

# Topical application of adipose tissue derived mesenchymal stem cells (ADMSCs) reduced cerebral edema in experimental traumatic brain injury (TBI)- a preliminary study

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## Research

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# Abstract

## Background:

Our previous studies showed that topical application of mesenchymal stem cells (MSCs) improved functional recovery in rat traumatic brain injury (TBI) model, and hypoxic precondition further enhanced the therapeutic effects of MSCs. There was no previous study on the attenuation of cerebral edema by MSCs.

## Objective:

We investigated whether topical application of normoxic and hypoxic MSCs could reduce cerebral edema in an experimental TBI model.

## Methods:

2 million normoxic (N=24) and hypoxic (N=24) MSCs were applied topically to exposed cerebral cortex in a controlled cortical impact (CCI) model. The MSCs were fixed in position with fibrin glue. No treatment was given to control animals (TBI only: n=24). After surgery, four animals in each group were sacrificed daily (day 1 to day 6) for edema evaluation. Normal animals without TBI were used as reference (n=4). The expressions of GFAP, AQP4 and MMP9 were also investigated by immunofluorescence staining and RT-PCR at day 3.

## Results:

The edema peaked within 3 days after TBI. Compared with the control, hypoxic MSCs reduced brain water content significantly ( $p < 0.05$ ). Both hypoxic and normoxic MSCs downregulated the expression of MMP9 and normalized AQP4 distribution to astrocytes end feet.

## Conclusion:

Our preliminary study showed that topical application of hypoxic MSCs suppressed both vasogenic and cytotoxic edema formation.

# Background

Brain edema was defined as 'an expansion of brain volume which increases intracranial pressure, impairs cerebral perfusion and causes additional ischemic injuries' [1]. For decades, edema has been a target in the treatment of traumatic brain injury. Conventional managements to reduce cerebral edema include: i) General measures: optimal head and neck position, adequate oxygenation, maintenance of normotension, management of fever and hyperglycaemia, nutritional support; ii) Specific interventions: controlled hyperventilation, osmotherapy(mannitol, hypertonic saline), corticosteroid administration and pharmacological coma(barbiturates); iii) Surgical procedures: cerebrospinal fluid (CSF) drainage,

decompressive craniectomy, decompressive laparotomy [2]. However, these passive treatments can induce complications such as renal failure and acute respiratory distress syndrome [2]. Classically, there are two types of edema associated with traumatic brain injury, namely vasogenic edema and cytotoxic/cell edema [3,4]. Vasogenic edema is caused by disruption of blood brain barrier (BBB) after TBI, resulting in water movement from blood vessels to extracellular space and an increase in brain water content. Cytotoxic edema, also known as cellular or ionic edema, is defined as 'cellular failure with disrupted ionic pump with anaerobic metabolism' which is caused by ischemia after TBI [5].

Mesenchymal stem cells (MSCs) are able to self-renewal and differentiate into multiple cell lineages. To accelerate functional recovery, MSCs bio-modulate local microenvironment, suppress inflammatory response, promote angiogenesis and regeneration in addition to the trans-differentiation after transplantation [6]. Our recent study showed that hypoxic preconditioning of MSCs enhanced tissue repair and functional recovery in experimental TBI [7]. To our knowledge, there are no reports on the attenuation of cerebral edema by MSCs.

We would like to investigate whether topical application of MSCs could reduce cerebral edema in a rodent model of TBI.

## Methods

MSCs were derived from subcutaneous adipose tissue of male SD rats. Cultured under either normoxic (18% O<sub>2</sub>) or hypoxic (5% O<sub>2</sub>), all MSCs expressed CD29, CD90 but not CD45. The MSCs had adipogenic, chondrogenic and osteogenic differentiation potentials in vitro under specific culture conditions. Female SD rats, aging 10-12 weeks were used for the study (Laboratory Animal Services Center, CUHK).

Traumatic brain injury was induced by controlled cortical impact (CCI) [7] (Figure 1). After surgery, 2 million normoxic (n=24) and hypoxic (n=24) MSCs were applied topically to the exposed brain cortex. A thin layer of fibrin glue was used to keep cells in position. No treatment was given to animals in control group (n=24) (Table 1).

Four animals in each group were sacrificed daily until day 6, normal animals were used as reference (n=4). For each animal, the injured hemisphere was collected and dried in the oven for 36 hours. Wet weight and dry weight were measured respectively. Brain water content was calculated according to the formula: (wet weight- dry weight)/wet weight %.

Hydrated paraffin tissue slices were stained by anti-GFAP (Abcam, Cambridge, UK) (marker of astrocytes) and anti-AQP4 (Abcam, Cambridge, UK) (marker of water channel on astrocytes) antibody. Real-time PCR was used for the determination of MMP9 (Abcam, Cambridge, UK) (marker of vasogenic edema) expression.

## Results

### Hypoxic MSCs reduced brain water content

The brain water content increased remarkably within 3 days after TBI (Fig.2) (Table 2). Then it reduced significantly in the hypoxic MSCs group ( $p < 0.05$  vs control); whereas no significant change was found in the normoxic group (Fig.3).

#### Hypoxic MSCs normalized the AQP4 distribution

Double florescent staining of GFAP and AQP4 indicated that AQP4 distribution was normalized to the end feet of astrocytes after either normoxic or hypoxic MSCs treatment, whereas positive fluorescent signal was observed around cell body of astrocyte in the control group (Fig.4).

#### Hypoxic MSCs down-regulated the expression of MMP-9

Expression of MMP9 was downregulated significantly in both normoxic ( $p < 0.05$  vs control) and hypoxic ( $p < 0.05$  vs control) groups on day 3. No significant difference was found between two different MSC groups (Fig.5) (Table 3).

## Discussion

The pathophysiology of brain edema involves vasogenic and cytotoxic cascades. Vasogenic edema is associated with blood brain barrier (BBB) integrity. Also, brain water content increases due to BBB dysfunction. In this study, we demonstrated that topical application of hypoxic MSCs reduced brain water content significantly on day 3. This effect was not observed in the normoxic MSCs treatment group. Previous study showed hypoxic preconditioning enhanced therapeutic effects of MSCs [7]. Thus, it was believed that the suppression effects against cerebral edema was stronger in the hypoxic MSC group. Animal studies showed upregulation of MMP9 in TBI [8,9]. MMP9 degrades extracellular matrix proteins, including neurovascular basal lamina and tight junction proteins of BBB [10]. The downregulation of MMP9 by MSC treatment could reduce edema formation and maintain BBB integrity [10]. AQP4, one of the water channel proteins, is a key factor in the development and resolution of cerebral edema [11]. Normally, it locates in the perivascular astrocyte end feet [12]. After traumatic brain injury occurs, astrocytosis is activated, the AQP4 signal is not limited at the end feet. It is also detected all reactive astrocytes [12]. Topical application of MSCs suppressed neuro-inflammation by triggering reactive astrocytosis at early phase [6]. In this study, topical MSCs normalized AQP4 distribution in astrocytes. In the tissue without MSCs treatment, the astrocytes underwent swelling after TBI, resulting in the opening and re-location of the water channel on the cell body. The topically applied MSCs contributed to the suppression of cerebral edema and the reduction of astrocytes swollen, shown as the normalization of the AQP4 distribution. Although the underlying mechanism is not yet clear, there are two hypotheses to explain the attenuation of cerebral edema. Firstly, numerous studies have shown that the transplanted MSCs were capable to express marker of astrocytes (GFAP) in vivo, which may help maintain BBB integrity [13]. Secondly, MSCs secrete soluble factors including growth factors, cytokines and chemokines, through paracrine activity [13], they might biomodulate the neuro-inflammation associated with cerebral edema.

However, we have some limitations in this study. First, we didn't measure brain water content at acute phase after TBI, such as 1h, 6h, 12h. Second, we didn't compare therapeutic effects of normoxic and hypoxic MSCs in acute phase. Third, we compare water content only in injured hemisphere instead of whole brain.

## Conclusion

In this study, we demonstrated topical application could deliver a large amount of MSCs safely and effectively to the injured brain. Hypoxic MSCs reduced brain water content 3 days after injury happened, both normoxic and hypoxic MSCs treatment downregulated pro-vasogenic edema gene expression and normalize water channel distribution in astrocytes.

## Abbreviations

ADMSCs: adipose tissue derived mesenchymal stem cells

BBB: blood brain barrier

CCI: controlled cortical impact

CSF: cerebrospinal fluid

MSCs: mesenchymal stem cells

TBI: traumatic brain injury

## Declarations

**Ethics approval:** All animal procedures were conducted in accordance with the guidelines in the Animals (Control of Experiments) Ordinance (Chapter 340), Department of Health, Hong Kong, and the study was approved by the Animal Experimentation Ethics Committee of The Chinese University of Hong Kong.

**Consent for publication:** Not applicable.

**Availability of data and materials:** Please contact the corresponding author if any data or materials are required.

**Competing interests:** There is no conflict of interests in this research.

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**Author contributions:** Hui MA, performed the experiments and wrote the manuscript. C.S.W. TONG, K.K. LO helped with the immunohistochemistry staining. Lian CUI and PK designed the study. PK LAM, George

Wong and WS POON supervised the study, provided language help and writing assistance. All authors reviewed and commented on the manuscript. All authors approved the final version of the manuscript.

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## Tables

Table 1. Animal groupings in the study.

Groupings	Number of animals	Treatment
Control	24	TBI without treatment
MSCs	24	TBI + 2 million MSCs
Hypoxic MSCs	24	TBI + 2 million hypoxic MSCs

Table 2. The average wet and dry weight of the injured hemisphere.

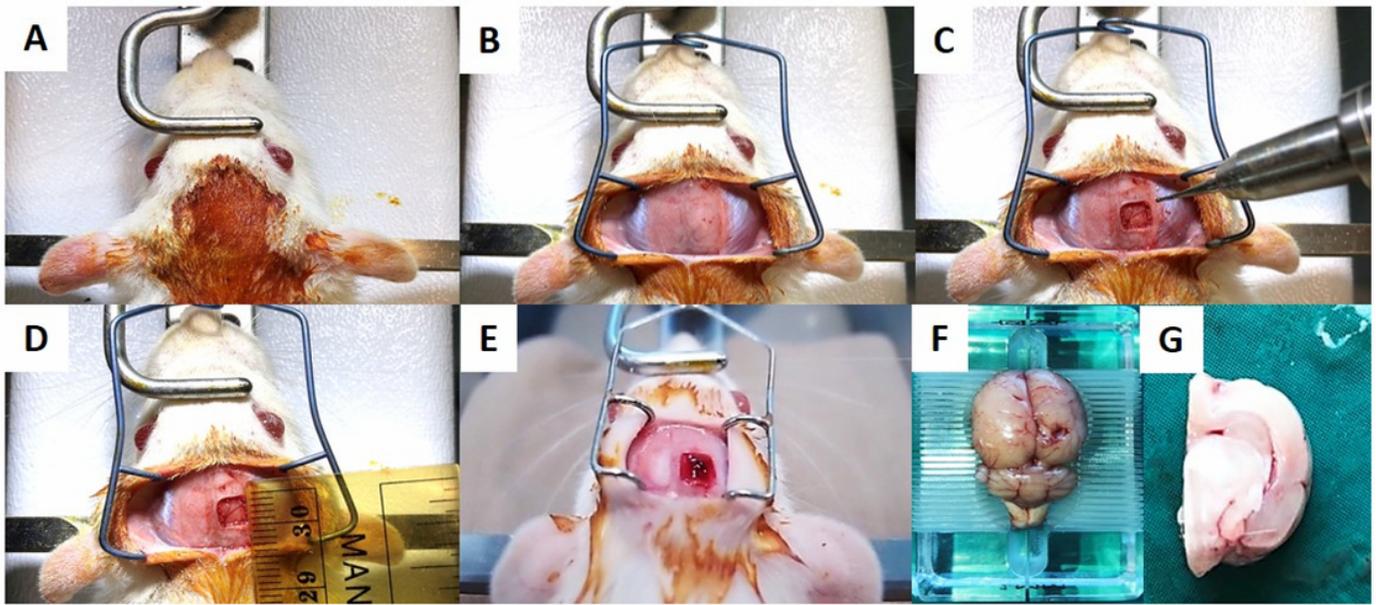
Groups/Time point	Wet weight (g)	Dry weight (g)	Water content (%)
Normal	0.73	0.17	76.88
Control-D1	0.73	0.16	78.00*
Control-D2	0.68	0.15	78.31*
Control-D3	0.70	0.15	77.92*
Control-D4	0.64	0.14	77.59
Control-D5	0.67	0.15	77.69
Control-D6	0.67	0.15	77.03
MSCs-D3	0.63	0.14	77.90
Hypoxic MSCs-D3	0.70	0.16	76.95#

\*p < 0.05 vs normal, # p < 0.05 vs control

Table 3. List of primers used in RT-PCR.

GAPDH	Forward AGA CAG CCG CAT CTT GT
	Reverse CTT GCC GTG GGT AGA GTC AT
MMP-9	Forward CCC CAC TTA CTT TGG AAA CGC
	Reverse ACC CAC GAC GAT ACA GAT GCTG

## Figures



**Figure 1**

The process of controlled cortical injury (CCI) and injured hemisphere collection. 1A-1E: process of CCI model on SD rat. F: right hemisphere was injured. G: the hemisphere harvested for brain water content measurement.

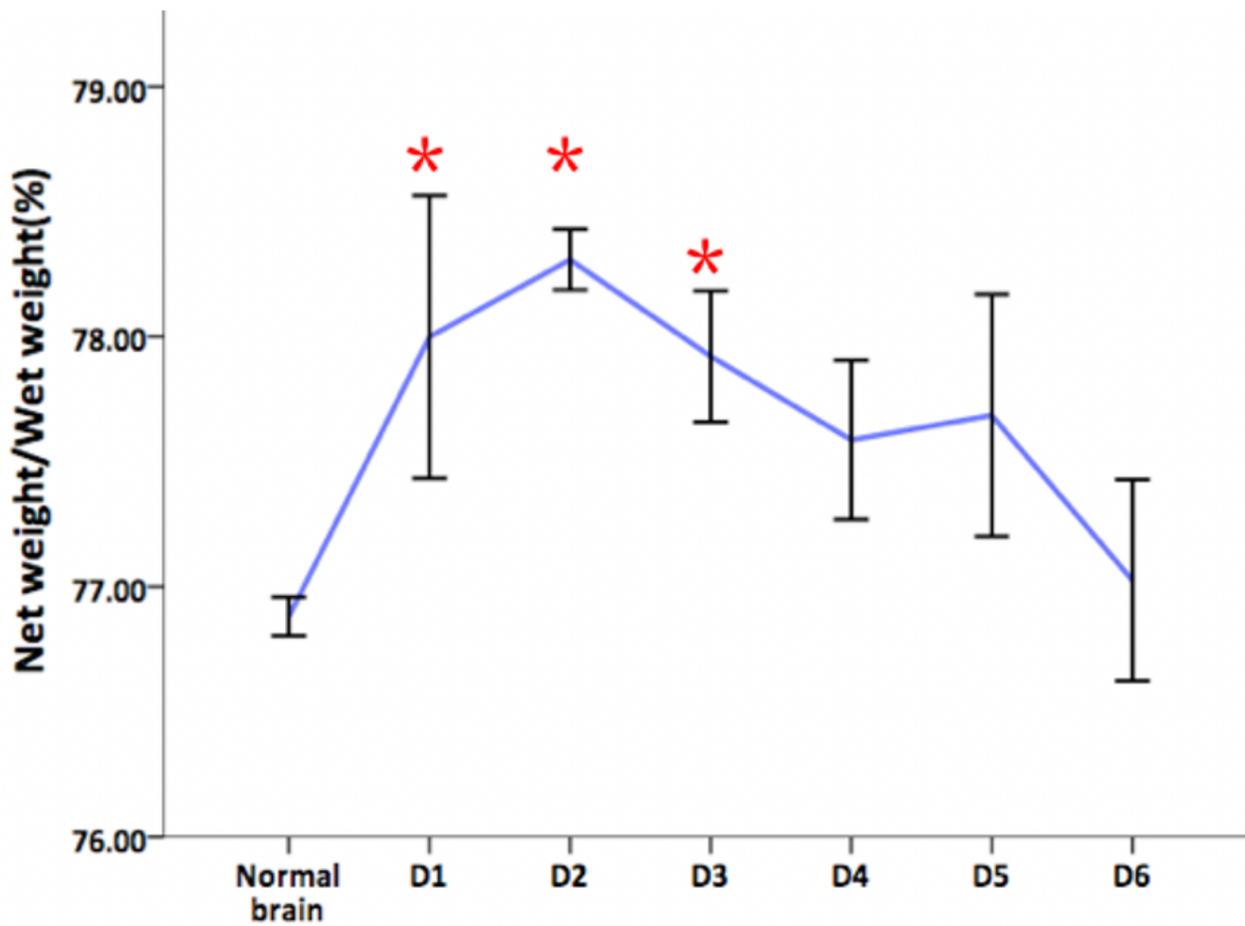


Figure 2

4 animals were sacrificed daily until day 6 after TBI. The injured hemisphere was harvested and measure brain water content. Compared with hemisphere from normal animals, the brain water content was significantly higher from day 1 to day 3 (\* p<0.05 vs control) (net weight = wet weight – dry weight)

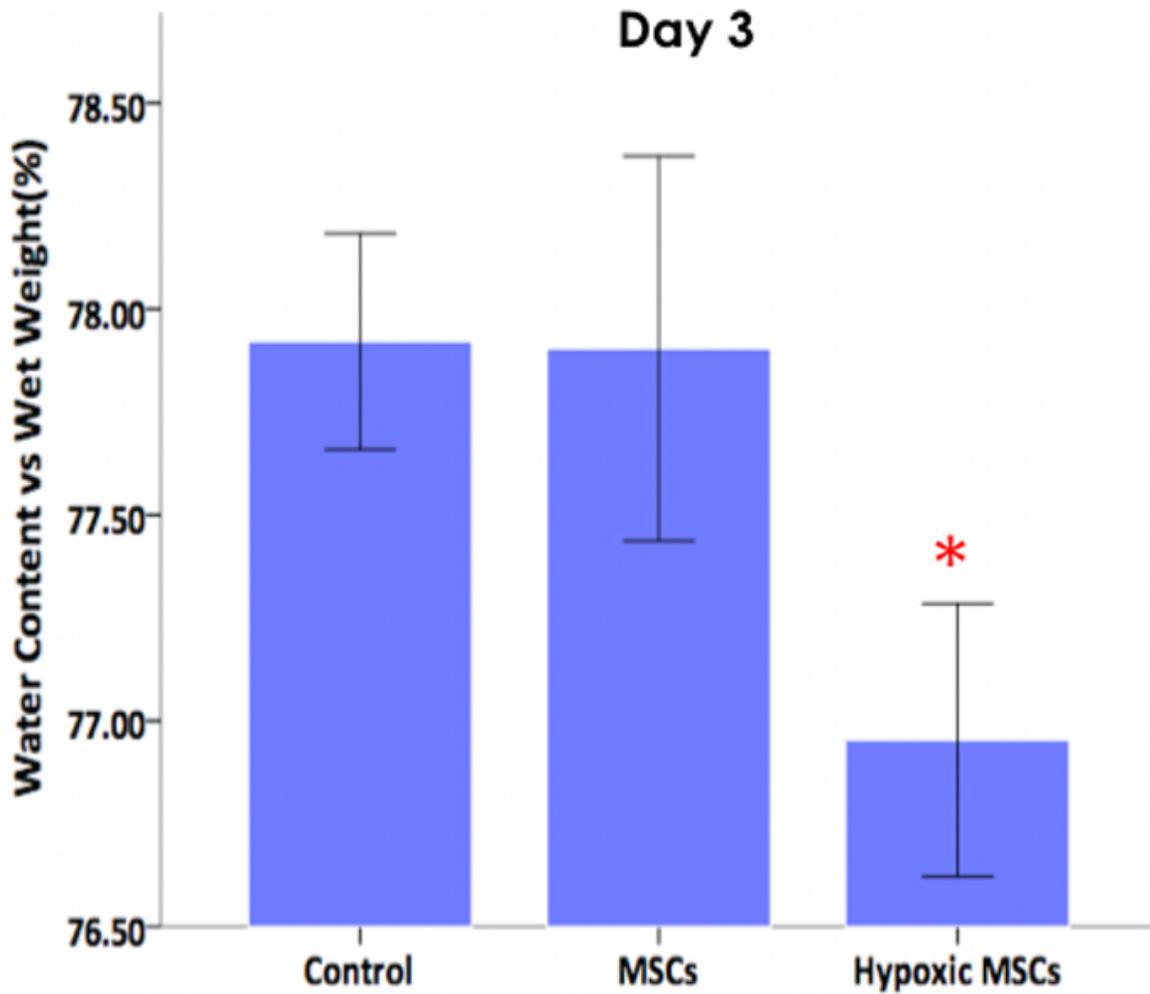
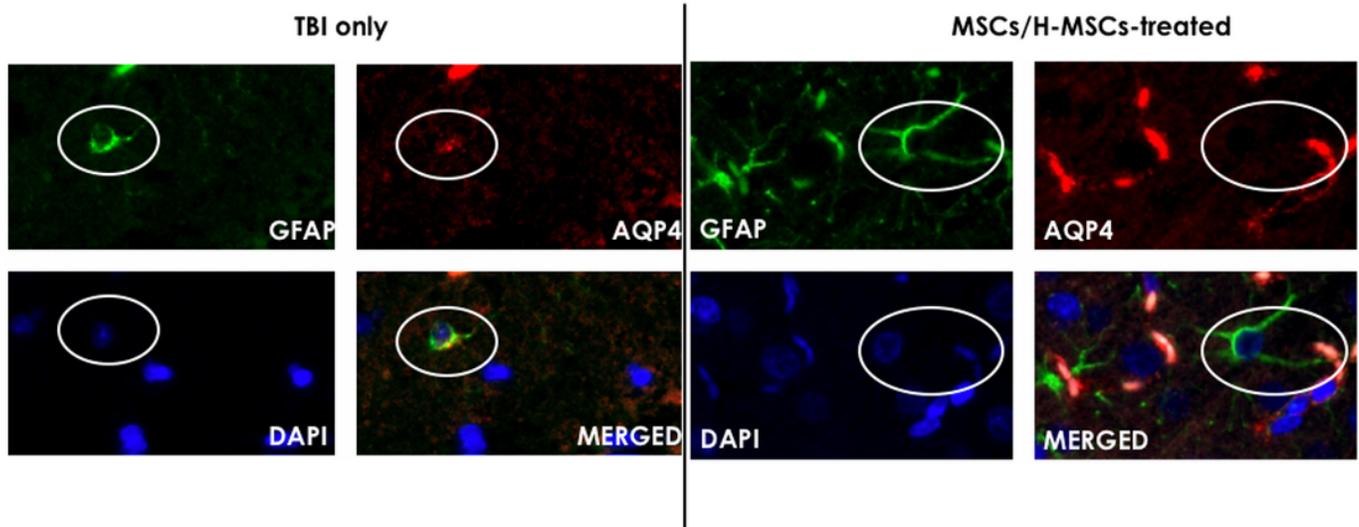


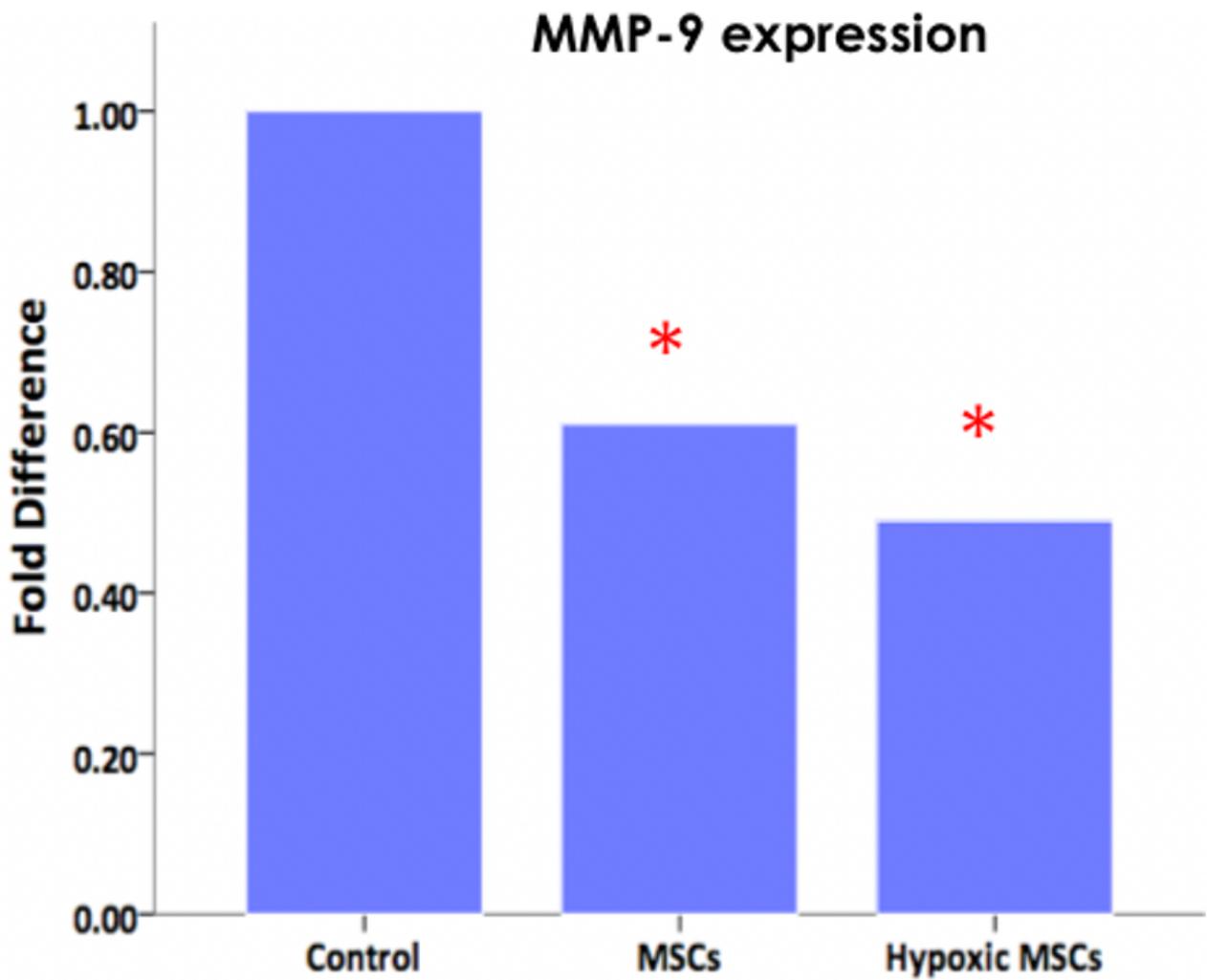
Figure 3

The injured hemispheres were harvested from animals in different groups on day 3. The hypoxic MSCs-treated brain showed lower water content than control (\* $p < 0.05$  vs control), no remarkable change was found between normoxic MSCs-treated brain and control.



**Figure 4**

Picture of double immune-florescent staining of GFAP and AQP4. After TBI, distribution of AQP4 signal spread to cell body of astrocytes. With treatment of normoxic/hypoxic MSCs, the signal located to end feet of astrocytes.



**Figure 5**

RT-PCR results indicated MMP9 expression was downregulated significantly after both normoxic and hypoxic MSCs transplantation (\* $p < 0.05$  vs control). No statistical difference was found between two cell treatments.