# Antiinflammatory Effect of Tetramethylpyrazine and Ferulic Acid

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**Tetramethylpyrazine (TMP) is one of the alkaloids contained in *Ligusticum wallichii* FRANCH (*L. wallichii*). Ferulic acid (FA) is a phenolic compound contained in *L. wallichii* and *Angelica sinensis* (OLIV.) DIELS (*A. sinensis*). The present study was carried out to examine the antiinflammatory effect and to elucidate the mode of the effect of TMP and FA. Both compounds significantly inhibited the edema induced by carrageenin, the increase of the dye leakage induced by acetic acid and the granuloma formation induced by cotton pellet. And also, TMP and FA inhibited the number of writhes induced by acetic acid. From these results, it is suggested that both compounds have the antiinflammatory effect and the analgesic effect, and both compounds exert an antiinflammatory effect at the early and the late stages of processes in the inflammatory pathology.**

**Keywords** antiinflammatory effect; tetramethylpyrazine; ferulic acid; *Ligusticum wallichii*; *Angelica sinensis*

*Ligusticum wallichii* FRANCH (*L. wallichii*) and *Angelica sinensis* (OLIV.) DIELS (*A. sinensis*) have been used in traditional Chinese medicine, especially as a homeostatic remedy for women's disorders and menoxenia, as well as an analgesic for dysmenorrhea, *etc.* in China.

In Japan, Tanaka *et al.* reported that the aqueous extract of *Angelica acutiloba* KITAGAWA (*A. acutiloba*) administered orally to mice inhibited writhes and the increase of vascular permeability induced by acetic acid, and the inhibitory effects were not due to the essential oil it contained.[1] Ke *et al.* reported that the aqueous extract of *L. wallichii* and *Cnidum officinale* MAKINO (*C. officinale*) administered orally inhibited writhes induced by acetic acid to mice and the edema induced by carragennin to rats.[2] On the other hand, Yamahara *et al.* reported that the methanol extract of *C. officinale* and *A. acutiloba* administered orally to mice inhibited writhes, but did not inhibit the increase of vascular permeability induced by acetic acid.[3]

Tetramethylpyrazine (TMP) is one of the alkaloids contained in *L. wallichii*. Ferulic acid (FA) is a phenolic compound contained in *L. wallichii* and *A. sinensis*.[4,5] TMP and FA showed inhibition of the uterus contractions and their combination synergically inhibited the contraction. TMP mainly acted on $\alpha$-adrenoceptor directly and FA partly acted on the oxytocin–receptor system in the uterus.[6,7]

Although many pharmacological studies of both compounds have been reported,[8-15] there have been very few antiinflammatory studies on this subject.

The present study was carried out to examine the antiinflammatory effect and to elucidate the mode of the effect of both compounds.

## Experimental

**Materials** TMP (Beijing Institute of the Pharmaceutical Industry of China) was dissolved in water, and FA (Shanghai First Reagent Factory of China) and indomethacin (Sigma) were dissolved with the same equivalent of sodium hydroxide solution in water for oral administration.

**Hind-Paw Edema Test** Male Wistar rats weighing 200—250 g were fasted for 16 h prior to experiments, but were supplied with water *ad libitum*. Carrageenin (Picnin-A, Zushikagaku Lab., Inc.) was suspended in 0.9% saline to make 1% (w/v) suspension.

The 0.05 ml suspension of carrageenin was injected subcutaneously into the right hind-paw 30 min after the test solutions had been administered orally.

The volume of the hind-paw was measured prior to administration of the test solutions by a water displacement transducer (LPU-0.1 A, Nihon Kohden). The hind-paw volumes were measured 30 min and 1 h after the suspension of carrageenin had been administered and then at intervals of 1 h for up to 6 h.

Control rats were treated similarly except that they received an equal volume of the vehicle. The results were expressed as percent increase in hind-paw volume due to swelling (%), as compared with the initial hind-paw volume.

**Acetic Acid-Induced Vascular Permeability Test** Male ddY mice weighing 20—25 g were fasted for 10 h prior to experiments, but were supplied with water *ad libitum*. Four percent pontamine sky blue solution in normal saline (w/v) was injected intravenously into the tail vein 40 min after the oral administration of test solutions.

After 30 min, 1% acetic acid solution in normal saline (w/v) was injected intraperitoneally, and after 20 min, mice were killed by dislocation of the neck and the abdominal wall was cut to expose the entrails. After washing of the entrails with saline, the washings were filtered through glass wool and collected in test tubes. To clear any turbidity due to protein, 0.1 ml of 1 N NaOH solution was added to each tube, and the absorbance was read at 590 nm in a spectrophotometer (model 200-10, Hitachi). Control mice were treated similarly, except that they received an equal volume of the vehicle.

The vascular permeability effects were expressed in terms of the amount of total dye ($\mu$g/animal) which leaked into the intraperitoneal cavity.

**Cotton Pellet Granuloma Inhibition Test** Male Wistar rats weighing 200—250 g were used. The rats were anesthetized with ether. After being sterilized in 2% acrinol, the back skin was cut and a sterile cotton pellet about $20 \pm 1.0$ mg was implantated subcutaneously into the shoulder. The cotton pellets together with granuloma were removed from the rats after 6 d. The increment in dry weight was obtained as a measure of granuloma formation.

From implantation to removal, the rats were administered the test compound every day by a stomach tube. The control rats were treated similarly, except that they received an equal volume of the vehicle.

The results were expressed as percent increment in dry weight due to granuloma formation (%), as compared with the initial cotton pellet weight.

**Acetic Acid-Induced Writhing Test** Male ddY mice weighing 20—25 g were fasted for 10 h, but were supplied with water *ad libitum*. A 0.7% solution of acetic acid in normal saline (w/v) was injected intraperitoneally 85 min after the test solutions had been administered orally. After 5 min, the number of writhes induced by the acetic acid solution was counted for 10 min. Control mice were treated similarly, except that they received an equal volume of the vehicle.

**Statistical Analysis** Data were expressed as the mean value $\pm$ standard error. All results were analyzed for variance by Bartlett's method. Significant differences were subsequently examined by Duncan's method.

## Results

**Effect on Carrageenin-Induced Hind-Paw Edema** TMP and FA inhibited dose-dependently carrageenin-induced edema. TMP, at 100 mg/kg, and FA, at 300 mg/kg, showed a lasting inhibition of the edema induced by carrageenin

TABLE I. Effect of TMP, FA and Indomethacin on the Paw Edema Induced by Carrageenin in Rats

| Time (min) | Increase of hind-paw volume (%) | | | | | |
|---|---|---|---|---|---|---|
| | Control (*p.o.*) ($n=8$) | TMP 30 mg/kg ($n=8$) | TMP 100 mg/kg | FA 100 mg/kg ($n=8$) | FA 300 mg/kg | Indomethacin 10 mg/kg ($n=8$) |
| 30 | 132.8±6.3 | 127.5±3.6[c] | 116.8±3.4[a] | 123.5±4.4 | 113.1±3.5[b] | 113.5±2.9[b] |
| 60 | 136.1±7.3 | 131.1±4.3[d] | 117.8±3.7[a] | 130.2±5.2[c] | 119.1±5.6[a] | 112.0±2.2[b] |
| 120 | 148.5±6.8 | 144.5±5.6[d] | 122.7±3.5[b] | 142.4±6.2[d] | 129.2±6.3[a] | 118.2±1.9[b] |
| 180 | 164.6±7.7 | 153.3±6.6[d] | 131.6±3.4[b] | 155.4±6.1[d] | 143.7±5.8[a] | 131.3±3.9[b] |
| 240 | 182.4±4.6 | 168.7±7.0[d] | 148.4±4.2[b] | 174.0±4.2[d] | 165.2±7.1[c] | 145.7±4.3[b] |
| 300 | 194.0±4.8 | 179.3±6.2[c] | 156.5±4.0[b] | 183.8±4.2[d] | 176.3±7.8[a] | 161.4±4.7[b] |
| 360 | 200.8±4.5 | 186.0±6.1[c] | 165.9±3.4[b] | 188.9±4.3[c] | 183.0±8.1[a] | 168.5±5.1[b] |

*a, b*) Significantly different from the control at $p<0.05$ and $p<0.01$, respectively. *c, d*) Significantly different from indomethacin at $p<0.05$ and $p<0.01$. All drugs were administered orally.

TABLE II. Effect of TMP, FA and Indomethacin on the Increased Vascular Permeability Induced by Acetic Acid in Mice

| Compounds | Dose (mg/kg *p.o.*) | No. of animals | Amount of leaked dye (μg/animals) |
|---|---|---|---|
| Control | | 6 | 342.1±16.3 |
| TMP | 30 | 6 | 288.6±11.3[a,c] |
| | 100 | 6 | 123.5± 7.6[b,c] |
| FA | 100 | 6 | 303.2±15.0[c] |
| | 300 | 6 | 142.4± 7.8[b,c] |
| Indomethacin | 10 | 6 | 220.9±12.6[b] |

*a, b*) Significantly different from the control at $p<0.05$ and $p<0.01$, respectively. *c*) Significantly different from indomethacin at $p<0.01$.

TABLE III. Effect of TMP, FA and Indomethacin on the Formation of Granuloma Tissue Induced by Cotton Pellet

| Compounds | Dose (mg/kg *p.o.*) | No. of animals | Weight gain (%) | Increment in dry weight (%) |
|---|---|---|---|---|
| Control | | 6 | 8.2±1.4 | 228.9±21.0 |
| TMP | 30 | 6 | 6.6±0.8 | 152.5±20.1[a] |
| | 100 | 6 | 7.0±1.5 | 81.5±21.0[b] |
| FA | 100 | 6 | 6.2±0.7 | 212.0±19.4[c] |
| | 300 | 6 | 7.0±1.8 | 105.2±19.8[b] |
| Indomethacin | 10 | 6 | 9.1±1.1 | 125.1±10.6[b] |

*a, b*) Significantly different from the control at $p<0.05$ and $p<0.01$, respectively. *c*) Significantly different from indomethacin at $p<0.01$.

during the 6 h period. Their inhibitory potency was about the same as that of indomethacin, at 10 mg/kg. The lower dose of TMP, at 30 mg/kg, and FA, at 100 mg/kg, did not show a significant inhibitory effect on the edema. The results are given in Table I.

**Effect on Acetic Acid-Induced Vascular Permeability Increase** TMP and FA inhibited dose-dependently the increase of dye leakage induced by acetic acid. The potency of TMP, and 100 mg/kg, and FA, at 300 mg/kg, was stronger than that of indomethacin, at 10 mg/kg. FA, at 100 mg/kg, did not show the significant inhibitory effect. The results are given in Table II.

**Effect of Cotton Pellet-Induced Granuloma Formation** TMP, at 30 mg/kg and 100 mg/kg, showed a dose-dependent inhibition on the cotton pellet-induced granuloma formation. FA, at 300 mg/kg, produced the inhibitory effect, but the dose of 100 mg/kg did not show significant inhibition.

TABLE IV. Analgesic Effect of TMP, FA and Indomethacin on the Writhing Symptom Induced by Acetic Acid in Mice

| Compounds | Dose (mg/kg *p.o.*) | No. of animals | No. of writhes (in 10 min) |
|---|---|---|---|
| Control | | 6 | 51.0±3.2 |
| TMP | 30 | 6 | 38.8±4.4[a,c] |
| | 100 | 6 | 19.3±2.3[b] |
| FA | 100 | 6 | 38.0±2.1[a,c] |
| | 300 | 6 | 25.8±4.9[b] |
| Indomethacin | 10 | 6 | 28.7±2.6[b] |

*a, b*) Significantly different from the control at $p<0.05$ and $p<0.01$, respectively. *c*) Significantly different from indomethacin at $p<0.05$.

Indomethacin, at 10 mg/kg, also showed the inhibitory effect and the potency was about the same as that of TMP, at 30 mg/kg and 100 mg/kg, and FA, at 300 mg/kg.

On the other hand, TMP, at 30 mg/kg and 100 mg/kg, FA, at 100 mg/kg and 300 mg/kg, and indomethacin, at 10 mg/kg, did not inhibit body weight gain in this experiment.

The results are given in Table III.

**Effect on Acetic Acid-Induced Writhe** TMP and FA reduced dose-dependently the number of writhes induced by acetic acid. The inhibitoty potency induced by TMP, at 100 mg/kg, and FA, at 300 mg/kg, was about the same as that of indomethacin at 10 mg/kg. The results are given in Table IV.

## Discussion

In the present study, it was found that TMP and FA significantly inhibited the edema induced by carrageenin, the increase of the dye leakage induced by acetic acid, the granuloma formation induced by cotton pellet and the number of writhes induced by acetic acid. TMP, FA and indomethacin, in the doses used in these experiments, have been found to be free from any apparent toxic symptoms, especially in the cotton pellet experiment, TMP, FA and indomethacin did not inhibit weight gain of the animals. Although it was reported that the intravenous $LD_{50}$ of TMP and FA were 416.0 mg/kg and 856.6 mg/kg in mice, respectively,[16] and that the orally lethal dose of TMP and FA were more than 300 mg/kg and 1000 mg/kg in rats, respectively.[6] The inhibitory potency induced by TMP, at 100 mg/kg, and FA, at 300 mg/kg, was about the same as that of indomethacin at 10 mg/kg.

It is well known that the development of edema induced by carrageenin and the increase of vascular permeability induced by acetic acid correspond to the early exudative stage of inflammation, one of the important processes of inflammatory pathology.[17,18] TMP and FA inhibited the edema induced by carrageenin and the increase of the vascular permeability induced by acetic acid in the present study, which shows that it exerts the antiinflammatory effect at the early exudative stage of inflammation.

Assay of mediators in the inflammatory exudate produced by carragennin in the rat showed the release of both histamin and bradykinin in the early phase, but that of prostaglandin $E_2$ only after two to three hours.[19-23] Moncada *et al.* reported that the carragennin induced edema was potentiated by prostaglandin $E_1$ and $E_2$.[22] Some pharmacological effects of TMP and FA have been reported in which both compounds inhibited biosynthesis of thromboxane $A_2$.[11,12,15]

From these results, it is suggested that the inhibitory effect of TMP and FA on carragennin induced edema and acetic acid induced increase of vascular permeability may be exerted partly through the inhibition of prostaglandin biosynthesis.

Swingle and Shideman reported that the proliferative phase of the inflammatory response in cotton pellet granuloma assay can be assessed by measurement of the increase in dry weight of the granuloma during days four through six after implantation.[24] TMP and FA inhibited on the cotton pellet-induced granuloma formation at day six after implantation, suggesting that both compounds also inhibit the late proliferative stage of inflammation.

TMP and FA also reduced dose-dependently the number of writhes induced by acetic acid. Although the inhibitory potency induced by both compounds was weaker than that of indomethacin, both compounds may also have an analgesic effect.

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