BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1994 Mar; 15 (2): 133—135

Inhibitory effect of tetrahydroberberine on platelet aggregation and thrombosis

XUAN Bo, WANG Wei[1], LI De-Xin (*Department of Pharmacology, [1]Department of Pathology, Nanjing Medical College, Nanjing 210029, China*)

ABSTRACT Tetrahydroberberine (THB), an alkaloid extracted from *Corydalis ambigua*, inhibited the rabbit platelet aggregation triggered by arachidonic acid (AA), ADP, and collagen with IC_{50} of 0.86, 1.31, and 1.10 mmol·L^{-1}, respectively. THB reduced the thromboxane B_2 (TXB_2) generation in rabbit platelet-rich plasma triggered by AA. THB 30 mg·kg^{-1}·d^{-1} ip for 3 or 5 d restrained the ADP-induced platelet aggregation in rats. THB 30 mg·kg^{-1}·d^{-1} ip for 1, 3, or 5 d inhibited the AA-induced platelet aggregation in rats. THB 15—30 mg·kg^{-1} iv showed an inhibition of venous thrombosis in rats. The results show that THB is a potent inhibitor of platelet aggregation in *vitro* and in *vivo* and is a promising antithrombotic drug.

KEY WORDS berbines; platelet aggregation; thromboxane B_2; thrombosis; aspirin; arachidonic acid; adenosine diphosphate; collagen

Tetrahydroberberine (THB) is an alkaloid extracted from Chinese herb, *Corydalis ambigua*. Our previous studies showed that THB protected ischemic myocardium[1,2]. There is a close relation between the function of platelet and myocardial infarction. This study was designed to disclose the effects of THB on platelet aggregation and thrombosis in rabbits and rats using aspirin (Asp) as reference.

MATERIALS

THB (North-East Pharmaceutical Factory) was dissolved in H_2SO_4 0.1 mmol·L^{-1} and diluted with normal saline (NS) adjusted to pH 5.0. Adenosine diphosphate (ADP) and AA were products of Sigma Co. TXB_2 RIA kit was purchased from Suzhou Medical College. Asp was dissolved in NS.

Received 1993-03-05 Accepted 1993-11-10

METHODS AND RESULTS

Effect on platelet aggregation *in vitro* Platelet-rich plasma (PRP) was obtained from blood of ♀ rabbits weighing 2.5±*s* 0.4 kg, anticoagulated with sodium citrate (3.8 %, 1:9, vol/vol), and centrifuged at 190×*g* for 8 min. The remaining red blood cell (RBC) precipitate of the sample was further centrifuged at 1800×*g* for 20 min to get platelet-poor plasma (PPP). The platelet counts of each PRP were adjusted to 4×10^8·ml^{-1}. The blood platelet aggregation test was performed with ADP[3]. PRP 0.3 ml was placed in a cuvette and stirred with drug or control solution at 37 C for 5 min, then the aggregation agent 30 μl was added (final concentration: AA 0.78 mmol·L^{-1}, ADP 2 μmol·L^{-1}, and collagen 30 μg·ml^{-1}). Aggregation was measured with a platelet aggregometer (Model: PAM-2). The transmission at maximal aggregation after an aggregating agent was recorded. THB caused a concentration-dependent inhibition of platelet aggregation. IC_{50} values for THB inhibiting platelet aggregation induced by AA, ADP, and collagen were 0.86, 1.31, and 1.10 mmol·L^{-1}, respectively. THB was more potent in inhibiting platelet aggregation induced by AA than by ADP and collagen (Tab 1).

Effect on platelet aggregation *in vivo*

Tab 1. Effects of tetrahydroberberine (THB) on rabbit platelet aggregations *in vitro*. $n=6-7$, $\bar{x}\pm s$. $^cP<0.01$ *vs* control.

Drug/mg·ml^{-1}		Platelet aggregation (% of full scale)	Inhibition (%)
Aggregation induced by AA 0.78 mmol·L^{-1}			
Control	0	71±4	—
Asp	0.10	24±9^c	—
THB	0.17	54±4^c	24
	0.33	26±8^c	63
	0.50	11±4^c	84
Aggregation induced by ADP 2 μmol·L^{-1}			
Control	0	65±7	—
Asp	1.00	10±4^c	—
THB	0.33	44±4^c	33
	0.50	26±7^c	59
	0.67	12±6^c	82
Aggregation induced by collagen 30 μg·ml^{-1}			
Control	0	55±7	—
Asp	1.00	7±3^c	—
THB	0.33	31±3^c	44
	0.50	18±4^c	67
	0.67	4±2^c	93

Sprague-Dawley rats weighing 230 ± s 24 g were injected with control solution and THB 30 mg·kg^{-1}·d^{-1} ip for 1, 3, or 5 d. After 0.5 h, the rats were anesthetized by ip urethane 1 g·kg^{-1} and blood was obtained from abdominal aorta. PRP and the blood platelet aggregation test were made as described above. THB 30 mg·kg^{-1}·d^{-1} ip for 3 or 5 d obviously inhibited the aggregation induced by ADP 2 μmol·L^{-1}. THB 30 mg·kg^{-1}·d^{-1} ip for 1, 3, or 5 d also dramatically reduced the platelet aggregation induced by AA 0.78 mmol·L^{-1} (Tab 2).

Effect on TXB_2 generation in PRP PRP was obtained from ♂ rabbits weighing 2.3±s 0.2 kg. PRP 0.3 ml and control or drug solution 10 μl were incubated at 37 °C for 5 min. AA (0.78 mmol·L^{-1}) was added. The mixture was stirred for 5 min, and then the reaction was terminated by placing the tube in an ice bath. TXB_2 in the supernatant was assayed using the RIA kit[4] after 1800×*g* for 20 min. THB 0.17—0.33 mg·ml^{-1}, similar to Asp (0.1 mg·ml^{-1}), markedly inhibited the TXB_2 generation in rabbit PRP induced by AA (Tab 3).

Tab 2. Effects of tetrahydroberberine (THB) 30 mg·kg^{-1}·d^{-1} ip on platelet aggregation induced by adenosine diphosphate (ADP) or arachidonic acid (AA). $n=10$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Drug/ 30 mg·kg^{-1}·d^{-1}		Plateler aggregation (% of full scale) ADP (2 μmol·L^{-1})	AA (0.78 mmol·L^{-1})
Control		64±6	77±8
THB	× 1 d	61±12^a	67±4^c
	× 3 d	47±13^c	64±6^c
	× 5 d	55±7^c	68±8^b

Tab 3. Effects of tetrahydroberberine (THB) on AA-induced TXB_2 in rabbit platelet-rich plasma. $n=7$, $\bar{x}\pm s$. $^cP<0.01$ *vs* control.

Drug/mg·ml^{-1}		Thromboxane B_2/pg·ml^{-1}
Control	0	5 793±1 165
Asp	0.10	1 413±597^c
THB	0.17	3 258±731^c
	0.33	2 183±425^c

Effect on venous thrombosis The model of venous thrombosis was derived from Reyers *et al*[5]. SD rats, weighing 220±*s* 18 g, were anesthetized by ip urethane 1 g·kg^{-1}. Half an hour before operation, the drug or control solution was injected via the sublingual vein. After 15 min, a midline abdominal incision was made and a tight ligature was applied to the inferior vena cava below the left renal vein level. The abdomen was then closed. Two

hours after the ligation, the abdomen was reopened. The thrombus in the inferior vena cava was gently picked out and weighed. It was then placed at 50 C for 20 h before measuring the dry weight. Similar to Asp, THB inhibited the formation of venous thrombosis (Tab 4).

Tab 4. Effects of tetrahydroberberine (THB) on venous thrombosis in rats. $n = 7$, $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Drug/ mg·kg^{-1}		Thrombus weight/mg Wet	Inhibition (%)	Dry	Inhibition (%)
Control	0	9.0±2.6	—	2.8±0.8	—
ASA	30	6.2±1.8^b	31.1	2.0±0.6^b	28.6
THB	15	5.8±2.0^b	35.6	1.8±0.6^b	35.7
	30	4.7±1.1^c	47.8	1.5±0.3^c	46.4

DISCUSSION

The results of the present study characterized THB as a potent inhibitor of platelet aggregation induced by a variety of agonists both *in vitro* and *in vivo*. THB was more effective on platelet aggregation induced by AA than by ADP in *vivo* as well as *in vitro*. THB 0.17 — 0.33 mg·ml^{-1} markedly reduced the TXB_2 generation in rabbit PRP induced by AA. The doses of THB (0.17 — 0.33 mg·ml^{-1}) were equal to those of THB in which THB inhibited platelet aggregation induced by AA. These findings revealed that THB decreased the synthesis of TXB_2, a more stable metablite of TXA_2, indicating that THB may affect the activity of cyclooxygenase or TXA_2 synthetase at this concentration. In order to prove THB's antithrombotic action, we studied THB with experimental model of venous thrombosis. The present data support that THB limits extension of venous thrombi.

The present study showed that THB is a potent antiplatelet and antithrombotic drug. The main mechanism may be due to its influence on AA metabolism. Therefore, THB appears to be a promising remedy in preventing myocardial reinfarction, stroke, or transient ischemic attacks.

REFERENCES

1 Xuan B, Li DX, Wang W. Protective effects of tetrahydroprotoberbernes on experimental myocardial infarction of rats. Acta Pharmacol Sin 1992; **13**: 167—71.

2 Zhou J, Xuan B, Li DX. Effects of tetrahydroberberine on ischemic and reperfused myocardium in rats. Acta Pharmacol Sin 1993; **14**: 130—3.

3 Born GVR. Aggregation of blood platelet by adenosine diphosphate and its reversal. Nature 1962; **194**: 927—9.

4 Wang ZY, Chen DC, He Y, Ruan CG. Radioimmunoassay of ^{125}I-laveled thromboxane. Acta Acad Med Suzhou 1986; **6**: 13—6.

5 Reyers I, Mussoni L, Donati MB, de Gaetano G. Failure of aspirin at different doses to modify experimental thrombosis in rats. Thromb Res 1980; **18**: 669—74.

133-135

四氢小檗碱对血小板聚集和血栓形成的抑制作用

R 971 965.2

宣 波, 王 伟[1], 李德兴 (南京医学院药理教研室, [1]病理教研室, 南京210029, 中国)

摘要 SD大鼠每天ip THB 30 mg·kg^{-1}共3 d或5 d后, 使ADP诱导的血小板聚集程度降低; 同剂量ip 1 d, 3 d或5 d后, 使AA诱导的血小板聚集程度降低. THB在体外抑制AA, ADP和胶原诱导的兔血小板聚集, IC_{50}分别为0.86, 1.31和1.10 mmol·L^{-1}. THB能抑制AA诱导的兔血小板生成血栓素B_2. THB 15 — 30 mg·kg^{-1} iv明显抑制大鼠静脉血栓的形成.

关键词 小檗碱; 血小板聚集; 血栓素B_2; 血栓形成; 阿司匹林; 花生四烯酸; 腺苷二磷酸; 胶原