INTRA AND EXTRACELLULAR CPPD CRYSTALS ARE A REGULAR FEATURE IN SYNOVIAL FLUID FROM UNINFLAMED JOINTS OF PATIENTS WITH CPPD RELATED ARTHROPATHY

KEY WORDS: CALCIUM PYROPHOSPHATE DIHYDRATE DEPOSITION, PSEUDOGOUT, CHONDROCALCINOSIS, SYNOVIAL FLUID

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ABSTRACT

Objectives: We aim to determine whether calcium pyrophosphate dihydrate (CPPD) crystals can be found in the synovial fluid (SF) of non-inflamed joints of patients with CPPD related arthropathy; if so, we also aim to determine whether they interact with cells and produce subclinical inflammation in this setting.

Methods: 74 SF samples were obtained from non-inflamed knees of 74 patients with CPPD related arthropathy. Identification of CPPD crystals and SF cell counts were done manually in undiluted samples, by means of a haematocytometric chamber. A supravital stain (Testsimplets, Boehringer Mannheim GmbH) was used to perform differential counts and to assess the presence of intracellular crystals.

Results: All 74 samples contained CPPD crystals. The mean cell count was 301.4 cells/µl (CI 216.6-386.4; range 22-2302.5). Mononuclear (MN) cells accounted for 83.2% (CI 80.4-86.1; range 43-99), the rest being polymorphonuclear (PMN) cells [16.8% (CI: 13.9-19.6; range 1-57)]. All the samples contained intracellular CPPD crystals, which were found in 24.0% (CI 20.1-27.9; range 1-78) of all the cells. Most of the intracellular crystals were inside MN cells [22.2% of all the cells (CI 18.5-25.9)], although some PMN also contained them [1.8% of all the cells (CI 1.1-2.4)].

Conclusions: CPPD crystals are normally found in SF of non-inflamed joints of patients with CPPD related arthropathy, and they interact with cells. The raised cell counts and percentage of PMN suggest mild subclinical inflammation in these joints.
INTRODUCTION

Calcium pyrophosphate dihydrate (CPPD) crystal related arthropathy is characterized by the presence of these crystals in synovial fluid (SF) samples obtained from the joints of the patients at the time of inflammation (1). Clinical features include acute episodes of self-limited arthritis (which have been known as pseudogout), and a chronic degenerative arthropathy that resembles osteoarthritis (OA) (2,3).

The presence of CPPD crystals in the joint cavity appears as an essential factor for the induction of joint inflammation. CPPD crystals are found in the SF of patients during acute attacks (1). Injection of the crystals in canine joints induces an acute inflammatory response (4,5). “In vitro” exposure of monocytes, synoviocytes and human neutrophils to CPPD crystals results in release of pro-inflammatory and chemotactic mediators (6,7,8,9,10). According to this model, which closely links presence of CPPD crystals with inflammation, the joints would be free of them outside the periods of clinical inflammation.

In gout, monosodium urate (MSU) crystals stay in the joint and are always found in the SF after they form, provided that patients have not received a long enough hypouricemic treatment (11,12). Moreover, in asymptomatic joints MSU crystals are actively phagocytosed (13), and associate to slightly raised cell counts and increased percentages of PMN leukocytes, suggesting the presence of mild subclinical inflammation (14,15).

Our aim in this study has been to determine whether CPPD crystals behave similarly as MSU crystals do in gout. Thus we have tried to establish whether: 1) CPPD crystals are found in SF samples from non-inflamed knees of patients with known CPPD related arthropathy; 2) phagocytosis of the crystals occurs in this setting; 3) cell count and percentage of polymorphonuclear (PMN) cells in the same SF samples are higher than normal, indicating some degree of subclinical inflammation in these asymptomatic joints. As an additional objective, we have tried to determine whether SF samples from patients with pain showed any difference in the presence of crystals, cellularity, percentage of PMN, and crystal phagocytosis, which could link pain with mild inflammation caused by CPPD crystals.
METHODS

Patients

Initially, 92 patients with CPPD related arthropathy were invited to enter the study. In every patient, the diagnosis had been previously established by identification of CPPD crystals in a SF sample. Seventy nine of them agreed, and informed consent was obtained from each one. At the time that SF samples were obtained, all these patients fulfilled the following criteria:

1) The time interval between the diagnostic arthrocentesis, and the sampling for the study was two months or longer.
2) For study purpose, presence of joint inflammation was defined as a combination of pain, swelling due to the presence of an effusion, with some restriction of motility, and the observation of over 2000 cells/µl in the SF. For all the patients studied, current joint inflammation was absent at the time of the study; however, it was accepted that some patients could present stable mild pain, or a small joint effusion, detectable by a positive bulge sign. Finally, it was required that patients had been free of joint inflammation or relevant increase in knee pain in the two months prior to the study.
3) Patients had been off colchicine or non-steroidal anti-inflammatory drugs (NSAIDs) for at least two months (acetaminophen was allowed for pain).

Radiological assessment was performed by means of standing fully extended anteroposterior and 30º flexion lateral views of the knee studied, to evaluate the presence of signs of osteoarthritis (joint space narrowing, osteophytes, or both), and radiological chondrocalcinosis.

After assessment, knee arthrocentesis was performed. 74 SF samples, obtained from 74 patients, were suitable for the study. 5 patients were excluded, as 2 samples from 2 patients were contaminated with blood (and found no suitable for the study), and no fluid was obtained in other 3 patients. Thus, 74 patients were finally included. Patients were classified, according to the presence of pain, in two groups: patients with chronic knee pain (n= 52) and without it (n=22) (Diagram 1). In the group of patients with pain, radiographic chondrocalcinosis, radiographic changes of OA, or both were observed in 4, 4, and 44 patients, respectively; in the group without pain, these findings were seen in 11, 0, and 12 patients, respectively; one patient’s radiograph did not show either chondrocalcinosis or radiographic changes of OA.

According to antecedents of previous joint inflammation, 33 patients referred previous pseudogout crises (23 in the knee, 10 in other joints). Joint inflammation had occurred in 20 of 52 patients, in the group with chronic knee pain, and in 13 of 22 patients, in the group without pain. The diagnostic arthrocentesis had been performed at that time, and the mean time interval between resolution of inflammation and the obtainment of SF samples for the study was 11.9 months (CI 95%: 6.3-17.4; range: 2-60). In the 41 patients that did not refer previous joint inflammation, the SF samples for the study were obtained two months after the diagnostic arthrocentesis had been performed.
Diagram 1. Classification of patients

- n = 92 patients with CPPD related arthropathy
  - n = 13 patients refused to participate
  - n = 79 patients accepted to enter the study
    - n = 5 patients excluded:
      - 2 patients: SF sample contaminated with blood
      - 3 patients: no SF sample obtained
    - n = 74 patients had a SF sample suitable for the study
      - n = 52 patients with chronic knee pain
        - Chondrocalcinosis (n = 4)
        - Radiographic changes OA (n = 4)
        - Both (n = 44)
      - n = 22 patients without pain
        - Chondrocalcinosis (n = 11)
        - Radiographic changes OA (n = 0)
        - Both (n = 10)
        - None (n = 1)
Synovial fluid samples

Knee arthrocentesis was performed by medial retropatellar approach, using a 1 ml plastic syringe and a 21 gauge needle, after routine aseptic precautions. SF analysis was performed immediately after extraction. Cell counts were done manually in undiluted SF and using a Neubauer haemocytometric chamber, as previously described (14). The same preparation was used to identify CPPD crystals: since most CPPD crystals are not birefringent (16), crystal detection was carried out by means of an ordinary microscope, and identification confirmed with a polarized light microscope provided with a first order red compensator (17). No other calcium crystals were sought in this study. Poor reproducibility has been reported for crystal identification in synovial fluid (18,19,20). However, reproducibility is related to the training of the observers, since we have found that after training crystal detection and identification is consistent (17). In our study, the investigator (AMS) who performed the SF analysis has long experience in crystal detection and identification of CPPD crystals, and his margin of error likely is small.

Differential cell counts were done using pre-stained slides (Testsimplets, Boehringer Mannheim GmbH) (21). CPPD crystals were searched in every cell and the percentage of cells with intracellular crystals was annotated, as supravital stain with Testsimplets does not modify the morphology and optic characteristics of the crystals (21).

Statistical analysis

The mean, 95% confidence interval (CI) for the mean and range were calculated for all the counts performed. Statistical analysis was performed using the non-parametric rank sum test, also known as the Mann-Whitney-Wilcoxon test for non parametric independent samples, to compare cell counts, PMN cell percentages, and percentages of cells with intracellular crystals, as number of patients was rather small and distributions were not normal.
RESULTS

Of the 74 patients studied, 27 were men and 47 women (ratio M/F: 0.57). Their mean age was 68.3 years (CI: 65.8-70.7; range 40-90). All 74 SF samples contained CPPD crystals. The mean cell count was 301.5 cells/µl (CI: 216.6-386.4; range: 22-2302.5) (Figure 1). MN cells accounted for 83.2% (CI: 80.4-86.1; range: 43-99), the remaining 16.8% being PMN (CI: 13.9-19.6; range 1-57). PMN cells were observed in all 74 samples (Figure 2). Cells with intracellular CPPD crystals were observed in all 74 samples. The mean percentage of cells containing crystals was 24.0% (CI: 20.1-27.9; range: 1-78); most of them were MN cells (22.2%; CI: 18.5-25.9; range 1-78), though some PMN also showed intracellular crystals (1.8%; CI: 1.1-2.4; range: 0-15) (Figure 3). The cells that contained crystals often contained more than one.

In the group of patients with chronic knee pain (Pain group), the mean percentage of cells with intracellular CPPD crystals was 23.7% (CI: 19.3-28.0; range: 1-59), and 24.7% (CI: 16.0-33.4; range: 2-78) in the group of patients without pain (Asymptomatic group) (p=0.93, ns). The mean cell count in the Pain group was 338.1 cells/µl (CI: 225.8-450.3; range: 22-2302.5), and in the Asymptomatic group was 215.0 cells/µl (CI: 105.8-324.2; range: 20-1130) (p=0.07, ns). PMN cells accounted for a mean of 17.5% (CI: 14.2-20.9; range: 1-57) in the Pain group, and for 15.0% (CI: 9.3-20.6; range 1-39) in the Asymptomatic group (p=0.28, ns). The results are resumed in Table 1.
Table 1: Cell counts in groups of patients with and without pain.

<table>
<thead>
<tr>
<th></th>
<th>Pain group (n=52)</th>
<th>Asymptomatic group (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% cells with crystals¹</td>
<td>23.7 % (CI: 19.3-28.0)</td>
<td>24.7 % (CI: 16.0-33.4)</td>
</tr>
<tr>
<td>Cell count ‡</td>
<td>338.1 (225.8-450.3)</td>
<td>215.0 (105.8-324.2)</td>
</tr>
<tr>
<td>% of PMN cells #</td>
<td>17.5 % (14.2-20.9)</td>
<td>15.0 % (9.3-20.6)</td>
</tr>
</tbody>
</table>

¹ Mean (95% CI).
‡ Cells / µl [mean (95% CI)].
DISCUSSION

We have studied 74 SF samples from non-inflamed knees of patients with CPPD related arthropathy, and we have found CPPD crystals in all of them. This indicates that they are regularly present in the joint cavity of these patients in absence of clinical inflammation. CPPD crystals were observed in samples from patients with previous inflammatory episodes, but also in samples from patients who never had arthritis. As crystals were found in all the studied samples, it would appear that after the crystals reach the joint cavity, periods free of crystals are rare, and crystals stay in the joints without causing any clinical symptom (some of these patients had not suffered arthritis ever, and they represent the group so-called lanthanic (22)). The regular presence of CPPD crystals in the joint cavity suggests either their slow clearance from the joint cavity, or a constant shedding from joint deposits, mainly the cartilage, or both. It has been reported that detection of CPPD crystals can be intermittent when successive SF analyses are performed in the same joint (23); if this is so, our results indicate that periods with crystals appear to be more common. Crystal shedding has been suggested as the mechanism for acute CPPD related arthritis (24); however, 51 of our patients had crystals (often many) in knees in which inflammation had never occurred. Thus, although shedding of new crystals may indeed have a role in triggering joint inflammation; it does not necessarily results in joint inflammation.

Cells containing intracellular CPPD crystals were found in all SF samples, indicating that phagocytosis is a regular feature in these non-inflamed joints. Most of these cells were MN, and frequently contained more than one crystal. The proportion of cells with intracellular crystals was rather high, and nearly reached 1 of every 4 cells counted. Altogether, these data indicate that the interaction between crystals and cells in these non-inflamed joints of patients with CPPD related arthropathy is active. Intracellular crystals were first described in inflamed joints with CPPD related arthropathy (1). Later on, phagocytosis of crystals has been described in SF samples obtained from patients with osteoarthritis (25). In patients with gout, intracellular MSU crystals are also a common feature in SF samples obtained from asymptomatic joints (13).

Cell count and percentage of PMN cells provide relevant information about that degree of joint inflammation. Normal joints have low cell counts, below 100 cells/µl (26). The mean cell count found here in non-inflamed joints is higher than the values reported for normal joints. Besides, SF samples from normal joints contain few PMN cells, usually none (27). As PMN leukocytes are not “resident” cells in the joint, their presence suggests inflammation. In our study, PMN cells were detected in all the samples, accounting for a mean of 16.8% of the cells. Both the raised cellularity in the SF fluid and the presence of PMN leukocytes is most likely indicative of the presence of low degree inflammation in these clinically non-inflamed joints of patients with CPPD related arthropathy. Of interest, cell counts and percentage of PMN leukocytes were similar in the knees of asymptomatic patients and in those with some chronic pain, suggesting that pain in these patients is not related to these findings.

Identification of crystals in SF samples obtained during inflammatory episodes has been the procedure for definite diagnosis of CPPD deposition disease (1). In absence of joint inflammation, a pool of criteria, based on combinations of clinical and radiological data, was proposed to establish a possible or probable diagnosis (28). The regular presence of CPPD crystals in SF samples obtained from asymptomatic joints shown here indicates that a definitive diagnosis of CPPD arthropathy can be reached at any time if needed, similarly as we have shown it can be done in gout (29).
In conclusion, our results show that CPPD crystals are normally found in SF samples obtained from non-inflamed knees of patients with CPPD deposition disease. The regular presence of intracellular crystals confirms interaction between crystals and cells in such joints in the absence of clinical inflammation. Mild subclinical inflammation is supported by the higher than normal cell count and the presence of PMN cells. These findings are similar to what is found in gout.

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Figure 1. Cell counts in the 74 patients.

Mean: 301.5 cells/µl
Figure 2. Percentage of PMN cells.

% of PMN cells

Mean: 16.8 %
Figure 3. Percentage of MN and PMN cells, with (+) and without (-) intracellular crystals.
Intra and extracellular CPPD crystals are a regular feature in synovial fluid from uninflamed joints of patients with CPPD-related arthropathy

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