

Colchicine in Avian Sodium Urate and Calcium Pyrophosphate Microcrystal Arthritis

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Abstract

The effect of single doses of colchicine on the acute arthritis elicited by the injection of microcrystalline sodium urate, calcium pyrophosphate or talcum into one intertarsal joint of chickens was functionally assessed. Colchicine was significantly active against the urate challenge. The arthritic inflammation induced by calcium pyrophosphate or talcum was reduced to a lesser degree. Colchicine may thus particularly affect inflammatory processes related to urate.

Attacks of acute gout in humans respond to colchicine [1-3]. On the other hand, pseudogout or chondrocalcinosis is resistant to this drug [4, 5]. Needle-shaped sodium urate crystals are found in the synovial cavities of patients with gout while in pseudogout, needle-, rod- and tablet shaped calcium pyrophosphate crystals cause the symptoms [6]. Moreover, intra-articular injection of microcrystals provoke both in man and dogs an acute inflammation which closely resembles the gouty attack [7-9].

We were interested to ascertain experimentally whether colchicine exerts its effect, as suggested by clinical experience, specifically in gout or whether the activity of this agent is independent of the type of microcrystal responsible for the elicitation of the arthritis.

Materials and methods

One-legged position test

The experimental model has been described previously [10, 11]. In short, microcrystals were injected into one intertarsal joint of three-week-old chickens. This causes the birds to lift the injected leg after a latency period. The time during which the birds stand on one leg within the observation time is likely to correspond to the intensity of the intrasynovial inflammation. Accordingly, antiarthritic drug activity may be expressed by the reduction of the one-legged standing time. We observed the stance of the chickens for three hours [10]. The average

one-legged standing time for each group of treated animals was then compared to the value of the corresponding control group and expressed as percent change.

Colchicine was administered subcutaneously before the intra-articular deposition of the crystals at the intervals indicated in Tables 2 and 3. The drug was dissolved in sterile isotonic saline and injected in a volume of 0.1 ml/100 g body weight. The controls received only saline.

Crystals

Some preliminary experiments were carried out to assess the most suitable type and concentration of microcrystals to elicit an arthritis of similar intensity. The following microcrystals were used: sodium urate (prepared according to [7]), commercially available calcium pyrophosphate dihydrate and talcum (magnesium hydroxypoly-silicate). The characteristics of the crystal suspensions used are given in Table 1. The needles or rods of the urate and

Table 1
Characteristics of the intratarsally injected microcrystal suspension.

Type of crystal	Shape and size	Proportion (%)	No. of microcrystals in 0.1 ml
Sodium urate	needles 1-5 μm	72	470×10^6
	needles 5-10 μm	16	
	needles 10-15 μm	7	
	needles 15-20 μm	5	
Calcium pyrophosphate	speckles 1-5 μm	86	480×10^6
	needles 5-10 μm	9	
	amorphous 5-10 μm crystals	5	
Talcum	speckles 1-5 μm	54	47×10^6
	needles 5-10 μm	12	
	amorphous 5-20 μm crystals	15	
	amorphous 20-50 μm crystals	19	

pyrophosphate crystals were of comparable appearance and measured 1–20 μm . Their forms corresponded to the shapes observed in aspirates from joints of patients with gout and pseudogout [4]. The urate crystals were somewhat thinner than pyrophosphate. A fraction of the latter were also tablet-shaped and broken. The relative composition of the different forms was similar with urate and pyrophosphate crystals. With talcum crystals, amorphous sizes were more frequent. The same batches of microcrystals were used throughout the experiment.

The crystals were suspended in 6% dextran in isotonic saline (Macrodex®) and injected in volumes of

0.1 ml. Either 2.25 g/percent sodium urate, 9 g/percent calcium pyrophosphate or 7 g/percent talcum were used. These concentrations led to a one-legged standing time of about 110 to 120 minutes (Fig. 1).

Results

Dose effect of crystals

With all three types of crystals, the intensity of the inflammatory response as expressed by the duration of the one-legged standing time was proportional to the quantity of the injected crys-

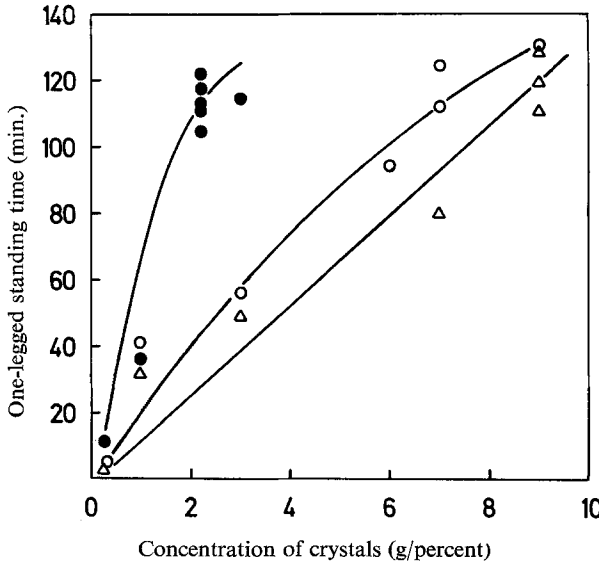


Figure 1
Relationship between the concentration of the crystal suspension injected into one intertarsal joint of chickens and their one-legged standing time. The points represent the mean values of groups of six chickens. ●—● sodium urate; Δ — Δ calcium pyrophosphate; ○—○ talcum.

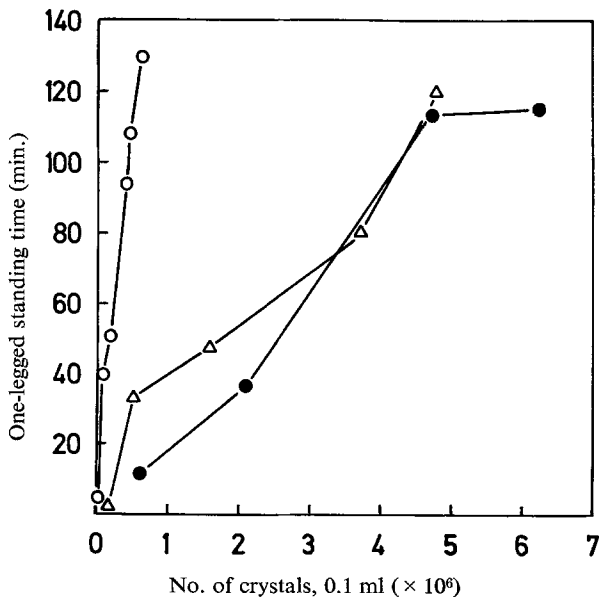


Figure 2
Relationship between the number of injected crystals and the one-legged standing time. Symbols as in Figure 1. The crystals were counted in a hemocytometer.

tals (Fig. 1). It was found that similar numbers of urate and pyrophosphate crystals caused inflammations of equal intensity. With both crystals, $470-480 \times 10^6$ particles caused a one-legged standing time of about 120 minutes. A tenfold smaller quantity of talcum crystals led to the same result. The relationship between the number of intra-articular crystals and the one-legged standing time is depicted in Figure 2.

Susceptibility of the microcrystal arthritis to colchicine

Two colchicine regimens were found to be significantly active. First, with an interval of five hours between the drug and the crystal injection,

1 mg/kg of colchicine reduced the one-legged standing time by 47% (Table 2). The most pronounced inhibition of the one-legged stance was observed with two doses of 0.5 mg/kg colchicine applied at twenty-four and five hours before the urate. It reduced the one-legged standing time by 60–65% (Tables 2 and 3). Interestingly, the colchicine regimen did not significantly affect the arthritis produced by calcium pyrophosphate (Table 3, groups 2 and 5) or talcum (Table 3, groups 3 and 5). With 1.5 mg/kg of colchicine, despite some reduction of the arthritic inflammation induced by calcium pyrophosphate or talcum, the preferential susceptibility of the sodium urate arthritis persisted (Table 3).

Table 2

Effect of colchicine on the microcrystal arthritis in chickens induced by sodium urate.

Group	No. of chickens	Colchicine dose (mg/kg)	Applied at hour	One-legged standing time		Reduction (%)
				Controls (min)	Colchicine (min)	
1	6	0.75	- 1	112±16	66±18	-41
2	6	1	- 5	113±13	60± 6	-47*
3	6	1	-24	121±24	82±10	-32
4	6	0.25	-44, -30, -20, - 6	121±17	106±17	-12
5	5	0.5	-24, - 5	106±19	37±11	-65*

The chickens were injected into the left intertarsal joint with 2.25 g/percent sodium urate. Colchicine was applied subcutaneously at the indicated dosages and time intervals before the crystal injection. The percentual reduction of the one-legged standing time on the right leg of the colchicine-treated groups was related to the equally sized control groups used in the same experiment. The reductions of the one-legged standing time of groups 2 and 5 differ significantly from the corresponding controls.*) $p < 0.05$, Student's *t*-test. The differences between the various control groups are statistically not significant. \pm = Standard deviation.

Table 3

Effect of colchicine on the microcrystal arthritis in chickens induced by sodium urate (U), calcium pyrophosphate (P) or talcum (T).

Group	No. of chickens	Type of microcrystal and concentration (g/percent)	Colchicine dose (mg/kg)	Applied at hour	One-legged standing time		Reduction (%)
					Controls (min)	Colchicine (min)	
1	12	U; 2.25	0.5	-24, -5	110±13	43± 4	-61*
2	12	P; 9	0.5	-24, -5	124±12	91±12	-27
3	12	T; 7	0.5	-24, -5	119±34	99±26	-17
4	12	U; 2.25	1.5	- 1	103±28	33± 9	-68*
5	9	P; 7	1.5	- 1	77±20	54±23	-30
6	9	T; 5	1.5	- 1	90±26	54±19	-40

See legend of Table 2. The chickens were injected into the left intertarsal joint with the microcrystals as indicated. Significant inhibition by colchicine was provided only in the arthritis induced by sodium urate.*) $p < 0.05$, Student's *t*-test.

Discussion

We have confirmed experimentally the clinical experience that the arthritis caused by calcium pyrophosphate or other microcrystals is less susceptible to colchicine therapy than gout, which is caused by sodium urate microcrystals. The inflammatory reaction induced by urate responds also to other agents besides colchicine [11, 12]. However, the antiarthritic activity of colchicine manifests itself to a greater degree in arthritic inflammations caused by urate than in those due to other microcrystals such as calcium pyrophosphate or talcum.

Modification of microcrystal arthritis by colchicine is thought to be related to a decreased immigration of polymorphonuclear leukocytes into the joint and to a functional impairment of crystal phagocytosis. More recently, the action of colchicine has been ascribed to its interference with cellular microtubular protein [13].

The observed effect of colchicine on the urate arthritis is difficult to explain by such an interference with unspecific processes applying to all types of microcrystal arthritis, because then arthritic lesions induced by the same number of pyrophosphate crystals ought to be equally sensitive to colchicine. As this is not the case, colchicine may particularly affect events related to urate, such as specific metabolites or mediator substances appearing in conjunction with uricolysis brought about by the leukocytes [14]. Differential membranolytic effects of sodium urate and calcium pyrophosphate microcrystals [15] also support the possibility that colchicine exerts specific effects on urate-induced changes. The difference may also be explained by the observation that colchicine inhibits the release of chemotactic activity following phagocytosis of sodium urate crystals but reduces it less after phagocytosis of calcium pyrophosphate crystals [16]. The release of chemotactic activity thus does not depend solely on microcrystal phagocytosis *per se* but is related to specific crystal effects.

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