Pharmacology of sinomenine, an anti-rheumatic alkaloid from Sinomenium acutum

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Abstract

The root and stem decoctions of Sinomenium acutum Rehd. et Wils. (formerly Sinomenium diversifolius Diels, one type of Fang-chi (Chinese)) have been used as a folk remedy for neuralgia and rheumatoid arthritis in many areas of the Far East. In Japan and China various viny plants have been identified as Fang-chi (Boi in Japanese) since antiquity. This uncertain nomenclature has made it difficult to evaluate the efficacy of the Fang-chi described in the classic literature. Among traditional Fang-chi plants only Sinomenium acutum has been demonstrated to contain the alkaloid sinomenine, which is now known to be effective in neuralgia and rheumatic diseases. Sinomenine is a unique plant alkaloid, as it potently releases histamine in association with degranulation of tissue mast cells in mammalian tissues. This action occurs preferentially in the skin and joint capsules. The released histamine is responsible for the dominant pharmacological actions of sinomenine, such as vasodilatation, increased vascular permeability, acceleration of the thoracic and peripheral lymph flow, contraction of plain muscles, increased peristalsis of the intestines, and stimulation of gastric acid secretion. At toxic doses of sinomenine, convulsive central excitation was observed in most laboratory animals. Clinical side effects encountered with high doses of injected sinomenine or of decocted Sinomenium acutum were: injection site flare, pruritus in the head and upper part of the body, edema around the lips and eyelids, and temporary cephalalgia. Most of these side effects were reduced by classical antihistamines (H1-receptor antagonists). Daily subcutaneous injections of sinomenine for more than one week produced an analgesic effect in mice. Granulation tissue growth and adjuvant arthritis induced in rats were both inhibited by daily injections of a small dose of sinomenine hydrochloride or histamine dihydrochloride. These inhibitory effects were mediated through histamine H2-receptors probably on fibroblasts (for granulation tissue growth) and on T-cells (for adjuvant arthritis), since these effects were clearly inhibited by the H2-antagonist burimamide but not by the H1-antagonist mepyramine. The anti-rheumatic effect on Sinomenium acutum are probably genuine and can probably be attributed to the histamine-releasing properties of sinomenine.

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Abstract: The root and stem decoctions of *Sinomenium acutum* Rehd. et Wils. (formerly *Sinomenium diversifolius* Diels, one type of Fang-chi (Chinese)) have been used as a folk remedy for neuralgia and rheumatoid arthritis in many areas of the Far East. In Japan and China various vine plants have been identified as Fang-chi (Boi in Japanese) since antiquity. This uncertain nomenclature has made it difficult to evaluate the efficacy of the Fang-chi described in the classic literature. Among traditional Fang-chi plants only *Sinomenium acutum* has been demonstrated to contain the alkaloid sinomenine, which is now known to be effective in neuralgia and rheumatic diseases. Sinomenine is a unique plant alkaloid, as it potently releases histamine in association with degranulation of tissue mast cells in mammalian tissues. This action occurs preferentially in the skin and joint capsules. The released histamine is responsible for the dominant pharmacological actions of sinomenine, such as vasodilatation, increased vascular permeability, acceleration of the thoracic and peripheral lymph flow, contraction of plain muscles, increased peristalsis of the intestines, and stimulation of gastric acid secretion. At toxic doses of sinomenine, convulsive central excitation was observed in most laboratory animals. Clinical side effects encountered with high doses of injected sinomenine or of decocted *Sinomenium acutum* were: injection site flare, pruritus in the head and upper part of the body, edema around the lips and eyelids, and temporary cephalalgia. Most of these side effects were reduced by classical antihistamines (H₁-receptor antagonists). Daily subcutaneous injections of sinomenine for more than one week produced an analgesic effect in mice. Granulation tissue growth and adjuvant arthritis induced in rats were both inhibited by daily injections of a small dose of sinomenine hydrochloride or histamine dihydrochloride. These inhibitory effects were mediated through histamine H₂-receptors probably on fibroblasts (for granulation tissue growth) and on T-cells (for adjuvant arthritis), since these effects were clearly inhibited by the H₂-antagonist burimamide but not by the H₁-antagonist mepyramine. The anti-rheumatic effect of *Sinomenium acutum* are probably genuine and can probably be attributed to the histamine-releasing properties of sinomenine.

INTRODUCTION

*Sinomenium acutum* Rehd. et Wils. (Fam. Menispermaceae) is a wild plant found in the warm valleys of southern Japan. This plant is hardy with twin-
ing vines sometimes growing to a height of 20 feet. The stems and roots are stripped and hairless. The leaves are long-stalked, ovate or multilobed, acuminate, usually cordate at the base, palmated to 5 to 7 divisions, and the surfaces glabrous and brilliant (Fig. 1). In summer the flower stalks grow from the axilla of the leaves. The flowers are small, light greenish, and organized in slender panicles. The drupes are compressed and bluish black. The roots and stems in cross sections are whitish and are characterized by a very distinct radiating pattern of ducts. The decocted dried roots and stems of this plant have been used as a folk remedy for neuralgia and rheumatoid arthritis since antiquity in Japan. This plants is now included in the Japanese pharmacopoeia under the name of Sinomeni Caulis et Rhizoma or Bōi.

The plant Fang-chi (防己), probably Sinomenium acutum, is mentioned in the Chinese Shen Nung Pen Ts’ao (神農本草經, “Herbal”)* (1) which was probably written about the first century B.C., during the Western Han dynasty. Fang-chi was recommended for the treatment of many diseases such as rheumatism, fevers, dropsies and pulmonary diseases in the Chin K’uei Yao Lüeh (金鶏要略, “Synopsis of the Golden Chamber”) written by Chang Chung-ching (2) in the same dynasty and also in the Ming I Pien Lu (明便秘錄,

* Shen Nung is the popularly ascribed mythical emperor who reigned in 2838-2698 B.C. Shen Nung Pen Ts’ao, 3 volumes, contains descriptions of 365 kinds of herbs (1).
Pharmacology of Sinomenine

"Formulas of Famous Physicians") compiled by T'ao Hung-ching (3) of the Liang dynasty 502 A.D. and which is said to be the first official pharmacopoeia in China. Among the prescriptions of Chang Chung-ching four contained Fang-chi. The Shen Nung Pen Ts'ao (1) distinguishes between the plant Han Fang-chi (漢防已) and Mu Fang-chi (木防已). The former plant is probably Sinomenium acutum, since it was prescribed for diseases now considered to be rheumatoid arthritis and neuralgia, while the latter was prescribed for many other diseases (2-4). In subsequent periods, varieties of viny plants have been used for the original plant for medical treatment in China and in Japan, as many Menispermaceae plants closely resemble each other in taxonomy. This has confused the identity and efficacy of Fang-chi among traditional physicians of Chinese medicine for a long time (5, 6). Nevertheless, it is curious that the original plant has continued to be used as a folk remedy for rheumatic diseases in many districts of Japan (7).

In 1920 an alkaloid sinomenine was isolated from Sinomenium acutum (8). This alkaloid has been demonstrated to be effective in relieving the pains of rheumatoid arthritis and neuralgia in extensive clinical trials (9-12). Pharmacological studies on this plant have been conducted at several institutions in Japan and China. The special interest of this alkaloid is the presence of a histamine-releasing feature that is probably important in the mechanism of the anti-rheumatic effect. The present paper presents a brief survey of pharmacological studies on this alkaloid, with special reference to its anti-rheumatic effect.

SINOMENIUM ALKALOIDS

In 1909 Jujiro Honda (13), then professor of pharmacology at Okayama Medical School, presented a paper at a meeting of the Okayama Medical Association on chemical and pharmacological properties of two toxic ingredients that he extracted from Sinomenium acutum, but a complete account of this presentation does not remain. In 1917 Ishikawa (14) of Kyushu University remarked briefly in a note that a picrotoxin-like convulsant action was found in frogs injected in the lymph sac with a crystalline ingredient obtained from this plant. During this early period, Taguchi and Ohta (1919) (15) conducted investigations on this plant under Prof. Kurata Morishima, at the Pharmacological Department of Kyoto University. They described the circumstances of their entering into this investigation: "February, 26, 1916, Mr. S. Masagaki (probably a stranger) visited Prof. Morishima and asked the professor earnestly to study this plant. The younger brother of the visitor suffered from rheumatoid arthritis for many years and had difficult walking. This
younger brother took the decoction of this plant several times, with a few days interval. A dramatic relief occurred.” Taguchi and Ohta obtained a crystalline colorless ingredient from the extracts of the roots and stems of *Sinomenium acutum* using the procedure of Stas and Otto. These early investigators found that this crystalline induced convulsions in frogs. In the middle of these studies they moved to Kitasato Institute in Tokyo, where they isolated two kinds of convulsant alkaloid: cocculin C_{17}H_{29}NO_5 \cdot 3H_2O (15) (later kukolin C_{16}H_{20}NO_3 \cdot 3H_2O (16, 17)) and diversin C_{16}H_{20}NO_4 (16, 18). The former was a crystalline and the latter amorphous. Ishiwari (8) at Kyoto University continued the same line of study after the departure of Taguchi and Ohta. He isolated from roots and stems a crystalline alkaloid and estimated the molecular formula as C_{16}H_{19}NO_3 \cdot H_2O, and called the compound sinomenine. This molecular formula was later corrected but the generic name sinomenine was maintained.

The chemical structure of sinomenine with the molecular formula of C_{19}H_{23}O_4N was established as shown in Fig. 2 by Kondo and Ochiai (19), and Goto and his colleagues (20) (cf. reviews in refs. 21, 22). These studies on sinomenine marked the first step in a series of chemical studies in Japan on the alkaloids of *Menispermaceae* and other plants. Several alkaloids of *Sinomenium acutum* other than sinomenine have been successively isolated and the chemical structures established. Most of these studies were conducted by Goto and Tomita and their colleagues. The isolated alkaloids included disinomenine (23, 24), sinacutine (25), tuduranine (26–28), acutumine (29), acutumidine (29), magnoflorine (30), sinoacutine (31), michelalbine (32) and stepharine (32). The neutral substances β-sitosterol and stigmasterol were also isolated (33). The sinomenine content in the plant is 1.0–2.0% as a base in water-extracts (34) or 1.09% as hydrochloride (35). The percentages of other alkaloids are not known. Sinomenine has not been isolated in other *Menispermaceae* plants. Pharmacological studies of *Sinomenium* alkaloids have thus far been limited to sinomenine and magnoflorine. The claimed chemical properties of diversine and parasinomenine have not been confirmed, and the reported alkaloid isosinomenine has been shown to be an artefact (36).
Decoction Sinomenium acutum has been used widely in the treatment of rheumatic diseases in Kyushu, Shikoku, Kinki and other districts of Japan and in Korea and Formosa with favorable reputations for remedy (7). The usual daily dose is prepared from 8-15 g of dried cut roots and stems. The remarkable beneficial effects of sinomenine were first reported scientifically by Ishiwrari (9) and Takaori (10) in 1921, and since then, a considerable clinical literature (12) has confirmed their findings. Sinomenine administered subcutaneously (s.c.) or orally (p.o.) was remarkably effective in relieving pain, swelling and other symptoms of acute and chronic rheumatoid arthritis and reduced pain in many types of neuralgia, such as sciatic neuritis, lumbalgia and muscular rheumatism. The s.c. injections were more effective than oral usage. Injections were usually started at 20 mg (dosages are expressed as hydrochloride) once daily and were increased by 10-15 mg every three to four days thereafter. Beneficial effects have been noted after two or three injections, and most acute phase symptoms subsided after eight to nine administrations, near a daily maximum dose of 90 - 100 mg. In chronic types of arthritis, swelling and oppressive pain in the affected joints decreased gradually, and the margins of the articular movements were increased. After the initial injection, a relapse in pain usually occurred but on repeated injections the periods of relief were prolonged. For oral usage, 30 - 120 mg per day in three divided doses has been recommended.

Side effects. An itchy flare of 4-5 cm in diameter appeared occasionally around the s.c. injection site. Pruritus and facial edema occurred with large doses of sinomenine (s.c.) or decocted Sinomenium acutum. The pruritus was predominant in the upper parts of the body and the edema remarkable around the eyelids and lips. Cephalalgia was encountered infrequently. These symptoms were temporary and abatement usually followed in about 30 min. There is a case report of a serious side effect in a patient who was probably injected sinomenine into the subcutaneous vein by error (37).

Edema and itching induced by sinomenine were suppressed experimentally by the usual antihistamines (38). The usual antihistamines did not interfere with the therapeutic effects of sinomenine on experimentally-induced arthritis or on granulation tissue growth, as stated later in more detail. Prior to 1935 when clinical usage of sinomenine was common among physicians, antihistamines were not available.

PHARMACOLOGICAL PROPERTIES OF SINOMENINE

Properties inherent to the morphinoid chemical structure. Sinomenine is the
only morphinoid base so far obtained from *Menispermaceae* plants. The phenanthropiperidine moiety in its chemical structure is shared with morphine and other natural opium alkaloids and with semisynthetic opiate derivatives. Sinomenine possesses certain pharmacological properties similar to those of morphine and related compounds. At large doses sinomenine elicits convulsant-type central excitation which is also found in morphine and its surrogates (39). The most prominent pharmacological property of sinomenine is histamine release. This action is also present in morphine, codeine, apomorphine (40), ethylmorphine (41, 42) and meperidine (43, 44). Meperidine is a synthetic opioid containing \(\gamma\)-phenyl-\(N\)-methoxypiperidine in structure like morphine and sinomenine. One distinct difference between sinomenine and morphine or congeners is that sinomenine may be less toxic due to the absence of prominent central nervous actions that are inherent in morphine, and because the major pharmacological and probably therapeutic effects of sinomenine are due to the potent histamine-releasing property, while for the morphine-type analgesics, histamine release is a side effect of clinical usage.

**Toxicity.** Subtoxic doses of sinomenine (s.c. or p.o.) resulted in decreased motility in mice, rats, rabbits, dogs and monkeys (8, 45). At toxic or lethal doses (s.c. or intravenous (i.v.)) this apparent sedation was soon followed by an increased reflex excitability or by clonic-tetanic convulsions in frogs (8), mice (8, 45, 46), rabbits (8) and dogs (47-49). After the seizure, the animals showed general muscular weakness and slow respiration, leading in some cases to fatal asphyxia.

Dogs after receiving a s.c. injection of 3-5 mg/kg of sinomenine, licked their lips, then scratched around the ear lobes, neck and jaw, where intense itching seemed to be present. At 10 - 40 mg/kg (s.c.) pruritic giant edema appeared in the face forming several vertical stripes in the snout. The animals violently scratched the face and ear lobes and rubbed their bodies against a burrowed hole. Urticaria-like macular erythemas were also visible in the breast and abdominal skin areas. At these dose levels profuse salivation, vomiting and defecation were not uncommon. The vomiting did not appear to be due to the direct action of sinomenine on the chemoreceptor trigger zone (CTZ), since direct application of 1% sinomenine onto the caudal angle of exposed rhomboid fossa did not provoke vomiting while apomorphine induced vomitings (47). Increased peristalses of the intestines were observed through the abdominal walls of dogs lying on their sides. At these dose levels reflex excitability increased. At doses of 60 mg/kg or more (lethal dose), the animals laid down and were able only to stagger ataxically when placed on their feet. Respiration was initially accelerated but soon became weak and slow. Increased reflex excitability gradually intensified.
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The animals cried aloud, and then manifested trismus and convulsive movements in the extremities. This was soon followed by a generalized clonic-tetanic convulsion initiated spontaneously or with inciting events, and occasionally culminated in opisthotonus. With seizures at short intervals, the animals prostrated and died in respiratory paralysis. Sinomenine injected i.v. at 2.5 or 3 mg/kg produced these symptoms more acutely than s.c. injection at the same dose and frequently led to death (47-49). Autopsy findings included congestion of the mesenteric veins and the liver (47, 50, 51). Cats receiving 10 mg/kg sinomenine s.c. scratched their heads and ear lobes and licked or bit areas of the body. Defecation also occurred (47).

In rats, after single intraperitoneal (i.p.) injection of 50 mg/kg, vascular dilatation or reddening was observed around the ears, mouth, snout, paws, and scrotum (52). The animals scratched their faces with the forelegs. These hyperemic areas were initially bright pink, changing progressively to blue as respiration failed. Edema gradually developed in hyperemic areas. These changes were quite obvious on visual inspection, and they were indistinguishable from the effects produced by an i.p. injection of egg-white, dextran or compound 48/80. Convulsions were not observed in rats even at 300 mg/kg, i.p. (52). A single injection of sinomenine at this dose to a group of rats reduced the histamine content of the abdominal skin by 32.5% compared to the initial level. When sinomenine was injected for 6 consecutive days starting at the initial dose of 50 mg/kg X 2 and increased daily 50 mg/kg X 2 (total dose of 2,100 mg/kg), an 88.2% reduction in histamine content was found in the abdominal skin. The symptoms, however, decreased gradually after subsequent injections in spite of increased dosages (52). Rats injected 40 - 80 mg/kg daily, i.p. for 14 consecutive days showed no body weight or food consumption effects. No changes in the blood picture or histology were observed (45).

Mice injected with sinomenine at 300-400 mg/km, i.p. showed reddening of the snout, ear lobes, feet and tail; edema was prominent in the distal extremities. Clonic-tetanic convulsions were observed at these dosages (46, 53, 54). Skin reactions produced in rabbits, mice (46), rats (55) and dogs (48) were reduced by appropriate doses of standard antihistamines, such as mepyramine and diphenhydramine. In mice at the same dosage, these antihistamines did not prevent sinomenine deaths (46). Rabbits (8, 53, 56) and guinea pigs (56) responded with convulsions to high doses (s.c. or i.v.) of sinomenine.

The toxic symptoms of sinomenine, especially on the skin, resembled those of compound 48/80. One difference is that at high doses sinomenine caused convulsions while compound 48/80 resulted in severe prostration in most examined animals (57). This suggests that the convulsive actions of
**Table 1. Lethal doses and minimum effective doses (convulsion, hypotension and other reactions) of sinomenine hydrochloride**

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>Lethal dose (mg/kg)</th>
<th>Reference</th>
<th>Minimum effective dose for indicated reaction (mg/kg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog</td>
<td>Lymph sac</td>
<td>500 8</td>
<td>300 Convulsion 8</td>
<td>600 58</td>
<td>500 Convulsion 58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>650 53</td>
<td></td>
<td>650 53</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>s.c.</td>
<td>400 58</td>
<td>300 Convulsion 54, 59</td>
<td>483* 46</td>
<td>50† Marked skin reaction 46</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>580* 45</td>
<td></td>
<td>p.o. 53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>i. v.</td>
<td>250 53</td>
<td></td>
<td>i. v. 53</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>p.o.</td>
<td>964† Sedation 45</td>
<td></td>
<td>p.o. 50† Sedation 52, 60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>i. p.</td>
<td></td>
<td></td>
<td>50† Marked skin reaction 52, 60</td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>s.c.</td>
<td>350 56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit</td>
<td>s.c.</td>
<td>250 8</td>
<td></td>
<td>280 58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>i. v.</td>
<td>120 58</td>
<td>5 Hypotension 50</td>
<td>150 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>i. cyst.</td>
<td>1.2 Convulsion 56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>s.c.</td>
<td>60 47</td>
<td>40-60 Convulsion 47</td>
<td>&gt;60 53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>i. v.</td>
<td>= 5 51</td>
<td>0.3 Hypotension 51</td>
<td>0.5 Hypotension 47</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>s.c.</td>
<td>10 Convulsion 47</td>
<td></td>
<td>1 Hypotension 61</td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>p.o.</td>
<td>95† Sedation 45</td>
<td></td>
<td>13.5† Prostration 45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>i. v.</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* LD₅₀, † without convulsion.

Sinomenine may not be related to the release of histamine. Table 1 shows the lethal doses of sinomenine for different species of laboratory animals and the minimum effective doses for inducing convulsion and hypotension.

Cardiovascular actions. Sinomenine decreased the contraction force and frequency of excised or perfused frog heart. Rabbit auricular vessels dilated when the perfusion fluid contained serum (47, 53). Intravenous injection of sinomenine induced hypotension in dogs (>0.1 mg/kg, irreversible at 3 - 5 mg/kg) (47, 48), cats (>0.1 mg/kg) (61, 62) and rabbits (>5 mg/kg) (50). In dogs an increase occurred in the volume of the liver, intestines and extremities, and the portal pressure rose at an early stage of hypotension (50, 51). Likewise
Pharmacology of Sinomenine

in cats an increase was observed in the portal pressure and the liver volume (61). The flow of thoracic lymph was markedly accelerated in dogs (50, 51), cats (61) and rabbits (50) in this order, and cervical lymph was increased in dogs (51). The lymph became rich in protein content, and the blood and lymph did not coagulate in sinomenine-shocked dogs (50, 51) and cats (61). Tachyphylaxis of these sinomenine actions was marked, especially in dogs (50, 51). When a small amount of sinomenine (0.05 ml of 0.1 – 0.3% solution) was injected intracutaneously (i.c.), the human skin responded with a triple response which was characteristic in that the wheal grew more slowly extending more prominent pseudopod-like processes along cutaneous veins than by histamine. A brief pain that was followed by itching of about 10 min duration usually occurred at the injection site. Occasional cephalalgia was encountered, due probably to the extention of cranial vascular walls.

Effects on plain muscles. Intravenous injection of sinomenine increased the rhythmic contraction of the uterus, which culminated in contracture at very high doses in cats and rabbits but this action was much weaker than that of quinine (63). An increase of intestinal peristalsis has been experienced in man after sinomenine injection or ingestion of the plant decoction. Gastric acid secretion increased after s.c. injection of sinomenine in dogs (38).

Histamine-releasing action. After i.v. injection of sinomenine in dogs, histamine and heparin levels elevate markedly in blood and lymph plasma, as first reported from this laboratory (51, 62, 64, 65). These actions of sinomenine were proved to be responsible for the hypotension, the rise in the portal pressure, the increased volumes of the liver and extremities, and the incoagulability of blood and lymph produced by this substance; this explains the development of tachyphylaxis in all these reactions (51). Cardiovascular changes were completely inhibited by pretreatment with antihistamines such as mepyramine, although the hypotension was partially inhibited (65).

In dogs i.v. injection of sinomenine depleted histamine of the skin preferentially (62, 64, 66). This response differed from compound 48/80 which releases liver histamine more easily in dogs (62, 64, 66). After sinomenine injection in dogs, edema or itchy erythema was prominent in the ear lobes, eyelids, around the mouth and nose, and in the areolae of nipples and genital-anal regions, where abundant histamine and mast cells are contained (49). Joint capsule histamine was released more readily than skin histamine (49). In tissues depleted of histamine, mast cells were disrupted at various stages, such as granule discharge, fragmentation and disappearance of cells. In the recovery process, a few mast cells appeared initially but gradually increased in population and granule density. For complete recovery of the cell population the usual period required was more than 40 days for the skin and about 2
weeks for the joint capsules. This coincides with the recovery course of the depleted histamine in the respective tissues (67). Occasional temporary headaches after sinomenine injection may be due to histamine release.

Fourteen (51) to 21 percent (68) of mongrel dogs were found to be refractory to both sinomenine and compound 48/80 (cross-tolerance). These non-reactive dogs responded normally to polyvinylpyrrolidone and tween 20 and showed normal sensitivity to histamine. The incidence of non-reactors was similar in both sexes (68).

The mechanism of histamine release by sinomenine has been studied (69–73, reviews 74, 75).

Analytic and local anesthetic effect. Daily s.c. injection of histamine in mice caused analgesia which reached maximum after 6–8 days of injection. Similar analgesia developed under the same injection schedule with sinomenine. When the injection of histamine and that of sinomenine were alternated daily at doses causing about the same degree of analgesia, neither agent produced an analgesic effect. The development of analgesia by histamine or sinomenine was not blocked by the concurrent use of an antihistamine mepyramine. The hypothermic effect of s.c. injection of histamine or sinomenine gradually decreased on repeated daily injections. Such desensitization of the hypothermic effect did not appear when histamine and sinomenine were injected on alternate days. In mice receiving repeated injections of sinomenine, the sensitivity to histamine increased as measured by the hypothermic effect (76). The close similarity between the development of analgesia and the desensitization of the hypothermic effect suggests the presence of histamine receptors in the sensory nerve endings that is similar to the skin vessels, and that histamine liberated by physical stimuli is a possible mediator of cutaneous pain in a manner similar to its mediation of vasodilatation. Sinomenine has a local anesthetic action as shown by i.c. injection or by instillation onto the conjunctiva (77, 78).

Anti-inflammatory and anti-allergic actions. Sinomenine inhibited eggwhite edema (52) but not carrageenin edema in rat paws (79). Daily injections to rats of sinomenine at high dose suppressed both the increase in the weight of croton-oil granuloma pouch and its histological inflammatory changes (60). Canine anaphylactic shock was prevented by an i.v. or s.c. injection of sinomenine (48) although this effect was not evident in guinea pigs (80). A recent report indicates that a small amount of histamine inhibits the allergic histamine release from human basophils (81). We have shown that histamine and also sinomenine inhibit certain types of inflammation, as described in more detail later.

Other pharmacological actions. Sinomenine lowered rabbit body temperature
Pharmacology of Sinomenine raised by heat puncture or adrenaline injection (8). Since the body temperature is also lowered by sinomenine in normal mice (76), the hypothermic effect of sinomenine may be due largely to the dilatation of cutaneous vessels by released histamine. Sinomenine produced hypo- and hyperglycemia in rabbits depending upon the dosage (82). In rabbits daily injections of sinomenine caused neutrophilia while erythrocyte count and hemoglobin concentration remained unchanged (83). Old Chinese medical books describe the diuretic effect of Han Fang-chi and Mu Fang-chi, but this effect has not been demonstrated so far in sinomenine (8) and trilobine (84). The latter alkaloid is an active principle of Cocculus trilobus D.C. which is one kind of Mu Fang-chi.

Absorption and excretion of sinomenine and the fate of released histamine. The absorption of sinomenine was estimated in rats by the amount of urinary histamine. Sinomenine was rapidly absorbed by s.c., i.v. and i.p. injections, but hardly absorbed by intramuscular (i.m.) injection. Peroral absorption was slower but better than by i.m. injection (85). This is consistent with the experimental results of sinomenine on gastric acid secretion (38). In rabbits, urinary excretion of sinomenine depended upon the s.c. dosage injected, but the excretion gradually decreased on repeated injections (86). A major part of released histamine converts to 1,4-methylimidazole acetic acid and imidazole acetic acid riboside; the remaining part is mostly excreted as free histamine but a small quantity of histamine remains for several hours in tissues (76, 85). The latter histamine probably has the therapeutic effects of sinomenine or Bod, as discussed in the next paragraph.

PHARMACOLOGICAL PROPERTIES OF SINOMENINE PROBABLY CONNECTED TO THE ANTI-RHEUMATIC EFFECT

1. Inhibitory effect of histamine and sinomenine on connective tissue growth. A characteristic pathological feature of rheumatoid arthritis is the thickening of the articular soft tissues. Synovial edema accompanied by cellular infiltration is the first sign of disease which is followed by synovial growth, formation of granulation tissue, accumulation of inflammatory exudates in articular cavities, and then fibrous or bony ankylosis of the joints. Hence, the suppression of connective tissue growth (granuloma formation) appears therapeutically important.

To study the effects of sinomenine and histamine on connective tissue growth, we (87, 88) placed filter-paper disks, soaked in 7% aqueous formaldehyde solution, in the subcutaneous tissue of each axillary and inguinal area of male Wistar rats under sterile conditions. On the seventh day after the operation, the granulomas including the filter-paper disks were extirpated.
To intensify the pink hue of the granulation tissue, the animals were killed by carbon monoxide poisoning.

**Effects of histamine and sinomenine.** Subcutaneous injections of 0.05 mg/kg of histamine twice a day (the dose of histamine is expressed as base, but for other drugs as salt or ester) decreased the weight of dry-defatted granulomas and their contents in hydroxyproline and hexosamine. These inhibitory effects became more marked when the dose was increased to 1 mg/kg/day. The hydroxyproline and hexosamine values per milligram of dry-defatted weight of granuloma were not significantly altered by histamine and other drugs tested with the exception of prednisolone at large dose. The gain in body weight during the 7-day period from disk implantation to the removal of granuloma was increasingly reduced by increasing dose of histamine, but this effect attained statistical significance only at doses higher than 1 mg/kg/day. Thymus weight was not affected at any dose.

The effects of sinomenine were tested at two different doses. In rats given large doses of sinomenine before disk implantation and during granuloma growth period*, the histamine content of the abdominal skin at the time of granuloma removal was markedly decreased to a level of 6.7 ± 0.8 μg/g wet wt. of tissue (mean ± S.E.M., N=6) compared to 23.1 ± 0.8 μg/g (N=4) in the control group. The formation of granuloma was inhibited by 55.7% in terms of weight by this treatment. When a relatively small dose of sinomenine (15 mg/kg twice a day) was administered from the implantation day to one day before the removal of granulomas without pretreatment of sinomenine, the histamine content of abdominal skin was 25.3 ± 3.6 μg/g (N=4) on the day of granuloma extirpation. In spite of the absence of an effect on skin histamine content, this small dose of sinomenine inhibited granuloma formation at the same rate as a larger dose.

Aminoguanidine bicarbonate, a potent histaminase inhibitor, produced a marked inhibitory effect on granuloma formation at a s.c. dose of 10 mg/kg/day. The inhibition of granuloma growth by 0.5 mg/kg/day of prednisolone (i.m.) was less marked than 0.1 mg/kg/day of histamine (s.c.). This dose of prednisolone, however, strikingly inhibited the growth of rats and caused a decrease in thymus weight. Phenylbutazone was ineffective at a daily dose of 30 mg/kg.

**Effects of burimamide and mepyramine.** Burimamide is known to specifically block histamine H2-receptors and to antagonize responses to histamine,

* The rats were pretreated before the disk implantation by i.p. injection of sinomenine for 6 days: on the first day 50 mg/kg x 2, increasing by 50 mg/kg x 2 daily, until a final dose of 300 mg/kg x 2 (total dose = 2,100 mg/kg). From the implantation day, 300 mg/kg was injected once daily.
such as stimulation of gastric acid secretion (89). When 5 mg/kg of burimamide was injected s.c. 30 min prior to the injection of 0.05 mg/kg of histamine, this compound clearly antagonized the inhibitory effect of histamine on the formation of granuloma and even enhanced its growth as revealed by the increased granuloma weight and hydroxyproline and hexosamine contents. Burimamide also blocked the inhibitory effect of sinomenine and aminoguanidine. Mepyramine, the classical antagonist of histamine $H_1$-receptors (89), was ineffective in blocking the inhibitory effect of histamine at a similar dosage as that used for burimamide. The hydroxyproline and hexosamine values per milligram of dry-defatted weight of granuloma were not markedly altered by histamine antagonists.

**Histological studies.** In granulomas obtained from rats treated with 0.05 mg/kg of histamine twice a day, tissue layers of densely populated fibroblasts were narrower than those of control animals. The formation of blood vessels in the granulation tissue appeared to be also poor in the treated rats. These morphological effects were entirely or largely counteracted when burimamide was injected concurrently with histamine. In sinomenine- and aminoguanidine-treated rats the morphological pattern of granuloma formation was similar to that observed in histamine-treated rats.

**Comment.** It has been shown that activation of $H_2$-receptors leads to an increase in the level of cyclic AMP in lymphocytes (90, 91), leukocytes (81), gastric tissue (92) and cardiac tissue (93). It is also known that the growth of cultured fibroblasts is inhibited by the increase in intracellular level of cyclic AMP (94). Therefore, the histamine inhibition of the granuloma growth may be induced by a rise in the cyclic AMP level of fibroblasts through the activation of $H_2$-receptors. We have recently observed an increase in the cyclic AMP content of granulomas in rats after a s.c. injection of histamine (95).

2. **Inhibitory effect of histamine and sinomenine on adjuvant arthritis**

The rat adjuvant arthritis shows a close similarity to the human rheumatoid arthritis in clinical signs. A dose of 0.05 ml of adjuvant mixture containing 0.25 mg of finely-ground killed *Mycobacterium tuberculosis* was administered i.d. under the volar surface of the left hind paw of male inbred Sprague-Dawley rats. The volume of each hind paw was measured prior to adjuvant injection and once daily thereafter.

**Effects of histamine and sinomenine.** Sinomenine was injected i.p. at a dose of 15 mg/kg twice daily from the day of adjuvant injection (day 0). Sinomenine did not inhibit the primary inflammation in the adjuvant-injected paw which reached maximum on day 5 or day 6, but significantly suppressed swelling of the secondary inflammation which appeared in the adjuvant-
Fig. 3. Effect of histamine on adjuvant-induced lesions in the rat hind paw. Histamine was injected at 5 mg/kg, s.c., twice a day from day 5 to day 10. The control rat received 0.9% saline. Roentgenographs were taken on day 25. a, Saline-treated rat; b, histamine-treated rat. The adjuvant-injected paw is on the left in both figures (97).

noninjected paw at day 10 or later. Histamine administered s.c. at a dose of 5 mg/kg twice daily also had a similar inhibitory effect. In the vehicle-treated control group, marked swelling and ankylosis of tarsal and other pedal joints were visible. Fusion of joints and destruction of bones and cartilages were observed in roentgenograms. These changes were more marked in the adjuvant-injected paw but were also observed in the adjuvant-noninjected paw. In groups treated with sinomenine or histamine, these radiological changes were also clearly inhibited (96, 97) (Fig. 3).

Histamine markedly suppressed the appearance of the secondary inflammation of adjuvant arthritis when injected for 6 consecutive days from day 5.
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to day 10. Histamine injection prior to or after this critical 6-day period produced no significant inhibition of the secondary inflammatory response. Histamine had no suppressive effect on the secondary lesion when administered after the establishment of the lesion. Therefore, the histamine effect on the secondary lesions appears to be based on a different mechanism from the usual anti-inflammatory drugs.

Effects of burimamide and mepyramine. When burimamide at 5 mg/kg was injected simultaneously with histamine of the same dosage, a complete blockage of histamine inhibition was found in the secondary inflammatory response and in histological and radiological examinations. Mepyramine at the same dosage showed no such blocking effect.

Comment. Rat adjuvant arthritis has been reported as being caused by cell-mediated immunity, mainly from evidence of the passive transfer of this disease with living lymph node cells (98). The most effective period of histamine administration to prevent the appearance of the secondary lesion of adjuvant arthritis coincides closely with the effective period reported for some immunosuppressants (99, 100).

Since the inhibitory effect of histamine on adjuvant arthritis is not antagonized by mepyramine and is completely blocked by burimamide, the histamine effect appears to be mediated through $H_2$-receptors. Histamine causes an increase in the cyclic AMP level of lymphocytes through the activation of $H_2$-receptors (91). In connection with this effect, histamine inhibits responses to antigen-stimulation, such as the proliferation and differentiation of lymphoid cells, immunoglobulin production and the cytotoxic activity of lymphocytes (101). Our findings together with the histamine effects on lymphoid cells described by others (99) suggest that the suppressive effect of histamine in adjuvant arthritis may be related to the inhibition of T-lymphocyte proliferation. The catecholamine release by histamine is blocked by $H_1$-receptor antagonists (102). Therefore, the catecholamines released by histamine do not seem to play an important role in the histamine inhibition of adjuvant arthritis.

From the inhibitory effects of histamine on adjuvant arthritis and granulation-tissue growth, it appears likely that the antirheumatic properties of sinomenine is mediated through histamine released from mast cells.

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