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# Therapeutic effects of human urocortin-1, -2 and -3 in intracerebral hemorrhage of rats

Hock-Kean Liew [a], Li-Chuan Huang [b,c], Hui-I Yang [a], Hsiao-Fen Peng [a], Kuo-Wei Li [a,d], Andy Po-Yi Tsai [e], Shin-Yuan Chen [f,g], Jon-Son Kuo [e,g], Cheng-Yoong Pang [a,c,*]

[a] Department of Medical Research, Buddhist Tzu Chi General Hospital, Hualien, Taiwan, ROC
[b] Department of Radiology, Buddhist Tzu Chi General Hospital, Hualien, Taiwan, ROC
[c] Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan, ROC
[d] Department of Life Science and Institute of Biotechnology, National Dong Hwa University, Hualien, Taiwan, ROC
[e] Institute of Pharmacology and Toxicology, Tzu Chi University, Hualien, Taiwan, ROC
[f] Department of Neurosurgery, Buddhist Tzu Chi General Hospital, Tzu Chi University, Hualien, Taiwan, ROC
[g] Neuro-Medical Scientific Center, Buddhist Tzu Chi General Hospital, Hualien, Taiwan, ROC

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ABSTRACT

Urocortin exerts neuroprotective effects in intracerebral hemorrhage (ICH) of rats. For pre-clinical trial, we intended to study the neuroprotective efficacy of human UCN (hUCN)-1, -2 and -3 in treating ICH rats. ICH was induced by infusing bacterial collagenase VII (0.23 U in sterile saline) to the striatum. The hUCN-1, -2, and -3 were administrated (2.5 μg/kg, i.p.) at 1 h after ICH insult, respectively. Neurological deficits were evaluated by modified Neurological Severity Scores. Brain edema and hematoma expansion was evaluated by coronal T2-WI and DWI magnetic resonance imaging on 1, 3, 6, 24, and 56 h after ICH insult. Blood–brain barrier permeability was evaluated by Evans blue assay on day 3 after ICH. Brain lesion volume was evaluated by morphormetric measurement on day 7 after ICH. Our results demonstrated that the hUCN-1 significantly reduced hematoma, blood–brain barrier disruption and neurological deficits on day 3, and brain lesion volume on day 7 after ICH insult. The prediction of secondary structure of the hUCNs clarifies that the percentage of alpha-helix, random coil and extended strand between rat-UCN (rUCN)-1 and hUCN-1 are the same. The structure similarity between human- and rat-UCN-1 may be one of the reasons that both can exert similar therapeutic potential in ICH rats.


## 1. Introduction

Urocortin (UCN), a 40-amino-acid endogenous neuropeptide, belongs to the corticotrophin releasing hormone (CRH) family. Three mammalian CRH-like paralogs have been identified: UCN-1 (or simply UCN), UCN-2 and UCN-3 (Vaughan et al., 1995). Each of them is phylogenetically distinct, having unique anatomical distribution under the control of different genes (Fekete and Zorrilla, 2007). The physiological effect of UCN includes suppression of appetite, anxiogenic effect on behavior, and regulation of cardiovascular function (Oki and Sasano, 2004). A recent study demonstrated that UCN-1 may stimulate the hypothalamic CRF production through activation of the HPA axis, which is mediated by CRF-R1 (Bagosi et al., 2014), while UCN-2 and UCN-3 may modulate the hypothalamic CRF production in time-dependent and dose-dependent manner (Bagosi et al., 2013).

UCN, nevertheless, has also been claimed to be a potentially protective or therapeutic drug for heart ischemia/reperfusion injuries *in vitro* or *in vivo* (Brar et al., 2000; Gordon et al., 2003; Lawrence et al., 2002; Liu et al., 2005), as well as for various neuronal or ICH injuries as demonstrated by our studies (Huang et al., 2011, 2012; Liew et al., 2012a,b).

Spontaneous intracerebral hemorrhage (ICH) accounts for 10 to 15% of all strokes. It is the deadliest stroke (Greenberg et al., 2010) associated with high morbidity and mortality (Broderick et al., 1999). Its prevalence in Asia is about 20 to 30%, higher than that in Europe. The primary injury of ICH is caused by hematoma expansion with time up to 24–48 h (Kazui et al., 1996), causing mechanical disruption of the brain parenchyma (Kazui et al., 1996) and accelerated neurological deterioration (Brott et al., 1997). The secondary injury of ICH is caused by coagulation, hemolysis and hemoglobin breakdown (Kazui et al., 1996). The coagulation cascades produce and activate the thrombin immediately in the brain after ICH (Xi et al., 2006), leading to activation of the microglia to induce BBB breakdown, early brain edema, and neuronal and glial cell death (Lee et al., 1996; Wang and Tsirka, 2005; Yenari et al., 2006). Currently, no effective treatment can reduce the ICH injury.

Our previous study indicates that (1) UCN promotes the survival of dopaminergic neurons by inhibiting HDAC activity *in vitro* (Huang

* Corresponding author at: Department of Medical Research, Buddhist Tzu Chi General Hospital, No. 707, Section 3, Chung-Yang Road, Hualien 97002, Taiwan, ROC. Tel.: +886 3 8561825x5613; fax: +886 3 8573710.
*E-mail address:* cypang@mail.tcu.edu.tw (C.-Y. Pang).

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et al., 2011), (2) UCN decreases inflammatory reaction in LPS-induced microglial activation *in vitro* (Wang et al., 2007), (3) UCN promotes differentiation of neural stem cells both *in vitro* and *in vivo* (Huang et al., 2012), and (4) UCN protects rats' brain from ICH stroke by reducing brain edema, blood–brain barrier disruption and regional neuroinflammation (Liew et al., 2012a,b).

In order to develop the human UCN as a clinical therapeutic agent for the ICH injuries, we compared the therapeutic or neuroprotective efficacy of the human UCNs (hUCN-1, hUCN-2, and hUCN-3) in an ICH rat model.

## 2. Materials and methods

### 2.1. Animals

The experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and experimental protocols were approved by the Affidavit of Approval of Animal Use Protocol Board Tzu Chi Hospital (Approval No.102-03-1). All efforts were made to minimize suffering and number of animal used. Animals were housed under a 12-hour light/dark cycle with free access to food and water.

### 2.2. Chemicals

Sodium pentobarbital, hUCN-1 & -3 were from Sigma-Aldrich (St Louis, MO, USA), isoflurane was from Abbott (Kent, UK), and hUCN-2 was from GenScript (NJ, USA).

### 2.3. Experimental protocol

Male Sprague Dawley rats (350–400 g) were assigned randomly to four experimental groups: ICH + saline (n = 6), ICH + hUCN-1 (2.5 μg/kg, n = 6), ICH + hUCN-2 (2.5 μg/kg, n = 6), and ICH + hUCN-3 (2.5 μg/kg, n = 6). The dosage of 2.5 μg/kg was used based on our previous investigations (Liew et al., 2012a,b).

### 2.4. ICH induction and drugs administration

The ICH is induced by stereotaxic infusion of bacterial collagenase VII-S (0.23 U in 1.0 μL sterile saline, Sigma-Aldrich, USA) (MacLellan et al., 2007). Briefly, rats were anesthetized with pentobarbital (50 mg/kg, IP), bacterial collagenase VII-S was infused into the right striatum of rats over a period of 10 min. One hour after the ICH induction, the ICH rats were intra-peritoneally injected with saline, hUCN-1, hUCN-2, and hUCN-3, respectively (Fig. 1).

### 2.5. Evaluation of brain penetration of fluorescently labeled hUCNs

To evaluate the penetration of hUCN-1, -2 and -3 through the blood–brain barrier (BBB), hUCN-1, -2, and -3 were labeled with Alexa Fluor 488 dye, respectively, using a Microscale Protein Labeling Kit (A30006, Invitrogen, USA) according to the manufacturer's instructions. The entrance of human UCNs into the brain was evaluated on brain slices according to the published method (Liew et al., 2012a,b). Briefly, the fluorescently labeled hUCN-1, -2, and -3 was administered intraperitoneally 1 h post-ICH. Three hours after injection of the fluorescently labeled human UCNs, the rats were re-anesthetized with pentolbarbitol (50 mg/kg i.p.), and their brains were removed immediately and sectioned to 20 μm thickness with a cryostat. To confirm the entrance of human UCNs peptide into the brain, slices were first incubated overnight at 4 °C with UCN primary antibody (1:100; Catalog No. U4757, Sigma, CA, USA). The slices were then washed with PBS and incubated for 1 h with secondary antibody (anti-rabbit-rhodamine, Jackson ImmunoResearch, West Grove, PA, USA) at room temperature. After rinsing with PBS buffer, the slices were examined under a fluorescence

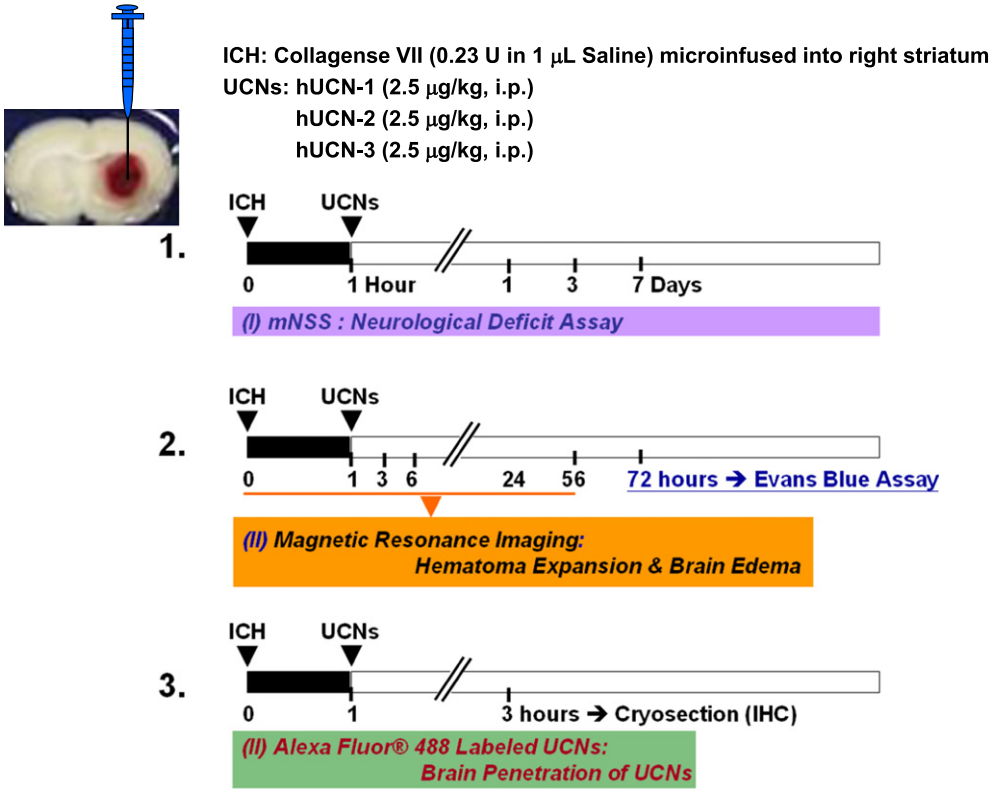


**Fig. 1.** Schematic diagrams of treating ICH rats with human UCNs. ICH is induced by infusion of bacterial collagenase VII into the striatum. ICH = intracerebral hemorrhage; mNSS = modified Neurological Severity Scores; and UCNs = urocortins.