# Antiplatelet and Antithrombotic Effects of the Diterpene Spiramine Q from *Spiraea japonica* var. *incisa*

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***Abstract:*** **Spiramine Q, a diterpene, was isolated from a Chinese herbal plant *Spiraea japonica* var. *incisa* Yu. Born's and Wan HY's methods were used to investigate effects of spiramine Q on rabbit platelet aggregation and serotonin release, respectively. Its antithrombotic effect in mice was also evaluated by Myers' method. Spiramine Q selectively inhibited arachidonic acid-induced platelet aggregation *in vitro* or *ex vivo*, and decreased serotonin secretion from rabbit platelets. Spiramine Q (5 mg/kg) decreased the mouse mortality caused by injection of 80 mg/kg arachidonic acid in the tail vein. The results suggested that spiramine Q showed potent antiplatelet and antithrombotic activites.**

*Spiraea japonica* var. *incisa* Yu, a Chinese herbal plant abundant in Yunnan Province, has been used for cerebral hemiplegia, anti-inflammation and analgesia in folk and ethnic medicine (1). Spiramine Q, a $C_{20}$-skeleton diterpenoid alkaloid (Fig. **1**), was isolated from this source (2). To screen new drugs that can prevent and cure cardiac and cerebral thromboembolic diseases in combination with platelet hyperfunction, we found that spiramine Q showed antiplatelet and antithrombotic activities.

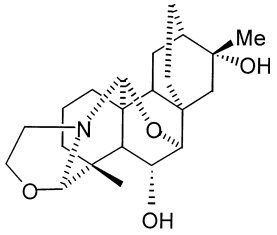


**Fig. 1** Structure of spiramine Q.

*In vitro*, spiramine Q significantly inhibited arachidonic acid-induced platelet aggregation in a concentration-dependent manner. The $IC_{50}$ value was 18.7 ± 4.1 $\mu$M. However, it had no effect on adenosine diphosphate- or platelet activating factor-induced aggregation. Aspirin significantly suppressed arachidonic acid-induced aggregation with an $IC_{50}$ of 28.4 ± 5.2 $\mu$M (Table **1**).

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Spiramine Q at 2.5 mg/kg (6.7 $\mu$M/kg) administered intravenously markedly inhibited arachidonic acid-induced aggregation by 92.8 ± 2.3% 10 min after administration. This strong inhibition lasted for 120 min ($P < 0.05$ vs. 0 min). Spiramine Q (5 mg/kg, 13.3 $\mu$M/kg) had an inhibitory effect within 10 – 240 min after injection (Table **2**). Neither spiramine Q nor aspirin had any influence on adenosine diphosphate- or platelet activating factor-induced platelet aggregation (data not presented).

Spiramine Q selectively reduced arachidonic acid-caused platelet serotonin release with a lower $IC_{50}$ value of 21.7 compared to that of aspirin (33.4 $\mu$M) (Table **3**). Both of them, however, had no influence on serotonin release caused by adenosine diphosphate or platelet activating factor (data not shown).

Within 15 min after injection of 80 mg/kg arachidonic acid in the mouse tail vein, the mortality was 86.6, 13.3, and 26.7% in control group, 5 mg/kg (13.3 $\mu$M/kg) spiramine Q and 5 mg/kg (27.8 $\mu$M/kg) aspirin treated groups, respectively (Table **4**).

Spiramine Q inhibited platelet aggregation induced by arachidonic acid but showed no significant inhibition on adenosine diphosphate- or platelet activating factor-induced aggregation *in vitro* or *ex vivo*. This suggested that spiramine Q exerted a selective inhibitory effect on arachidonic acid-induced platelet aggregation. *In vitro*, the $IC_{50}$ of spiramine Q was lower than that of aspirin and *ex vivo* spiramine Q at 5 mg/kg (13.3 $\mu$M/kg) produced a nearly equal inhibitory effect to that of aspirin 10 mg/kg (55.6 $\mu$M/kg). Obviously, spiramine Q as compared with aspirin was more active in inhibiting platelet aggregation based on their molar levels per kg body weight.

On activation, serotonin is released from platelets leading to further aggregation. In this study, spiramine Q selectively suppressed arachidonic acid-induced serotonin secretion from platelets in a concentration-dependent manner, and showed a more potent effect than aspirin. This might contribute to its stronger antiplatelet activity.

At 13.3 $\mu$M/kg spiramine Q markedly decreased the mouse mortality challenged by intravenous injection of arachidonic acid in the tail vein, giving a lower mortality than that of 27.8 $\mu$M/kg aspirin. This demonstrated that spiramine Q had a stronger antithrombotic effect. Sudden death induced by arachidonic acid is probably due to pulmonary thrombosis and ensuing hypoxia, as massive platelet aggregation occurs in the pulmonary vessels (3). Thromboxane $A_2$ plays an important role in arachidonic acid-induced platelet aggregation (4), and is a major mediator in arachidonate-mediated thrombosis (5). Spiramine Q has a potent inhibition effect on platelet aggregation both *in vitro* and *ex vivo*. We speculate that the antithrombotic effect of spiramine Q might result from its antiplatelet effect. Further studies are required to evaluate the mechanism of the antiplatelet effect of spiramine Q. In conclusion, spiramine Q, a diterpene isolated from *Spiraea japonica* var. *incisa* Yu, should become a promising plant drug with antiplatelet and antithrombotic activities.

## Materials and Methods

The roots of *Spiraea japonica* var. *incisa* Yu were collected in Dali City, Yunnan Province, China. The voucher specimen was

**Table 1** Effects of spiramine Q on rabbit platelet aggregation induced by arachidonic acid (AA), adenosine diphosphate (ADP), or platelet-activating factor (PAF) *in vitro*.

| Drug/$\mu$M | Platelet aggregation/% AA | | ADP | | PAF | |
|---|---|---|---|---|---|---|
| | spiramine Q | aspirin | spiramine Q | aspirin | spiramine Q | aspirin |
| 0 | 72.5 ± 3.6 | 71.4 ± 2.5 | 62.3 ± 4.1 | 59.4 ± 2.8 | 61.5 ± 3.7 | 62.6 ± 2.9 |
| 5 | 65.7 ± 2.8 | 69.4 ± 2.8 | 62.1 ± 3.4 | 58.3 ± 2.1 | 59.7 ± 2.5 | 60.7 ± 3.2 |
| 10 | 47.4 ± 3.2* | 56.5 ± 3.5* | 61.5 ± 2.5 | 57.3 ± 3.4 | 60.4 ± 2.6 | 61.3 ± 2.6 |
| 20 | 32.1 ± 2.1** | 42.6 ± 2.5* | 60.3 ± 3.2 | 58.4 ± 2.5 | 59.4 ± 2.7 | 62.2 ± 2.3 |
| 40 | 26.5 ± 2.8** | 30.5 ± 2.4** | 59.5 ± 2.8 | 60.1 ± 1.7 | 59.7 ± 3.3 | 59.4 ± 3.6 |
| 80 | 12.3 ± 2.3** | 25.7 ± 1.8** | 61.2 ± 1.7 | 57.4 ± 3.1 | 58.9 ± 3.6 | 61.8 ± 2.7 |

Expressed as mean ± SD of 5 rabbits.

Values are significantly different from the control at *P < 0.05, **P < 0.01 by Student's t-test.

**Table 2** Effects of spiramine Q intravenously on rabbit platelet aggregation induced by arachidonic acid.

| Drug/mg/kg | Platelet aggregation/% 0 | 10 | 30 | 60 | 90 | 120 | 180 | 240 min |
|---|---|---|---|---|---|---|---|---|
| Spiramine Q | | | | | | | | |
| 2.5 | 74.5 ± 2.6 | 4.5 ± 1.5** | 2.5 ± 1.2** | 5.0 ± 1.8** | 14.0 ± 2.5** | 45.8 ± 3.2* | 67.2 ± 3.5 | 71.5 ± 2.1 |
| 5.0 | 75.5 ± 3.4 | 2.5 ± 1.2** | 0.0 ± 0.0** | 2.5 ± 1.5** | 4.8 ± 1.7** | 19.6 ± 2.2** | 40.5 ± 3.2* | 59.8 ± 2.7* |
| Aspirin | | | | | | | | |
| 10.0 | 72.6 ± 2.8 | 2.5 ± 1.7** | 0.0 ± 0.0** | 3.6 ± 1.1** | 5.4 ± 1.9** | 22.6 ± 2.3** | 42.6 ± 3.1* | 56.4 ± 3.6* |

Expressed as mean ± SD of 6 rabbits.

Values are significantly different from 0 min at *P < 0.05, **P < 0.01 by Student's t-test.

**Table 3** Effect of spiramine Q on arachidonic acid-induced serotonin release from rabbit platelets *in vitro*.

| Drug/$\mu$M | Serotonin/nM/$10^9$ platelets Spiramine Q | Aspirin |
|---|---|---|
| 0 | 1.9 ± 0.2 | 1.8 ± 0.2 |
| 5 | 1.6 ± 0.2 | 1.7 ± 0.4 |
| 10 | 1.2 ± 0.1* | 1.3 ± 0.2* |
| 20 | 0.9 ± 0.1* | 1.1 ± 0.1* |
| 40 | 0.6 ± 0.1** | 0.7 ± 0.2** |
| 80 | 0.4 ± 0.1** | 0.6 ± 0.1** |

Expressed as mean ± SD of 5 rabbits.

Values are significantly different from the control at *P < 0.05, **P < 0.01 by Student's t-test.

**Table 4** Preventive effect of spiramine Q against mouse sudden death caused by injection of 80 mg/kg arachidonic acid in the tail vein.

| Drug | Dose (mg/kg) | Died/total | Mortality (%) |
|---|---|---|---|
| Saline | – | 13/15 | 86.6 |
| Spiramine Q | 5 | 2/15 | 13.3* |
| Aspirin | 5 | 4/15 | 26.7* |

Saline and the drugs were given intraperitoneally 30 min before injection of 80 mg/kg arachidonic acid in the mouse tail vein.

"Died" denotes the number of animals that died 15 min after injection of arachidonic acid; "total" denotes the number of animals used in the study.

*P < 0.05, significantly different from the result for the same volume of saline group.

deposited in the Herbarium of the Department of Phytochemistry, Kunming Institute of Botany, the Chinese Academy of Sciences, P. R. China (Number: 95078). Spiramine Q was supplied by Professor Xiaojiang Hao (HPLC; purity >97.8%). Its optical rotation value is $[\alpha]_D^{25}$: $-70^\circ$ ($CHCl_3$, *c* 0.84) (2). It was dissolved in distilled water, adjusting its pH to about 6.5 with 0.1 MHCl.

Arachidonic acid, adenosine diphosphate, and platelet-activating factor were all from Sigma. Arachidonic acid was diluted in 100 mM $Na_2CO_3$ before use. Adenosine diphosphate was dissolved in phosphate buffer solution. Platelet activating factor was dissolved in Tris-NaCl buffer containing 0.25% bovine serum albumin. Serotonin was dissolved in distilled water, as a standard.

Healthy rabbits of either sex weighing 2.0–3.0 kg were used for platelet aggregation and male ICR mice (about 25 g) for thrombosis. Blood from the rabbit carotid artery was anticoagulated with 3.8% sodium citrate solution (9/1, v/v). Platelet-rich plasma and platelet-poor plasma were obtained by the centrifuging the blood at 1000 and 3000 rev/min, respectively, for 10 min. Platelet-poor plasma was used as the reference for platelet aggregation or to adjust the platelet count in platelet-rich plasma ($5 \times 10^{11}$ cell/L).

Platelet aggregation in plasma was monitored by Born's method (6) with an aggregometer (model SH-93, Shanghai Biochem. Equipment Co.) at 37 °C. The maximal aggregation was recorded (final concentration: arachidonic acid 0.35 mM, adenosine diphosphate 3 $\mu$M, and platelet activating factor

7.2 nM). Percentage inhibition by drugs was calculated according to the formula:

Inhibition of aggregation (%) = (A – B) / A × 100

where A = maximum change of turbidity in control added and B = maximum change of turbidity in sample added.

Rabbits were randomly divided into 4 groups of 6. Group A: the same volume of saline, groups B and C: 2.5 and 5 mg/kg spiramine Q, and group D: 10 mg/kg aspirin. All the above substances were injected in rabbit ear marginal vein, respectively. Platelet-rich plasma and platelet-poor plasma were prepared before injection and 10, 30, 60, 90, 120, 180, and 240 min after injection. Platelet aggregation was monitored.

Serotonin release was measured after (7) with a spectrofluorophotometer (model RF5000, SHIMADZU Co, Japan).

Mice were divided into 3 groups of 15. Groups A and B: 5 mg/kg spiramine Q and aspirin, respectively, and group C: 0.9% of saline. All the above substances were intraperitoneally injected. After 30 min, 80 mg/kg arachidonic acid was injected into the mouse tail vein. The mortality was evaluated within 15 min after injection of arachidonic acid according to Myers's method (8). The difference in the data between the treated groups and control group was analyzed by $X^2$ test.

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