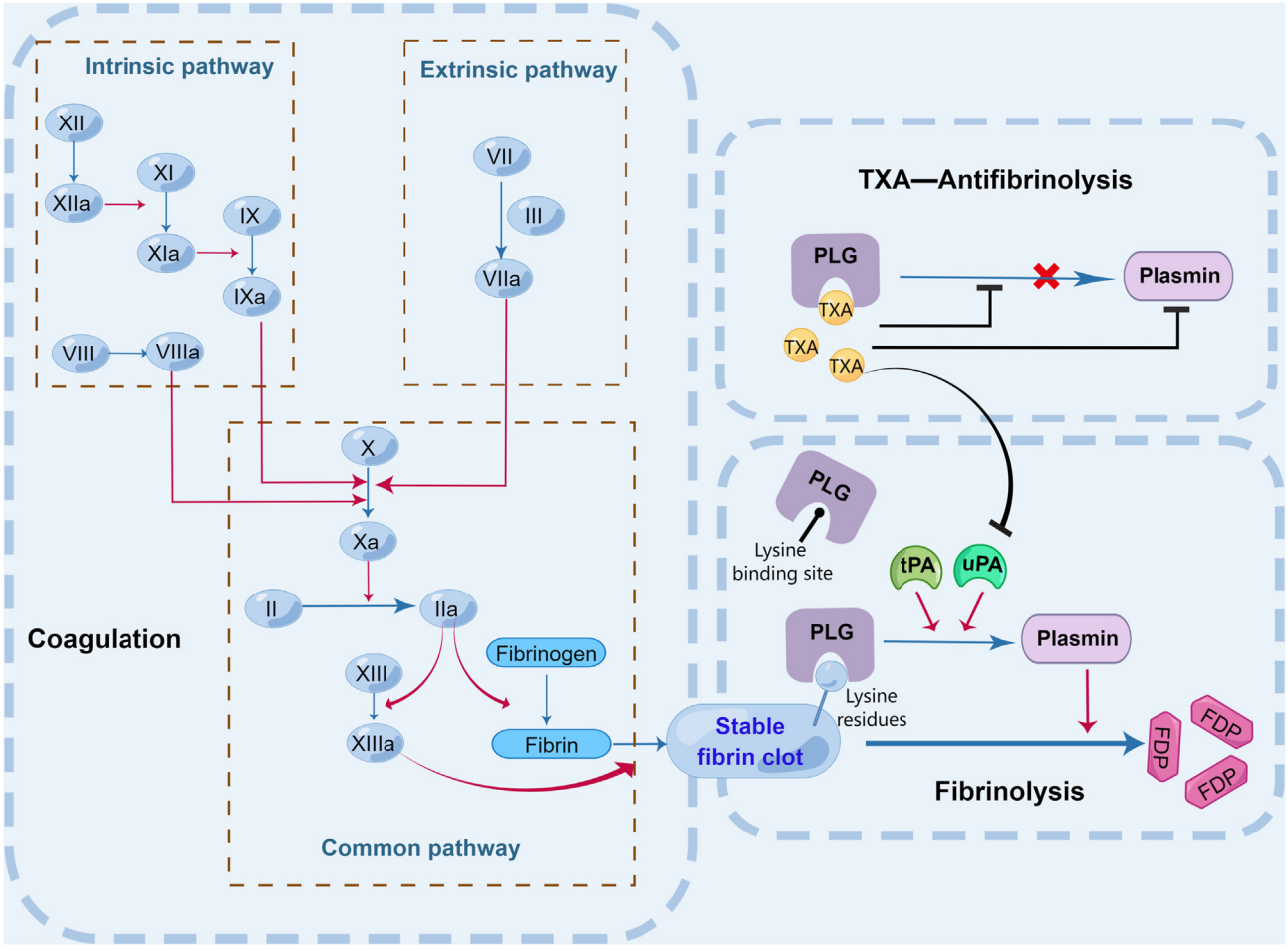

Figure 1 Coagulation cascade and tranexamic acid (TXA) action. Coagulation is a complex cascade process which is usually initiated by the surface-mediated intrinsic or tissue-mediated extrinsic pathway. Both pathways ultimately converge on the common pathway of coagulation in which the soluble fibrinogen is converted into fibrin, producing a stable fibrin clot. At the same time, fibrinolysis is activated. There are lysine residues on the surface of fibrin and lysine binding sites on plasminogen (PLG). During normal fibrinolysis, PLG binds to fibrin and subsequently is converted to plasmin in the presence of tissue PLG activators (tPA) and urokinase PLG activators (uPA). Afterwards, plasmin degrades the fibrin clot into soluble fibrin degradation products (FDPs). When TXA is present, fibrinolysis is inhibited. On the one hand, TXA as an analogue of lysine, competitively blocks the lysine binding sites in PLG and prevents binding of PLG to the fibrin. On the other hand, TXA also acts as a direct inhibitor of plasmin and uPA. The figure was created by Figdraw (ID: AWWUAa4f44).

subsequently inhibits the activation of PLG to plasmin. Therefore, TXA can potentially reduce the mortality of trauma patients due to its ability to reversibly bind and hence impair the fibrinolytic action of PLG.

PLG has furthermore been implicated in playing a role in the physiological regulation of a variety of biological processes[82-84], including inflammation, immune response and bone metabolism. At a concentration higher than 10 mmol/L, TXA can also directly inhibit plasmin activity[85], suggesting that TXA itself may function as a weak plasmin inhibitor. As a PLG inhibitor as well as a weak plasmin inhibitor, TXA has thus been postulated to exert multiple roles beyond its anti-fibrinolytic effect as described in detail below.

4.2. *Plasminogen-dependent mechanisms*

As described above, previous work has demonstrated a variety of different effects of TXA on immune responses and bone metabolism, thus impacting skeletal health. However, the underlying mechanisms of action are largely still unknown. In this regard, PLG, the principal target of TXA, has been implicated to play a role in the regulation of a variety of biological processes[82-84], including inflammation, immune response, and bone metabolism. Studies reported that TXA inhibits plasmin activity *via* binding to the primary catalytic (S1) pocket of the enzyme with a dissociation constant (K_d) of 25 mmol/L, which is much higher than the K_d of 1.1 μmol/L for TXA and high-affinity binding sites of PLG[85,86]. Therefore, TXA can act as a potent PLG inhibitor as well as a comparatively weak plasmin inhibitor. Based on its pleiotropic functions, the inhibition of PLG may be one of the key mechanisms accounting for TXA action as described below.

4.2.1. *Plasminogen in inflammation and immune response*

The TXA target PLG regulates inflammation and immunity in both fibrin-dependent and -independent manners (Fig. 2). In the fibrin-dependent manners, PLG is activated to plasmin and then degrades fibrin into FDPs. Fibrin is known as an immune modulator, which can stimulate multiple innate immune cell components, including neutrophils and macrophages[87]. During

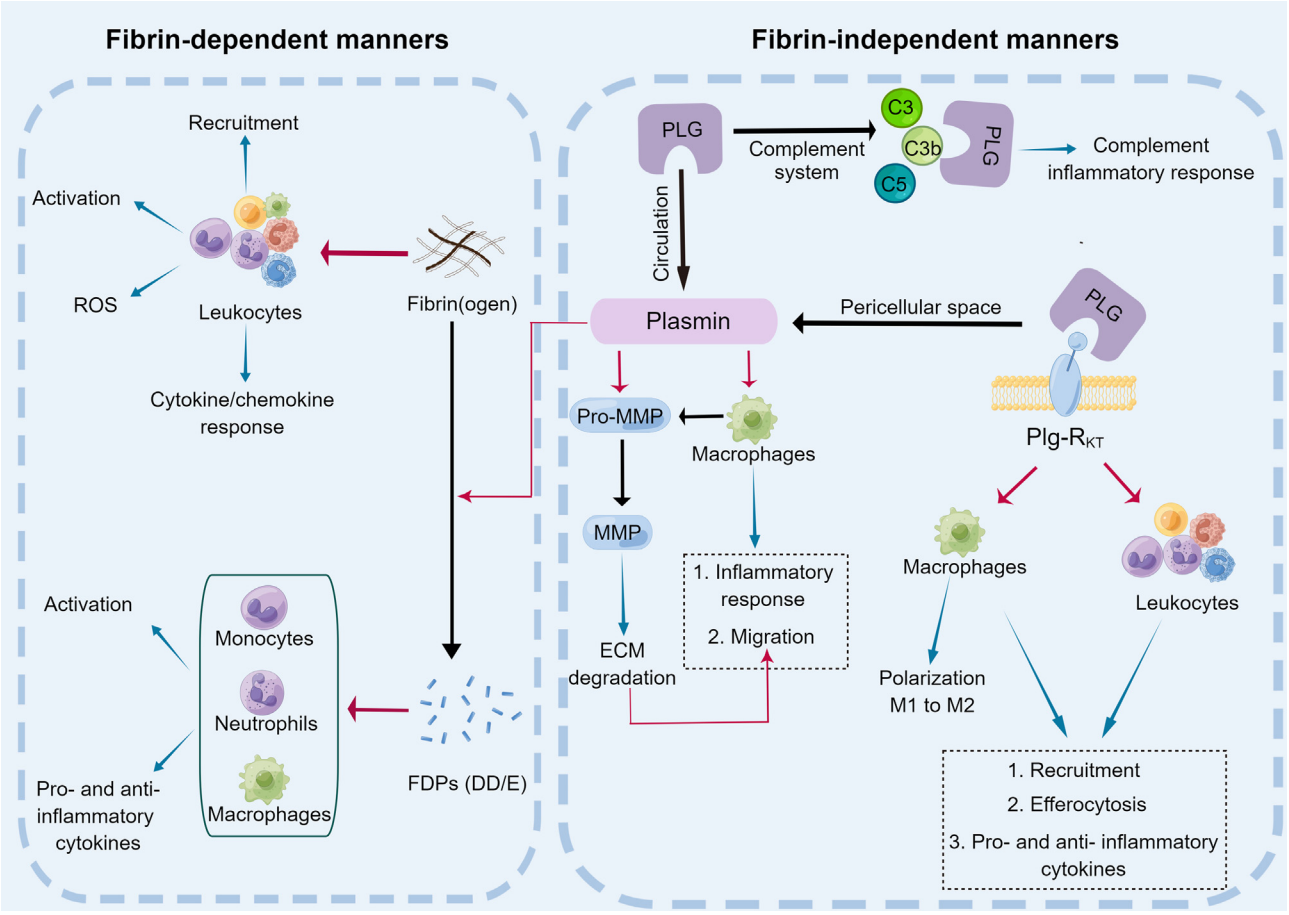

Figure 2 Plasminogen (PLG) actions on inflammatory responses. The actions of PLG can be classified into fibrin-dependent (left) and -independent (right) manners. In the fibrin-dependent manner, PLG is converted into plasmin, subsequently degrading fibrin into soluble fibrin degradation products (FDPs). In this process, both fibrin(ogen) and FDPs have been demonstrated to be involved in inflammation. Fibrinogen and fibrin regulate the inflammatory response of leukocytes by enhancing cytokine/chemokine responses, inducing cell recruitment and increasing levels of reactive oxygen species (ROS). FDPs are composed of various of fragments, among which the fragments D-dimers and E (DD/E) have been reported to activate and stimulate the production of pro- and anti-inflammatory cytokines in macrophages, monocytes and neutrophils. In the fibrin-independent manner, PLG regulates inflammation *via* multiple pathways. First, PLG can bind to several complement proteins including C3, C3b, and C5 through lysine residues and thus acts as a complement inhibitor, which is involved in the complement inflammatory response. Second, PLG binds to the PLG receptors such as Plg-R_{KT}, which results in the activation of intracellular signaling pathways and the conversion of itself to plasmin. On the one hand, Plg-R_{KT} directs the localization of plasmin generation to the pericellular space. Plasmin promotes extracellular matrix (ECM) degradation by activating matrix metalloproteinases (MMPs), and enhances inflammatory response and migration of macrophages. On the other hand, Plg-R_{KT} in leukocytes and macrophages is involved in regulating their phenotype and inflammatory functions. The figure was created by Figdraw (ID: APSIO441a8).

tissue inflammation, repair and regeneration, circulating monocytes and tissue-resident macrophages are recruited onto the sites of inflammation[88], and are considered the master regulators involved in tissue healing among leukocytes[89,90]. Soluble fibrinogen has been reported to stimulate high levels of tumor necrosis factor-α in macrophages[91,92]. In contrast, macrophages demonstrated anti-inflammatory properties with enhanced secretion of IL-10 when cultured on fibrin gels[91]. While FDPs are generally considered to induce mostly pro-inflammatory events, potential anti-inflammatory effects were also reported[93], highlighting the complexity of the fibrinolytic pathway as an immune regulator through PLG. In this regard, TXA was shown to inhibit the fibrin-dependent manners potently through its anti-fibrinolytic effect, thereby regulating of inflammation and immune response.

PLG has also been proposed to interact with components of the immune system and contribute to regulating inflammatory responses in the fibrin-independent manner[79]. For example, PLG was reported to participate in the regulation of complement system[94], which is an integral part of the innate immunity. Some bacteria such as *Moraxella catarrhalis*[95], *Haemophilus influenzae*[96], and *Borrelia burgdorferi*[97] bind to host PLG and degrade C3b and C5, thereby escaping from the innate host defense. Mechanistically, Barthel et al.[94] reported that PLG binds to C3, C3b, and C5 *via* lysine residues, and cleaves and degrades C3b and C5 when activated to plasmin. Moreover, PLG in the proteolytically inactive form was also shown to enhance factor I-mediated C3b inactivation. Therefore, PLG and its active form plasmin act as complement inhibitors, which influence the effects of antibodies and phagocytic cells such as pathogen clearance and

immune response. As mentioned above, TXA as an analogue of lysine, blocks the lysine binding sites in PLG and prevents it from activation. In this regard, TXA is potentially involved in the regulation of the complement cascade *via* inhibiting the activity of PLG and plasmin, whose relevance to skeletal diseases, however, remains to be determined.

PLG can also interact with various leukocytes including monocytes, macrophages, and dendritic cells through directly binding to PLG receptors. At least 12 distinct PLG receptors have been described to date[98], many of which are expressed on monocytes and macrophages, such as annexin A2, α-enolase, and Plg-R_{KT}. Plg-R_{KT} was discovered in 2010 by Andronicos et al.[99] and has been gaining particular interest over the last years. Miles et al.[100] found a remarkable defect in macrophage recruitment in *Plg-R*$_{KT}$ knockout mice. Furthermore, the capacity of macrophages for PLG binding in *Plg-R*$_{KT}^{-/-}$ mice was significantly decreased, suggesting that Plg-R_{KT} is essentially required for PLG binding and macrophage migration. Vago et al.[101] demonstrated that both $Plg^{-/-}$ and *Plg-R*$_{KT}^{-/-}$ mice show impaired recruitment of mononuclear cells to the pleural cavity and increased percentages of inflammatory M1-like macrophages during pleurisy. In another peritonitis model, deficiency of PLG[102] and Plg-R_{KT}[103,104] inhibited macrophage recruitment and lymphocyte recruitment as well. Thaler et al.[104] indicated that pro-inflammatory monocyte and macrophage subsets express higher Plg-R_{KT} which contributed significantly to their migration and recruitment to the inflammatory area. Several *in vitro* studies demonstrated that PLG interacts with the receptors on monocytes and macrophages and stimulate cytokine release[101,105-108]. Further, PLG influences the innate immune response by promoting dendritic cell-[109] and macrophage-[101,110] phagocytosis through PLG receptors. Hence, PLG receptors play a key role in the fibrin-independent impact of PLG on inflammation and immune responses. Here, it was shown that the C-terminal lysine of Plg-R_{KT} exposed on the cell surface can interact with PLG, thus activating it[99]. Therefore, TXA is likely to inhibit the activation of Plg-R_{KT} by blocking PLG, which could be one of the mechanisms underlying the regulatory functions of TXA in immune and inflammatory responses also relevant to the skeletal system.

4.2.2. *Plasminogen in bone metabolism and repair*

To date, multiple cellular and animal studies have documented evidence that PLG is critical for bone metabolism. Mice lacking PLG were reported to exhibit early degeneration and biomechanical incompetency of the axial skeleton, in addition to developing severe osteoporosis[111]. The degenerative changes in $Plg^{-/-}$ mice were completely prevented by additional fibrinogen-deficiency. From a mechanistic point of view, increased deposition of fibrin and osteoclast number were observed in the skeleton of $Plg^{-/-}$ mice, and fibrinogen *in vitro* promoted osteoblast the receptor activator of nuclear factor-κB ligand expression and stimulated osteoclastogenesis. The collective results suggest that PLG plays an important role in bone remodeling, particularly bone resorption, through regulating fibrin deposition. Similarly, Kanno et al.[84] reported a significantly reduced BMD and increased bone resorption in $Plg^{-/-}$ mice, which were rescued by plasmin injections. *In vitro*, osteoclastogenesis of bone marrow-derived cells from $Plg^{-/-}$ mice was significantly increased and the expression of osteoprotegerin in osteoblasts was suppressed, which was reversed by plasmin treatment. However, plasmin alone could also inhibit the increased osteoclastogenesis and induce osteoblastic osteoprotegerin expression in wild-type cells, suggesting that plasmin directly stimulates osteoblasts and osteoclasts without the activation of fibrin. Taken together, PLG was shown to regulate bone metabolism in both fibrin-dependent and -independent manners.

In terms of bone regeneration, there are several animal studies available investigating the effects of PLG on fracture repair[112,113]. Kawao et al.[112] demonstrated that the repair processes following a femoral bone defect were delayed in $Plg^{-/-}$ mice, and that angiogenesis, chondrogenesis and bone formation were impaired at the damaged site. Another noteworthy observation was the decrease in macrophages at the site of bone damage in $Plg^{-/-}$ mice, which supports the above-mentioned studies on PLG and macrophage recruitment[101,102]. A recent publication by Wang et al.[113] confirmed that $Plg^{-/-}$ mice exhibited impaired trabecular and cortical bone structure and delayed fracture healing. In addition, the authors reported a greatly reduced thickness of periosteum in $Plg^{-/-}$ mice. *In vitro* experiments showed that PLG increased cell proliferation, migration and survival in periosteal mesenchymal progenitors through activation of cysteine-rich angiogenic inducer 61, which suggests a novel role of PLG in bone repair.

Along with the direct effects of PLG on bone metabolism, the fibrinolytic activity mediated by PLG appears also essential for efficient fracture repair. As seen in other injuries, a bone fracture initiates the coagulation cascade followed by the deposition of a fibrin matrix, which is considered a vital component of the bone regenerative process. Yuasa et al.[114] generated mice lacking fibrinogen globally and found that fibrin deposition was beneficial for limiting hemorrhage following bone fracture. However, neither a difference in soft callus formation and vascularization nor in hard callus formation and remodeling between fibrinogen-deficient and wild-type mice was observed. Therefore, it seems that fibrin is not essentially required for fracture repair. In addition, studies demonstrated that $Plg^{-/-}$ mice, which failed to clear fibrin from the fracture site, exhibited delayed bone fracture repair with severely impaired callus formation, remodeling, and vascularization[112-114]. In this regard, knockdown of fibrinogen decreases fibrin deposition in the fracture callus and partially restored fracture repair in $Plg^{-/-}$ mice[114]. Besides, Kawao et al.[112] described that fibrinogen depletion induced by procoagulatory batroxobin did not restore impaired angiogenesis or recruitment of osteoblasts at the defect site during bone repair in $Plg^{-/-}$ mice. Therefore, it suggests that PLG is essential for bone repair in mice involving both fibrinolytic and non-fibrinolytic activity. In conclusion, TXA may potentially regulate bone metabolism and repair either in a fibrinolytic-dependent manner, or by inhibiting the activity of PLG and plasmin in their function as direct signaling molecules. However, future experimental studies are warranted to further determine the precise role of each mechanism in skeletal health and disease.

4.3. *Plasminogen-independent mechanisms*

4.3.1. *TXA as an inhibitor of uPA*

Although TXA is primarily considered as a PLG inhibitor, it may also exert specific effects independent of PLG and fibrinolysis. This phenomenon is commonly referred to as “off-target” effects of TXA[115]. In regard to the coagulation system, a recent study reported that TXA is an active-site inhibitor of uPA which attenuates uPA activity with an inhibitory constant of 2 mmol/L[86]. Therefore, TXA-mediated inhibition of uPA activity in clinical applications is possible in some cases, for instance when large

doses of TXA are used or renal clearance is impaired. Within fibrinolysis, uPA binds to its receptors (uPAR) on the cell surface and activates PLG to plasmin, resulting in fibrinolysis[116]. Apart from that, however, uPA is also directly involved in several physiological and pathophysiological processes including tumor invasion and metastasis, immunity and bone metabolism[117,118], marking uPA as an important off-target molecule of TXA.

In this regard, several genetic experiments have been performed to investigate the effects of uPA on fracture healing. Kawao et al.[119] induced bone defect models in *uPA*$^{-/-}$ and demonstrated that uPA contributes to the bone repair process at early time points. Compared with wild-type mice, the *uPA*$^{-/-}$ mice showed delayed bone repair, impaired angiogenesis and decreased number of macrophages at the defect sites on Days 4−6 after operation. Popa et al.[120] demonstrated that *uPA*$^{-/-}$ mice exhibit a higher proportion of cartilage in the fracture callus on Day 14, and that healing is accompanied by a decreased number of osteoclasts and reduced angiogenesis in the fracture callus. uPA and its receptor uPAR are expressed by both osteoblasts[121] and osteoclasts[122]. uPAR knockout mice display increased BMD and decreased length of long bones which results in a reduced capability to sustain mechanical loading on the tibia. At the cellular level, the osteogenic potential of osteoblasts was increased and the formation of osteoclasts was decreased in uPAR-deficient mice[123]. Besides, Anaraki et al.[124] reported that the expression of macrophage colony-stimulating factor from osteoblasts was inhibited by uPAR loss, and that the uPAR-deficiency impaired osteoclastogenesis of macrophages. Furthermore, the same group also suggested that uPAR mediates the osteogenic differentiation of mesenchymal stromal cells *via* the complement C5a receptor[125].

In cartilage, chondrocytes were also shown to express uPAR and uPA. The secretion of uPA by chondrocytes is actively controlled by inflammatory cytokines[126], and the binding of uPA and uPAR in chondrocytes results in pericellular proteolysis, playing an important role in cartilage degradation[127]. Furthermore, a study of experimental canine osteoarthritis showed that uPA activity was increased significantly during disease progression with an increase in osteoclasts in the subchondral bone[128]. Altogether, strong evidence shows that both uPA and uPAR are involved in bone repair and osteoarthritis, which may be one of the relevant "off-target" pathways how TXA may potentially modulate skeletal diseases.

4.3.2. *Other "off-target" receptors of TXA*

Although the safety of TXA application in clinical settings is well recognized, several side effects were observed in practical application, including seizures, back pain and myoclonus. Most of TXA-associated seizures have been reported in patients undergoing major cardiac interventions such as cardiopulmonary bypass surgery[129-134]. In contrast, myoclonus and back pain occurred only when TXA was accidently injected intrathecally[135]. In this regard, TXA application to the cortex or cisterna magna caused generalized

Table 3 Summary of expression and functions of TXA "off-target" receptors in bone cells.

"Off-target" receptors	Reported expression in bone cells	Reported functions on bone metabolism	Ref.
NMDA receptor	Osteoblasts	Promoting osteoblast differentiation and mineralization	144,145,151
	Osteoclasts	Promoting osteoclastogenesis	146,151,152
	Chondrocytes	Promoting chondrogenesis and regulating mechanotransduction	147,150
	Macrophages	Mediating anti-inflammatory response	148
$GABA_A$ receptor	Osteoblasts	Not reported	153,154
	Chondrocytes	Promoting proliferation of chondrocytes	155
	Macrophages	Mediating anti-inflammatory response and regulating polarization of macrophages	156−158
AMPA receptor	Osteoblasts	Promoting osteoblast differentiation and mineralization	151,159
	Osteoclasts	No influence on osteoclastogenesis	151,160
	Chondrocytes	Not reported	161
	Macrophages	Promoting inflammatory response	162
KA receptor	Osteoblasts	Not reported	159
	Osteoclasts	Promoting osteoclast resorptive function	160
Gly receptor	Chondrocytes	Involved in chondrocyte apoptosis	163
	Macrophages	Mediating anti-inflammatory response	164

TXA, tranexamic acid; NMDA, *N*-methyl-D-aspartate; $GABA_A$, γ-aminobutyric acid type A; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; KA, kainate; Gly, glycine.

seizures in experimental animals[136], which suggested that the proconvulsant effects of TXA may result from its actions on the central nervous system (CNS) by PLG-independent mechanisms.

To date, several studies have offered insights into the mechanisms underlying seizures caused by TXA, and several receptors involved have been found. TXA is also a structural analog of glycine, which is a major inhibitory neurotransmitter in the CNS. Glycine is an agonist at glycine (Gly) receptors and an obligatory co-agonist of *N*-methyl-D-aspartate (NMDA) receptors[137]. Studies indicate that TXA acts as a competitive antagonist of Gly receptors[135,138] and NMDA receptors[115] in the CNS, which may contribute to the proconvulsant effects of TXA. The γ-aminobutyric acid type A ($GABA_A$) receptors[139], another major inhibitory compound in the CNS, can also be blocked by TXA in seizure-prone CNS structures like the amygdala and the hippocampus[135,140,141]. Besides, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors and kainate (KA) receptors, both belonging to the ionotropic glutamate receptor family along with NMDA receptors, have been reported to be inhibited by TXA as well. Important to note, it still remains unclear how TXA inhibits the $GABA_A$, AMPA and KA receptors since they lack a glycine binding site[115,142], and further pharmacological characterization is warranted.

Despite this lack of knowledge, at least five "off-target" receptors have been identified to date, including NMDA, $GABA_A$, AMPA, KA, and Gly receptors. As a matter of fact, they are not only expressed in the CNS but can also be found in peripheral tissues including the skeleton. In particular, some of these "off-target" receptors are expressed on the surface of bone cells and have been suggested to participate in the regulation of bone metabolism. For example, NMDA receptors are expressed on osteoblasts[143-145], osteoclasts[143,146], chondrocytes[147] and macrophages[148]. Experimental studies have shown that activation of NMDA receptors stimulates osteoblast differentiation[144,145] and enhances the osteoblast response to stretching[149]. Antagonists of the NMDA receptor could dose-dependently inhibit osteoclast formation through the NF-κB pathway[146]. NMDA receptors are also essential for chondrogenesis, as blockade inhibits the differentiation of chondroprogenitor cells[147] and the hyperpolarization response of chondrocytes to mechanical stimulation[150]. Additionally, Mantuano et al.[148] demonstrated that the NMDA receptors mediated the anti-inflammatory response of tPA in macrophages. The expression of the other receptors on bone cells and their functions on bone metabolism are summarized in Table 3. Collectively, while TXA was shown to exert pleiotropic functions in the skeletal system, it remains unclear which of these actions are mediated in a PLG-dependent manner and which are transduced through "off-target" receptors.

5. Conclusions

TXA represents an anti-fibrinolytic agent with high efficacy and safety. With its widespread clinical applications, pharmacological effects beyond its anti-fibrinolytic action were noted. To date, several clinical trials have been published, providing evidence for favorable pharmacological effects including modulation of pro-inflammatory immune responses and reduction of post-operative complications, apart from reducing blood loss and transfusion requirements. Several *in vitro* experiments have revealed the effects of TXA on the differentiation and function of cells pivotal for skeletal health, such as osteoblasts, osteoclasts and macrophages. While few *in vivo* studies provided inconclusive results, TXA was shown to beneficially affect the course of osteoporosis and osteoarthritis in pre-clinical animal models. In this regard, although TXA is primarily known as a PLG inhibitor, accumulating evidence suggests that some of the pharmacologic effects in the skeleton are mediated through "off-target" receptors.

In trauma and orthopedic surgery, the available clinical data allows the conclusions that TXA can be safely applied off-label both systemically and topically in settings, where enhanced blood loss is to be expected. In terms of novel indications of TXA use including fracture healing, osteoarthritis progression, or osteoporosis, further and thorough pre-clinical studies are required to justify future RCTs for efficacy and safety testing in every day clinical practice. TXA should not be administered to patients predisposed to thromboembolic events in skeletal medicine, although available data does not support a higher rate of respective complications in patients receiving TXA.

Therefore, it will be critical to determine the strengths and weaknesses of TXA in skeletal health. This can only be achieved through further experimental studies and subsequent evaluation in clinical trials. Based on its excellent safety profile, pharmacological efficacy, mechanisms of action, and low cost, it must be rigorously tested whether TXA can benefit additional indications for patients with common skeletal diseases.

Acknowledgments

This work was supported by the German Research Foundation (KE 2179/9-1, Germany). Figures were made by Figdraw (https://www.figdraw.com).

Author contributions

Weixin Xie: Writing-Original Draft, Methodology, Investigation, Visualization. Antonia Donat: Writing-Original Draft, Investigation. Shan Jiang: Writing-Original Draft. Anke Baranowsky: Writing-Reviewing and Editing. Johannes Keller: Conceptualization, Supervision, Writing-Reviewing and Editing. All authors have approved the final article.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Cieza A, Causey K, Kamenov K, Hanson S, Chatterji S, Vos T. Global estimates of the need for rehabilitation based on the Global Burden of Disease study 2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2021;**396**:2006−17.
2. Zhu L, Tang Y, Li X, Keller E, Yang J, Cho J, et al. Osteoclast-mediated bone resorption is controlled by a compensatory network of secreted and membrane-tethered metalloproteinases. *Sci Transl Med* 2020;**12**:eaaw6143.
3. Hadjidakis D, Androulakis I. Bone remodeling. *Ann N Y Acad Sci* 2006;**1092**:385−96.
4. Schindeler A, McDonald MM, Bokko P, Little DG. Bone remodeling during fracture repair: the cellular picture. *Semin Cell Dev Biol* 2008;**19**:459−66.
5. Ginaldi L, Di Benedetto MC, De Martinis M. Osteoporosis, inflammation and ageing. *Immun Ageing* 2005;**2**:14.
6. Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat Rev Cancer* 2013;**13**:759−71.

7. Greene MA, Loeser RF. Aging-related inflammation in osteoarthritis. *Osteoarthritis Cartilage* 2015;**23**:1966−71.
8. Loi F, Córdova LA, Pajarinen J, Lin TH, Yao Z, Goodman SB. Inflammation, fracture and bone repair. *Bone* 2016;**86**:119−30.
9. Franceschi C, Garagnani P, Parini P, Giuliani C, Santoro A. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol* 2018;**14**:576−90.
10. Massera D, Biggs ML, Walker MD, Mukamal KJ, Ix JH, Djousse L, et al. Biochemical markers of bone turnover and risk of incident diabetes in older women: the cardiovascular health study. *Diabetes Care* 2018;**41**:1901−8.
11. Dou C, Ding N, Zhao C, Hou T, Kang F, Cao Z, et al. Estrogen deficiency-mediated M2 macrophage osteoclastogenesis contributes to M1/M2 ratio alteration in ovariectomized osteoporotic mice. *J Bone Miner Res* 2018;**33**:899−908.
12. Zhang H, Cai D, Bai X. Macrophages regulate the progression of osteoarthritis. *Osteoarthritis Cartilage* 2020;**28**:555−61.
13. Wu CL, Harasymowicz NS, Klimak MA, Collins KH, Guilak F. The role of macrophages in osteoarthritis and cartilage repair. *Osteoarthritis Cartilage* 2020;**28**:544−54.
14. Vi L, Baht GS, Soderblom EJ, Whetstone H, Wei Q, Furman B, et al. Macrophage cells secrete factors including LRP1 that orchestrate the rejuvenation of bone repair in mice. *Nat Commun* 2018;**9**:5191.
15. Hegde V, Jo JE, Andreopoulou P, Lane JM. Effect of osteoporosis medications on fracture healing. *Osteoporos Int* 2016;**27**:861−71.
16. Davis AJ, Smith TO, Hing CB, Sofat N. Are bisphosphonates effective in the treatment of osteoarthritis pain? A meta-analysis and systematic review. *PLoS One* 2013;**8**:e72714.
17. Eriksen EF, Shabestari M, Ghouri A, Conaghan PG. Bisphosphonates as a treatment modality in osteoarthritis. *Bone* 2021;**143**:115352.
18. Abramoff B, Caldera FE. Osteoarthritis: pathology, diagnosis, and treatment options. *Med Clin North Am* 2020;**104**:293−311.
19. Jevsevar DS, Brown GA, Jones DL, Matzkin EG, Manner PA, Mooar P, et al. The American Academy of Orthopaedic Surgeons evidence-based guideline on: treatment of osteoarthritis of the knee, 2nd edition. *J Bone Jt Surg Am* 2013;**95**:1885−6.
20. Baranowsky A, Appelt J, Tseneva K, Jiang S, Jahn D, Tsitsilonis S, et al. Tranexamic acid promotes murine bone marrow-derived osteoblast proliferation and inhibits osteoclast formation *in vitro*. *Int J Mol Sci* 2021;**22**:449.
21. Hiramoto K, Oikawa H, Yamate Y, Sato EF. Tranexamic acid protects ovary and testis functions and ameliorates osteoporosis in mice. *Pharmacology* 2020;**105**:652−61.
22. Xie W, Jiang S, Donat A, Knapstein PR, Albertsen LC, Kokot JL, et al. Tranexamic acid attenuates the progression of post-traumatic osteoarthritis in mice. *Am J Sports Med* 2024;**52**:766−78.
23. Roberts I, Shakur H, Afolabi A, Brohi K, Coats T, Dewan Y, et al. The importance of early treatment with tranexamic acid in bleeding trauma patients: an exploratory analysis of the CRASH-2 randomised controlled trial. *Lancet* 2011;**377**:1096−101.
24. Kirsch JM, Bedi A, Horner N, Wiater JM, Pauzenberger L, Koueiter DM, et al. Tranexamic acid in shoulder arthroplasty: a systematic review and meta-analysis. *JBJS Rev* 2017;**5**:e3.
25. Wang C, Xu GJ, Han Z, Ma JX, Ma XL, Jiang X, et al. Topical application of tranexamic acid in primary total hip arthroplasty: a systemic review and meta-analysis. *Int J Surg* 2015;**15**:134−9.
26. Fillingham YA, Ramkumar DB, Jevsevar DS, Yates AJ, Shores P, Mullen K, et al. The efficacy of tranexamic acid in total knee arthroplasty: a network meta-analysis. *J Arthroplasty* 2018;**33**:3083−9.e4.
27. Del Rosario E, Florez-Pollack S, Zapata L, Hernandez K, Tovar-Garza A, Rodrigues M, et al. Randomized, placebo-controlled, double-blind study of oral tranexamic acid in the treatment of moderate-to-severe melasma. *J Am Acad Dermatol* 2018;**78**:363−9.
28. Johnson SM, Tsang D, Dansby M, Allen C. New and off-label uses of tranexamic acid. *AACN Adv Crit Care* 2021;**32**:237−42.
29. U.S. Food and Drug Administration. *Drug Approval Package-Lysteda (tranexamic acid) tablets*. 2010. Updated May 6, 2010. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022430_lysteda_toc.cfm. [Accessed 8 March 2024].
30. European Medicines Agency. *Antifibrinolytic medicines − referral*. 2012. Updated November 6, 2013. Available from: https://www.ema.europa.eu/en/medicines/human/referrals/antifibrinolytic-medicines. [Accessed 8 March 2024].
31. Therapeutic Goods Administration. *Australian Public assessment report: tranexamic acid*. 2010. Updated December 15, 2010. Available from: https://www.tga.gov.au/resources/auspar/auspar-tranexamic-acid. [Accessed 8 March 2024].
32. Pharmaceuticals and Medical Devices Agency. *MHLW Pharmaceuticals and Medical Devices Safety Information FY2013 No. 302*. 2013. Updated Aug 7, 2013. Available from: https://www.pmda.go.jp/english/safety/info-services/drugs/medical-safety-information/0012.html. [Accessed 8 March 2024].
33. Shakur H, Roberts I, Bautista R, Caballero J, Coats T, Dewan Y, et al. Effects of tranexamic acid on death, vascular occlusive events, and blood transfusion in trauma patients with significant haemorrhage (CRASH-2): a randomised, placebo-controlled trial. *Lancet* 2010;**376**:23−32.
34. Morrison JJ, Dubose JJ, Rasmussen TE, Midwinter MJ. Military application of tranexamic acid in trauma emergency resuscitation (MATTERs) study. *Arch Surg* 2012;**147**:113−9.
35. CRASH-3 trial collaborators. Effects of tranexamic acid on death, disability, vascular occlusive events and other morbidities in patients with acute traumatic brain injury (CRASH-3): a randomised, placebo-controlled trial. *Lancet* 2019;**394**:1713−23.
36. Valle EJ, Allen CJ, Van Haren RM, Jouria JM, Li H, Livingstone AS, et al. Do all trauma patients benefit from tranexamic acid?. *J Trauma Acute Care Surg* 2014;**76**:1373−8.
37. Harvin JA, Peirce CA, Mims MM, Hudson JA, Podbielski JM, Wade CE, et al. The impact of tranexamic acid on mortality in injured patients with hyperfibrinolysis. *J Trauma Acute Care Surg* 2015;**78**:905−11.
38. Li ZJ, Fu X, Xing D, Zhang HF, Zang JC, Ma XL. Is tranexamic acid effective and safe in spinal surgery? A meta-analysis of randomized controlled trials. *Eur Spine J* 2013;**22**:1950−7.
39. Luo W, Sun RX, Jiang H, Ma XL. The efficacy and safety of topical administration of tranexamic acid in spine surgery: a meta-analysis. *J Orthop Surg Res* 2018;**13**:96.
40. Zhang P, He J, Fang Y, Chen P, Liang Y, Wang J. Efficacy and safety of intravenous tranexamic acid administration in patients undergoing hip fracture surgery for hemostasis: a meta-analysis. *Medicine* 2017;**96**:e6940.
41. Zhang S, Xiao C, Yu W, Long N, He F, Cai P, et al. Tranexamic acid safely reduces hidden blood loss in patients undergoing intertrochanteric fracture surgery: a randomized controlled trial. *Eur J Trauma Emerg Surg* 2022;**48**:731−41.
42. Zhou XD, Zhang Y, Jiang LF, Zhang JJ, Zhou D, Wu LD, et al. Efficacy and safety of tranexamic acid in intertrochanteric fractures: a single-blind randomized controlled trial. *Orthop Surg* 2019;**11**:635−42.
43. Chen F, Jiang Z, Li M, Zhu X. Efficacy and safety of perioperative tranexamic acid in elderly patients undergoing trochanteric fracture surgery: a randomised controlled trial. *Hong Kong Med J* 2019;**25**:120−6.
44. Tian S, Shen Z, Liu Y, Zhang Y, Peng A. The effect of tranexamic acid on hidden bleeding in older intertrochanteric fracture patients treated with PFNA. *Injury* 2018;**49**:680−4.
45. Jordan M, Aguilera X, González JC, Castillón P, Salomó M, Hernández JA, et al. Prevention of postoperative bleeding in hip fractures treated with prosthetic replacement: efficacy and safety of fibrin sealant and tranexamic acid. A randomised controlled clinical trial (TRANEXFER study). *Arch Orthop Trauma Surg* 2019;**139**:597−604.

46. Ma H, Wang H, Long X, Xu Z, Chen X, Li M, et al. Early intravenous tranexamic acid intervention reduces post-traumatic hidden blood loss in elderly patients with intertrochanteric fracture: a randomized controlled trial. *J Orthop Surg Res* 2021;**16**:106.
47. Nikolaou VS, Masouros P, Floros T, Chronopoulos E, Skertsou M, Babis GC. Single dose of tranexamic acid effectively reduces blood loss and transfusion rates in elderly patients undergoing surgery for hip fracture: a randomized controlled trial. *Bone Jt J* 2021;**103-B**:442−8.
48. Sen RK, Attar MU, Saini G, Tripathy SK. Safety and efficacy of perioperative tranexamic acid infusion in acetabular fracture fixation: a randomized placebo-controlled double-blind prospective study. *Injury* 2022;**53**:3361−4.
49. Ekinci M, Ok M, Ersin M, Günen E, Kocazeybek E, Sırma SÖ, et al. A single dose of tranexamic acid infusion is safe and effective to reduce total blood loss during proximal femoral nailing for intertrochanteric fractures: a prospective randomized study. *Ulus Travma Acil Cerrahi Derg* 2022;**28**:1627−33.
50. Huang J, Guo H, Huang W, Tan X, Huang H, Zeng C. Topical application of tranexamic acid can reduce postoperative blood loss in calcaneal fractures: a randomized controlled trial. *J Foot Ankle Surg* 2022;**61**:1056−9.
51. Shen J, Yang Z, Fu M, Hao J, Jiang W. The influence of topical use of tranexamic acid in reducing blood loss on early operation for thoracolumbar burst fracture: a randomized double-blinded controlled study. *Eur Spine J* 2021;**30**:3074−80.
52. Cuff DJ, Simon P, Gorman RA. Randomized prospective evaluation of the use of tranexamic acid and effects on blood loss for proximal humeral fracture surgery. *J Shoulder Elbow Surg* 2020;**29**:1627−32.
53. Khiabani K, Ahmadfar M, Labafchi A, Gosheh MR, Samieirad S. Is preoperative administration of tranexamic acid effective on blood loss reduction in mandibular fracture surgeries? A triple-blind randomized clinical trial. *J Oral Maxillofac Surg* 2021;**79**:429.e1−7.
54. Wang W, Duan K, Ma M, Jiang Y, Liu T, Liu J, et al. Tranexamic acid decreases visible and hidden blood loss without affecting prethrombotic state molecular markers in transforaminal thoracic interbody fusion for treatment of thoracolumbar fracture-dislocation. *Spine* 2018;**43**:E734−9.
55. Sharaby MMF, El-Deeb YM. Is intravenous tranexamic acid effective in reduction of blood loss during pelvic and acetabular surgery?. *Int Orthop* 2022;**46**:1721−9.
56. Xu JW, Qiang H, Li TL, Wang Y, Wei XX, Li F. Efficacy of topical *vs* intravenous tranexamic acid in reducing blood loss and promoting wound healing in bone surgery: a systematic review and meta-analysis. *World J Clin Cases* 2021;**9**:4210−20.
57. Yu SP, Hunter DJ. Intra-articular therapies for osteoarthritis. *Expert Opin Pharmacother* 2016;**17**:2057−71.
58. Fenwick A, Antonovska I, Pfann M, Mayr J, Wiedl A, Nuber S, et al. Does tranexamic acid reliably reduce blood loss in proximal femur fracture surgery?. *Eur J Trauma Emerg Surg* 2023;**49**:209−16.
59. Chandran S, Sasidharan S. A clinical analysis of pre-operative tranexamic acid and wound closure without suction drain in decreasing the blood loss in surgical treatment of fractures of hip. *Int J Sci Study* 2018;**6**:85−8.
60. Maniar RN, Pradhan P, Bhatnagar N, Maniar A, Bidwai R, Bindal P. Role of suction drain after knee arthroplasty in the tranexamic acid era: a randomized controlled study. *Clin Orthop Surg* 2019;**11**:73−81.
61. Maliarov A, Newman N, Sabouret P, Al-Shakfa F, Chergui S, Lavoie F. Suction drainage in total knee replacement does not influence early functional outcomes or blood loss: a randomized control trial. *Arthroplasty* 2023;**5**:8.
62. Draxler DF, Yep K, Hanafi G, Winton A, Daglas M, Ho H, et al. Tranexamic acid modulates the immune response and reduces postsurgical infection rates. *Blood Adv* 2019;**3**:1598−609.
63. Zhang S, Xu H, Xie J, Cao G, Lei Y, Pei F. Tranexamic acid attenuates inflammatory effect and modulates immune response in primary total knee arthroplasty: a randomized, placebo-controlled, pilot trial. *Inflammopharmacology* 2020;**28**:839−49.
64. Yazdi H, Klement MR, Hammad M, Inoue D, Xu C, Goswami K, et al. Tranexamic acid is associated with reduced periprosthetic joint infection after primary total joint arthroplasty. *J Arthroplasty* 2020;**35**:840−4.
65. Klement MR, Padua FG, Li WT, Detweiler M, Parvizi J. Tranexamic acid reduces the rate of periprosthetic joint infection after aseptic revision arthroplasty. *J Bone Jt Surg Am* 2020;**102**:1344−50.
66. Hong GJ, Wilson LA, Liu J, Memtsoudis SG. Tranexamic acid administration is associated with a decreased odds of prosthetic joint infection following primary total hip and primary total knee arthroplasty: a national database analysis. *J Arthroplasty* 2021;**36**:1109−13.
67. Lacko M, Jarčuška P, Schreierova D, Lacková A, Gharaibeh A. Tranexamic acid decreases the risk of revision for acute and delayed periprosthetic joint infection after total knee replacement. *Jt Dis Relat Surg* 2020;**31**:8−13.
68. Drain NP, Gobao VC, Bertolini DM, Smith C, Shah NB, Rothenberger SD, et al. Administration of tranexamic acid improves long-term outcomes in total knee arthroplasty. *J Arthroplasty* 2020;**35**:S201−6.
69. Sukeik M, Alshryda S, Powell J, Haddad FS. The effect of tranexamic acid on wound complications in primary total Hip arthroplasty: a meta-analysis. *Surgeon* 2020;**18**:53−61.
70. Karaduman ZO, Arıcan M, Turhan Y, Turhal O, Orhan Z, Gamsızkan M. Systemic tranexamic acid promotes bone healing in a rat model of femur fracture. *Jt Dis Relat Surg* 2020;**31**:432−9.
71. Çevik HB, Eceviz E, Çilingir Kaya ÖT, Ercan F, Çeçen GS. The effect of topical and systemic tranexamic acid on fracture healing in rats. *Acta Orthop Traumatol Turc* 2020;**54**:207−12.
72. Balkanlı B, Çopuroğlu C, Çopuroğlu E. The effects of intravenous and local tranexamic acid on bone healing: an experimental study in the rat tibia fracture model. *Injury* 2020;**51**:2840−5.
73. Wagenbrenner M, Heinz T, Horas K, Jakuscheit A, Arnholdt J, Mayer-Wagner S, et al. Impact of tranexamic acid on chondrocytes and osteogenically differentiated human mesenchymal stromal cells (hMSCs) *in vitro*. *J Clin Med* 2020;**9**:3880.
74. Parker JD, Lim KS, Kieser DC, Woodfield TBF, Hooper GJ. Is tranexamic acid toxic to articular cartilage when administered topically? What is the safe dose?. *Bone Jt Lett J* 2018;**100-B**:404−12.
75. Tuttle JR, Feltman PR, Ritterman SA, Ehrlich MG. Effects of tranexamic acid cytotoxicity on *in vitro* chondrocytes. *Am J Orthop* 2015;**44**:E497−502.
76. Ambra LF, de Girolamo L, Niu W, Phan A, Spector M, Gomoll AH. No effect of topical application of tranexamic acid on articular cartilage. *Knee Surg Sports Traumatol Arthrosc* 2019;**27**:931−5.
77. Chu CR. The effects of tranexamic acid on joint inflammation and cartilage health in anterior cruciate ligament injured patients. ClinicalTrials.gov identifier: NCT03552705. 2018. Updated September 21, 2023. Available from: https://clinicaltrials.gov/study/NCT03552705. [Accessed 8 March 2024].
78. Weisel JW, Litvinov RI. Mechanisms of fibrin polymerization and clinical implications. *Blood* 2013;**121**:1712−9.
79. Keragala CB, Medcalf RL. Plasminogen: an enigmatic zymogen. *Blood* 2021;**137**:2881−9.
80. Hayakawa M, Maekawa K, Kushimoto S, Kato H, Sasaki J, Ogura H, et al. Hyperfibrinolysis in severe isolated traumatic brain injury may occur without tissue hypoperfusion: a retrospective observational multicentre study. *Crit Care* 2017;**21**:222.
81. Faraoni D, Van Der Linden P. A systematic review of antifibrinolytics and massive injury. *Miner Anestesiol* 2014;**80**:1115−22.
82. Miles LA, Ny L, Wilczynska M, Shen Y, Ny T, Parmer RJ. Plasminogen receptors and fibrinolysis. *Int J Mol Sci* 2021;**22**:1712.
83. Baker SK, Strickland S. A critical role for plasminogen in inflammation. *J Exp Med* 2020;**217**:e20191865.
84. Kanno Y, Ishisaki A, Kawashita E, Chosa N, Nakajima K, Nishihara T, et al. Plasminogen/plasmin modulates bone metabolism by regulating the osteoblast and osteoclast function. *J Biol Chem* 2011;**286**:8952−60.

85. Law RHP, Wu G, Leung EWW, Hidaka K, Quek AJ, Caradoc-Davies TT, et al. X-ray crystal structure of plasmin with tranexamic acid-derived active site inhibitors. *Blood Adv* 2017;**1**:766−71.
86. Wu G, Mazzitelli BA, Quek AJ, Veldman MJ, Conroy PJ, Caradoc-Davies TT, et al. Tranexamic acid is an active site inhibitor of urokinase plasminogen activator. *Blood Adv* 2019;**3**:729−33.
87. Flick MJ, Du X, Prasad JM, Raghu H, Palumbo JS, Smeds E, et al. Genetic elimination of the binding motif on fibrinogen for the *S. aureus* virulence factor ClfA improves host survival in septicemia. *Blood* 2013;**121**:1783−94.
88. Jin H, Liu K, Tang J, Huang X, Wang H, Zhang Q, et al. Genetic fate-mapping reveals surface accumulation but not deep organ invasion of pleural and peritoneal cavity macrophages following injury. *Nat Commun* 2021;**12**:2863.
89. Brancato SK, Albina JE. Wound macrophages as key regulators of repair: origin, phenotype, and function. *Am J Pathol* 2011;**178**:19−25.
90. Koh TJ, DiPietro LA. Inflammation and wound healing: the role of the macrophage. *Expert Rev Mol Med* 2011;**13**:e23.
91. Hsieh JY, Smith TD, Meli VS, Tran TN, Botvinick EL, Liu WF. Differential regulation of macrophage inflammatory activation by fibrin and fibrinogen. *Acta Biomater* 2017;**47**:14−24.
92. Smiley ST, King JA, Hancock WW. Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. *J Immunol* 2001;**167**:2887−94.
93. Jennewein C, Tran N, Paulus P, Ellinghaus P, Eble JA, Zacharowski K. Novel aspects of fibrin(ogen) fragments during inflammation. *Mol Med* 2011;**17**:568−73.
94. Barthel D, Schindler S, Zipfel PF. Plasminogen is a complement inhibitor. *J Biol Chem* 2012;**287**:18831−42.
95. Singh B, Al-Jubair T, Voraganti C, Andersson T, Mukherjee O, Su YC, et al. *Moraxella catarrhalis* binds plasminogen to evade host innate immunity. *Infect Immun* 2015;**83**:3458−69.
96. Barthel D, Singh B, Riesbeck K, Zipfel PF. *Haemophilus influenzae* uses the surface protein E to acquire human plasminogen and to evade innate immunity. *J Immunol* 2012;**188**:379−85.
97. Koenigs A, Hammerschmidt C, Jutras BL, Pogoryelov D, Barthel D, Skerka C, et al. BBA70 of *Borrelia burgdorferi* is a novel plasminogen-binding protein. *J Biol Chem* 2013;**288**:25229−43.
98. Draxler DF, Sashindranath M, Medcalf RL. Plasmin: a modulator of immune function. *Semin Thromb Hemost* 2017;**43**:143−53.
99. Andronicos NM, Chen EI, Baik N, Bai H, Parmer CM, Kiosses WB, et al. Proteomics-based discovery of a novel, structurally unique, and developmentally regulated plasminogen receptor, Plg-RKT, a major regulator of cell surface plasminogen activation. *Blood* 2010;**115**:1319−30.
100. Miles LA, Baik N, Lighvani S, Khaldoyanidi S, Varki NM, Bai H, et al. Deficiency of plasminogen receptor, Plg-R, causes defects in plasminogen binding and inflammatory macrophage recruitment *in vivo*. *J Thromb Haemost* 2017;**15**:155−62.
101. Vago JP, Sugimoto MA, Lima KM, Negreiros-Lima GL, Baik N, Teixeira MM, et al. Plasminogen and the plasminogen receptor, Plg-R, regulate macrophage phenotypic, and functional changes. *Front Immunol* 2019;**10**:1458.
102. Gong Y, Hart E, Shchurin A, Hoover-Plow J. Inflammatory macrophage migration requires MMP-9 activation by plasminogen in mice. *J Clin Invest* 2008;**118**:3012−24.
103. Lighvani S, Baik N, Diggs JE, Khaldoyanidi S, Parmer RJ, Miles LA. Regulation of macrophage migration by a novel plasminogen receptor Plg-R KT. *Blood* 2011;**118**:5622−30.
104. Thaler B, Baik N, Hohensinner PJ, Baumgartner J, Panzenböck A, Stojkovic S, et al. Differential expression of Plg-R and its effects on migration of proinflammatory monocyte and macrophage subsets. *Blood* 2019;**134**:561−7.
105. Syrovets T, Lunov O, Simmet T. Plasmin as a proinflammatory cell activator. *J Leukoc Biol* 2012;**92**:509−19.
106. Syrovets T, Jendrach M, Rohwedder A, Schüle A, Simmet T. Plasmin-induced expression of cytokines and tissue factor in human monocytes involves AP-1 and IKKbeta-mediated NF-kappaB activation. *Blood* 2001;**97**:3941−50.
107. Zalfa C, Azmoon P, Mantuano E, Gonias SL. Tissue-type plasminogen activator neutralizes LPS but not protease-activated receptor-mediated inflammatory responses to plasmin. *J Leukoc Biol* 2019;**105**:729−40.
108. Li Q, Laumonnier Y, Syrovets T, Simmet T. Plasmin triggers cytokine induction in human monocyte-derived macrophages. *Arterioscler Thromb Vasc Biol* 2007;**27**:1383−9.
109. Borg RJ, Samson AL, Au AEL, Scholzen A, Fuchsberger M, Kong YY, et al. Dendritic cell-mediated phagocytosis but not immune activation is enhanced by plasmin. *PLoS One* 2015;**10**:e0131216.
110. Das R, Ganapathy S, Settle M, Plow EF. Plasminogen promotes macrophage phagocytosis in mice. *Blood* 2014;**124**:679−88.
111. Cole HA, Ohba T, Nyman JS, Hirotaka H, Cates JMM, Flick MJ, et al. Fibrin accumulation secondary to loss of plasmin-mediated fibrinolysis drives inflammatory osteoporosis in mice. *Arthritis Rheumatol* 2014;**66**:2222−33.
112. Kawao N, Tamura Y, Okumoto K, Yano M, Okada K, Matsuo O, et al. Plasminogen plays a crucial role in bone repair. *J Bone Miner Res* 2013;**28**:1561−74.
113. Wang L, Yao L, Duan H, Yang F, Lin M, Zhang R, et al. Plasminogen regulates fracture repair by promoting the functions of periosteal mesenchymal progenitors. *J Bone Miner Res* 2021;**36**:2229−42.
114. Yuasa M, Mignemi NA, Nyman JS, Duvall CL, Schwartz HS, Okawa A, et al. Fibrinolysis is essential for fracture repair and prevention of heterotopic ossification. *J Clin Invest* 2015;**125**:3117−31.
115. Lecker I, Wang DS, Kaneshwaran K, Mazer CD, Orser BA. High concentrations of tranexamic acid inhibit ionotropic glutamate receptors. *Anesthesiology* 2017;**127**:89−97.
116. Whyte CS, Mutch NJ. uPA-mediated plasminogen activation is enhanced by polyphosphate. *Haematologica* 2021;**106**:522−31.
117. Su SC, Lin CW, Yang WE, Fan WL, Yang SF. The urokinase-type plasminogen activator (uPA) system as a biomarker and therapeutic target in human malignancies. *Expert Opin Ther Targets* 2016;**20**:551−66.
118. Gonias SL. Plasminogen activator receptor assemblies in cell signaling, innate immunity, and inflammation. *Am J Physiol Cell Physiol* 2021;**321**:C721−34.
119. Kawao N, Tamura Y, Horiuchi Y, Okumoto K, Yano M, Okada K, et al. The tissue fibrinolytic system contributes to the induction of macrophage function and CCL3 during bone repair in mice. *PLoS One* 2015;**10**:e0123982.
120. Popa NL, Wergedal JE, Lau KHW, Mohan S, Rundle CH. Urokinase plasminogen activator gene deficiency inhibits fracture cartilage remodeling. *J Bone Miner Metab* 2014;**32**:124−35.
121. Rabbani SA, Gladu J, Mazar AP, Henkin J, Goltzman D. Induction in human osteoblastic cells (SaOS2) of the early response genes fos, jun, and myc by the amino terminal fragment (ATF) of urokinase. *J Cell Physiol* 1997;**172**:137−45.
122. Daci E, Udagawa N, Martin TJ, Bouillon R, Carmeliet G. The role of the plasminogen system in bone resorption *in vitro*. *J Bone Miner Res* 1999;**14**:946−52.
123. Furlan F, Galbiati C, Jorgensen NR, Jensen JEB, Mrak E, Rubinacci A, et al. Urokinase plasminogen activator receptor affects bone homeostasis by regulating osteoblast and osteoclast function. *J Bone Miner Res* 2007;**22**:1387−96.
124. Kalbasi Anaraki P, Patecki M, Tkachuk S, Kiyan Y, Haller H, Dumler I. Urokinase receptor mediates osteoclastogenesis *via* M-CSF release from osteoblasts and the c-Fms/PI3K/Akt/NF-κB pathway in osteoclasts. *J Bone Miner Res* 2015;**30**:379−88.
125. Kalbasi Anaraki P, Patecki M, Larmann J, Tkachuk S, Jurk K, Haller H, et al. Urokinase receptor mediates osteogenic differentiation of mesenchymal stem cells and vascular calcification *via* the complement C5a receptor. *Stem Cells Dev* 2014;**23**:352−62.
126. Del Rosso M, Margheri F, Serratì S, Chillà A, Laurenzana A, Fibbi G. The urokinase receptor system, a key regulator at the

intersection between inflammation, immunity, and coagulation. *Curr Pharm Des* 2011;**17**:1924−43.
127. Milner JM, Patel A, Rowan AD. Emerging roles of serine proteinases in tissue turnover in arthritis. *Arthritis Rheum* 2008;**58**:3644−56.
128. Lavigne P, Benderdour M, Lajeunesse D, Reboul P, Shi Q, Pelletier JP, et al. Subchondral and trabecular bone metabolism regulation in canine experimental knee osteoarthritis. *Osteoarthritis Cartilage* 2005;**13**:310−7.
129. Kalavrouziotis D, Voisine P, Mohammadi S, Dionne S, Dagenais F. High-dose tranexamic acid is an independent predictor of early seizure after cardiopulmonary bypass. *Ann Thorac Surg* 2012;**93**:148−54.
130. Murkin JM, Falter F, Granton J, Young B, Burt C, Chu M. High-dose tranexamic acid is associated with nonischemic clinical seizures in cardiac surgical patients. *Anesth Analg* 2010;**110**:350−3.
131. Sharma V, Katznelson R, Jerath A, Garrido-Olivares L, Carroll J, Rao V, et al. The association between tranexamic acid and convulsive seizures after cardiac surgery: a multivariate analysis in 11529 patients. *Anaesthesia* 2014;**69**:124−30.
132. Keyl C, Uhl R, Beyersdorf F, Stampf S, Lehane C, Wiesenack C, et al. High-dose tranexamic acid is related to increased risk of generalized seizures after aortic valve replacement. *Eur J Cardio Thorac Surg* 2011;**39**:e114−21.
133. Manji RA, Grocott HP, Leake J, Ariano RE, Manji JS, Menkis AH, et al. Seizures following cardiac surgery: the impact of tranexamic acid and other risk factors. *Can J Anaesth* 2012;**59**:6−13.
134. Koster A, Börgermann J, Zittermann A, Lueth JU, Gillis-Januszewski T, Schirmer U. Moderate dosage of tranexamic acid during cardiac surgery with cardiopulmonary bypass and convulsive seizures: incidence and clinical outcome. *Br J Anaesth* 2013;**110**:34−40.
135. Ohashi N, Sasaki M, Ohashi M, Kamiya Y, Baba H, Kohno T. Tranexamic acid evokes pain by modulating neuronal excitability in the spinal dorsal horn. *Sci Rep* 2015;**5**:13458.
136. Lecker I, Wang DS, Whissell PD, Avramescu S, Mazer CD, Orser BA. Tranexamic acid-associated seizures: causes and treatment. *Ann Neurol* 2016;**79**:18−26.
137. Paul P, de Belleroche J. The role of D-serine and glycine as co-agonists of NMDA receptors in motor neuron degeneration and amyotrophic lateral sclerosis (ALS). *Front Synaptic Neurosci* 2014;**6**:10.
138. Lecker I, Wang DS, Romaschin AD, Peterson M, Mazer CD, Orser BA. Tranexamic acid concentrations associated with human seizures inhibit glycine receptors. *J Clin Invest* 2012;**122**:4654−66.
139. Sigel E, Steinmann ME. Structure, function, and modulation of $GABA_A$ receptors. *J Biol Chem* 2012;**287**:40224−31.
140. Irl H, Kratzer S, Schwerin S, Kochs E, Blobner M, Schneider G, et al. Tranexamic acid impairs hippocampal synaptic transmission mediated by gamma aminobutyric acid receptor type A. *Eur J Pharmacol* 2017;**815**:49−55.
141. Kratzer S, Irl H, Mattusch C, Bürge M, Kurz J, Kochs E, et al. Tranexamic acid impairs γ-aminobutyric acid receptor type A-mediated synaptic transmission in the murine amygdala: a potential mechanism for drug-induced seizures?. *Anesthesiology* 2014;**120**:639−49.
142. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, et al. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev* 2010;**62**:405−96.
143. Itzstein C, Cheynel H, Burt-Pichat B, Merle B, Espinosa L, Delmas PD, et al. Molecular identification of NMDA glutamate receptors expressed in bone cells. *J Cell Biochem* 2001;**82**:134−44.
144. Hinoi E, Fujimori S, Yoneda Y. Modulation of cellular differentiation by *N*-methyl-D-aspartate receptors in osteoblasts. *FASEB J* 2003;**17**:1532−4.
145. Li JL, Zhao L, Cui B, Deng LF, Ning G, Liu JM. Multiple signaling pathways involved in stimulation of osteoblast differentiation by *N*-methyl-D-aspartate receptors activation *in vitro*. *Acta Pharmacol Sin* 2011;**32**:895−903.
146. Merle B, Itzstein C, Delmas PD, Chenu C. NMDA glutamate receptors are expressed by osteoclast precursors and involved in the regulation of osteoclastogenesis. *J Cell Biochem* 2003;**90**:424−36.
147. Matta C, Juhász T, Fodor J, Hajdú T, Katona É, Szűcs-Somogyi C, et al. *N*-Methyl-D-aspartate (NMDA) receptor expression and function is required for early chondrogenesis. *Cell Commun Signal* 2019;**17**:166.
148. Mantuano E, Azmoon P, Brifault C, Banki MA, Gilder AS, Campana WM, et al. Tissue-type plasminogen activator regulates macrophage activation and innate immunity. *Blood* 2017;**130**:1364−74.
149. Li JL, Cui B, Qi L, Li XY, Deng LF, Ning G, et al. NMDA enhances stretching-induced differentiation of osteoblasts through the ERK1/2 signaling pathway. *Bone* 2008;**43**:469−75.
150. Salter DM, Wright MO, Millward-Sadler SJ. NMDA receptor expression and roles in human articular chondrocyte mechanotransduction. *Biorheology* 2004;**41**:273−81.
151. Lin TH, Yang RS, Tang CH, Wu MY, Fu WM. Regulation of the maturation of osteoblasts and osteoclastogenesis by glutamate. *Eur J Pharmacol* 2008;**589**:37−44.
152. Peet NM, Grabowski PS, Laketic-Ljubojevic I, Skerry TM. The glutamate receptor antagonist MK801 modulates bone resorption *in vitro* by a mechanism predominantly involving osteoclast differentiation. *FASEB J* 1999;**13**:2179−85.
153. Fujimori S, Hinoi E, Yoneda Y. Functional $GABA_B$ receptors expressed in cultured calvarial osteoblasts. *Biochem Biophys Res Commun* 2002;**293**:1445−52.
154. Takahata Y, Takarada T, Hinoi E, Nakamura Y, Fujita H, Yoneda Y. Osteoblastic γ-aminobutyric acid, type B receptors negatively regulate osteoblastogenesis toward disturbance of osteoclastogenesis mediated by receptor activator of nuclear factor κB ligand in mouse bone. *J Biol Chem* 2011;**286**:32906−17.
155. Tamayama T, Maemura K, Kanbara K, Hayasaki H, Yabumoto Y, Yuasa M, et al. Expression of $GABA_A$ and $GABA_B$ receptors in rat growth plate chondrocytes: activation of the GABA receptors promotes proliferation of mouse chondrogenic ATDC5 cells. *Mol Cell Biochem* 2005;**273**:117−26.
156. Reyes-García MG, Hernández-Hernández F, Hernández-Téllez B, García-Tamayo F. $GABA_A$ receptor subunits RNA expression in mice peritoneal macrophages modulate their IL-6/IL-12 production. *J Neuroimmunol* 2007;**188**:64−8.
157. Zhang B, Vogelzang A, Miyajima M, Sugiura Y, Wu Y, Chamoto K, et al. B cell-derived GABA elicits IL-10^+ macrophages to limit anti-tumour immunity. *Nature* 2021;**599**:471−6.
158. Januzi L, Poirier JW, Maksoud MJE, Xiang YY, Veldhuizen RAW, Gill SE, et al. Autocrine GABA signaling distinctively regulates phenotypic activation of mouse pulmonary macrophages. *Cell Immunol* 2018;**332**:7−23.
159. Hinoi E, Fujimori S, Takemori A, Kurabayashi H, Nakamura Y, Yoneda Y. Demonstration of expression of mRNA for particular AMPA and kainate receptor subunits in immature and mature cultured rat calvarial osteoblasts. *Brain Res* 2002;**943**:112−6.
160. Szczesniak AM, Gilbert RW, Mukhida M, Anderson GI. Mechanical loading modulates glutamate receptor subunit expression in bone. *Bone* 2005;**37**:63−73.
161. Wang L, Hinoi E, Takemori A, Yoneda Y. Release of endogenous glutamate by AMPA receptors expressed in cultured rat costal chondrocytes. *Biol Pharm Bull* 2005;**28**:990−3.
162. Cheng XL, Ding F, Li H, Tan XQ, Liu X, Cao JM, et al. Activation of AMPA receptor promotes TNF-α release *via* the ROS−cSrc−NFκB signaling cascade in RAW264.7 macrophages. *Biochem Biophys Res Commun* 2015;**461**:275−80.
163. Dai M, Sui B, Xue Y, Liu X, Sun J. Cartilage repair in degenerative osteoarthritis mediated by squid type II collagen *via* immunomodulating activation of M2 macrophages, inhibiting apoptosis and hypertrophy of chondrocytes. *Biomaterials* 2018;**180**:91−103.
164. Liu C, Liu X, Xue Y, Ding T, Sun J. Hydrolyzed tilapia fish collagen modulates the biological behavior of macrophages under inflammatory conditions. *RSC Adv* 2015;**5**:30727−36.