Please cite this article in press as: Wang L et al. Preclinical efficacy of human Albumin in subarachnoid hemorrhage. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2016.12.033

Neuroscience xxx (2017) xxx–xxx

PRECLINICAL EFFICACY OF HUMAN ALBUMIN IN SUBARACHNOID HEMORRHAGE

LIUMIN WANG,[a,b†] MEIYING LI,[c†] YI XIE,[a] LILI XU,[a] RUIDONG YE[a*] AND XINFENG LIU[a*]

[a] *Department of Neurology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China*

[b] *Department of Neurology, Xianlin Hospital of Nanjing Drum Tower Hospital, Nanjing, China*

[c] *Department of Neurology, Maanshan People's Hospital, Maanshan, China*

Abstract—Human Albumin is a unique pleiotropic protein with multiple properties. Previous clinical and laboratory studies have indicated a possible beneficial effect of Albumin in subarachnoid hemorrhage (SAH). The present study aimed to further define the preclinical characteristics of Albumin. SAH was induced by endovascular perforation in Sprague–Dawley rats. In the dose-escalation study, Albumin ranging from 0.02 g/kg to 1.0 g/kg was intravenously infused immediately after SAH. In the therapeutic window study, 1.0 g/kg Albumin was administered at 0 h, 2 h, 4 h or 8 h after SAH. Physiologic variables were monitored in different Albumin treatment regimens. One day after SAH, neurological scores, SAH scores, blood–brain barrier permeability, neural degeneration and apoptosis were examined. The efficacy of Albumin for SAH was also determined in female rats and in spontaneously hypertensive rats. We found that 0.2 g/kg and 1.0 g/kg Albumin significantly attenuated sensorimotor deficits, brain edema, IgG leakage, and neuronal degeneration after SAH. The benefits of Albumin existed even when the administration was delayed to 4 h after SAH onset. No significant difference was found between 0.2 g/kg and 1.0 g/kg Albumin groups. In female rats and spontaneously hypertensive rats, Albumin likewise improved neurological outcomes and early brain injury. In conclusion, Albumin could reduce both cerebral lesions and functional deficits in the early stage of SAH. The beneficial regimen occurs within a favorable therapeutic window and is reproducible in different high-risk subjects. © **2016 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: human albumin, subarachnoid hemorrhage, neurovascular dysfunction, blood–brain barrier, behavior.

INTRODUCTION

Subarachnoid hemorrhage (SAH) is associated with high mortality and poor prognosis (Connolly et al., 2012). However, few therapies are available in clinical settings. A number of drugs effective in animals have failed to be proved successful in human trials. One of the reasons accounting for this disparity may be the flawed methodology in both laboratory and clinical studies (Sehba et al., 2012). Omitting quality characteristics in animal studies will lead to an overestimation of the efficacy of candidate drugs (Philip et al., 2009; Minnerup et al., 2010). As such, a more rigorous preclinical evaluation, including the assessment of prolonged therapeutic window and the employment of animals with comorbidity (Sehba et al., 2012), is required for the preclinical therapy investigations of SAH.

Human Albumin (Alb) is known to have unique intravascular neuroprotective effects. It is able to reduce brain edema (Belayev et al., 2001), enhance neuronal survival (Belayev et al., 1999), and maintain blood–brain barrier (BBB) integrity in the cerebrovascular diseases (Belayev et al., 2005). The Albumin in Subarachnoid Hemorrhage (ALISAH) pilot study revealed that 1.25 g/kg/d Alb treatment is safe in patients and may produce a better outcome (Suarez et al., 2012). We previously demonstrated Alb improves long-term neurobehavioral sequelae after SAH through neurovascular remodeling (Xie et al., 2015). However, the preclinical characteristics of Alb have not been fully defined. Thus, the present study aimed to characterize the effects of different Alb treatment regimens for SAH. In particular, we examined the dose–response relationship and therapeutic window of Alb, and investigated whether this protection occurs in female rats and spontaneously hypertensive rats (SHR).

EXPERIMENTAL PROCEDURES

Animals

All experimental protocols were approved by the Jinling Hospital Animal Care and Use Committee and conducted in accordance with the recommendations in the guideline published in the National Institutes of Health guide for the Care and Use of Laboratory Animals. A total of 180 male SD rats weighting 270–320 g, 50 female SD rats weighting 230–250 g and 55

*Corresponding authors at: Department of Neurology, Jinling Hospital, Nanjing University School of Medicine, East Zhongshan Road, Nanjing 210002, Jiangsu, China. Fax: +86-25-84664563.
E-mail addresses: yeruid@gmail.com (R. Ye), xfliu2@vip.163.com (X. Liu).

† The first two authors contributed equally to this work.

Abbreviations: Alb, human albumin; ALIAS, the Albumin in acute stroke; ALISAH, the albumin in subarachnoid hemorrhage; ANOVA, analysis of variance; BBB, blood–brain barrier; FJC, Fluoro-Jade C; MABP, mean blood pressure; SAH, subarachnoid hemorrhage; SD, Sprague–Dawley; SHR, spontaneously hypertensive rat; TUNEL, terminal deoxynucleotide transferase-mediated dUTP nick end labeling.

http://dx.doi.org/10.1016/j.neuroscience.2016.12.033

Table 1. Physiologic variables in dose–escalation study

	Dose–escalation study					
	Baseline	Vehicle	Alb 0.02	Alb 0.1	Alb 0.2	Alb 1.0
PH	7.41 ± 0.02	7.38 ± 0.01	7.39 ± 0.02	7.38 ± 0.01	7.39 ± 0.02	7.41 ± 0.01
P_{O2}, mmHg	105 ± 3	103 ± 4	102 ± 5	98 ± 3	99 ± 4	102 ± 3
P_{CO2}, mmHg	38.1 ± 0.8	39.0 ± 1.3	37.4 ± 0.8	39.4 ± 0.7	38.8 ± 0.9	41.2 ± 1.2
Plasma glucose, mg/dl	138 ± 4	137 ± 5	138 ± 3	128 ± 4	145 ± 5	135 ± 5
Hematocrit, %	41.4 ± 0.7	42.3 ± 0.9	41.4 ± 0.8	41.5 ± 0.7	39.5 ± 0.7	34.1 ± 0.9***
MABP, mmHg	105 ± 5	101 ± 3	106 ± 4	99 ± 4	102 ± 5	97 ± 4
Plasma oncotic pressure, mOsm/kg	295 ± 3	296 ± 3	299 ± 1	295 ± 1	294 ± 2	294 ± 2
Plasma colloid oncotic pressure, mmHg	18.6 ± 0.5	17.7 ± 0.4	19.3 ± 0.3	20.0 ± 0.4**	22.6 ± 0.4***	24.8 ± 0.5***

Date are presented as the mean ± S.E.M (n = 5–8 each group), $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$vs vehicle group.

SHR weighting 240–280 g were used. Sample size was determined by power analysis based on pilot experiments (significance level 0.05, power 80%). All animals were group-housed with 12-h light/dark cycle at 24 °C and given free access to food and water.

Treatment groups

Animals were randomly assigned into the following 2 experiments: experiment I was performed to determine the dose–escalation relationship and therapy window of Alb for the treatment of SAH. For dose–escalation study, animals were randomly assigned to following treatment groups: 20% Alb was administered through tail vein immediately after SAH at the doses of 0.02 g/kg, 0.1 g/kg, 0.2 g/kg or 1.0 g/kg, vehicle animals received a similar volume of sodium chloride (0.9%, 5 ml/kg). For therapeutic window study, Alb was administrated through tail vein immediately or at 2 h, 4 h, 8 h after SAH onset, vehicle (0.9% sodium chloride, 5 mL/kg) was administered immediately after onset of SAH. Experiment II was performed to investigate the therapy effects of Alb in female and spontaneously hypertensive rats. 0.2 g/kg and 1.0 g/kg Alb were administered intravenously immediately both in female and spontaneously hypertensive rats, vehicle animals received a similar volume of sodium chloride (0.9%, 5 ml/kg).

SAH rat model

The endovascular perforation model was established as described previously with slight modification (Bederson et al., 1995). Briefly, with 1% pentobarbital anesthesia, a 4.0 monofilament nylon suture was inserted through right common carotid artery and internal carotid artery to perforate the junction of the middle and anterior cerebral artery. Sham-operated rats underwent the same procedures without perforation. Rectal temperature was maintained at 37 ± 0.5 °C during surgery. The right femoral artery was cannulated, and physiological parameters, including arterial blood gases (pH, Pco_2 and Po_2), plasma glucose, mean blood pressure (MABP), hematocrit, plasma osmotic pressure and plasma colloid oncotic pressure, were monitored. Colloid oncotic pressure (COP) was calculated by equation as the following: $COP = [5.3192\ A/G - 2.2252\ (A/G)^2 + 0.2939\ (A/G)^3]\ TP$ (Nematbakhsh et al., 2006).

SAH grade

SAH grade was blindly assessed at 24 h after SAH (Sugawara et al., 2008). Briefly, the brain basal cistern was divided into six segments. Each segment was scored from 0 to 3 according to the amount of blood in corresponding segment. Total scores range from 0 to 18.

Neurological scores

Neurological scores were blindly evaluated at 24 h post-SAH by modified Garcia score (Garcia et al., 1995). An 18-point scoring system consists of six sensorimotor tests including spontaneous activity, spontaneous movements of all limbs, movements of forelimbs, climbing, trunk and vibrissa touch.

Brain water content

Brains were harvested at 24 h after SAH and divided into left and right hemisphere. Then, the tissue samples were weighed on an electronic analytical balance immediately and again after drying in an oven at 105 °C for 24 h. Brain water content (%) was calculated as [(wet weight-dry weight)/wet weight] × 100% (Ye et al., 2011a).

Histology

At 24 h after SAH, rats were anesthetized and intracardially perfused with 200 ml physiological buffer solution followed by 400 ml 4% paraformaldehyde (pH 7.4). Brains were removed and postfixed in 4% paraformaldehyde for 4–8 h, then dehydrating. Once finished dehydrating, brains were embedded into optimal cutting temperature compound and frozen. Then the coronal sections 14 μm thick were cut on a Leica CM1950 cryostat and stored at −80 °C. Sections were fixed in 4% paraformaldehyde for 15 min at room temperature before IgG staining, Fluoro-Jade C staining and TUNEL staining.

Please cite this article in press as: Wang L et al. Preclinical efficacy of human Albumin in subarachnoid hemorrhage. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2016.12.033