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Account/Revue

Shedding light on the morphology of calcium oxalate monohydrate crystallites present in kidney biopsies in the case of hyperoxaluria

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ABSTRACT

Hyperoxaluria corresponds to an excessive urinary excretion of oxalate anions. Hyperoxaluria may be associated with the presence of calcium oxalate monohydrate crystals in kidney tissue and in some cases may lead to renal failure. In this contribution, a set of ten kidney biopsies corresponding to patients affected by hyperoxaluria from various origins such as primary hyperoxaluria or gastrointestinal disease has been investigated through μFourier transform infrared (FTIR) spectroscopy and Field Emission scanning Electron Microscopy (FE-SEM). The complete set of results indicates that if the deposits are mainly constituted of calcium oxalate monohydrate, some of them are made of calcium phosphate apatite, an observation which underlines the use of physicochemical techniques instead of the classical staining procedures. Moreover, FE-SEM observations clearly show a diversity of the crystallite morphology. Such diversity suggests changes in the composition of the milieu along the nephron and different interactions between calcium oxalate crystals and ions or macromolecules such as osteopontin and/or Tamm–Horsfall protein for example. This approach may help the clinician to understand more deeply the biochemical parameters which determine the formation of calcium oxalate monohydrate crystallites in kidney tissue and define the corresponding etiology.

1. Introduction

Hyperoxaluria is a metabolic disorder that may lead in some cases to end-stage renal failure and is defined as the excessive urinary excretion of oxalate anions (greater than 50 mg/1.73 m^2 per 24 h) [1]. This disease is characterized by calcium oxalate deposition first in kidney tissue and in the case of severe decrease of the glomerular filtration rate in other tissues including bones, heart and vessels. Hyperoxaluria constitutes a multifactorial disease and among the different aetiologies related to hyperoxaluria, we can quote lethal genetic disorders of the glyoxylate metabolism (the primary hyperoxalurias) [2–8], gastrointestinal diseases including cancers, inflammatory bowel diseases and their

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surgical therapy (secondary enteric hyperoxaluria [9]), cystic fibrosis, and overconsumption/production of oxalate or its precursors from exogenous sources. Bariatric surgery [10] and intestinal lymphangiectasia [11] may also lead to hyperoxaluria.

Primary hyperoxalurias (PHs) are related to inborn errors in the metabolism of glyoxylate and oxalate [2–8] and lead to recurrent nephrolithiasis, nephrocalcinosis, systemic oxalosis, and renal failure. Aside from combined liver/kidney transplantation, no curative treatment exists. Calcium oxalate monohydrate (COM) kidney stones related to PH exhibit a peculiar morphology at the macroscopic and the mesoscopic scale [12–14]. Crystallite morphologies play definitely a major role in the classification of kidney stones [13,15–17] and thus in the definition of their etiology.

This contribution is devoted to the characterization of ectopic calcifications present in kidney of patients suffering from severe hyperoxaluria [18–21]. If ectopic calcifications can be probed using a usual procedure based on staining, it is at the cost of limited information on their chemical nature [22–24]. To go beyond this drastic limitation, different techniques can be used [25–28]. Here, we have selected μFourier Transform Infra-Red (FTIR) spectroscopy [29–36] and Field Emission Scanning Electron Microscopy (FE-SEM) [37–39] in order to characterize the crystalline phases forming abnormal deposits and to describe precisely their topology and structure.

2. Material and methods

Ten kidney biopsies were investigated (Table 1). The biological samples came from Tenon Hospital (Paris, France). For tissue embedded in paraffin, the paraffin was chemically removed in order to improve the crystal detection under the microscope. Each sample was only named with a study number, without indication of the name of the patient or potential identification data.

2.1. μFTIR microspectroscopy

The starting point of this investigation was given by μFTIR data in order to localize and determine the chemical nature of the pathological deposits present in kidney biopsy [39]. To fullfil IR reflexion, kidney biopsies were deposited on low-e microscope slides (MirrIR, Kevley Technologies, Tienta Sciences, Indianapolis). FTIR hyperspectral images were recorded with a Spectrum Spotlight 300 FTIR imaging system (Perkin Elmer Life Sciences, France), with a spatial resolution of 6.25 μm and a spectral resolution of 8 cm^{-1}. Each spectral image, covering a substantial part of the biopsy, consisted of about 30 000 spectra.

IR microspectroscopy was also performed on an IN10MX microscope (Thermo Scientific) for recording large maps. All spectra were collected in ultrafast mode using a 50 μm × 50 μm aperture. The spectra were collected in the 4000–800 cm^{-1} mid-IR range at a resolution of 16 cm^{-1} with one spectrum per pixel. Data analysis of IR spectra and chemical images was performed using OMNIC software (Thermo Scientific).

Table 1
Etiology corresponding to the selected kidney biopsies.

Sample	Etiology
B274	Primary hyperoxaluria diagnosed after kidney transplantation
B279	Excessive consumption of rhubarb
B292	Ethylene glycol intoxication
B305	Nephrocalcinosis after kidney transplantation
B317	Nephrocalcinosis after kidney transplantation
B329	Primary hyperoxaluria
B364	Cystic fibrosis
B379	Bariatric surgery
B380	Stomach cancer
B386	Nephrocalcinosis after kidney transplantation

2.2. Field Emission Scanning Electron Microscopy

A Zeiss SUPRA55-VP SEM was used for observation of the microstructure [37,39]. This field-emission “gun” microscope (FE-SEM) operates at 0.5–30 kV. High-resolution observations were obtained by using 2 secondary electron detectors: an in-lens SE detector and an Everhart-Thornley SE detector. Measurements were taken at low voltage (between 0.5 and 2 kV) without the usual deposits of carbon at the surface of the sample. Energy Dispersive X-ray (EDX) experiments can also be performed. In order to perform Ca cartography, the FE-SEM was operated at 12 kV.

3. Results and discussion

Quite recently, calcium oxalate compounds have been the subject of different publications [40–44] which have been dedicated to the insertion of heavy elements in their structure [45] or to their synthesis [46]. From a chemical point of view, the synthesis of amorphous COM can be considered a breakthrough in the chemistry of calcium oxalate compounds [47]. Among the different investigations we can quote the study of the adhesion and internalization between African green monkey kidney epithelial cells (before and after oxidative damage by hydrogen peroxide) and COM nanocrystals [48] or the internalization into MDCK renal tubular cells of COM crystals by endolysosomes [49]. As underlined by H. Shiraga et al. [50], although normal urine is frequently supersaturated with respect to calcium oxalate, most humans do not form stones. Inhibitors are among the multiple factors that may influence the complex process of urinary stone formation.

Regarding diseases related to calcium oxalate crystal deposition that lead to a significant loss of the kidney function, prevalent attention has been paid to hyperoxaluria. Although numerous excellent investigations exist [9,10,51–53], little attention has been paid to the crystallite morphology of COM ectopic calcification. This justifies the efforts undertaken here to describe the topology of these abnormal mineral deposits as well as their spatial repartition. In order to do so, the first step is to localize the COM deposits in the kidney biopsy.