[Chem. Pharm. Bull. 30(5)1844—1850(1982)]

Pharmacological Studies of Ignavine, an Aconitum Alkaloid

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(Received July 7, 1981)

Pharmacological properties, especially, analgesic, antipyretic, antiinflammatory, sedative and antidiuretic activities, of ignavine, one of the main alkaloids in Aconite root, are discussed on the basis of the results of blind screening. Ignavine showed antiinflammatory activity in the acetic acid–induced writhing and carrageenin paw edema tests at doses of 50 and 100 mg/kg *p.o.*, which did not produce undesirable effects such as sedation, motor incoordination, muscle relaxation, hypothermia, ulceration or antidiuresis. Pharmacological properties of mesaconitine are also discussed; mesaconitine showed antiinflammatory, antipyretic and weak sedative activities.

Keywords——ignavine; mesaconitine; aconite alkaloids; antiinflammatory activity; sedative activity

Aconite root has been used for thousands of years in Eurasia as a powerful poison, an arrow poison and a drug. The alkaloids of this plant, aconitine, mesaconitine and jesaconitine, are extremely deadly poisons, and a number of pharmacological papers concenrning them have appeared.[1–4] Hikino *et al.*[5] reported recently that aconitine and mesaconitine have antiinflammatory activity. Since ancient times, the Chinese have processed Aconite root in order to decrease its toxicity for safe administration, *i.e.*, by transforming the main component aconitines to benzoylaconines or pyroaconitines.[6] Chinese medicine uses the drug to treat weak constitution, poor metabolism, dysuria, cardiac weakness, gout, rheumatism in the limbs, neuralgia and chill, while Western medicine uses it for making a liniment as an anodyne in chronic rheumatism and neuralgia. It was also suggested that there was no relationship between the pharmacological activity of benzoylaconines and the activity of the extracts of Aconite root practically used in Chinese medicine.[7] The possibility remains that substances contained in Aconite root, not aconitines, had the pharmacological effects considered to be produced by Chinese medicine.

Ignavine, one of the low-toxicity alkaloids contained in *Aconitum japonicum* Thunb and *Aconitum subcuneatum* Nakai, was first isolated by Ochiai *et al.*[8–11] *Aconitum japonicum* Thunb and *Aconitum subcuneatum* Nakai, whose main components are ignavine and mesaconitine, grow naturally in Japan and have been used as a drug in Chinese medicine practised in Japan. Neuropharmacological observations in mice[12] showed the possibility that ignavine had weak sedative, analgesic, antiinflammatory, antidiuretic, parasympatholytic, vasodilative and ganglion blocking actions. The present study was an attempt to confirm the weak sedative, analgesic, antiinflammatory and antidiuretic activities of ignavine by blind screening, but only antiinflammatory activity was confirmed. Mesaconitine and low toxicity alkaloids contained in Aconite root (hypognavine, isohypognavine, kobsine and pseudokobsine) were also studied.

Materials and Methods

Neuropharmacological Observations in Mice——For blind screening to determine the pharmacological spectrum of activities of ignavine, neuropharmacological observations in mice were made. Ignavine was intraperitoneally administered to male mice (ddY strain), weighing 20—22 g, in ascending logarithmic doses

(from a dose inducing symptoms shown by control animals to a dose approximately equal to LD_{50}). Mice thus treated were observed for 2 h according to the methods of Takagi *et al.*,[12] and overnight mortality was recorded.

Acute Toxicity in Mice——Male mice, weighing 20—22 g, were used to determine the intraperitoneal and peroral LD_{50}s. Mortality was recorded 72 h after administration. LD_{50}s were calculated by the up-and-down method.

Drugs suspended in saline with tween 80 were given orally in the following tests.

Writhing induced by 0.7% Acetic Acid——Male mice in groups of 6, weighing 22—24 g, were given ignavine, followed after 30 min by intraperitoneal injection of 0.1 ml/10 g body weight of 0.7% acetic acid. The number of writhings per mouse was recorded for a period of 10 min, beginning 10 min after administration of acetic acid. Aminopyrine was used as a reference agent.

Tail Pressure Test——The method used was that described by Takagi *et al.*[13] Groups of 6 male mice, weighing 22—24 g, were tested every 30 min for 2 h after administration of ignavine. Aminopyrine was used as a control agent.

Hypothermia Test——Male mice in groups of 6, weighing 24—26 g, with 37—38°C rectal temperature, were given ignavine. After administration, the change in rectal temperature was recorded every 30 min with a thermometer; the mice were kept at a room temperature of 23°C and a relative humidity of 60%. Aminopyrine was used as a control agent.

Antipyretic Test——Male mice in groups of 6, weighing 24—26 g, with 37—38°C rectal temperature, were given 20% yeast (0.05 ml/10 g body weight) orally, followed after 15 h by ignavine. The change in rectal temperature was recorded every 30 min. Aminopyrine was used as a reference agent.

Capillary Permeability Test——The procedures employed were similar to those reported by Whittle.[14] Male mice in groups of 6, weighing 24—26 g, were given ignavine, followed after 30 min by intravenous injection of 5% solution of PSB (0.1 ml), then after 5 min by intraperitoneal injection of 0.2 ml per mouse of 0.7% acetic acid. After a further 20 min the mice were killed by dislocation of the neck. The viscera was washed with distilled water. The combined washings were filtered through glass wool and made up to 10 ml in a graduated test tube. The amount of dye was determined with a spectrophotometer at 620 nm. Phenylbutazone was used as a reference agent.

Carrageenin Paw Edema Test——According to the method of Winter *et al.*,[15] 1% carrageening solution (0.1 ml) was injected as a phlogistic agent into the tissues of the plantar surface of the hind paw of male Wistar rats (weighing 100—120 g) in groups of 5. One and 3 h after the carrageenin treatment, the volume of the foot was determined. Ignavine was given 30 min before the carrageenin treatment. Aspirin was used as a reference agent.

Erosion in Starved Animals——Male Wistar rats, weighing 150—180 g, were starved in groups of 5 for 20 h before the administration of ignavine. Aspirin was used as a control agent. Eight h later, the rats were sacrificed by dislocation of the neck. The stomach of each animal was removed, inflated by injection of 1 ml of a formalin solution and immersed in 1% formalin solution for 10 min. The stomach was incised along the greater curvature and examined for lesions developed in the glandular portion. The length of each ulcer (mm) was measured individually under a dissecting microscope with a grid (10X). Male mice, weighing 22—24 g, were examined in groups of 6 under the same conditions.

Potentiation of Hexobarbital Sleeping Time——Male mice, weighing 20—22 g, were given ignavine in groups of 6, followed 30 min by an intraperitoneal injection of 70 mg/kg of hexobarbital sodium. The duration of loss of the righting reflex was recorded. Chlorpromazine (CPZ) was used as a reference agent.

Rotating Rod Test——A wooden rod with a diameter of 3.2 cm was rotated at a speed of 12 rpm. The number of mice which fell in 3 min was taken to express motor incoordination, since only mice which had stayed on the rotating rod for more than 3 min in two successive trials were used. The test was performed 4 times: every 30 min for 2 h after administration of ignavine. CPZ was used as a control agent. Male mice, weighing 24—26 g, were used in groups of 6.

Activity Wheel Test——A mouse was placed in the activity wheel cage 30 min after the administration of ignavine. Counting was continued for 1 h and the result was recorded as a percent of the control value obtained for the same animal when it was treated with saline at the same hour on the day before the test. Male mice, weighing 24—26 g, were used in groups of 6. CPZ was used as a reference agent.

Diuretic and Natriuretic Test——Groups of 3 male mice, weighing 26—28 g, were starved of food and water for 15 h, then placed in a metabolic cage for 2 h after the administration of 0.3 ml/10 g of body weight of drug for the collection of a 2 h sample of urine. Three groups were used. Theophylline was used as a control agent.[16]

Results

Neuropharmacological Observations

From the number of survivors in each group, the intraperitoneal LD_{50} of ignavine in

mice was calculated approximately to be between 20 and 50 mg/kg. At doses between 5 and 20 mg/kg of ignavine, characteristic behavior was observed: weak decreases in grooming, spontaneous movement, pain responses in tail clip and hot plate tests and grip tone, piloerection and writhing syndrome were noted within 3 min after injection. These effects disappeared within 1 h after the injection. Decreases in urination and fecal excretion were also noted for 2 h after the injection.

Acute Toxicity

Table I shows oral and intraperitoneal LD_{50}s of the alkaloids. After a dose of 500 mg/kg of ignavine administered orally, weak decreases in spontaneous movement, rectal temperature and pain response, and piloerection were noted for 1 h. After the intraperitoneal injection of

Table I. Acute Toxicity of Aconite Alkaloids

Compounds	Route	
	p.o.	*i.p.*
Ignavine	>500 mg/kg	22.8 mg/kg
Hypognavine	54.8 mg/kg	11.4 mg/kg
Isohypognavine	91.3 mg/kg	16.4 mg/kg
Kobsine	>500 mg/kg	188.2 mg/kg
Pseudokobsine	>500 mg/kg	98.2 mg/kg
Mesaconitine	2.2 mg/kg	0.23 mg/kg

more than 22.8 mg/kg, writhing was seen within a few min and the belly of the mouse touched the floor. Convulsions were seen every few min and death occurred 20 min after the injection. Similar behavioral changes were observed after lethal doses of hypognavine, isohypognavine, mesaconitine, kobsine and pseudokobsine.

Writhing induced by Acetic Acid

Ignavine at a dose of 100 mg/kg, hypognavine at a dose of 20 mg/kg and mesaconitine at a dose of 1 mg/kg had significant inhibiting effects on writhing (Table II).

Table II. Effect of Aconite Alkaloids on Writhing Induced by 0.7% Acetic Acid

Compounds	Dose (mg/kg)			Aminopyrine 200
	0	100	200	
Ignavine	35.0±4.2	13.7±3.9[a)]	14.1±3.8[a)]	5.8±3.2[a)]
Kobsine	32.5±5.1	17.3±3.5	15.8±5.7	0[a)]
Pseudokobsine	35.6±5.2	19.7±4.8	17.5±6.1	2.8±2.8[a)]
	0	20	40	
Hypognavine	38.1±4.0	11.8±3.5[a)]	12.7±5.2[a)]	1.3±1.1[a)]
Isohypognavine	42.8±3.8	42.6±5.7[a)]	38.2±6.2	1.0±1.0[a)]
	0	0.5	1	
Mesaconitine	33.6±4.0	17.5±6.1	12.1±3.6[a)]	3.0±1.8[a)]

The number of writhings per mouse is indicated as the mean±S.E. *a*); Significantly different from control (Student's *t*-test, $p<0.01$ and *b*); ($p<0.05$).

Tail Pressure Test

Significant changes of the maximum pain threshold were not seen during 2 h after administration of ignavine and mesaconitine (Table III).

Hypothermia Test

Ignavine at a dose of 400 mg/kg and mesaconitine at doses of 0.5 and 1 mg/kg were found to reduce the normal rectal temperature of mice significantly (Fig. 1).

TABLE III. Effect of Ignavine on Pain Response in the Tail Pressure Test

Compounds	Dose (mg/kg)					Aminopyrine
	0	0.5	1	100	200	50
Ignavine	78.8±3.7			95.4±5.8	102.4±9.4	158.0±10.7[a]
Mesaconitine	73.6±2.6	82.4±5.3	95.6±9.2			150.2±11.5[a]

Maximum pain threshold (mmHg) per mouse is indicated as the mean±S.E. For symbols, see Table II.

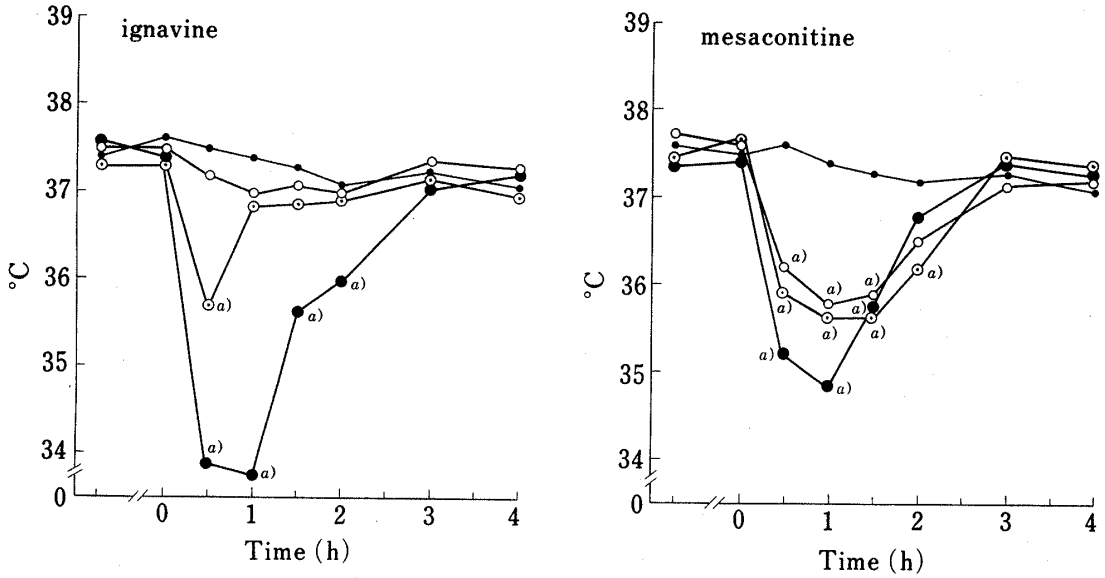

Fig. 1. Effect of Ignavine on Rectal Temperature of Mice

—·—: control, —●—: aminopyrine 200 mg/kg, —○—: ignavine 200 mg/kg, —⊙—: ignavine 400 mg/kg. —○—: mesaconitine 0.5 mg/kg, —⊙—: mesaconitine 1 mg/kg.

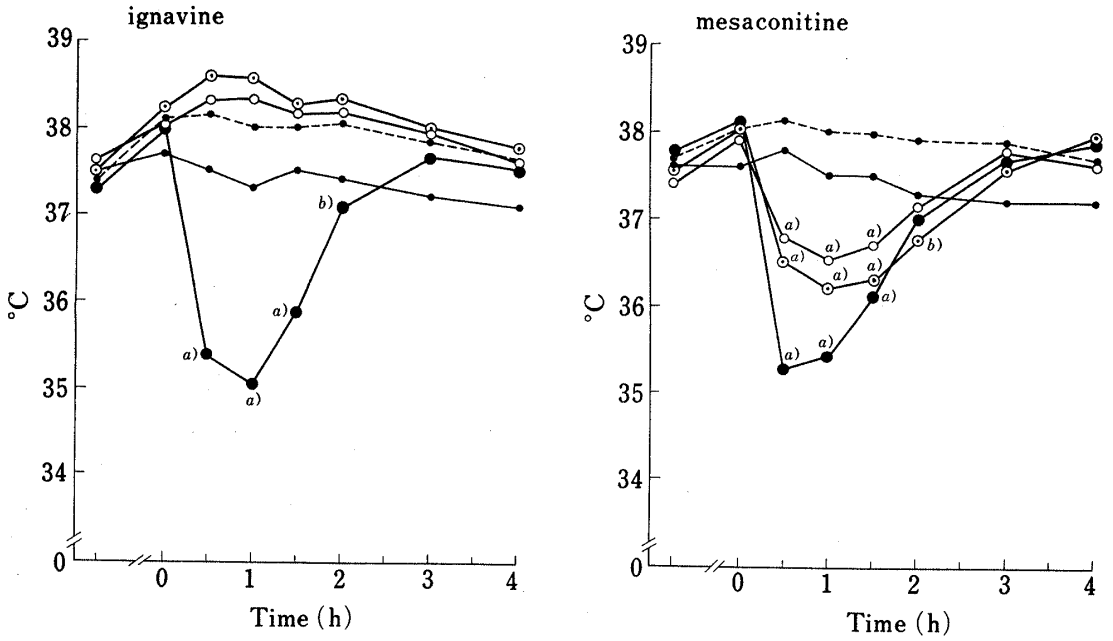

Fig. 2. Effect of Ignavine on Rectal Temperature of Yeast-treated Mice

—·—: control, —·—: yeast, —●—: yeast+aminopyrine 200 mg/kg, —○—: yeast+ignavine 100 mg/kg, —⊙—: yeast+ignavine 200 mg/kg. —○—: yeast+mesaconitine 0.5 mg/kg, —⊙—: yeast+mesaconitine 1 mg/kg.

Antipyretic Test

Mesaconitine at doses of 1 and 0.5 mg/kg was found to reduce the rectal temperature of yeast-treated mice significantly, but ignavine at doses of 100 and 200 mg/kg had no effect (Fig. 2).

Capillary Permeability Test

Ignavine and mesaconitine did not significantly inhibit the acetic acid-induced increase in capillary permeability (Table IV).

Carrageenin Paw Edema Test

As shown in Table V, ignavine at doses of 50 and 100 mg/kg and mesaconitine at a dose of 0.5 mg/kg significantly suppressed the development of rat paw edema examined 3 h after carrageenin application.

Erosion in Starved Animals

Ignavine and mesaconitine did not induce ulcers in starved rats and mice (Table VI).

Table IV. Effect of Ignavine on Capillary Permeability

Compounds	Dose (mg/kg) 0	0.5	1	100	200	Phenylbutazone 200
Ignavine	0.54±0.06			0.45±0.05	0.39±0.04	0.32±0.05[a]
Mesaconitine	0.52±0.05	0.54±0.08	0.40±0.05			0.34±0.04[a]

The absorbance of PSB leaked into peritoneal cavity through acetic acid–induced dye leakage is expressed as the mean ± S.E. For symbols, see Table II.

Table V. Effect of Ignavine on Carrageenin–Induced Paw Edema

Compounds	Dose (mg/kg) 0	0.25	0.5	50	100	Aspirin 200
Ignavine	1.71±0.06			1.51±0.03[b]	1.36±0.07[a]	1.25±0.03[a]
Mesaconitine	1.71±0.06	1.49±0.06	1.45±0.06[b]			1.25±0.03[a]

Figures indicate the ratio of the foot volume measured in the same animal before the administration and 3 h after the administration, expressed as the mean ± S.E. For symbols, see Table II.

Table VI. Effect of Ignavine on Erosin in Starved Animals

VI-I Compounds	Dose (mg/kg) 0	0.25	0.5	50	100	Aspirin 200
Rats						
Ignavine	0.20±0.20			0.10±0.10	0.60±0.20	7.62±2.21[a]
Mesaconitine	0.20±0.20	0	0			7.62±2.21[a]

VI-II Compounds	Dose (mg/kg) 0	0.5	1	100	200	Aspirin 200
Mice						
Ignavine	0			0	0	0
Mesaconitine	0	0	0			0

Ulcer index is expressed as the mean ± S.E. For symbols, see Table II.

Potentiation of Hexobarbital Sleeping Time

Ignavine and mesaconitine had no effect on the duration of sleep induced by hexobarbital (Table VII).

Rotating Rod Test

Mesaconitine at a dose of 1 mg/kg induced slight inhibition of motor coordination, observed 30 min after administration (Table VIII). Ignavine had no effect.

Activity Wheel Test

Mesaconitine at a dose of 1 mg/kg produced a significant decrease in motor activity (Table IX), but ignavine did not.

Diuretic and Natriuretic Tests

Neither ignavine nor mesaconitine changed the volume of urine, or the quantity of Na^+ and K^+ in the urine.

TABLE VII. Effect of Ignavine on Hexobarbital Anesthesia

Compounds	Dose (mg/kg) 0	0.5	1	100	200	CPZ 2
Ignavine	32.7±4.8			26.6±4.2	22.3±3.5	59.8±7.2[a]
Mesaconitine	29.7±3.1	35.4±3.0	39.5±2.8			52.4±4.9[a]

Duration of loss of the righting reflex is indicated as the mean time (min) ± S.E.
For symbols, see Table II.

TABLE VIII. Effect of Ignavine on Motor Coordination in the Rotating Rod Test

Compounds	Dose (mg/kg) 0	0.5	1	100	200	CPZ 3
Ignavine	0			0	0	5[b]
Mesaconitine	0	1	4			5[b]

The number of mice which failed to stay on the rotating rod for 3 min, 30 min after drug administration, is indicated. Mice were used in groups of 6. For symbols, see Table II.

TABLE IX. Effect of Ignavine on Motor Activity in the Activity Wheel Test

Compounds	Dose (mg/kg) 0	0.5	1	100	200	CPZ 2
Ignavine	118±16			124±16	92±18	45±12[a]
Mesaconitine	127±21	78±15	62±11[b]			56±11[a]

Percent counts are indicated as the mean ± S.E. For symbols, see Table II.

TABLE X. Effect of Ignavine on Urinary Volume and Na^+, K^+ Excretions in Saline-Loaded Mice

Compounds	Dose (mg/kg)	Urinary volume g/animal	Na^+ Excretion μeq/2 h/animal	K^+ Excretion μeq/2 h /animal
control		0.52±0.06	83.4± 9.8	74.2± 7.5
Ignavine	100	0.48±0.04	78.5± 8.4	73.8± 6.8
	200	0.48±0.05	76.3± 7.5	69.8± 5.4
Mesaconitine	0.5	0.41±0.04	68.4± 8.4	70.1± 6.2
	1	0.38±0.03	64.9± 7.6	60.3± 5.8
Theophylline	30	0.87±0.08[b]	140.4±13.8[a]	97.2±10.8

For symbols, see Table II.

Discussion

Ignavine and mesaconitine were tested for sedative, analgesic, antipyretic and antiinflammatory activities in mice. Analgesic, antipyretic and antiinflammatory activities were examined by means of tests of acetic acid-induced writhing, hypothermia, antipyretic activity, capillary permeability, carrageenin paw edema, and erosion in starved antimals. Inhibition of the writhing syndrome was observed at doses of 100 mg/kg of ignavine and 1 mg/kg of mesaconitine, while inhibition of carrageenin paw edema was seen at doses of 50 mg/kg of ignavine and 0.5 mg/kg of mesaconitine. Ignavine and mesaconitine had no inhibitory effect on the acetic acid-induced increase of capillary permeability and no effect on erosion in starved animals. Ignavine was found to reduce the normal rectal temperature at a high dose. Hypothermia and antipyretic activities of ignavine were not recognized at doses which produced inhibitions of the writhing syndrome and carrageenin paw edema, but mesaconitine did show activity at the same doses. Analgesic activity was examined by the tail pressure test, but ignavine and mesaconitine had no activity.

Ignavine had no influence on the duration of hexobarbital–induced sleep, motor coordination or motor activity, but mesaconitine had inhibitory effects on motor coordination and motor activity. Neither ignavine nor mesaconitine had antidiuretic activity. From these results, ignavine presumably has antiinflammatory activity which is not accompanied by undesirable effects such as sedation, motor incoordination and ulceration. Mesaconitine has antiinflammatory, antipyretic and sedative activities. It may be assumed that the antiinflammatory activity shown by Aconite root used in Chinese medicine, *e.g.* to treat rheumastism, is due to the action of ignavine. The relationship between the pharmacological activity of ignavine and the activity of the extracts of Aconite root practically used in Chinese medicine may require further investigation, however.

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