MICROANALYSIS OF PATHOLOGICAL CRYSTALS AND URINARY CALCULI

Kiyoko Kaneko,1 Shin Fujimori,2 Naoyuki Kamatani,3 Hisashi Yamanaka,3 Noriko Yamaoka,2 and Leo Akaoka2

1Central Laboratory of Analytical Biochemistry
2Department of Internal Medicine
Teikyo University School of Medicine
3Institute of Rheumatology
Tokyo Women’s Medical College
Tokyo, Japan

INTRODUCTION

Pathological crystals are known to cause crystal-induced arthritis (1). The identification of crystals is necessary on the diagnosis of the disease (2). The identification of crystals is usually carried out with a polarizing microscope. However, it is difficult to determine the composition of crystals with the microscope. X-ray analysis is recommended on the determination of crystals.

Urinary calculi are often found in patients with gout or hypouricaemia (3). Infrared (IR) spectroscopy is a routine analytical method in studying urinary stones (4). It is described that best suited methods for the analysis of calculi are X-ray diffractometry and IR spectroscopy (5).

In this study, micro area analysis with X-ray diffractometer was carried out to determine fine structures of such small materials as pathological crystals and urinary calculi. Infrared analysis was also done on those small materials and was compared with micro area X-ray analysis.

MATERIALS AND METHODS

Materials

Crystalline materials from patients with arthritis, gout or hyperuricemia, were examined. White subcutaneous nodes were extirpated from patients with arthritis nodosa, which proved to be calcium deposit with the X-ray radiographic inspection. Urinary calculi were...
excreted in the urine from patients with gout or hyperuricemia. Synovial fluid with crystals were collected by a puncture needle from a patients with gout. After synovial fluid was incubated at 37°C for 60 min. with papain following 30 min. incubation with hyaluronidase, suspension was centrifuged for 1 min. at 11,000 g. Pellets containing crystals were dried and were examined.

**Analysis**

A micro area X-ray diffractometer (JEOL JDX-8030, DX-MAP2, Tokyo, Japan) with microscope was used in all experiments. Each specimens were analyzed with micro beam X-ray on several spots of those surface and cross sections. The X-ray diffraction pattern is recorded with an X-ray goniometer and is represented as an intensity as a function of twice the diffraction angle (2θ) curve. Analytical conditions were as follows, target: Cu; filter: Ni; voltage: 40 kV; current: 40 mA; diameter of the collimator: 100 μm. The diffraction pattern was compared with the data registered in JCPDS (Joint Committee on Powder Diffraction Standards) database. An infrared spectrophotometer (JASCO A-302, Tokyo, Japan) was used in IR analysis.

**RESULTS**

**Subcutaneous Nodes**

Subcutaneous nodes were washed several times with distilled water and were dried over 2 days. In Figure 1, the X-ray diffraction pattern of a white subcutaneous node (calcium deposit) is shown. When several spots of the calcium deposit were analyzed on the surface or the cross section, the obtained diffraction patterns were similar in every area analyzed. X-ray diffraction patterns agreed well with hydroxyapatite registered in JCPDS database, which was shown as (A) in Figure 1. IR spectra also supported those results. Furthermore, IR spectra additionally indicated carbonate groups in the deposits.

**Monosodium Urate Crystals in Synovial Fluid**

Synovial fluid from joints with inflammation contains many biological compounds, such as leukocytes, proteins, and proteoglycans. When synovial fluid from a patients with gout was examined by a polarizing microscope, a number of needle-shaped crystals and leukocytes were observed. Crystals were estimated to be monosodium urate (MSU) from their negative birefringence. Crystalline pellets were isolated according to Materials and Methods, and X-ray diffraction was carried out (Figure 2). The crystals were analyzed precisely with X-ray diffractometer even with much amount of interfering substances. The diffraction pattern was well in agreement with MSU which was shown as (C) in Figure 2. IR analysis could not determine the crystals in synovial fluid, because of a large amount of organic materials.

**Urinary Calculi**

A urinary calculus, which was analyzed with IR spectrophotometry first, was shown to contain mainly (more than 95%) calcium oxalate. But IR spectrophotometry could not distinguish clearly with two calcium oxalate salts; calcium oxalate monohy-