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# Effect of dipfluzine on platelet aggregation and thrombus formation

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**ABSTRACT** Dipfluzine (Dip) is a novel diphenylpiperazine calcium channel blocker first synthesized in China. Effects of Dip on experimental thrombosis and platelet aggregation were studied *in vitro* and *in vivo* compared with cinnarizine (Cin). Dip 1 and 2 $mg \cdot kg^{-1}$ iv and incubated in 1—100 $\mu mol \cdot L^{-1}$ *in vitro* inhibited dose- or concentration-dependent rabbit platelet aggregation induced by ADP and by arachidonic acid (AA), respectively. Dip 2.5—10 $mg \cdot kg^{-1}$ iv and 50—100 $mg \cdot kg^{-1}$ ip inhibited the thrombosis in rats. Dip 10 $mg \cdot kg^{-1}$ iv and 200 $\mu mol \cdot L^{-1}$ depressed the *in vitro* thrombosis. These results suggest that attenuation of disturbed platelet-vessel wall reaction associated with platelet activation and vasoconstriction may be a main factor involved in the antithrombotic action of Dip, and that the effects of Dip were more potent than those of Cin.

**KEY WORDS** dipfluzine; cinnarizine; platelet aggregation; thrombosis

Platelets are deposited in the microvasculature during focal cerebral ischemia[1]. Abundant platelet-released products at the site of thrombus formation may contribute to ischemic brain injury[2]. Dipfluzine {1-diphenyl-methyl-4-[3-(4-fluorobenzoyl)]-piperazine, Dip}, a new derivative of cinnarizine (Cin) first developed by Department of Chemistry, Beijing University, provided potent protection against the symptoms and electrolyte alterations after bilateral carotid artery ligation[3]. This study was to determine whether Dip exerted some effects against thrombosis and platelet aggregation.

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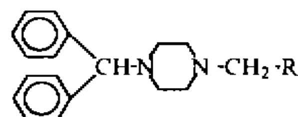

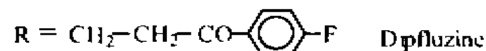


## MATERIALS AND METHODS

**Chemicals** Dip and Cin synthesized by Department of Chemistry, Beijing University, were dissolved in 2% tartaric acid solution containing 20% dimethylacetamide (solvent). Drugs were injected iv or ip and a same amount of the solvent was used as control. Solvent, drugs, and stock solutions of arachidonic acid (AA, Sigma) and adenosine 5'-diphosphate (ADP, Sigma) were diluted to phosphate buffer (pH 7.4) 0.1 $mmol \cdot L^{-1}$ before use.

**Experiments on platelet aggregation**

***In vitro*** Platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were prepared from the blood of New Zealand rabbits ($n=8$, either sex, 2.3 $\pm s$ 0.4 kg), anticoagulated with 3.8% sodium citrate (9:1, vol:vol). The blood or PRP was exposed only to plastic or siliconized tube. The platelet counts of each PRP were adjusted to $4 \times 10^8$ $cells \cdot ml^{-1}$.

PRP 0.8 ml was placed in a cuvette and stirred with 0.1 ml solvent or drugs (final concentrations of Dip or Cin: 0.1, 1, 10, or 100 $\mu mol \cdot L^{-1}$) at 37 ℃ for 10 min, then aggregating agent 0.1 ml was added (final concentration: AA 200 $\mu mol \cdot L^{-1}$ and ADP 9.3 $\mu mol \cdot L^{-1}$). The curves of AA- and ADP-induced platelet aggregation were recorded in an aggregometer (model BS-631, Beijing Biochemical Instrument Factory). The aggregation rate was measured by turbidimetry[4], and the percentage of inhibition of

platelet aggregation rate was calculated in comparison with the aggregation rates obtained in the presence of Dip, Cip, or solvent.

*In vivo* New Zealand rabbits ($n$ = 28, either sex, 2.8 ± $s$ 0.5 kg) divided in 4 groups were injected iv solvent 1 ml·kg$^{-1}$, Dip 1 and 2 mg·kg$^{-1}$, and Cin 2 mg·kg$^{-1}$, respectively. Fresh blood was obtained from each rabbit through cardiac puncture just before and 15 min after iv drugs. PRP preparation and platelet aggregation were made according to the procedures mentioned above.

**Experiments on thrombosis**

*In vitro* Wistar rats ($n$=42, either sex, 258 ± $s$ 33 g) were divided into 7 groups, and injected iv solvent 1 ml·kg$^{-1}$, Dip or Cin 2.5, 5, and 10 mg·kg$^{-1}$. Fresh blood 1.8 ml was obtained from each rat through cardiac puncture 15 min after iv drugs and placed in a siliconized plastic ring. The *in vitro* thrombosis of rats was performed on Chandler's rotating loop system[5].

Fresh blood 1.8 ml was obtained from New Zealand rabbits through cardiac puncture and placed in a siliconized plastic ring with 0.1 ml solvent or drugs (final concentrations of Dip or Cin: 2, 20, or 200 μmol·L$^{-1}$). The *in vitro* thrombosis of rats was induced in Chandler's rotating loop system[5].

*In vivo* Wistar rats ($n$=70, either sex, weighing 310 ± 19 g) were anesthetized with 2.5 % sodium pentobarbital 50 mg·kg$^{-1}$ ip. An extracorporeal shunt (a casing pipe filled with saline, which consisted of 3 plastic pipes and a preweighed 5 cm silk thread was inserted into the middle plastic pipe) was placed between the right jugular vein and left carotid artery. The circulation of blood was established 15 min after iv Dip or Cin and induced an increase in the weight of the thread due to deposition of thrombus. After 15 min, the silk thread was weighed and thrombus wet weight was calculated[6].

Wistar rats ($n$=30, either sex, weighing 293 ± $s$ 26 g) were anesthetized with sodium pentobarbital 1 h after iv solvent or Dip. The right carotid artery was isolated up to 15 mm long. Thrombosis was induced by electric stimulation of 1.6 mA for 7 min. Thrombosis was indicated by occlusion time which was determined by a sudden decrease of the arterial surface temperature after blocking the blood flow by the thrombus induced[7].

## RESULTS

**Experiments on platelet aggregation**

*In vitro* The minimal effective concentrations of Dip were 1 μmol·L$^{-1}$ in inhibiting the ADP-induced aggregation and 10 μmol·L$^{-1}$ in inhibiting AA-induced aggregation. But the same concentrations of Cin did not have such an effect (Tab 1).

**Tab 1. Effects of Dip and Cin incubated in platelet-rich plasma on ADP- and arachidonic acid (AA)-induced rabbit platelet aggregation.** $n$=7, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* solvent.

| Drug/μmol·L$^{-1}$ | | Platelet aggregation rate ADP | AA |
|---|---|---|---|
| Solvent | | 0.49 ± 0.04$^a$ | 0.61 ± 0.03$^a$ |
| Dipfluzine | 0.1 | 0.47 ± 0.06$^a$ | 0.61 ± 0.02$^a$ |
| | 1.0 | 0.43 ± 0.03$^b$ | 0.60 ± 0.02$^a$ |
| | 10.0 | 0.43 ± 0.04$^b$ | 0.53 ± 0.06$^b$ |
| | 100.0 | 0.39 ± 0.04$^c$ | 0.45 ± 0.02$^c$ |
| Cinnarizine | 0.1 | 0.46 ± 0.06$^a$ | 0.58 ± 0.05$^a$ |
| | 1.0 | 0.46 ± 0.07$^a$ | 0.59 ± 0.03$^a$ |
| | 10.0 | 0.43 ± 0.05$^b$ | 0.58 ± 0.05$^a$ |
| | 100.0 | 0.42 ± 0.02$^c$ | 0.53 ± 0.05$^b$ |

*In vivo* Dip iv 1–2 mg·kg$^{-1}$ and Cin iv 2 mg·kg$^{-1}$ inhibited AA-induced rabbit platelet aggregation. When administered iv 2 mg·kg$^{-1}$, Dip depressed ADP-induced aggregation, but Cin did not (Tab 2).

**Experiments on thrombosis**

*In vitro* Dip and Cin of each 10 mg·kg$^{-1}$ iv or incubated in 200 μmol·L$^{-1}$ decreased the weights (wet and dry) and length of the thrombus (Tab 3).

*In vivo* Both Dip (2.5–10 mg·kg$^{-1}$) and Cin (5–10 mg·kg$^{-1}$) reduced the thrombus weight dose-dependently. However, when administered iv 2.5 mg·kg$^{-1}$, Dip depressed the thrombosis, but Cin did not (Tab 4).

**Tab 2. Effects of Dip and Cin iv in rabbits on ADP- and AA-induced rabbit platelet aggregation. $n=7$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* solvent.**

| Drug/ mg·kg$^{-1}$ | | | Adenosine diphosphate | | Arachidonic acid | |
|---|---|---|---|---|---|---|
| | | | Aggregation rate | Inhibition rate | Aggregation rate | Inhibition rate |
| Solvent | | Before | 0.50±0.08 | | 0.59±0.05 | |
| | | After | 0.49±0.09 | 0.00±0.02$^a$ | 0.59±0.05 | 0.00±0.03$^a$ |
| Dip | 1 | Before | 0.49±0.06 | | 0.60±0.08 | |
| | | After | 0.48±0.07 | 0.02±0.03$^a$ | 0.55±0.07 | 0.04±0.03$^b$ |
| | 2 | Before | 0.48±0.08 | | 0.57±0.07 | |
| | | After | 0.43±0.08 | 0.04±0.03$^b$ | 0.51±0.09 | 0.07±0.05$^c$ |
| Cin | 2 | Before | 0.47±0.06 | | 0.57±0.07 | |
| | | After | 0.44±0.05 | 0.02±0.04$^a$ | 0.54±0.07 | 0.03±0.02$^b$ |

**Tab 3. Effects of Dip and Cin iv in rats and incubated in rabbit blood on thrombosis *in vitro*. $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$; *vs* solvent.**

| Drug/μmol·L$^{-1}$ | | Thrombus in Chandler's loop Length /cm | Wet weight /mg | Dry weight /mg |
|---|---|---|---|---|
| Injected iv in rats ($n=7$) | | | | |
| Solvent | | 4.6±1.9$^a$ | 139±38$^a$ | 45±13$^a$ |
| Dip | 2.5 | 4.1±1.3$^a$ | 135±42$^a$ | 36±5$^a$ |
| | 5.0 | 3.5±1.6$^a$ | 111±63$^a$ | 32±6$^a$ |
| | 10.0 | 2.7±0.4$^b$ | 85±13$^c$ | 26±5$^c$ |
| Cin | 2.5 | 4.0±1.5$^a$ | 135±26$^a$ | 38±11$^a$ |
| | 5.0 | 3.7±1.1$^a$ | 116±55$^a$ | 30±13$^a$ |
| | 10.0 | 2.8±0.8$^b$ | 76±18$^c$ | 24±6$^c$ |
| Incubated in rabbit blood ($n=10$) | | | | |
| Solvent | | 5.1±2.3$^a$ | 147±53$^a$ | 42±17$^a$ |
| Dip | 2 | 5.6±2.3$^a$ | 140±40$^a$ | 32±9$^a$ |
| | 20 | 5.0±2.5$^a$ | 136±38$^a$ | 30±12$^a$ |
| | 200 | 3.1±1.4$^b$ | 92±28$^b$ | 21±7$^c$ |
| Cin | 2 | 4.3±1.0$^a$ | 136±43$^a$ | 38±8$^a$ |
| | 20 | 4.2±1.6$^a$ | 125±38$^a$ | 31±10$^a$ |
| | 200 | 3.3±0.3$^b$ | 103±23$^b$ | 25±6$^b$ |

Both of Dip (50—100 mg·kg$^{-1}$) and Cin (100 mg·kg$^{-1}$) ip prolonged the occlusion time. When administered ip 50 mg·kg$^{-1}$, Dip inhibited *in vivo* thrombosis, but Cin did not (Tab 5).

**Tab 4. Effects of Dip and Cin iv on thrombosis in rats. $n=10$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$; *vs* solvent.**

| Drug/mg·kg$^{-1}$ | | Thrombus wet weight/mg | Inhibition/% |
|---|---|---|---|
| Solvent | | 42.3±4.9 | |
| Dipfluzine | 2.5 | 35.6±6.3$^b$ | 16.3 |
| | 5.0 | 34.0±3.0$^c$ | 19.6 |
| | 10.0 | 29.7±5.0$^c$ | 29.8 |
| Cinnarizine | 2.5 | 41.6±4.5$^a$ | 1.6 |
| | 5.0 | 35.4±6.3$^b$ | 16.3 |
| | 10.0 | 32.7±2.6$^c$ | 22.6 |

**Tab 5. Effects of Dip and Cin ip on arterial thrombosis in rats. $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$ *vs* solvent.**

| Drug/mg·kg$^{-1}$ | | Occlusion time/min |
|---|---|---|
| Solvent | | 15.2±1.5 |
| Dipfluzine | 50 | 17.5±1.6$^b$ |
| | 100 | 18.2±1.9$^b$ |
| Cinnarizine | 50 | 15.8±1.6$^a$ |
| | 100 | 17.9±1.8$^b$ |

## DISCUSSION

The present study confirmed that Dip, either *in vitro* or *in vivo*, inhibited the ADP- and AA-induced rabbit platelet aggregation and thrombosis in experimental thrombosis models, and these effects of Dip were more potent than those of Cin.

Platelet activation plays an important role

in thrombosis. But we noticed that *in vitro* antithrombotic activity of Dip (Tab 3) was less potent than its antiplatelet effects (Tab 1). The results suggested that inhibitory action of Dip on the platelet activity may not be a primary factor of its antithrombotic action.

The main difference between *in vivo* and *in vitro* thrombosis was that the former involved a disturbed platelet-vessel wall interaction associated with platelet activation and vasoconstriction. In the present study, the inhibitory effects of Dip on *in vivo* thrombosis were more potent than those on *in vitro* thrombosis. In addition, for inhibiting *in vivo* thrombosis Dip was more potent than Cin, and for depressing *in vitro* thrombosis Dip was as potent as Cin. These results suggested that attenuation of disturbed platelet-vessel wall reaction may be a main factor involved in the antithrombotic action of Dip. Considering these together with data of previous studies in which the vasodilatory effect of Dip was more potent than that of Cin either *in vitro*[8] or *in vivo*[9], we suggested that the antithrombotic effect of Dip might be contributable in part to its vasodilatory action.

In view of the fact that a disturbed platelet-vessel wall interaction is crucial in the etiology of cerebral ischemic injury, antiplatelet aggregation and antithrombotic effect of Dip were to be effective in the prevention and treatment of cerebral vascular disease and to be one of the mechanisms by which Dip improved the cerebral ischemia[3].

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## 双苯氟嗪对血小板聚集和血栓形成的影响

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A **摘要** 在体内外实验中观察我国首创合成的新钙拮抗剂双苯氟嗪(Dip)对血小板聚集和实验性血栓形成的作用. Dip iv 1-2 mg·kg$^{-1}$和1-100 μmol·L$^{-1}$体外温育可剂量或浓度依赖性地抑制 ADP 和 AA 诱发的家兔血小板聚集. Dip iv 2.5-10 mg·kg$^{-1}$和 ip 50-100 mg·kg$^{-1}$可抑制大鼠体内血栓形成; Dip iv 10 mg·kg$^{-1}$和200 μmol·L$^{-1}$体外给药可抑制体外血栓形成. 提示，减轻紊乱的血小板与血管壁反应是其抗血栓作用的主要因素，Dip 的作用强于 Cin.

**关键词** 双苯氟嗪; 桂利嗪; 血小板聚集; 血栓形成