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Nanobacteria: An Infectious Cause for Various Human Diseases

[1]K. Abo-EL-Sooud, [1]M.M. Hashem and [2]A.Q. Gab-Allaha
[1]Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt
[2]The Holding Company for Biological Products and Vaccines VACSERA, Egypt

Abstract: Background: Calcium phosphate is deposited in many diseases, but formation mechanisms remain tentative. Nanobacteria are newly found alone replicating particles that have been detected in mineral stones forming diseases. Nanobacteria were the first (may still be the only ones) calcium-phosphate mineral containing particles isolated from human blood. **Results:** The stimuli for calcium salt deposition in patients with these circumstances are unclear, but nidi (meaning that biomineralization is taking place out of chemical equilibrium) for precipitation and crystallization are needed. Several important human diseases have calcium phosphate deposition as a distinctive, e.g. atherosclerosis and cardiovascular diseases, stone formation in kidneys, polycystic kidney disease, gall-bladder, salivary, venous and gingival locations, other urological diseases, e.g. prostatitis, many cancers and various forms of autoimmune diseases. **Conclusion:** In this review, we discuss fully the possible etiological relationships of nanobacteria in the pathogenesis of each previous disease.

Key words: Nanobacteria, urolithiasis, prostatitis, atherosclerosis, ovarian cancer

INTRODUCTION

Nanobacteria appear as self-propagating calcifying molecular complexes found in bovine and human blood and blood products. These nanoparticles are active nidi forming calcium phosphate mineral under subsaturation level of calcium and/or phosphate. Nanobacteria have unique characteristics in different regards, appear as self-propagating calcifying macro-molecular complexes found in bovine and human blood and blood products, and were published as an infectious cause for pathological calcification (Kajander and Ciftcioglu, 1998). Several important human diseases have calcium phosphate deposition as a hallmark, e.g., atherosclerosis and cardiovascular diseases, stone formation in kidneys, gall-bladder, salivary, venous and gingival locations, other urological diseases, e.g., prostatitis, many cancers and various forms of autoimmune diseases and arthritis. Several hypotheses regarding the pathogenesis of calcification in soft tissues have been proposed, including: (i) calcium deposit is formed on degrading cells and/or apoptotic bodies (Vu *et al*., 1998); (ii) nanobacterial-like particles (PLP) surround themselves with sphere-like structures from deposited calcium Ciftcioglu and Kajander, 1998; Miller *et al*., 2004); (iii) induction of supersaturated calcium liquid is used as a building block for passive calcium sedimentation with or without participation of phospholipids or proteoglycans (Kim, 1976; Poggi *et al*., 2001) and (iv) smooth muscle cells undergo bone-like differentiation and hyper phosphonemia is stimulated by vascular calcification (Giachelli *et al*., 2005).

Furthermore, these organisms were found to produce a biofilm containing hydroxyl apatite or carbonate, preventing their effective staining. The stimuli for calcium salt deposition in patients with these conditions are unclear but nidi (meaning that biomineralization is taking place out of chemical equilibrium) for precipitation and crystallization are needed even under supersaturation conditions (Carson, 1998). Nanobacteria are carbonate apatite forming, cytotoxic bacteria recently discovered in human and bovine blood and blood products (Kajander *et al*., 1997; Ciftcioglu *et al*., 1997; Kajander and Ciftcioglu, 1998). Calcific kidney stones in humans are located on renal papillary surfaces and consist of an organic matrix and crystals of calcium oxalate and/or calcium phosphate (Khan, 1997). It has been stated that the core of 67% of calcium oxalate stones contains calcium phosphate (Abraham and Smith, 1987). Nanobacteria are thought to play an important role in extraskeletal calcifying diseases including stones formation, urolithiasis and polycystic kidney disease (Kajander *et al*., 2003). Nanobacteria are phylogenetically close relatives of mineral forming bacteria (Kajander *et al*., 1997). These particles have been isolated from kidney stones and urine of patients with renal

Corresponding Author: K. Abo-EL-Sooud, Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Giza 12211, Egypt

lithiasis (Ciftcioglu *et al*., 1999), renal fluid taken from patients with polycystic kidneys. It has been speculated that nanobacteria may be the spherical deposits found in the kidneys of patients who suffer from kidney stones (Vogel, 1998; Bradbury, 1998). Nanobacteria are thought to play an important role in extraskeletal calcifying diseases including stones formation, urolithiasis and polycystic kidney disease (Kajander *et al*., 2003), atherosclerosis (Jelic *et al*., 2007), periodontal disease (Ciftcioglu *et al*., 2003), rheumatoid arthritis (Cassell, 1998) and prostatitis (Bock *et al*., 1989; Geramoutsos *et al*., 2004). Two strains, one of *Nanobacterium sanguineum* and the other of Nanobacterium sp., were isolated from kidney stones and human and bovine sera, respectively. Phylogenetic analysis based on comparison of 16S ribosomal DNA (rDNA) sequences has placed the nanobacteria isolated from fetal calf serum into the α_2 subgroup of *Proteobacteria* (Kajander *et al*., 1997), closely related to *Thiobacillus*, a water contaminant and *Agrobacterium* and *Rhizobium* which are plant associated bacteria.

Nanobacteria is the nucleating agents for kidney stones: Kidney stone formation (nephrolithiasis) composed of a variety of minerals amalgamated with proteins (Pak, 1998). Regardless of their mineralogy, two factors are fundamental in kidney stone development: supersaturation with respect to the forming mineral phase and crystal nucleation. Research has concentrated in understanding the metabolic and environmental factors which produce an abnormal urinary composition causing supersaturation (Moe, 2006). Less is known about the controls on the nucleation of crystals, which grow to form kidney stones. Ten years ago, the claim that nanobacteria promote the nucleation of kidney stones provoked much controversy. Nanobacteria are calcium phosphate (apatite)-covered organic entities, which have been proposed to be the smallest known living organisms (Kajander and Ciftcioglu, 1998). Nanobacteria seem to occur in the majority of kidney stones (Ciftcioglu *et al*., 1999) but have also been observed in other human and animal materials (Kajander, 2006) as well as in sediments (Folk, 1993). Based on the presence of apatite in the core of most kidney stones, on the widespread occurrence of nanobacteria in kidney stones and on the *in vitro* formation of kidney stone-like apatite in the presence of nanobacteria, nanobacteria have been indicated as the nucleating agents of kidney stones (Kajander *et al*., 2003). Traditionally, only struvite and some carbonate apatite stones are thought to be infectious in origin and are linked to alkalinity and phosphate production by urea-splitting bacteria (Rodman, 1999). According to Ciftcioglu *et al*. (1999), instead, the formation of virtually all kidney stones is infectious because nanobacteria cause their nucleation. Moreover, they analyzed kidney stones from different groups such as calcium oxalate, calcium phosphate, uric acid, calcium hydrogen phosphate-dihydrate, magnesium ammonium phosphate hexahydrate and all of them had small amounts of apatite. Their experiments continued and they decided to isolate the supposed nanobacteria from the real kidney stones and she analyzed them *in vitro*. The surprising result was that the nanobacteria created stones *in vitro*. An external contamination was rejected because they created some control cultures and they remained without any change.

In vitro animal studies have revealed calcific stone formation by nanobacteria, as well as stone formation following nanobacteria inoculation of rat kidneys (Kajander *et al*., 2003). An *in vitro* study on human kidney stones has demonstrated the presence of nanobacteria on their surfaces (Ciftcioglu *et al*., 1999). Despite these supportive basic science reports, the role of nanobacteria in stone pathogenesis is controversial and clinical evidence in humans is lacking. Nanobacteria antigens have been reported in 97% of human kidney stones (Kajander *et al*., 1997; Ciftcioglu *et al*., 1999). Apparently, these nanobacteria surround themselves with a mineral coating and can serve as nidi for the genesis of renal calculi (Garcia Cuerpo *et al*., 2000). Aqel (2008) stated that the incidence of anti-nanobacterial Antibodies was examined and an attempt to culture these nanobacteria from Jordanian patients with urolithiasis was also done. Despite the fact of strict application of the methods described by previously Drancourt *et al*. (2003); Khullar *et al*. (2004) and Miller *et al*. (2004), cultures of nanobacteria were not obtained from kidney stones. The study therefore wonders if there is a culture parameters not mentioned in publications that could explain discrepancy between our results and those previously reported. However, a significant controversy has erupted over the existence and significance of nanobacteria as living or non living particles (Abbott, 1999, 2000; Drancourt *et al*., 2003).

Link between the early calcium deposition in placenta and nanobañterial-like infection: Extensive calcium deposition in placenta named as Pathological Placental Calcification (PPC), could have serious negative consequences for the adequate growth of embryos. The hypothesis that the molecular basis of PPC development consists of nanobacteria-induced calcification in infected female placenta (Agababov *et al*., 2007). Electron microscopy findings support this hypothesis. The initial stage of micro-calcification may originate from the external

surface of individual nanobacteria-like particles found mainly in placental extracellular matrix, where initial calcium deposition occurs as a needle surface deposition or as an amorphous-like surface precipitate. Further calcific propagation in placenta takes place in the newly formed macro-cavities, which are characterized by low electron density, possibly reflecting its liquid content around calcium deposition. The micro-cavities contain free nanobacterial-like particles, which may relate to atypical Gram-negative bacteria but not to apoptotic bodies by morphological characters and DNA/RNA distribution. We hypothesize that the increased placental calcification might be caused, at least in part, by nanobacterial infection. Thus, it can be assumed that the nanobacteria are involved in the formation of placental micro-cavities in which a supersaturated calcium environment is maintained. This effect may result from the ability of calcium phosphate crystals to induce matrix metalloproteinases (Sun *et al*., 2002), the activity of which leads to extracellular matrix degradation followed by micro-cavity formation. The placental depositions are composed of calcium phosphate (Poggi *et al*., 2001). These depositions are arranged predominantly near the basement membrane, probably due to its ability to act as placental calcium pump (Kasznica and Petcu, 2003). Calcium and phosphorus are actively transported via the placenta throughout gestation, making the fetus relatively hypercalcemic and hyperphosphatemic (Pitkin, 1985).

Nanobacteria: A possible etiology for type III prostatitis

Of all types of chronic prostatitis in men type III has the highest incidence, accounting for 60 to 90% of those with prostatitis (Millan-Rodriguez *et al*., 2006). However, the etiology of type III prostatitis remains unclear and thus, it is difficult to treat the disease. Bacterial pathogens have not been found in prostatic tissue, urine or prostatic fluid by conventional culture but prostatic inflammation or inflammatory markers are often identified (Wood and Shoskes, 2006). This suggests the existence of yet unknown infectious pathogens and the importance of identifying these pathogens to diagnose and treat type III prostatitis. Nanobacteria, a kind of recently discovered bacteria, are thought to be associated with type III prostatitis pathogenesis (Shoskes *et al*., 2005; Zhou *et al*., 2008).

Shen *et al*. (2010) postulated that the pathogenesis of prostatic calculi involves a certain mechanism: (1) Nanobacteria form calcifications and mineral deposition cores. (2) Prostatic epithelial membrane is damaged by nanobacterial infection, causing exposure of tissue components that may form crystal cores (Anderson, 1988; Wiessner *et al*., 2001). (3) Nanobacteria mix with prostatic secretions (Ciftcioglu *et al*., 2002). (4) With urine backflow high metabolite concentrations increase topical calcium and phosphate and even cause calcium and phosphate saturation, accelerating the formation of crystals with nanobacteria or their debris as cores. No prostatic calculi were found within the first 8 weeks after nanobacteria infusion, suggesting a correlation between calculus formation and time.

Zhou *et al*. (2008) selected a total of 48 patients with chronic pelvic pain syndrome for whom conventional therapy had failed and randomly divided into two groups, one receiving anti-nanobacterial treatment and the other receiving a placebo. The nanobacteria were isolated and cultured from expressed prostatic secretions and urine samples before and after treatment. The morphologic features were recorded and 16s rRNA gene expression was determined. The curative effect was evaluated by the nanobacteria-positive rate and symptomatic changes using the National Institutes of Health Chronic Prostatitis Symptom Index. They found that after anti-nanobacterial treatment, the nanobacteria-positive rates had decreased from 62.5 to 16.7% in the expressed prostatic secretions and from 12.5 to 0% in the urine samples after prostatic massage. In the patients receiving a placebo, the positive rates had no obvious change in either the expressed prostatic secretions or the urine samples after prostatic massage. The result revealed that the 16s rRNA gene sequence from the nanobacteria in the patients with chronic pelvic pain syndrome was 97%, similar to that of the known nanobacteria with identity (97%). After anti-nanobacteria treatment, the Chronic Prostatitis Symptom Index scores decreased significantly. In contrast, no change in the Chronic Prostatitis Symptom Index scores was seen after placebo treatment. They concluded that nanobacterial infection might be an important etiologic factor of type III prostatitis. Anti-nanobacteria treatment could be an effective therapy against refractory type III prostatitis.

The role of nanobacteria in degenerative aortic valve stenosis: Atherosclerosis is an inflammatory disease caused in part by abnormal lipid metabolism within the arterial wall (Ross and Glomset, 1976). Calcification of human arterial tissue within atherosclerotic plaques is a common occurrence, increases with age and is a strong predictor of cardiovascular and all-cause mortality (Greenland *et al*., 2004). There are hypotheses that microbes contribute to pathological tissue calcifications. Recently isolated an infectious agent, nanobacteria appear to be self-replicating in culture, are nanometer-scale bacteria.

Nanobacteria, like *Bartonella* spp. and *Brucella* spp. are very small, slow-growing, Gram-negative proteobacteria (Kajander *et al*., 1997) that produce slime in order to increase their chances of survival in a colony. Nanobacteria have been demonstrated in many human degenerative tissues (Kajander and Ciftcioglu, 1998; Ciftcioglu *et al*., 2003; Khullar *et al*., 2004). Thus, nanobacteria may also play a role in the pathogenesis of sclerotic valve degeneration (Jelic *et al*., 2007). On the other hand, nanobacteria such as *Chlamydia* spp. may only have a high affinity to fatty tissue degeneration or plaque formation, without playing an active role in their pathogenesis.

Calcification of human arterial tissue within atherosclerotic plaques is a common occurrence, increases with age and is a strong predictor of cardiovascular and all-cause mortality (Blacher *et al*., 2001; Greenland *et al*., 2004). Several hypotheses regarding the pathogenesis of vascular calcification have been proposed, including (1) that crystals deposit on degrading senescent cells (matrix vesicles) (Hsu and Camacho, 1999 ; Kim and Trump, 1975) (2) that nucleate amorphous hydroxylapatite on phospholipids and proteoglycans (Kim and Trump, 1975) (3) that cellular alkaline phosphatases and/or phosphate-specific channels are stimulated resulting in critical escalation of local saturation levels (Shioi *et al*., 1995; Tintut and Demer, 2001) and (4) and that smooth muscle cells undergo bonelike differentiation (Tintut *et al*., 1998). Calcified hard plagues are now the common form of coronary heart disease but were surprisingly a clinical rarity 100 years ago (Meade, 2001). Calcified plaques can lead to acute myocardial infarct, because apatite (calcium phosphate mineral) exposed to blood activates a thrombotic cascade. Nanobacteria were the first (may still be the only ones) calcium-phosphate mineral containing particles isolated from human blood. Radioactively labeled nanobacteria were shown to accumulate in rabbit aorta and aortic valve, although their main elimination route was excretion via kidneys into urine (Akerman *et al*., 1997). This study already pointed to the potential role that nanobacteria could have in atherosclerosis, heart valve calcification and kidney stone formation. Nanobacteria were present and actively involved in the processes:(1). Nanobacteria were shown to be active nidi forming the right type of calcified mineral. Active nidus means a center of calcification that can mediate calcium-phosphate mineral formation under non-saturating calcium and phosphate concentrations. In fact, nanobacteria are so good in doing this that they can consume all free calcium and/or phosphate from their culture medium, whichever is first consumed to zero (Ciftcioglu *et al*., 1999). (2). Nanobacteria have and release endotoxin (Hjelle *et al*., 2000) and thereby stimulate chronic local inflammatory reaction in atherosclerotic plaque. (3) Nanobacteria have been shown to infect humans and infections last possibly lifelong. 4. Almost 100% of atherosclerotic patients in USA and in Finland have antinanobacteria antibodies in their serum, whereas in healthy blood donors antinanobacteria antibodies are present in about 15% (see web pages of Nanobacteria Minisymposium held at Kuopio last year). 5. Nanobacteria have been shown to be susceptible to several antibiotics and sequestering agents (Ciftcioglu *et al*., 2002).

Presence of nanobacteria in psammoma bodies of ovarian cancer: Over the last years, several studies have addressed the question of the pathogenic origin of these deposits, which still remains obscure. In a study conducted by Maki *et al*. (2000) utilizing immunohistochemistry and in-situ hybridization, the glycoprotein osteopontin was identified as a possible factor causing the development of psammoma bodies via the accumulation of calcium phosphate in serous adenocarcinomas of the ovary. Within this, biomineralization and the process of stone formation has been recently associated with the detection of 80-500 nm-sized calcium salt-precipitating organisms named nanobacteria (Ciftcioglu *et al*., 1999; Dorrell, 1999; Kajander and Ciftcioglu, 1998). Although there is much controversy concerning their archetypical origin, (Cisar *et al*., 2000) these needle shaped, Gram-negative and filterable particles have been demonstrated to form a calcium phosphatecontaining shell, thereby presenting a novel model for tissue calcification (Ciftcioglu *et al*., 1999; Kajander *et al*., 2003; Ciftcioglu *et al*., 2003). Nanobacteria are able to infect phagocytosing cells via receptor-mediated internalization (Kajander and Ciftcioglu, 1998; Kim *et al*., 2004). They have been shown to exert cytotoxic effects on fibroblasts (Kajander and Ciftcioglu, 1998) and appear to be involved in the development of kidney stones (Ciftcioglu *et al*., 1999; Dorrell, 1999; Kajander *et al*., 2001) independent of elevated urinary pH values and urease/alkaline phosphatase activity. It has thus been suggested that the biogenic apatite layer present on the cellular surface might act as a nidus promoting the process of crystallization and formation of calcified deposits (Ciftcioglu *et al*., 1999; Kajander and Ciftcioglu, 1998; Kajander *et al*., 2001). Hudelist *et al*. (2004) evaluated a possible pathogenic link between the development of psammoma bodies and nanobacteria infection. They found that a 100% concordance between the expression of nanobacteria and the presence of psammoma bodies in malignant ovarian tumors and

several lines of evidence proposed the involvement of nanobacteria in the process of biomineralization. Consequently, they concluded that nanobacterial infection of malignant ovarian tissue contributes to mechanisms leading to the formation of calcified deposits known as psammoma bodies in malignant ovarian tumors.

Relation between nanobacteria and periodontal diseases: Periodontal diseases, including gingivitis and periodontitis, have been described as inflammation of the supporting tissues of the teeth. The main cause of periodontal disease is dental plaque. If dental plaque is not eliminated of dental surface, mineralized dental plaque (calculus) occur. The mineralization process of calculus is similar to that of other ectopic calcifications, such as kidney stones and gallstones. The presence of a certain type of microorganism discovered during the last decade in various pathogenic calcification such as renal stones, atherosclerotic plaques. This microorganism is nanobacterium that has unique characteristics in different regards (Demir, 2008). The mineral combinations in oral fluids (saliva, gingival crevice fluid), oral microorganisms, and oral pH have effects on the mineralization causing dental calculus. Calculus formation is facilitated by an alkaline oral environment, which increases the precipitation of minerals from the surrounding oral fluids (saliva and gingival crevice fluid) (Mandel, 1995; Carranza, 1996). At neutral pH (7.0) saliva is supersaturated with calcium phosphate with most of the phosphate present in either the mono-or di-hydrogen phosphate form. The nucleating role of the microorganisms in the formation of dental calculus shows similarities to that of nanobacteria in calcification. What is more significant is that the presence of an alkali environment is essential for nanobacteria to cause calcification as is the case for dental calculus to occur. Kajander and Ciftcioglu (1998) were able to grow tumor size plaque in medium that had a pH of 7.6. They found that calcium carbonate could not form if the pH is under 7.4. These two significant conditions support the idea that nanobacteria may be present in the formation and in the contents of dental calculus.It was determined in the studies made on the prevalence of dental calculus, it was found to have higher prevalence in men than in women (Mandel and Gaffar, 1986; Beiswanger *et al*., 1989). Prevalence similar to that of dental calculus was determined in a study conducted on the distribution of nanobacteria. Hjelle *et al*. (2000) was able to randomly recover nanobacteria from the urine of 30% of male and 10% of female young, healthy, control subjects. It is an interesting situation that Tetracycline, which is effective on nanobacteria, has been being used in the treatment of periodontal diseases for many years.

Link between nanobacteria and HIV-infected patients: Sommer (2004) stated that nanobacteria seem to have an ideal size for effective surface covering deposition by self-assembly, driven by energetically favorable conditions and the strongly hydrophilic nature of the apatite mineral. *In vivo* the tendency to attach to tissue surfaces could be elevated by slime containing calcium and phosphate, produced by stressed nanobacteria. Massive physiological changes in the blood milieu, as for example, represented by the multitude of opportunistic infections described in HIV, (Klatt, 2003) may stimulate nanobacteria to slime production. Slime-assisted perineurial deposition of a large number of stressed nanobacteria, as presumably realized in peripheral neuropathy, could progressively cut signal transduction along the enclosed bundle of nerve fibers, primarily via metabolic isolation of the perineurium and second by destroying the elasticity of the perineurial envelope, restricting the vital capability of the perineurium to stretch and deform. Sommer (2004) established a link was between nanobacteria and HIV and the presence of nanobacteria in HIV-infected patients is indicated by the interrelation of seven experimental findings: (1) The perineurium was virtually coated with apatite in many of the diabetic patients afflicted with peripheral neuropathy (Kalimo *et al*., 1981; King *et al*., 1988). (2) Nanobacteria are protected by a porous mineral shell consisting of apatite (Ciftcioglu and Kajander, 1998). (3) Exposed to physiological or biomechanical stress, nanobacteria have been observed to produce *in vitro* a slime promoting rapid colony formation by attachment one to another and/or to surfacessa mechanism proposed to be instrumental in inducing various forms of pathogenic calcification (Sommer and Franke, 2002). (4) HIV-infected patients presented extreme conditions of pathogenic calcification (Nadler *et al*., 2003). (5) The situation of numerous HIV-infected patients is additionally burdened by peripheral neuropathysa disease frequently associated with diabetes mellitus. (6) Low-level light was found to compensate stress in cultured nanobacteria (Sommer *et al*., 2002a). (7) Peripheral neuropathy has been reported to dramatically ameliorate with low level light therapy (Sommer *et al*., 2002b).

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