

Original Research Article

Sevoflurane induces ho-1-mrna expression by regulating P13K/Akt/P70S6K signaling pathway and affects neuronal apoptosis

Zhiqiang Lu¹, Yan Wei², Jiandang Xu¹, Yinghua Tang^{1*}

¹Department of Anesthesiology, Shanghai No. 8 People's Hospital, Shanghai 200235, ²Department of Anesthesiology, Shanghai Pudong New Area People's Hospital, Shanghai 201299, China

*For correspondence: **Email:** ri5wvb@163.com

Sent for review: 5 February 2022

Revised accepted: 13 May 2022

Abstract

Purpose: To determine the effect of sevoflurane (SE) on neuronal apoptosis, and the mechanism involved.

Methods: Sixty healthy male Sprague-Dawley rats were assigned to control, model and SE groups. Sham surgery was performed in control group, while middle cerebral artery infarction (MCAO) was established in model group. The expression of HO-1 mRNA was determined by quantitative real-time polymerase chain reaction (qRT-PCR). Apoptosis, autophagy and protein content of P13K/Akt/P70S6K signaling pathway were assessed by Western blot assay.

Results: Apoptosis was significantly lower in SE rats, relative to model rats. There were markedly higher protein levels of LC3 II / I, beclin-1, bad, Bcl-2 and caspase-3 in model and SE groups than in control rats ($p < 0.05$). The HO-1 mRNA was significantly up-regulated in model and SE groups, relative to controls, but it was significantly up-regulated in SE group, relative to model rats ($p < 0.05$). The expression levels of phosphorylated proteins of the P13K/Akt /P70S6K signal-related proteins in model and SE groups were significantly up-regulated, relative to control, but elevated in SE mice, when compared to model mice ($p < 0.05$).

Conclusion: SE improves the behavior of MCAO rats, reduces cerebral infarction, and inhibits apoptosis and autophagy of nerve cells by regulating autophagy and apoptosis-related proteins through a mechanism that may be related to the induction of HO-1-mRNA expression by regulating P13K/Akt /P70S6K signal pathway. This provides new insights for the development of anti-neuronal apoptosis drugs.

Keywords: Sevoflurane, P13K/Akt/P70S6K signaling pathway, HO-1-mRNA, Neuronal cells, Apoptosis

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Cerebrovascular diseases are associated with high rate of disability; they are primary causes of mortality in China, and they seriously endanger

the lives and health of Chinese people [1]. Stroke comprises two types: hemorrhagic stroke and ischemic stroke, with ischemic stroke accounting for about 80 % of strokes [2]. Cerebral vascular embolism, severe trauma and arterial

malformation may lead to ischemic stroke which may result in severe physical disability, and impairment of intelligence and other functions, thereby seriously endangering patients' lives [3]. Early restoration of blood supply is key to the treatment of ischemic stroke. However, a sudden restoration of blood flow in ischemic brain tissue can lead to ischemia reperfusion injury (I/R) in local brain tissue [4].

Complex mechanisms underlie I/R injury which can lead to autophagy and apoptosis, and damage to cell function. Sevoflurane (SE) is an inhaled anesthetic frequently used in clinical practice, with the advantages of rapid induction, low irritation and rapid absorption [5]. Liu *et al* [6] found that SE alleviated cerebral ischemia-reperfusion injury, but the relevant mechanism was not determined. In this study, 60 healthy male SD mice were used as experimental animals to investigate the mechanism involved in the effect of SE on neuronal cell apoptosis.

EXPERIMENTAL

Animals

Sixty selected healthy male Sprague Dawley (SD) mice with average age of 8 ± 1 weeks and a mean weight of 260 ± 10 g were provided by Beijing Weitonglihua Experimental Animal Center (license no. SCXKJING 2006-0009). The mice were maintained in SPF animal room at room temperature of 23 ± 1 °C and relative humidity of 55 ± 5 %, and were allowed *ad libitum* access to feed and drinking water. The mice were handled in line with the relevant standards of the Regulations on the Management of Experimental Animals.

Ethical approval

This research was approved by the Animal Ethical Committee of Shanghai Pudong New Area People's Hospital (approval no. 20200879), and conducted according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985) [7].

Establishment of rat MCAO model

The mice were fasted for 8 h before surgery and anesthetized *via* administration of chloral hydrate (10 %). The right common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) were dissected in the middle of the neck, and the CCA, ECA and ICA were exposed. The ICA and ECA were ligated, and a small incision was made at the bifurcation of CCA. Then, ICA and nylon wires were ligated,

and perfusion was resumed after occlusion for 120 min. Spontaneous breathing was maintained during the operation.

Animal grouping and treatment

Sixty (60) rats were assigned to control, model and SE groups. Model mice were the established MCAO model. The SE group was subjected to inhalation of 2 % sevoflurane for 15 min at the same time of reperfusion.

Evaluation of indicators/parameters

Water maze test

Morris Water Maze was used. First, a positioning trial was carried out: the rats were put in a pond at various starting points, and the time from entering the water to finding the underwater platform was noted. If platform location was unsuccessful in 60 s, the rat was led to it, and a recording of 60 s was made. Training was carried out four times daily for each rats at 15 - 20 min spacing, and the mean time was the result for that day. Each rat was removed from the platform after 10 s, and towed dry.

Space exploration experiment

Two days after the positioning navigation experiment training, the rats were trained to explore space for 60 s. They were put into water from the 1st quadrant, and the number of crossings of the original platform within 60 seconds was recorded.

Determination of cerebral infarction area

Deep anesthesia was given with pentobarbital sodium which was injected intraperitoneally. Animal sacrifice was done *via* decapitation. Excised brain tissues were frozen at -20 °C for 30 min. Then, 5 coronal brain slices were stained in TTC solution (10g/L) at 37 °C for 10 min, and fixed and preserved in FPS. Healthy brain tissue was reddish in color, while infarct tissue was gray. The infarct area in each was calculated.

Nerve cell apoptosis

Brain tissue was paraffin-embedded and sectioned, dewaxed in xylene and dehydrated in gradient alcohol. Apoptosis assay kits were used to measure apoptosis in nerve cells. In this assay, normal nucleus was blue, and apoptotic nucleus was brownish-yellow. Cell apoptosis was calculated for each group.

The formation of autophagosome vacuoles was examined with electron microscopy. The brain tissues were excised, cleaned and fixed with PBS, dehydrated in alcohol gradient, embedded in paraffin and sectioned; stained with uranium acetate for 30 min, and with lead citrate for 10 min, and examined using transmission electron microscopy. The sections were photographed, and the number of autophagosomes was counted.

The HO-1 mRNA expression level was determined using RT-fluorescence quantitative PCR. The extraction of total RNA was done using TRizol method. The RNA concentration was determined using ultraviolet spectrophotometry, followed by reverse-transcription to cDNA. Fluorescence signal was collected at 72 °C. The levels of HO-1 mRNA were determined with real-time quantitative Polymerase Chain Reaction (PCR).

Immunoblot assay

Total protein was extracted from tissue sections from each group by homogenization in RIPA lysis buffer. The homogenates were centrifuged at 12000 g for 5 min, and the supernatants containing total proteins were collected. The total protein concentration in each lysate was quantitatively determined using BCA method. Thereafter, equal amounts of protein were resolved using SDS-polyacrylamide gel electrophoresis, followed by electro-transfer to polyvinylidene difluoride (PVDF) membrane. Following the membrane transfer, each membrane was rinsed with TBST washing solution for 3 min to wash off excess membrane transfer solution. Then, the membrane was incubated with Western blocking solution (5 % skimmed milk powder) at room temperature for 2 h to block non-specific binding of the blot. This was followed by incubation overnight at 4 °C with appropriate primary antibodies diluted with primary antibody diluent in appropriate proportions. Thereafter, the membranes were incubated with horse radish peroxidase-linked secondary antibody (diluted 1:10000) at room temperature for 2 h. The protein bands were subjected to ECL, while Quantitative One Grayscale analysis software was used for determination of relative protein expression levels, with β -actin as standard.

Statistical analysis

The SPSS 20.0 software package was employed for data analysis. Normally-distributed data are expressed as mean \pm SD. One-way ANOVA was used to compare multiple groups, while paired

comparison was done with SNK-Q test. Values of $p < 0.05$ indicated statistically significant differences.

RESULTS

Morphology of neuronal cells at different times of hypoxia and different concentrations of sevoflurane

When hypoxic, the volume of spindle like neurons increased, or the number of spindle like cells increased gradually with the prolongation of hypoxia. Compared with hypoxia for 0 h, the proportion of activated cells increased gradually after hypoxia for 2, 4, 6, 8 and 12 h (Figure 1).

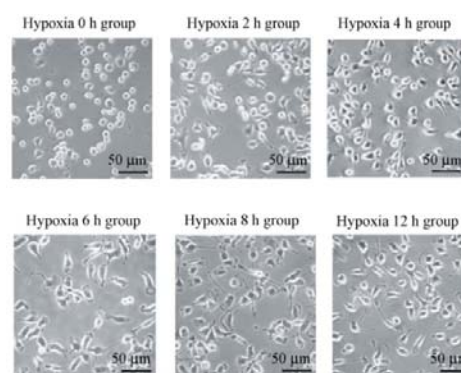


Figure 1: Morphological changes in neuronal cells under different hypoxia times

When the concentration of sevoflurane was 0 %, the volume of neuronal cells increased, and there were more cells with branch-like or spindle-like changes. Compared with 0 % sevoflurane treatment group, the proportion of activated cells in 2 %, 4 % and 6 % sevoflurane treatment groups increased gradually in a dose-dependent manner (Figure 2).

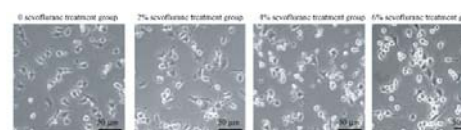


Figure 2: Morphological changes of neuronal cells in each group

Behavioral characteristics of rats

At each same time point, escape latency values in model and SE rats were markedly higher than the corresponding control value, but escape latency was lower in SE rats than in model rats. The frequency of crossing of the platform was markedly lower in model rats than in control rats, but it was higher in SE rats than in model rats,

with the difference being statistically significant ($p < 0.05$; Table 1).

Cerebral infarction area

There were higher areas of cerebral infarction in model and SE mice than in control mice, but infarction area was lower in SE mice than in model mice, as shown in Table 2 and Figure 3.

Apoptosis level

Apoptosis levels were higher in model and SE groups than in controls, but apoptosis was lower in SE rats than in model rats, with statistical significance ($p < 0.05$; Table 3).

Apoptotic and autophagic proteins

The protein expression levels of LC3 ii/i, Beclin-1, Bad, Bcl-2 and Caspase-3 in model and SE rats were significantly up-regulated, relative to those in control group, while they were significantly lower in SE group than in model group ($p < 0.05$; Table 5).

mRNA levels of heme oxygenase 1

The mRNA levels of heme oxygenase 1 in model and SE groups were markedly up-regulated, relative to control value, but HO-1 mRNA level was higher in SE mice than in model rat, with statistical significance ($p < 0.05$; Table 6).

P13K/Akt/p70S6K proteins

Protein levels of p-PI3K, p-Akt and p-P70S6K were up-regulated in model mice and SE mice, relative to control mice, while these protein levels were higher in mice in SE group than in model mice, as shown in Table 7.

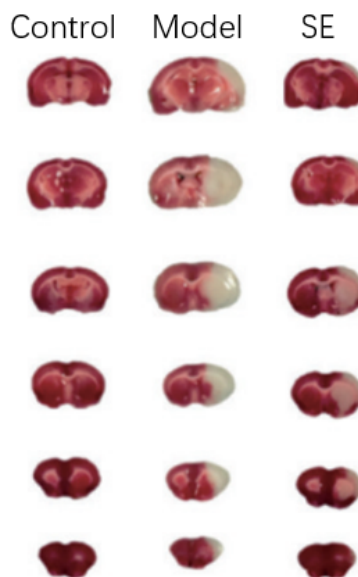


Figure 3: Cerebral infarct area

Table 1: Comparison of behavioral characteristics of rats amongst the groups ($\bar{x} \pm s$)

Group	Escape latency			Number of platform crossings (n)
	Day 1	Day 3	Day 5	
Control	48.16±4.16	16.25±1.85	5.68±3.12	4.65±0.35
Model	57.89±1.26 ^a	49.85±2.13 ^a	44.28±3.46 ^a	1.35±0.25 ^a
SE	50.46±2.46 ^{ab}	24.56±1.89 ^{ab}	9.78±4.15 ^{ab}	3.78±0.35 ^{ab}
F	62.204	1493.154	692.811	570.790

^{a,b} $P < 0.05$, ^avs control; ^bvs model

Table 2: Cerebral infarction areas (mean ± SD)

	Control	Model	SE	F	P
Cerebral infarction areas	1.182±0.661	42.786±2.152 a	25.744±4.031 ab	593.800	< 0.001

Table 3: Apoptosis levels (mean ± SD)

	Control	Model	SE	F	P
Apoptosis level	1.742±0.526	15.331±4.218 ^a	7.406±1.754 ^{ab}	66.100	< 0.001

Table 4: Number of autophagosome vacuoles (mean ± SD)

Item	Control	Model	SE	F	P-value
Number of autophagosome vacuoles	1.588±0.302	19.544±3.766 ^a	13.527±2.205 ^{ab}	130.900	<0.001

Table 5: Apoptotic and autophagic proteins (mean \pm SD)

Variable	Control	Model	SE	F	P-value
LC3- II/ I	0.724 \pm 0.115	2.088 \pm 0.883 ^a	0.898 \pm 0.200 ^{ab}	19.85	< 0.001
Beclin-1	0.901 \pm 0.096	3.779 \pm 0.905 ^a	2.324 \pm 0.385 ^a	213.70	< 0.001
Bad	1.033 \pm 0.272	3.406 \pm 0.988 ^a	2.111 \pm 0.955 ^a	22.31	< 0.001
Bcl-2	5.672 \pm 0.648	2.883 \pm 0.723 ^a	3.998 \pm 0.615 ^a	44.76	< 0.001
Caspase-3	0.855 \pm 0.197	4.497 \pm 0.524 ^a	3.186 \pm 0.211 ^a	285.20	< 0.001

Table 6: mRNA levels of heme oxygenase 1(HO-1) (mean \pm SD)

Item	Control	Model	SE	F	P
HO-1	0.214 \pm 0.047	0.385 \pm 0.096 ^a	1.247 \pm 0.205 ^{ab}	172.10	< 0.001

Table 7: P13K/Akt/p70S6K proteins (mean \pm SD)

Variable	Control	Model	SE	F	P-value
p-PI3K	0.522 \pm 0.121	0.831 \pm 0.135 ^a	1.192 \pm 0.172 ^{ab}	41.57	< 0.001
p-Akt	0.637 \pm 0.054	0.812 \pm 0.076 ^a	1.255 \pm 0.163 ^a	86.33	< 0.001
p-P70s6K	0.574 \pm 0.069	0.799 \pm 0.149 ^a	1.078 \pm 0.138 ^a	54.02	< 0.001

DISCUSSION

With recent pronounced increases in population of the aged, incidents of stroke have shown increasing trend year by year, thereby seriously endangering people's health and bringing heavy economic burden to families and society [8]. Therefore, there is need to focus on how to alleviate the brain injury of stroke patients and reduce the incidence of disability. Ischemic stroke causes depolarization, necrosis, inflammation and apoptosis of neurons in the corresponding parts of the brain, and it may lead to hemiplegia, consciousness disorder and aphasia. Sevoflurane (SE) has been a frequently used inhalation anesthetic in recent years. Recent studies have found that SE produced a protective effect on the brain, which may be related to its antioxidant, neurotransmitter regulation and suppression of neuronal death [9].

In the present study, the behavior of MCAO rats was tested using positioning navigation test and space exploration test. It was found that escape latency was markedly higher in MCAO rats than in healthy rats, while the number of platform crossings was significantly lower than that of normal mice. Thus, SE improved the behavioral ability of MCAO mice. This suggests that SE may slow down impairment of learning and impairment of memory in MCAO rats, which is similar to the research results of Wang *et al* [10].

Autophagy is a process of cellular self-catabolism, while apoptosis is a process of programmed cell death. In ischemia reperfusion injury, autophagy and apoptosis of neurons are significantly increased, resulting in cerebral infarction [11]. In this study, cerebral infarct zone,

percentage apoptosis, and the number of autophagosome vacuoles in MCAO rats were markedly raised, relative to values in normal rats. Sevoflurane (SE) reduced the cerebral infarction area, percentage apoptosis, and the number of autophagosome vacuoles in MCAO mice. These results indicate that SE inhibited autophagy and apoptosis, and reduced neuron injury. Autophagy is regulated by autophagy-related proteins, with LC3 II/I as one of the important proteins that mediate the process. Beclin-1 is not only an important autophagy-related protein, it also mediates apoptosis by interacting with Bcl-2 lentinus edodes [12].

Apoptosis is a process generated by the hypoxia, radiation and cytotoxins. In ischemia-reperfusion injury, the cytoplasm is abnormally increased and can bind bcl-3; Bad is inserted into the mitochondrial membrane to form apoptotic bodies which promote cell apoptosis by activating caspase-3 protein [13]. In this study, LC3 II/I, Beclin-1, Bad, Bcl-2 and Caspase-3 proteins in MCAO rats were significantly higher than those in normal rats, while SE inhibited the expression of autophagy and apoptosis proteins in MCAO rats. These results suggest that SE may protect nerve cells by inhibiting apoptosis and autophagy through regulation of autophagy and apoptosis-related proteins.

It is known that heme oxygenase 1 is an important part of the response of brain cells against oxidative stress. It has been found that an increase in HO-1 mRNA level increased SOD activity, reduced MDA and NO contents, and improved spatial cognitive learning ability of mice to a certain extent [14]. It was found that the level of HO-1 mRNA in MCAO rats was markedly

raised, relative to that in normal mice, and SE increased the expression of HO-1 mRNA. These data imply that SE up-regulated HO-1 mRNA, which is similar to the results of Yang *et al* [15].

Activated PI3K is an important protein which regulates neuronal survival after ischemic brain injury and it inhibits apoptosis [16]. The important downstream target of PI3K, Akt is a key factor for nerve cell survival after ischemic brain injury, and it modulates Bcl-2 and caspase-associated apoptotic proteins. Moreover, P70s6k is a protein synthesis regulator which is crucial for regulating proteins, cell cycle, and DNA synthesis, and promoting cell survival [17]. Previous studies have found that P13K/Akt/p70S6K is an important pathway for protein synthesis; it responds to various physiological or pathological signals and regulates the transcription of HO-1 and other genes [18]. In this study, SE increased the expressions of P-PI3K, P-Akt and P-P70S6K-related proteins in MCAO mice. These results suggest that SE protected nerve cells and suppressed apoptotic changes in the cells through a process which may be associated with HO-1-mRNA expression induced by regulating P13K/Akt/p70S6K signaling pathway.

CONCLUSION

This study shows that SE improves the behavioral characteristics of MCAO rats, reduces cerebral infarction in rats, and inhibits neuronal apoptosis and autophagy by regulating autophagy and apoptosis-related proteins. Thus, the mechanism of action may be related to HO-1-mRNA expression induced by SE via regulation of P13K/Akt/p70S6K signaling pathway. This provides a new idea for the development of an anti-neuronal apoptosis drug.

DECLARATIONS

Acknowledgement

This study was supported by the Foundation of Research on Technology and Quality Standards of Mixture Formula for Regulating Stomach and Releasing Excessive Turbid (no. ZRQN1509, 030029031 to Wei Jin).

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article and all liabilities

pertaining to claims relating to the content of this article will be borne by the authors. Zhongmin Deng and Guijun Wei designed the study, supervised the data collection, and analyzed the data. Lei Qiu interpreted the data and prepared the manuscript for publication. Huifei Lu supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

- Xin Y, Zhang X, Li J, Gao H, Li J, Li J, Hu W, Li H. New insights into the role of mitochondria quality control in ischemic heart disease. *Front Cardiovasc Med* 2021; 8: 774619.
- Sikalias N, Karatzas T, Alexiou K, Mountzalia L, Demonakou M, Kostakis ID, Zacharioudaki A, Papalois A, Kouraklis G. Intermittent ischemic preconditioning protects against hepatic ischemia-reperfusion injury and extensive hepatectomy in steatotic rat liver. *J Invest Surg* 2018; 31(5): 366-377.
- Zhang FW, Tong J, Yan YS, Chen QQ, Zhao XP. ω -3 Polyunsaturated Fatty Acid Postconditioning Protects the Isolated Perfused Rat Heart from Ischemia-Reperfusion Injury. *Cardiorenal Med* 2018; 8(3): 173-182.
- Zheng N, Liu F, Lu H, Zhan Y, Zhang M, Guo W, Ding G. Schisantherin A protects against liver ischemia-reperfusion injury via inhibition of mitogen-activated protein kinase pathway. *Int Immunopharmacol* 2017; 47: 28-37.
- Geng YJ, Wu QH, Zhang RQ. Effect of propofol, sevoflurane, and isoflurane on postoperative cognitive dysfunction following laparoscopic cholecystectomy in elderly patients: A randomized controlled trial. *J Clin Anesth* 2017; 38:165-171.
- Liu B, Xia J, Chen Y, Zhang J. Sevoflurane-Induced Endoplasmic Reticulum Stress Contributes to Neuroapoptosis and BACE-1 Expression in the Developing Brain: The Role of eIF2 α . *Neurotox Res* 2017; 31(2): 218-229.
- World Health Organization. *Principles of laboratory animal care*. WHO Chron 1985; 39: 51-56.
- Mard SA, Akbari G, Dianat M, Mansouri E. Protective effects of crocin and zinc sulfate on hepatic ischemia-

- reperfusion injury in rats: a comparative experimental model study. *Biomed Pharmacother* 2017; 96: 48-55.
9. Xiao Q, Ye Q, Wang W, Xiao J, Fu B, Xia Z, Zhang X, Liu Z, Zeng X. Mild hypothermia pretreatment protects against liver ischemia reperfusion injury via the PI3K/AKT/FOXO3a pathway. *Mol Med Rep* 2017; 16(5): 7520-7526.
 10. Wang Z, Cui R, Wang K. Effects of sevoflurane pretreatment on the apoptosis of rat H9c2 cardiomyocytes and the expression of GRP78. *Exp Ther Med* 2018; 15(3): 2818-2823.
 11. Yang F, Liao J, Pei R, Yu W, Han Q, Li Y, Guo J, Hu L, Pan J, Tang Z. Autophagy attenuates copper-induced mitochondrial dysfunction by regulating oxidative stress in chicken hepatocytes. *Chemosphere* 2018; 204: 36-43.
 12. Chen X, Gao F, Lin C, Chen A, Deng J, Chen P, Lin M, Xie B, Liao Y, Gong C, et al. mTOR-mediated autophagy in the hippocampus is involved in perioperative neurocognitive disorders in diabetic rats. *CNS Neurosci Ther* 2021; 16.
 13. Wang L, Yang JR, Yang LY, Liu ZT, Rao JM, Liu LF. Expression of Ki-67, Bcl-2, Bax and caspase-3 in benign prostatic hyperplasia combined with prostatitis and their significances. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2008; 33(3):222-226.
 14. Balaban CL, Rodríguez JV, Tiribelli C, Guibert EE. The effect of a hydrogen sulfide releasing molecule (Na₂S) on the cold storage of livers from cardiac dead donor rats. A study in an ex vivo model. *Cryobiology* 2015; 71(1): 24-32.
 15. Yang S, Ohe R, Aung NY, Kato T, Kabasawa T, Utsunomiya A, Takakubo Y, Takagi M, Yamakawa M. Comparative study of HO-1 expressing synovial lining cells between RA and OA. *Mod Rheumatol* 2021; 31(1): 133-140.
 16. Cai CX, Buddha H, Castelino-Prabhu S, Zhang Z, Britton RS, Bacon BR, Neuschwander-Tetri BA. Activation of Insulin-PI3K/Akt-p70S6K Pathway in Hepatic Stellate Cells Contributes to Fibrosis in Nonalcoholic Steatohepatitis. *Dig Dis Sci* 2017; 62(4): 968-978.
 17. Cai CX, Buddha H, Castelino-Prabhu S, Zhang Z, Britton RS, Bacon BR, Neuschwander-Tetri BA. Activation of Insulin-PI3K/Akt-p70S6K Pathway in Hepatic Stellate Cells Contributes to Fibrosis in Nonalcoholic Steatohepatitis. *Dig Dis Sci* 2017; 62(4): 968-978.
 18. Peng R, Wang S, Wang R, Wang Y, Wu Y, Yuan Y. Antifibrotic effects of tanshinol in experimental hepatic fibrosis by targeting PI3K/AKT/mTOR/p70S6K1 signaling pathways. *Discov Med* 2017; 23(125): 81-94.