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Effect of Urinary Protease Inhibitor (Ulinastatin) on Cardiopulmonary Bypass: A Meta-Analysis for China and Japan

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Abstract

Objectives: A meta-analysis was conducted to investigate the effects of ulinastatin treatment on adult patients undergoing cardiac surgery under cardiopulmonary bypass (CPB).

Methods: Seven electronic databases were searched for reports of randomized, controlled trials conducted up to February 2014 in which patients undergoing cardiac surgery with CPB were administered ulinastatin in the perioperative period.

Results: Fifty-two studies with 2025 patients were retained for analysis. The results showed that the ulinastatin can attenuate the plasma levels of pro-inflammatory cytokines and enhance the anti-inflammatory cytokine levels in patients undergoing cardiac surgery with CPB. Meanwhile, the ulinastatin had a significant beneficial effect on myocardial injury. The mean differences (MD) and 95% confidence intervals (95% CI) of biochemical markers were −63.54 (−79.36, −47.72) for lactate dehydrogenase, −224.99 (−304.83, −145.14) for creatine kinase, −8.75 (−14.23, −3.28) for creatine kinase-MB, and −0.14 (−0.20, −0.09] for troponin I (all $P<0.01$). However, neither hemodynamics nor cardiac function improved significantly, except that the MD and 95% CI of mean arterial pressure were 2.50 (0.19, 4.80) ($P=0.03$). There were no statistically significant differences in the use of inotropes, postoperative bleeding, postoperative complications, the intensive care unit (ICU) stay, and the hospital stay; however, the frequency of auto resuscitation increased significantly (OR 1.98, 95%CI 1.19 to 3.30, $P<0.01$), the duration of intubation (MD −1.58, 95%CI −2.84 to −0.32, $P<0.01$) and the duration of mechanical ventilation (MD −3.29, 95%CI −4.41 to −2.17, $P<0.01$) shortened significantly in patients who were treated with ulinastatin.

Conclusions: Ulinastatin can reduce the plasma levels of pro-inflammatory cytokines and elevate anti-inflammatory cytokine in patients from China and Japan undergoing cardiac surgery with CPB. Ulinastatin treatment may have protective

effects on myocardial injury, and can increase the frequency of auto resuscitation, shorten the duration of intubation and mechanical ventilation.

Introduction

A systemic inflammatory response (SIR) and multiple-organ ischemia/reperfusion injury often occur after open-heart surgery carried out under cardiopulmonary bypass (CPB) [1]. The activation of the complement cascade and fibrinolytic system by multiple physical, chemical, and biological stimuli in CPB, along with neutrophil activation and the generation of a sequential inflammatory cascade, contribute to a series of postoperative complications [2, 3], such as post-surgical bleeding, acute lung injury, acute respiratory distress syndrome, cardiac insufficiency, and acute renal injury [4, 5]. This results in prolongation of mechanical ventilation and ICU or hospital stay, and increases costs.

Ulinastatin is a broad-spectrum hydrolase inhibitor purified from the urine of healthy men. As a serine protease, ulinastatin is able to inhibit various inflammatory proteases, including trypsin, chymotrypsin, and neutrophil elastase and plasmin. Thus, it is considered an effective anti-inflammatory molecule, and it has been widely used clinically in China, Korea, and Japan to treat pancreatitis, rheumatoid arthritis, sepsis, and other inflammatory diseases [6, 7]. In recent years, ulinastatin has been used to prevent postoperative complications and post-pump organ injury in patients undergoing cardiac surgery with CPB [4, 8, 9]. Xu and colleagues found that high-dose ulinastatin can reduce pulmonary injury, improve pulmonary function after CPB, and shorten the duration of intubation and intensive care unit (ICU) stay [8]. However, Park and colleagues observed no significant effect of ulinastatin on major organ dysfunction, SIR, or other postoperative complications [9]. Since the effect of ulinastatin on patients' responses to CPB and the associated potential challenges and complications are not yet clear, we conducted a systematic review and meta-analysis to assess studies that used ulinastatin in order to evaluate its effect in patients undergoing open-heart surgery with CPB.

Methods

Search strategy

The electronic databases PubMed, EMBASE (using OVID), ISI, ACS, Cochrane Controlled Trials Registry, China National Knowledge infrastructure, and Wanfang Data were searched from their inception up to February 2014. The keywords and terms used were (''ulinastatin'' OR ''protease inhibitor'' OR ''UTI'' OR ''hydrolase inhibitors'') AND (''cardiopulmonary bypass'' OR ''cardiac surgery'' OR ''open heart surgery''). Papers in the English or Chinese language

were included. All articles were imported into EndNote ×6 software to delete duplicates.

Study selection criteria

The inclusion criteria for the primary studies were as follows: (1) randomized, placebo-controlled clinical trials; (2) adults undergoing open-heart surgery employing CPB; (3) ulinastatin was used perioperatively; (4) CPB time or aortic cross-clamping time were reported; (5) outcomes of interest included, ie, the inflammatory markers (TNF-α, IL-6, IL-8, and IL-10), the myocardial biomarkers: lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase-MB (CK-MB) and troponin I (TnI), the hemodynamic parameters heart rate (HR), mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP) and central venous pressure (CVP), cardiac index and left ventricular ejection fraction (LVEF) as markers of cardiac function, the numbers of patients needing inotropes and auto resuscitation, the postoperative bleeding, the postoperative complications, the duration of mechanical ventilation, the intubation time, and the lengths of ICU stay and hospital stay.

The exclusion criteria for the primary studies were as follows: (1) review, abstract, or case report; (2) animal or cell study; (3) not open-heart surgery; (4) pediatric cardiac surgery; (5) total number of patients <20, and (6) published in any language other than English or Chinese.

Study selection and data extraction were performed by two independent reviewers (YZ and ZW). Disagreements were resolved through consensus.

Data extraction

A data collection form was designed before the data were extracted. The articles finally included were assessed independently by two reviewers (YZ and ZW). The extracted data included: (1) first author and year of publication; (2) total number of patients, number of patients in the ulinastatin and control groups, gender, age, body weight, and body surface area; (3) CPB time, and aortic cross-clamping time in both groups; (4) data regarding outcomes of interest, as described above, in the ulinastatin and control groups. When the results of the trial were reported as median and quartile, the Stela Pudar-Hozo method was used to estimate the mean and standard deviation [10].

Assessment of study quality

Two reviewers (YZ and ZW) independently assessed the risk of bias, using the tool described in the Cochrane Handbook for Systematic Reviews of Interventions [11]. The risk of bias was rated as low, high, or unclear with regard to: (1) sequence generation; (2) allocation concealment; (3) blinding; and (4) completeness of outcome data.

Statistical methods

Review Manager Software (version 5.02 for Windows; The Cochrane Collaboration, 2009) was used to perform the meta-analysis, and STATA 12.0 (Stata Corp, College station, TX) was used to evaluate the publication bias (Egger's test and Begg's test). In accordance with the Review Manager Handbook, odds ratio (OR) or mean difference (MD), and their 95% confidence interval (CI) were used to estimate the effective value. Statistical heterogeneity was examined by Cochrane Q-test (significant at $P<0.1$) and the I^2 value. For comparisons with $P<0.1$ or $I^2<50\%$, a fixed-effect model was used; otherwise, a random-effect model was adopted. If necessary, a sensitivity analysis was also performed to evaluate the influences of individual studies on the final effect. All P-values were two-sided, and $P<0.05$ was considered significant.

Results

Search results and characteristics

Of the initial 3252 records identified, 2159 remained after duplicates were removed. Two thousand thirty-five articles were excluded because they were not clinical trials (in Japanese, review, animal studies, cell studies, comment, etc.). Of the 124 articles reviewed at the full-text level, 72, including 44 pediatric studies, 13 observational studies, 10 retrospective studies, and 5 non-open heart surgery studies were excluded. Finally, 52 articles were eligible for this meta-analysis [4, 8, 9, 12–61] (Fig. 1), of which 11 were in English. All were published between 1996 and 2013 (Table 1).

A total of 2025 patients were included in our meta-analysis; 1013 received ulinastatin and 1012 received a placebo saline solution. The groups showed no significant differences with respect to gender [OR (95% CI) were 0.85 (0.69, 1.04), P=0.12], age [MD (95% CI) were -0.14 (−0.58, 0.31), P=0. 55], CPB time [MD (95% CI) were −0.95 (−2.34, 0.44), P=0.18], or aortic cross-clamping time [MD (95% CI) were −0.48 (−1.52, 0.57), P=0.37] (Table 2). As no heterogeneity existed among these studies, a fixed-effect model was adopted.

Risk of bias of included trials

Overall, 2 trials had a low risk of bias; 47 trials had a moderate risk of bias, due to insufficient information about the sequence generation process, concealed allocation methods, or blinding, and incomplete addressing of outcome data; and 3 trials [4, 26, 29] had a high risk of selection bias, as the patients' ID numbers, the order of admission, or specific groups of people were used for the generation of the randomization sequence (Fig. 2, S1 Figure).

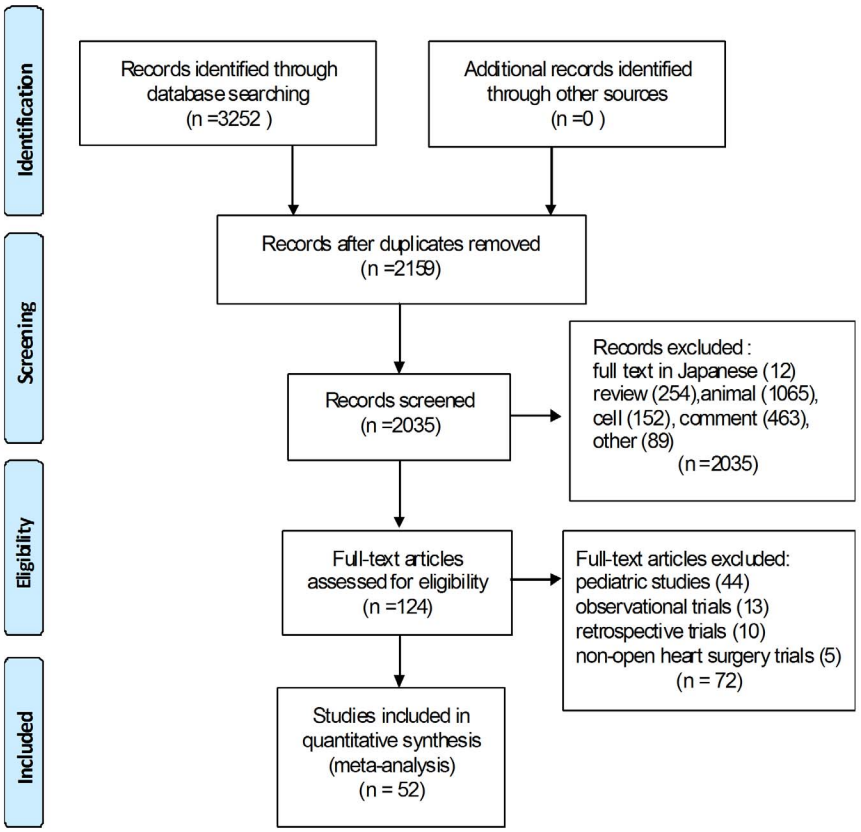

Fig. 1. Flow chart showing how eligible studies were identified.

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Efficacy of ulinastatin on patients with cardiopulmonary bypass

Inflammatory mediators (TNF-α, IL-6, IL-8 and IL-10)

At first, we perform a meta-analysis to assess the changes of these inflammatory mediators at twenty-four hours postoperatively. The heterogeneities were found, and the random-effect model was used to perform meta-analysis. Pooled analysis showed the significant differences existed in TNF-α (MD-7.53, 95%CI [−9.45, −5.61], $P<0.01$), IL-6 (MD-32.87, 95%CI [−39.53, −26.20], $P<0.01$), IL-8 (MD-2.40, 95%CI [−3.40, −1.41], $P=0.02$) and IL-10 (MD14.49, 95%CI [4.57, 24.42], $P=0.02$) between the ulinastatin and control groups (Table 2). Sensitivity analysis showed that the overall effect was not changed by omitting some studies until these heterogeneities became acceptable (heterogeneity: $P>0.10$; $I^2<50\%$) (Table 3).

Myocardial Biomarkers (LDH, CK, CK-MB, and TnI)

We assessed these myocardial biomarkers: LDH changes in 4 trials, CK changes in 4 trials, CK-MB changes in 12 trials, and TnI changes in 8 trials. The meta-analysis demonstrated that all biomarkers significantly reduced in the ulinastatin group compared with the placebo groups. The MD (95% CI) values were −63.54 (−79.36, −47.72) for LDH ($P<0.01$), −224.99 (−304.83, −145.14) for CK

Table 1. Main characteristics of the eligible studies.

Author(year)	No. of patients	Gender (male)		Age(year)		CBP time(min)		ACC time(min)		Ulinastatin (IU/kg)
Groups	Total	U	C	U	C	U	C	U	C	
Bao HJ (2006)	30	NA	NA	29.19±9.4	27.5±14.7	70.4±22.0	73.7±18.9	48.2±16.4	49.3±17.3	15000
Chen BJ (2011)	130	31	31	42.11±5.66	43.26±5.92	69.41±26.53	70.12±26.03	48.74±22.5	50.22±24.2	10000
Chen P (2003)	60	17	15	42±11	41±12	115±43	111±47	79±34	77±37	9×10^6 IU/c
Chen RW (2007)	30	NA	NA	3.4±1.1	3.2±1.2	25.4±9.2	28.5±7.5	20.7±6.0	20.2±7.1	10000
Chen TT (2013)	60	14	12	49.8±10.8	50.4±10.0	102.5±20.7	99.5±22.6	74.7±20.5	69.0±20.4	12,000
Chen Y (2005)	24	6	9	10.25±5.53	9.58±7.09	46.26±6.58	47.37±17.22	25.08±6.44	27.26±10.16	10000
Gao XG (2012)	65	18	16	43±2	44±2	NA	NA	NA	NA	NA
Hiyama A (1997)	18	NA	NA	54±10	52±5	145±14	186±23	82±10	107±15	6×10^5 IU/c
J BY (2007)	30	11	11	57.4±6.6	56.8±6.1	108±29	117±34	60±22	67±23	1×10^6 IU/c
Ji HW (2009)	36	NA	NA	60±8	59±9	120±19	105±16	74±16	66±11	1×10^6 IU/c
Jiang B (2012)	40	9	10	34±12	35±11	100±29	103±38	68±24	71±25	12000
Jiang CB (2009)	40	NA	NA	35.6±8.3	37.4±9.2	112.6±23.9	109.2±19.4	81.1±16.3	79.4±15.8	20000
Kawamura T (1996)	22	NA	NA	63±11	54±17	159±27	159±47.4	97±26	101±32.6	6000
Kuang X (2004)	40	NA	NA	30.19±19.4	28.5±14.7	70.4±22	74.7±18.9	49.2±16.4	50.5±17.3	15000
Li J (2010)	30	6	8	43±8	45±9	129±26	131±28	97±22	99±23	20000
Li JL (2009)	40	10	12	5.3±3.2	4.8±3.5	90.88±51.30	90.68±50.2	75.6±19.02	72±20.67	10000
Li LW (2004)	26	NA	NA	NA	NA	NA	NA	NA	NA	12000
Li MR (2005)	40	4	6	33.7±13.1	34.3±12.6	57.2±38.1	56.5±37.6	35.5±23.6	36.2±22.1	12000
Li Q (2012)	60	14	14	40.5±5.92	46.3±6.25	59.5±19.9	61.6±19.1	44.7±17.0	45.5±16.5	10000
Li WD (2009)	56	14	16	17.8±7.6	17.8±8.1	41.9±5.8	42.3±6.7	26.3±3.7	25.7±3.3	12000
Li YC (2004)	20	4	4	50.5±7.6	46.7±10.8	92.8±21	98.3±25.8	63.5±19.1	69.6±22.2	12000
Liu JD (2009)	36	8	9	51.8±10.6	50.4±10	95.9±22.4	98.6±20.9	69.2±21.7	67.9±19.4	12000
Liu Y (2005)	20	4	5	32.1±8.7	31.2±9.5	71.9±19.5	73.5±18.1	52±12.5	53.2±10.3	3×10^5 IU/c
Meng DM (2002)	20	NA	NA	NA	NA	NA	NA	NA	NA	12000
Nakanishi K (2006)	28	12	11	62±9	61±10	150±37	135±39	118±32	104±32	5000
Oh SY (2012)	60	20	16	67±19	60±12	99±25	96±22	74±19	76±21	1×10^6 IU/c
Park JB (2013)	110	10	27	55.37±14.22	47.55±13.86	125.85±37.58	116.91±32.40	78.13±27.64	70.83±27.00	5000
Qi XY (2010)	60	27	24	60.3±5.2	61.3±4.7	121.7±26.3	108.8±25.6	68.7±19.6	57.2±24.1	1×10^6 IU/c
Qiang Z (2004)	30	7	6	12±5.4	11.8±5.2	87±28	82±27	51±20	46±21	20000
Ren BH (2004)	30	8	7	42.4±5.64	44.06±5.96	93.6±16.4	94.87±16.49	48.27±10.99	54.67±9.09	12000
Shao B (2006)	16	3	3	44.0±5.29	43.13±5.37	96.75±14.06	98.25±15.96	66.50±7.60	64.63±8.73	12000
Shu Q (2005)	27	7	8	3.63±1.81	4.45±4.04	46.43±7.06	45.67±8.86	22.14±3.66	24.45±8.08	15000
Song JE (2011)	48	8	9	54±16.3	52±17.7	170±29.6	172±26.6	102±22.2	105±24.4	5,000
Song JE (2013)	24	4	6	58±17	59±15	150±44	139±42	104±39	91±44	5,000
Sugita T (2002)	22	NA	NA	5.2±3.4	4.9±3.8	96.1±61.3	117.7±61.6	56.3±44.1	69.5±44.3	5000
Sun CY (2003)	20	5	6	19.7±5.6	16.3±4.4	59.9±7.6	71.9±7.2	46.2±7.2	49.8±8.6	8000
Sun CY (2003)-1	20	6	6	19.9±5.1	16.3±4.4	64.2±8.8	71.9±7.2	40.2±7.8	49.8±8.6	16000
Wang DJ (2005)	30	7	6	35.9±12.4	39.1±14.3	68.2±28.1	82.1±34.2	48.8±24.6	57.7±29.5	20000
Wang X (2012)	24	6	5	47.3±16.8	45.6±18.2	71.3±18.6	69.2±19.1	49.9±18.3	48.1±17.2	15000
Wang YQ(2008)	42	10	9	7.5±3.7	6.9±2.8	NA	NA	NA	NA	20000
Wei L(2009)	25	NA	NA	35.1±14.1	30.2±10.6	78.5±26.6	72.1±35.8	48.7±24.5	42.6±27.8	20000

Table 1. Cont.

Author(year)	No. of patients	Gender (male)		Age(year)		CBP time(min)		ACC time(min)		Ulinastatin (IU/kg)
Groups	Total	U	C	U	C	U	C	U	C	
Xu CE(2013)	36	15	16	54.8±8.9	53.2±7.3	235.5±25.9	247.2±20.3	87.3±23.7	89.4±24.6	20,000
Xu KQ (2004)	40	8	11	46.1±12.3	45.7±11.8	71.09±11.42	70.28±10.74	40.88±9.75	41.71±8.92	10000
Xu KQ (2004)-1	40	8	11	47.2±10.9	45.7±11.8	69.87±10.91	70.28±10.75	41.67±9.43	41.71±8.92	20000
Xue QH (2006)	30	6	7	48.8±7.57	43.2±8.38	96±35	86±22	68±28	61±19	6000
Yu XY (2009)	28	NA	NA	48.89±8.74	52.33±9.17	58.11±18.16	56.11±18.92	43.33±15.75	42.56±16.21	8000
Yu XY (2009)-1	28	NA	NA	53.22±9.74	52.33±9.17	55.78±17.72	56.11±18.92	42.22±17.44	42.56±16.21	12000
Zhang JW (2004)	24	NA	NA	NA	NA	NA	NA	NA	NA	5000
Zhang JW (2004)-1	24	NA	NA	NA	NA	NA	NA	NA	NA	10000
Zhang XL (2007)	30	7	6	43.8±12.5	42.2±11.8	83.4±16.5	84.1±15.7	54.5±12.7	52.7±14.5	20000
Zhao QF (2007)	30	8	9	30±11	29±11	57±16	54±18	35±12	35±16	10000
Zhao QF (2007)-1	30	7	9	29±10	29±11	58±16	54±18	36±12	35±16	20000
Zhao Y (2009)	40	8	11	32±7	32±8	98±8	98±5	56±6	61±7	20000
Zhong JY (2007)	30	10	9	42±9	51±11	118.4±28.3	117.7±27.7	79.7±34.6	77.6±29	20000
Zhou Q (2010)	40	NA	NA	NA	NA	NA	NA	NA	NA	15000
Zou DQ (2005)	24	6	7	35±5	36±6	89±11	87±12	56±7	52±8	24000

Abbreviations: U, Ulinastatin; C, Control; CPB, Cardiopulmonary bypass; ACC, aortic cross clamping; OR, odds ratio; MD, mean difference; IU/c, Unit per case; NA, not available.

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(P<0.01), −8.75 (−14.23, −3.28) for CK-MB (P<0.01) and −0.14 (−0.20, −0.09) for TnI (P<0.01) (Table 2). As there was significant heterogeneity in the CK-MB values (P<0.01, I^2=91%), a random-effect model was adopted. Sensitivity analysis showed that the overall effect was not changed by omitting 6 studies until these heterogeneities became acceptable (heterogeneity: P>0.10; I^2<50%) (Table 3) [4].

Hemodynamic Parameters (HR, MAP, MPAP, and CVP)

Parameters relating to hemodynamics were measured in some trials after CPB. The meta-analysis results showed that ulinastatin had no significant effect on any of these parameters except MAP. The MD (95% CI) values were −1.63 (−4.11, 0.84) for HR (P=0.20), 0.84 (−0.64, 2.32) for MPAP (P=0.26), 0.89 (−0.07, 1.84) for CVP (P=0.07), Although MAP showed a statistically significant increase—MD (95% CI) 2.50 (0.19, 4.80), P=0.03—the clinical significance was unclear. Since there was no significant heterogeneity among these analyses, a fixed-effect model was adopted (Table 2).

Table 2. Meta-analysis of interesting outcomes between ulinastatin and control group in patients with CPB.

Outcomes	Studies (n)	Ulinastatin (n)	Control (n)	Heterogeneity		Meta-analysis Model	OR*/MD (95%CI)	*P*
				I^2	P			
Gender(male)	39	393	420	0%	1.00	M-H, Fixed	0.85 [0.69, 1.04]*	0.12
Age(year)	48	980	978	9%	0.29	IV, Fixed	−0.14 [−0.58, 0.31]	0.55
CBP time(min)	47	998	998	17%	0.15	IV, Fixed	−0.95 [−2.34, 0.44]	0.18
ACC time(min)	46	958	963	7%	0.34	IV, Fixed	−0.48 [−1.52, 0.57]	0.37
TNF-α	15	349	334	92%	<0.01	Random	−7.53 [−9.45, −5.61]	<0.0-1
IL-6	14	324	309	80%	<0.01	Random	−32.87 [−39.53, −26.20]	<0.0-1
IL-8	12	287	271	94%	<0.01	Random	−2.40 [−3.40, −1.41]	0.02
IL-10	4	55	55	86%	<0.01	Random	14.49 [4.57, 24.42]	0.02
LDH (IU/L)	4	87	78	42%	0.16	IV, Fixed	−63.54 [−79.36, −47.72]	<0.0-1
CK(IU/L)	4	63	63	0%	0.99	IV, Fixed	−224.99 [−304.83, −145.14]	<0.0-1
CK-MB(ng/ml)	12	274	291	91%	<0.01	IV, Random	−8.75 [−14.23, −3.28]	<0.0-1
TnI(ng/ml)	8	175	197	6%	0.39	IV, Fixed	−0.14 [−0.20, −0.09]	<0.0-1
HR(beats/min)	8	175	200	0%	0.65	IV, Fixed	−1.63 [−4.11, 0.84]	0.20
MAP(mmHg)	8	136	133	0%	0.58	IV, Fixed	2.50 [0.19, 4.80]	0.03
MPAP(mmHg)	2	43	41	0%	0.37	IV, Fixed	0.84 [−0.64, 2.32]	0.26
CVP(cmH_2O)	2	43	41	0%	0.52	IV, Fixed	0.89 [−0.07, 1.84]	0.07
Cardiac Index($L/min/m^2$)	3	57	55	11%	0.32	IV, Fixed	−0.18 [−0.42, 0.06]	0.14
LVEF (%)	2	44	44	0%	0.47	IV, Fixed	1.32 [−2.63, 5.26]	0.51
Auto Resuscitation(n)	9	175	166	0%	0.85	M-H, Fixed	1.98 [1.19, 3.30]*	<0.0-1
Patients Inotrope (n)	5	132	123	0%	0.71	M-H, Fixed	0.98 [0.52, 1.85] *	0.96
Postoperative Bleeding(ml)	6	136	162	61%	0.03	IV, Random	14.98 [−69.10, 99.07]	0.73
Complications(n)	7	467	545	0%	0.50	M-H, Fixed	0.68 [0.44, 1.04]*	0.07
MVT(h)	12	305	292	94%	<0.01	IV, Random	−3.29 [−4.41, −2.17]	<0.0-1
ITT(h)	7	167	195	57%	0.03	IV, Random	−1.58 [−2.84, −0.32]	<0.0-1
ICU Stay(h)	13	276	303	68%	<0.01	IV, Random	−2.17 [−8.13, 3.80]	0.48
Hospital Stay(d)	3	55	55	0%	0.72	IV, Fixed	0.43 [−1.93, 2.79]	0.72

Abbreviations: CPB, cardiopulmonary bypass; ACC, aortic cross-clamping; LDH, lactate dehydrogenase; CK-MB, creatine kinase-MB; TnI, troponin I; HR, heart rate; MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; CVP, central venous pressure; LVEF, left ventricular ejection fraction; MVT: mechanical ventilation time; ITT: intubation time; ICU, intensive care unit; OR*, Odds Ratio; M-H, Mantel-Haenszel; IV, Inverse Variance; MD, mean difference; CI, confidence interval.

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Cardiac Function (Cardiac index and LVEF)

Only 3 trials reported the cardiac index and 2 trials the LVEF postoperatively. The meta-analysis showed no significant effects on either parameter: MD (95% CI) were −0.18 (−0.42, 0.06) for cardiac index (P=0.14), and 1.32 (−2.63, 5.26) for

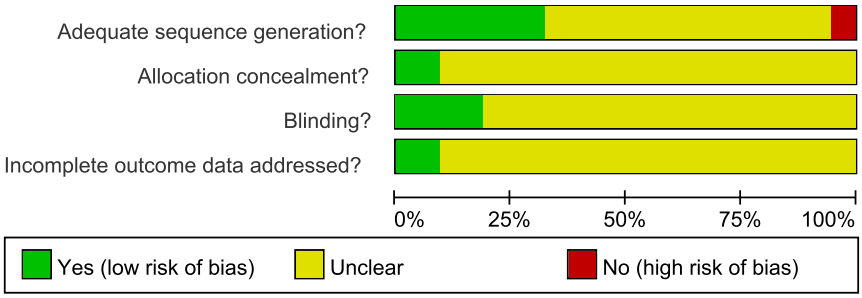

Fig. 2. Methodological quality graph summarizing the risk of bias from all included studies.

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left ventricular ejection fraction (P=0.51). As there was no significant heterogeneity among these analyses, a fixed-effect model was adopted (Table 2).

Important Clinical Outcomes (Including the number of heart auto resuscitations, the number of patients needing inotropes, the postoperative bleeding volume, and the number of complications)

These important clinical outcomes have aroused the concern of clinicians. The results showed that the frequency of autoresuscitation increased significantly (OR 1.98, 95%CI 1.19 to 3.30, P<0.01) in the ulinastatin group, but there were no statistically significant differences in the number of patients needing inotropes (OR: 0.98, 95%CI 0.52 to1.85, P=0.96), the postoperative bleeding (MD 14.98,

Table 3. Sensitivity analyses of high heterogeneity outcomes in meta-analysis.

Heterogeneity outcomes	Omitted (excluded) studies	Ulinastatin (n)	Control (n)	Heterogeneity		Meta-analysis model	Outcomes	
				I^2	*P*		MD (95%CI)	*P*
TNF-α(pg/ml)	7[15, 17, 18, 36, 48, 57, 59]	137	136	47%	0.06	IV, Fixed	−0.78 [−1.03, −0.52]	<0.01
IL-6(pg/ml)	5[18, 33, 36,44 53]	226	216	42%	0.08	IV, Fixed	−1.41 [−1.62, −1.20]	<0.01
IL-8(pg/ml)	4[17, 49, 51, 57]	169	163	42%	0.09	IV, Fixed	−0.69 [−0.92, −0.47]	<0.01
IL-10(pg/ml)	2[46, 55]	25	25	0%	0.61	IV, Fixed	2.39 [1.63, 3.14]	<0.01
CK-MB(ng/ml)	6[9, 12, 13, 14, 18, 27]	123	116	42%	0.11	IV, Fixed	−14.19 [−16.01, −12.37]	<0.01
MVT(h)	4[8, 17, 21, 22]	172	169	47%	0.06	IV, Fixed	−2.32 [−2.71, −1.93]	<0.01
ITT(h)	1[9]	126	126	40%	0.14	IV, Fixed	−2.43 [−2.93, −1.92]	<0.01
Postoperative Bleeding(ml)	1[21]	116	142	0%	0.79	IV, Fixed	56.79 [−2.09, 115.67]	0.06
ICU stay(h)	1[8]	258	285	0%	0.58	IV, Fixed	1.69 [−1.32, 4.70]	0.27

Abbreviations: TNF-α, tumor necrosis factor-α; IL, interleukine; CK-MB, creatine kinase-MB; ITT: intubation time; MVT, mechanical ventilation time; ICU, intensive care unit; IV, Inverse Variance; MD, mean difference; CI, confidence interval.

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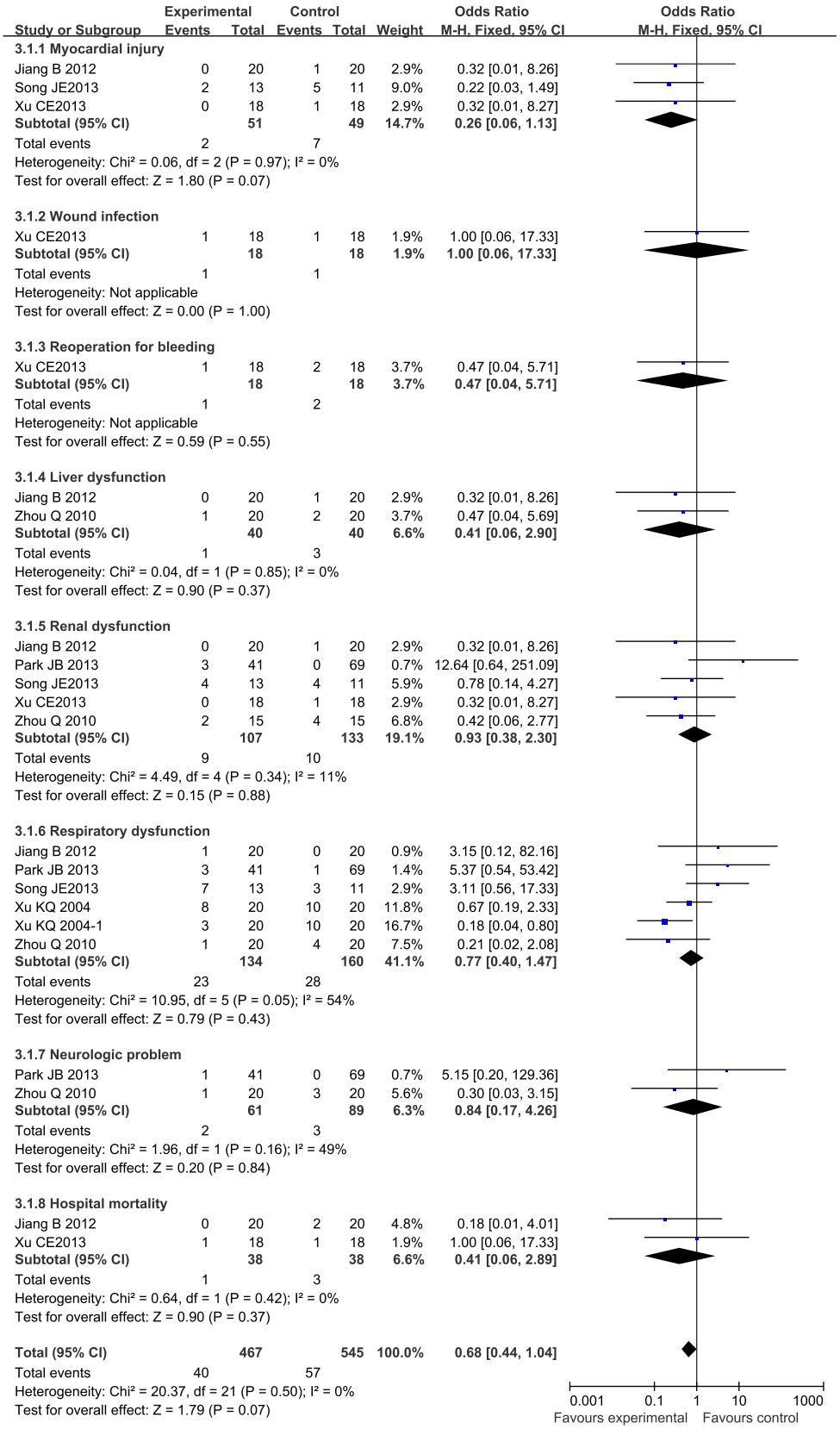
Experimental
Control
Odds Ratio
Odds Ratio
Study or Subgroup Events Total Events Total Weight M-H, Fixed, 95% CI
M-H, Fixed, 95% CI
3.1.1 Myocardial injury
Jiang B 2012 0 20 1 20 2.9% 0.32 [0.01, 8.26]
Song JE2013 2 13 5 11 9.0% 0.22 [0.03, 1.49]
Xu CE2013 0 18 1 18 2.9% 0.32 [0.01, 8.27]
Subtotal (95% CI) 51 49 14.7% 0.26 [0.06, 1.13]
Total events 2 7
Heterogeneity: Chi² = 0.06, df = 2 (P = 0.97); I² = 0%
Test for overall effect: Z = 1.80 (P = 0.07)
3.1.2 Wound infection
Xu CE2013 1 18 1 18 1.9% 1.00 [0.06, 17.33]
Subtotal (95% CI) 18 18 1.9% 1.00 [0.06, 17.33]
Total events 1 1
Heterogeneity: Not applicable
Test for overall effect: Z = 0.00 (P = 1.00)
3.1.3 Reoperation for bleeding
Xu CE2013 1 18 2 18 3.7% 0.47 [0.04, 5.71]
Subtotal (95% CI) 18 18 3.7% 0.47 [0.04, 5.71]
Total events 1 2
Heterogeneity: Not applicable
Test for overall effect: Z = 0.59 (P = 0.55)
3.1.4 Liver dysfunction
Jiang B 2012 0 20 1 20 2.9% 0.32 [0.01, 8.26]
Zhou Q 2010 1 20 2 20 3.7% 0.47 [0.04, 5.69]
Subtotal (95% CI) 40 40 6.6% 0.41 [0.06, 2.90]
Total events 1 3
Heterogeneity: Chi² = 0.04, df = 1 (P = 0.85); I² = 0%
Test for overall effect: Z = 0.90 (P = 0.37)
3.1.5 Renal dysfunction
Jiang B 2012 0 20 1 20 2.9% 0.32 [0.01, 8.26]
Park JB 2013 3 41 0 69 0.7% 12.64 [0.64, 251.09]
Song JE2013 4 13 4 11 5.9% 0.78 [0.14, 4.27]
Xu CE2013 0 18 1 18 2.9% 0.32 [0.01, 8.27]
Zhou Q 2010 2 15 4 15 6.8% 0.42 [0.06, 2.77]
Subtotal (95% CI) 107 133 19.1% 0.93 [0.38, 2.30]
Total events 9 10
Heterogeneity: Chi² = 4.49, df = 4 (P = 0.34); I² = 11%
Test for overall effect: Z = 0.15 (P = 0.88)
3.1.6 Respiratory dysfunction
Jiang B 2012 1 20 0 20 0.9% 3.15 [0.12, 82.16]
Park JB 2013 3 41 1 69 1.4% 5.37 [0.54, 53.42]
Song JE2013 7 13 3 11 2.9% 3.11 [0.56, 17.33]
Xu KQ 2004 8 20 10 20 11.8% 0.67 [0.19, 2.33]
Xu KQ 2004-1 3 20 10 20 16.7% 0.18 [0.04, 0.80]
Zhou Q 2010 1 20 4 20 7.5% 0.21 [0.02, 2.08]
Subtotal (95% CI) 134 160 41.1% 0.77 [0.40, 1.47]
Total events 23 28
Heterogeneity: Chi² = 10.95, df = 5 (P = 0.05); I² = 54%
Test for overall effect: Z = 0.79 (P = 0.43)
3.1.7 Neurologic problem
Park JB 2013 1 41 0 69 0.7% 5.15 [0.20, 129.36]
Zhou Q 2010 1 20 3 20 5.6% 0.30 [0.03, 3.15]
Subtotal (95% CI) 61 89 6.3% 0.84 [0.17, 4.26]
Total events 2 3
Heterogeneity: Chi² = 1.96, df = 1 (P = 0.16); I² = 49%
Test for overall effect: Z = 0.20 (P = 0.84)
3.1.8 Hospital mortality
Jiang B 2012 0 20 2 20 4.8% 0.18 [0.01, 4.01]
Xu CE2013 1 18 1 18 1.9% 1.00 [0.06, 17.33]
Subtotal (95% CI) 38 38 6.6% 0.41 [0.06, 2.89]
Total events 1 3
Heterogeneity: Chi² = 0.64, df = 1 (P = 0.42); I² = 0%
Test for overall effect: Z = 0.90 (P = 0.37)
Total (95% CI) 467 545 100.0% 0.68 [0.44, 1.04]
Total events 40 57
Heterogeneity: Chi² = 20.37, df = 21 (P = 0.50); I² = 0%
Test for overall effect: Z = 1.79 (P = 0.07)
0.001 0.1 1 10 1000
Favours experimental Favours control

Fig. 3. Forest plot of complications in patients who received ulinastatin versus controls in open-heart surgery with CPB. OR, Odds Ratio; M-H, Mantel-Haenszel; MD, mean difference; CI, confidence interval.

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95%CI −69.10 to 99.07, P=0.73), or the postoperative complications (MD 0.68, 95%CI 0.44 to 1.04, P=0.07) (Table 2). Postoperative complications, including the number of cases with myocardial injury, wound infection, reoperation for bleeding, liver dysfunction, renal dysfunction, respiratory dysfunction, and neurological problems, as well as the in-hospital mortality, are shown in detail in Fig. 3. Heterogeneity was seen only in the postoperative bleeding (P=0.03, I^2=61%), where a random-effect model was adopted. Sensitivity analysis showed that the overall effect was not changed by omitting one study (MD 56.79, 95%CI −2.09 to 115.67, P=0.06) (Table 3).

Duration of Mechanical Ventilation and Intubation

Eleven trials reported mechanical ventilation time (MVT), and 7 trials reported intubation time (ITT). The meta-analysis results showed that the durations of mechanical ventilation and intubation were significantly shortened in the ulinastatin group compared with the placebo group; MD (95% CI) values were −3.29 (−4.41, −2.17) and −1.58 (−2.84, −0.32) respectively (P<0.01) (Table 2). Significant heterogeneities were found (heterogeneity: P=0.03, I^2=57% in the duration of intubation and P<0.01, I^2=94% in the duration of mechanical ventilation), and a random-effect model was adopted. Sensitivity analysis showed that the overall effects on the durations of intubation and mechanical ventilation were not altered significantly (still P<0.01) (Table 3).

Length of ICU and Hospital Stays

Finally, we assessed the effect of ulinastatin on the length of the postoperative ICU and hospital stays. Thirteen studies reported the length of stay in the ICU, and 3 studies reported the length of stay in the hospital. The meta-analysis indicated that there were no significant differences in the duration of either ICU stay (P=0.48) or hospital stay (P=0.72) between the ulinastatin and placebo groups: the MD (95% CI) values were −2.17 (−8.13, 3.80) and 0.43 (−1.93, 2.79), respectively. Significant heterogeneity was found in the ICU stay analysis (heterogeneity: P<0.01, I^2=68%), and a random-effect model was adopted (Table 2). After one obviously different study was omitted, the heterogeneity became non-significant (heterogeneity: P=0.58, I^2=0%), and the same result was still obtained (P=0.27) (Table 3).

Publication bias

We assessed the publication biases in the analysis of the above 10 studies, Publication biases for two interesting outcomes (TnI, MVT) were detected by Begg's test or Egger's test (P<0.05).

Discussion

Many studies have claimed that the postoperative complications of cardiac surgery are associated with the activation of inflammatory mediators and the occurrence of systemic inflammatory response syndrome (SIRS). Protease inhibitors have been used clinically to treat many diseases, such as HCV [62], HIV [63], and H1N1 virus [64], and also have a potential role as anti-tumor agents [65, 66] and parasiticides [67]. Ulinastatin is a multivalent serine protease inhibitor. It is a glycosylated protein composed of 143 amino acid residues and weighted from 62 kDa to 72 kDa, injected to treat various inflammatory diseases [68]. In CPB, it has been widely used to block the inflammatory cascade with a view to prevent postoperative complications. Several animal studies found that mediators of inflammation after CPB reduced significantly with ulinastatin treatment [69]. The same findings were demonstrated in human studies [70, 71].

As inflammatory mediators and proinflammatory cytokines, TNF-α, IL-6, IL-8, and PMNE can lead to an inflammatory cascade by acting on other inflammatory cytokines or influencing the actions of each other. In this study, we found that in patients undergoing cardiac surgery with CPB, the preoperative administration of ulinastatin significantly reduced the levels of these inflammatory mediators, as compare with those in the control group, although not to the baseline preoperative levels at twenty-four hours postoperatively. Moreover, IL-10, also known as the human cytokine synthesis inhibitory factor, is an anti-inflammatory cytokine that is capable of inhibiting the synthesis of proinflammatory cytokines such as IFN-γ, IL-2, IL-3, and TNF-α. The levels of IL-10 increase in response to increased levels of proinflammatory cytokines [72]. In our study, the levels of IL-10 also increased after CPB; however, this increase was greater in the ulinastatin groups than in the control groups. Therefore, we consider that the anti-inflammatory function of ulinastatin occurs by blocking the inflammatory cascade rather than by clearing of inflammatory mediators.

In general, the key clinical and prognostic parameters, such as hemodynamics, organ function, duration of mechanical ventilation, and length of ICU or hospital stays, have received more attention from surgeons. Some studies showed that ulinastatin can reduce the risk of myocardial injury in an animal model [73, 74] and in patients undergoing CPB [5], but other studies found that this effect was not remarkable [9, 12, 14]. In this meta-analysis, we focused on biomarkers of myocardial damage and some important clinical outcomes. The results showed a significant decrease in these biomarkers (including LDH, CK, CK-MB, and TnI) in patients treated with ulinastatin. However, we did not find a significant improvement in cardiac function (cardiac index and LVEF) or hemodynamic parameters (MPAP, CVP). Although there was a statistically significant increase in MAP, the clinical significance was not certain, because the magnitude of the increase in the MD value was only 2.5 mmHg. This meta-analysis also showed that ulinastatin may increase the frequency of heart auto resuscitation, and shorten the duration of mechanical ventilation and intubation, but does not change the number of patients needing inotropic agents, the volume of

postoperative bleeding, or the complication rate, and does not shorten the ICU or hospital stay.

Cardiac insufficiency, such as postoperative low cardiac output or even cardiogenic shock, is the most common complication after cardiac surgery with CPB. Regional myocardial ischemia/reperfusion injury in the process of aortic cross clamping and opening, together with the apoptosis or necrosis of cardiomyocytes, are thought to be critical reasons for cardiac insufficiency [75, 76]. Additionally, the activation of inflammatory mediators, finally leading to SIRS, is also thought to be an important factor [76]. These biomarkers, especially CK-MB and cTnI, are predictive factors that reflect myocardial injury [19]; cTnI is the most sensitive indicator of minor myocardial damage and has superior cardiac specificity to CK-MB [77]. Therefore, the protective effect of ulinastatin on myocardium may be related with inhibition or elimination of the inflammatory mediators and alleviation of SIRS. However, cardiac function was not obviously improved in the ulinastatin group, while hemodynamic parameters, which are affected by many factors closely related with cardiac function, blood volume, and systemic inflammatory reaction, also did not improve significantly. Another interesting finding was that ulinastatin did not reduce the number of patients who needed inotropic agents, again suggesting that it did not ameliorate cardiac function, although it may have a benefit in limiting myocardial damage.

Successful auto resuscitation can avoid electrical defibrillation, which may cause damage to the myocardium. Many factors, such as the cardiac conduction system, blood electrolytes, and acid-base balance, are related with the heart's auto resuscitation rate [78]. Nine trials reported data concerning heart auto resuscitation intraoperatively. Overall, the auto resuscitation rate in the ulinastatin group was 80% (140/175), significantly higher than in the control group 66.87% (111/166): OR=1.98, 95%CI (1.19, 3.30), $P<0.05$. Ulinastatin's beneficial effect on the heart's autoresuscitation rate may be associated with its alleviation of damage in cardiomyocytes, including the cells of the cardiac conduction system.

We found no significant difference in complications (including key organ function, postoperative bleeding, wound infection and hospital mortality) between the 2 groups according to the current data. However, very few studies in this meta-analysis reported these data; thus, more studies and a larger sample size will be needed before we can discuss the relationship between ulinastatin and complications.

In our study, the outcomes of interest were improved by ulinastatin compared with placebo control as regards the duration of mechanical ventilation and intubation, whereas no effect was noted on the length of ICU or hospital stays. According to a sensitivity analysis, the outcomes were not changed. Some studies found that ulinastatin can reduce pulmonary injury and improve pulmonary function [8, 42], perhaps via a reduction in inflammatory mediators. However, these clinical outcomes are influenced by many factors and a prospective, multicenter, randomized controlled trial is mandated to elucidate these matters.

The heterogeneity of some subgroup analyses was significant. Possible reasons are that the ulinastatin dosage may affect the result, and different dosages were used to investigate the effects. The doses of ulinastatin ranged from 5000 U/kg to 20,000 U/kg, with total doses reported to range from 600,000 U to 1,000,000 U. It seems that a higher dosage produced better effects [8], but no studies discussed the dose-effect relationship. In addition, different drug delivery protocols were used. In some studies, the ulinastatin was administered 3 times (preoperative, operative, and postoperative) [13]; in other studies, it was given twice (preoperatively and postoperatively) [42], or delivered only once by being added to the CPB pump prime [9] or via continuous intravenous infusion [79]. Some researchers think that ulinastatin preprocessing (used preoperatively) can achieve more advantages [80]. In order to explain the effect of ulinastatin adequately, a subgroup analysis is required, taking into account the methods of administration or dosage, but this was not possible in the present study because of the limited quantity of data.

Our study had some limitations and potential biases. First, in our meta-analysis, only randomized controlled trials in English and Chinese were included. However, ulinastatin was allowed first in Japan and is now widely used in Asia. As many studies have been published in the Japanese or Korean languages, there may have been bias owing to the language restriction. Second, in some of these randomized controlled trials, the aspects of the study design relating to patient inclusion, sequence generation, allocation concealment, and blinding were not reported clearly. In some trials, although the designs were reported, the methods adopted were different. Therefore, the quality of these trials was uneven, and this unevenness may have been a main source of heterogeneity. Third, data relating to the functional parameters of main organs were not reported adequately, so we were not able to analyze the effects of ulinastatin treatment on organ function after CPB. Fourth, the data regarding outcomes of interest did not include the same variables in every study, and some data needed to be estimated; thus, the accuracy of some results was affected to some extent. Last, the numbers of patients and studies included were insufficient, especially for subgroup analysis, so a further meta-analysis without language restrictions should be performed.

Summary of findings

The proinflammatory cytokines (TNF-α, IL-6 and IL-8) were significantly reduced and the anti-inflammatory cytokine IL-10 were significantly increased at 24th hour of plasma levels in the ulinastatin-treated patients from China and Japan undergoing open-heart surgery with CPB.

Ulinastatin treatment had no significant effect on hemodynamics, cardiac function, patients' need for inotropes, complications, or postoperative course (ICU and hospital stay).

Ulinastatin treatment may have protective effects on myocardial injury, and can increase the frequency of auto resuscitation, shorten the duration of intubation and mechanical ventilation.

Supporting Information

S1 Figure. Risk of bias summary: review authors' judgments about each risk of bias item for each included study.
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S1 PRISMA Checklist.
doi:10.1371/journal.pone.0113973.s002 (DOC)

Author Contributions

Conceived and designed the experiments: YZ ZZ. Performed the experiments: YZ ZW XDD. Analyzed the data: YZ ZW. Contributed reagents/materials/analysis tools: YZ ZZ. Wrote the paper: YZ ZW YC.

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