Propionibacterium acnes – pathogenicity and possible role in intervertebral disc herniation

Propionibacterium acnes – chorobotwórczość i rola w przepuklinie krążka międzykręgowego

Key words: Propionibacterium acnes, lower back pain, disc degeneration disease, lumbar disc herniation

Słowa kluczowe: Propionibacterium acnes, dolegliwości bólowe lędzwiowego odciinka kręgosłupa, choroba degeneracyjna krążka międzykręgowego, przepuklina krążka międzykręgowego

PROPIONIBACTERIUM ACNES – CHARACTERISTICS OF THE SPECIES

Propionibacterium acnes is an anaerobic Gram-positive rod-shaped bacterium. It is slow-growing and belongs to skin microbiome, but is also the pathogenic factor of acne vulgaris. Its older names include Bacillus acnes and Corynebacterium acnes. Within the species P. acnes, around 100 strains have been described; the genome of many has been completely sequenced. A single bacterium is 0.4–0.5 μm wide and 0.8–0.9 μm long [1]. Under light microscope it can most often be seen in groups of two or bigger chains. It has no flagella, thus no capability of active movement and it does not form spores [2]. Both in vitro and in vivo it forms biofilm, in anaerobic conditions it can survive 8 month. The fact that the bacteria can be found in macrophages of alveoli, proves that it persists within phagocytes [3].
The bacterium lives mainly in the sebaceous glands of the hair follicle, but its presence has also been reported in gastro-intestinal tract. Pathogenic strains cause release of cytokines from sebocytes and inflammation [4]. Study of various polysaccharide chains in the cell wall have led to subdivision of P. acnes species into the following groups: IA, IB, IC, II and III. Group IA is responsible for acne vulgaris, other groups are isolated from foci of opportunistic inflammation, e.g. postoperative infection of hip joint [5].

On solid culture media, incubated 4-5 days anaerobically it forms round, elevated colonies, 1.5 to 5 mm in diameter. Initially white, in time they turn light pink. Propionibacterium acnes is aerotolerant anaerobic bacteria, it survives in oxygen but its growth is retarded. Its optimal growing temperature is 37°C, in room temperature the growth slows down, at 45°C – stops altogether. The bacterium prefers neutral pH, but even with optimal conditions the growth proceeds at a slow pace. It uses organic compounds (sugars, fibers, pectins) to produce energy in the process of fermentation, the products being, among others: propionic acid, acetic acid and carbon dioxide. Propionibacterium genome sequencing, completed in 2004 revealed that the genetic material of the bacterium consists of 2560 base pairs, with high percentage of guanine and cytosine [6].

The presence of some enzymes, that break down certain substrates is used for the identification of P. acnes in biochemical test. The enzymes are catalase, nitrite reductase (NADH), tryptophanase. Some of the strains are capable of beta-hemolysis [7]. Propionibacterium has considerable demands for medium nutrients. Most frequently used are: bovine broth, casein peptones, yeast extract, KH₂PO₄, cysteine, hemin, some vitamins, glucose and sodium thioglycolate.

PATHOGENICITY

Propionibacterium acnes is associated with the pathogenesis of acne vulgaris. The bacteria proliferate in comedones, where the conditions are moderately anaerobic. Using lipase they break down components of sebum to produce energy. Additionally, inflammatory factors (IL-1β, IL-8, IL-12, TNF-α) are produced and stimulate chemotaxis of leukocytes, that die in the tissue forming pustules [8].

Moreover, studies have shown that P. acnes can be found in granulomas in sarcoidosis, keratitis, discitis, endocarditis, osteomyelitis and endophthalmitis [9]. In cases of sarcoidosis, where the bacteria have been identified, remission has been observed after minocycline treatment (broad-spectrum tetracycline antibiotic widely used for acne [10]). However, there are two hypothesis explaining the efficiency of this therapy – antimicrobial and immunoregulative action of minocycline is emphasized. P. acnes is also associated with SAPHO (Synovitis, Acne, Pustulosis, Hyperostosis, Osteitis) syndrome, where immunological complexes antibody-P. acnes antigen are suspected to deposit in bone and joint [11]. After Staphylococci P. acnes is the most frequent cause of infection of cerebrospinal liquid shunts (9% of all cases), where they form biofilm. It is recommended to obtain material for anaerobic culture from the infected shunt [12]. The bacterium is also reported to cause cerebral abscesses as a late postoperative complication of neurosurgery.
Propionibacterium acnes – pathogenicity and possible role in intervertebral disc herniation

P. acnes has been isolated from the canal of tooth root, dental pulp and periodontium [13]. Cases of endocarditis caused by P. acnes are rare and most often occur around foreign material (artificial valve, annuloplasty rings, pacemaker electrodes). In 79% of endocarditis cases analyzed by Sohail and Gray, prosthetic material was the source of the infection [14]. Bacterial inflammation of the eye ball, a very serious complication after a lens replacement operation in patients with cataract, are almost always caused by Gram-positive bacteria (91%); P. acnes is sometimes the responsible pathogen, as it is for corneal ulcerations and conjunctivitis. When diagnosing inflammations of hip joint endoprothesis, it is recommended to use ultrasound to break up the biofilm from the infected prosthesis, than to incubate the samples for time long enough for the slow-growing bacterial species to form colonies. As is presented below, P. acnes is the most frequently identified anaerobic agent in spondylodiscitis, postoperative or after an invasive procedure, i.e. epidural anesthesia.

ANTIBIOTIC TREATMENT

Usually Propionibacterium acnes is susceptible to a large number of antibiotics, and its infections are efficiently cured with simple therapeutic schemes. The bacterium is resistant to metronidazole and partly to aminoglycosides. In recent years, a decrease in efficiency of clindamycin, erythromycin and minocycline is being observed due to their excessive use in acne [15].

LUMBAR DISC HERNIATION – SHORT CHARACTERISTICS

Low back pain (LBP) is familiar to almost every adult [16]. Lumbar disc herniation (LDH) is a condition affecting the spine as consequence of trauma, injury or without evident cause (idiopathic). Tears in the outer layer (annulus fibrosus) lead to bulging out of the soft, central part of the disc (nucleus pulposus). In the ventral part of the spine annulus fibrosus is reinforced by the ligamentum longitudinale anterior, in the dorsal part, from the inside of the spinal canal – by the ligamentum longitudinale posterior. Consequently, the posterior-lateral portion of the ring is the locus minoris resistantiae, where herniation most commonly occurs.

Intervertebral disc is a bradytrophic tissue – it is not supplied by capillary vessels, but through diffusion. Nucleus pulposus is a mix of water and aggrecan-proteoglycan gel, suspended on a matrix made of type II collagen and elastin fibers. Annulus fibrosus is composed of 15-25 laminae made of type I collagen [17]. If the nucleus (comprising in 80% of water) loses its elasticity (so-called ‘black disc disease’) its function as shock-absorber decreases. The causes are: genetic predisposition, asymmetric load, weak paravertebral muscles, pregnancy. Modern lifestyle – little activity, sitting work, postural deformities, addictions – have a share in the occurrence of the condition. Possibly, childhood infections, when the intervertebral disc are supplied by capillaries, play a part in the degeneration [18]. The sequence leading to the disc degeneration: until the disc is supplied by circulation (age of 20), daily activity causes transitory dehydration, during sleep it is replenished. At 30-40
years, dehydration proceeds irreversibly, the disc loses elasticity, tears occur in the *annulus*. The final stage is stabilization through ossification, thickening of the discs and decreased concentration of inflammatory proteins [19]. Thus, 30-40 years-old patients are more likely to suffer from LBP, than 60-year-olds. Disc degeneration detected in diagnostic imaging does not have to be painful. MRI based studies showed that 30% of young people with bulging disc had no clinical symptoms [20].

Disc herniation (*prolapsus disci intervertebralis*) advances gradually, previous stage being *protrusion* of the nuclear masses into the fissures in the ring. Finally, degenerated disc becomes fibrous and scarred, intervertebral space becomes narrower, osteophytes grow at the edges of the vertebrae and the whole motor unit becomes stiff [21]. The direct cause of pain is the compression of the spinal cord, spinal nerves, release of inflammatory factors and acidic metabolites of the nucleus. The disc itself is not enervated, unlike posterior longitudinal ligament, periosteum, facet joints (*ramus meningaeus nervi spinalis*) [22].

Symptoms differ depending on localization and affected tissues – from symptomless through severe pain radiating into the regions enervated by the compressed nerves to Cauda Equina Syndrome, requiring quick surgical intervention. Other complaints are: diffuse pain in thighs, knees, feet, paresthesia, tingling, tickling, burning, numbness, increased muscle tonus, dystonia. The onset is usually rapid, connected with an abrupt movement of bending or rotating of the spine. It can also be caused by static load, such as prolonged sitting. Professional drivers can suffer from LBP, due to vibrations of the car (4-5 Hz) [23]. The pain is especially intense, when patient is sitting or at the beginning of walking, increases at coughing, sneezing and any increase of abdominal pressure. In examination, reflexes are below normal and stretch tests for nerve roots L5-S1 (Lasegue’s, Bragard’s Fajersztajn-Krzemicki’s sign) or L4 (Mackiewicz’s sign) are positive [24]. In the last one (also referred to as ‘reverse Lasegue’) knee flexion is performed, patient lying face down. Pain in the knee or front part of the thigh suggests pathology in the femoral nerve. Pain of the lumbar spine at pressing the chin to the chest is known as Negrie’s sign.

Disc herniation it the cause in 90% of sciatica [25]. It occurs when compression affects L3-L5 lumbar or S1-S3 sacral nerve roots or the sciatic nerve. Lumbar pain radiates to the buttock and lower limb impeding walking. In such cases we speak of *claudicatio caudae equinae*, if pain occurs after a short distance (similarly to vascular *claudicatio intermittens* [26]). Particularly severe, central disc herniation is also the most common cause of the Cauda Equina Syndrome. Cauda Equina is bundle of nerve roots L2 to coccygeal nerve, that hangs in the spinal canal. Segments S2-S4 of the spinal cord form pudendal nerve, which is a part of sacral plexus. This mixed nerve carries motor, sensory, parasympathetic and sympathetic fibers; when it is compressed erectile dysfunction, urinary retention and incontinence, bowel dysfunction result.

The first diagnostic tool in degenerative disc disease should be lumbar spine x-ray. It can rule spondylololisthesis and bone neoplasm out. More precise information about bone structures is delivered by computed tomography (CT), and magnetic resonance imaging (MRI) is best for nervous tissue.
Reports about postoperative spondylodiscitis due to Propionibacterium acnes were published over 30 years ago. In 1983, Burki and al. described a case of L4-L5 disc degeneration (confirmed in x-ray, CT and increased radioisotope uptake in scintigraphy) in a 49-year-old female patient treated with epidural corticosteroid infiltrations for sciatica. Microbiologic culture and serologic antigen identification revealed bacterial origin of the inflammation. Identified pathogens were Peptococcus Constellatus and Propionibacterium acnes [27]. In 1987 Noble and Overman presented a case of a male patient who developed similar symptoms 4 weeks after discectomy. The authors present a literature review and summarize P. acnes related bone infections. Nobel and Overman distinguish four categories: infection after a surgery or an invasive procedure, infection in an immunodeficient patient, infection in a patient without obvious predisposition and infection where P. acnes as causative agent is uncertain. The first category predominates [28].

A controversy was provoked by the study Association between sciatica and Propionibacter acnes, by Stirling and al. published in 2001 in The Lancet. It concerned 36 patients, with no history of an infection in the previous 6 months, who had discectomy. In 19 out of 36 (52.7%) patients the obtained disc samples gave positive microbiological cultures (Propionibacterium acnes – x16, coagulase-negative Staphylococcus - x 2, Corynebacterium propinquum - x1). None of the patients in the control group (operated for other spine conditions: scoliosis, trauma, myeloma) were tested positive (0 out of 14 patients – 0%). Additionally, the authors used ELISA to detect lipid S (Gram-positive bacteria cell wall component) in the serum of the patients. Both methods put together gave the following results:

- positive culture + positive serology - 7/36 (19,4%) patients
- positive culture + negative serology - 12/36 (33,3%) patients
- negative culture + positive serology - 1/36 (2,8%) patients
- negative culture + negative serology - 16/36 (44,4%) patients

Neither living nor dead bacteria could be found in Gram-staining of intervertebral disc samples and microscopic examination [29].

Another article in favor of the role of P. acnes in the inflammation around herniated disc, published in 2013, reports the results of the study of a Danish-English research group (Rollason, McDowell, Albert and al.). Under strict aseptic regime, 5 disc samples were harvested from 64 patients who underwent discectomy. After incubation in anaerobic conditions, P. acnes was detected in 24 patients (37,5%). Using polymerase chain reaction (PCR), recA gene (P. acnes housekeeping gene, necessary for vital functions of the bacterium, continuously expressed) was sequenced. Additionally, scientist used immunofluorescent microscopy and mouse monoclonal antibodies QUBPa1 and QUBPa2, that attach to strains IA, II and partly IC, but do not adhere to IB and III. Both techniques allowed to determine the phylogenetic groups, that the isolated P. acnes populations belonged to. Two conclusions drawn by Rollason and al. speak against contamination as the source of the bacteria.
Firstly, in 16 patients *P. acnes* was identified in at least 2 samples of the disc (according to the Infectious Diseases Society of America (IDSA) criteria, it proves the infection [30]). Secondly, the populations had the following distribution: II (52%), IA (28%), III (11%), IB (9%), IC (0%), so the phylogroups rarely isolated from acne and skin, and frequently found in blood, soft tissues and prosthetic material (IB, II, III) were widely represented. The authors remark that *P. acnes* could enter the bloodstream during teeth brushing or a dental procedure. Neovascularization taking place around the herniated disc allows the colonization of that region [31].

Another research group obtained positive culture in 48.2% (40 among 83 patients operated from the anterior retroperitoneal approach, lumbar disc replacement). However, the grown bacteria were more varied (18 *P. acnes*, 16 coagulase-negative *Staphylococcus*, 3x *Staphylococcus aureus*, 3x Gram-negative bacilli, 3x *Micrococcus*, 3x *Corynebacterium*, 1x *Microbacterium*, 1x *Brevibacterium*, 1x *Rothia dentocariosum*, 1x *Enterococcus faecalis*, 1x *Streptococcus intermedius*). Patients with previous spine interventions were not excluded from the study – more than half of them had had a discography or discectomy [32].

<table>
<thead>
<tr>
<th>Authors</th>
<th>Journal and publication date</th>
<th>Number of patients</th>
<th>Number of positive microbiological cultures</th>
<th>Number of <em>P. acnes</em> cultures</th>
<th>Incubation time</th>
<th>Previous spine interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stirling A, Worthington T and al.</td>
<td>The Lancet, June 2001</td>
<td>36</td>
<td>19/36 (52.7%)</td>
<td>16/36 (44.4%)</td>
<td>21 days</td>
<td>16/36 (44%) epidural injections</td>
</tr>
<tr>
<td>Albert HB, Lambert P and al.</td>
<td>European Spine Journal, April 2013</td>
<td>61</td>
<td>28/61 (45.9%)</td>
<td>24/61 (39.3%)</td>
<td>7 days</td>
<td>none</td>
</tr>
<tr>
<td>Rollason J, McDowell A and al.</td>
<td>BioMed Research International, August 2013</td>
<td>64</td>
<td>27/64 (42.2%)</td>
<td>24/64 (37.5%)</td>
<td>7 days</td>
<td>none</td>
</tr>
<tr>
<td>Arndt J, Charles YP and al.</td>
<td>Journal of Spinal Disorders &amp; Techniques, October 2012</td>
<td>83</td>
<td>40/83 (48.2%)</td>
<td>18/83 (21.7%)</td>
<td>10 days</td>
<td>49/83 (59%) discography 24/83 (28.9%) discectomy</td>
</tr>
<tr>
<td>Agarwal V, Golish SR and al. [33]</td>
<td>Journal of Spinal Disorders &amp; Techniques, August 2011</td>
<td>52</td>
<td>10/52 (19.2%)</td>
<td>7/52 (13.5%)</td>
<td>5 days</td>
<td>17/52 (32.7%) epidural injections</td>
</tr>
<tr>
<td>Carricajo A, Nuti C and al</td>
<td>Journal of Hospital Infection, July 2007</td>
<td>54</td>
<td>4/54 (7.4%)</td>
<td>2/54 (3.7%)</td>
<td>20 days</td>
<td>none</td>
</tr>
</tbody>
</table>
Other scientists try to find a connection between \textit{P. acnes} and edema in the endplates of the vertebrae adjacent to the degenerated disc – so called Modic changes. They were first described in 1988 by Michael T. Modic; they can be observed in T1 and T2-weighted MRI (hypo- and hyperdense foci respectively) and they correlate with LBP [34,35]. Trials of antibiotic treatments were conducted (100-day therapy amoxycillin-clavulanic acid (500 mg/125 mg) every 8 hours) [36].

A very important contribution in the discussion about the causative role of low-virulence bacteria in LBP, are studies which show the high probability of contamination of the tissue samples during the surgery. French scientific group from CHU Bellevue Saint-Etienne clinical hospital, led by Anne Carricajo, designed their experiment similarly to the studies mentioned before, but additionally used air monitoring equipment and harvested tissue samples from ligamentum flavum and back muscles (\textit{Musculus erector spinae}). The study group was 54 patients undergoing lumbar discectomy, with no previous epidural or spinal surgeries. The results were as follows:

- 2 out of 54 (3.7%) patients – positive \textit{P. acnes} cultures from disc tissue
- 4 out of 54 patients (7.4%) – positive microbiological cultures from disc tissue (\textit{P. acnes} x 2, Anaerobic Streptococci x 1, \textit{Actinomyces sp.} + Coagulase-negative Staphylococci x 1).
- 12 out of 54 (22.2%) patients - \textit{Ligamentum flavum} and muscle tissue samples positive cultures (\textit{P. acnes}; importantly, all patients with positive disc culture had positive muscle/ligament tissue samples, as well)
- 4 out of 54 (7.4%) patients - laminar flow controls positive cultures (3x \textit{P. acnes}, 1x Anaerobic Streptococci)

The authors point out, that two facts speak against the inflectional hypothesis: absence of inflammation markers (CRP, leukocytes) and absence of conditions predisposing to low-virulence bacterial infection (compromised immunity or foreign material, e.g. \textit{fixateur externe})[37].

\textbf{CONCLUSIONS}

- \textit{Propionibacterium acnes} is a commensal bacterium, also identified in various pathologic conditions, although contamination or coincidental presence (so-called innocent bystander) cannot be dismissed.
- Although \textit{P. acnes} is usually susceptible to the majority of standard antibiotics, growing resistance is being observed.
- The analyzed literature reports positive \textit{P. acnes} cultures in 3.7% to 44.4% of examined disc tissue samples.
- Causative role of \textit{P. acnes} in lower back pain (LBP) and Modic changes has been put forward.
REFERENCES


ABSTRACT

The article is a literature review, summarizing up-to-date information about the Gram-positive bacillus Propionibacterium acnes and its pathogenicity. We present the results of several studies on the role of P. acnes in the degenerative disc disease related pain. Connection between microbial agents and lumbar disc herniation, as well as type I Modic changes (vertebral end-plate edema visualized in MRI) has resulted in trials of experimental implementation of antibiotic treatment in those patients. We present the standpoint of the advocates of infection hypothesis as well as the suggestions that the positive bacterial cultures are the result of a contamination.

STRESZCZENIE

Praca przeglądowa, zbierająca aktualne informacje o Gram-dodatniej laseczce Propionibacterium acnes i jej chorobotwórczości. Przedstawiony zostaje aktualny stan badań dotyczących udziału P. acnes w wywoływaniu dolegliwości bólowych w dyskopatii lędźwiowej. Wiązanie dyskopatii lędźwiowej, zmian Modic’a typu I (obręczki blaszki granicznej kręgu widocznego w MRI) i bólu lędźwiowego odcinka kręgosłupa (ang. low back pain) z drobnoustrojami, stało się podstawą do prób eksperymentalnego wdrożenia terapii antybiotykowej u tych pacjentów. Przedstawiamy
stanowisko autorów skłaniających się ku hipotezie infekcyjnej i prace mające wykazać, że jest to wynik zanieczyszczenia.

Artykuł zawiera 27156 znaków ze spacjami