Inhibitory Effects of Tetramethylpyrazine and Ferulic Acid on Spontaneous Movement of Rat Uterus *in Situ*

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Tetramethylpyrazine is one of the alkaloids contained in *Ligusticum wallichii* FRANCH. Ferulic acid is a phenolic compound contained in *Ligusticum wallichii* FRANCH and *Angelica sinensis* (OLIV.) DIELS. The present study was carried out to examine the effect of tetramethylpyrazine and ferulic acid and the combined effect of both compounds on spontaneous uterine contractions in rats *in situ*. Tetramethylpyrazine and ferulic acid showed an inhibitory effect on uterine movement when given perorally and intravenously, respectively. The combination of both compounds, at doses individually insufficient to inhibit, synergistically inhibited uterine contraction.

It was found that tetramethylpyrazine and ferulic acid inhibited uterine contractions and the inhibitory effect induced by the combination of both was due to the potentiation.

Keywords tetramethylpyrazine; ferulic acid; spontaneous uterine movement; inhibitory effect; combination; synergism; *Ligusticum wallichii*; *Angelica sinensis*

Introduction

Tetramethylpyrazine (TMP) is one of the alkaloids contained in *Ligusticum wallichii* FRANCH (*L. wallichii*). Ferulic acid (FA) is a phenolic compound contained in *Liguisticum wallichii* FRANCH and *Angelica sinensis* (OLIV.) DIELS (*A. sinensis*).[1,2] *L. wallichii* and *A. sinensis* are compatible and have been frequently used in traditional Chinese medicine, especially as a homeostatic remedy for women's disorders and menoxenia, as well as an analgesic for dysmenorrhea, *etc.*

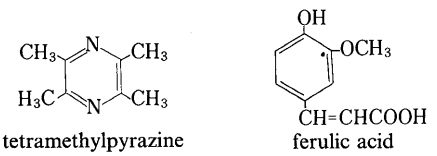
There have been a lot of reports in which essential oil (ligustilide, butylidenephthalide, butylphthalide, *etc.*) obtained from *L. wallichii* and *A. sinensis* showed a central depressive effect, an antispasmodic effect, a uterine relaxing effect, *etc.*[3-7] Also, many pharmacological effects of TMP, one of the effective compounds of *L. wallichii*, and FA, one of the effective compounds of both crude drugs, have been reported in which both compounds inhibited heart excitability, platelet aggregation and biosynthesis of thromboxane A_2, and dilated the coronary artery.[8-15]

However, there have been few pharmacological reports about TMP and FA on spontaneous uterine movement. The present study was carried out to examine the effect of TMP and FA, and their combined effect on the spontaneous movement of rat uterus *in situ*.

Materials and Methods

Materials TMP (Beijing Institute of Pharmaceutical Industry of China) was dissolved in water for oral administration (*p.o.*) and in 0.9% saline solution for intravenous administration (i.v.). FA (Shanghai First Reagent Factory of China) was dissolved with the same equivalent carbonic acid in water (*p.o.*) and in 0.9% saline solution (i.v.). The chemical structures of TMP and FA are shown in Fig. 1.

Methods Adult virgin female Wistar rats weighting 180—230 g were used. Only those rats that were in estrus of the estrous cycle were used. Their estrous stages were determined by vaginal smear tests in the morning before rats were selected. In the case of oral administration, rats were fasted for 16 h. The rats were anesthetized with urethane (1.5 g/kg, i.p.). Tracheotomy was performed, and then one jugular vein was cannulated for administering i.v. injection of the test solution. The uterine horns were exposed at the cervical junction by a low abdominal incision over the bladder area. One horn was lifted up gently and griped with a small clip 1 cm away from the cervical junction. The clip was joined to a force-displacement transducer, with which the spontaneous uterine contractions were recorded isometrically and the contractile activities were integrated (EI-601G, Nihon Koden) on a polygraph (RM-6200, Nihon Kohden) in connection. Each preparation was subjected to an initial load of 1 g.



tetramethylpyrazine ferulic acid

Fig. 1. Chemical Structures of Tetramethylpyrazine and Ferulic Acid

Statistical Analysis The average integral value for 5 min before the administration of the test solution was taken as 100%. Response to the test compound was expressed in terms of percentages to the average integral value before the administration. All results were expressed as the mean value ± S.E. and were analyzed for variance by Bartlett's method. Their significant differences were subsequently examined by Duncan's method. The ID_{50} value was obtained by Finney's probit analysis.

Results

Effect of TMP and FA by Oral Administration The oral administration of TMP, FA and papaverine produced a dose-dependent decrease in the frequency and the amplitude of spontaneous uterine contractions. Typical recordings of the inhibitory effect of TMP, at 100 and 300 mg/kg, FA, at 300 and 1000 mg/kg, and papaverine, at 30 mg/kg, on the uterine contractions are given in Fig. 2. TMP and FA decreased uterine contractions for 10—15 min after administration. TMP, at 100 mg/kg, maximally inhibited the contractions by about 35% and the inhibitory duration was about 45 min. TMP, at 300 mg/kg, showed a stronger inhibition and the inhibitory duration was beyond 120 min. FA, at 300 mg/kg, maximally inhibited the contractions by about 30% and the uterine contractions returned to their original level about 45 min later. The effect of FA, at 1000 mg/kg, was stronger and the duration was beyond 60 min. Papaverine, at 30 mg/kg, inhibited the uterine contractions by about 30% and lasted for 45 min.

Effect of TMP and FA by Intravenous Injection The intravenous injection of TMP and FA produced a dose-dependent decrease in the frequency, the amplitude and the tone of the uterine contraction.

Spontaneous uterine contractions were inhibted immediately after over 10 mg/kg of TMP and over 30 mg/kg of FA were given. Both maximal inhibitory effects appeared

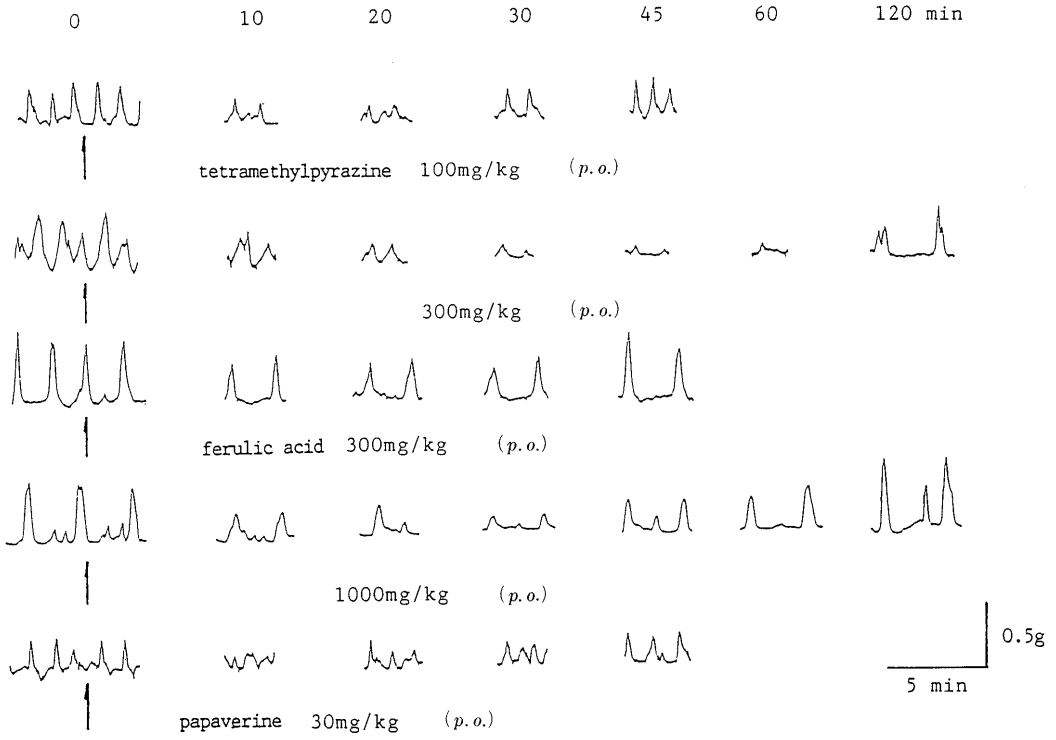


Fig. 2. Inhibitory Effect of Tetramethylpyrazine, Ferulic Acid and Papaverine on Spontaneous Uterine Contractions in Rats

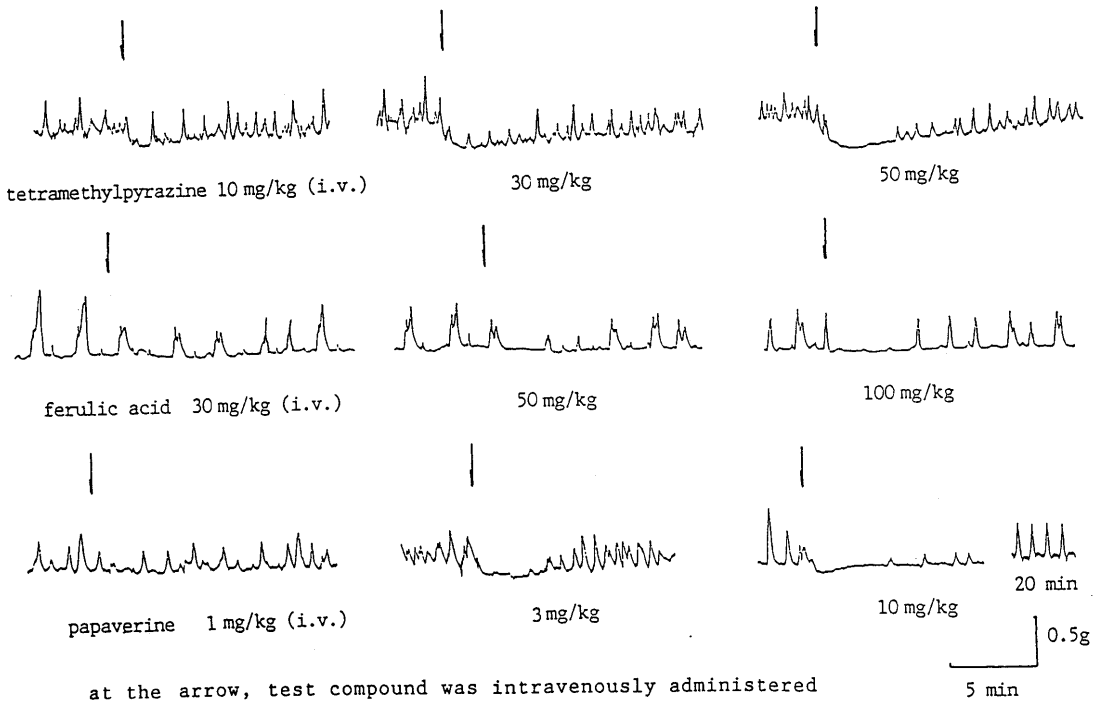


Fig. 3. Inhibitory Effect of Tetramethylpyrazine, Ferulic Acid and Papaverine on Spontaneous Uterine Contractions in Rats

in 2—3 min after the administration, then returned to the original level gradually. The inhibitory potency and the recovery time depended on the given doses. TMP, at 3 mg/kg, and FA, at 10 mg/kg, did not produce significant inhibition.

TMP, at 50 mg/kg, and FA, at 100 mg/kg, inhibited the uterine contractions significantly. In some cases, uterine contractions were inhibited completely for 2—5 min after the administration of these doses, and returned to the original level completely. In all preparation, TMP, at 100 mg/kg, and FA, at 300 mg/kg, completely inhibited the uterine contractions for over 10 min. (data are not shown in Fig. 3, Tables I and II).

Papaverine, at 1, 3 and 10 mg/kg, inhibited the con-

TABLE I. Inhibitory Effect of Tetramethylpyrazine and Papaverine on Spontaneous Uterine Contractions in Rats

Time (min)	Control ($n=5$)	Tetramethylpyrazine				Papaverine	
		(3 mg/kg, $n=5$)	(10 mg/kg, $n=5$)	(30 mg/kg, $n=5$)	(50 mg/kg, $n=5$)	(1 mg/kg, $n=5$)	(3 mg/kg, $n=5$)
1	105.6± 6.9	73.5±11.9[a]	59.2±11.9[b]	51.0±10.3[b]	47.8± 7.0[c]	63.5± 6.7[b]	75.0±9.0
2	91.5±10.3	78.3±17.2	58.3±13.5	55.1±12.9	27.9±11.3[b]	69.6±13.4	64.7±7.8[b]
3	96.5± 4.1	85.3±10.4	61.0±17.8	55.2±11.5[a]	35.0±11.7[b]	62.9± 5.5[b]	68.4±4.6[b]
4	102.2± 2.9	90.6±14.4	70.0±11.3[a]	59.9±12.1[a]	34.6± 9.6[b]	74.2± 3.5[c]	65.9±5.9[a]
5	105.4± 5.3	87.8± 9.3	66.4±12.6[a]	70.7±15.3	48.0± 8.7[c]	85.4± 9.1	78.4±6.3[a]
6	101.9± 4.5	86.9±15.2	77.6± 8.3[a]	71.2±10.6[a]	53.4± 7.4[c]	72.6± 9.5[a]	77.9±3.8[b]
7	87.6± 6.8	106.4±16.8	86.1±10.0	72.8±12.4	55.9± 8.3[a]	74.1± 7.3	81.8±3.0
8	96.8± 5.0	90.4±13.1	76.2±13.3	71.6±12.4	63.7± 8.4[b]	77.1± 7.3	78.1±5.4[a]
9	89.9± 6.4	102.8± 6.4	86.4± 9.1	82.3±10.3	73.2± 7.5	82.7± 5.3	81.9±4.4
10	99.0± 2.0	103.3±11.8	93.6± 1.3	84.5±10.2	69.6± 7.2[a]	81.0± 7.2	82.0±4.8
15	97.1± 4.0	104.6± 5.0	96.1± 3.3	84.2± 7.4	88.6± 4.8	95.2± 5.1	92.3±3.0
30	100.3± 8.5	111.5±10.1	101.2± 0.9	99.0± 2.1	113.0± 9.8	108.4± 8.7	102.0±4.5

a) $p<0.05$. *b*) $p<0.01$. *c*) $p<0.001$ *vs.* control. Drugs were administered intravenously at time 0. Values shown are mean±S.E. (%) to the value at time 0 (0.40±0.04, 0.41±0.04, 0.41±0.04, 0.45±0.02, 0.50±0.06, 0.39±0.03, 0.43±0.02 g, respectively).

TABLE II. Inhibitory Effect of Ferulic Acid and Pavaverine on Spontaneous Uterine Contractions in Rats

Time (min)	Control ($n=5$)	Ferulic acid				Papaverine	
		(10 mg/kg, $n=5$)	(30 mg/kg, $n=5$)	(50 mg/kg, $n=5$)	(100 mg/kg, $n=5$)	(1 mg/kg, $n=5$)	(3 mg/kg, $n=5$)
1	94.1± 6.5	90.7±5.1	67.3± 8.0[a]	65.1± 8.9[a]	67.3± 6.5[a]	62.0± 8.0[a]	76.0±9.0
2	106.8±12.4	95.7±5.7	67.3± 6.5[a]	63.0± 6.5[a]	40.3±11.4[b]	58.8±11.1[a]	64.7±7.8[b]
3	101.9±10.7	97.9±2.6	67.6± 4.5[a]	62.3± 4.2[a]	46.3±12.5[b]	65.7± 8.0[a]	68.4±4.6[b]
4	101.2± 7.0	97.4±4.4	69.2± 6.2[b]	65.6± 6.2[b]	46.5± 8.8[b]	69.4±11.8[a]	65.9±6.0[b]
5	93.6± 5.1	93.8±6.7	77.3± 6.7[a]	66.3± 7.2[a]	51.8± 7.0[b]	84.3±11.6	78.4±6.3[a]
6	101.8± 2.2	95.0±4.1	75.4± 7.6[a]	57.4±11.1[a]	62.8± 8.4[b]	82.5±11.2	77.9±3.8[a]
7	102.2± 2.0	88.7±3.1[b]	73.4± 9.5[a]	57.5± 8.9[b]	71.1± 3.5[c]	83.4±11.1	81.8±3.0[b]
8	99.1± 1.8	97.3±5.3	75.2±10.5	78.5±14.5	70.9± 7.0[a]	86.7±10.1	88.1±5.5
9	103.0± 4.2	102.0±6.4	89.9± 5.3	59.8±17.2	79.0± 8.8[a]	89.6±10.9	82.0±4.5[b]
10	100.0± 4.2	100.0±6.0	85.6± 1.4[a]	82.1± 8.5	87.8± 4.9	95.8±10.0	92.8±3.8
15	94.1± 7.1	99.1±1.8	108.1± 8.7	95.4± 2.9	101.0± 8.2	97.9± 5.1	92.3±3.2
30	102.7± 6.0	100.4±5.5	99.4± 4.4	103.1± 4.5	104.6± 2.0	107.4± 6.0	102.2±3.5

a) $p<0.05$. *b*) $p<0.01$. *c*) $p<0.001$ *vs.* control. Drugs were administered intravenously at time 0. Values shown are mean±S.E. (%) to the value at time 0 (0.46±0.03, 0.46±0.04, 0.39±0.03, 0.39±0.03, 0.46±0.02, 0.41±0.04, 0.43±0.02 g, respectively).

tractions and the inhibitory potency was stronger than that of TMP and FA. The inhibitory duration of papaverine, at 10 mg/kg, was about 20 min. After injected intravenously with over 3 mg/kg of papaverine, the rats were short of breath and after papaverine, at 10 mg/kg, was given, the rats even momentarily stopped breathing. However, the intravenous injection of TMP, at 100 mg/kg, and FA, at 300 mg/kg, did not depress the breath.

Typical recordings of responses to TMP, at 10, 30 and 50 mg/kg, and to FA, at 30, 50 and 100 mg/kg, are shown in Fig. 3. All results are summarized in Tables I and II, respectively.

ID_{50} of TMP, FA and papaverine, taking the maximal inhibitory potency within 10 min after the administration as an index, are shown in Table III.

Combined Effect of TMP and FA by Intravenous Injection As TMP and FA inhibited spontaneous uterine contractions, respectively, an experiment of the combined effect of TMP and FA on the contractions was carried out in order to study their synergic effect. Neither TMP, at 2 mg/kg, nor FA, at 6 mg/kg, showed an inhibitory effect, however, a combination of one-half of both doses produced an inhibitory effect on the contractions, especially in the amplitude. The inhibitory potency was about 15%.

TABLE III. ID_{50} Values of Tetramethylpyrazine, Ferulic Acid and Papaverine on Spontaneous Uterine Contractions in Rats

Compounds	ID_{50} (mg/kg, i.v.)
Tetramethylpyrazine	18.58 (5.49— 62.95)
Ferulic acid	47.90 (17.79—129.02)
Papaverine	4.51 (0.76— 26.60)

Inhibitory duration was about 4 min after i.v. injections and it returned to the original level 5—10 min later. These results showed that the combination of the low dose of TMP and FA synergistically inhibited the contractions. All results are summarized in Table IV.

In another combination experiment, a combined dose of TMP, at 9.3 mg/kg, and FA, at 24 mg/kg, in doses of 1/2 ID_{50}, was selected. The maximal inhibitory potency induced by the combination of a 1/2 ID_{50} of TMP and FA reached 57.6% and was greater than the algebraic sum of the individual actions, showing that inhibition was due to potentiation. The results are summarized in Table V.

Discussion

TMP and FA showed an inhibitory effect on spontaneous

TABLE IV. Synergism of no Effective Dose of Tetramethylpyrazine and Ferulic Acid on Inhibiting Spontaneous Uterine Contractions in Rats

Time (min)	Control ($n=7$)	Tetramethylpyrazine 2 mg/kg ($n=7$)	Ferulic acid 6 mg/kg ($n=7$)	Combination TMP 1 mg/kg, FA 3 mg/kg ($n=7$)	Papaverine 3 mg/kg ($n=7$)
1	98.5±2.3	93.0±3.8	97.3±2.9	87.0±4.9	71.7±7.5[b]
2	98.2±1.7	88.4±2.9	97.2±1.7	84.5±4.0[a]	62.3±6.3[b]
3	97.8±1.9	93.7±2.3	92.6±1.9	84.2±4.3[b]	65.3±4.2[b]
4	101.1±2.5	92.8±3.9	97.3±1.7	85.2±1.7[b]	70.3±6.0[b]
5	101.1±2.5	95.0±3.3	95.2±8.3	93.2±3.7	75.3±6.8[b]
6	97.6±2.5	96.8±2.6	103.2±6.0	91.6±4.2	75.8±4.0[b]
7	97.7±2.9	105.1±6.3	107.0±5.9	100.9±1.8	83.2±6.2
8	95.8±3.8	100.8±6.2	107.3±5.4	94.1±5.9	88.7±6.9
9	95.9±3.8	91.2±3.4	106.7±6.0	101.8±4.2	81.8±3.6[b]
10	97.7±3.0	102.5±4.3	109.5±7.2	101.1±5.1	91.8±3.8
15	96.0±2.9	102.5±4.2	104.6±7.0	103.5±3.7	91.5±3.8
30	98.9±5.7	102.1±2.3	115.8±7.1	108.5±3.0	100.3±2.9

a) $p<0.05$. *b*) $p<0.01$ *vs.* control. Drugs were administered intravenously at time 0. Each value indicates the mean±S.E. (%) to the value at time 0 (0.38±0.03, 0.35±0.02, 0.34±0.02, 0.36±0.03, and 0.40±0.03 g, respectively).

TABLE V. Potentiation of One-Half the ID_{50} of Tetramethylpyrazine and Ferulic Acid on Inhibiting Spontaneous Uterine Contractions in Rats

Compounds	Dose (mg/kg, i.v.)	No. of animals	Maximal inhibition (%)
Tetramethylpyrazine	9.3	4	28.0±6.0
Ferulic acid	24.0	4	18.6±5.2
Combination		4	57.6±4.5

uterine contractions in estrous rat *in situ* preparation, after given perorally or intravenously.

The inhibitory potency of TMP was stronger than that of FA, though the inhibitory duration was about the same. Papaverine also showed an inhibitory effect on spontaneous uterine contractions and the potency was stronger than that of both compounds.

It was reported that the intravenous LD_{50} of papaverine is 33.1 mg/kg,[16] TMP is 416.0 mg/kg and FA is 856.6 mg/kg in mice,[17] respectively. In light of the proportion of LD_{50} to ID_{50}, therapeutic indexes of TMP and FA (22.4 and 17.2, respectively) are greater than that of papaverine (7.4).[18] Because of that, TMP and FA are safer than papaverine. In the present experiment, we found that rats were short of breath after being intravenously injected with over 3 mg/kg of papaverine and in the cases given papaverine at 10 mg/kg, they momentarily stopped breathing. But the intravenous injection of TMP, at 100 mg/kg, and FA, at 300 mg/kg, did not affect the breathing of the rats. It is suggested that TMP and FA may be better agents for inhibiting uterine motility than papaverine.

In the combination experiment, we observed that the combined effect of lower doses of TMP with FA showed a synergic effect in inhibiting uterine contraction. Because the effect was not definitively due to addition or potentiation, a further experiment, using a combination of one-half the ID_{50} of TMP and FA, was carried out. The maximal inhibitory potency within 10 min after the administration was over 50%. It is known that if a certain dose of drug A and another dose of drug B produce the same effect quantitatively and the combination of one-half the dose of each drug produces a stronger effect than drug A or drug B used individually produces, the effect implies potentiation. From the above results, it is suggested that the combination of TMP with FA should, at least, produce potentiation.

Because it was reported that TMP is contained only in 1/10 millions in *L. wallichii*,[19] FA is 0.02% in *L. wallichii*[6,20] and 0.1% in *A. sinensis*,[5] both compounds may have a minor effect in a prescription involving *L. wallichii* and *A. sinensis*. Despite that, TMP or FA or their combination, as an effective compound, may be an effective and safe agent in the treatment of uterine hypercontractility and cramping pain with primary dysmenorrhea.

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