의학 석사 학위 논문

Anti-Inflammatory Mechanism of Fimasartan, a Novel Angiotensin Ⅱ Receptor Antagonist, on Astrocytes Stimulated by Hemolysate

용혈물 처리된 성상교세포에 작용하는 fimasartan의 항염증 기전에 관한 연구

2014년 7월

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의학과 뇌신경과학 전공

양 슈 리

A thesis of the Master Degree

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July 2014

Graduate program of neuroscience
Seoul National University
College of Medicine

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지도교수 윤병우

이 논문을 의학 석사 학위 논문으로 제출함

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양 슈 리

양슈리의 의학석사 학위 논문을 인준함

2014년 7월

위 원 장 _____ (인)

부위원장 _____ (인)

위 원 _____ (인)

Anti-Inflammatory Mechanism of Fimasartan, a Novel Angiotensin Ⅱ Receptor Antagonist, on Astrocytes Stimulated by Hemolysate

by

Xiu Li Yang

A thesis submitted to the Department of Medicine in partial fulfillment of the requirements for the Degree of Master of Science in Medicine (Neuroscience) at Seoul National University College of Medicine

June 2014

Approved by Thesis Committee:

Professor	_____	Chairman
Professor	_____	Vice chairman
Professor	_____	

Abstract

Anti-Inflammatory Mechanism of Fimasartan, a Novel Angiotensin Receptor Antagonist, on Astrocytes Stimulated by Hemolysate

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Background: Intracerebral hemorrhage (ICH), which is a subtype of stroke, is a kind of devastating disease. Accumulating evidences indicate that ICH-induced inflammation represents a key role leading to the secondary brain damage. Hemolysate which was proved to contribute to brain injury of ICH, can produce a variety of pro-inflammatory cytokines. Angiotensin Ⅱ receptor blockers are mostly used for anti-hypertension therapy and has been reported to decrease brain inflammation. As fimasartan is one of novel angiotensin Ⅱ receptor blockers, we investigated whether fimasartan modulates inflammatory effects on ICH.

Method: We stimulated astrocytes with hemolysate to induce a hemorrhagic environment as in vitro model of ICH. To analyze the immune-modulatory effects of

fimasartan, we pretreated the drug on hemolysate stimulated astrocytes in different concentrations and time points. Anti-inflammatory cell signals such as Akt, ERK, IκBα (inhibitor of NF-κB) were assessed with western blotting and the pro-inflammatory enzyme cyclooxygenase-2 (COX-2) was evaluated using RT-PCR method. To examine the drug cytotoxicity, cell counting assay was carried out.

Results: Astrocytes stimulated with hemolysate increased the phosphorylation of Akt (25.9 fold, $P<0.01$) at 1h, ERK1/2 (15.4 fold, $P<0.05$) at 20min and promoted degradation of IκBα (2.9 fold, $P<0.01$) at 12h. We also found that the activation of these signaling pathways up-regulated the expression of the COX-2. In comparison with non-treated group, astrocytes stimulated with hemolysate increased COX-2 expression up to 71% ($p<0.01$). To observe the anti-inflammatory effects of fimasartan, we treated astrocytes with hemolysate with or without pre-condition of fimasartan. The group pretreated with fimasartan in hemolysate stimulated astrocytes down-regulated Akt, ERK, NF-κB signaling pathways. Furthermore, COX-2 mRNA expression was also decreased consistently.

Conclusion: Fimasartan, as a new ARB, can modulate hemolysate induced inflammatory effects of astrocytes. It may be considered as a candidate for inflammatory regulator associate with ICH. Further study, such as in vivo study, will be administrated to verify our hypothesis.

Key words: Fimasartan, Hemolysate, Intracerebral hemorrhage, Inflammation, Astrocyte

Student Number: 2012-22673

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Introduction

Hemorrhage stroke generates from the rupture of blood vessels (1), including intracerebral hemorrhage (ICH) and aneurismal subarachnoid (SAH). Hemorrhage stroke triggers many adverse events and sever disability (2). Unfortunately there are no effective therapies for improving survival and quality of life (3). Many efforts to search effective therapies underlying pathophysiology of ICH have been devoted, especially inflammation is considered as an important mechanism of the secondary brain related to ICH.

Hemolysate, as one of hemoglobin breakdown products, can be transported into cells, considered as a spasminogen in SAH and participate in brain edema formation after ICH (4, 5). Hemolyste treatment on cells as a characterized model of hemorrhage in vitro studies have been well established (6, 7). It is well established that exposure to hemolysate leads to pro-inflammation and apoptosis after ICH (8-10).

Angiotensin Ⅱ receptor blockers (ARBs), are mostly used in the hypertension treatment and exert pleiotropic effects to protect cerebral injury, including amelioration of inflammation of brain (11). Fimasartan potassium trihydrate is a novel, non-peptide angiotension Ⅱ receptor antagonist, with beneficial effects of antihypertension, which was approved by Korean Food and Drug Administration

KFDA) through phase Ⅲ clinical studies in 2010 (12). Previous study has reported cardioprotection of fimasartan (13). However, whether fimasartan has effect on hemorrhage stroke remains unknown, and the associated molecular mechanisms have not been completely elucidated. Since ARBs have been proved to have good anti-inflammatory effects on cerebral vasculature (14) , the present study attempts to investigate whether fimasartan has anti-inflammatory effects on ICH.

NF-κB is one of the classic transcription factors associated with immune and inflammatory responses. NF-κB exists in the cytoplasm combined with the inhibitory protein IκB in quiescent cells (15). The phosphorylation of Akt and ERK, regulates the activatin of NF-KB through IκB degradation. The activation of NF-κB contributes to the pro-inflammatory gene expression, such as the induction of COX-2, which is an important inflammatory enzyme in brain (15). Cyclooxygenase (COX) is a rate-limiting enzyme inprostanoid biosynthesis, induced by proinflammatory stimuli in many diseases (16).

In the present study, we focused on the signalling pathways associated with inflammation induced by hemolysate. Moreover, astrocytes are responsible for inflammatory reactions in the brain, we investigated the anti-inflammatory effects of fimasartan on hemolysate-induced astrocytes.

Materials and Methods

The study was designed to perform in two stages. The western blot assay and RT-PCR method were used. Firstly, we treated astrocytes with hemolysate to stimulate the inflammatory response, and then we observed the inflammatory cell signalling pathways through western blot assay. Secondly, we pretreated astrocytes with fimasartan and examined the effects of fimasartan on the same cell signalling pathways and mRNA of COX-2 by RT-PCR assay.

Astrocyte cell culture

The mouse brain astrocytes (Astrocytes Type Ⅰ clone; ATCC, CRL-2541) were maintained in Dulbecco's modified eagle's medium (WELGENE Inc. Daegu, Republic of Korea) supplemented with 10% fetal bovine serum, 10,000 Units/ml penicillin, and 10,000 ug/ml streptomycin. Medium was changed once a day. Cells were incubated in a humidified incubator maintained at 37℃ in a 5% CO_2 and 95% air atmosphere. Before the experiment, astrocytes (1×10^6) were starved overnight in a 0.2% serum medium, and then incubated with or without 10% hemolysate for indicated time in the pretreatment with absence or presence of 30ng/ml fimasartan for 12h, after which they were harvested for further analysis.

Preparation of hemolysate

Hemolysate was prepared from fresh rat arterial blood by using the method as described previously (17). Heparinized rat arterial blood was centrifuged at 2,500G for 15 minutes at 4℃, and the supernant was aspirated. The erythrocyte properties were washed three times with cold saline solution, and lysed by four freeze/thaw cycling procedures. Following a 1:1 dilution with PBS, the debris of erythrocytes were centrifuged at 31,000G for 15minutes, and then the supernant was collected and stored at -80℃. The concentration of hemolysate was determined by measuring spectra of hemoglobin on spectrophotometer. Absorbance peaks at 540 and 576 nm were observed to confirm the existence of oxyhemoglobin (18). The concentration of OxyHb of the 100% hemolysate was 10.92nm. We used the 10% hemolysate by volume of cell media.

Cell viability analysis

The fimasartan was obtained from Boryung Pharm. Co. (Seoul, Republic of Korea). Cytotoxicity of fimasartan was evaluated by cell counting kit-8 assay (Enzo® Life Sciences, NY, USA). We seeded 5×10^4 cells into 96 well plates, incubated at 37℃ for 24h, and gave a fresh change medium. Astrocytes were then treated with or without of various concentrations of fimasartan for an additional 24h. At that point, 10ul CCK-8 solution was added to each well further incubated for 3h. The absorbance of color was assayed at 450nm using an ELISA reader. Astrocytes incubated in the control medium were supposed to be 100% viable.

Western blot analysis

Astrocytes culture medium was discarded and washed once with cold (4℃) phosphate buffer saline (PBS). The cell pellets were collected by centrifugation. The pellets were lysed by protein extraction reagent, and incubated on ice for 30min. The cell debris was removed by micro-centrifugation. Protein concentration was determined by the Bradford reagent (Bio-Rad, Hercules, CA). Equal amounts of nuclear protein extracts (15ug) were separated by on 8-10% sodium SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and then were transferred onto a polyvinylidene difluoride (PVDF) filters. The blots were blocked with 5% skim milk in PBS with 0.1% Tween-20 for 1h at room temperature (RT). Extracellular signal-regulated kinase (ERK: Santa Cruz Biotechnology, Santa Cruz, CA, USA), Akt (Santa Cruz Biotechnology), phospho-ERK, phospho-Akt (1:1,000 dilution, Cell Signaling Technology, Danvers, MA, USA), IκBα (1:1,000 dilution, Cell Signaling Technology, Danvers, MA, USA) as the primary antibodies were incubated at 4℃ for overnight. Anti-β-actin antibody (1:10,000 dilution Sigma-Aldrich, St. Louis, MO, USA) was used as an internal control. Immunoreactive proteins were detected by ECL autoradiography system.

Real time reverse transcription polymerase chain reaction (Real-time RT-PCR)

For analysis of mRNA expression by real time PCR, astrocytes were harvested after the specific treatment and total RNA was isolated by the TRIzol® reagent kit (Invitrogen, La Jolla, CA, USA) followed by the manufacturer's instructions. 1ug of

total RNA was reverse transcribed into cDNA according to RT PreMix kit (AccuPower RT PreMix®, BIONEER). cDNA synthesis reaction was performed under 42℃, 60min, 94℃, 5min. Taqman probes for COX-2 RNA (Assay ID: Mm03294838_ g1) were obtained from Applied Biosystems. Quantitative polymerase chain reaction (PCR) was performed by the TaqMan real-time PCR method on an ABI 7500 Real Time PCR Systerm (Perkin-Elmer Applied Biosysterms, Lincoln, CA, USA). All the PCR reactions were performed in triplicate, and the relative amount of gene expression was measured by the relative standard curve method with the dilution cDNA samples, comparative threshold (Ct) by normalizing to GAPDH.

Statistical Analysis

The results were expressed as the mean ± standard deviation or standard error of the mean through the statistics of three independent cell preparations. Significance was detected by two-tailed unpaired Student's *t*-test. The differences at probability value of less than 0.05 were considered to be significant.

Results

Fimasartan had no significantly effects on astrocytes viability

To determine whether the exibition of fimasartan anti-inflammatory effects were caused by the decreased astrocytes viability, cell viability was evaluated by CCK-8 assay. As shown in Fig.1, astrocytes were exposed to various concentrations of fimasartan (30-1000ng/ml) for 24h. The results revealed that fimasartan treatment has no significant cytotoxicity, and in our study anti-inflammatory effects were achieved with less than 30ng/ml of fimasartan.

Hemolysate stimulated the activation of PI3K-Akt, MAPK and degradarion of IκBα on astrocytes.

The PI3K-Akt, MAPK are known to play a key role on intracellular signalling pathways of the inflammatory responses (19). To study the role of Akt, ERK and NF-κB in astrocytes activation by hemolysate, we treated astrocytes with 10% hemolysate in a time-dependent manner. Notably, a short time exposure (20min) of astrocyte to hemolysate triggered the obvious phosphorylation of the Akt and ERK, with the peak time of p-Akt at 1h (25.9-fold versus control, ** $p<0.01$) (Fig. 2A) and p-ERK at 20min (15.4-fold versus control, * $p<0.05$) (Fig. 2B). The up-regulations were gradually attenuated consistently with the time-dependent of hemolysate

stimuli. Cytoplasmic NF-κB is activated by IκBα degradation, which is modulated by the phosphorylation of MAPKs and PI3K/Akt. (20). We found significant degradation of IκBα was at 12h after hemolysate treatment. (2.9-fold versus control, ** p<0.01) (Fig. 1C).

Fimasartan suppressed hemolysate-induced phosphorylation of Akt in concentration dependent manner.

Based on the point time of Akt activation, we selected a minimum effective concentration of fimasaran (Fig.2A). We pretreated astrocytes with fimasartan for 12h in a dose dependent manner before astrocytes incubation with hemolysate for 20min. Akt phosphorylation was significantly elevated 29.8-fold compared with that in control group (*** p<0.001) after stimulated by hemolysate, however it was inhibited by fimasartan from 30ng/ml with 2.68-fold compared with that in hemolysate alone treatment group (* p<0.05), 100ng/ml of fimasartan inhibited p-Akt with 3.3-fold (* p<0.05) (Fig.3B). Therefore, we selected 30ng/ml as proper experimental concentration of fimasartan. In addition, the inhibition effects were not caused by the cytotoxicity of fimasartan because the cell viability in these concentration ranges had no impairment (Fig.1).

Fimasartan inhibited hemolysate-induced Akt, ERK1/2 phosphorylation in astrocytes.

To investigate whether fimasartan affects Akt, ERK1/2 levels, astrocytes were

pretreated with fimasartan for 12h before incubation hemolysate for periods from 20min to 1h. We found that Akt, ERK1/2 phosphorylation were significantly activated after hemolysate alone stimuli. The level of p-Akt was 185-fold higher than control at 20min, 218-fold increased 1h. (* $P<0.05$), (Fig.4B). The expression of p-ERK1/2 was increased 7.05-fold compared with control at 20min, (** $p<0.01$) and 5.37-fold 1h, (* $p<0.05$), (Fig.4C). However, comparing the non-treatment of fimasartan group, we found a strong trend toward a decrease of p-ERK1/2, p-Akt at 20min and 1h. As the figures 4A, 4B shown, p-Akt was decreased 37-fold (# $p<0.05$); p-ERK1/2 was decreased 3.75-fold at 20min and 1.34-fold at 1h (# $p<0.05$). The amount of non-phosphorylated kinases were unaffected by hemolysate. (Fig.4A).

Fimasartan delayed the hemolysate-induced IkB-α degradation in astrocytes.

Previous studies have shown that PI3K-Akt and MARK regulate activation of NF-κB in response to inflammation (15, 21). To analyze whether the ability of fimasartan to modulate NF-κB signaling pathway, astrocytes were stimulated with hemolysate for 6h and 12h after pretreated with 30ng/ml fimasartan for 12h. As shown in Fig. 4A, hemolysate led to a degradation of IκBα at 6h, the level of IκBα decreased 0.58-fold and 0.62-fold at 12h compared to control group (** $p<0.01$). It is interesting to find that when we pretreated fimasartan, the degradation of IκBα was significantly delayed from 6h to 12h. The level of IκBα on 12h was inhibited 0.5-fold compared to fimasartan alone treatment group (## $p<0.01$) (Fig.5B). As reported previously (22), only when IκB kinase degradation, the NF-κB translocation to the nucleus and activate inflammatory response. Based on our result, we can confirm fimasartan

inhibited NF-κB nuclear translocation in a time-dependent manner.

Fimasartan down-regulated the gene expression of COX-2 induced by hemolysate.

NF-κB plays a well-known role in the regulation of inflammation and modulate a much number of genes, such as COX-2 (23). To determine whether the modulation of inflammation by fimasartan was related to the COX-2, we analyzed the mRNA expression of proinflammatory enzyme COX-2 gene. Astrocytes were pretreated by fimasrtan with different concentration (10, 30, 100ng/ml) for 12h, then incubated with 10% hemolysate for 18h. The COX-2 mRNA expression was increased 71% than that in control group after stimulation of hemolysate (## $p<0.01$). In contrast, this increasing tendency was consistently down-regulated by fimasartan in a dose-dependent manner. When fimasartan was added with 10, 30, 100ng/ml, COX-2 expression was decreased by approximately 3%, 10% and 40%, compared with hemolysate alone treatment group. (Figure 6). It indicated that fimasartan at a high concentration of 100ng/ml obviously inhibited the induction of COX-2 mRNA. (** $p<0.01$).

Figure1.

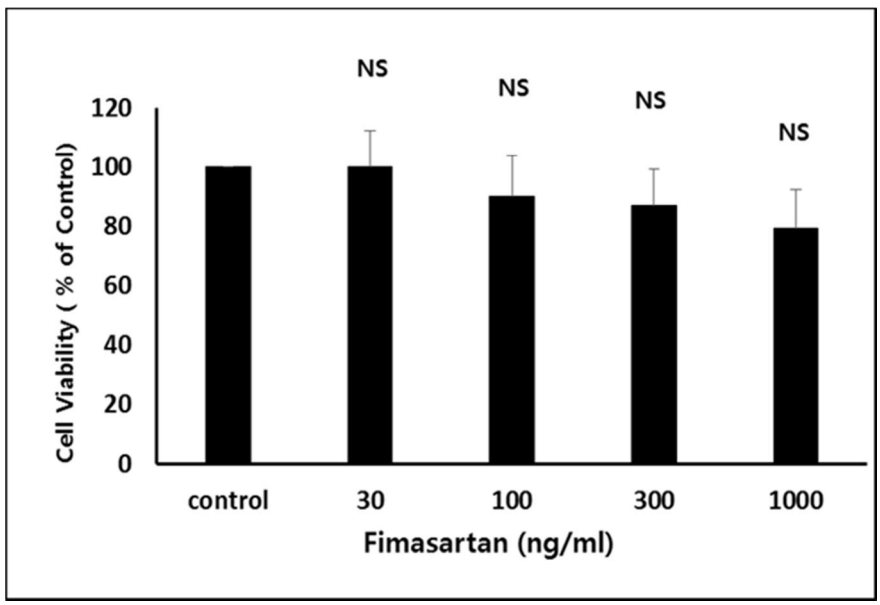
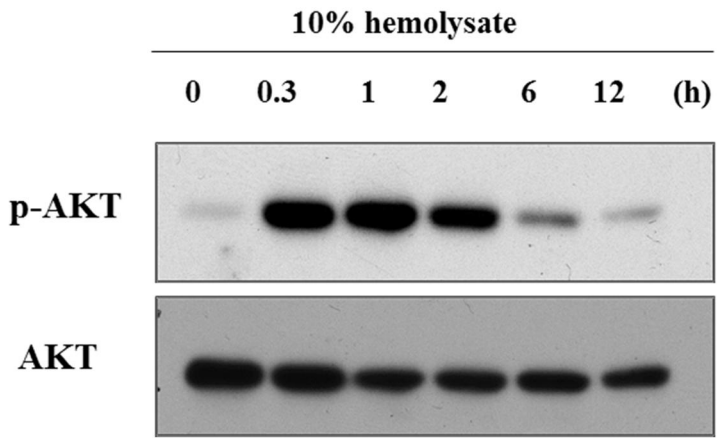


Figure 1. Cell viability assay in astrocytes. Cell viability was evaluated using CCK-8 assay as method described. Astrocytes were treated with or without various concentrations of fimasartan (30-1000ng/ml) for 24h. Results are given as a percentage of viable cells related to untreated controls. The data represents the mean ± S.E. for five independent experiments performed with triplicates.

Figure2A.


10% hemolysate
0
0.3
1
2
6
12
(h)
p-AKT
AKT

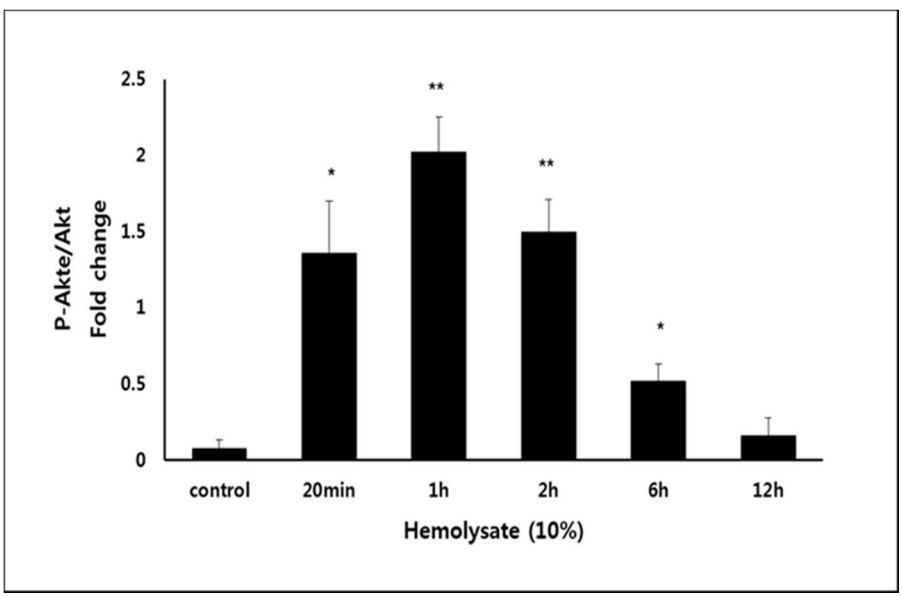
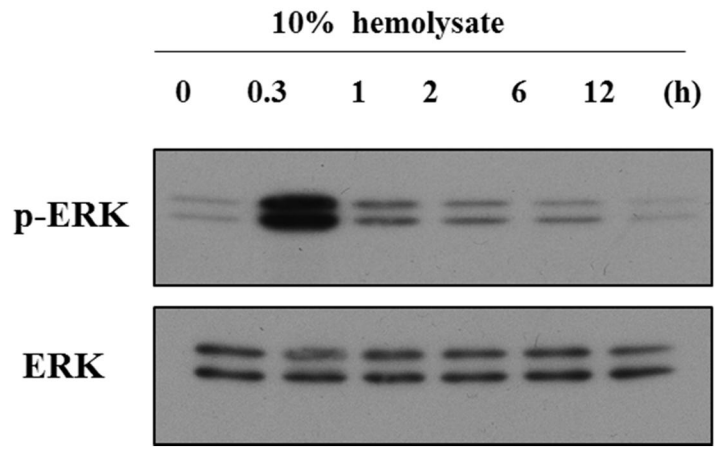

P-Akte/Akt
Fold change
2.5
2
1.5
1
0.5
0
control
20min
1h
2h
6h
12h
Hemolysate (10%)

Figure2B.


10% hemolysate
0
0.3
1
2
6
12
(h)
p-ERK
ERK

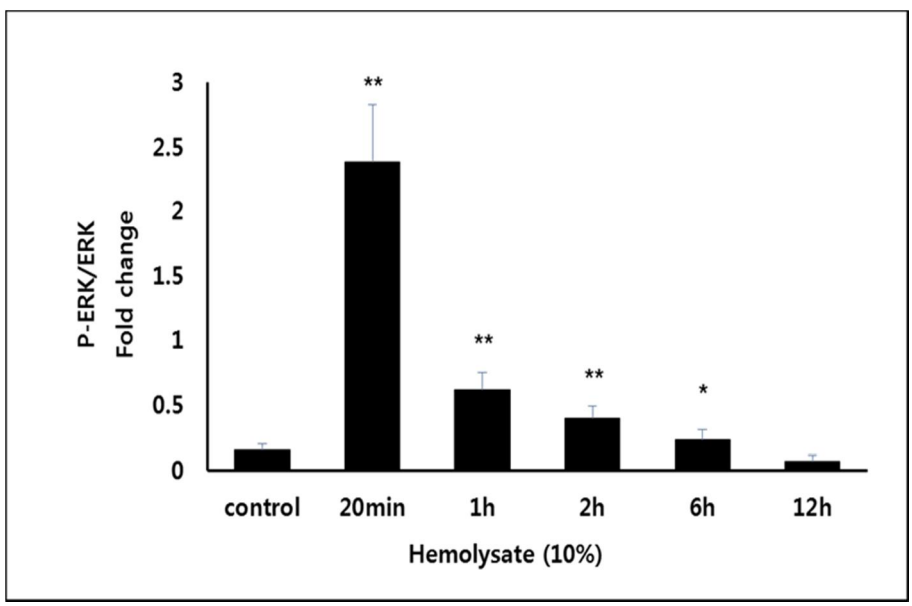
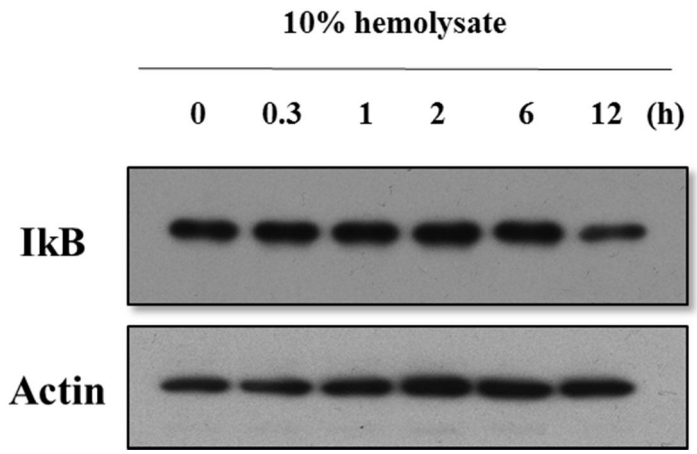

P-ERK/ERK
Fold change
3
2.5
2
1.5
1
0.5
0
**
**
**
*
control
20min
1h
2h
6h
12h
Hemolysate (10%)

Figure2C.


10% hemolysate
0
0.3
1
2
6
12
(h)
IkB
Actin

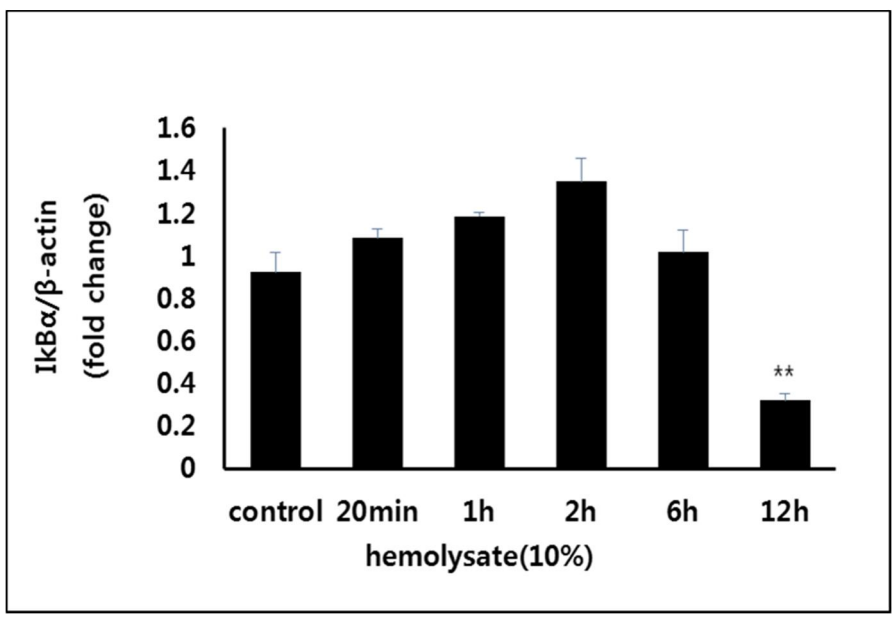

IkBα/β-actin
(fold change)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
control
20min
1h
2h
6h
12h
hemolysate(10%)

Figure2. Hemolysate promoted the phosphorylation of Akt, ERK1/2 and degradation of IκBα on astrocytes. Cells were treated with 10% hemolysate in different time points (0, 20min, 1h, 2h, 6h, 12h), incubated at 37℃.The activation of phosphorylation of Akt, ERK and the degradation of IKBα were detected on western blotting by using specific antibodies. β-actin protein was used as internal control. Similar results were observed for at least five different times. The intensity of the bands was quantified by scanning densitometry, standardized with respect to β-actin protein. Values represent the mean ± S.E. fold change compared with untreated cells, pooled from five independent experiments. ** $P < 0.01$ by two-tailed Student's *t* test indicates significant difference from untreated cells.

Figure3A.

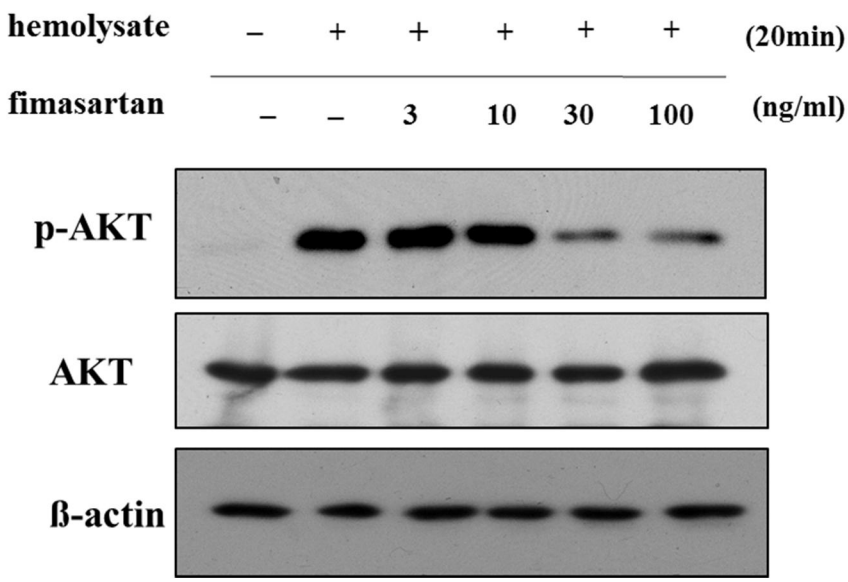


Figure3B.

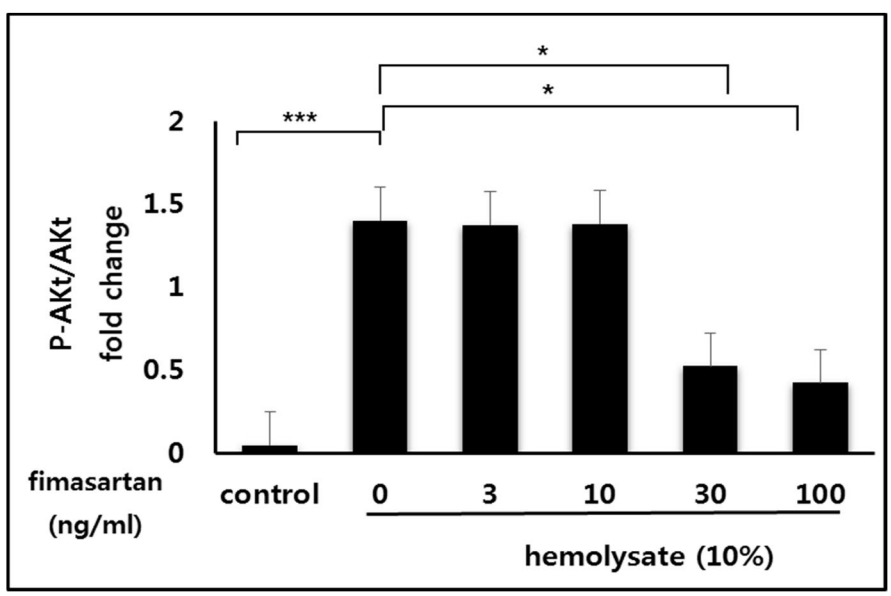


Figure 3. Fimasartan inhibited hemolysate-induced phosphorylation of Akt from 30ng/ml. Expression of phosphorylation of Akt was detected by western blotting using specific antibodies in astrocytes. The intensity of the bands was quantified by scanning densitometry and expressed as fold change compared with untreated cells. Values represent the mean ± SEM for three independent experiments that performed in triplicates. *** $P < 0.001$ versus control group; * $P < 0.05$ versus hemolysate alone treatment group.

Figure4A.

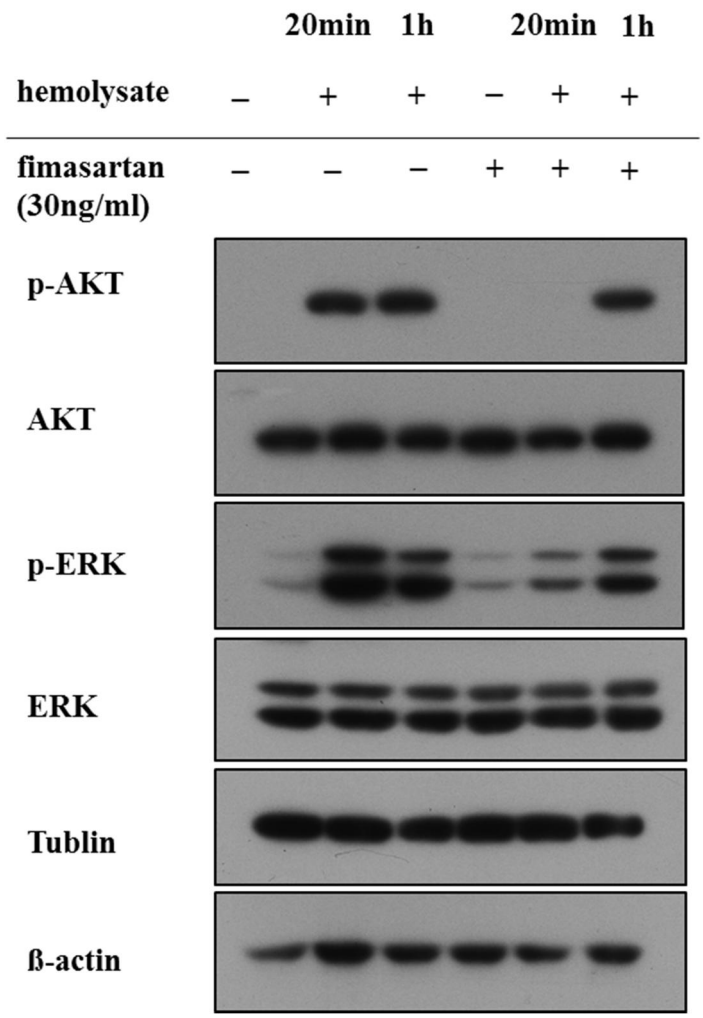

20min
1h
20min
1h
hemolysate
–
+
+
–
+
+
fimasartan (30ng/ml)
–
–
–
+
+
+
p-AKT
AKT
p-ERK
ERK
Tublin
ß-actin

Figure4B.

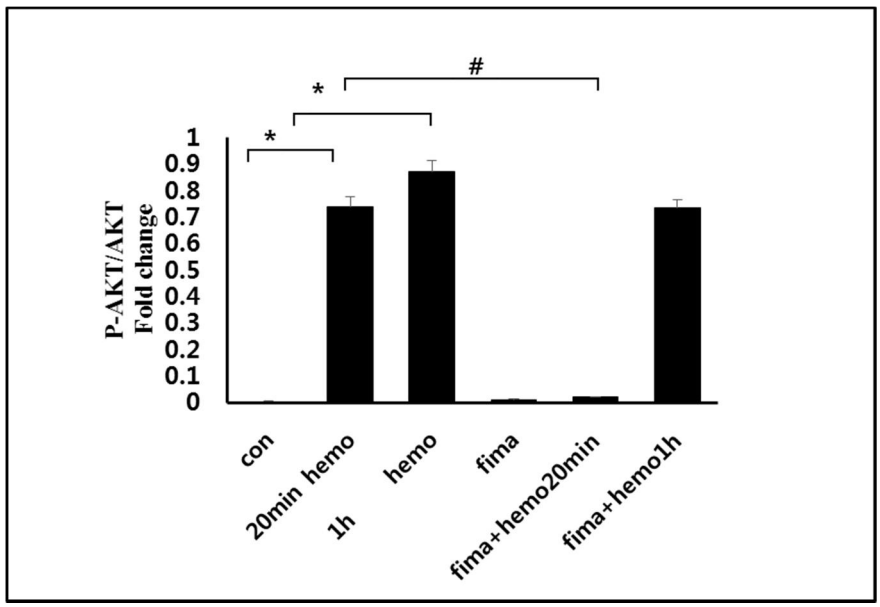


Figure4C.

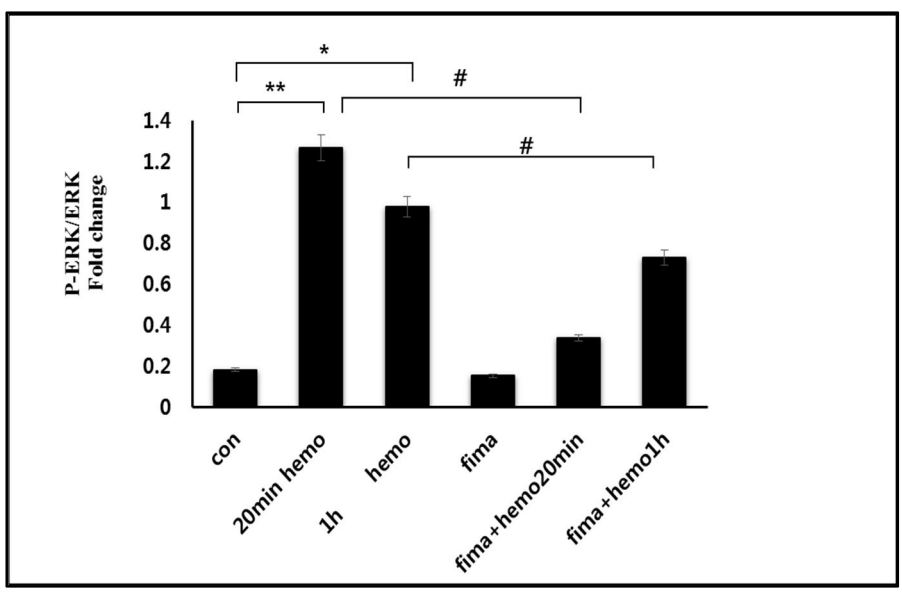


Figure 4. Fimasartan down-regulated hemolysate-induced AKT, ERK phosphorylation in astrocytes. The cells were divided into 4 groups. One is control group and second group was treated with hemolysate for 20min and 1h respectively. The third group was pretreated with fimasartan (30ng/ml) for 12h alone; the last group was treated hemolysate in 20min and 1h before fimasrtan treatment. Quantification of the expression of p-Akt/ total Akt and p-ERK/ERK was evaluated by scanning densitometry of bands, standardized with respect to β-actin protein. The values represent the mean ± S.E fold change compared with untreated cells for three independent experiments. * $P < 0.05$, ** $p < 0.01$ versus control group, # $p < 0.05$ versus hemolyste alone treatment group.

Figure 5A.

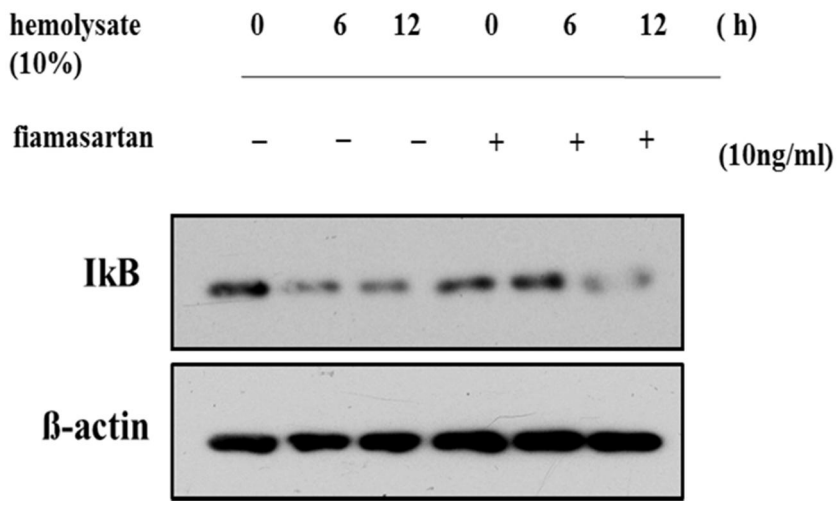


Figure5B.

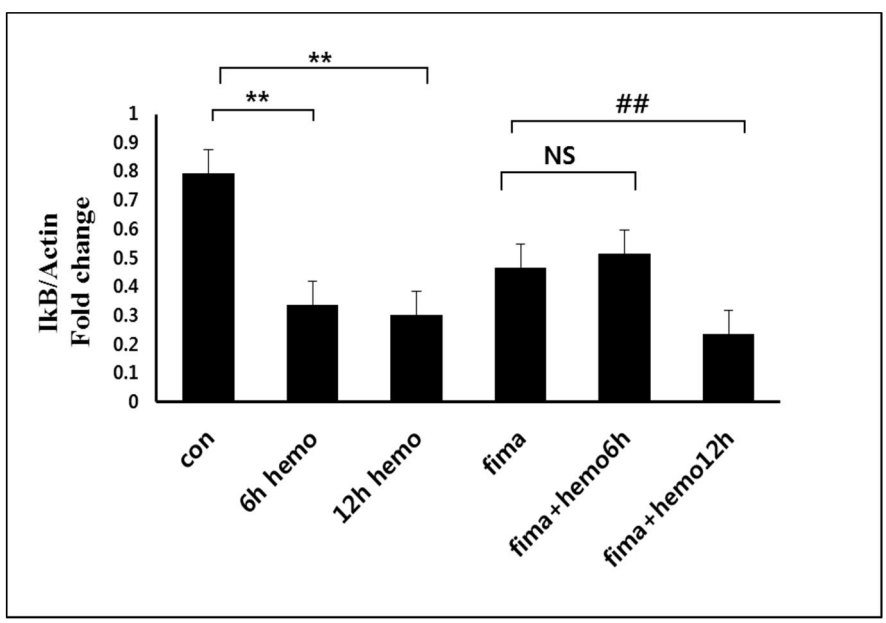


Figure5. Fimasartan delayed the hemolysate induced IκB-α degradation in astrocytes. The cells were divided into 4 groups as described in Figure 5. Astrocytes were treated with fimasartan and hemolysate for the long time (6h, 12h). Attenuated IκBα degradation was detected by western blotting. Values represent the mean ± S.E. in at least three independent experiments. ** $p<0.01$ versus control group, # # $p<0.01$ versus fimasartan alone group.

Figure 6.

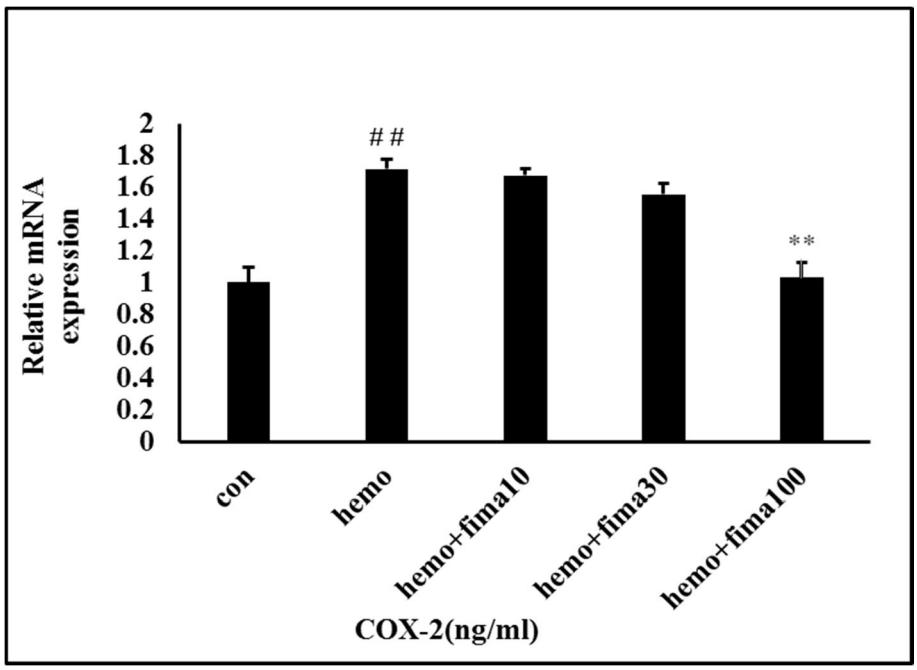


Figure 6. Fimasartan inhibited the expression of hemolysate induced the expression of COX-2 mRNA on astrocytes. Astrocytes were incubated in the absence or presence of hemolysate for 18h, or in combination pretreated with different concentration (10, 30, 100ng/ml) of fimasartan for 12h. The COX-2 expression was assessed by real-time RT-PCR. Results were expressed as a fold induction in at least three experiments. ## $P < 0.01$ versus to control; ** $P < 0.01$ versus to hemolysate stimuli alone.

Discussion

Our study investigated the activation of inflammatory signalling pathways by hemolysate and associated gene expression. Hemolysate induced an immunological response in astrocytes in time dependent manner. Under indicated conditions, we assessed the anti-inflammatory effects of fimasartan through blocking the PI3K-Akt, MAPK and NF-κB pathways and modulating the COX-2 expression.

Previous evidences suggest that inflammation is a determinant role of secondary brain damage after ICH (24). To understand the inflammation that happens after ICH, many preclinical and clinical studies were carried out. (25) Depending on the deeper research of the inflammatory roles of ICH, we propose inflammatory signalling cascades as target of translation research in hemorrhage stroke.

It is well known that ARBs have pleiotropic effects including ameliorate brain inflammatory activity. (26, 27) It was revealed candesartan decreases the acute inflammation of brain on normotensive rats; Telmisartan modulates inflammation and oxidative response of endothelial cells. Based on these previous studies, ARBs are proved to modulate brain inflammation by several parallel mechanisms (11). Fimasartan is a new angiotensin Ⅱ receptor antagonist with selectivity for the AT1 receptor subtype. Previous study suggested that fimasartan has cardioprotection effects, (13) however to the best of our knowledge no study investigate fimasartan effect on ICH. Our study assessed the fimasartan inhibited the inflammatory

response induced by hemolysate. We clarified a new molecular mechanism as new targets for anti-inflammatory therapy based on the ICH pathophysiology. Therefore, we propose that fimasartan can contribute to reduction of inflammatory effects in stroke.

This study targets inflammatory response of astrocytes after ICH. We observed several classical signaling pathways of inflammation on astrocytes to find the pathology of ICH injury. Several reviews illustrated that astrocytes participate in innate immune reactions and play a key role in the CNS response to inflammation. (28-30) Although recent studies have focused on the astrocyte-neuron interaction, more researchers find astrocytes have more endogenous antioxidants than neurons. Activated astrocytes can produce many cytokines which are major modulator of the inflammatory response (31).

The NF-κB is one of the classical transcription factor and a key regulator of inflammatory genes. Blocking the NF-κB pathway leads to anti-inflammatory effects (32). Our study demonstrated that fimasartan inhibited the IκBα degradation, phosphorylation of Akt and ERK induced by hemolysate. It is well known that MAPKs and Akt play an important regulatory role in the production of various pro-inflammatory factors. MAPKs and Akt provide a potential therapeutic target for prevention of different inflammatory disease (19). Through focusing these signaling system, fimasartan may be a novel therapy to the inflammatory of stroke. COX-2 gene expression is associated with attenuating IKappaB degradation (33). COX-2 are important cytokines associated with inflammation and suppression of COX-2 mRNA may play an important role in the anti-inflammatory response. Up-regulation of COX-2 and iNOS mRNA in astrocytes stimulated by hemolysate has

been reported in the previous study (34). Recently, fimasartan inhibits iNOS via attenuating NF-κB on macrophage has been reported (35). In our study, increasing of COX-2 mRNA activated by hemolysate in astrocytes could be reversed by pretreated fimasartan with dose dependent manner (Fig.7). These results proved that fimasartan inhibit COX-2 through the suppression of IKappaB degradation.

Red blood cell (RBC) lysis causes brain injury through increasing the blood-brain barrier permeability and edema formation in hemorrhage stroke. (36) Hemolysate, as the species of breakdown of erythrocytes, was used in animal model to study SAH at the past decade years. (17) Recently, a number of studies have showed that hemolysate contributes to the secondary damage of ICH. (37, 38) Our study is the first time to examine that hemolysate induces the classical effectors of inflammatory signalling cascade (Fig.2) which is consistent with ICH. Although our study have revealed some positive clues to prove the hypothesis, hemolysate as a vitro model, has many limitations compared to animal studies. Since the mechanisms of neurotoxicity of hemolysate have not been fully understood, further researches are needed to clarify them.

Taken together, our results suggest that fimasartan as one of the new angiotensin I I AT1 receptor antagonist, suppresses hemolysate-induced inflammation in astrocytes via PI3K-Akt, MAPK and NF-κB and COX-2 pathways. Fimasartan would be a potential therapeutic strategy that targets with inflammation of intracerebral hemorrhage.

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초 록

서론: 뇌출혈은 뇌졸중의 한 종류로서 치명적인 질병이다. 축적된 증거에 의하면 뇌출혈로 인한 염증반응은 이차 뇌 손상을 유발하는데 있어서 중요한 역할을 한다. 이런 뇌 손상은 뇌출혈로 인한 용혈물 형성에 의한 것임을 이미 입증이 되었다. 뿐만 아니라 형성된 용혈물은 다양한 염증성 싸이토카인 분비를 촉진한다. 엔지오텐신 Ⅱ수용체 차단제는 고혈압 치료제로 알려져 있고 뇌에서의 함염증 반응이 보고 되었다. Fimasartan은 새로운 엔지오텐신 Ⅱ수용체 차단제로 알려져 있고 본 연구에서는 fimasartan의 뇌출혈에서의 염증조절 효과를 알아보고자 하였다.

방법: 성상교세포를 용혈물로 처리하여 뇌출혈의 in vitro 모델을 만들었다. 염증반응을 유발한 후 fimasartan의 염증 조절반응을 확인하기 위하여 다른 농도로 처리를 하였고 부동한 시간에서 그 반응을 분석하였다. 이런 조절 반응은 western blotting 통하여 항염증 관련 세포 신호전달 물질인 Akt, ERK, IκBα의 인산화를 확인하였고, RT-PCR 방법을 사용하여 염증관련 유전자인 COX-2 발현 양상을 비교 분석하였다. Fimasartan의 세포독성을 확인하기 위하여 cell counting assay를 실행하였다.

결과: 용혈물을 처리한 성상교세포에서 Akt의 인산화(25.9 배, P<0.01)와 ERK1/2인산화(15.4 배, P<0.05) 가 현저히 증가하고, 처리 12시간후 IκB α (2.9 배, P<0.01) 현저한 degradation을 확인하였다. 뿐만 아니라 염증 관련 유전자인 COX-2의 발현이 71% 증가하였다. Fimasartan을 처리한 그룹의 경우 이런 염증관련 세포 내 신호전달 물질의 인산화가 감소 되었고, COX-2 발현도 감소되었다. 특히 100ng/ml의 fimasartan 처리 그룹에서 COX-2 발현이 40% (p<0.01) 감소 되었다.

결론: Fimasartan은 엔지오텐신 II 수용체 차단제로써 용혈물에 의한 성상교세포 내의 염증반응을 억제하는 것을 확인하였다. 이러한 효과는 fimasartan이 뇌출혈에 의한 염증반응 조절 후보물질로의 가능성을 제시하였다.

핵심단어: Fimasartan, 용혈물, 뇌출혈, 염증반응, 성상교세포

학번: 2012-22673