Prostatic Stones: Evidence of a Specific Chemistry Related to Infection and Presence of Bacterial Imprints

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Abstract

Prostatic stones are a common condition in older men in industrialized countries. However, aging appears not to be the unique pathogenesis of these calcifications. Our morpho-constitutional investigation of 23 stone samples suggested that infection has a significant role in the lithogenic process of prostate calcifications, even without detection of infection by clinical investigation. Most stones (83%) showed bacterial imprints and/or chemical composition, suggestive of a long-term infection process. Chronic infection may induce persistent inflammation of the tissue and secondarily, a cancerization process within a few years. Thus, the discovery of prostate calcifications by computerized tomodensitometry, for example, might warrant further investigation and management to search for chronic infection of the prostate gland.

Introduction

Prostate cancer is the second most frequent cause of mortality due to cancer in males in the United States [1]. Transurethral resection, radical prostatectomy, radiation therapy and hormone therapy are the usual prostate cancer treatments [2]. Prostate removal leads to the observation of prostatic calculi. Because almost 99% of surgically removed prostates contain stones, these stones are generally considered clinically insignificant [3]. Therefore, only a few papers have investigated these calcifications.

We noted the large chemical diversity of these prostatic stones. Carbonated calcium phosphate apatite (carbahpatite; CA) seems to be the major component, but several investigations show the presence of other mineral phases such as calcium oxalate monohydrate and dihydrate [4], brushite [5], and whitlockite [6]. More recently, several other mineral phases previously not reported in prostatic calculi were octacalcium phosphate pentahydrate and amorphous carbonated calcium phosphate [7]. Such chemical diversity indicates significant variations in the local biochemical, which may be linked to different conditions.

A previous investigation involving stone culture revealed that infected calculi in the prostate were implicated in relapsing urinary tract infection [6]. This work aimed to assess a possible relationship between infection and prostatic calculi, taking into account the chemical and structural characteristics of such calculi. We combined chemical analysis with Fourier transform infra-red (FTIR) spectroscopy and structural investigation at the mesoscopic scale by scanning electron microscopy (SEM). FTIR spectroscopy has helped us to examine the presence of chemical phases involved in infection of other organs [8,9]. Moreover, SEM observations allowed for assessing the presence of bacterial imprints on prostatic calculi [10]. FTIR spectroscopy and SEM have been used in several studies of pathological calcifications [11,12,13,14,15].

Materials and Methods

Samples

We investigated 23 prostatic stones obtained from the Saint-Louis and Tenon hospitals in Paris. The calcifications were collected from the prostate after radical prostatectomy or transurethral resection. The mean age of the patients was 71 years (range 33 to 87 years). All participants gave their verbal consent for use of the material. Samples were examined without knowledge of the name of the patient or other identifying data. Ethical approval for the study was obtained from the ethics committee of Tenon Hospital.

FTIR Spectroscopy

The FTIR spectroscopy was performed at Tenon Hospital. Each sample was analysed in absorbance mode on a Bruker Vector 22 spectrometer by accumulation of 32 spectra between 4000 and 400 cm⁻¹, with resolution 4 cm⁻¹ and time acquisition 1 sec/spectrum. The analysis was as previously described [16]. For each sample, the inner and surface compositions were established. The compounds were identified by comparing them to reference spectra [17].

SEM

Each prostatic stone was observed by Field-effect SEM (Zeiss SUPRA55-VP with an Everhart-Thornley secondary electron detector). To maintain sample integrity, each measurement was
Results

Stones were imaged at similar magnification for comparison.

Table 1. Major phases in inner and peripheral layers (minor phases in italics) in 23 samples of prostate stones, along with presence of urinary infection and bacterial imprints.

| N° | Age | Core | Periphery | Urinary infection | Bacterial imprints |
|----|-----|------|-----------|-------------------|-------------------|
| 1  | 81  | WK>ACCP>CA>PROT | CA>OCP>ACCP>PROT | ND | Yes |
| 2  | 68  | CA>ACCP>>MAP, COM, PROT | CA>>ACCP>PROT | ND | Yes |
| 3  | 78  | CA>>COD>PROT>COM | CA>>COD, PROT | Yes | No |
| 4  | 78  | CA>>COD>COM>>MAP, PROT | CA>>PROT>COD | No | Yes |
| 5  | 79  | CA>>WK>PROT | CA>>WK>PROT | ND | Yes |
| 6  | 81  | CA>>COD>>COM, PROT | CA>>PROT, COM, COD | No | No |
| 7  | 53  | CA>>COD, PROT, COM | CA>>COD, PROT, COM | Yes | No |
| 8  | 63  | CA>>PROT>COM | CA>>PROT>COM | Yes | Yes |
| 9  | 69  | WK>CA>ACCP>PROT>COM, COD | WK>CA>ACCP | Yes | Yes |
| 10 | 71  | CA>WK>>PROT>COM | PROT>CA>ACCP>>WK | Yes | Yes |
| 11 | 42  | CA>>Br>WK>COD>PROT, COM | CA>>WK>PROT | No | Yes |
| 12 | 58  | CA>>PROT | CA>>PROT | ND | No |
| 13 | 70  | ACCP>CA>>PROT>COM | ACCP>CA>>PROT | No | Yes |
| 14 | 58  | WK>CA>ACCP>PROT | WK>CA>ACCP | ND | Yes |
| 15 | 76  | CA>>WK>OCP>PROT, ACCP | PROT>CA>OCP>WK, ACCP | No | Yes |
| 16 | 59  | ACCP>CA>>PROT, MAP | PROT>ACCP>CA | ND | Yes |
| 17 | 75  | WK>PROT>ACCP>CA>CO | WK>ACCP>PROT>CA | Yes | Yes |
| 18 | 35  | WK>>CA>ACCP>COM, PROT | CA>WK>ACCP, PROT | No | No |
| 19 | 77  | WK>ACCP>>CA>PROT | CA>PROT, ACCP, WK | ND | Yes |
| 20 | 68  | CA>>Br>COD>PROT>COM | CA>>Br>PROT | No | Yes |
| 21 | 72  | WK>CA>ACCP | CA>WK>PROT, ACCP | ND | Yes |
| 22 | 74  | ACCP>CA>>WK, PROT | PROT>ACCP>CA | No | Yes |
| 23 | 67  | WK>CA>ACCP>PROT>COM | WK>CA>PROT | No | Yes |

Br = brushite, ACC = amorphous carbonated calcium phosphate, CA = carabapatite (carbonated calcium phosphate), COD = calcium oxalate dihydrate, COM = calcium oxalate monohydrate, OCP = octacalcium phosphate pentahydrate, Prot = proteins, WK = whitlockite. ND: not determined.

Table 2 shows the proportion and frequencies of the main chemical phases in 23 prostatic stones.

Table 2. Nature and frequency of main chemical phases in 23 prostatic stones.

| Chemical phase | Core phase (%) | Main core phase (%) | Peripheral phase (%) | Main peripheral phase (%) |
|----------------|----------------|---------------------|----------------------|--------------------------|
| Carbapatite    | 21 (91%)       | 12 (52%)            | 17 (68%)             | 14 (62%)                 |
| Whitlockite    | 11 (48%)       | 8 (35%)             | 7 (30%)              | 4 (17%)                  |
| Amorphous carbonated calcium phosphate | 6 (26%) | 3 (13%) | 5 (22%) | 1 (4%) |
| Octacalcium phosphate pentahydrate | 1 (4%) | 0 | 2 (9%) | 0 |
| Brushite       | 2 (9%)         | 0                   | 1 (4%)               | 0                        |
| Proteins       | 4 (17%)        | 0                   | 8 (35%)              | 4 (17%)                  |

Taken at low voltage (≤2 keV). Stones were imaged at similar magnification for comparison.
core of 11 stones (48%) and as the main component in 8 (35%) but was the main component of the stone surface in only 4 stones (17%). ACCP was identified in the core of 6 stones (26%) and was the main phase in 3 (13%). ACCP was the main component of the stone surface in only 1 stone (4%).

Representative SEM images of normal stones and those with bacterial imprints (size about 500 nm) on the surface and in the core of stones are in Figure 1.
Discussion

Our SEM study revealed a high occurrence of bacterial imprints (78%) in 23 prostatic stones, which reveals a past or present infection of the prostate tissue; however, urinary tract infection was detected in only 6 (26%) cases. The large difference between number of reported infections and markers of infection within stones implies that aging may not be the only cause of prostatic calcifications. Infection and a lithogenic process induced by infection may play a role in most of the 99% of surgically removed prostate-containing stones.

The bacterial imprints had a specific spherical shape, which suggests infection by cocci germs. More precisely, the grape-like clustering, shape and size are common with staphylococci infection [18]. In 2 cases of proven infection, the species was *Staphylococcus aureus*. Nevertheless, these imprints were not seen on all stone surfaces. Similarly to kidney stones, the bacteria can imprint on a particle surface such as CA or ACCP but not other crystal types such as whitlockite, octacalcium phosphate or brushite. Indeed, the size of CA and ACCP crystals is smaller than that of other phases, such as struvite [19]. As well, SEM examination was restricted to some parts of small and partial samples collected during prostate removal. However, all stones contained at least 15% CA or ACCP, so a careful observation by SEM allows for detection of bacterial imprints on the surface of these minerals.

We discuss only the major phases. As previously reported, the main compounds of prostatic stones are calcium phosphate [20]. The most common and abundant phase is CA, well known as a common form of ectopic calcification in the kidney [21], vascular system [22,23] or breast [24]. Particular crystalline phases, namely whitlockite and ACCP, a marker of infection stones in the urinary tract, were identified in the core of 17 (74%) prostatic stones. Whitlockite is an infrequent component of kidney stones and has been associated with chronic urinary tract infection in most calculi from women [8]. This phase has been also found in infections such as tuberculosis [9]. These different markers (imprints, specific phases, etc.) led to the conclusion that 63% of calculi (19 stones, p<0.0001) could be linked to an infection process.

The deleterious consequences of chronic infection and inflammation have been well described in the cellular model [25] and chemical model [26]. A number of papers have highlighted the relation between infection-related inflammation and cancer in various organs such as stomach, liver, lung, colon or bladder [27,28]. The same reasoning may be applied to prostatic stones. Moreover, recent studies suggest epidemiological and pathologic links between benign prostate hypertrophy and prostate cancer [29]. Chronic infection, as well as the resulting stones, may induce persistent inflammation and could contribute to prostatic hypertrophy. In fact, the inflammatory process, associated with tumor phenomena, seems to influence the formation and evolution of these concretions [30]. We previously reported a high content of proteins in prostate stones [7]. Lactoferrin was found among proteins identified in both corpora amylacea and stones. This protein is considered a marker of inflammation and infiltration by neutrophil polynuclear factors and is implicated in the carcinogenesis process [30].

The clinical interest of this paper is to draw attention to the high occurrence of asymptomatic infection of the prostate.

History of urinary tract infection and risk of renal cell carcinoma have been found to be related. As reported by Parker et al. [31], analysis of epidemiological data suggests a positive association of history of urinary tract infection and renal cell carcinoma development. Similar results were reported by MacLaughin et al. [32] and by Meares for prostate tissue [6]. Clearly more research is needed to establish a relationship because intratissue infection necessitates antibiotic treatment during several weeks. Our data suggest that the presence of stones or calcifications within the prostate could indicate chronic, often asymptomatic infection, the consequence of which remains to be assessed for medical management.

Conclusions

Prostatic stones are often considered to have no clinical significance, but the use of SEM showed for the first time the high frequency of bacterial imprints in these stones. Moreover, our data underline the specific chemistry of calcium phosphate phases, particularly the preponderance of whitlockite and ACCP in these calcifications. These results demonstrate the high occurrence of bacterial infections in the prostate, often without any clinical symptoms.

Inflammation induced by an infection may lead to cancerization of the tissue. Early detection of prostatic calcifications or stones could suggest a search for asymptomatic chronic infection. If an infection is detected, medical management and antibiotic treatment could avoid chronic inflammation of the tissue and further deleterious consequences. Thus, we suggest that discovery of prostatic calcifications by imaging such as computerized tomosdensitometry might warrant further investigations and management to search for chronic infection of the prostate gland.

Author Contributions

Conceived and designed the experiments: AD PM DB MD. Performed the experiments: AD PM DB MD. Analyzed the data: AD PM DB MD. Contributed reagents/materials/analysis tools: AD PM DB MD.

References

1. Jemal A, Siegel R, Xu J, Ward E (2010) Cancer statistics, 2010. CA Cancer J. Clin. 60: 27–200.
2. Oh S, Shin S, Janneke R (2012) ETIV, 4 and 5: An oncogenic subfamily of ETS transcription factors. Biochimica et Biophysica Acta 1826: 1–12.
3. Sonderegard G, Vetter M, Christensen PO (1987) Prostatic calculi. Acta Pathol Microbiol Immunol Scand A. 95: 141–145.
4. Hou THS, Lin SY, Lin CC, Cheng WT (2011) Preliminary feasibility study of FTIR microscopic mapping system for the rapid detection of the composed components of prostatic calculi. Urol. Res. 39: 165–170.
5. Kato H, Ogasawa A (1987) Large brushite stone in a dilated prostatic urethra. The Journal of Urology 138: 124–125.
6. Meares EM (1974) Infection Stones of Prostate Gland. Urology 4: 560–567.
7. Desombre A, Meria P, Bazin D, Foy E, Rouziere S, et al. (2011) Revisiting the chemical diversity in prostatic calculi: An SEM and FT-IR investigation. Progres en Urologie 21: 940–945.
8. Maurice-Estape L, Levilain P, Lacour B, Daudon M (1999) Crystalline phase differentiation in urinary calcium phosphate and magnesium phosphate calculi. Scan J. Urol Nephrol 33: 299–305.
9. Lagier R, Baud CA (2003) Magnesium whitlockite, a calcium phosphate crystal of special interest in pathology. Pathology, Research and Practice 199: 329–335.
10. Carpenter X, Daudon D, Traxer O, Jungers P, Mazuyas A, et al. (2009) Relationships Between Carbonation Rate of Carbapatite and Morphologic Characteristics of Calcium Phosphate Stones and Etiology. Urology 75: 968–975.
11. Daudon M, Jungar P, Bazin D (2008) Peculiar Morphology of Stones in Primary Hypercalcuria. New England Journal of Medicine, 359: 100–102.
12. Li C, Ebenstein D, Xu C, Chapman C, Saloner D, et al. (2003) Biochemical characterization of atherosclerotic plaque constituents using FTIR spectroscopy and histology. Biomed. Mater. Res. 64: 197–206.
13. Mendelssohn R, Hassankhani A, DiCarlo E, Boskey A (1989) FT-IR microscopy of endochondral ossification at 20 mu spatial resolution. Calcif Tissue Int. 44: 20–24.
14. Bazin D, Daudon M (2012) Pathological calcifications and selected examples at the medicine solid-state physics interface. J. Phys. D. Appl. Phys. 45: 383001–383010.
15. Bazin D, Daudon M, Combes Ch, Rey Ch (2012) Characterization and some physicochemical aspects of pathological microcalcifications. Chem Rev. DOI: 10.1021/cr200068d.
16. Estepa L, Daudon M (1997) Contribution of Fourier transform infrared spectroscopy to the identification of urinary stones and kidney crystal deposits. Biospectroscopy 3: 347–369.
17. Quy-Dao N, Daudon M (1997) Infrared and Raman Spectra of Calculi. Elsevier.
18. Freeman-Cook L, Freeman-Cook K (2005) Staphylococcus Aureus Infections, Chelsea House Publications.
19. Bazin D, André G, Weil R, Matzen G, Emmanuel V et al. (2012) Absence of bacterial imprints on struvite-containing kidney stones: a structural investigation at the mesoscopic and atomic scale. Urology 79: 786–790.
20. Sutor DJ, Wooley SE (1974) The Crystalline composition of prostatic calculi. British Journal of Urology 46: 533–535.
21. Dessombz A, Bazin D, Dumas P, Sandt C, Sule-Suso J, et al. (2011) Shedding Light on the Chemical Diversity of Ectopic Calcifications in Kidney Tissues: Diagnostic and Research Aspects. PLoS ONE 6.
22. Dorfmüller P, Bazin D, Aubert S, Weil R, Brisset F, et al. (2010) Crystalline ultrastructures, inflammatory elements and neangiogenesis are present in inconspicuous aortic valve tissue. Cardiology Research and practice, 685926.
23. Reid JD, Andersen ME (1993) Medial calcification (whitlockite) in the aorta. Atherosclerosis. 101: 213–224.
24. Kopans D, Gavenonis S, Halpern E, Moore R (2011) Calcifications in the breast and digital breast tomosynthesis. Breast J. 17: 638–644.
25. Karin M, Lawrence T, Nizet V (2006) Innate Immunity Gone Awry: Linking Microbial Infections to Chronic Inflammation and Cancer. Cell 124: 823–835.
26. Hussain P, Harris C (2007) Inflammation and cancer: An ancient link with novel potentials. Int. J. Cancer 121: 2373–2380.
27. Engels EA (2008) Inflammation in the development of lung cancer: epidemiological evidence. Expert Rev Anticancer Ther. 8: 605–613.
28. Grivennikov SI, Greten FR, Karin M (2010) Immunity, inflammation, and cancer. Cell 140: 883–899.
29. Alcaraz A, Hammerer P, Tabaro A, Schroder FH, Castro R (2009) Is there evidence of a relationship between benign prostate hyperplasia and prostate cancer? Findings of a literature review. Eur Urol 55: 864–875.
30. Stamos KS, Wilson BA, De Marzo AM, Isaacs WB (2009) Acute inflammatory proteins constitute the organic matrix of prostatic corpora amylacea and calculi in men with prostate cancer. PNAS 106: 3443–3448.
31. Parker AS, Cerhan JR, Lynch CF, Bradley BC, Cantor KP (2004) History of urinary tract infection and risk of renal cell carcinoma. Am J Epidemiol 159: 42–48.
32. McLaughlin JK, Mandel JS, Blot WJ, Schuman LM, McEl ES, et al. (1984) A population-based case control study of renal cell carcinoma. J. Natl Cancer Inst. 72: 273–284.