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Original Article

Efficacy of human urinary kallidinogenase in ischemic stroke in animal models: a meta-analysis

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Abstract: Background: Accumulating experimental and clinical evidence suggests that human urinary kallidinogenase (HUK) improves stroke outcome. Here, a systematic review and meta-analysis was conducted to evaluate the effects of HUK on cerebral ischemia in animal models. Methods: A systematic search of the literature published before June 2018 was performed using PubMed, Embase, Medline, and Cochrane Library. The outcome was assessed using the infarct volume and heterogeneity was analyzed using Cochrane Library's RevMan 5.3. Results: From the 471 studies that were initially examined, a total of nine studies were selected. When compared with the control group data, HUK therapy resulted in an overall 4.52% reduction in infarct size (95% confidence interval: 3.66-5.39, $P < 0.00001$). Subgroup analysis showed that maximal neuroprotective effects were reached when HUK was administered immediately after middle cerebral artery occlusion (MCAO). Conclusions: HUK had a neuroprotective effect in animal models of MCAO, especially in animals administered HUK immediately after MCAO.

Keywords: Human urinary kallidinogenase, cerebral ischemia, stroke, meta-analysis

Introduction

Stroke is one of the most common causes of mortality and disability worldwide; moreover, with 87% of strokes being ischemic [1]. Currently the only clinically validated therapy for acute ischemic stroke is intravenous administration of tissue-type plasminogen activator within 3 hours of onset [2]. As few therapeutic options are available for the treatment of ischemic stroke, searching for new interventions to reduce ischemic brain damage has been a major research endeavor. Using experimental focal cerebral ischemia models (most often middle cerebral artery occlusion [MCAO]) that mimic acute stroke under strict control, studies have provided information on the pathophysiological mechanisms of stroke and developed novel stroke therapies during the recent decades [3].

The kallikrein-kinin system (KKS), which consists of kallikrein, kininogen, kininase, kinin, and kinin receptors, is an indispensable inflammatory modification system in vivo [4]. Human tissue kallikrein has been shown to exert protective effects in ischemic stroke patients by inhibiting apoptosis and inflammation following cerebral ischemic injury [5]. Human urinary kallidinogenase (HUK) is a tissue kallikrein extracted from urine with a substrate preference for cleaving low-molecular-weight kininogen to release kinins. Consequently, vasoactive kinins trigger a series of biological effects by activating bradykinin B1 and B2 receptors [6]. China's State Food and Drug Administration approved HUK as a new state category I drug for the therapy of acute ischemic stroke, and it has been used since 2012.

During the past few decades, several clinical studies have revealed that HUK promotes cerebral perfusion, boosts cerebral glucose metabolism, extenuates brain edema, and blocks post-stroke inflammatory cascades. However, the efficacy of HUK for ischemic stroke has not been clarified in previous preclinical and clinical studies. The progress of HUK research *in vivo* has been limited by the small sample size and different experimental conditions. In this paper,

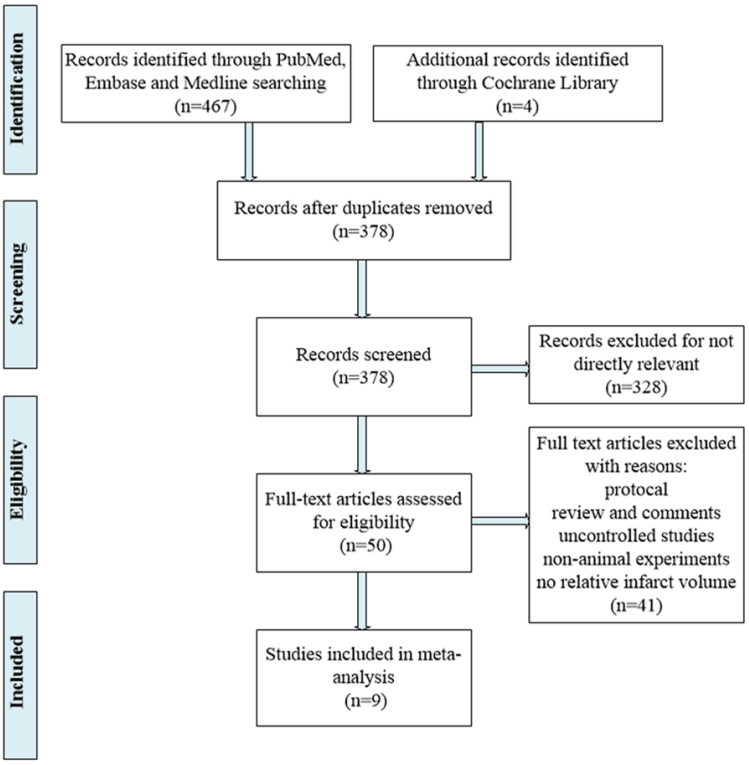

Figure 1. Flow diagram of study selection process. Nine papers were included in this study.

a systematic review and meta-analysis was performed to evaluate the therapeutic effect of HUK in animal models of MCAO. These findings may provide evidence for clinical judgment in the future.

Materials and methods

Methods

A meta-analysis was performed according to the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) guidelines [7].

Database and literature search strategies

A systematic search of the literature from January 1960 to June 2018 was conducted using PubMed, Embase, Medline, and Cochrane Library. The keywords used for the search were "kallikrein" OR "Urinary Kallidinogenase" AND "isch(a)emia" OR "stroke" OR "infarct" OR "middle cerebral artery occlusion (MCAO)".

Study selection and inclusion criteria

Studies were independently screened by two investigators (MFX and BWX). Study inclusion criteria were: 1) kallikrein used to treat ischemic stroke induced by MCAO; 2) study design including a control group; and 3) infarct sizes compared between groups. Study exclusion criteria were: 1) secondary study; 2) clinical studies; 3) studying a disease other than MCAO; and 4) kallikrein therapy not used. A third investigator (XZQ) resolved any disagreement between the primary screening investigators.

Quality of data

Study quality with a total of 10 points was assessed using the criteria by Macleod et al., awarding points for: (a) publication after peer review; (b) temperature control instruction; (c) randomly divided into the treatment or control groups; (d) ischemia was blindly induced; (e) outcome was blindly scored; (f) used an anesthetic without intrinsic neuroprotection; (g) used an appropriate animal model; (h) use of and appropriate sample size; (i) compliance with animal rights protection regulations; (j) made a statement about potential conflicts of interest [8, 9]. Each study was assessed by two investigators (MFX and BWX) and the group median was calculated.

Data extraction

Data from all reports was extracted independently by two investigators (MFX and BWX), in accordance with the inclusion criteria described above (**Figure 1**). Comparisons were performed between the HUK treatment and control groups from the selected studies. For each comparison, the mean outcome, standard deviation, and number of animals in each group were examined. The data collected included time of ischemia, mode of HUK administration, timing of HUK administration, dosage of HUK, the infarct volumes, timing of treatment initiation, and outcome detection. When the results were presented graphically, the numerical data were requested from the original authors or extracted using the image analysis software Image J

(National Institutes of Health, Bethesda, MD, USA).

Data analysis

Statistical analysis was performed using the RevMan 5.3 software (Cochrane Library, London, UK). Data were aggregated using weighted mean differences and a fixed effects model. Subgroups were defined based on different experimental conditions and the source of heterogeneity was then analyzed. $P < 0.01$ was set as significance level for multiple comparisons.

Results

Study inclusion and characteristics

Nine publications [10-18] were identified describing the effects of HUK in focal cerebral ischemia that met the inclusion criteria (**Table 1**). These publications included nine full articles published from 2006 to 2018; eight studies used rats (six Sprague-Dawley rats and two Wistar rats) and one study used mice (Kunming mice). The MCAO models from eight studies were injected with human tissue kallikrein (Techpool Bio-Pharma Co. LTD, Guangdong, China) with a molecular weight of approximately 43 kD, and only one study used adenovirus-carrying human tissue kallikrein cDNA (Ad.CMV-TK). Injection of HUK was through tail vein in all included studies, and all publications reported relative infarct volume. Treatment initiation ranged from 0 to 24 hours after ischemia induction, and the outcome detection time ranged from 3 hours to 28 days.

Efficacy

The detection time of the outcome in MCAO ranged from 3 hours to 28 days. Protective effects were observed at different detection time points. In particular, **Figure 2** shows that HUK treatment decreased infarct volume to the greatest extent 24 hours after occlusion (χ^2 = 43.69, df = 4, $P < 0.00001$, I^2 = 91%).

Kallikrein treatment elicited a 4.52% reduction of infarct size (95% confidence interval (CI): 3.66-5.39%, $P < 0.00001$) with significant heterogeneity (χ^2 = 136.98, df = 8, $P < 0.00001$, I^2 = 94%). All treatment subtypes (repetitive or single administration) exhibited protective effects (**Figure 3**). It showed that single administration of HUK (9.22% reduction; 95% CI: 7.79-10.65%, $P < 0.00001$) has a better impact on decreasing effect size when compared to repetitive administration (1.77% reduction; 95% CI: 0.68-2.87%, $P < 0.002$).

Treatment of HUK ranged from 0 to 24 hours after the onset of ischemia, and protective effects were found for all treatment timings (**Figure 4**). However, the maximum neuro-protective effect was observed immediately after reperfusion (10.56% reduction; 95% CI: 8.54-12.58%, $P < 0.00001$).

Quality of studies

The median included quality score was 6 (range 4 to 7). None of the included studies declared masked induction of ischemia and masked assessment of outcome. Six studies stated temperature control and three stated random allocation to HUK treatment or control groups. All of the studies reported the use of appropriate anesthetics and animal models. The overall quality score table is included as Table S1.

Discussion

The current meta-analysis supports the neuroprotective effects of HUK in cerebral ischemia-reperfusion (I/R) injury. Except for one study using adenovirus carrying human tissue kallikrein cDNA, the rest of the studies used HUK protein directly, and all studies were performed by intravenous injection of the tail vein. Delayed treatment of HUK was found to not be as effective as immediate treatment, which was consistent with previous guidelines that recommend rapid identification, emergency care, and early rehabilitation for acute ischemic stroke [19, 20]. It is important to highlight that single HUK treatment is more effective than repetitive administration, which might be relative to the detection time points selected. Additional studies are required to define the HUK therapeutic window and course after cerebral I/R injury. Moreover, animal species, route of administration, and pharmaceutical dosage are also important variables influencing the outcome. Unfortunately, the limited data show a non-significant trend towards differential variables.

Plasma and tissue kallikrein, a subgroup of serine protease, cleave kininogens to form kinins and have been recommended as an attractive target linking several pathological hallmarks to cerebral ischemic damage [21]. Previous stud-

Table 1. Characteristics of the animal studies included in the meta-analysis

	Year	Author	Species	Time of ischemia#	Route of HUK treatment	Injection region	Dosage	Time of onset	Outcome	Behavioral Test	Detection time
1	2015	Han	SD	2 h	Human urinary kallidinogenase	Tail vein	$1.6*10^{-2}$ PNA U/kg	Immediately after reperfusion	3*	Longa Score	3 h, 1 d, 3 d, 7 d and 14 d
2	2008	Ling	SD	2 h	Human urinary kallidinogenase	Tail vein	$1.6*10^{-2}$ PNA U/kg	24 h after occlusion, daily	3*	Bederson scores	3 d, 7 d, 14 d and 28 d
3	2010	Chen	Kunming mice	2 h	Human urinary kallidinogenase	Tail vein	$2.0*10^{-2}$ PNA U/kg	24 h after reperfusion	3*	NSS	6 h, 24 h, 48 h and 72 h
4	2016	Dong	SD	2 h	Human urinary kallidinogenase	Tail vein	$8.75*10^{-3}$ PNA U/kg	30 min after occlusion	1*	-	72 h
5	2006	Xia	SD	1 h	Ad.CMV-TK	Tail vein	10^{11} PFU	8 h after reperfusion	3*	Bederson scores	3 d, 7 d and 14 d
6	2016	Shi	SD	1.5 h	Human urinary kallidinogenase	Tail vein	$1.6*10^{-2}$ PNA U/kg	Immediately after reperfusion	3*	NSS	24 h
7	2009	Tang	Wistar rats	2 h	Human urinary kallidinogenase	Tail vein	1 mg/kg	Immediately afterocclusion	3*	NSS	24 h
8	2018	Liang	Wistar rats	2 h	Human urinary kallidinogenase	Tail vein	$1.6*10^{-2}$ PNA U/kg	24 h after occlusion, daily	3*	Bederson scores	3 d and 7 d
9	2017	Yang	SD	2 h	Human urinary kallidinogenase	Tail vein	$8.75*10^{-3}$ PNA U/kg	0 and 12 h after reperfusion	3*	Longa Score	24 h

#All studies used the middle cerebral artery occlusion (MCAO) models. 1*: Infarction size; 2*: Neurobehavioral score; 3*: Infarction size combined Neurobehavioral score. PFU = Plaque-forming units. h = hours; d = days.

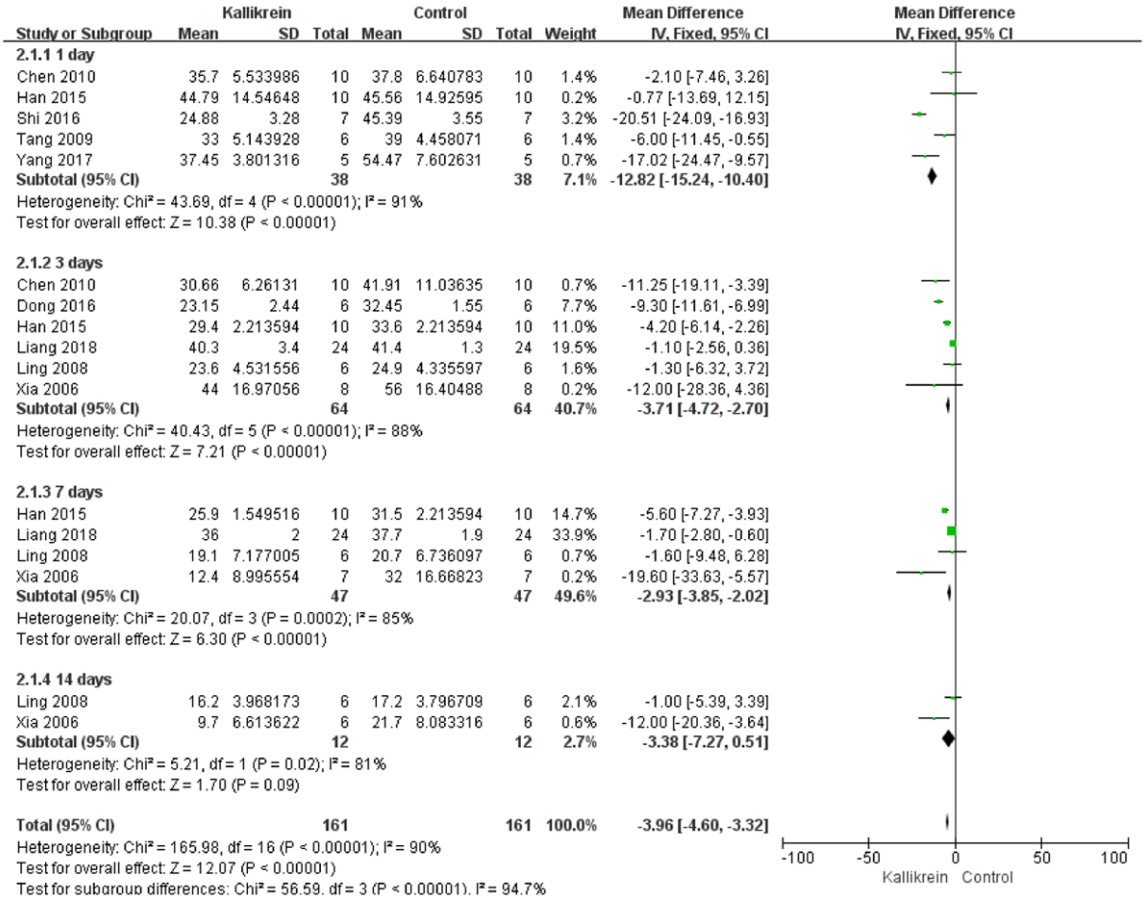

Figure 2. Evaluation of relative infarction size and 95% CI at different detection time. HUK treatment decreased infarction volume by 12.82% compared with controls at 1 day after occlusion with significant heterogeneity.

ies have suggested that delivery of the kallikrein protein or gene plays a beneficial role after cerebral I/R injury, such as reducing infarct size and inhibiting inflammation and cell apoptosis [10, 22, 23]. However, KKS activation, especially kinins and their receptors, has been thought to induce pro-inflammatory responses, which aggravates ischemic injury [24]. These results may help explain why kallikrein exerts biological functions through kinin B2-receptor signaling without kinin formation [25].

A previous meta-analysis by Zhang et al. assessed the efficacy of HUK for acute ischemic stroke patients, which included 24 trials and 2433 patients [26]. Patients treated with HUK had a greater neurological score and a better recovery than patients who received placebo. Considering these results, it is essential to introduce animal experiments to study potential mechanisms underlying HUK treatment and evaluate its biological value. Although current focal or global cerebral ischemia models fail to precisely mimic human ischemia, animal models have no additional pathological changes and can be well controlled with highly consistent pathological effects. In addition, animal models offer opportunities to study the effects of HUK on the brain, improve our understanding of the complex physiopathologic cascades, and identify possible therapeutic targets [27]. Preclinical data from animal models of ischemic stroke indicate the development of therapeutic strategies and help facilitate their translation to clinical practice.

Some limitations in the present study should be mentioned. First, the included studied showed a high degree of heterogeneity because of limited data. Only nine animal studies were pooled to assess the effects of HUK. Second, the subgroup analysis was not well implemented because of the lack of statistical power. Third,

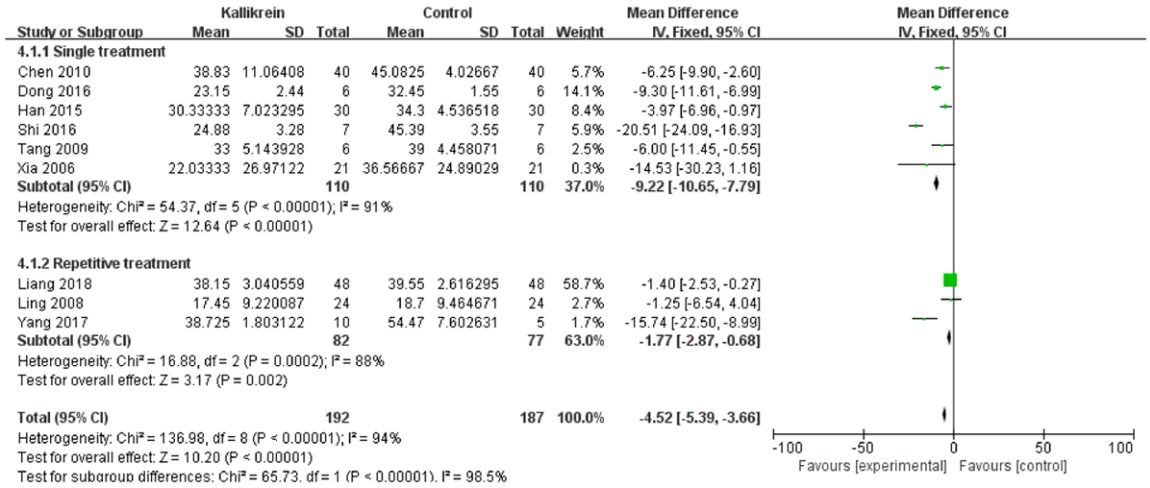

Figure 3. Evaluation of mode of HUK administration (single or repetitive) and 95% CI.

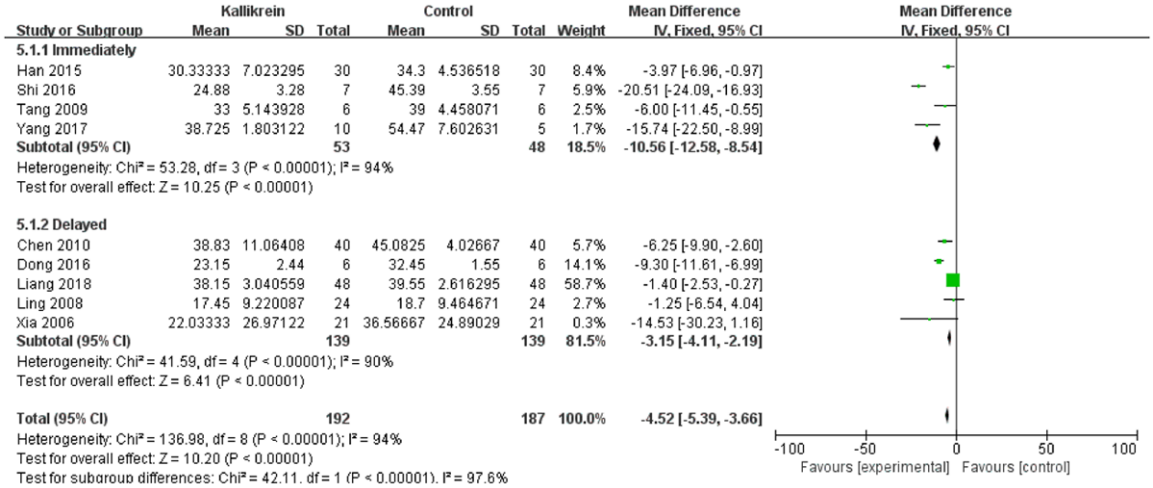

Figure 4. Evaluation of the timing of initiation of HUK treatment and 95% CI.

possible adverse side effects of HUK were not reported in these experiments, making it difficult to determine the comprehensive effects of HUK. Finally, the quality of most included studies was not ideal.

Conclusion

In conclusion, the current study reports that administration of HUK decreases the infarct size in animal models of MCAO. The neuroprotective effects for MCAO models reached a maximum when HUK therapy was implemented immediately after stroke and tended to be the highest 24 hours after I/R injury. These findings may provide comprehensive evidence for the neuroprotective effects of HUK therapy in MCAO models and may be important to future clinical trials.

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Disclosure of conflict of interest

None.

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Table S1. Quality Scores for Included Papers

Year	Author	Publication after peer review	Temperature control instruction	Random allocation to treatment or control	Ischemia was blindly induced	Outcome was blindly scored	Anesthetic without intrinsic neuroprotection	Appropriate animal model	Appropriate sample size	Compliance with animal welfare standards	Conflict of interest	Total
2015	Han	1	0	0	0	0	1	1	0	1	1	5
2008	Ling	1	1	1	0	0	1	1	1	1	0	7
2010	Chen	1	1	1	0	0	1	1	0	1	1	7
2016	Dong	1	0	1	0	0	1	1	1	1	1	7
2006	Xia	1	0	0	0	0	1	1	1	0	0	4
2016	Shi	1	1	0	0	0	1	1	0	1	1	6
2009	Tang	1	1	0	0	0	1	1	1	1	0	6
2018	Liang	1	1	0	0	0	1	1	1	1	1	7
2017	Yang	1	1	0	0	0	1	1	1	1	0	6