



# Study of the influence and molecular mechanism of ticagrelor on cerebral ischemia reperfusion injury in rats

Gui-Fa Chen 

General Medicine Department, Huizhou Third People's Hospital in Guangdong Province, Huizhou City, Guangdong Province, 516002

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## ABSTRACT

**Objective:** To study the influence and molecular mechanism of ticagrelor on cerebral ischemia reperfusion injury in rats. **Methods:** SD rats were selected as experimental animals and divided into control group, model group, ticagrelor group and clopidogrel group, cerebral ischemic reperfusion injury models were made, then ticagrelor group were given intragastric administration of 150 mg ticagrelor, clopidogrel group were given intragastric administration of 90 mg clopidogrel. 1 week after intervention, the brain water content as well as the contents of oxidative stress molecules and inflammatory factors were measured. **Results:** Water content in brain, MDA, Ox-LDL, NF-kB, TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in brain tissue as well as TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in serum of model group were significantly higher than those of control group while SOD, GSH-Px and Prdx6 contents in brain tissue were significantly lower than those of control group; water content in brain, MDA, Ox-LDL, NF-kB, TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in brain tissue as well as TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in serum of ticagrelor group and clopidogrel group were significantly lower than those of model group while SOD, GSH-Px and Prdx6 contents in brain tissue were significantly higher than those of model group; water content in brain, MDA, Ox-LDL, NF-kB, TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in brain tissue as well as TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in serum of ticagrelor group were significantly lower than those of clopidogrel group while SOD, GSH-Px and Prdx6 contents in brain tissue were significantly higher than those of clopidogrel group. **Conclusion:** Ticagrelor can be more effective in inhibiting oxidative stress response and inflammatory response, and reducing the cerebral ischemia reperfusion injury than clopidogrel.

## 1. Introduction

Acute cerebral infarction is a clinically common cerebrovascular disease, and both ischemia hypoxia and ischemia-reperfusion can cause brain tissue damage[1,2]. Antiplatelet agents are the common drugs for clinical treatment of cerebral infarction, and can effectively inhibit the platelet aggregation and activation and relieve the brain tissue damage caused by ischemia hypoxia and ischemia-reperfusion[3]. Clopidogrel is a kind of irreversible ADP

receptor antagonist, which is inactive and needs to be metabolized by the liver CYP450 enzyme after it enters the body to produce active products and exert antiplatelet effect[4]. So the antiplatelet effect of clopidogrel is influenced by the catalytic activity of CYP450 enzyme, and the clinical application is significantly different. Ticagrelor is a new type of anti-platelet agent, which can be used without metabolic activation after taken orally, and can be reversibly combined with P2Y<sub>12</sub> receptors and antagonize platelet aggregation. Besides, ticagrelor can also inhibit cellular uptake of adenosine, which further increases the plasma concentrations of adenosine and reduces the brain injury through the biological effect of adenosine[5]. In the following study, the influence and molecular mechanism of ticagrelor on cerebral ischemia reperfusion injury in rats were analyzed.

✉ Corresponding author: Gui-Fa Chen, General Medicine Department, Huizhou Third People's Hospital in Guangdong Province, Huizhou City, Guangdong Province, 516002.

Tel: 0752-2583768; 13620491502

E-mail: wenguoquan84@163.com

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## 2. Experimental materials

### 2.1 Experimental animals

A total of 40 SPF adult male SD rats with body mass 250-300 g were purchased at the laboratory animal center of Sun Yat-sen University Zhongshan School of Medicine, and the animal license number SCXK (yue) 2011-0029. They were randomly divided into the control group, the model group, the ticagrelor group and the clopidogrel group, 10 in each group. Animal experiments passed the hospital's ethical review and the procedures were followed for animal testing and the treatment of animals after death.

### 2.2 Reagents and instruments

Ticagrelor was provided by AstraZeneca AB in Sweden, clopidogrel was provided by Sanofi-Aventis (Hangzhou) Pharmaceutical Co., Ltd., enzyme-linked immunosorbent assay kit was purchased from Sino Biological Inc. in Beijing, radioimmunoprecipitation kits were bought from Nanjing Jiancheng Bioengineering Institute, and the microplate reader for enzyme-linked immunosorbent experiments and the spectrophotometer for radioimmunoprecipitation experiments were from Bio-rad Company.

### 2.3. Experimental methods

#### 2.3.1 Animal experiment methods

Model group, Ticagrelor group and clopidogrel group were made into the animal models according to the following methods: after anesthesia by intraperitoneal injection of chloral hydrate, a median neck incision was made to separate right common carotid artery, internal carotid artery and external carotid artery, the distal end of external carotid artery was ligatured, electric coagulation pen was used to fuse and then free the external carotid artery, ophthalmic scissors were used to make a small hole in the external carotid artery, the pre-made suture was put in, extended to the internal carotid artery via the external carotid artery and inserted upwards until there was a resistance, and the artery was properly ligatured to cause cerebral ischemia; the suture was removed two hours later to cause the reperfusion in ischemic brain tissue. Control group were anesthetized to make surgical incision in accordance with the same procedure without inserting the suture.

Four groups of rats began to receive intervention immediately after the model was made, and the ticagrelor group received intragastric administration of 150 mg/kg ticagrelor, once per day; the clopidogrel group received intragastric administration of 90 mg/kg clopidogrel, once a day; the control group and the model group received intragastric administration of same volume of saline, once a day.

#### 2.3.2 Brain water content detection methods

1 week after intervention, rats were killed by decapitation, brain tissue was collected to weigh the wet weight, and then dried in the oven at 110-115 °C until the weight was constant, the dry weight was weighed, and the water content was calculated by the formula (wet weight - dry weight)/wet weight × 100%.

#### 2.3.3 Molecule detection methods

1 weeks after the intervention, rats were killed by decapitation to collect the brain tissue and serum specimens, radioimmunoprecipitation kits were used to detect MDA, SOD and GSH-Px contents in brain tissue, and enzyme-linked immunosorbent assay kits were used to detect Ox-LDL, Prdx6 and NF-κB contents in brain tissue as well as TNF-α, IL-1β and IL-6 contents in serum.

### 2.4 Statistical methods

SPSS 20.0 software was used to input data, differences in measurement data among four groups were by variance analysis and  $P < 0.05$  indicated statistical significance in differences.

## 3. Results

### 3.1 Water content in brain of four groups of rats

Water contents in brain of four groups of rats was as follows: water content in brain of control group was (74.9±9.3)%, water content in brain of model group was (85.1±10.2)%, water content in brain of clopidogrel group was (81.4±11.4)% and water content in brain of ticagrelor group was (77.4±9.7)%. Variance analysis showed that water content in brain of model group was significantly higher than that of control group; water contents in brain of ticagrelor group and clopidogrel group were significantly lower than that of model group; water content in brain of ticagrelor group was significantly lower than that of clopidogrel group. Differences in pair-wise comparison of water contents in brain were statistically significant among four groups of rats ( $P < 0.05$ ).

### 3.2 Oxidative stress molecule contents in brain tissue of four groups of rats

Analysis of oxidative stress molecules MDA, Ox-LDL, SOD, GSH-Px and Prdx6 contents in brain tissue of four groups of rats was as

**Table 1.**

Oxidative stress molecule contents in brain tissue of four groups of rats.

Groups	n	MDA	Ox-LDL	SOD	GSH-Px	Prdx6
Control group	10	1.85±0.25	8.39±1.15	26.84±3.58	36.82±5.29	8.48±1.03
Model group	10	6.84±0.93 <sup>†</sup>	25.28±4.41 <sup>†</sup>	9.39±1.14 <sup>†</sup>	13.24±1.67 <sup>†</sup>	2.68±0.36 <sup>†</sup>
Clopidogrel group	10	4.02±0.57 <sup>#</sup>	16.53±1.93 <sup>#</sup>	15.20±1.94 <sup>#</sup>	19.49±2.46 <sup>#</sup>	4.41±0.68 <sup>#</sup>
Ticagrelor group	10	2.77±0.41 <sup>#&amp;</sup>	11.25±1.85 <sup>#&amp;</sup>	21.37±3.26 <sup>#&amp;</sup>	27.59±3.25 <sup>#&amp;</sup>	6.52±0.89 <sup>#&amp;</sup>

<sup>†</sup>: compared with control group, differences were significant,  $P<0.05$ ; <sup>#</sup>: compared with model group, differences were significant,  $P<0.05$ ; <sup>&</sup>: compared with clopidogrel group, differences were significant,  $P<0.05$ .

follows: MDA and Ox-LDL contents in brain tissue of model group were significantly higher than those of control group while SOD, GSH-Px and Prdx6 contents were significantly lower than those of control group; MDA and Ox-LDL contents in brain tissue of ticagrelor group and clopidogrel group were significantly lower than those of model group while SOD, GSH-Px and Prdx6 contents were significantly higher than those of model group; MDA and Ox-LDL contents in brain tissue of ticagrelor group were significantly lower than those of clopidogrel group while SOD, GSH-Px and Prdx6 contents were significantly higher than those of clopidogrel group. Differences in pair-wise comparison of MDA, Ox-LDL, SOD, GSH-Px and Prdx6 contents in brain tissue were statistically significant among four groups of rats ( $P<0.05$ ).

### 3.3 Inflammation-related molecule contents in brain tissue and serum of four groups of rats

Analysis of inflammation-related molecules NF-kB, TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in brain tissue of four groups of rats was as follows: NF-kB, TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in brain tissue of model group were significantly higher than those of control group; NF-kB, TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in brain tissue of ticagrelor group and clopidogrel group were significantly lower than those of model group; NF-kB, TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in brain tissue of ticagrelor group were significantly lower than those of clopidogrel group. Differences in pair-wise comparison of above inflammatory factor contents in brain tissue were statistically significant among four groups of rats ( $P<0.05$ ).

Analysis of inflammation-related molecules TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in serum of four groups of rats was as follows: TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in serum of model group were significantly higher than those of control group; TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in serum of ticagrelor group and clopidogrel group were significantly lower than those of model group; TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in serum of ticagrelor group were significantly lower than those of clopidogrel group. Differences in pair-wise comparison of above inflammatory factor contents in brain tissue were statistically significant among four groups of rats ( $P<0.05$ ).

**Table 2.**NF-kB, TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in brain tissue of four groups of rats (ng/mL).

Groups	n	NF-kB	TNF- $\alpha$	IL-1 $\beta$	IL-6
Control group	10	3.22±0.47	13.25±1.85	7.49±0.92	0.93±0.11
Model group	10	12.15±1.98 <sup>*</sup>	45.62±7.24 <sup>*</sup>	30.29±4.51 <sup>*</sup>	2.68±0.36 <sup>*</sup>
Clopidogrel group	10	7.74±0.93 <sup>#</sup>	26.73±3.57 <sup>#</sup>	21.32±4.24 <sup>#</sup>	1.83±0.22 <sup>#</sup>
Ticagrelor group	10	5.48±0.71 <sup>#&amp;</sup>	16.68±2.05 <sup>#&amp;</sup>	13.42±1.86 <sup>#&amp;</sup>	1.36±0.17 <sup>#&amp;</sup>

<sup>\*</sup>: compared with control group, differences were significant,  $P<0.05$ ;

<sup>#</sup>: compared with model group, differences were significant,  $P<0.05$ ; <sup>&</sup>:

compared with clopidogrel group, differences were significant,  $P<0.05$ .

**Table 3.**

Comparison of serum inflammatory factor contents among four groups of rats (ng/mL).

Groups	n	TNF- $\alpha$	IL-1 $\beta$	IL-6
Control group	10	25.92±3.85	11.32±1.67	9.24±1.52
Model group	10	67.53±9.31 <sup>*</sup>	32.48±5.62 <sup>*</sup>	27.48±4.27 <sup>*</sup>
Clopidogrel group	10	44.25±7.14 <sup>#</sup>	20.13±2.95 <sup>#</sup>	18.39±2.04 <sup>#</sup>
Ticagrelor group	10	32.42±4.49 <sup>#&amp;</sup>	15.62±2.24 <sup>#&amp;</sup>	13.21±1.52 <sup>#&amp;</sup>

<sup>\*</sup>: compared with control group, differences were significant,  $P<0.05$ ;

<sup>#</sup>: compared with model group, differences were significant,  $P<0.05$ ; <sup>&</sup>:

compared with clopidogrel group, differences were significant,  $P<0.05$ .

## 4. Discussion

Both clopidogrel and ticagrelor belong to antiplatelet agents, which can antagonize P2Y12 receptor and inhibit platelet activation and aggregation, and are used for the treatment of cerebral infarction and myocardial infarction. Clopidogrel cannot exert antiplatelet effect until it generates active products after the catalysis of liver CYP450 enzyme, and the efficacy for antiplatelet therapy is greatly affected by the catalytic activity of CYP450 enzyme[6]. Ticagrelor is a new ADP receptor P2Y12 antagonist, which is a non-precursor drug, can be functional directly after entering into the body without being metabolized, and has characteristics of faster onset and less affected by the catalytic activity of liver CYP450 enzyme[7,8]. Studies have reported that ticagrelor can be more effective than clopidogrel to alleviate myocardial ischemia-reperfusion injury, but it is not yet clear about the ticagrelor effects on cerebral ischemia reperfusion injury[9,10]. Cerebral edema is the macro performance after cerebral ischemia reperfusion injury, and in order to define the effects of different P2Y12 antagonists on cerebral ischemia reperfusion injury, the cerebral edema after ischemia reperfusion was analyzed in the study, and the comparison of brain tissue water content showed that the water content in brain of model group increased significantly, and

the water content in brain of ticagrelor group and clopidogrel group were lower than that of model group; in addition, the water content in brain of ticagrelor group was lower than that of clopidogrel group. This means that both ticagrelor and clopidogrel can alleviate ischemia-reperfusion injury and brain edema, and ticagrelor is better than clopidogrel in reducing ischemia-reperfusion injury.

Oxidative stress is an important pathologic factor that causes cerebral ischemia reperfusion injury, a large amount of oxygen free radicals are generated in the process of ischemia reperfusion, and will cause lipid peroxidation, and generate the corresponding products MDA, Ox-LDL[11]. At the same time, antioxidant enzymes SOD, GSH-Px, and Prdx6 are continuously consumed in the process of eliminating oxygen free radicals[12]. In the study, analysis of oxidative stress molecules after cerebral ischemia reperfusion injury showed that the contents of oxidation products in brain tissue of model group increased significantly while the contents of antioxidant enzymes decreased significantly. This indicates that there is significant oxidative stress response in brain tissue during the ischemia reperfusion process. Ticagrelor treatment of cerebral infarction can not only improve blood perfusion by antiplatelet effect, but can also inhibit cellular uptake of adenosine and increase the content of adenosine in plasma to dilate blood vessels and reduce oxidative stress through the biological effect of adenosine. In order to define whether ticagrelor improved the oxidative stress reaction to alleviate ischemia-reperfusion injury of brain tissue, the contents of oxidative stress molecules in brain tissue after ischemia-reperfusion were analyzed in the study, and the results showed that MDA and Ox-LDL contents in brain tissue of ticagrelor group and clopidogrel group were significantly lower than those of model group while SOD, GSH-Px and Prdx6 contents were significantly higher than those of model group, and these changes in ticagrelor group were more significant than those in clopidogrel group. This means that both ticagrelor and clopidogrel can reduce the oxidative stress response in brain tissue during ischemia reperfusion, and ticagrelor is better than clopidogrel in reducing oxidative stress reaction.

Studies on myocardial ischemia reperfusion have shown that ticagrelor has inhibitory effect on the inflammatory response in the process of myocardial ischemia reperfusion; the levels of a variety of inflammatory factors in serum and myocardial tissue significantly reduce after ticagrelor intervention. The inflammatory response mediated by the nuclear transcription factor NF- $\kappa$ B plays an important role in the process of cerebral infarction and cerebral ischemia reperfusion injury[13]. Ischemia-reperfusion stimulation can cause NF- $\kappa$ B to be massively expressed and transfer into the nucleus, which can be combined with gene promoter regions to increase the expression of inflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$  and IL-6[14,15]. In the study, analysis of the contents of these inflammatory factors in brain tissue and serum showed that the contents of nuclear transcription factor NF- $\kappa$ B and corresponding expression products in brain tissue as well as the contents of corresponding cytokines in serum of model group were significantly higher than those of control group. This indicates that there is significant inflammatory response during the cerebral ischemia reperfusion process. Further analysis of the ticagrelor effect on the inflammatory response in the process of cerebral ischemia reperfusion injury showed that NF- $\kappa$ B, TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in brain tissue as well as TNF- $\alpha$ , IL-1 $\beta$  and IL-6 contents in serum of ticagrelor group and clopidogrel group were significantly lower than those of model group, and these changes in ticagrelor

group were more significant than those in clopidogrel group. This means that both ticagrelor and clopidogrel can improve the brain tissue inflammation during ischemia reperfusion, and ticagrelor is better than clopidogrel in inhibiting inflammation.

Ticagrelor is able to reduce cerebral ischemia reperfusion injury, and is superior to clopidogrel to reduce the cerebral ischemia reperfusion; inhibiting oxidative stress and inflammatory response are the functional molecular mechanisms of ticagrelor.

## References

- [1] Nolte CH, Audebert HJ. Management of acute ischemic stroke. *Dtsch Med Wochenschr* 2015; **140**(21): 1583-1586.
- [2] Lai PM, Du R. Association between s100b levels and long-term outcome after aneurysmal subarachnoid hemorrhage: systematic review and pooled analysis. *PLoS One* 2016; **11**(3): e0151853.
- [3] Niu PP, Guo ZN, Jin H, Xing YQ, Yang Y. Antiplatelet regimens in the long-term secondary prevention of transient ischaemic attack and ischaemic stroke: an updated network meta-analysis. *BMJ Open* 2016; **6**(3): e009013.
- [4] Tada K, Ito K, Hamauchi A, Takahashi K, Watanabe R, Uchida A, et al. Clopidogrel-induced thrombotic microangiopathy in a patient with hypocomplementemia. *Intern Med* 2016; **55**(8): 969-973.
- [5] Wang Y, Minematsu K, Wong KS, Amarenco P, Albers GW, Denison H, et al. Ticagrelor in acute stroke or transient ischemic attack in asian patients: from the SOCRATES trial. *Stroke* 2017; **48**(1): 167-173.
- [6] Li Z, Wang Y, Zhao X, Liu L, Wang D, Wang C, et al. Treatment Effect of clopidogrel plus aspirin within 12 hours of acute minor stroke or transient ischemic attack. *J Am Heart Assoc* 2016; **5**(3): e003038.
- [7] Johnston SC, Amarenco P, Albers GW, Denison H, Easton JD, Evans SR, et al. Ticagrelor versus aspirin in acute stroke or transient ischemic attack. *N Engl J Med* 2016; **375**(1): 35-43.
- [8] Zhai Ting, Hua Sheng-yu. Research advances in platelet ADP receptors and their antagonists. *Tianjin J Tradit Chin Med* 2017; **34**(4): 280-284.
- [9] Findik O, Kunt AT, Yazir Y, Yardimoglu M, Yilmaz SG, Aydin U, et al. Ticagrelor attenuates apoptosis of lung and myocardial cells induced by abdominal aorta ischemia/reperfusion. *In Vivo* 2016; **30**(3): 243-249.
- [10] Liu Xiao-Gang, Liu Yu-Feng, Gu Ye, Hu Li-Qun. Effect of different p2y12 receptor antagonist on ischemic reperfusion inflammatory factors and myocardial infarct size in rats. *Chin J Arterioscl* 2016; **24**(6): 571-575.
- [11] Atik I, Kozaci N, Beydilli I, Avci M, Ellidag H, Kesapli M. Investigation of oxidant and antioxidant levels in patients with acute stroke in the emergency service. *Am J Emerg Med* 2016; **34**(12): 2379-2383.
- [12] Lorente L, Martin MM, Perez-Cejas A, Abreu-Gonzalez P, Ramos L, Argueso M, et al. Association between total antioxidant capacity and mortality in ischemic stroke patients. *Ann Intensive Care* 2016; **6**(1): 39.
- [13] Simani L, Naderi N, Khodaghali F, Mehrpour M, Nasoohi S. Association of long-term atorvastatin with escalated stroke-induced neuroinflammation in rats. *J Mol Neurosci* 2017; **61**(1): 32-41.
- [14] Ge Jian-bin, Lu Hong-jian, Song Xin-jian, Li Mei, Chen Dan-dan, Wu Feng. Protective effects of LBP on cerebral ischemia reperfusion injury in mice and mechanism of inhibiting NF- $\kappa$ B, TNF-, IL-6 and IL-1 $\beta$ . *China J Chin Materia Medica* 2017; **42**(2): 326-331.
- [15] Xu R, Yin X, Xu W, Jin L, Lu M, Wang Y. Assessment of carotid plaque neovascularization by contrast-enhanced ultrasound and high sensitivity C-reactive protein test in patients with acute cerebral infarction: a comparative study. *Neurol Sci* 2016; **37**(7): 1107-1112.