Effects of ibuprofen on amyloid β-protein fragment 1 – 40-induced p38 MAP kinase signal pathway and caspase cascades in rat hippocampus *in vivo*

FAN Ying[1], JIN Ying[1*], YAN En-Zhi[1], YANG Jing[2], ZONG Zhi-Hong[3], QI Zhi-Min[1]

(1. *Department of Pharmacology*, 2. *Department of Biochemistry*, *Jinzhou Medical College*, *Jinzhou*　121001, *China*; 3. *Department of Biochemistry*, *China Medical University*, *Shengyang*　110001, *China*)

Abstract；**AIM**　To observe the neuroprotective effect and protective mechanisms of ibuprofen on amyloid β-protein fragment 1 – 40 ($A\beta_{1-40}$)-induced neurotoxicity in rat hippocampus. **METHODS**　Rats were given ibuprofen (15 mg·kg^{-1} daily, ig) for 3 weeks prior to icv single dose of $A\beta_{1-40}$ (5 μL, 1 mmol·L^{-1}). Six hours after $A\beta_{1-40}$ injection, Western blotting was used to determine the expressions of phospho-MAP kinase kinase (MKK) 3/MKK6, phospho-p38 MAP kinase, phospho-MAP kinase activating protein kinase 2 (MAPKAPK2), heat-shock protein 27 (Hsp27), procaspase 9, 3, and 7 cleavage, and poly (ADP-ribose) polymerase (PARP) cleavage in hippocampal CA1 region. **RESULTS**　Intracerebroventricular injection of $A\beta_{1-40}$ induced an increase in phosphorylated MKK3/MKK6 and p38 MAP kinase expressions in hippocampal CA1. These increases, in combination with reduced phospho-MAPKAPK2 and phospho-Hsp27 expressions, mediated $A\beta_{1-40}$-induced the activation of caspases cascades. Ibuprofen (15 mg·kg^{-1}·d^{-1}, 3 weeks) significantly prevented $A\beta_{1-40}$-induced increases in phosphorylated MKK3/MKK6 and p38 MAP kinase expressions. In addition, $A\beta_{1-40}$-induced decreases in phosphorylated MAPKAPK2 and Hsp27 expressions were abrogated by ibuprofen. $A\beta_{1-40}$-induced changes in activation of caspases cascades were inhibited by ibuprofen. **CONCLUSION**　Ibuprofen prevents $A\beta_{1-40}$-induced neurotoxicity through suppression of phosphorylated MKK3/MKK6 and p38 MAP kinase expressions and the up-regulation of phospho-Hsp27 expression.

Key words；ibuprofen；amyloid beta-protein；MAP kinase signaling system；mitogen-activated protein kinase；heat-shock proteins；caspases

Alzheimer disease (AD) is a neurological disorder[1]. Brains of individuals with AD manifest two characteristic lesions；extracellular amyloid β-protein (Aβ) deposition and intracellular neurofibrillary tangles[2]. However, it is still unclear how Aβ causes cell damage. There is compelling evidence that Aβ deposition is associated with a local inflammatory response, which is initiated by the activation of microglia and the recruitment of astrocytes. These cells secrete a number of cytokines and neurotoxic products that may contribute to neuronal degeneration and cell death. Numerous studies have shown that Aβ-induced neuronal death demonstrate signs of apoptosis[3-4]. However, the signal transduction mechanism by which these changes occur remain largely unknown.

The mammalian mitogen-activated protein (MAP) kinases can be subdivided into the extracellular signal-regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs), and the p38 MAP kinase. JNKs and p38 MAP kinase are also called stress activated protein kinases (SAPKs). These pathways appear to be activated by a wide variety of cellular stresses inclu-

ding heat shock, lipopolysaccharides (LPS), and inflammatory cytokines. It has also been demonstrated that p38 MAP kinase pathway is hyperactive in the AD brain[5]. Activation of p38 MAP kinase is mediated by the upstream MAP kinase kinases, referred to as mitogen-activated protein kinase kinase (MKK)3 and MKK6. In addition, there is the potential for crosstalk between JNKs and p38 MAP kinase pathways because of MKK4 (SEK1) which has been shown to activate both p38 MAP kinase and JNKs[6]. Activated p38 MAP kinase phosphorylates MAP kinase activating protein kinase 2 (MAPKAPK2), which phosphorylates the 27 ku heat shock protein (Hsp27)[7] and activating transcription factor 2 (ATF 2)[8]. It has been shown that Hsp27 prevents cell death by a wide variety of agents that cause apoptosis. It is a molecular chaperone with interaction with a large number of proteins. Recent evidence has shown that Hsp27 regulates apoptosis through the interaction with key components of the apoptotic signaling pathway, in particular, those involved in caspases activation and apoptosis[9].

Epidemiological and basic research suggests that nonsteroidal anti-inflammatory drugs (NSAIDs) should protect against the most common forms of AD. Lim *et al*[10] first reported the effects of ibuprofen on amyloid pathology in AD transgenic mice. Chronic administration of ibuprofen in the diet significantly decreases $A\beta$ immunoreactive plaques, total $A\beta$ burden, interleukin 1β (IL-1β) levels and astrocytosis in Tg2576 mice. Yan *et al*[11] also found that oral administration of ibuprofen for 4 months significantly reduces the levels of SDS-soluble $A\beta_{1-42}$ and, to a lesser extent, $A\beta_{1-40}$ and decreases the extent of microglia activation in Tg2576 mice. Importantly, the maximum reduction in cerebral insoluble $A\beta$ requires early and chronic ibuprofen treatment. Some evidence indicated that ibuprofen may alter the conformation of $A\beta$ peptides exerting anti-aggregation activity *in vitro*[12-13]. In addition, it has been shown that

ibuprofen binds and activates peroxisome proliferator activator receptor (PPAR)γ in microglia and THP-1 monocytes, preventing microglia- and monocyte-mediated neurotoxicity *in vitro*[14]. Our previous report showed that ibuprofen has the inhibitory effects on $A\beta_{25-35}$-induced p38 MAP kinase phosphorylation and neuronal apoptotic death in rat hippocampus[15]. These previous reports prompted us further to examine the effects of ibuprofen on $A\beta$-induced MKK3/MKK6-p38 MAP kinase-Hsp27 signal pathway and caspases cascades. Therefore, we investigated the effects of ibuprofen on $A\beta_{1-40}$-induced phosphorylation levels of the MKK/MKK6 and p38 MAP kinase as well as MAPKAPK2 and Hsp27 in rat hippocampus.

1   MATERIALS AND METHODS

1.1   Materials

$A\beta_{1-40}$ (Product №: A2326, Lot: 014K08131, Cas №: 144409-99-4, Sigma Chemical Co, St Louis, MO, USA) was resuspended at a concentration of 1 mmol·L^{-1}. To obtain the aggregated form of $A\beta_{1-40}$, the peptide solution was placed in an incubator at 37℃ for 48 h. Phospho-MKK3/MKK6 (Ser189/207, #9231), phospho-p38 MAP kinase (Thr180/Tyr182, #9211), phospho-MAPKAPK-2 (Thr334, #3404), phospho-Hsp27 (Ser82, #2401), caspase 3 (#9662), caspase 9 (Human Specific, #9502), caspase 7 (#9492), PARP (#9542), cleaved PARP (ASP330, #9541), and anti-rabbit IgG, HRP-linked antibodies and biotinylated protein ladder detection pack (#7727) were purchased from Cell Signaling (Beverly, MA, USA). IL-1β antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). β-Actin antibody was from Sigma Chemical Co. SeeBlue Plus2 Pre-stained Standard (Catalog №: LC5925) was from Invitrogen Life Technologies (USA). The enhanced chemiluminescence kit was from Pierce Biotechnology Inc. (Rockford, IL, USA).

Ethylene diamine tetraacetic acid（EDTA）and phenylmethanesulfonyl fluoride（PMSF）was from Merck Biosciences.

1.2　Animal treatment and administration

Sprague-Dawley rats（weighing 200 – 220 g，Experimental Animal Center of Jinzhou Medical College），maintained at an ambient temperature of 22 – 24℃ under a 12 h：12 h light-dark cycle，were randomly divided into 3 groups：control，$A\beta_{1-40}$，$A\beta_{1-40}$ + ibuprofen. Rats in $A\beta_{1-40}$ + ibuprofen group were given ibuprofen（15 mg·kg^{-1}），ig，for 3 weeks prior to icv single dose of $A\beta_{1-40}$ injection. The dose of ibuprofen was chosen on the basis of published literature and our preliminary experiments. The rats were anesthetized with chloral hydrate（300 mg·kg^{-1}）and placed in a stereotaxic apparatus. The rats were injected icv with $A\beta_{1-40}$（5 μL，1 mmol·L^{-1}）or saline solution by means of a Hamilton microsyringe. The injection lasted 5 min and the needle with the syringe was left in place for 2 min after the injection for the completion of the drug infusion. Control rats were injected with saline solution. The rats were sacrificed by cervical dislocation 6 h after $A\beta_{1-40}$ injection. Hippocampal slices（500 μm-thick）were prepared and immediately frozen on dry ice. The CA1 region was microdissected using previously described method[16] for Western blot.

1.3　Western blot

Western blot was performed for the analysis of phospho-MKK3/MKK6，phospho-p38 MAP kinase，phospho-MAPKAPK2，phospho-Hsp27，caspases 9，7，3，and PARP. The fresh hippocampal CA1 region was homogenized in RIPA buffer〔1% Triton，0.1% SDS，0.5% deoxycholate，1 mmol·L^{-1} EDTA，20 mmol·L^{-1} Tris（pH 7.4），150 mmol·L^{-1} NaCl，10 mmol·L^{-1} NaF，0.1 mmol·L^{-1} PMSF〕and insoluble materials were removed by centrifugation at 12 000 ×g for 20 min at 4℃. Protein concentrations were quantified by the method of Lowry et al. Tissue samples were equalized for protein concentration. Proteins were resolved by 10% –

12% SDS-PAGE，transferred on nitrocellulose membranes. Gels were also loaded with colored molecular weight markers to assess electrophoretic transfer，and biotinylated protein ladder marker to estimate molecular weights of bands of interest. The membranes were blocked by 3% BSA in TBS（pH 7.6）for 1 h and incubated overnight at 4℃ with suitably diluted primary antibodies. After extensive washing with TTBS，the membranes were incubated with anti-rabbit IgG，HRP-linked antibody plus anti-biotin antibody for 1 h at room temperature. The blots were detected using the enhanced chemiluminescence（ECL）reaction. After visualization by ECL，all of the nitrocellulose strips were reprobed with β-actin antibody to ensure equal loading of protein on all SDS-PAGE gels. Immunoreactive blots were incubated with alkaline phosphatase-conjugated anti-mouse IgG antibody for 1 h. Finally，the blots were developed with the alkaline phosphatase substrate O-dianisidine tetrazotized along with β-naphthyl acid phosphate. Quantification of protein bands was achieved by Chem Image 5500 software.

1.4　Statistical analysis

All data were presented as $\bar{x} \pm s$. Statistical analysis was carried out with t-test，which was provided by SPSS 11.5 statistical software. The level of significance was accepted as $P < 0.05$.

2　RESULTS

2.1　Ibuprofen inhibiting $A\beta_{1-40}$-induced increase in phospho-MKK3/MKK6 and phospho-p38 MAP kinase expressions

As shown in Tab 1 and Fig 1A，basal levels of hippocampal phospho-MKK3/MKK6 were very low. Intracerebroventricular injection of preaggregated $A\beta_{1-40}$ led to a significant increase in phospho-MKK3/MKK6 protein expression. Ibuprofen（15 mg·kg^{-1}）significantly inhibited $A\beta_{1-40}$-induced increase in phospho-MKK3/MKK6 expression. In addition，the protein expression of phospho-p38 MAP kinase was

significantly elevated by $A\beta_{1-40}$. The $A\beta_{1-40}$-induced increase in expression of phospho-p38 MAP kinase was partly reversed by ibuprofen (Fig 1B).

Tab 1.　Effects of ibuprofen on $A\beta_{1-40}$-induced increase in phospho-MKK3/MKK6 and phospho-p38 MAP kinase expressions

Group	$A_{\text{Target protein}} : A_{\beta\text{-Actin}}$	
	phospho-MKK3/ MKK6	phospho-p38 MAP kinase
Control	0.116 ± 0.012	0.085 ± 0.008
$A\beta_{1-40}$	0.898 ± 0.099 **	0.336 ± 0.034 **
$A\beta_{1-40}$ + ibuprofen	0.704 ± 0.072 #	0.245 ± 0.038 #

MKK：mitogen-activated protein kinase kinase. MAP：mitogen-activated protein. The rats were given saline or ibuprofen (15 mg·kg^{-1} daily, ig) for 3 weeks prior to icv $A\beta_{1-40}$ (5 μL, 1 mmol·L^{-1}) injection. Rats in $A\beta_{1-40}$ and $A\beta_{1-40}$ + ibuprofen were injected icv with $A\beta_{1-40}$. Control rats were injected icv with saline. Hippocampal CA1 area was dissected 6 h after injection for the analysis of phospho-MKK3/MKK6 and phospho-p38 MAP kinase level. Each value was measured by densitometric analysis of Western blotting and was expressed by the density ratio of the band to β-actin. $\bar{x} \pm s$, $n = 4$. ** $P < 0.01$, compared with control；# $P < 0.05$, compared with $A\beta_{1-40}$.
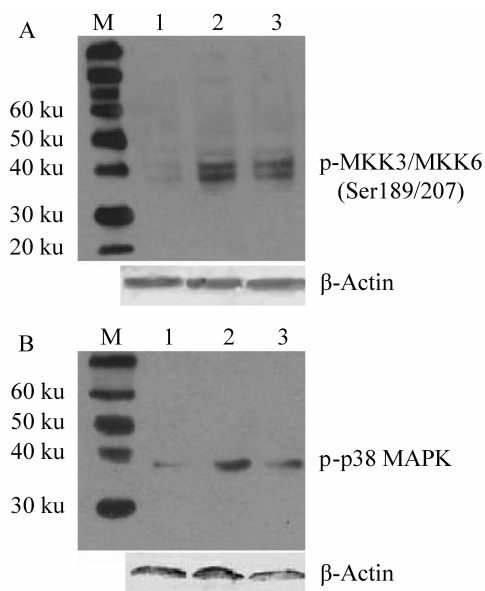
Fig 1.　Representative Western blot in the experiment of Tab 1. Lane 1：control；lane 2：$A\beta_{1-40}$-treated；lane 3：$A\beta_{1-40}$ + ibuprofen 15 mg·kg^{-1}.

2.2　Effects of ibuprofen on $A\beta_{1-40}$-induced phosphorylated MAP kinase activating protein kinase 2 and the phosphorylated heat-shock protein 27 expressions

As shown in Tab 2 and Fig 2A, phosphorylated MAPKAPK2 in hippocampal CA1 prepared from $A\beta_{1-40}$-treated rats was significantly reduced compared to that in control rats. This $A\beta_{1-40}$-induced decrease in phosphorylated MAPKAPK2 was partly reversed by ibuprofen. The results also showed that intracerebroventricular injection of $A\beta_{1-40}$ led to the decrease in Hsp27 phosphorylation (Tab 2, Fig 2B), being consistent with change of MAPKAPK2 phosphorylation induced by $A\beta_{1-40}$. Ibuprofen (15 mg·kg^{-1}) completely reversed the effect of $A\beta_{1-40}$ on phosphorylated Hsp27.

Tab 2.　Effects of ibuprofen on $A\beta_{1-40}$-induced decrease in phospho-MAPKAPK2 and phospho-Hsp27 expression

| Group	$A_{\text{Target protein}} : A_{\beta\text{-Actin}}$	
	phospho-MAPKAPK2	phospho-Hsp27
Control	0.619 ± 0.087	1.681 ± 0.203
$A\beta_{1-40}$	0.052 ± 0.014 **	1.082 ± 0.063 **
$A\beta_{1-40}$ + ibuprofen	0.487 ± 0.074 ##	1.602 ± 0.094 ##

MAPKAPK 2：MAP kinase activiating protein kinase 2. Hsp27：27 ku heat-shock protein. The experimental scheme was the same as indicated in Tab 1. $\bar{x} \pm s$, $n = 4$. ** $P < 0.01$, compared with control；## $P < 0.01$, compared with $A\beta_{1-40}$.

2.3　Ibuprofen inhibiting $A\beta_{1-40}$-induced increase in interleukin 1β protein expression

The sample immunoblot and mean data in Tab 3 and Fig 3 indicated that the expression of IL-1β in hippocampal sample prepared from $A\beta_{1-40}$-treated rats was significantly increased compared to samples from control rats. Ibuprofen (15 mg·kg^{-1}) markedly inhibited $A\beta_{1-40}$-induced increase in IL-1β expression.

2.4　Ibuprofen inhibiting $A\beta_{1-40}$-induced caspase 9 activation

The results showed that intracerebroventricular injection of preaggregated $A\beta_{1-40}$ led to the processing of inactive procaspase 9 into their
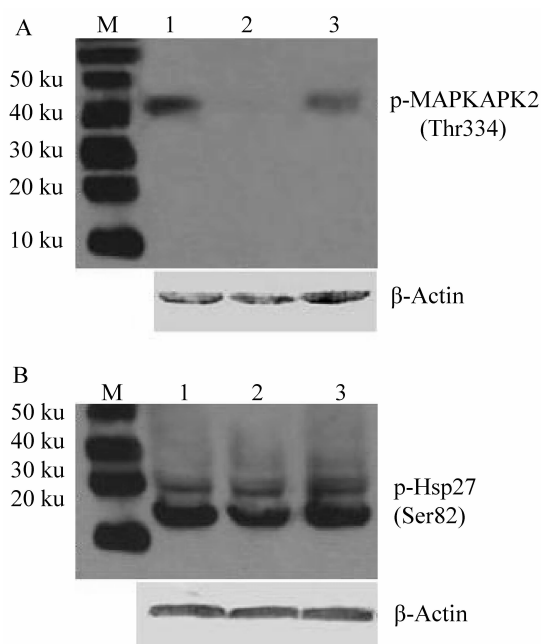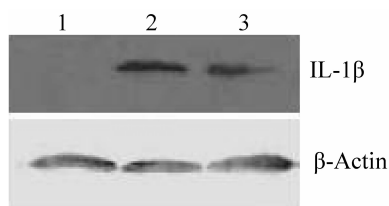
Fig 2. Representative Western blot in the experiment of Tab 2. Lane 1：control；lane 2：$A\beta_{1-40}$-treated；lane 3：$A\beta_{1-40}$ + ibuprofen.

Tab 3. Effect of ibuprofen on $A\beta_{1-40}$-induced increase in IL-1β protein expression

Group	$A_{IL-1\beta}: A_{\beta\text{-Actin}}$
Control	0.023 ± 0.005
$A\beta_{1-40}$	$0.563 \pm 0.078^{**}$
$A\beta_{1-40}$ + ibuprofen	$0.204 \pm 0.041^{\#\#}$

The experimental scheme was the same as indicated in Tab 1. $\bar{x} \pm s$, $n = 4$. $^{**}P < 0.01$, compared with control；$^{\#\#}P < 0.01$, compared with $A\beta_{1-40}$.

Fig 3. Representative Western blot in the experiment of Tab 3. Lane 1：control；lane 2：$A\beta_{1-40}$-treated；lane 3：$A\beta_{1-40}$ + ibuprofen.

active forms. Procaspase 9 proteolysis was confirmed by the increase of a 37 ku fragment in hippocampal CA1. Ibuprofen（15 mg·kg^{-1}）significantly prevented $A\beta_{1-40}$-induced pro-

caspase 9 cleavage（Tab 4，Fig 4）.

Tab 4. Effect of ibuprofen on $A\beta_{1-40}$-induced procaspase 9 cleavage

Group	$A_{Caspase\ 9}: A_{\beta\text{-Actin}}$
Control	0.211 ± 0.038
$A\beta_{1-40}$	$0.588 \pm 0.062^{**}$
$A\beta_{1-40}$ + ibuprofen	$0.297 \pm 0.051^{\#\#}$

The experimental scheme was the same as indicated in Tab 1. $\bar{x} \pm s$, $n = 4$. $^{**}P < 0.01$, compared with control；$^{\#\#}P < 0.01$, compared with $A\beta_{1-40}$.
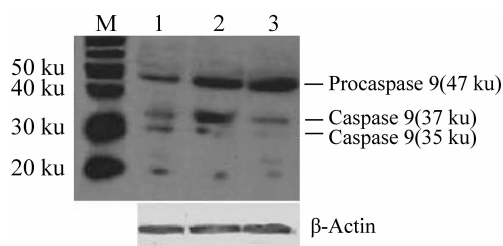
Fig 4. Representative Western blot in the experiment of Tab 4. The active fragment of caspase 9 is indicated as caspase 9（37 ku）. Lane 1：control；lane 2：$A\beta_{1-40}$-treated；lane 3：$A\beta_{1-40}$ + ibuprofen.

2.5 Ibuprofen preventing $A\beta_{1-40}$-induced procaspase 3 and procaspase 7 cleavage

Caspase 9 activity is responsible for procaspase 3 and procaspase 7 activation（executioner caspases）by proteolytic cleavage. Procaspase 3 and 7 processing was investigated by using Western blot. Tab 5 and Fig 5 showed that $A\beta_{1-40}$ induced procaspase 3 processing and caspase 3 activation by the appearance of p19 fragments. Similarly, caspase 7 was also cleaved to its p20 active form in $A\beta_{1-40}$-treated rat hippocampus. Ibuprofen（15 mg·kg^{-1}）inhibited caspase 3 and caspase 7 activation induced by $A\beta_{1-40}$.

## 2.6 Ibuprofen reducing poly（ADP-ribose）polymerase（PARP）cleavage

During apoptosis, PARP is one of the earliest targets for active caspase 3 which results in the formation of an 89 ku C-terminal fragment

Tab 5.   Effects of ibuprofen on $A\beta_{1-40}$-induced procaspase 3 and procaspase 7 activation

Group	$A_{\text{Target protein}} : A_{\beta\text{-Actin}}$			
	Procaspase 3	Caspase 3	Procaspase 7	Caspase 7
Control	1.33 ± 0.08	0.0434 ± 0.0013	0.27 ± 0.09	0.111 ± 0.012
$A\beta_{1-40}$	2.4 ± 0.5 *	0.176 ± 0.012 **	1.64 ± 0.10 **	1.11 ± 0.13 **
$A\beta_{1-40}$ + ibuprofen	1.96 ± 0.22	0.011 ± 0.002 ##	0.71 ± 0.09 ##	0.097 ± 0.015 ##

The experimental scheme was the same as indicated in Tab 1. $\bar{x} \pm s$, $n = 4$. $^* P < 0.05$, $^{**} P < 0.01$, compared with control; $^{\#\#} P < 0.01$, compared with $A\beta_{1-40}$.
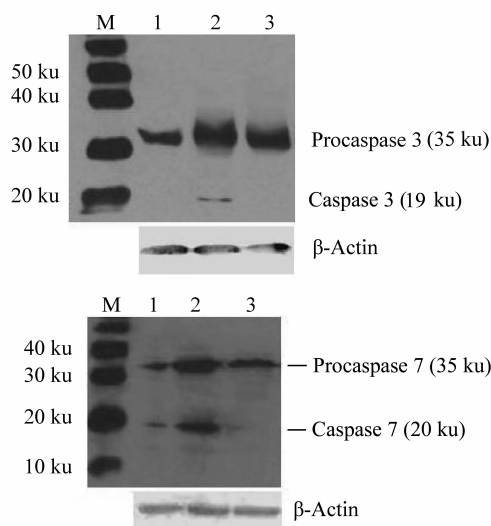
Fig 5.   Representative Western blot in the experiment of Tab 5. β-Actin was analyzed as a sample loading control. Lane 1: control; lane 2: $A\beta_{1-40}$-treated; lane 3: $A\beta_{1-40}$ + ibuprofen at 15 mg·kg^{-1}.

containing the catalytic domain and a 24 ku fragment that binds DNA ends[17]. As shown in Tab 6 and Fig 6, the expression level of intact PARP (116 ku) was significantly lower in hippocampal CA1 region prepared from Aβ-treated

Tab 6.   Effect of ibuprofen on $A\beta_{1-40}$-induced PARP cleavage

Group	$A_{\text{Target protein}} : A_{\beta\text{-Actin}}$	
	Intact PARP	Cleaved PARP
Control	0.229 ± 0.024	0.125 ± 0.038
$A\beta_{1-40}$	0.103 ± 0.016 **	0.250 ± 0.029 *
$A\beta_{1-40}$ + ibuprofen	0.191 ± 0.013 ##	0.160 ± 0.04 #

The experimental scheme was the same as indicated in Tab 1. $\bar{x} \pm s$, $n = 4$. $^* P < 0.05$, $^{**} P < 0.01$, compared with control; $^{\#} P < 0.05$, $^{\#\#} P < 0.01$, compared with $A\beta_{1-40}$.

animals and was associated with the appearance of the 89 ku fragment of PARP. In ibuprofen-treated animals, the expression level of intact PARP (116 ku) was higher while the expression of the 89 ku fragment was lower when compared with that of Aβ-treated animals.
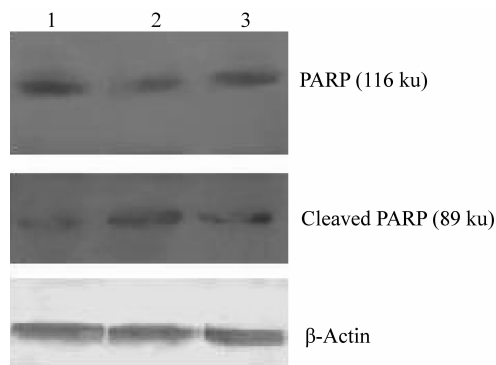
Fig 6.   Representative Western blot in the experiment of Tab 6. Lane 1: control; lane 2: $A\beta_{1-40}$-treated; lane 3: $A\beta_{1-40}$ + ibuprofen at 15 mg·kg^{-1}.

3   DISCUSSION

We report here that $A\beta_{1-40}$ induced an increase in phosphorylated MKK3/MKK6 and p38 MAP kinase expressions in hippocampal tissue. This increase, in combination with enhanced IL-1β protein expression, mediated Aβ-induced the activation of the pro-apoptotic pathways, the caspase cascades. Ibuprofen significantly prevented $A\beta_{1-40}$-induced increase in phosphorylated MKK3/MKK6 and p38 MAP kinase expressions. In addition, ibuprofen also inhibited $A\beta_{1-40}$-induced increase in IL-1β protein expression. Similarly, ibuprofen remarkably in-

hibited Aβ$_{1-40}$-induced the activation of pro-caspase 9 and the subsequent procaspase 3 and procaspase 7, and cleavage of PARP. One of the most interesting aspects of the present results was that the discrepancy between the changes in p38 MAP kinase and the corresponding phospho-MAPKAPK2. It is obvious from Fig 1 B and Fig 2 that intracerebroventricular injection of Aβ$_{1-40}$ elevated phospho-p38 MAP kinase expression, but reduced its substrates, phospho-MAPKAPK2 and phospho-Hsp27 protein expression. Ibuprofen reversed Aβ$_{1-40}$-induced these changes.

Activation of p38 MAP kinase is involved in neuronal response to various stresses[18], and this kinase is closely related to hyperphosphorylated tau protein in AD[19]. Our result and previous studies demonstrated that Aβ-induced increase in p38 MAP kinase activation was accompanied by the increase in IL-1β[20]. Overexpression of IL-1β observed in AD brain contributes to the neuronal dysfunction of AD, particularly those involved in formation of neurofibrillary tangle and loss of synapse. In addition, IL-1β upregulates expression and stimulates the processing of the Aβ precursor protein, resulting in amyloidogenic fragments in neurons[21]. In this way, IL-1β may sustain and enhance plaque formation. Alternatively, Aβ, phospho-tau or IL-1β may be the stressors of p38 MAP kinase/JNK. Positive feedback loops may be present in the AD brain whereby the initial stressor is amplified via MAP kinase pathway activation. Ibuprofen can inhibit p38 MAP kinase, thereby blocking this vicious cycle.

One of the p38 MAP kinase substrates is MAPKAPK2, which phosphorylates Hsp27[8]. In this study, we investigated the effect of Aβ$_{1-40}$ on phosphorylated MAPKAPK2 and Hsp27 and the effect of ibuprofen. Surprisingly, our results showed that intracerebroventricular injection of Aβ$_{1-40}$ reduced phosphorylated MAPKAPK2 and Hsp27 protein expressions. Ibuprofen significantly prevented Aβ$_{1-40}$-induced decrease in phosphorylated MAPKAPK2

and Hsp27 protein expression. This might be the other mechanism by which ibuprofen inhibited Aβ$_{1-40}$-induced hippocampal neurotoxicity. In recent years, evidence has showed that Hsp27 has cellular protection against the central nervous system. First, as a molecular chaperone, Hsp27 is active in the formation and maintenance of the native conformation of cytosolic protein[22] and stabilization of the actin filaments which make up the cytoskeleton of the cell[23]. Secondly, Hsp27 functions in neuroprotection through anti-apoptotic actions, particularly on the mitochondrial pathway of caspase-dependent cell death. One of the main mechanisms of caspase activation involves the release of cytochrome c from mitochondria. Cytochrome c interacts and binds with the Apaf-1 resulting in an apoptosome. The apoptosome recruits and activates procaspase 9 to caspase 9 which recruits, cleaves and activates procaspase 3 and procaspase 7. It is these caspases that mediate the death through selective proteolysis. Hsp27 inhibit the release of cytochrome c by suppressing Bid, a proapoptotic member of the Bcl-2 family. Hsp27 acts to prevent the activation of procaspase 3 directly or via a mechanism similar to Bcl-2 which delays PARP cleavage and procaspase 3 activation[24-25]. Therefore, this has led to the hypothesis that Hsp27 may be useful in the treatment of neurodegenerative diseases.

In summary, our finding indicated that ibuprofen reduced caspases 9, 3, and 7 expressions and activities and PARP cleavage. This inhibitory effect of ibuprofen on caspase-dependent cell death might occur through reduction of inflammatory cytokine IL-1β production and p38 MAP kinase activity and increase of Hsp27 expression in rat hippocampus.

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布洛芬对淀粉样 β 蛋白片段 1～40 引起的大鼠海马 p38 MAP 激酶 信号传导通路及半胱氨酸天冬氨酸蛋白酶级联的影响

范　莹[1]，金　英[1]，闫恩志[1]，杨　菁[2]，宗志红[3]，齐志敏[1]

（锦州医学院 1. 药理教研室，2. 生物化学教研室，辽宁 锦州　121001；3. 中国医科大学
生物化学教研室，辽宁 沈阳　110001）

摘要：目的　研究布洛芬对淀粉样 β 蛋白片段 1－40（$A\beta_{1-40}$）所致大鼠海马损伤的保护作用及作用机制。**方法**　大鼠 ig 给予布洛芬 15 $mg \cdot kg^{-1}$，连续应用 3 周，脑室内一次性注射 $A\beta_{1-40}$（5 μL，1 mmol · L^{-1}），注射后 6 h 快速取海马 CA1 区，Western 免疫印迹法观察磷酸化丝裂原激活的蛋白激酶的激酶 3/6（MKK3/MKK6）、磷酸化丝裂原激活的蛋白激酶 p38（p38 MAP 激酶）、磷酸化 MAPK 活化的蛋白激酶 2（MAPKAPK2）、磷酸化热休克蛋白 p27（Hsp27）、半胱氨酸天冬氨酸蛋白酶（caspase）9，3，7 以及 ADP-核糖聚合酶（PARP）的蛋白表达水平的改变。**结果**　脑室内注射 $A\beta_{1-40}$ 可引起海马 CA1 区磷酸化的 MKK3/MKK6 和磷酸化 p38 MAP 激酶表达明显增加，但可使磷酸化 MAPKAPK2 和磷酸化的 Hsp27 表达降低，这些改变伴随有 caspase 级联的激活。此外，也发现 $A\beta_{1-40}$ 可使海马 CA1 区完整的 PARP 蛋白表达明显减少，而劈切 PARP（分子量为 89 ku）表达明显增加。布洛芬可明显对抗 $A\beta_{1-40}$ 引起的磷酸化的 MKK3/MKK6 和磷酸化 p38 MAP 激酶表达增加，上调磷酸化 MAPKAPK2 和磷酸化的 Hsp27 表达水平，同时抑制 $A\beta_{1-40}$ 引起的 caspase 级联激活和 PARP 的劈切。**结论**　布洛芬通过抑制 $A\beta_{1-40}$ 引起的磷酸化的 MKK3/MKK6 和磷酸化 p38 MAP 激酶表达，明显增加以及上调磷酸化的 Hsp27 表达水平，对抗 $A\beta_{1-40}$ 引起的海马的神经细胞损伤。

关键词：布洛芬；淀粉样 β 蛋白；MAP 激酶信号系统；有丝分裂素激活蛋白激酶类；热休克蛋白类；半胱氨酸天冬氨酸蛋白酶

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