

Comparison of tranexamic acid pharmacokinetics after intra-articular and intravenous administration in rabbits

Gen-xiang Rong¹, Chen-lin Shen², Bin-jie Gui^{1*}, Hao Yin³ and Zhi Tang¹

¹Department of the joint and reconstructive microsurgery, The First Affiliated Hospital of Anhui Medical University, Hefei Anhui, PR China

²Institute for Liver Diseases of Anhui Medical University (AMU), Anhui key Laboratory of Bioactivity of Natural Products, School of pharmacy, Anhui Medical University, Hefei, PR China

³Chromatography and Mass Spectrometry Lab, Hefei National Laboratory for Physical Sciences at Microscale, University of Science and Technology of China, Hefei, Anhui, PR China

Abstract: Tranexamic Acid (TXA) is commonly administered in total knee arthroplasty for reducing blood loss. There has been a growing interest in the topical use of TXA except intravenous use for prevention of bleeding in TKA. The aim of this study was to develop and validate a HPLC-MS method to detect TXA and apply to compare the pharmacokinetic profile of TXA after intravenous (IV) and topical intra-articular (IA) application of TXA at a dose of 20 mg/kg in rabbits. In order to prove intra-articular administration is better than that of intravenous administration from the point of rabbit pharmacokinetic. Two groups of rabbits (n=6/group) respectively received TXA intra-articularly or intravenously. Blood samples were collected at scheduled time. The concentration of TXA in plasma was determined by a validated HPLC-MS method. Excellent linearity was found between 0.015 and 70.0 µg/ml with a lower limit of quantitation (LLOQ) of 0.015 µg/ml ($r > 0.99$); moreover, all the validation data including accuracy and precision (intra- and inter-day) were all within the required limits. The pharmacokinetic parameters in IA and IV group were: C_{max} : 30.65 ± 3.31 VS 54.05 ± 6.21 µg/ml ($p < 0.01$); $t_{1/2}$: 1.26 ± 0.05 VS 0.68 ± 0.13 h ($p < 0.05$); AUC_{0-t} : 42.98 ± 7.73 VS 23.39 ± 4.14 µg/ml · h ($p < 0.01$), time above the minimum effective concentration (%T > MEC): $1.5-2.2$ VS $0.7-1.2$ h ($p < 0.05$). HPLC-MS method is suitable for TXA pharmacokinetic studies. The results demonstrated that topical intra-articular application of TXA showed a reduced peak plasma concentration and prolonged therapeutic drug level compared with intravenous TXA from the point of rabbit pharmacokinetic.

Keywords: Tranexamic acid, intra-articular, rabbit, hplc-ms, pharmacokinetics.

INTRODUCTION

Nowadays, Total knee arthroplasty (TKA) has become one of the most effective methods to solve the end-stage knee joint disease. It can relieve the pain and enhance the knee function, which greatly improves the life quality of patients. Postoperative blood loss is one of the most common complications in TKA. The major causes of postoperative blood loss following TKA can be attributed to surgical trauma, which stimulates fibrinolysis. The latter is further enhanced by tourniquet release at the end of the surgical procedure that leads to an increase in fibrinolytic activity simultaneously, which in turn causes a rebound effect in the immediate postoperative period, with a significant increase in blood loss (Fukuda *et al.*, 2007, Lozano *et al.*, 2008). Tranexamic acid (TXA) is a synthetic derivative of the amino acid lysine (Dunn and Goa, 1999). that exerts its antifibrinolytic effect through the reversible blockade of lysine binding sites on plasminogen molecules. Another possible effect of TXA is the protection of platelets based on its antiplasmin effect and the inhibition of the platelet activation factor (O'Brien *et al.*, 2000). It is well established that tranexamic acid can reduce the loss of blood during TKA,

but the ideal route of administering TXA still has no final consensus (Alvarez *et al.*, 2008, Goodet *et al.*, 2003). The most commonly administration route for TXA in published studies regarding TKA is intravenous and intra-articular. The effectiveness of the intravenous or intra-articular regimens of TXA in reducing blood loss during TKA in patients has been evaluated by several studies. Most of which have shown that no difference of clinical efficacy and thromboembolic complications were found between two routes (Hegde *et al.*, 2013, Patel *et al.*, 2014, Soni *et al.*, 2014). However, Seo (Seo *et al.*, 2013) pointed out that compared with intravenous administration, intra-articular administration of TXA seems to be more effective in terms of reducing blood loss and transfusion frequency. On the contrary, Sarzaeem MM (Sarzaeem *et al.*, 2014) reported that on the sides of reducing hemoglobin drop and transfused units, intravenous injection of TXA appears to be more effective; while intra-articular administration of TXA is more effective in terms of reducing postoperative wound drainage. Several studies proposed the assumption that using TXA intra-articularly can minimise the systemic effects on inducing hypercoagulable states (Wong *et al.*, 2010), and due to its ease of use and lower cost, intra-articular administration has become one of most popular methods for reducing blood loss and transfusion requirement in TKA (Alshryda

*Corresponding author: e-mail: ayguibinjie@126.com

et al., 2013). The intravenous pharmacokinetic parameters of TXA are well known, however, this is not the case when it is administered intra-articularly. No information is available on the pharmacokinetics of intra-articular TXA, which remains largely unsafe and still unable to confirm the hypothesis that topical intra-articular application provides a maximum concentration of TXA at the bleeding site, and is associated with little or no systemic absorption of the TXA, which need to be established as soon as possible.

In our present study, in order to prove that whether intra-articular administration is better than that of intravenous from the point of rabbit pharmacokinetic, we firstly established and validated a HPLC-MS method to determinate TXA in rabbit plasma, and then successfully applied it to pharmacokinetics study after intra-articular and intravenous administration of TXA at a dose of 20 mg/kg in rabbits. The pharmacokinetic parameters were calculated and compared between the two routes, in order to prove intra-articular administration is better than that of intravenous from the point of rabbit pharmacokinetic. We hope to provide more evidence and recommendation for clinical use of TXA rationally.

MATERIALS AND METHODS

Chemicals and reagents

TXA (fig. 1, purity >99%, batch number: 100174-201203) was obtained from National Institutes for Food and Drug Control. Formic acid, acetonitrile and methanol were HPLC grade, and purchased from the company of Fisher Scientific. And other reagents used were of analytical grade. Double deionized water was generated by a Milli-Q® academic ultra-pure water purification system supplied by Millipore (Shanghai, China).

Animals

Adult rabbits (♀ and ♂, n=16, weighing 2.25-2.50kg) were purchased from the Experimental Animal Center of Anhui Medical University. The study protocol was approved by the Ethics Committee for Scientific Research of Anhui Medical University. Rabbits were housed in a controlled room with a humidity of 50-60% and temperature of 20-25°C with water and food freely available.

Experimental design

Sixteen rabbits were randomly divided into IA and IV administration groups with 8 rabbits each. IA group received a single dose of TXA (20mg/kg) following local intra-articular administration, IV group was given a single dose of TXA (20 mg/kg) by intravenous administration via the marginal ear vein. Serial blood samples (1.0ml) were collected from ear vein into heparinized tubes at scheduled time points for the pharmacokinetic evaluation of TXA. In brief, blood samples were drawn before (0h) and at 2, 5, 10, 15, 30min and 1, 2, 4, 6, 8, 12 and 24h for

IV and IA group after drug administration. Plasma samples were separated by centrifugation at 3000 g for 15 min at 4°C within 30min and stored at -80°C until analysis.

Preparation of Stock Solutions, Quality Control and Calibration Standard Samples

Primary stock solution of TXA was prepared with a concentration of 1000µg/ml in deionized water. The primary solutions were further diluted with deionized water as appropriate to get the working standard solution of 0.15, 0.5, 1.5, 5, 15, 50, 200 and 700µg/ml for TXA. The working solutions of TXA were spiked to blank rabbit plasma to obtain calibration standards of 0.015, 0.05, 0.15, 0.5, 1.5, 5.0, 20.0 and 70.0µg/ml for TXA. Quality control (QC) samples at three concentrations of 0.03, 1.2 and 55µg/ml were prepared in the same fashion. The stock solutions, calibration standards and quality control samples were stored in amber vials at -20°C.

Quantification of TXA in rabbit plasma

The HPLC-MS method was used to determine the TXA concentrations in rabbit plasma. A 100µl aliquot plasma of rabbit was taken into a 1.5ml Eppendorf tube, then deproteinated with 400µl acetonitrile. The mixture was vortexed and centrifuged at 14000g for 10 min. Then, the aqueous supernatant was transferred into an auto-sampler vial and 10µl aliquot was subsequently injected into the HPLC-MS system (ThermoFisher Scientific, USA). The separation was performed isocratically (0.1% formic acid water/ acetonitrile, 60:40v/v) at a flow rate of 0.20 ml/min using a Hypersil GOLD C18 column (150 × 2.1 mm, i.d. 3µm) maintained at 25°C. The total run time was 5 min per sample. TXA was detected by a LTQ-Orbitrap XL mass spectrometer (ThermoFisher Scientific, USA) equipped with an electrospray ionization (ESI) source in the positive ion mode. The source parameters of the mass spectrometer were optimized and maintained as follows: capillary voltage 35 v; capillary temperature 320°C; source current 2.83µA; source voltage 4.7 kv; aux gas flow rate 5 arb units; sheath gas flow rate 20 arb units; vaporizer temp 84°C. Parent protonated [M+H]⁺ ions of tranexamic acid (m/z 158.0) was trapped with a mass resolution of 1.0 atomic mass unit (amu) (fig. 1). The Xcalibur software (version 2.1.0, ThermoFisher Scientific, USA) was used to acquire and quantitate chromatographic data.

Method validation procedure

The HPLC-MS method was fully validated on the basis of FDA guidelines for specificity, linearity, precision, accuracy, recovery, lower limit of quantitation (LLOQ), and stability. Specificity was assessed by analyzing blank rabbit plasma, blank rabbit plasma samples containing spiked TXA, and rabbit plasma after single use of TXA, to ensure that no endogenous interference at the retention time of TXA. LLOQ (0.015µg/ml) was calculated as the lowest concentration in the curve of linear calibration.

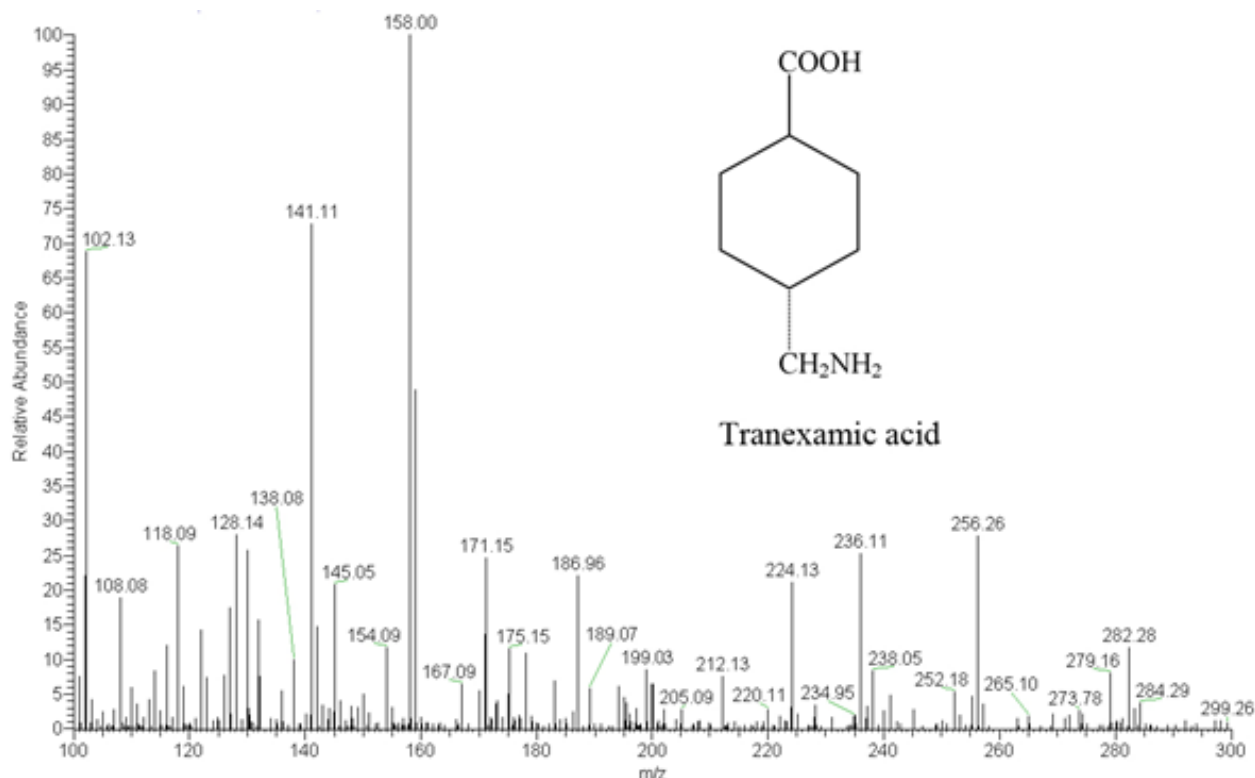


Fig. 1: Full-scan MS spectra of 1.0 µg/ml TXA in methanol. Chemical structures of TXA is also presented.

Table 1: Accuracy, precision, matrix effect, and method recovery data for plasma QC samples of TXA.

Nominal Conc.(µg/ml)	Accuracy (%) ^a		Precision (%) ^b		Matrix effect	Method recovery
	Intra-day	Inter-day	Intra-day	Inter-day		
0.03	94.1±5.7	102.3±9.8	7.3±0.5	11.5±0.9	95.4±6.3	94.2±5.6
1.2	104.1±8.3	92.3±7.2	8.2±0.7	6.2±0.7	103.1±5.9	89.1±9.7
55	97.5±9.1	104.8±5.1	10.7±0.6	10.1±0.8	94.7±9.3	90.2±7.9

^aExpressed as mean measured concentrations /nominal concentrations×100%. ^bExpressed as the relative standard deviation.

Five samples independent of standards was used to establish the LLOQ. The signal intensity of the LLOQ was ≥ 10 fold blank response. Calibration curve was obtained by least-squares linear regression (weighted $1/x^2$) of the peak area of TXA (Y-axis) versus the nominal analyte plasma concentrations (X-axis) ranging from 0.015 to 70 µg/ml for TXA, calibration curves for TXA in rabbit plasma were executed on three consecutive days. When determine the intra-day accuracy and precision, we analyze six replicates of each QC sample ($n=6$) at low (0.03 µg/ml), medium (1.2 µg/ml), and high (55 µg/ml) concentration levels extracted with a set of standards in one batch. When determine the inter-day accuracy and precision, the same procedure was repeated on three consecutive days with new samples. Matrix effect was determined by comparing the peak area of the neat solution spiked into blank matrix samples after the precipitation step with those of the neat solution at low (0.03 µg/ml), medium (1.2 µg/ml), and high (55 µg/ml) concentration levels (five samples for each concentration level). Recovery was determined by comparing peak areas

obtained after injection of the processed QC samples with those achieved by direct injection of the same amount of drug in mobile phase at low (0.03 µg/ml), medium (1.2 µg/ml) and high (55 µg/ml) concentration levels (five samples for each level). The stability of TXA in rabbit plasma was evaluated at following conditions: -80°C storage, room temperature storage (25°C), 3 freeze-thaw cycles, and at different steps in the sample preparation process. Each condition was tested with QC samples with low (0.03 µg/ml), medium (1.2 µg/ml), and high (55 µg/ml) concentration levels triplicately and the reference was fresh samples. Stock solution of TXA was evaluated at -20°C and room temperature (25°C).

Data processing

We use DAS (Drugs and Statistics, version 3.0) software to calculate pharmacokinetic parameters of TXA by the noncompartmental method. The directly obtained pharmacokinetic parameters of TXA were the peak plasma concentration (C_{max}) and its corresponding time (T_{max}). AUC_{0-t} and AUC_{0-∞} are the area under curve to t

Table 2: Stability of TXA in stock solution, in plasma, and in post-processing plasma sample

Nominal	Pre-processing	Post-processed	3 freeze-thaw cycles	Long term	Stock solution	
Conc. ($\mu\text{g/ml}$)	(25°C, 12h)	(4°C, 24h)	(-80°C~25°C)	(-80°C, 60 days)	(-20°C, 30 days)	(25°C, 24h)
0.03	92.4 $\pm$ 5.9	92.5 $\pm$ 4.9	90.3 $\pm$ 7.2	89.1 $\pm$ 5.5	95.3 $\pm$ 6.4	103.5 $\pm$ 4.7
1.2	106.8 $\pm$ 6.3	97.5 $\pm$ 5.4	103.8 $\pm$ 8.1	96.3 $\pm$ 4.7	101.6 $\pm$ 7.2	92.3 $\pm$ 5.3
55	97.7 $\pm$ 8.8	101.2 $\pm$ 6.4	96.3 $\pm$ 6.8	91.6 $\pm$ 7.1	91.0 $\pm$ 5.2	94.7 $\pm$ 8.1

Table 3: Comparison between the mean pharmacokinetic parameters of TXA after IA or IV administration of TXA in rabbits

Pharmacokinetic parameters	Intra-articular (n=8)	Intravenous (n=8)
C_{max} ($\mu\text{g/ml}$)	30.65 $\pm$ 3.31**	54.05 $\pm$ 6.21
T_{max} (h)	0.19 $\pm$ 0.05*	0.05 $\pm$ 0.03
$t_{1/2}$ (h)	1.26 $\pm$ 0.05*	0.68 $\pm$ 0.13
MRT (h)	1.34 $\pm$ 0.12*	0.69 $\pm$ 0.12
AUC_{0-t} ($\mu\text{g}\cdot\text{h/ml}$)	42.98 $\pm$ 7.73**	23.39 $\pm$ 4.14
$\text{AUC}_{0-\infty}$ ($\mu\text{g}\cdot\text{h/ml}$)	43.45 $\pm$ 8.00**	23.43 $\pm$ 4.13
V_d (L/kg)	0.76 $\pm$ 0.17*	1.04 $\pm$ 0.38
Cl (L/h/kg)	0.47 $\pm$ 0.09*	0.87 $\pm$ 0.13
AUMC_{0-t} ($\mu\text{g}\cdot\text{h}^2/\text{ml}$)	54.25 $\pm$ 11.32**	16.15 $\pm$ 5.19
$\text{AUMC}_{0-\infty}$ ($\mu\text{g}\cdot\text{h}^2/\text{ml}$)	58.80 $\pm$ 14.43**	16.46 $\pm$ 5.08

* $P < 0.05$, ** $P < 0.01$ versus intravenous group, C_{max} : maximum observed plasma concentration, T_{max} : time to reach the maximum concentration, $t_{1/2}$: terminal half-life, MRT: mean retention time, AUC_{0-t} : area under the plasma concentration-time curve from zero to the last measurable concentration, $\text{AUC}_{0-\infty}$: area under the concentration-time curve from zero to infinity, V_d : apparent volume of distribution, Cl: total plasma clearance, AUMC_{0-t} : area under the first moment curve from zero to t the last measurable concentration, $\text{AUMC}_{0-\infty}$: area under the first moment curve from zero to infinity, IA: intra-articular, IV intravenous.

and to infinity, AUMC is the area under the first moment curve. Elimination rate (k_e) was the slope of the linear regression of the log-transformed concentration values versus time date in the terminal phase. And elimination half-life ($t_{1/2}$) was calculated as $\ln 2/k_e$. All the experimental datum are shown as mean $\pm$ standard error. Student's unpaired two-tail t-test was used to analyze these datum with 95% confidence interval. All statistical analyses were performed using SPSS 17.0.

RESULTS

Bioanalytical method validation

The HPLC-MS method we used to detect and quantitate TXA in rabbit plasma met all of the validation criteria per the FDA Guidance. The mean retention time for TXA in rabbit plasma was 1.94 min (fig. 2). In rabbit plasma, the recovery and matrix effect have no relations with TXA concentration (table 2). The mean matrix effect was 96.2 $\pm$ 6.3% and the mean recovery of TXA was 91.6 $\pm$ 7.8%, respectively, which can indicate there is no matrix effect in our method. The assay was linear over the range 0.015–70 $\mu\text{g/ml}$ for the TXA and the calibration curve could be described by the equation: $Y = 3.4 \times 10^{-7} X - 9.25 \times 10^{-2}$ ($r^2 = 0.9991$). The LLOQ was 0.015 $\mu\text{g/ml}$ with a relative standard deviation (RSD) of 9.7%. Intraday precision and accuracy ranged from 4.3 to 11.7%, and 87.4 to 108.6%, respectively. Interday precision and accuracy ranged from 3.6 to 12.4%, and 89.3 to 109.1%, respectively (table 1). The results of the stability study indicated that stock

solutions of TXA was stable for at least one day when stored at indoor temperature (25°C) and for at least 30 days in a refrigerator (-20°C) (table 2). TXA was also stable in plasma after short term storage for 24h at +4 °C and at room temperature (+25°C, RSD < 7.4%, compared with freshly prepared QC samples at three levels) and after long term storage for 2 months at -80°C (RSD < 8.6%). There was no significant difference of the TXA plasma concentrations in the QC samples after three freeze-thaw cycles (RSD < 9.1%). And compared to the initial value, there was no significant changes when processed samples were kept at +25°C or +4°C for 24h, (RSD < 7.4%, and RSD < 8.8%).

Pharmacokinetic analysis

The mean rabbit plasma concentration of TXA versus time profiles after IV and IA administration was presented in fig. 3. The main pharmacokinetic parameters of TXA including T_{max} , C_{max} , AUC_{0-t} , and $t_{1/2}$ of TXA were given in table 3. The plasma TXA concentration increased rapidly and peaked at 0.05 h after IV administration, while the peak concentration of TXA reached at 0.19 h following IA administration. TXA was eliminated from the systemic circulation with the mean $t_{1/2}$ values of 1.26 h and 0.68 h ($p < 0.01$) for IA and IV group. There was a significant reduction in C_{max} , and a markedly increase in AUC_{0-t} following IA administration ($p < 0.01$ for C_{max} , and $p < 0.01$ for AUC_{0-t}), and time above the minimum effective concentration ($T > \text{MEC}$) necessary to inhibit fibrinolysis (5–10 $\mu\text{g/ml}$) was 1.5–2.2 hours in IA group,

but just 0.7-1.2 hours in IV group. Which could indicate that the slower rate of plasma TXA efflux from the joint cavity to systemic circulation after IA administration compared to IV administration.

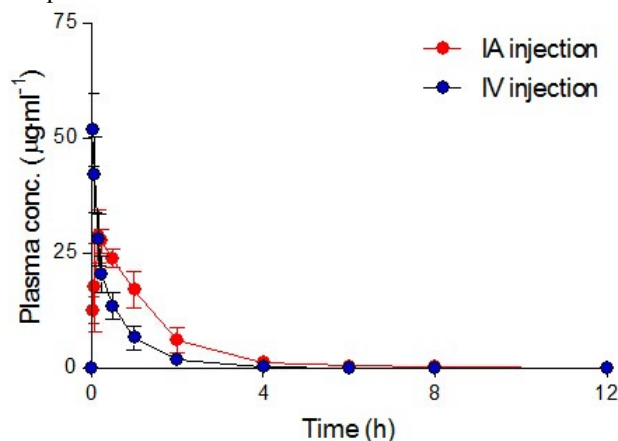


Fig. 2: Representative LC-MS chromatograms of a pooled blank plasma sample (A), a spiked plasma containing 80 µg/ml TXA (B), a plasma sample from a rabbit obtained at 5 minutes after IV administration of 20 mg/kg TXA (C), or from a rabbit obtained at 15 minutes after IA administration of 20 mg/kg TXA (D).

DISCUSSION

Studies (Kalairajah *et al.*, 2005, Sehat *et al.*, 2000) have shown that the average postoperative blood loss in TKA can be ranged from 1450 to 1790 ml. Besides increasing the risk of cardiovascular disease in older patients, bleeding into the soft tissues surrounding the knee would increase pain, stiffness, and length of recovery following surgery. Furthermore, accumulation of blood in the knee joint also can lead to swelling, delay wound healing and increase the risk of infection (Pulido L *al.*, 2008). About one third of patients who underwent TKA need blood transfusion ranging from 200 to 600 ml (Alshryda *et al.*, 2013). Therefore, these patients will be exposed to the risks of transmitting bloodborne pathogens, hemolysis, transfusion-related injuries, renal impairment or failure, and even increased mortality (Blajchman *et al.*, 2005, Fuller *et al.*, 2009). Mostly, they constitute a well-established risk factor for the development of periprosthetic joint infection (Pulido *et al.*, 2008). Multi-center clinical trial (Poeran *et al.*, 2014) have shown that up to 69% patient's transfusion requirements were reduced in TKA with the administration of TXA. Meta-analysis (Kagoma *et al.*, 2009, Yang *et al.*, 2012, Zhou *et al.*, 2013) also showed that administrating TXA can reduce blood loss by 300 to 500 ml, and reduce the rate of transfusion requirements about 23% to 47%. Recently, the use of TXA has become one of the most effective methods to reduce blood loss in TKA. Cap and other researchers (Cap *et al.*, 2011) have suggested this safe and inexpensive drug should be included into trauma clinical practice guidelines and treatment protocols. But,

the administration route and dosage for TXA are different in different studies. So some researchers also indicated that recommending TXA as a routine drug in TKA was too early (Poeran *et al.*, 2014). The guide of the European Anesthesiology Society recommended the use of TXA in orthopedic surgery and established the dosage pattern for intravenous TXA at 20-25 mg/kg body weight (Aguilera-Roig *et al.*, 2014), but no recommendation for intra-articular administration. In order to avoid dosage difference, the doses of tranexamic acid chosen for the current investigation were all at 20 mg/kg body weight.

When TXA is administered intravenously with healthy volunteers, its half-life was 2 hours (Eriksson *et al.*, 1974). Early pharmacokinetic study has shown that the minimum effective plasma concentration of TXA required to inhibit fibrinolysis in human was 5-10 µg/ml (Dunn and Goa, 1999). After a dose of 15 mg/kg intravenously with healthy volunteers, the plasma concentration can still remain above the effective plasma concentration for more than 4-6 hour (Andersson *et al.*, 1965). But the intra-articular pharmacokinetic parameters of TXA are not known so far. In this study, we firstly established the plasma concentration-time profile in rabbit plasma after intra-articular administration of 20 mg/kg TXA.

The pharmacokinetic parameters in our study have shown that the average peak concentration (C_{max}) of TXA was 54.05 µg/ml intravenously, but when it was administered topically by intra-articular, C_{max} was 30.65 µg/ml. Lower C_{max} of TXA can minimise the systemic effects on inducing hypercoagulable states. Thus, 20mg/kg TXA appears to be relatively safer when administered intra-articularly than intravenously from the point of pharmacokinetic, which proves the hypothesis proposed by some researchers. The half-life of TXA in rabbit plasma was 0.68 hours intravenously, but 1.26 hours intra-articularly. Shorter half-life is one of the shortcomings of intravenous injections of TXA. Therefore, there is a clinical demand for a longer half-life method to maintain the efficacy. We found that the half-life of TXA was significantly increased when administered topically by intra-articular, which can offer a safe and convenient sustained-release platform to deliver TXA, and overcome the shortcoming of common injections of TXA. After a dose of 20mg/kg TXA in rabbits, plasma concentration above the effective plasma concentration (5-10µg/ml) was about 0.7-1.2 hours for intravenously, but approximately 1.5-2.2 hours for intra-articularly. Furthermore, TXA can be detectable in plasma after 8 hours by intra-articularly, but only 4 hours intravenously. So, intra-articular administration of TXA can prolong haemostasis time, and increase the clinical effect. In conclusion, intra-articular administration of TXA is more effective and safer compared with intravenous administration in their respective pharmacokinetic characteristics.

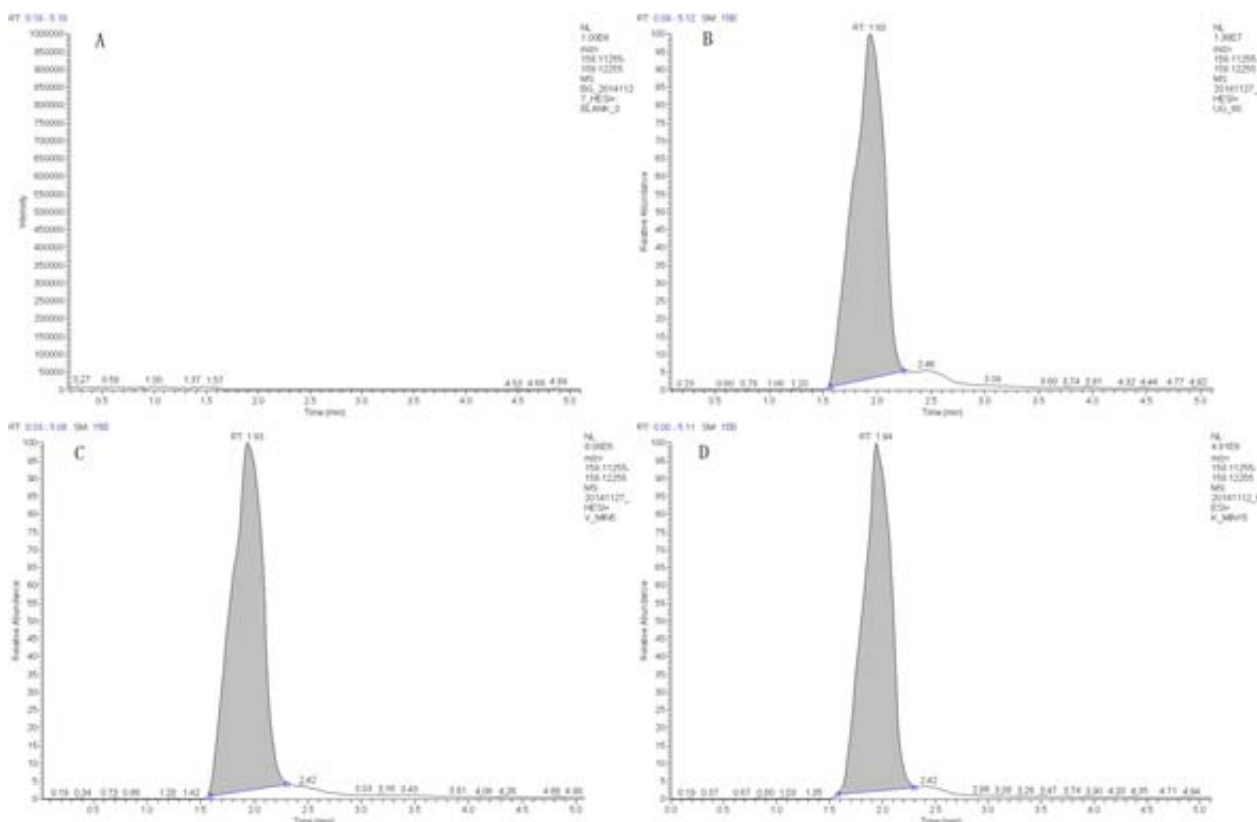


Fig. 3: Mean ($\pm$ SD) plasma concentration-time profile in rabbit plasma after IV and IA administration of 20mg/kg TXA.

A number of analytical methods have been published for the assay of TXA in biological samples. To assay TXA in blood, there are many methods have been established, including reversed-phase HPLC with ultraviolet or fluorescent detection (Huertas-Perez *et al.*, 2007), gas chromatography (GC)(Vessman and Stromberg, 1977) and capillary electrophoresis (CE)(Lin *et al.*, 2005). However, because of the post-column or pre-column derivatization procedure, those methods are cumbersome and cannot meet large sample analysis. What's more, all those methods have complex procedure, which could result in large assay variations (Grassin Delyle *et al.*, 2010). In our study, a new HPLC-MS method was established and validated to detect TXA in blood sample. And because of its ease of sample preparation, small sample size requirement, short turn-around time, and high sensitivity and selectivity (Chang *et al.*, 2004), this new HPLC-MS method is advantageous over existing methods and very fit for clinical pharmacokinetic studies. In our study, the results of accuracy, precision, and recovery of this assay method are all met with FDA's acceptance criteria.

The main limitation of the current study is conducted in animals, the pharmacokinetic parameters in rabbits may be somewhat different compared with healthy volunteers or patients. It has been reported that the half-life time of intravenous administrated tranexamic acid is about 2

hours in human (Eriksson *et al.*, 1974), while it is 0.68 hours in this study. This discrepancy may be caused by the conditions, methods, and objects of the two studies. It is indicated that more pharmacokinetic studies are needed in the future to provide related exposure information.. Secondly, all the experiments are performed on healthy rabbits. TXA is administered topically at an intra-articular just using an injector. Which has no destruction of knee articular cavity, including articular blood vessels and bone surface. However, in TKA, the knee articular cavity is opened, small blood vessels could be destroyed, tibial tubercle and supracondylar femoral osteotomy are conducted. TXA could penetrate into blood easily through destroyed blood vessels and bone surface. And the inflammation and other complications after surgery could significantly affect the pharmacokinetics of intra-articularly administrated tranexamic acid. It would be more informative if the experiments are performed on the animals with surgical trauma on the knee joints. Thirdly, we do not detect the concentration of TXA in knee articular cavity. Thus we cannot prove that intra-articular application provide a maximum concentration of tranexamic acid at the bleeding site, and cannot know the time which remain above the effective plasma concentration in knee articular cavity. Above all, the study is limited because of the experimental design, but it also can suggest that intra-articular administration of TXA is

better than intravenous from the point of rabbit pharmacokinetic.

CONCLUSION

In summary, a sensitive HPLC-MS method was developed and validated for the determination of TXA in rabbit plasma and was successfully applied to pharmacokinetic studies after IA and IV administration. Compared to intravenous administration, TXA intra-articular administration has reduced peak plasma concentration, prolong the half-life and effective concentration time, increase AUC_{0-t}. Which can minimise the systemic effects on inducing hypercoagulable states, and also prolong haemostasis time. These findings partly suggest the hypothesis that intra-articular administration of TXA is safer and more effective than intravenous from the point of rabbit pharmacokinetic. Of course, more pharmacokinetic studies are urgently needed to confirm the conclusion further more.

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