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Nephrotoxicity induced by drugs: The case of foscarnet and atazanavir—A SEM and μFTIR investigation

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ABSTRACT

Biopsies of native or transplanted kidneys in patients suffering chronic or acute renal failure are commonly stained for tissue examination and search for possible crystal deposits which are then identified by polarizing microscopy and staining by von Kossa's method revealing mainly calcium deposits. Renal biopsies presumably containing crystal deposits were analyzed with a Spotlight 400 μFTIR (Fourier Transform Infra Red) imaging system in the mid-infrared spectral range to obtain infrared maps of tissue slides at high spatial resolution, down to 10 microns. When required, an optional ATR imaging accessory was used, improving the spatial resolution by a factor four, down to 3 microns at 1000 cm^{-1}. Among the 685 renal biopsies, 72% contained abnormal non-proteic material. Among them, 2.16% contained drug crystals (triamterene, *N*-acetylsulfadiazine, ciprofloxacine, indinavir, atazanavir, foscarnet, and vancomycine). We focused on foscarnet and atazanavir deposits. In the case of foscarnet-induced renal failure, two types of crystals were found in one patient. They were located in different parts of the nephron: sodium and/or calcium phosphonoformate crystals within glomerules and carbapatite in the proximal tubular cells. By contrast, atazanavir was only found in the tubular lumen of the nephron.

A precise identification of crystal deposits is essential for the diagnosis of an unexplained renal failure. Common histological procedures clearly fail to identify crystals deposits accurately. In most cases, light and polarizing microscopic examination should be completed by FTIR analysis.

1. Introduction

As assessed by several authors [1–3], renal dysfunction and injury secondary to medications are common, and can present as a subtle injury and/or overt renal failure. Renal injury associated with drugs may involve several parts of the kidney: glomeruli, tubules, interstitium and blood vessels. Drug-induced acute renal failure (ARF) was reported to account for 20% of all ARF cases [4]. Drug-induced nephrolithiasis accounts for 1–2% of all renal calculi [5]. Regarding children, C. Glanzmann et al. [6] have noticed that some drugs are associated with acute renal dysfunction in pediatric intensive care, especially some critical medication groups, such as betalactam antibiotics,

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glucocorticoids, opioids, and non-steroid anti-inflammatory drugs.

Among the different manifestations of drug-induced nephrotoxicity, we can quote acid–base abnormalities, electrolyte imbalances, urine sediment abnormalities, proteinuria, pyuria, hematuria, and, most commonly, a decline in the glomerular filtration rate [2]. Different mechanisms of drug-induced acute kidney injury (AKI) can be described such as prerenal AKI, glomerulonephritis, tubulopathies, interstitial nephritis, vascular nephropathies, crystalluria, etc.

Numerous drugs including foscarnet, atazanavir, tenofovir [7], vancomycin [8], gentamicin [9], taxol [10], cisplatin [11], and nucleotides [12] were reported as nephrotoxic drugs. Among drugs that are able to induce crystals or stones, we must distinguish between (1) poorly soluble drugs with high urine excretion that favors crystallization of the drug or its metabolites in urine and (2) drugs that provoke urinary crystals and calculi as a consequence of their metabolic effects [4,13–15]. In the two cases, the chemical complexity of this particular etiology i.e. the fact that drugs as well as their metabolites may be involved calls for the use of physicochemical techniques such as Fourier transform infrared microspectroscopy (μFTIR) to identify accurately the substances accumulated in the tissue [16,17].

The aim of this contribution is to assess the chemical nature of drugs and their metabolites as well as their localization in the kidney (presence in the glomerulus or in the tubule). To attain this goal, a set of kidney biopsies has been selected in order to consider nephrotoxicity by foscarnet and atazanavir. The chemical characterization of abnormal deposits will be performed through vibrational spectroscopies such μFTIR. The morphology of the deposits at the micrometer scale will be described through Field Emission Scanning Electron Microscopy (FE-SEM).

2. Materials and methods

2.1. Samples

685 kidney biopsies were investigated. The biological samples came from various hospitals from France, Canada, Belgium, Switzerland, Italy, and North Africa. All biopsies were examined in Tenon Hospital (Paris–France). Five microns slices of the biopsies were deposited either on CaF_2 plates or on low-e microscope slides (MirrIR, Kevley Technologies, Tienta Sciences, Indianapolis) in order to perform μFTIR experiments. In the case of Raman observation, the samples were deposited on classical glass supports used at the hospital. 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 by a study number, without indication of the name of the patient or potential identification data.

2.2. Materials

As already described in a previous publication [18], a part of the experiments (n=24 biopsies) were carried out at SOLEIL-Synchrotron (Saint-Aubin, Gif-sur-Yvette, France) on the SMIS beamline. The synchrotron radiation-μFTIR mappings were collected in reflection mode using an Infrared microscope (Thermo/Nicolet Nic-Plan) coupled to a μFTIR spectrometer (Thermo Nicolet MAGNA-IR 550). The IR microscope is equipped with a motorized sample stage (precision 1 mm) and a liquid nitrogen cooled mercury cadmium telluride (MCT − 250 mm) detector. Most of the analysis and maps presented here were achieved with a projected area on the sample of $6 \times 6\ \mu m^2$ and a step size of 6 μm, and each spectrum was acquired after 64 accumulations at 8 cm^{-1} spectral resolution. Data acquisition and processing were performed using OMNIC software (Version 7.4, Thermo-Scientific). The compounds were identified by comparing them to reference spectra [19].

IR microspectroscopy was 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 4000–700 cm^{-1} mid-IR range at a resolution of 8 cm^{-1} with one spectrum per pixel. Data analysis of IR spectra and chemical images was performed using OMNIC software (Thermo Scientific). Other biopsies were analyzed, in our hospital department in the Service des Explorations Fonctionnelles Multidisciplinaires (Tenon hospital – Paris), with the Spotlight 400 FTIR imaging System in the mid-infrared spectral range to obtain infrared maps of tissue slides at high spatial resolution, down to 10 microns. When required, an optional ATR imaging accessory was used, improving the spatial resolution by a factor four, down to 3 microns at 1000 cm^{-1}.

3. Results and discussion

A very limited proportion of patients receiving drugs develops crystalluria and, sometimes, acute renal failure due to tubular obstruction by drug crystals. This suggests that the formation of drug-induced crystal involves an interplay of risk factors specific either of the drug itself (high dose, high urinary excretion, low solubility product, long-term treatment without supervision, size and crystal forms) (Fig. 1) and of the patient (inadequate diuresis, urinary stasis, antacids, uricosurics, anti inflammatory drugs, urinary pH, and urinary tract infection) [20].

Drugs, which may crystallize in urine are not so numerous [21] and belong to various pharmacological groups: antibacterial drugs, protease inhibitors, analgesics, antihypertensive agents, antacids, and some other drugs. We found a number of these drugs in renal deposits. Note that hypercalciuria and nephrolithiasis may result from calcium supplements, especially when associated with vitamin D [22].

At the hospital, the classical analysis procedure starts by observation through optical microscope underlining the presence of birefringent or non-refringent deposits. This criterion is not sufficient to ensure the precise identification of the compound. Several drugs are present as birefringent crystals, namely triamterene, *N*-acetylsulfadiazine, ciprofloxacine, indinavir, and atazanavir while others are non-refringent, namely foscarnet or vancomycine. To identify precisely the chemical nature of the drugs, μFTIR