



ORIGINAL ARTICLE

Safety and efficacy of cyclooxygenase-2 inhibition for treatment of primary hypertrophic osteoarthropathy: A single-arm intervention trial

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Received 28 June 2018; received in revised form 27 September 2018; accepted 2 October 2018
Available online 31 October 2018

KEYWORDS

COX-2 inhibitor;
Primary hypertrophic
osteoarthropathy;
Prostaglandin;
Treatment

Abstract *Background:* Primary hypertrophic osteoarthropathy (PHO) is a rare disease involving joint, bone and skin. Two underlying genes responsible for this disease—hydroxyprostaglandin dehydrogenase (*HPGD*) and solute carrier organic anion transporter family, member 2A1 (*SLCO2A1*)—are both associated with aberrant accumulation of prostaglandin E2 (PGE2). Cyclooxygenase-2 (COX-2) is a key enzyme in PGE2 synthesis. This study was intended to evaluate the safety and efficacy of COX-2 inhibitor in the treatment of PHO. *Methods:* We recruited patients presenting to Peking Union Medical Hospital between January 2009 and December 2016 who were diagnosed with PHO. Participants were given the COX-2 inhibitor etoricoxib (60 mg once daily) and followed up for 9 months. Gene analysis was performed at baseline. The following data were collected at baseline and during treatment: visual analogue score (VAS), volume of the distal middle finger (VDMF), knee joint circumference (KJC), serum and urinary levels of prostaglandin E2 (PGE2) and PGE metabolite (PGE-M) and serum levels of inflammatory markers.

Abbreviations: PHO, Primary hypertrophic osteoarthropathy; HPGD, hydroxyprostaglandin dehydrogenase; SLCO2A1, solute carrier organic anion transporter family, member 2A1; PGE2, prostaglandin E2; COX, Cyclooxygenase.

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<https://doi.org/10.1016/j.jot.2018.10.001>

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Results: A total of 27 patients were recruited, including seven patients with PHO type I (PHOAR1) carrying *HPGD* gene mutations and 20 patients with PHO type II (PHOAR2) carrying *SLCO2A1* gene mutations. After treatment with etoricoxib, the majority of patients experienced resolution of symptoms including pachydermia (60.9%), joint swelling (100%), digital clubbing (74.1%) and hyperhidrosis (55.0%). In both the PHO subtypes, serum and urinary levels of PGE2 were elevated at baseline and declined sharply upon treatment. For PHOAR1 patients, serum and urinary PGE-M levels were relatively low and demonstrated minimal response to COX-2 inhibition. Among PHOAR2 patients, mean serum and urinary levels of PGE-M presented at a high level at baseline and were normalized after 3 months of treatment. No severe adverse effects were reported during the study period.

Conclusions: We found COX-2 inhibitor to be safe and effective for the treatment of PHO in our cohort.

The translational potential of this article: The underlying genes responsible for PHO suggest COX inhibitor as potential therapy, and our study demonstrates the efficacy and safety of this treatment.

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Introduction

Primary hypertrophic osteoarthropathy (PHO), also known as pachydermoperiostosis, is a rare congenital disease. This disease is characterized by the triad of digital clubbing, periostosis and pachydermia, as well as additional features including arthritis, hyperhidrosis and congenital heart disease [1]. In recent years, two underlying genes have been revealed to be responsible for PHO: hydroxyprostaglandin dehydrogenase (*HPGD*), which encodes 15-hydroxyprostaglandin dehydrogenase (15-PGDH) [2], and solute carrier organic anion transporter family, member 2A1 (*SLCO2A1*), which encodes a prostaglandin transporter [3]. According to these molecular findings, PHO is categorized into two subtypes: (1) hypertrophic osteoarthropathy, primary, autosomal recessive, type 1 (PHOAR1; OMIM 259100), caused by *HPGD* deficiency; and (2) hypertrophic osteoarthropathy, primary, autosomal recessive, type 2 (PHOAR2; OMIM 614441), caused by *SLCO2A1* deficiency.

Both *HPGD* and *SLCO2A1* genes encode components of the prostaglandin E2 (PGE2) catabolic pathway. PGE2 degradation is a two-step process: (1) cellular uptake via active transport facilitated by organic anion transporters encoded by *SLCO2A1*, *SLCO3A1* and *SLCO4A1*; (2) PGE2 degraded into 15-oxe-PGE2 by 15-PGDH in cytosol and further degraded by prostaglandin reductase into 13, 14-dihydro-15-keto PGE2 and 13, 14-dihydro-15-keto PGA2 [4–7]. Both *HPGD* and *SLCO2A1* deficiency can independently lead to failure of PGE2 degradation, resulting in elevated levels of PGE2 [2,8].

As with other rare diseases, evidence-based treatment options for PHO are limited due to the small number of patients. Before the discovery of its underlying causes, treatments were mostly focused on alleviation of symptoms, including nonsteroidal antiinflammatory drugs (NSAIDs), pamidronate and tamoxifen citrate to relieve painful osteoarthropathy [9–12], botulinum toxin type A injection and plastic surgery to improve cosmetic

appearance [13] and arthroscopic synovectomy and radio-synoviorthesis for the management of recurrent arthritis [14]. However, given the knowledge that PGE2 may play an important role in the pathogenesis of PHO, cyclooxygenase (COX) inhibition may serve as a potential therapeutic option [15].

Cyclooxygenase is an important enzyme in PGE2 synthesis. There are two isoforms of COX, COX-1 and COX-2. COX-1 is a constitutive enzyme expressed in most tissues, generating “housekeeping” prostaglandins. COX-2 is inducible and highly regulated by a range of factors, generating prostaglandins in inflammatory and neoplastic disorders [16]. Thus, COX-2–selective inhibitors, which inhibit the COX-2 enzyme and thereby suppress PGE2 biosynthesis, represent promising treatment options for PHO.

To study the efficacy and safety of COX-2 inhibitor treatment in this disease, we conducted this single-arm intervention study of COX-2 inhibitor therapy in patients with PHO.

Participants and methods

Study population

We recruited patients with PHO presenting in the Department of Endocrinology at Peking Union Medical Hospital, from January 2006 to Dec 2016. Diagnoses of PHO were initially made based on clinical presentation including digital clubbing, pachydermia, joint swelling, palmar and plantar hyperhidrosis and X-ray findings such as periostosis and acroosteolysis, with exclusion of secondary causes including lung diseases, cardiovascular diseases, inflectional arthritis, rheumatoid arthritis and malignancy. Cases were subsequently confirmed by *HPGD* or *SLCO2A1* gene analysis. Inclusion criteria included the following: (1) clinical and genetic diagnosis of PHO; (2) age ≥ 16 years; (3) no medication use within the past week and (4) provision of

informed consent. Patients with any of the following conditions were excluded: (1) age <16 years; (2) hypersensitivity to any component of the drug; (3) congestive heart failure (New York Heart Association grade II-IV); (4) uncontrolled hypertension; (6) established ischaemic heart disease, peripheral artery disease and/or cerebrovascular disease; (7) severe hepatic dysfunction; (8) active peptic ulceration or gastrointestinal bleeding and (9) an estimated creatinine clearance <30 mL/min. Patients would be excluded if they took medications including steroids or other NSAIDs during the trial. Standard care of medications was permitted. Informed consents were obtained from all the participants. Permissions for publication of biochemical and genetic data and photographs were also obtained from the patients. The study was approved by the ethics committee of Peking Union Medical Hospital and carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Study design and outcome measures

In this single-arm intervention trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02438709) ID NCT02438709), all patients enrolled were given a uniform regimen of a selective COX-2 inhibitor (etoricoxib, 60 mg once daily; Merck Sharp & Dohme Corp, USA) and evaluated at five time points (at baseline and at 1, 3, 6 and 9 months).

For each participant, a detailed medical history was obtained. Bone and joint pain was assessed using the visual analogue score (VAS). The volume of the distal middle finger (VDMF) was measured by displacement method with a 50-mL graduated cylinder, as shown in Fig. 1. Both left and right fingers were each measured twice, and the average value was defined as the VDMF. The intraassay coefficient of variation (CV) of this method was 3.17%, whereas the interassay CV was 3.04%. The knee joint circumference (KJC) was assessed just at the base of the patella using a measuring tape while the patient stood upright with knees fully extended. Both knees were measured twice, and the average value was used as the KJC in the data analysis. The intraassay CV for KJC measurement was 1.48%, and the interassay CV was 0.52%. The VAS, VDMF and KJC were evaluated at baseline and during follow-up. A normal range for VDMF and KJC was obtained by measuring these parameters in 20 normal persons (10 women and 10 men). Decreasing of VDMF and KJC by more than 5% was defined as responding to treatment. Responding rate was estimated by the proportion of patients with relived symptom out of patients with the very symptom (e.g., joint swelling).

Fasting blood samples and morning urine samples were collected and stored at -80°C until the time of batch analysis (first thaw) at baseline and each follow-up time point. DNA was isolated from blood samples using the QIAamp DNA Blood Maxi Kit (QIAGEN KK, Tokyo, Japan) according to the manufacturer's protocol at baseline. Both *HPGD* and *SLCO2A1* exons were analyzed by Sanger sequencing. Suspected novel mutations were confirmed by comparison with the DNA sequences from 100 unrelated healthy Chinese individuals. Serum and urinary PGE2 and PGE-M (13, 14-dihydro-15-keto PGA2 and 13, 14-dihydro-15-

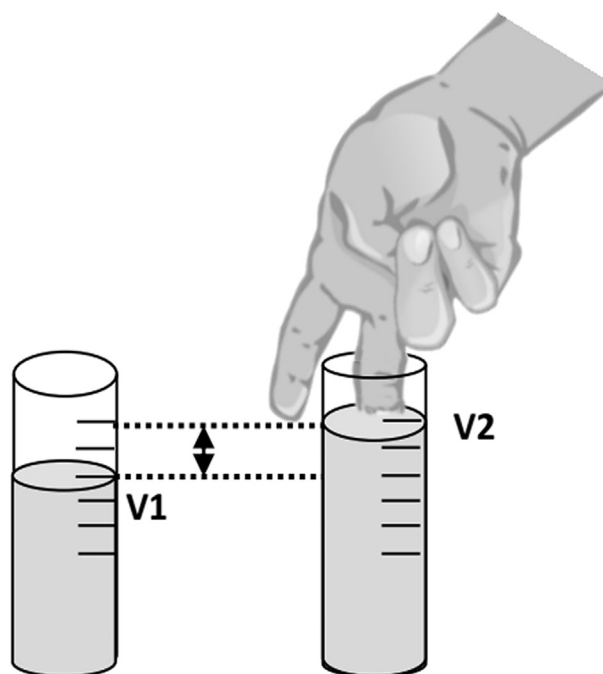


Fig. 1 Measurement of the volume of distal part of middle finger (VDMF). First, 35 mL of water was added into a 50-mL graduated cylinder (V1); then, the patient inserted his or her middle finger vertically into the water, with the distal interphalangeal joint crease just at the water surface. The new water surface level was marked as V2. The VDMF for each patient was defined as the volume of water displaced by the distal tip of the middle finger ($V2-V1$).

keto PGE2) were measured by competitive enzyme-linked immunosorbent assay (ELISA) using PGE2 and PGE-M ELISA kits (Cayman Chemicals, Ann Arbor, Michigan) according to the manufacturer's protocol. Urinary PGE2 and PGE-M levels were normalized to urinary creatinine levels. Samples of 14 age-matched healthy individuals (7 females and 7 males) who were not on any medications were enrolled as healthy controls at baseline with informed consent obtained. Serum levels of the bone resorption marker, C-terminal telopeptide of type I collagen (beta-CTX), were measured by a computer-controlled automatic analyzer (Roche cobas e 601). Routine blood testing including platelet, erythrocyte sedimentation rate (ESR), high-sensitive C-reactive protein (hsCRP) and the bone formation marker alkaline phosphatase (ALP) was performed by standard methods at the central laboratory of Peking Union Medical Hospital.

Bone mineral density (BMD) of lumbar spine 1–4 and proximal femur was measured via dual-energy X-ray absorptiometry (Prodigy Advance; GE Lunar Corporation, USA). Plain radiographs of the hands (posteroanterior view) and knee joints (anteroposterior view) were performed by the same experienced radiologist to assess radiographic change over time at baseline and 6 months. Periostosis was defined as increased thickness of bone cortex. Osteophytes was defined as bony projections along joint margins. Acroosteolysis was graded as 0 (no resorption), 1 (small amount of resorption at the terminal tuft), 2 (resorption of most of the distal tip of the terminal tuft), 3 (resorption of

most of the terminal tuft), and 4 (complete resorption of the terminal tuft) [17].

Data regarding adverse events were collected at each time point through clinical assessments including routine blood and urine testing, liver and kidney function tests, routine stool testing including fecal occult blood test and blood pressure measurement. Self-reported information regarding adverse events including stomach pain and discomfort, constipation, diarrhoea, headache, palpitation, increased blood pressure, shortness of breath and other discomforts was also systematically collected during follow-up.

Statistical analysis

Normally distributed data are expressed as means \pm standard deviation. Nonparametric data are expressed as medians (interquartile range). Comparisons between time points or groups were analyzed using the two-tailed Fisher's exact test for categorical variables and the two-tailed Mann–Whitney U test or analysis of variance for continuous variables as indicated. All analyses were performed using SPSS (Release 19; SPSS, Chicago, IL, USA). For all the two-tailed tests mentioned previously, a p value < 0.05 was considered significant.

Results

Study populations

A total of 27 patients were prospectively enrolled. Sequencing revealed *HPGD* mutations in 7 patients and *SLCO2A1* mutations in 20 patients (Supplemental Table 1). All the participants were of Han ethnicity. The mean age was 26.5 years, and there was only one female (P19). Patients presented with a range of clinical manifestations including digital clubbing (27/27, 100%), periostosis (27/27,

100%), joint swelling (22/27, 81.5%), hyperhidrosis (20/27, 74.1%) and pachydermia (23/27, 85.2%). Twenty-four patients took etoricoxib strictly as prescribed during the 9-month period, two patients discontinued treatment for one week and one discontinued treatment for one month during the study period (all due to personal affairs resulting in delay of follow-up) (Fig. 2).

Changes in symptoms and signs after etoricoxib treatment

Most patients reported a resolution of symptoms during the course of treatment (Tables 1 and 2 and Fig. 3). Improvement in digital clubbing was observed among 20 out of 27 patients (74.1%), with time to resolution varying from one week to 12 months. The VDMF decreased significantly by 3 months compared with baseline (7.01 ± 1.31 vs. 8.71 ± 2.53 mL, $p = 0.029$) and remained constant in the following 6 months (Fig. 4A). Responding rate for joint swelling was 100% (22/22). KJC showed a decrease in the first three months and then kept constant for the following 6 months (Fig. 4B). Bone and joint pains were significantly improved at one month, with VAS changing from 2.41 ± 2.14 to 0.73 ± 1.07 ($p = 0.004$). Pachydermia (14/23, 60.9%) and sweating (11/20, 55.0%) were also improved. No significant difference in clinical changes was observed between PHOAR1 and PHOAR2 subtypes. During longer follow-up, one patient (P20) discontinued COX-2 inhibitor treatment after 12 months of therapy and developed recurrent symptoms including pachydermia and joint swelling within one month.

Changes in PGE2 and PGE-M levels after etoricoxib treatment

Serum and urinary PGE2 and PGE-M changes are shown in Table 1. PHOAR1 and PHOAR2 patients presented with

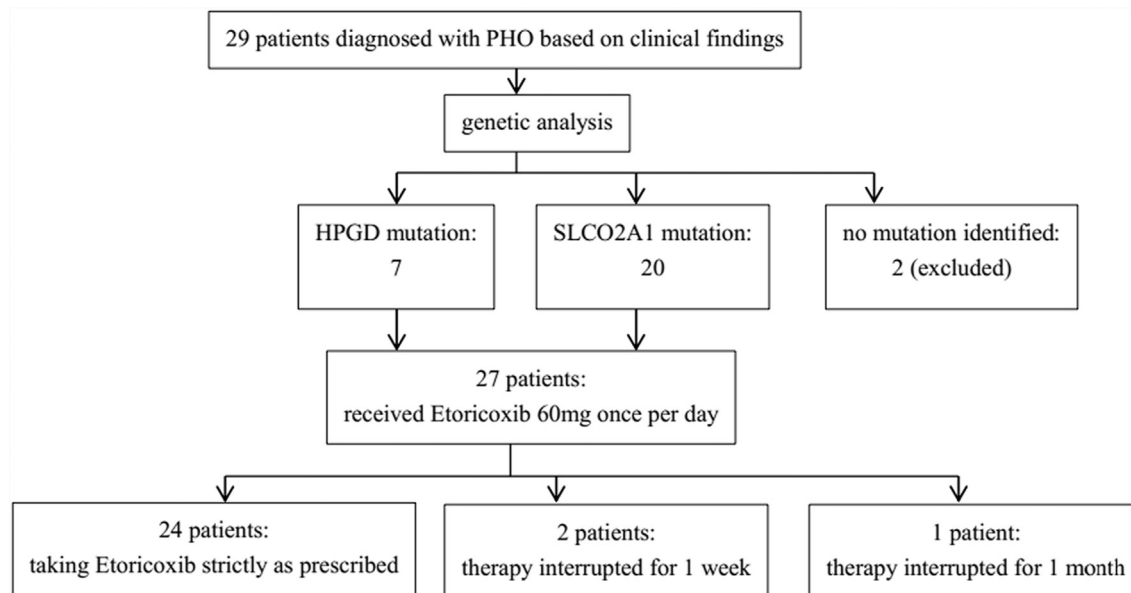


Fig. 2 Flow diagram of patient recruitment, treatment and adherence. PHO = primary hypertrophic osteoarthropathy.

Table 1 Change in clinical characteristics and biochemical markers after 1 and 3 months of etoricoxib treatment for two subtypes of primary hypertrophic osteoarthropathy (PHO) patients.

Patients	Parameters	Baseline	1 month	3 months	6 months	9 months
All patients (N = 27)	VDMF (ml)	8.71 ± 2.53	8.13 ± 2.01	7.01 ± 1.31 ^a	7.48 ± 1.91 ^a	6.90 ± 1.66 ^a
	KJC (cm)	39.46 ± 5.15	38.46 ± 4.37	36.98 ± 3.00	37.07 ± 3.53	36.62 ± 3.29
	VAS	2.41 ± 2.14	0.73 ± 1.07 ^a	0.35 ± 0.79 ^a	0.13 ± 0.35 ^a	0.25 ± 0.71 ^a
	ESR (mm/h)	12.68 ± 9.78	5.57 ± 6.31 ^a	4.80 ± 5.23 ^a	4.84 ± 6.08 ^a	2.95 ± 2.46 ^a
	hsCRP (mg/L)	14.56 ± 13.64	5.27 ± 8.02 ^a	12.56 ± 16.48	4.12 ± 6.76 ^a	1.85 ± 2.28 ^a
	PLT (× 10 ⁹ /L)	274.15 ± 67.81	231.90 ± 55.10 ^a	220.92 ± 54.45 ^a	214.20 ± 54.26 ^a	211.86 ± 63.08 ^a
	ALP (U/L)	106.2 ± 54.9	96.4 ± 45.3	106.3 ± 48.1	104.3 ± 42.6	98.2 ± 28.3
	Beta-CTX (ng/mL)	0.98 ± 0.75	0.74 ± 0.34	0.68 ± 0.30	0.71 ± 0.38	0.53 ± 0.27
	Femoral neck BMD (g/cm ²)	0.98 ± 0.12	—	—	1.01 ± 0.10	—
	Lumbar spine BMD (g/cm ²)	1.18 ± 0.15	—	—	1.16 ± 0.13	—
	Serum PGE2 (pg/mL)	346.6 (181–797.1)	294.2 (178.6–427.9) ^a	194.6 (62.6–317.9) ^a	42.6 (18.1–125.6) ^a	36.1 (13.7–123.7) ^a
	Urinary PGE2 (ng/mmol cr)	562.5 (119.7–1205.4)	100.9 (48.4–386.4) ^a	114.6 (37.4–208.0) ^a	51 (22.9–91.1) ^a	39.8 (23.5–103.7) ^a
	Serum PGE-M (pg/mL)	10.9 (2.7–42.7)	5.7 (3.8–61.3)	7.4 (4.9–13.1)	1.7 (1.1–5.2)	4.5 (1.2–8.4)
	Urinary PGE-M (ng/mmol cr)	92.9 (11.6–709.3)	23.2 (12.9–93.8)	7.9 (4.8–22.2)	12.5 (6.1–51.3)	23.6 (9.4–34.8)
PHOAR1 (N = 7)	VDMF (ml)	9.20 ± 2.41	8.75 ± 2.29	7.31 ± 1.70	7.73 ± 1.85	7.48 ± 1.91
	KJC (cm)	39.09 ± 4.92	39.08 ± 5.15	36.56 ± 2.10	38.07 ± 4.68	37.74 ± 4.74
	VAS	4.36 ± 1.84	1.14 ± 1.21 ^a	0.57 ± 1.13 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a
	ESR (mm/h)	10.86 ± 6.31	2.71 ± 2.87 ^a	2.00 ± 0.82 ^a	2.17 ± 0.75 ^a	2.17 ± 0.75 ^a
	hsCRP (mg/L)	7.76 ± 7.30	1.67 ± 2.23 ^a	5.46 ± 9.65 ^a	0.59 ± 0.37 ^a	0.87 ± 0.75 ^a
	PLT (× 10 ⁹ /L)	225.25 ± 17.84	205.71 ± 43.10	189.00 ± 1.41	188 ± 0.00	184 ± 0.00
	ALP 9 U/L)	88.5 ± 19.1	96.0 ± 69.8	94.8 ± 38.7	115.0 ± 0.00	110.0 ± 0.00
	Beta-CTX (ng/mL)	0.78 ± 0.41	0.63 ± 0.30	0.70 ± 0.44	0.43 ± 0.06	0.38 ± 0.08
	Femoral neck BMD (g/cm ²)	0.96 ± 0.12	—	—	1.02 ± 0.02	—
	Lumbar spine BMD (g/cm ²)	1.19 ± 0.16	—	—	1.25 ± 0.12	—
	Serum PGE2 (pg/mL)	233.1 (217.3–407.3)	138.0 (74.9–224.1)	127.1 (58.0–190.8)	28.85 (5.6–56.3) ^a	39.8 (13.5–50.6)
	Urinary PGE2 (ng/mmol cr)	691.7 (534.9–1369.3)	221.1 (142.0–346.3)	227.3 (88.6–471.5)	39.9 (14.3–56.3) ^a	46.9 (12.2–338.5) ^a
	Serum PGE-M (pg/ml)	1.3 (0.2–3.7)	1.1 (0.8–2.8)	1.4 (0.3–2.8)	0.8 (0.1–1.6)	1.0 (0.2–1.2)
	Urinary PGE-M (ng/mmol cr)	6.1 (2.5–12.6)	12.9 (5.8–18.9)	7.3 (4.7–7.9)	6.7 (5.6–12.5)	8.8 (4.3–12.3)
PHOAR2 (N = 20)	VDMF (ml)	8.39 ± 2.43	7.85 ± 2.04	6.84 ± 1.22	6.83 ± 1.64	6.70 ± 1.66
	KJC (cm)	39.30 ± 4.93	38.17 ± 4.15	37.08 ± 3.21	36.73 ± 3.16	36.25 ± 2.72
	VAS	1.63 ± 1.67	0.34 ± 0.82 ^a	0.13 ± 0.33 ^a	0.11 ± 0.32 ^a	0.11 ± 0.46 ^a
	ESR (mm/h)	15.33 ± 11.82	7.00 ± 7.13 ^a	5.15 ± 5.55 ^a	6.08 ± 7.06 ^a	3.31 ± 2.90 ^a
	hsCRP (mg/L)	19.54 ± 15.07	7.06 ± 9.29	12.92 ± 17.32	5.72 ± 7.71 ^a	2.34 ± 2.65 ^a
	PLT (× 10 ⁹ /L)	289.15 ± 71.40	246.00 ± 57.12	229.92 ± 56.01	220.75 ± 60.33	216.50 ± 67.78
	ALP (U/L)	111.3 ± 59.7	99.4 ± 45.3	106.3 ± 48.1	102.7 ± 45.8	95.8 ± 31.0
	Beta-CTX (ng/mL)	1.05 ± 0.88	0.84 ± 0.34	0.67 ± 0.27	0.93 ± 0.38	0.63 ± 0.31

(continued on next page)

Table 1 (continued)

Patients	Parameters	Baseline	1 month	3 months	6 months	9 months
	Femoral neck BMD (g/cm ²)	1.00 ± 0.13	—	—	1.00 ± 0.11	—
	Lumbar spine BMD (g/cm ²)	1.18 ± 0.15	—	—	1.15 ± 0.12	—
	Serum PGE2 (pg/mL)	280.1 (181.0–545.5)	356.0 (280.3–396.3)	196.6 (59.9–348.1) ^a	52.8 (23.7–129.9) ^a	33.9 (13.7–123.8) ^a
	Urinary PGE2 (ng/mmol cr)	800.1 (145.6–1150.7)	74.8 (46.2–944.3)	116.0 (38.5–139.0)	51.2 (34.7–120.1) ^a	39.8 (23.6–60.8) ^a
	Serum PGE-M (pg/mL)	17.5 (4.3–57.2)	5.7 (3.7–39.3)	7.0 (4.3–12.5)	2.6 (1.4–8.9) ^a	4.7 (2.3–8.9)
	Urinary PGE-M (ng/mmol cr)	147.4 (36.9–744.7)	57.7 (15.9–156.4)	27.5 (20.9–1476.8) ^a	33.8 (12.3–215.8) ^a	34.6 (23.4–45.2)

ALP = alkaline phosphatase; beta-CTX = C-terminal telopeptide of type I collagen; ESR = erythrocyte sedimentation rate; hsCRP = high-sensitive C-reactive protein; KJC = knee joint circumference; PGE2 = prostaglandin E2; PGE-M = prostaglandin E metabolite; PHOAR1 = hypertrophic osteoarthropathy, primary, autosomal recessive, type 1; PHOAR2 = hypertrophic osteoarthropathy, primary, autosomal recessive, type 2; PLT = platelet count; VAS = visual analogue score of bone and joint pain; VDMF = volume of distal part of middle finger. Normal range: VDMF, 3.5–5 mL; KJC, 33–35.5 cm; VAS 0; ESR, 0–15 mm/h; hsCRP, 0–3.00 mg/L; PLT, 100–300 × 10⁹/L; ALP, male: 30–120 U/L and female: 27–107 U/L; CTX, 0.26–0.512 ng/mL; serum PGE2, 29–547 pg/mL; urinary PGE2, 31–280 ng/mmol cr; serum PGE-M, 1–22 pg/mL; serum PGE2/PGE-M ratio, 61–172; urinary PGE-M, 8–51 ng/mmol cr; urinary PGE2/PGE-M ratio, 0.4–11.5. Data normally distributed are expressed as means ± standard deviation (SD). Nonparametric data are expressed as median (interquartile range). ^a*p* < 0.05 compared with baseline.

similarly elevated urinary PGE2 levels [median, 562.5 (119.7–1205.4) ng/(mmol creatinine)] and mildly elevated serum PGE2 levels [median, 346.6 (181.0–797.1) pg/ml] at baseline, which both decreased significantly by 3 months, and then remained in the normal range for the following treatment period (Fig. 4C). By contrast, for PGE-M levels, there was a marked difference between the two subtypes. PHOAR1 patients had low serum PGE-M levels of 1.3 (0.2–3.7) pg/ml and low urinary PGE-M levels of 6.1 (2.5–12.6) ng/(mmol creatinine) and showed a limited response to COX-2 inhibitor treatment. On the other hand, PHOAR2 patients showed slightly higher serum PGE-M levels of 17.5 (4.3–57.2) pg/ml (*p* = 0.099) and extremely high urinary PGE-M levels of 147.4 (36.9–744.7) ng/(mmol creatinine) (*p* = 0.023), which both decreased sharply upon COX-2 inhibitor treatment and were normalized by three months.

Changes in inflammatory markers after etoricoxib treatment

Levels of inflammatory markers were elevated in most patients at baseline and decreased concurrently with symptom alleviation after initiation of treatment (Table 1). Levels of hsCRP were four times above normal at baseline and declined rapidly to twice the upper limit of normal by one month. Mean ESR levels were mildly elevated at baseline (12.68 ± 9.78 mm/h), declined significantly at one month (5.57 ± 6.31 mm/h, *p* = 0.001) and three months (4.80 ± 5.23 mm/h, *p* = 0.001) and remained low thereafter through nine months (Fig. 4E). Platelet counts, which are acute phase reactants, were at the upper limit of normal at baseline (274.15 ± 67.81 × 10⁹/L), decreased significantly at one month (231.90 ± 55.10 × 10⁹/L, *p* = 0.009) and three months (220.92 ± 54.45 × 10⁹/L, *p* = 0.009) and remained constant between three and nine months (Fig. 4F). Baseline values and changes in inflammatory marker levels were similar in the two PHO subtypes.

Changes in bone turnover markers' levels after etoricoxib treatment

At baseline, serum beta-CTX levels were elevated at 0.98 ± 0.77 ng/ml, and ALP level was at the upper limit of normal at 107.84 ± 54.75 U/L. These markers both showed no significant change after COX-2 inhibitor treatment. No differences in bone turnover marker levels or patterns of change were found between the two subtypes of PHO.

Change in BMD and X-ray findings after etoricoxib treatment

BMD at the lumbar spine 1–4 and femoral neck was within the normal range at baseline and did not change significantly during follow-up. X-rays of the hands and knees showed periostosis (100%), osteophytes (59.3%), joint space narrowing (51.9%) and acroosteolysis (11.1%) at baseline, all of which did not change significantly in the 9-month follow-up period.

Table 2 Responding rate to COX-2 inhibitor treatment for clinical symptoms for primary hypertrophic osteoarthropathy patients at different time points.

Patients	Clinical symptoms	1 month	3 months	6 months	9 months
All patients (N = 27)	Clubbing	13/27 (48.1%)	18/27 (66.7%)	18/27 (66.7%)	19/27 (70.4%)
	Joint swelling	21/22 (95.5%)	22/22 (100%)	22/22 (100%)	22/22 (100%)
	Pain	22/27 (81.55%)	25/27 (92.6%)	27/27 (100%)	27/27 (100%)
	Pachydermia	13/23 (56.5%)	14/23 (60.9%)	14/23 (60.9%)	14/23 (60.9%)
PHOAR1 (N = 7)	Clubbing	3/7 (42.9%)	4/7 (57.1%)	4/7 (57.1%)	4/7 (57.1%)
	Joint swelling	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)
	Pain	6/7 (85.7%)	6/7 (85.7%)	7/7 (85.7%)	7/7 (85.7%)
	Pachydermia	3/7 (42.9%)	4/7 (57.1%)	4/7 (57.1%)	4/7 (57.1%)
PHOAR2 (N = 20)	Clubbing	10/20 (50.0%)	14/20 (70.0%)	14/20 (70.0%)	15/20 (75.0%)
	Joint swelling	15/16 (93.8%)	16/16 (100%)	16/16 (100%)	16/16 (100%)
	Pain	16/20 (80.0%)	19/20 (95.0%)	20/20 (100%)	20/20 (100%)
	Pachydermia	10/16 (62.5%)	10/16 (62.5%)	10/16 (62.5%)	10/16 (62.5%)

PHOAR1 = hypertrophic osteoarthropathy, primary, autosomal recessive, type 1; PHOAR2 = hypertrophic osteoarthropathy, primary, autosomal recessive, type 2.

Note: responding was defined by >5% decrease of volume of the distal middle finger (for clubbing), >5% decrease of knee joint circumference (for joint swelling), improved visual analogue score (for bone and joint pain) and patient self-reported improved pachydermia.

Data are expressed as responding patient number/number of patients who carry the symptom (percentage).

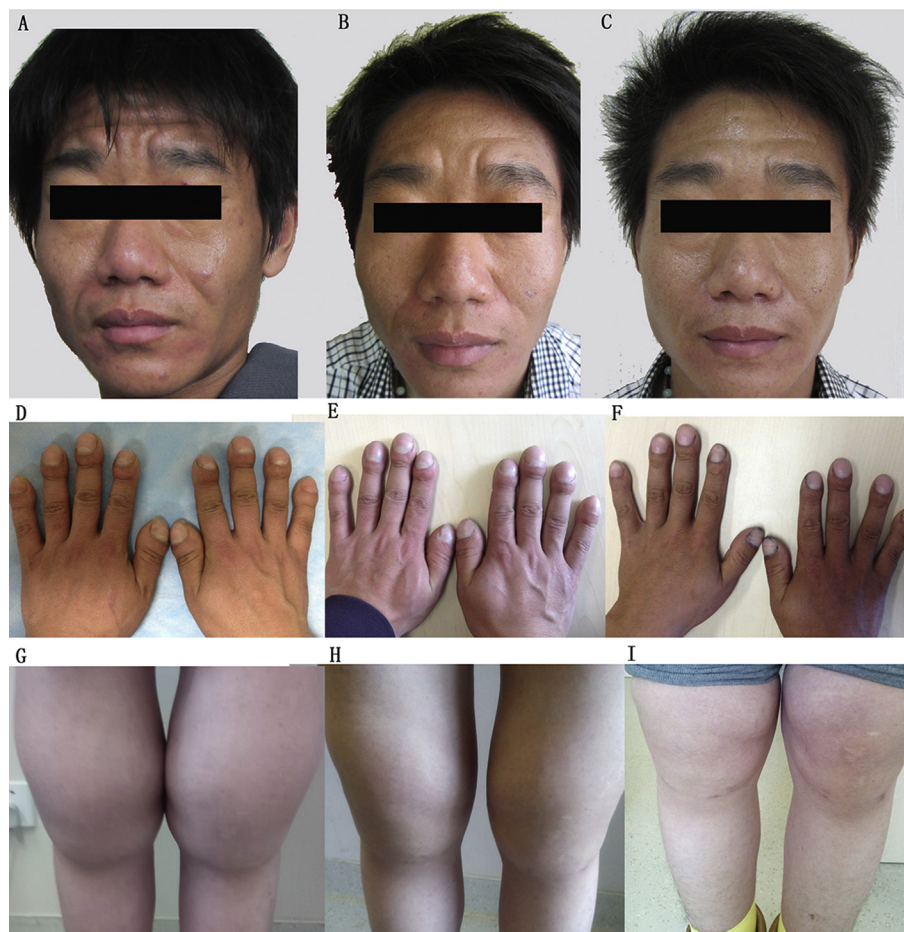


Fig. 3 Changes in physical signs of PHO among study participants after COX-2 inhibitor treatment. A, B & C show facial change for P4, demonstrating deep wrinkles at baseline (A) which gradually decreased by 1 month (B) and 6 months (C). D, E & F show changes in digital clubbing for P9. Note the enlargement of the distal fingers at baseline (D), which gradually improved by 1 month (E) and 6 months (F). G, H & I show knee joint swelling in P15, which also improved gradually from baseline (G) to 1 month (H) and 6 months (I). COX-2 = cyclooxygenase-2; PHO = primary hypertrophic osteoarthropathy.

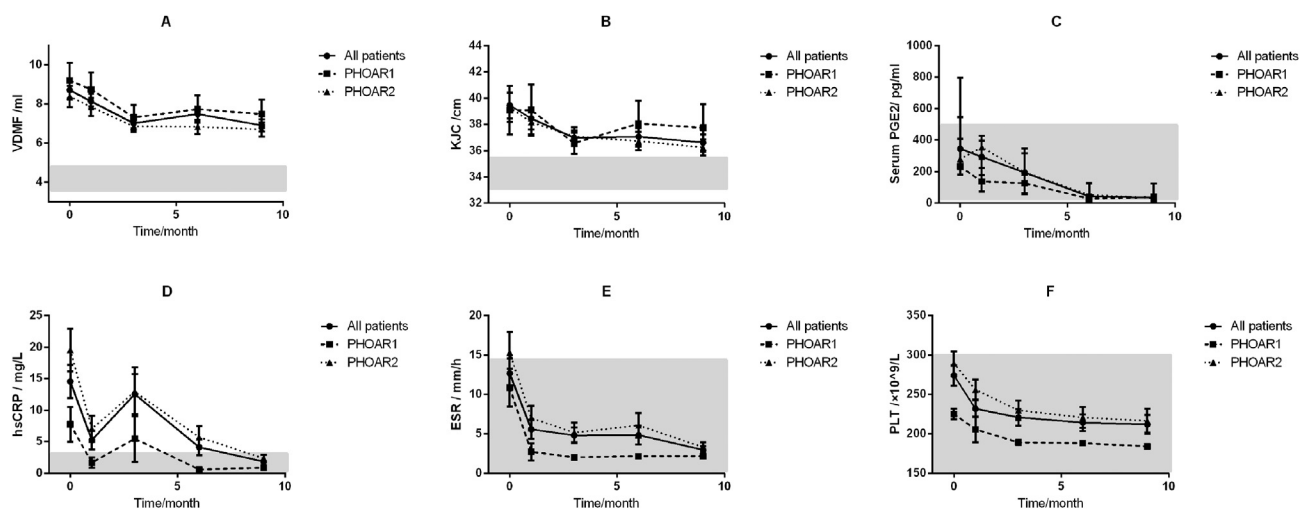


Fig. 4 Change in clinical signs and laboratory markers over 9 months of COX-2 inhibitor treatment among patients with PHO. (A) Volume of distal part of middle finger (VDMF). (B) Knee joint circumference (KJC). (C) Serum PGE2. (D) High-sensitivity C-reactive protein. (E) Erythrocyte sedimentation rate. (F) Platelet count. The shaded grey zones represent the normal reference ranges for the tests depicted.

COX-2 = cyclooxygenase-2; PHO = primary hypertrophic osteoarthropathy.

Adverse effects

No severe adverse effects were reported or observed throughout the study period. Mild adverse effects primarily involved the digestive system, just in line with expectations for COX-2 inhibitors: Five patients (18.5%) had a positive fecal occult blood test which resolved with or without proton pump inhibitor treatment, four patients (14.8%) reported loose stools once, 3 patients (11.1%) reported self-limiting stomach upset and 1 patient reported occasional gastroesophageal reflux. No abnormalities in liver enzyme levels, blood pressure, influenza-like symptoms or asthenia were found during the clinical trial.

Discussion

Although PHO has been recognized for more than a century, research on the treatment of PHO is still limited because of the rare nature of the disease. Our study systematically evaluated the safety and efficacy of COX-2 inhibitor in the treatment of PHO and explored genetic and biological correlations that may help illuminate the underlying mechanisms of this disease.

In this study, we evaluated COX-2 inhibition as a targeted treatment method. We found an increase of serum and urinary PGE2 levels in both subtypes of PHO. It is hypothesized that sustained high levels of PGE2 may result in the clinical features of PHO. COX is a key enzyme in PGE2 synthesis. COX-2 inhibitors can suppress COX-2-derived PGE2 synthesis and lower PGE2 levels from upstream [18,19], making it a potentially efficient drug for PHO. Etoricoxib is a novel COX-2 inhibitor with improved biochemical selectivity over that of other selective COX-2 inhibitors [20]. Consistent with a recent study [21], the serum and urinary PGE2 in both PHOAR1 and PHOAR2 patients were normalized after 3-month COX-2 inhibitor

treatment in our study, demonstrating its efficacy to treat this disease based on pathogenic considerations.

In contrast, levels of PGE-M, the 15-PGDH pathway end metabolite of PGE2, were different between the two PHO subtypes in our cohort. The finding of extremely low levels of serum and urinary PGE-M in PHOAR1 patients is in agreement with a prior study [2]. As anticipated, COX-2 inhibitor treatment did not normalize PGE-M levels in PHOAR1 patients. Consistent with reported results [8], an increase in urinary PGE-M levels in PHOAR2 patients was found in our study. As there are other prostaglandin transporters other than SLCO2A1 [22], we infer that the excessive PGE2 finds other ways into SLCO2A1-deficient cells and is further degraded into PGE-M. In a previous study [23], Guda et al. found that neither treatment with sulindac (150 mg twice daily for 4 weeks) nor celecoxib (200 mg twice daily for 6 weeks) could lead to a normalization of urinary PGE-M levels in PHOAR2 patients, which suggested limited efficacy of NSAIDs in PHOAR2 patients. Our longer period of follow-up enabled us to observe that both serum and urinary PGE-M levels were normalized after 3-month treatment with etoricoxib 60 mg daily in SLCO2A1-deficient patients. Therefore, our findings demonstrated the ability of COX-2 inhibition to normalize both urinary PGE2 and PGE-M levels in PHOAR2 patients. Despite the difference in PGE-M in two subtypes of PHO, both PHOAR1 and PHOAR2 patients showed good response to COX-2 inhibitor, suggesting the more important role of PGE2 in developing clinical symptoms of PHO. According to the results, it is recommended to use COX-2 inhibitors in the treatment of both PHOAR1 and PHOAR2 patients for symptom relieving.

Inflammatory marker levels including hsCRP and ESR were found to be high at baseline and subsequently declined after COX-2 inhibitor treatment, signifying a decline in inflammation with decreasing levels of PGE2.

In contrast to prior studies [24], in our cohort, bone turnover markers failed to correspond with PHO disease

activity. In addition, X-rays showed no obvious bone changes at 3 months, suggesting that the early relief of digital clubbing may be secondary to reduction of oedema and connective tissue swelling rather than involvement of periostosis.

During longer follow-up, we found that one patient (P20) developed recurrent symptoms after 1 month of discontinuing COX-2 inhibitor treatment. As COX-2 inhibitor only reduces PGE2 levels temporarily but not changes the underlying mutant genes, the recurrence is predictable. Therefore, sustained COX-2 therapy appears to be necessary to maintain disease remission at least within the first year, and longer studies are necessary to evaluate whether therapy can be weaned off at a later point in the course of PHO.

In this study, we enrolled both PHOAR1 and PHOAR2 patients, making it possible to compare the manifestations and response to treatment between these two subtypes. We collected detailed clinical data from baseline through 9 months, giving us a chance to see both short-term and intermediate-term effects of COX-2 inhibitor treatment. However, this study still has some limitations: 1) Owing to the rare nature of PHO, we chose to conduct a single-arm intervention trial and therefore did not enrol a control group. (2) Although a sample size of 27 is large for a rare disease, it is still relatively small for a clinical trial; therefore, future studies should be conducted confirm or add to our findings. (3) Finally, the follow-up period was 9 months in this study, which was still too short to see the long-term impact of etoricoxib on PHO.

In conclusion, our study demonstrated the efficacy and safety of COX-2-selective inhibitor in the treatment of PHO. This treatment successfully decreased PGE2 levels and relieved PHO symptoms, highlighting the role of PGE2 in the pathogenesis of PHO. In addition, we demonstrated different levels of PGE-M between the two subtypes of PHO, adding to the mechanistic understanding of this disease.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Funding/support statement

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors, and no material support of any kind was received.

Acknowledgements

The authors thank the patients for their involvement in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jot.2018.10.001>.

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