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The Protein Phosphatase Agonist Reaneuomine(CRM-2102), A New Drug for The Treatment of Alzheimer's Disease

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The Protein Phosphatase Agonist Reaneuomine(CRM-2102), A New Drug for The Treatment of Alzheimer's Disease

Alzheimer's disease(AD) is a neurological disorder characterized by memory, cognitive and language dysfunction and behavioral impairment caused by chronic progressive central nervous system degeneration.

According to the China Alzheimer's Disease Report 2021, led by Shanghai Ruijin Hospital, there were 13,243,950 cases of AD and other dementias in China in 2019. The prevalence rate and mortality rate were slightly higher than the global average, and higher in females than in males. The prevalence rate (1188.9/100,000) and mortality rate (30.8/100,000) were higher in females than in males (669.3/100,000) and mortality rate (14.6/100,000), respectively.

At present, two classes of drugs are widely used in the clinical treatment of AD: cholinesterase inhibitors such as Donepezil and N-methyl-D-aspartate (NMDA) receptor antagonists such as Memantine. Donepezil can increase cholinergic nerve signals by inhibiting acetylcholinesterase and maintaining or increasing the level of acetylcholine, so as to improve the cognitive function of patients. Memantine decreased neurotoxic effects of excitatory amino acids and improved cognitive function by inhibiting NMDA receptor activity. However, these drugs are only symptomatic treatment, which can improve cognitive and memory impairment to some extent, but there is no drug that can stop or delay the progression of AD.

The release of neurotransmitters and the opening and closing of ion channels are associated with many protein phosphorylation and dephosphorylation processes, which are important links of information transmission in nerve cells. Under the

combined action of adenosine triphosphate (ATP) and protein phosphorylase (protein kinase, PK), protein phosphorylation is catalyzed, protein structure changes and becomes active protein. The activated protein continues to activate the downstream target protein, thus activating the whole cascade reaction to complete signal transduction. Protein dephosphorylation ensures that the signaling pathway can be closed in time, providing the basis for the next neural activity.

We believe that the occurrence and development of AD is related to Protein Phosphatase; PP) is closely related to functional decline. PP activity is reduced, the protein is not dephosphorylated and inactivated, the postsynaptic membrane continues to receive neurotransmitters released by the anterior membrane, and the signal channel cannot be closed in time, resulting in delayed nerve conduction and memory breakdown.

Amyloid precursorprotein (APP) is a widely existing essential bioactive substance. β-amyloid (Aβ) is produced by the hydrolysis of APP by β -secretase and γ -secretase. In vitro tests showed that Aβ inhibited the bioactivity of PP, and the inhibition intensity was closely related to the level of Aβ. The normal level of Aβ had limited effect on the activity of PP. Only when the level of Aβ reached A certain level, the activity of PP was inhibited, and with the increase of the concentration of Aβ, the inhibition of PP activity also increased.

In conclusion, the abnormal increase of Aβ inhibits PP activity, which is an important factor in the induction of AD.

The biological function of Tau protein (P-Tau) is to induce the aggregation of tubulin into microtubules and prevent its depolymerization, thus maintaining the

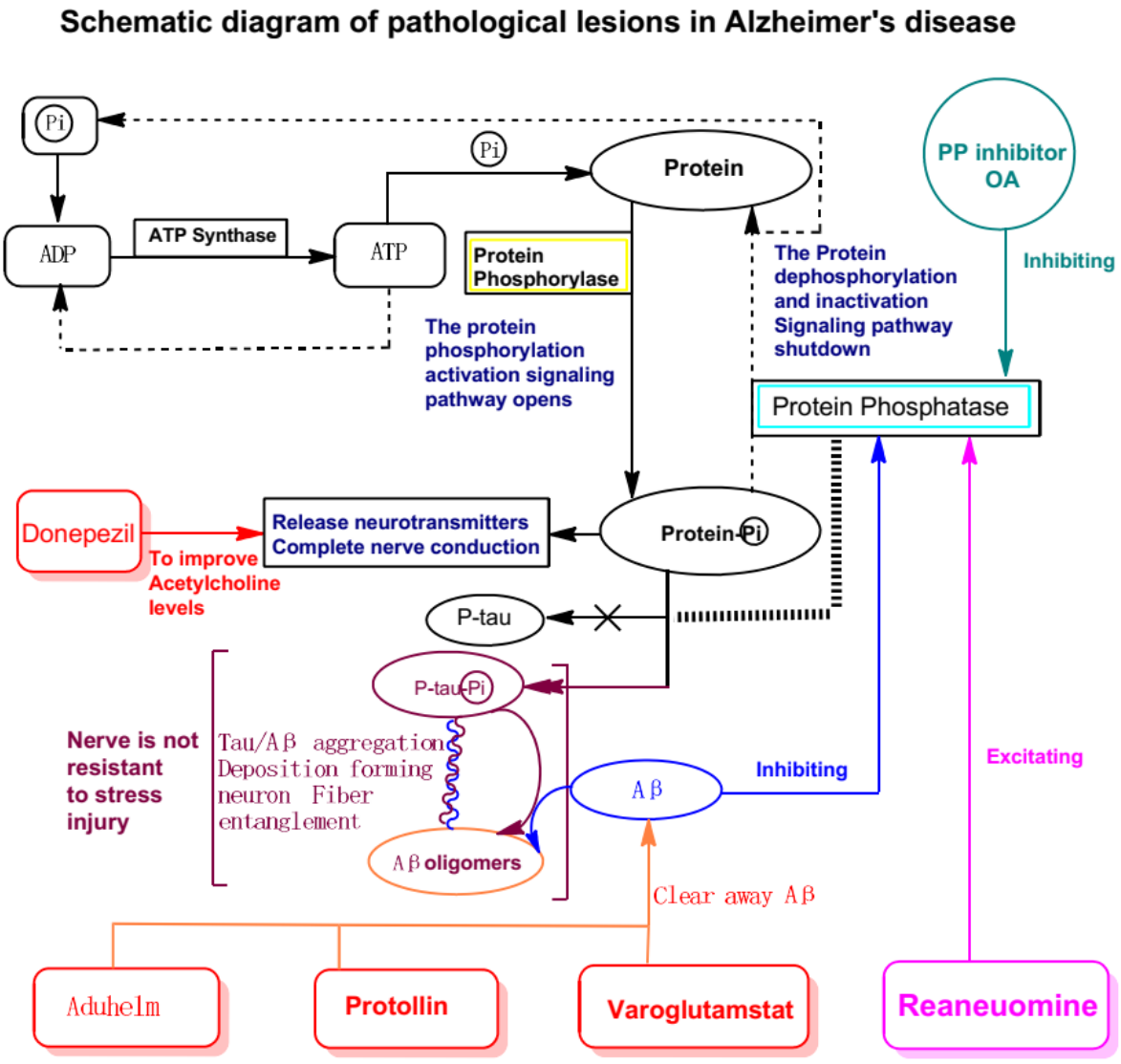
morphology and function of nerve cells. In order to restore PP bioactivity, the body sacrifices P-Tau and Aβ to form p-Tau /Aβ complex and deposit it in nerve cells, so as to reduce the level of Aβ and restore PP activity. This fluctuation in PP bioactivity due to changes in Aβ levels is shown in PATIENTS with AD in terms of good and bad memory.

Over time, p-Tau /Aβ complexes gradually burst nerve cells, causing irreversible neuroorganic lesions in AD patients. PET scans of the brains of patients with AD have confirmed that p-Tau /Aβ tangles (plaques) cause brain atrophy and brain nerve cell death.

PP Agonist catalyzes protein dephosphorylation to maintain normal neural activity by improving PP biological activity. In addition, increased PP activity can also promote p-Tau-Pi dephosphorylation, thus avoiding neurotoxic effects and nerve cell death mediated by tau hyperphosphorylation.

Protein phosphatase 2A(PP2A) is an important member of the protein phosphatase family, and the catalytic domain of its catalytic subunit is highly homologous with that of other members of the family. Reaneuomine (CRM-2102), as an agonist, has a strong excitatory effect on impaired PP2A and can significantly increase the biological activity of PP2A.

Therefore, the development and study of PP2A agonists can bring hope for the rehabilitation of AD patients.



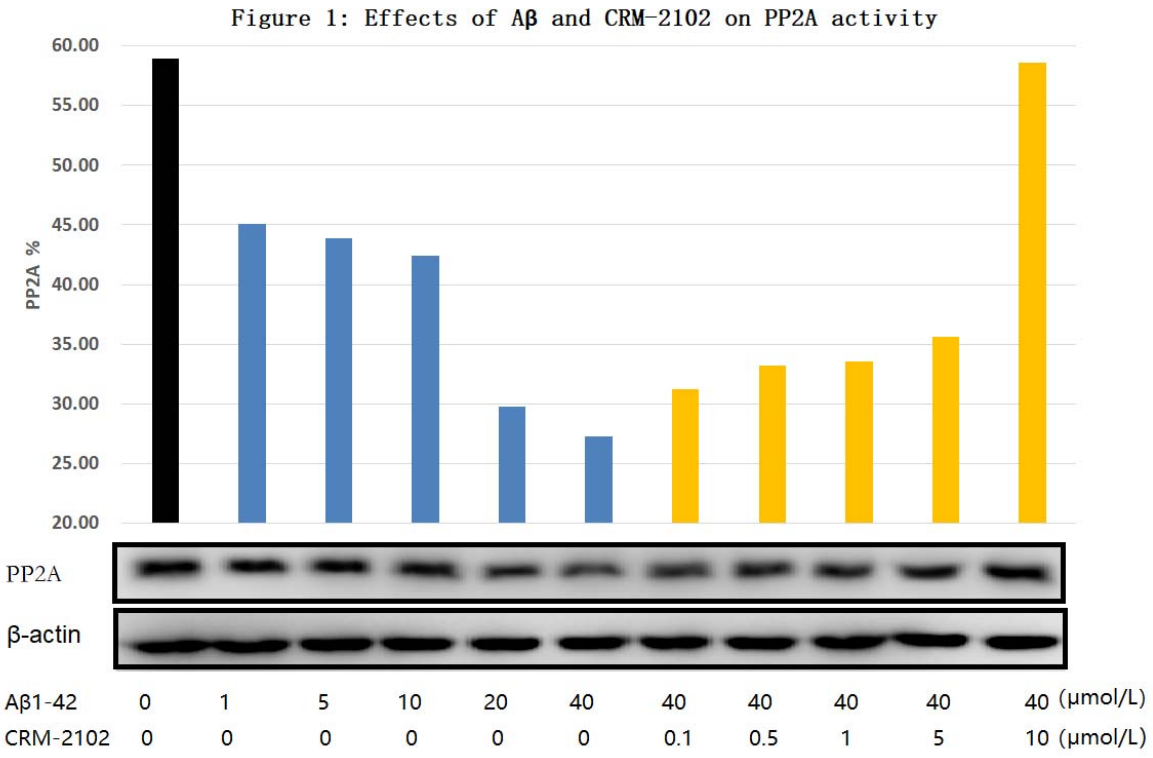
Inhibitory effect of Aβ on PP2A in PC12 cells and excitatory effect of CRM-2102 on PP2A

PC12 cells were cultured with 1, 5, 10, 20 and 40μmol/L Aβ, respectively, and incubated for 24h to extract the total protein. Western blot was used to detect the activity of PP2A. The results showed that the concentration of Aβ below 10μmol/L had little effect on the expression of PP2A in PC12 cells. The expression of PP2A in PC12 cells was significantly inhibited at concentrations above 10μmol/L, and the activity of PP2A was dose-dependently inhibited at concentrations above 10, 20 and 40μmol/L.

PC12 cells were adherentially cultured with four doses of CRM-2102, 0.1, 0.5, 1 and 5μmol/L, respectively, and incubated for 2h. Then, 40μmol/L Aβ was added and incubated for 24h, and the whole protein was extracted. Western blot was used to detect the activity of PP2A. The results showed that four doses of CRM-2102, 0.1, 0.5, 1 and 5μmol/L, could increase the expression of PP2A protein in PC12 cells induced by Aβ, and showed A quantitative effect. CRM-2102 at 10μmol/L significantly increased the expression level of PP2A protein (58.59%), which was almost the same as that of normal cells (58.95%).

Table 1: Effects of Aβ and CRM-2102 on PP2A protein expression level in PC12 cells

group	1	2	3	4	5	6	7	8	9	10	11
$A\beta_{1-42}$(μmol/L)	0	1	5	10	20	40	40	40	40	40	40
CRM-2102(μmol/L)	0	0	0	0	0	0	0.1	0.5	1	5	10
Protein(μg/μL)	3.15	2.89	2.67	2.91	2.78	2.80	3.06	2.60	2.84	2.62	2.73
PP2A	2069	1606.5	1628.1	1498.8	1018.9	914.16	1025.7	1129.7	1180.1	1284.7	2192.1
β-actin	3509.5	3563.5	3711.3	3533.8	3423.5	3354.1	3283.7	3404.8	3521.7	3607.8	3741.2
PP2A(%)	58.95	45.08	43.87	42.41	29.76	27.26	31.24	33.18	33.51	35.61	58.59



Excitatory effect of CRM-2102 on PP2A in PC12 cells

The total protein of PC12 cells was extracted and the activity of PP2A was detected by Western blot. The results showed that CRM-2102 significantly enhanced the activity of PP2A inhibited by okadaic acid (OA) in a dose-dependent manner.

Table 2：Effects of different concentrations of CRM-2102 on the activity of PP2A induced by OA in PC12 cells

group	Control	CRM-2102			
CRM-2102(μmol/L)	0	0	1	5	10
OA(nmol/L)	0	40	40	40	40
PP2A	1330.4	339.39	557.1	688.01	1130.4
β-actin	3616.5	4062.7	3933.6	3838.5	3874.6
PP2A（%）	37	8	14	18	29

Figure 2: Excitatory effect of CRM-2102 on PP2A

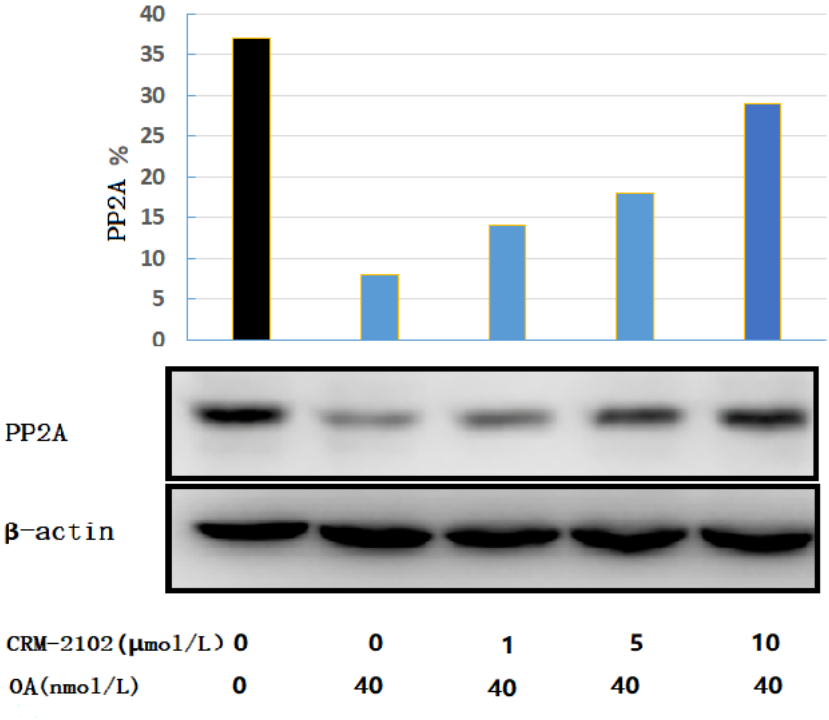
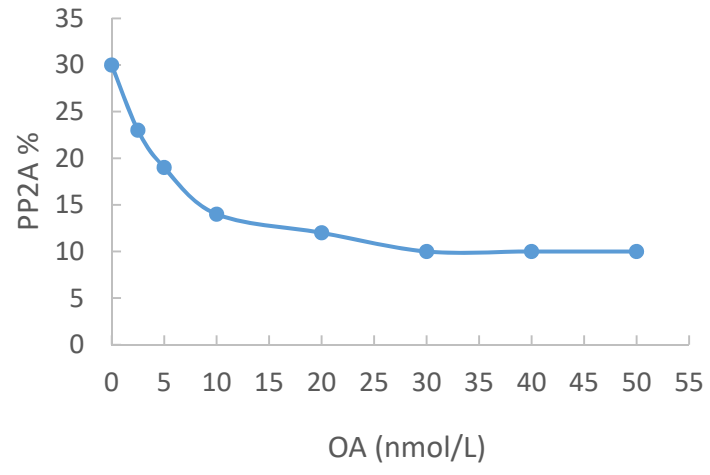


Table 3：Effect of OA on PP2A activity in PC12 cells

group	1	2	3	4	5	6	7	8
OA(nmol/L)	0	2.5	5	10	20	30	40	50
Protein(μg/μL)	1.9	1.87	1.94	1.89	1.95	1.89	1.9	1.93
PP2A	1096.1	822.36	711.31	527.03	434.01	393.47	389.63	367.89
β-actin	3655.9	3528.4	3835.6	3848.1	3760.3	3798.7	3768.3	3837.2
PP2A（%）	30	23	19	14	12	10	10	10

Figure 3： Inhibitory effect of OA on PP2A



Effects of CRM-2102 on tau phosphorylation in human neuroblastoma cells (SH-SY5Y cells)

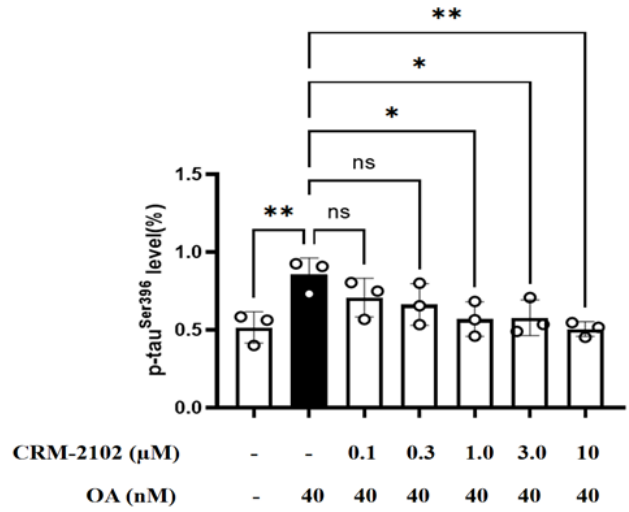
SH-SY5Y cells were cultured in CRM-2102 medium with different concentrations for 24 h, and then treated with 40 nmol/L OA for 24 h. The p-Tau^{ser396} level of SH-SY5Y cells was determined by Western Blot. The results showed that CRM-2102 could inhibit OA-induced tau protein phosphorylation, and the p-Tau^{ser396} level in 1 and 3 μmol/L CRM-2102 group was significantly different from that in OA control group ($P<0.05$). The level of P-Tau^{ser396} in 10 μmol/L CRM-2102 group was significantly different from that in OA control group ($P<0.01$).

Table 4: Effects of CRM-2102 on OA induced SH-SY5Y cells p-tau^{Ser396} ($\bar{x}$±SEM，n=3)

group		**p-tau^{Ser396}level(%）**
Control		0.450±0.021**
OA (negative control)		0.855±0.023
CRM-2102（μmol/L）	0.1	0.761±0.008
	0.3	0.663±0.027
	1.0	0.557±0.013*
	3.0	0.578±0.025*
	10	0.522±0.016**

*Compared with a negative control group: *P<0.05, **P<0.01.*

Figure 4: Effects of CRM-2102 on OA induced SH-SY5Y cells p-tau^{Ser396}



*Compared with a negative control group: *P<0.05, **P<0.01.*

Effects of CRM-2102 on the survival rate of OA and Aβ induced PC12 cells

The CCK8 cell proliferation detection method was used to incubate PC12 cells with different concentrations of CRM-2102 in vitro suspension for 2h, and then add 50nmol/L OA or 40μmol/L Aβ for 24h. The experimental results showed that CRM-2102 could significantly improve the survival rate of PC12 cells with OA or Aβ induced injury, and the survival rate of PC12 cells showed A dose-dependent increase.

Table 5：Effects of CRM-2102 on the survival rate of PC12 cells in OA induced injury model

Group		Mean±SD	Survival rate
Negative control		1.326±0.021	
OA(50nmol/L)		0.669±0.025	50.45%
CRM-2102 (μmol/L)	10	1.04±0.017*	78.43%
	5	0.967±0.018*	72.93%
	1	0.787±0.028*	59.35%
	0.5	0.676±0.016	50.98%

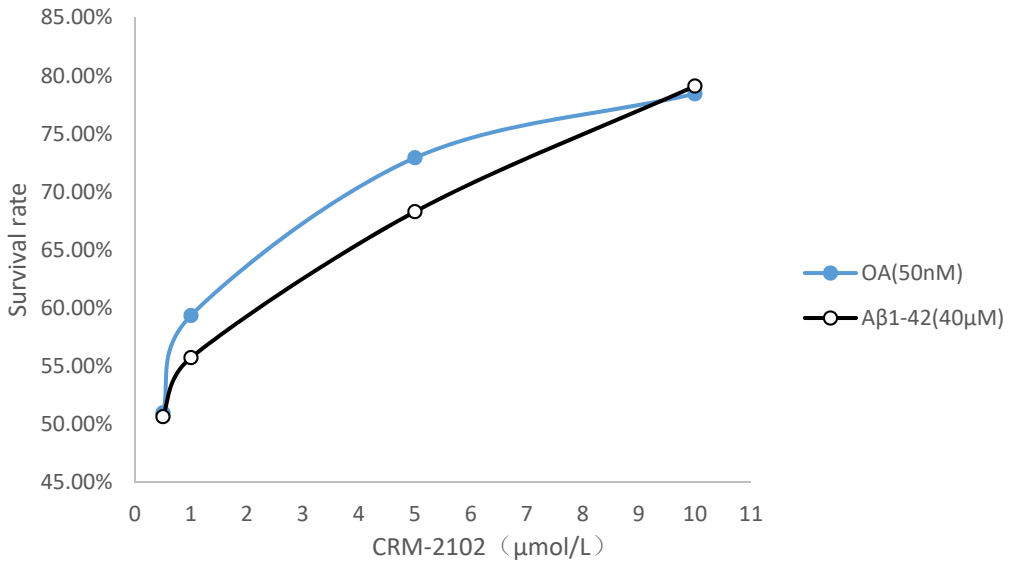
Compared with OA：**P <0.01*

Table 6：Effects of CRM-2102 on the survival rate of PC12 cells in Aβ induced injury model

Group		Mean±SD	Survival rate
Negative control		1.378±0.017	
$A\beta_{1-42}$(40μmol/L)		0.709±0.023	51.45%
CRM-2102 (μmol/L)	10	1.09±0.026*	79.10%
	5	0.941±0.018*	68.29%
	1	0.768±0.015*	55.73%
	0.5	0.698±0.024	50.65%

Compared with Aβ：*$P<0.01$*

Figure 5：Effects of CRM-2102 on the survival rate of PC12 cells



Effects of CRM-2102 on PP2A in hippocampus and cortex of mice

Mice（C57BL/6J,23g-25g, male）were injected with Aβ (4μg/ mouse) in lateral ventricles, and three doses of CRM-2102 (1.5 mg/kg, 4.5 mg/kg and 13.5 mg/kg) were given by gavage for 14 days. Western Blot was used to detect the expression of PP2A in hippocampus and cortex.

Compared with the model group, the expression of PP2A in the hippocampus of CRM-2102 mice in 13.5 mg/kg dose group was significantly increased(*P<0.05*), the expression of PP2A in the hippocampus of CRM-2102 mice in 1.5 mg/kg and 4.5 mg/kg groups was increased, but there was no significant difference (*P>0.05*). There was no significant difference in the expression level of PP2A in the cortex of mice between experimental groups (*P>0.05*).

The results showed that lateral ventricle injection of Aβ had no effect on the expression level of PP2A in the cortex of mice, but had A significant effect on the expression level of PP2A in the hippocampus of mice. Low and middle dose group of CRM - 2102 in mice hippocampal PP2A expression level increased to a certain extent, but no statistical difference; The expression level of CRM-2102 in high dose group was significantly increased, with statistical difference.

Table 5. PP2A expression in hippocampus and cortex of mice in each group ($\bar{x}$ ±SEM，n=5)

Groups		PP2A levels(%）	
		Hippocampus	Cortex
Sham-operated		1.132±0.074*	0.704±0.058
Model		0.576±0.045	0.699±0.085
CRM-2102（mg/kg）	1.5	0.904±0.039	0.782±0.058
	4.5	0.918±0.054	0.715±0.047
	13.5	1.202±0.077*	0.877±0.057

**P<0.05，Compared to the model group*

Figure 6： PP2A expression levels in hippocampus and cortex of mice