Infrared Microspectroscopy using Synchrotron Radiation (Sr $M_{\text{ftir}}$) and Infrared Microspectroscopy as New Tools for Rapid Detection of Ectopic Calcifications Associated with Peritoneal Dialysis

Agnieszka Pozdzik, Pieter Demetter, Monika Tooulou, Anwar Hamade, Joelle Nortier, Dominique Bazin, Michel Daudon

To cite this version:

Agnieszka Pozdzik, Pieter Demetter, Monika Tooulou, Anwar Hamade, Joelle Nortier, et al.. Infrared Microspectroscopy using Synchrotron Radiation (Sr $M_{\text{ftir}}$) and Infrared Microspectroscopy as New Tools for Rapid Detection of Ectopic Calcifications Associated with Peritoneal Dialysis. Journal of Nanomedicine and Nanotechnology, 2015, 6, pp.342. 10.4172/2157-7439.1000342 . hal-01293344

HAL Id: hal-01293344
https://hal.sorbonne-universite.fr/hal-01293344
Submitted on 24 Mar 2016

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution| 4.0 International License
Infrared Microspectroscopy using Synchrotron Radiation (Sr μFTIR) and Infrared Microscopy as New Tools for Rapid Detection of Ectopic Calcifications Associated with Peritoneal Dialysis

Agnieszka A Pozdzik1,*, Pieter Demetter3, Monika Toulou2, Anwar Hamade4, Joelle Nortier1,4, Dominique Bazin2,4 and Michel Daudon5

1Department of Nephrology, Dialysis and Renal Transplantation, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium
2Laboratory of Experimental Nephrology, Department of Biochimie, Faculty of Medicine, Université Libre de Bruxelles, Brussels, Belgium
3Department of Pathology, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium
4Laboratoire de Physique des Solides, Université Paris XI, Orsay, Paris, France
5Service d’Explorations Fonctionnelles, Hôpital Tenon, Paris, France

Abstract

The cardiovascular calcifications (CVC) represent a central complication of chronic kidney disease (CKD) responsible of high cardiovascular mortality particularly in patients on peritoneal dialysis. Unfortunately, electron beam and multislice computed tomography, planar X-ray, ultrasonography or cardiac echocardiography methods are not accurate to detect calcium phosphate microcrystals since of its small size. We investigated the ectopic calcifications by Von Kossa staining and infrared microspectroscopy using synchrotron radiation (SR μFTIR) and infrared microspectroscopy in formalin-fixed peritoneal tissues from 3 cases. Von Kossa staining allowed us to detect vascular calcifications only in one of 3 studied peritoneal biopsies. Vascular calcifications contained mainly carboxyapatite accordingly to the presence of the IR absorption bands positioned at 1030 cm⁻¹ (ν3), 960 cm⁻¹ (ν1). In all studied biopsy, we found several tissue microcrystals also composed by carboxyapatite.

To our knowledge, we report for the first time the usefulness of infrared microscopy and SR μFTIR for the assessment of calcium phosphate microcrystals and identification of biochemical composition of VC associated with CKD in peritoneal membrane from patients on peritoneal dialysis. SR μFTIR technique and infrared microspectroscopy are new, remarkable, and rapid tools for detection of early stage of ectopic calcifications associated with peritoneal dialysis. Investigation of calcium phosphate microcrystals by both methods might improve our understanding of early stage of CVC pathophysiology.

Keywords: Cardiovascular calcifications; Hyperphosphatemia; Ultrasonography; Microspectroscopy; Echocardiography

Introduction

The cardiovascular calcifications (CVC) represent a central complication of chronic kidney disease (CKD) responsible of high cardiovascular mortality particularly in patients on peritoneal dialysis. Hyperphosphatemia is a leading mediator [1]. Experimental data suggest that the true culprit of phosphate toxicity may be mediated by calcifying nanoparticles (CNP) containing calcium phosphate microcrystals and some protein. Indeed, increased level of CNP is independently associated with CVC in CKD patients [2,3]. Unfortunately, electron beam and multislice computed tomography, planar X-ray, ultrasonography or cardiac echocardiography methods are not accurate to detect calcium phosphate microcrystals since of its small size [1].

Materials and Methods

As ectopic calcifications have been reported in peritoneal tissue in patients on peritoneal dialysis, [4,5] we investigated the ectopic calcifications by Von Kossa staining and infrared microspectroscopy using synchrotron radiation (SR μFTIR) and infrared microspectroscopy in formalin-fixed peritoneal tissues. To do this, paraffin blocks from 3 cases of EPS available in the archives of pathology department were used. Tissue sections (4 um) were treated with a sliver nitrate solution. Silver ions substitute the calcium ions bounded to phosphates and are subsequently visualized by hydroquinone reduction to metallic silver producing a brown-black staining (von Kossa reaction) as previously reported.

Thereafter, the biochemical composition was determined by infrared microspectroscopy using synchrotron radiation in collaboration with Condensed Material Paris, National Center for Scientific Research (CNRS), College de France, Paris, France as follow (chemical composition of pathological microcalcifications can be accurately identified by FTIR spectroscopy through their IR absorption bands. The peritoneal tissue microcrystals were approached by infrared microspectroscopy as detailed previously [6]. This study was evaluated and approved by the local Ethic Committee (Erasme hospital No.: P2014/184) [7].

Results

Von Kossa staining allowed us to detect vascular calcifications only in one of 3 studied peritoneal biopsies but this technique is not able

*Corresponding author: Agnieszka A Pozdzik, Department of Nephrology, Dialysis and Renal Transplantation Erasme Hospital, Université Libre de Bruxelles, Route de Lennik 808, B-1070 Brussels, Belgium, Tel: +32-2-555-3334; Fax: ++32-2-555-6499; E-mail: Agnieszka.Pozdzik@erasme.ulb.ac.be

Received September 28, 2015; Accepted December 03, 2015; Published December 10, 2015


Copyright: © 2015 Pozdzik AA, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
to determine significantly their chemical composition. Interestingly, this biopsy provided from patient with the largest number of bacterial peritonitis (8 episodes) and peritoneal dialysis vintage (Figure 1) [8]. Apart from vascular areas no black staining was found in all three analyzed tissues. Vascular Calcifications composition contained mainly carboxypatite accordingly to the presence of the IR absorption bands positioned at 1030 cm⁻¹ (ν3), 960 cm⁻¹ (ν1) (Figure 2). In all studied biopsy, we found several tissue microcrystals also composed by carboxypatite (Figure 2).

**Discussion**

To our knowledge, we report for the first time the usefulness of infrared microscopy and SR μFTIR for the assessment of calcium phosphate microcrystals and identification of biochemical composition of VC associated with CKD in peritoneal membrane from patients on peritoneal dialysis. The von Kossa staining considered as specific for calcium although was not accurate for specify types of calcifications. Infrared microspectroscopy using synchrotron radiation and infrared microspectroscopy were more adapted as informs precisely that respectively vascular calcification and tissue microcrystals are composed by calcium phosphate (carboxypatite) independently of its sizes.

Calcium phosphate microcrystals precipitate in a passive way secondary of phosphate and/or of calcium super-saturation. This funding emphasizes once again, the crucial importance of strict control of phospho-calcium parameters in PD patients [2]. Indeed, carboxypatite may reflects two origins: 1) the metabolic disorders, mainly hyperphosphatemia and secondary hyperparathyroidism related to lost of renal function and 2) iatrogenic component related to treatment of above mentioned metabolic complications by high calcium load (calcium based phosphate binders) and/or supplementation of vitamin D [1,9].

Importantly calcium phosphate microcrystals but not soluble phosphate have been proposed to play a role in the local genesis of osteo-chondrogenetic transformation in cultured smooth muscle cells [10].

**Figure 1:** Photomicrographs demonstrating histopathological findings: parietal peritoneal biopsy reported the presence of tissue calcifications. (A) Positive control of Von Kossa staining (internal controls) (A), control case: normal peritoneum (B), case of acute peritonitis (Case control N° 3; C) and Case of encapsulating peritoneal sclerosis (EPS) (A) Abnormal calcifications in vessel vascular corresponded to intimal calcinosis. (B-C) Absence of vascular calcifications. (D) Von Kossa coloration demonstrating calcifications in media of vessels; in this method tissue section were treated with a silver nitrate solution and the silver is deposited by replacing the calcium reduced by the strong light, and thereby visualized as metallic (black) silver. (A-D)Von Kossa staining. Original magnifications :A-B-C: 20X et D : 40x.

**Figure 2:** Photomicrographs demonstrating technique applied for analysis of peritoneal tissue vascular calcifications.
Nano-sized complexes of calcium phosphate microcrystals serve as mineral chaperones for further calcification and have been proposed as a very early step of vascular calcification process. Moreover in vitro, calcium phosphate microcrystals induce apoptosis of smooth muscle cells, inflammation in the arterial intima such and increase in mineralized multicellular nodules [3]. The identification of carbapatite in our peritoneal biopsy samples is of great importance because it is formed preferentially within alkalin pH [2], frequently seen during bacterial infections. Therefore, this finding raises the issue of relation between the previous bacterial peritonitis and peritoneal vasculopathy in EPS.

Conclusion

In conclusion, SR μFTIR technique and infrared microspectroscopy are new, remarkable, and rapid tools for detection of early stage of ectopic calcifications associated with peritoneal dialysis. Investigation of calcium phosphate microcrystals by this method might improve our understanding of early stage of CVC physiopathology.

References