TOXICOLOGICAL STUDIES ON PIPEMIDIC ACID. V*. EFFECT ON DIARTHRODIAL JOINTS OF EXPERIMENTAL ANIMALS

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Abstract.....Pipemidic acid (PPA) orally given in a dose of 100 mg/kg/day or more was found to cause lame gait in immature beagle dogs of about 3 months old. Their diarthrodial joints were abnormal with increased synovial fluid and blister formation under the outer layer of the articular cartilage. However, such an abnormality was not found in dogs younger than 2 weeks or older than 12 months. The blisters were formed at the joint areas bearing the body weight at a time when PPA was considered to be present there. Nalidixic and piromidic acids, structural analogues of PPA, also caused abnormality similar to PPA. The severity of the arthropathy was slight with piromidic acid as compared with PPA and nalidixic acid. The gait abnormality was almost disappeared spontaneously even if medication was continued. The incidence of the arthropathy was not or rarely observed in any young rats, rabbits and monkeys.

Key words : pipemidic acid, arthropathy

INTRODUCTION

Pipemidic acid (PPA), 8-ethyl-5, 8-dihydro-5-oxo-2-(1-piperazinyl) pyrido [2, 3-*d*] pyrimidine-6-carboxylic acid trihydrate, is a newly synthesized antibacterial agent, which is active mainly against gram-negative bacteria including *Pseudomonas aeruginosa* and most of bacteria resistant to piromidic and nalidixic acids (Shimizu *et al.*, 1975 a).

In the studies using young adult animals, PPA has been reported to be a compound of low toxicity as determined by subacute toxicity tests in rats, dogs and monkeys (Senda *et al.*, 1975 a, b, c), and chronic toxicity tests in rats and dogs (Senda *et al.*, 1975 a, b). However, it was found that the compound, when administered to immature beagle dogs of about 3 months old, induced abnormal gait with

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alterations in diarthrodial joints.

The present study was performed to investigate this phenomenon in more detail.

MATERIALS AND METHODS

Experimental animals

Dogs : Dogs used were male and female beagles of 1 week - 18 months old bred in our laboratory or purchased from Japan E. D. M. INC., a male spitz and a male pomeranian of 2 months old each, and male and female mongrels about 3 months old.

Sucking dogs were kept under nursing of dams, and dogs of over 2 months old were housed in a cage or tethered individually in air-conditioned rooms kept at about 24°C. They were given commercial food DS (Oriental Yeast Co., Ltd.) and skim milk, and water *ad libitum*.

Rats : Male Sprague-Dawley rats of 3 weeks old used were obtained from CLEA Japan INC.. They were housed individually in an air-conditioned room kept at $24 \pm 2^\circ\text{C}$ of room temperature and $55 \pm 5\%$ of humidity, and given commercial food CE-2 (CLEA Japan INC.) and water *ad libitum*.

Rabbits : Male and female albino rabbits were housed in a cage individually and given commercial food RC-4 (Oriental Yeast Co., Ltd.) and water *ad libitum*.

Monkeys : Wild crab-eating monkeys (*Macaca irus*) and rhesus monkeys (*Macaca mulatta*) of 5-10 and 9-12 months of presumptive age, respectively, were imported from Southeast Asia. Monkeys were housed in a cage individually, and given commercial food AB (Oriental Yeast Co., Ltd.) and fruits, and water *ad libitum*.

Compounds

Pipemidic acid (Matsumoto *et al.*, 1975) and piromidic acid (Minami *et al.*, 1971) were synthesized at Research Laboratories, Dainippon Pharmaceutical Co., Ltd., and nalidixic acid was extracted from commercial tablets. The compounds suspended in 0.5% aqueous gum tragacanth were administered twice a day (9:00 a.m. and 4:00 p.m.) unless otherwise described.

Observation and laboratory tests

Animal behavior and appearance were observed in detail every day during experimental terms. Spontaneous electromyogram (EMG) and evoked EMG (H and M waves) were taken in some dogs without anesthesia, and biochemical analysis of blood samples was performed by an autoanalyzer (Technicon AutoAnalyzer SMA 12/60 -survey model- and basic type).

For autopsy, animals were sacrificed by venesection under anesthesia with pentobarbital sodium. Abnormality of all joints of the limbs and Articulationes atlantooccipitalis and atlantoaxialis opened carefully, was checked macroscopically or with a dissecting microscope. In some dogs their limbs were examined by X-ray rad-

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iography. A part of joint with abnormality was fixed with 10% formalin and prepared for histopathologic examination after decalcification with trichloroacetic acid. Vertical sections of the joints were stained with hematoxylin and eosin, toluidine blue or colloidal iron. PPA levels in plasma were determined by the thin-layer cup-plate method using *E. coli* Kp as an indicator organism (Shimizu *et al.*, 1975b).

RESULTS

1. Arthropathy in young beagle dogs receiving PPA

When young beagle dogs of 2.5–3.5 months of age were orally given 300mg or more of PPA/kg/day in two divided doses (hereinafter referred to as 300 mg of PPA/kg/day or 300 mg/kg/day), the animals showed abnormal bending in Articulationes manus and lame gait about 2 days after medication, and laid down on the side, making staggering gait when forced and broken down readily on loading within 3 days (Table 1). Such an abnormal state was the most marked on the 3rd–7th days after medication.

Such abnormal gait was not noted in a dose of less than 30 mg/kg/day. The abnormal gait manifested in massive doses was gradually recovered, even on further

Table 1. Arthropathy in young beagle dogs receiving pipemidic acid

Animal No.	Sex	Age (month)	Dosage (mg/kg × days)	Degree of lame gait**	Articulationes atlantooccipitalis & atlantoaxialis	Membrum anterior Articulationes				Membrum posterior Articulationes			
						humeri	cubiti	manus	mcp*	coxae	genus	pedis	mtp**
666	F	3.5	1,000 × 3	++	—	f+e	f+e	f	f	f+e	f+b	f	f
671	M	3.5	1,000 × 14	++	f+e	f+e	f+e	f+b	f+b	f+e	f+e	f+b	f+b
667	F	3.5	500 × 8	++	—	f+b	f+b	f+b	f	f+b	f+b	f+b	f+b
EJ4M1	M	3	300 × 90	++ → —	e	e	e	e	e	e	e	e	—
BC4M4	M	3	300 × 90	++ → —	e	e	e	b	e	e	e	e	b
IU1F1	F	2.5	300 × 90	++ → —	e	b	e	e	b	e	e	e	b
674	F	3.5	250 × 7	+	—	f+b	f+b	f	f	f+b	f+b	f+b	f
700	F	3.5	100 × 7	+	—	f+b	f'	f'	f'	f+b	—	f+b	f'
BC4M3	M	3	100 × 90	++ → —	e	e	e	e	—	e	b	—	—
HS2M2	M	3	100 × 90	++ → —	—	e	e	—	—	—	b	b	—
EJ4F1	F	3	100 × 90	++ → —	—	e	e	—	—	e	e	e	e
EL3M1	M	3	30 × 90	—	—	b	e	—	—	—	—	e	—
XX2M2	M	3	30 × 90	—	—	—	—	—	—	—	e	e	—
HS2F2	F	3	30 × 90	—	e	e	e	—	—	—	—	e	—
HS2M1	M	3	10 × 90	—	—	—	—	—	—	—	—	—	—
BC4M5	M	3	10 × 90	—	—	—	—	—	—	—	—	—	—
XX1F1	F	3	10 × 90	—	—	—	—	—	—	—	—	—	—

*) daily dosage(administered in two divided doses)

* : metacarpo-phalangeae

**) + : slight, ++ : marked, — : no abnormality

** : metatarso-phalangeae

+ + → — : Marked lame gait was found on the 3rd–7th days of medication, but recovered to nearly normal gait by 4 weeks.

+ → — : Slight lame gait was found from the 3rd day of medication, but disappeared gradually by 3 weeks.

f : increase of synovial fluid, f' : slight increase of synovial fluid, b : blister, e : erosion, — : no alteration

medication, to nearly normal state in about 4 weeks in all animals.

There was no abnormal finding in both of spontaneous and evoked EMGs taken in beagle dogs with gait disturbances.

Biochemical tests of blood samples collected during medication revealed no abnormal finding in such items as glucose, Ca^{++} , inorganic phosphorus, urea nitrogen, creatinine, cholesterol, total protein, albumin, A/G, total bilirubin, Al-P, LDH, GOT, GPT, Na^+ , K^+ and Cl^- .

Autopsy performed on the 3rd-14th days after medication disclosed the following abnormalities in dogs with gait disturbances; increased synovial fluid in synovial cavity of the four limbs, blisters of various sizes under the outer layer of articular cartilage (Photo. 1), some of which were broken to cause erosion on the surface (Photo. 2) and fragments of cleft surface cartilage in the fluid.

On microscopic examination, fibrous portions with regressive cells developed near the outer layer of articular cartilage with a general orientation parallel to the joint surface, where fluid substance was retained (Photo. 3). Microscopic alteration was hardly observed in the synovial membrane (Photo. 4).

As seen in Table 1, there was blister or erosion formation in joints related to dosage in cases medicated for 90 days, but there was no such increase of synovial fluid as noted in the early state of medication.

Histopathologically, the articular cartilage was thin at the injured portion and there were chondrocytes divided like giant cells in the peripheral cartilage tissue and uneven stroma in some surrounding area of the lesion. At autopsy on the 91 st

Table 2-1. Arthropathy in young beagle dogs receiving pipemidic acid
-Relationship of articular abnormality to the age of beagles-

No.	Animal Sex	Age (month)	Dosage (mg/kg [*] × days)	Degree of lame gait ^{**}	Articulationes atlantooccipitalis & atlantoaxialis	Membrum anterior Articulationes				Membrum posterior Articulationes			
						humeri	cubiti	manus	mcp*	coxae	genus	pedis	mtp**
481	F	17.5	1,000×14	—	—	—	—	—	—	—	—	—	—
482	F	17.5	1,000×14	—	—	—	—	—	—	—	—	—	—
486	F	16.5	1,000×14	—	—	—	—	—	—	—	—	—	—
728	F	12	1,000×7	—	—	—	—	—	—	—	—	—	—
734	M	12	1,000×7	—	—	—	—	—	—	—	—	—	—
735	F	12	1,000×7	—	—	—	—	—	—	—	—	—	—
741	F	12	1,000×7	—	—	—	—	—	—	—	—	—	—
759	M	12	1,000×7	—	—	—	—	—	—	—	—	—	—
795	M	9	1,000×7	—	—	f+b	—	—	—	—	b	—	—
796	M	9	1,000×7	—	—	—	—	—	—	e	—	—	—
800	F	9	1,000×7	—	—	—	—	—	—	—	—	—	—
868	M	6	1,000×7	++	f+e	f+e	f+b	f+b	—	f+e	f+b	—	f+b
889	M	6	1,000×7	++	f+b	f+e	—	—	—	f+e	f+b	—	—
899	M	6	1,000×7	++	f+e	f+e	f+b	f+b	—	f+e	f+e	f+b	—

^{*)} daily dosage (administered in two divided doses)

* : metacarpo-phalangeae

^{**)} ++ : marked, — : no abnormality

** : metatarso-phalangeae

f : increase of synovial fluid, b : blister, e : erosion, — : no alteration

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Table 2-2. Arthropathy in rounq beagle dogs receiving pipemidic acid
-Relationship of articular abnormality to the age of beagles-

No.	Animal Sex	Age (week)	Dosage (mg/kg*×days)	Degree of lame gait**	Articulationes atlantooccipitalis & atlantoaxialis	Membrum anterior				Membrum posterior			
						Articulationes				Articulationes			
						humeri	cubiti	manus	mcp*	coxae	genus	pedis	mtp**
587-2	F	4	500×7		f+b	f+e	f+e	f+b	f+b	f+e	f+e	f+b	f+b
212-2	F	4	500×7		f+b	f+e	f+b	f+b	f+e	f+e	f+e	f+b	f+b
146-2	F	4	500×7		f+b	f+e	f+e	f+e	f+e	f+e	f+e	f+e	f+b
578-1	F	4	100×7		f+b	f+b	f+b	—	f+b	f+b	f+e	f+b	f+b
212-1	F	4	100×7		f+b	f+e	f+e	f+b	f+b	f+b	f+b	f+b	f+b
146-1	M	4	100×7		f+b	f+b	f+b	—	f+e	f+b	f+e	f+b	f+b
219-2	F	2	45×30		—	—	—	—	—	—	—	—	—
219-3	M	2	45×30		—	—	—	—	—	—	—	—	—
326-3	F	2	45×30		—	—	—	—	—	—	—	—	—
219-1	F	2	15×30		—	—	—	—	—	—	—	—	—
326-1	M	2	15×30		—	—	—	—	—	—	—	—	—
326-2	M	2	15×30		—	—	—	—	—	—	—	—	—
731-1	M	1	100×7		—	—	—	—	—	—	—	—	—
731-2	F	1	100×7		—	—	—	—	—	—	—	—	—
731-3	F	1	100×7		—	—	—	—	—	—	—	—	—

°) baily dosage (administered in two divided doses)

* : metacarpo-phalangeae

°°) too young to check the abnormal gait.

** : matetarso-phalangeae

f : increase of synovial fluid, b : blister, e : erosion, — : no alteration

day of medication, neither the X-ray radiographic findings of appendicular skeleton nor the length of the femur and humerus gave any difference between medicated and control groups. Moreover, the cartilage of the trachea and epiphysis did not show any histopathological alterations.

2. Arthropathy in relation to the age of beagle dogs

Although PPA caused the abnormality in gait and joints in young beagle dogs of about 3 months old as shown in Table 1, it failed to cause such an abnormality in mature beagle dogs of 12 months or older as seen in Table 2.

When an oral dose of 1,000 mg of PPA/kg/day was given to beagle dogs of 9 months old for 7 days, there was no abnormal gait, nor, slight if any, abnormality in the articular cartilage. Beagle dogs of 6 months old orally receiving 1,000 mg of PPA/kg/day showed abnormality in gait within 3 days and in large joints such as Articulationes atlantooccipitalis, atlantoaxialis, humeri, cubiti, coxae and genus similar to that observed in beagle dogs of 2.5–3.5 months old, though arthropathy in Articulationes manus, pedis and other distal joints was low in incidence and severity. It was not possible to observe the gait of young beagle dogs of 4 weeks old, but autopsy demonstrated abnormality in the articular cartilage. However, the abnormality was not observed in younger dogs of 2 weeks or 1 week old.

3. Comparison of PPA and its analogues in the effect causing arthropathy

Effect of nalidixic acid (NA) and piromidic acid (PA), both PPA analogues, on arthropathy was examined in young beagle dogs of 2.5–4 months old (Table 3)

Table 3. Arthropathy in young beagle dogs receiving nalidixic acid(NA) and piromidic acid (PA)

No.	Animal Sex	Age (month)	Compounds & Dosage (mg/kg × days)		Degree of lame gait**	Articulationes atlantooccipitalis & atlantoaxialis	Membrum anterior Articulationes				Membrum posterior Articulationes			
							humeri	cubiti	manus	mcp*	coxae	genus	pedis	mtp**
673	M	3	NA	1,000 × 3	...	f+b	f+b	f+b	f+b	f+b	f+b	f+b	f+b	f
664	F	3.5	NA	500 × 7	...	f+b	f+e	f+b	f+b	f	f+b	f+b	f+b	f
679	F	3.5	NA	250 × 7	...	—	f+b	f+b	f	f	f+b	f+b	f+b	f
702	F	4	NA	100 × 7	+	—	f+b	f	f+b	f	f+b	f+b	f	f'
AA5M1	M	3	NA	30 × 90	—	—	e	—	—	—	—	e	—	—
CO3M2	M	2.5	NA	30 × 90	—	—	—	e	—	—	—	e	e	—
CT5F1	F	2.5	NA	30 × 90	—	—	—	—	e	—	—	b	—	—
676	M	2.5	PA	1,000 × 17	+	—	f+b	f+b	f+e	—	f+b	—	—	—
678	F	3	PA	500 × 18	—	—	—	—	—	—	—	—	—	—
AO6F2	F	2.5	PA	500 × 7	—	—	f+e	f+e	—	—	f+b	f+b	—	—
ES3F1	F	3.5	PA	500 × 7	—	—	f+b	f+b	—	—	f+b	f+b	f+b	—
AO6F3	F	3.5	PA	100 × 7	—	—	—	—	—	—	—	—	—	—

*) daily dosage (administered in two divided dosage)

* : metacarpo-phalangeae

**) + : slight, — : no abnormality

** : metatarso-phalangeae

***) Abnormality of gait could not be checked due to other severe toxic symptoms caused by NA.

f : increase of synovial fluid, f' : slight increase of synovial fluid, b : blister, e : erosion, — : no alteration

and compared with PPA (Table 1).

In the case of PPA, abnormal gait was marked in a dose of 300 mg/kg/day or more, slight at 100 mg/kg/day, and not found at 30 mg/kg/day or less. Autopsy disclosed arthropathy in the limbs in a dose of 100 mg/kg/day or more, less frequent manifestation of blisters and erosions in a dose of 30 mg/kg/day, and no abnormal finding in the joints in a dose of 10 mg/kg/day, even if PPA was given for a long period of 90 days.

A dose of 250 mg of NA/kg/day or more is toxic to the dog, and toxic symptoms prevented us from observation for gait disturbance. However, autopsy findings disclosed increased synovial fluid, blister (Photo. 5) or erosion formation in a dose of 100 mg/kg/day or more as seen with PPA, and the formation of blister or erosion was also low frequency in a dose of 30 mg/kg/day for 90 days like the same dose of PPA.

The severity of abnormality was slight with PA as compared with PPA and NA, and gait abnormality was slightly seen in a dose of 1,000 mg/kg/day, and sporadic blisters were sometimes observed. However, there were no abnormal gait and slight blister formation in a dose of 500 mg/kg/day. No abnormality was observed in gait and joints in a dose of 100 mg/kg/day for 7 days.

4. Recovery of arthropathy found in young beagle dogs

Although abnormal gait induced by PPA disappeared in about 4 weeks even on continuing medication, the course of recovery from abnormal gait was further

studied by stopping the medication at that time when the abnormality was pronounced.

When PPA was discontinued after given in a dose of 1,000 mg/kg/day for 1 week, the animals were restored to normal gait within about 1 week of discontinuation. Autopsy performed 30 days after the final medication did not disclose increase synovial fluid but blisters in the articular cartilage. Histopathological study revealed chondrocytes divided like giant cell appearing in the outer cartilage with blister, showing a picture of repair process (Sakakida *et al.*, 1972) (Photo. 6). At autopsy performed about 4 years after discontinuation, however, the areas at which blister or erosion seemed to be formed were still found as scars, although the repair process progressed than that in the cases of 30-day discontinuation.

5. Effect of PPA on other breeds of the dog and other animal species

5-1) Young spitz, pomeranian and mongrel dogs

When a spitz and a pomeranian of 2 months old were orally given PPA in a dose of 1,000 mg/kg/day for 4 days and in a dose of 500 mg/kg/day for 3 days, respectively, abnormal gait, increased synovial fluid, and blister formation in the outer layer of articular cartilage were observed as in beagle dogs. Similar abnormalities were noted in a young mongrel dog of the presumptive age of 3 months which was given PPA in a dose of 500 mg/kg/day for 3 days.

5-2) Young rats

When 5 male rats of Sprague-Dawley strain of 3 weeks old were orally given PPA in a dose of 1,000 mg/kg/day for 20 days, any abnormality in joints was not detected.

5-3) Young rabbits

When PPA was orally given to groups of albino rabbits of 4 weeks old each consisting of 2 males and 1 female in doses of 30, 100 and 300 mg/kg/day, once a day for 30 days, there was no abnormality in joints. Administration of more than 300 mg/kg/day was not possible because of its toxicity.

5-4) Adult monkeys

When five crab-eating monkeys of 5-10 years of presumptive age were given PPA in a dose of 1,000 mg/kg/day for 30 days intragastrically via nose, there was abnormality probably due to premedication in articular cartilage surface of Articulationes humeri and coxae, but no abnormality in joints ascribable to PPA.

5-5) Young monkeys

When three of male crab-eating monkeys of 5-10 months of presumptive age, and three of rhesus monkeys (2 males and 1 female) of 9-12 months of presumptive age were given PPA in a dose of 1,000 mg/kg/day every day intragastrically via nose, there was no abnormal finding in behavior and locomotion. Autopsy performed on the 30th day of medication failed to prove any abnormality in such joints as Articulationes humeri, cubiti, carpi, coxae, genus and pedis of all animals. However,

discussing-microscopic examination incidentally demonstrated a small change like blister or erosion in the articular cartilage surface of digits of some animals.

6. Relation of exercise load to arthropathy

In consideration of the fact that blisters formed in young dogs were apt to develop in such joint area under mechanical pressure as articular cartilage in dogs of particular months of age, it is inferred that PPA exerts certain effect on the articular cartilage in the growing stage, and leads to blister formation in association with mechanical pressure onto it. In order to prove this inference, a young beagle dog was fixed its forefoot with plaster bandage to keep some of the joints pressure-free and given PPA in a dose of 1,000 mg/kg/day for 3 days. As a result, there was blister formation in the joints subjected to pressure, but no blisters were noted in the joints protected from pressure (Table 4).

Further, on the assumption that blister formation takes place when pressure is put only while PPA is present in articular cartilage, young dogs were given PPA and kept hung with their shoulder up so that the joints of the forefeet should be free from pressure for a certain period of time during which PPA was considered to exist in the articular cartilage, and then examined if blister formation takes place in the joints of forefeet. The levels of PPA in the articular cartilage were estimated from its plasma levels because it has been disclosed by macroautoradiography that the formers change in parallel with the latters (Hashimoto *et al.*, 1975).

In the first, a single dose of 500 mg of PPA/kg was given to dogs without

Table 4. Relation of pressure load to blister formation in a young beagle dog* receiving pipemidic acid

Dog No.	Dosage (mg/kg* × days)	Articulatio atlanto- occipitalis	Membrum anterior Articulationes							Membrum posterior Articulationes																					
			humeri	cubiti	manus	interphalangeae					coxae	genus	pedis	interphalangeae																	
						I	II	III	IV	V				II	III	IV	V														
XX1M1	1000×3	—	L**	17×10	7×5	—	—	—	—	—	10×7	13×10	10×8	4×4	2×1	2×1	2×2	2×2													
				7×5	7×2							8×7							7×4												
			R	8×8	5×5							—							—	2×2	3×3	2×1	—	10×6	12×12	3×3	10×10	2×2	3×3	2×2	1×1
				3×3	2×1																				10×5	4×3					

^o) daily dosage (administered in two divided doses)

* : male, 4 months old

** : a left front leg blocked with plaster bandage

Figures represent length and width of blisters in millimeter

L : left R : right

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any restriction for 24 hours and their joints were examined. Obvious abnormality was noted in the outer layer of articular cartilage in both of fore and hind legs to a similar extent. PPA level in plasma taken 5 hours postadministration was found to be 31.5 $\mu\text{g/ml}$ on the average. In the second, dogs were kept their shoulder hung and free from pressure on forefeet immediately after PPA administration and subjected to autopsy after 24 hours. Obvious abnormality was observed in the articular cartilage in the hind legs, but blister formation was hardly noted in the forefeet. When dogs were hung in a similar fashion for 12 hours after PPA administration and then allowed to move freely, abnormality was milder and lower in incidence in the forefoot joints than the hind leg joints. However, blister formation was hardly found in the forefeet when dogs were hung similarly for 24 or 48 hours and then allowed to move freely for 24 hours (Table 5). Average PPA levels measured 24 and 48 hours after administration were lower than 2.5 $\mu\text{g/ml}$ and lower than the assay limit (1.6 $\mu\text{g/ml}$), respectively.

Based on the above results, it is considered that the potential of blister form-

Table 5. Relation of exercise load to arthral abnormality in young dogs receiving pipemidic acid at a dose of 500 mg/kg

State of dogs		Animal					Membrum anterior Articulationes					Membrum posterior Articulationes				
Hanging of forefeet	Free movement	No.	Sex	Age (month)	Body weight (kg)		humeri	cubiti	manus	metacarpo-phalangeae		coxae	genus	pedis	metatarso-phalangeae	
(hour)	(hour)									II III IV V					II III IV V	
—	0-24	GB6F3	F	3.0	3.3		rb-1	b-3	rb-1 b-1	— — — —		rb-2	b-4	rb-1 b-1	— — — —	
—	0-96	GB6F4	F	3.0	3.3		b-1	b-2	— — — —			b-3	b-3	— — — —		
		DY3F3	F	3.5	3.5		—	—	— — — —			—	e-3 b-3	rb-3	b-1	b-1 — —
		931	Fe	3.0	4.5		—	—	— — — —			b-1	—	— — — —		
0-24	—	Mo.11 * ¹	Fe	2-4* ²	1.3		—	—	— — — —			rb-1	e-2	— — — —		
		Mo.12 * ¹	Fe	2-4* ²	1.8		e-1	—	— — — —			b-1	e-2	— — — —		
		BA4F2	Fe	3.0	3.5		rb-1 rb-3	e-1 rb-1 b-2	— — b-1 — —			rb-2	e-4 b-3	e-3	b-1	— — — —
0-12	12-36	Mo.13 * ¹	Fe	2-4* ²	2.7		b-2	—	— — — —			b-2	e-1	e-2	b-1	b-1 b-1 —
		Mo.14 * ¹	F	2-4* ²	3.1		b-2	—	— — — —			rb-1 b-1	e-2 rb-3	— — — —		
		DY3F1	F	3.5	3.4		—	b-1	— — — —			b-2	e-4	e-3	b-1	b-1 — —
0-24	24-48	DX3F2	F	3.0	3.5		b-1	b-1	— — — —			e-1 b-1	e-2 b-5	b-2	b-1	b-2 rb-1 b-1 —
		BA4F1	F	3.0	3.7		—	—	— — — —			rb-1 b-1	e-3 b-4	e-3	b-1	— — b-1
		DY3F4	F	3.5	2.8		—	—	— — — —			rb-1 b-1	b-3	b-1	— — — —	
0-48	48-72	JUIF1	F	3.5	4.1		b-2	—	— — — —			e-1 b-4	e-3 b-2	e-3	e-1	e-1 b-1 b-1

* : distal end of Ossa metacarpalia or metatarsalia *¹ : mongrel *² : presumptive age

b : blister

rb : ruptured blister

e : erosion

Figures following b, rb or e indicate number of lesions.

ation develops with increased plasma level of PPA (possibly increase of PPA in the articular cartilage), in association with body weight pressure on the joints, and the potential disappears with decreased plasma level of PPA.

DISCUSSION

It was found that PPA induced abnormal gait in immature beagle dogs of about 3 months of age when serially administered in oral doses of 100 mg/kg/day or more. The abnormal gait was observed within 3 days after the medication, being the most pronounced in about 3–7 days, and spontaneously disappeared in about 4 weeks even on continuing medication. Dogs with abnormal gait showed no change in spontaneous and evoked EMGs, and blood biochemical analysis, but some changes in diarthrodial joints; i.e., increased synovial fluid in the synovial cavity and blister formation under the outer layer of the articular cartilage. This phenomenon was almost similar to the results reported recently by Ingham *et al* (1977). Blister was histopathologically a separated portion of articular cartilage with a general orientation parallel to the joint surface, where fluid substance was retained. X-ray radiographic and histopathological examination did not reveal abnormality in the length of femur and humerus, and cartilage of trachea and epiphysis in the dogs given PPA even in a dose of 300 mg/kg/day for 90 days. These findings were different from those of lathyrogenic compounds (Wawzonek *et al.*, 1955) and papain (Bentley, 1971) which were known to affect connective tissues including all cartilage as well as those in joints.

Arthropathy was induced in young beagle dogs of 2.5–6 months old but was not in dogs older than 12 months of age and dogs younger than 2 weeks of age. This result suggests that articular cartilage only at a certain developmental stage is susceptible to PPA as mentioned by Ingham *et al* (1977).

NA, an analogue of PPA, induced arthropathy to the same degree as PPA, while PA, another analogue of PPA, was considerably weak in inducing arthropathy. The results suggest that arthropathy-inducing effect is common to this group of compounds, though it is variable in intensity.

Abnormal gait disappeared within one week after discontinuation of medication and histopathological picture of the outer cartilage confirmed the repair process of the blisters once formed. However, blister scar was observed for a rather long period of time. Arthropathy was noted in young dogs of other breeds, but was not, rare if any, in young animals of other species such as rats, rabbits and monkeys. It is, therefore, considered that the induction of arthropathy by PPA is a phenomenon particularly frequent in young dogs of restricted age.

Blisters usually formed at the joints under body weight pressure but not when the joints were kept free from the pressure. Even under pressure, the blisters did not form at a time when the levels of PPA in the joints were considered to be lowered.

Arthropathy by pipemidic acid

It is considered from the experimental results that blister formation in arthral areas is possible only when the areas are exposed to both high level of PPA and body weight pressure. However, its detailed mechanism remains to be clarified.

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Arthropathy by pipemidic acid

Photo. 1 A blister (arrow) is observed on the head of the humerus of a dog (No. 667) receiving pipemidic acid at a dose of 500mg/kg/day for 8 days.

Photo. 2 An erosion of the articular cartilage on the head of the humerus is observed in a dog (No. 671) receiving pipemidic acid at a dose of 1,000 mg/kg/day for 14 days.

Photo. 3 Histopathological finding of the blister observed in the articular cartilage. Stained with toluidine blue at pH 7.0.

Photo.4 Histopathological finding of synovial cavity with small loose bodies and an increase of synovial fluid. No remarkable changes are observed in the synovial membrane. H-E stain

Photo. 5 Two blisters (arrows) are observed on the surface of the Trochlea femoris of a dog (No. 664) receiving nalidixic acid at a dose of 500mg/kg/day for 7 days.

Photo. 6 A recovery test of damaged articular cartilages. The dog (No.689) was given pipemidic acid at a dose of 1,000mg/kg/day for 7 days and discontinued the medication for 30 days. A repairing process such as the appearance of chondrocytes divided like giant cell is observed in the surroundings of the blister. Stained with toluidine blue at pH 7.0.