Colchicine on Sodium Urate-Induced Paw Swelling in Mice: Structure–Activity Relationships of Colchicine Derivatives (35207)

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(Introduced by J. Doull)

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The relationship between the molecular structure of colchicine and its unique anti-inflammatory activity in gout has been little investigated due, in part, to the lack of suitable animal models of acute gout. The finding (1) that acute attacks of gout depend on the presence of microcrystalline sodium urate in synovial fluid has led to the development of experimental models of the disease in dogs by intra-articular injection of microcrystalline monosodium urate (2), and in rats by subplantar injection of the urate crystals (3, 4). We have adapted the latter method to mice. By subplantar injections of microcrystalline sodium urate suspensions, we were able to induce a paw swelling, the extent of which was measured plethysomographically. Using this system, we have evaluated a number of colchicine derivatives and related compounds in an effort to determine which features of the colchicine molecule are necessary and/or sufficient for anti-inflammatory activity as expressed by suppression of sodium urate-induced paw swelling.

Materials and Methods. Colchicine purchased from various commercial sources was purified according to Ashley and Harris (5) and recrystallized from ethyl acetate. 1,2,3-Trimethoxybenzene (Aldrich Chemical Co.) was recrystallized from ethanol–water. Tropolone (Aldrich Chemical Co.) was sublimed *in vacuo*. Other compounds were prepared by literature methods: 2-methoxytropone (6), desacetamidocolchicine (7), desacetylcolohiceine (8), colchiceine (9), 4-cyanocolchicine (10), desacetylcolchicine tartrate (8), and isocolchicine (9).

Monosodium urate crystals were prepared according to Seegmiller *et al.* (1). Crystals were between 0.5 and 10 μ in length.

Female HA/ICR mice, obtained from A.R.A./Sprague-Dawley, Madison, Wisconsin and weighing between 20 and 35 g were used as the test animals. Substances to be tested were dissolved in saline. Colchiceine and 4-cyanocolchicine were dissolved in 95% ethanol such that the final solution contained 10% ethanol. 1,2,3-Trimethoxybenzene was dissolved in propylene glycol such that the final solution contained 25% of the cosolvent. Desacetamidocolchicine was finely powdered and suspended in a mixture of 400 mg of Tween 80 and 1 g of carboxymethylcellulose jelly (4.5%) and then made up to 10 ml with saline. Desacetylcolchiceine was dissolved in a small amount of dilute HCl and treated with dilute NaOH solution and 0.1 *M* phosphate buffer (pH 7) to give a final solution of pH 5–6. Drug solutions or suspensions were injected intraperitoneally. Control animals were treated with the same quantity of saline containing the appropriate solvent or suspending agent which was used to prepare the drug solution or suspension. One hr after treatment with the drug or saline solution, each animal was injected in the subplantar region of the left hind paw with 0.02 ml of a suspension of 50 mg of monosodium urate crystals in 1 ml of saline. The right hind paw received 0.02 ml of saline and served as a control. Plethysmographic measurements of paw volumes were made over a period of several hours using the procedure of Uyeki *et al.* (11). Percentage of swelling was calculated by the formula:

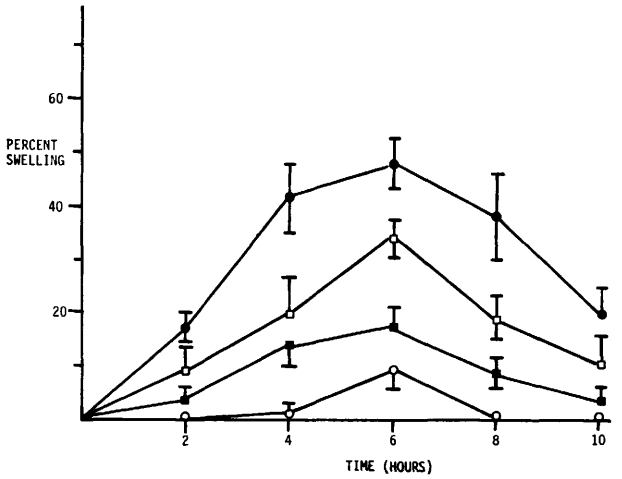

FIG. 1. Temporal curve showing development of paw swelling in mice following subplantar injections of 0.02 ml of suspensions of different sodium urate concentrations: (●) 50 mg/ml; (□) 25 mg/ml; (■) 12.5 mg/ml; (○) 6.25 mg/ml. Each group consisted of five animals.

$$\frac{\text{Left paw} - \text{right paw (mm of pen deflection)}}{\text{Right paw (mm of pen deflection)}} \times 100.$$

Results. Subplantar injection of a suspension of microcrystalline sodium urate into the hind paw of mice produced a paw swelling, the extent of which was quantitated by plethysmographic measurements. Figure 1 indicates that maximum swelling was reached in 6 to 8 hr; by 24 hr the swelling was almost gone. A single injection of 2 mg/kg of colchicine (I), ip, 1 hr previous to sodium urate administration dramatically suppressed the resulting swelling as shown in Fig. 2.

There was no significant difference in paw swelling between control animals and animals treated with trimethoxybenzene (II) up to 150 mg/kg, 2-methoxytropone (III, R = OCH_3) in doses up to 50 mg/kg, tropolone (III R = OH) in doses up to 50 mg/kg, colchiceine (V) in doses up to 10 mg/kg or isocolchicine (VIII) in doses up to 50 mg/kg. The time course of paw swelling for

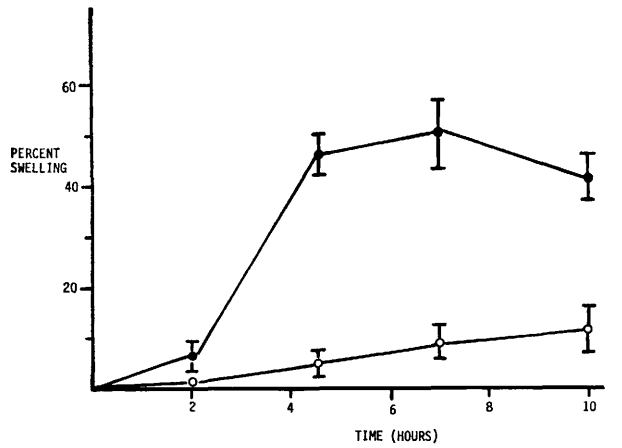

FIG. 2. Effect of 1 hr pretreatment of colchicine (2 mg/kg) on development of mouse paw swelling induced by subplantar injection of 0.02 ml of a suspension of 50 mg/ml of sodium urate in saline: (●) percentage of paw swelling in animals receiving no drug; (○) percentage of paw swelling in colchicine-treated animals. Each group consisted of five animals.

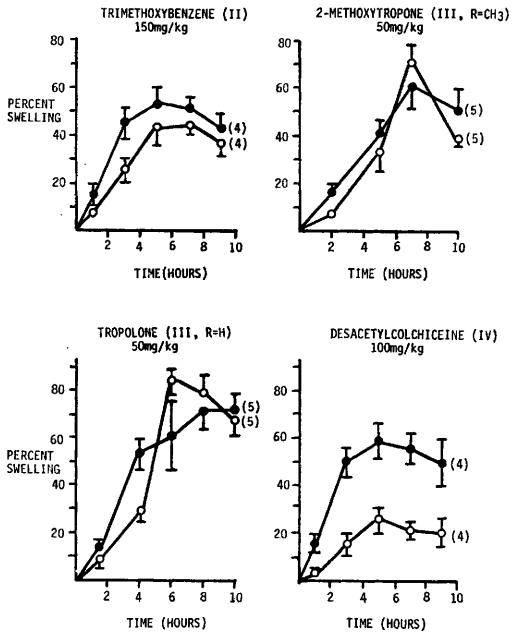

FIG. 3. Effect of 1 hr pretreatments with trimethoxybenzene, 2-methoxytropone, tropolone, and desacetylcolchiceine on development of mouse paw swelling induced by subplantar injections of 0.02 ml of a suspension of 50 mg/ml of sodium urate in saline: (●) percentage of paw swelling in animals receiving no drugs; (○) percentage of paw swelling in drug-treated animals. Number of animals used is given in parentheses.

the highest dose of these drugs is shown in Figs. 3 and 4.

Paw swelling of animals treated with desacetylcolchiceine (IV) at 100 mg/kg, 4-cyanocolchicine (VI) at 10 mg/kg, and desacetylcolchicine tartrate (VII) at 15 mg/kg was significantly different from controls at $p<0.05$. The course of the swelling for each of these drugs is shown in Figs. 3 and 4. Desacetylcolchicine tartrate (VII) and 4-cyanocolchicine (VI) were both tested for activity at 2 mg/kg. At that dose, neither substance exhibited any significant activity. Pretreatment of the animals with 25 mg/kg of desacetylcolchiceine (IV) did not result in inhibition of paw swelling.

Desacetamidocolchicine was of special interest in this work since it is known to be a more potent antimitotic agent than colchicine (12–14). The effect of desacetamidocolchicine on paw swelling is shown in Fig. 5. Colchicine suppression of paw swelling in this experiment was significantly different than controls at 4, 6, 7.5, and 10 hr. Suppression of the swelling by 100 mg/kg of desacetamidocolchicine was significant at 6, 7.5, and 10 hr while response to the 10 mg/kg dose was significant only at 10 hr. At no point was the response to the 10 mg/kg dose significant. An experiment using only 2 mg/kg of desacetamidocolchicine was run initially in expectation of a potent suppressive effect on the paw swelling. However, this dose was completely ineffective.

Discussion. Following the discovery that acute attacks of gout are dependent on the presence of microcrystalline sodium urate in the synovial fluid, experimental models of acute gout were developed. In dogs and nongouty humans it was found that intra-articular injection of microcrystalline sodium urate resulted in an inflammatory response which resembled acute gout (1, 2). This

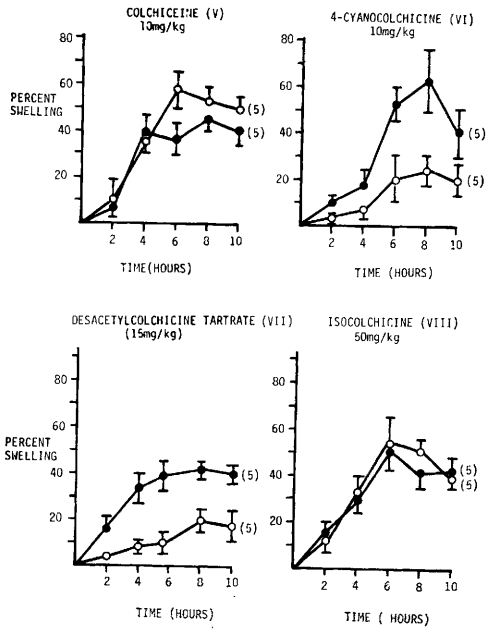

FIG. 4. Effect of 1 hr pretreatments with 4-cyanocolchicine, desacetylcolchicine tartrate, colchiceine, and isocolchicine on development of mouse paw swelling induced by subplantar injection of 0.02 ml of a suspension of 50 mg/ml of sodium urate in saline. (●) percentage of paw swelling in animals receiving no drug; (○) percentage of paw swelling in drug-treated animals. Number of animals used is given in parentheses.

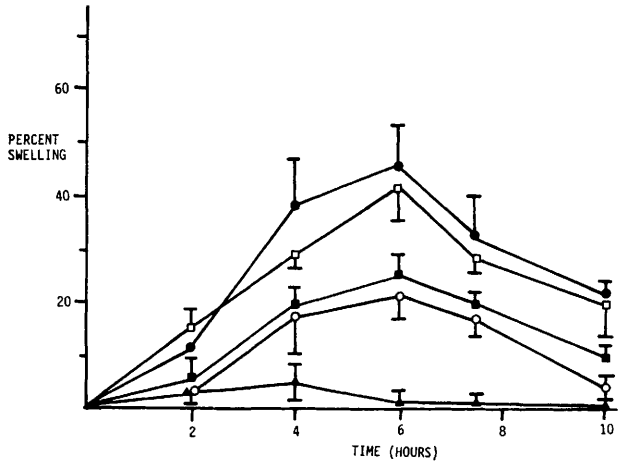

FIG. 5. Comparison of desacetamidocolchicine (DAAC) and colchicine on mouse paw swelling induced by subplantar injection of 0.02 ml of a suspension of 50 mg/ml of sodium urate in saline. (●) animals receiving no drug (5); (□, ■, ○) animals receiving 10 (5), 50 (5), and 100 (4) mg/kg of DAAC, respectively; (▲) animals receiving 2 mg/kg of colchicine (5). Number of animals used is given in parentheses.

inflammation responded to colchicine and other anti-inflammatory agents. Injection of microcrystalline sodium urate into the subplantar region of the rat paw produced a paw swelling which was measured by means of a contact measuring device (3) or by weighing the amputated paw (4). Using the paw immersion technique (11) we have measured the paw swelling generated by subplantar injection of microcrystalline sodium urate in mice (Fig. 1). The time course of the swelling is similar to that reported for the urate-induced inflammation in dogs and nongouty humans (15). Furthermore, the swelling was strikingly inhibited by pretreatment of the animals with colchicine (Fig. 2). Thus, it seemed that the sodium urate-induced paw swelling in mice would provide a convenient and reasonable model for examing structure–activity relationships of colchicine and related compounds. Nevertheless, it would be presumptuous to extrapolate the results obtained with this animal model to potential activity in humans. This point is emphasized by the fact that desacetylcolchiceine (IV) is as effective as colchicine against human gout (16) but is much less effective than colchicine in our model system.

Although the structure of colchicine (I) has been known for several years, there has been a paucity of research concerning the structural features of colchicine which make it unique as an antigout agent. With our paw-swelling model of urate-induced inflammation in mice, we have attempted to analyze some structural features of colchicine for their contribution to the drug's anti-inflammatory activity. Some of the compounds examined for activity represent portions of the skeletal structure of colchicine, while others retain the basic carbon skeleton but possess modified functional groups (Fig. 6).

Introduction of a cyano substituent into the four position of the benzene ring (VI) reduced the activity against the paw swelling. Since this compound was the only 4-substituted derivative of colchicine tested it is not possible to decide whether it is the presence of a substitutent at the four position which reduces activity, or whether the nature of the substituent itself is the determining factor.

The loss of activity observed on conversion of the tropone methoxyl of colchicine (I) to a hydroxyl (colchiceine, V) parallels the finding of Wallace that colchiceine is inactive against human gout (16). This result suggests that a methoxyl group in the tropone ring is probably necessary for activity. Nevertheless, the anti-inflammatory activity is

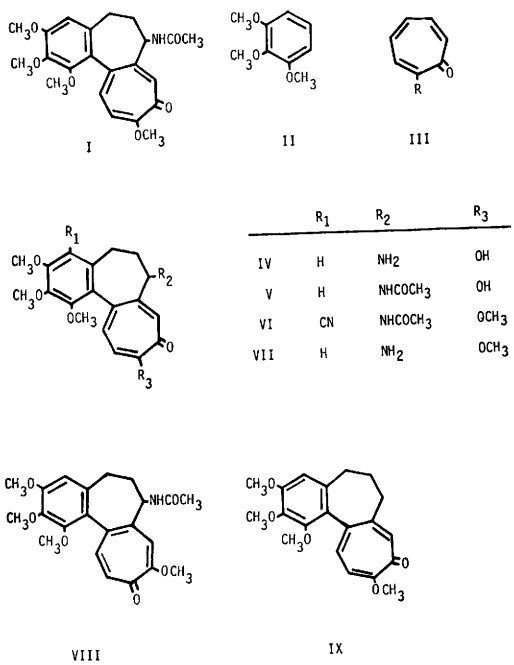

FIG. 6. Molecular structures of the various substances tested for their effect on sodium urate-induced paw swelling in mice: colchicine (I), trimethoxybenzene (II), 2-methoxytropone (III R = OCH_3), tropolone (III, R = OH), desacetylcolchiceine (IV), colchiceine (V), 4-cyanocolchicine (VI), desacetylcolchicine (VII), isocolchicine (VIII), desacetamidocolchicine (IX).

quite dependent on the substitution pattern in the tropone ring as suggested by the lack of activity of isocolchicine (VIII). In this compound, which retains all the chemical groupings of colchicine, the tropone carbonyl and methoxyl groups are interchanged.

That the nitrogen function and the tropone methoxyl group must both be present for activity is illustrated by the results obtained with desacetylcolchiceine (IV), desacetylcolchicine (VII) and desacetamidocolchicine (IX). When the acetyl group is removed from colchicine, to give desacetylcolchicine, activity is retained although somewhat diminished. Complete removal of the nitrogen function (desacetamidocolchicine, IX) resulted in a 50-fold decrease in activity. Although desacetylcolchicine (VII) retained some activity, replacement of the tropone methoxyl of this compound with a hydroxyl (to give desacetylcolchiceine, IV) reduced activity against paw swelling to one-fiftieth that of colchicine. Since either replacement of the tropone methoxyl group with a hydroxyl or removal of the *N*-acetyl group of colchicine reduces activity it might seem pointless to examine desacetylcolchiceine (IV) for its anti-inflammatory activity. However, desacetylcolchiceine is reported to be as effective in the treatment of gout as colchicine (16). In our animal model this substance suppressed the urate-induced swelling only at a dose 50 times that of colchicine. Desacetylcolchiceine, by virtue of the free amino group and the tropolone hydroxyl is amphoteric; this property is not found in the nonionizable colchicine molecule. Consequently desacetylcolchiceine, depending on the pH of the local biophase environment, could conceivably bear either a positive or negative charge. This is not possible with colchicine at any imaginable physiological pH. Because of this difference in chemical properties then, it is quite possible that desacetylcolchiceine may act in humans by a different mechanism than does colchicine. There is some evidence that desacetylcolchiceine does *not* produce its effect in humans in the same manner as does colchicine (17). Hence, it is possible that the mechanism through which desacetylcolchiceine acts in humans is absent or not significant in our mouse model.

Results obtained with these compounds indicate that the methoxytropone system and the nitrogen function of colchicine are at least necessary for anti-inflammatory activity in our system. It is tempting to suggest that the trimethoxyphenyl moiety is also necessary for activity. However, it may be possible that either a simple benzene ring or one or more methoxyl or hydroxyl groups on the ring would suffice.

Among current theories concerning colchicine's antimitotic and anti-inflammatory activities, disruption of cytoplasmic microtubule systems by binding of colchicine to a protein subunit of the microtubules appears to be a common denominator (18). Hence it is of interest to compare the known antimitotic activities of the colchicine derivatives we have tested with their effect on the sodium urate-induced paw swelling. Both colchiceine

(V) and isocolchicine (VIII) are much weaker antimitotic agents than colchicine (19), and both substances were at least 5 and 25 times, respectively, less active against the paw swelling. Desacetylcolchiceine (IV) is virtually devoid of antimitotic activity but is claimed to possess antigout activity in humans (16). In our animal model of gout this substance was only one-fiftieth as active as colchicine. Desacetylcolchicine (VII), which suppressed paw swelling but required a dose five times as great as colchicine, has an antimitotic activity of approximately one-third that of colchicine (13). In view of the correspondence between the antimitotic and anti-inflammatory activities of these drugs, it was surprising to find that desacetamidocolchicine (IX) at 50 times the dose of colchicine was only slightly active against the urate-induced paw swelling (Fig. 5). Desacetamidocolchicine has been shown to be 5 to 25 times more potent than colchicine as an antimitotic agent in three diverse systems, *i.e.*, murine mast cell tumor (12), chicken embryo fibroblasts (13), and onion root meristem (14). If the antimitotic activity of desacetamidocolchicine is the result of microtubule dissolution, as it is believed to be the case for colchicine (18), then it is possible, at least in our test system, that disruption of microtubules may not be the sole factor in the colchicine-mediated suppression of the urate-induced inflammation. Other explanations for the low activity of desacetamidocolchicine, such as a more rapid biotransformation and/or excretion, are of course also possible.

Summary. Several compounds representing various structural modifications of the colchicine molecule were examined for their anti-inflammatory activity in an experimental model of inflammation to determine which parts of the colchicine molecule are necessary for its anti-inflammatory activity. The experimental model of inflammation consisted of mouse paw swelling induced by a subplantar injection of a suspension of microcrystalline sodium urate. Paw swelling was then measured by the paw immersion technique. The results indicate that the methoxytropone moiety and the nitrogen function of colchicine are essential for anti-inflammatory activity. Complete removal of the nitrogen function of colchicine resulted in a compound with only one-fiftieth the activity of colchicine. Since this substance is known to be a more potent antimitotic agent than colchicine, these results suggest that the anti-inflammatory and antimitotic activities of these compounds are distinct at least in our test system.

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