Chlamydia pneumoniae and cardiovascular disease : could we treat cardiovascular disease with antibiotics ?

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Abstrak

Pada saat ini peranan Chlamydia pneumoniae dalam aterosklerosis masih kontroversial dan diabaikan oleh kebanyakan peneliti yang mempelajari patogenesis penyakit tersebut. Meskipun demikian sudah banyak penelitian seroepidemiologik dan kasus kelola yang membuktikan adanya hubungan antara infeksi C. pneumoniae dengan penyakit jantung koroner (PJK). Sebagian besar penelitian tersebut melaporkan odd-ratio paling kecil sebesar 2 dan beberapa penelitian menunjukkan odd-ratio yang makin bertambah tinggi sesuai dengan peningkatan kadar titer antibodi. C. pneumoniae juga sudah ditemukan didalam plak ateroma diberbagai negara baik dengan cara mikroskop elektron, PCR maupun "immunocytochemistry". Organisme tersebut dapat deteksi dalam lesi ateroma kurang lebih 10 kali lebih sering daripada dalam sampel kontrol dari jaringan arterial. Sampai sekarang hubungan sebab akibat antara C. pneumoniae dengan aterosklerosis masih belum jelas. Namun demikian terdapat beberapa mekanisme yang diduga dapat bekerja secara akut (yaitu menimbulkan instabilitas plak) maupun secara kronik (yaitu merangsang pertumbuhan plak ateroma). Beberapa studi pendahuluan melaporkan bahwa terapi dengan antibiotik golongan makrolid jangka pendek dapat menurunkan kejadian koroner rekuren pada penderita infark miokard akut dan angina pektoris tidak stabil yang disertai dengan kenaikan titer antibodi terhadap C. pneumoniae. Meskipun tampak menjanjikan, hasil tersebut diperoleh dari penelitian pilot yang berskala kecil, sehingga masih diperlukan penelitian berskala besar yang bersifat acak, tersamar ganda dan dikontrol dengan placebo untuk dapat menegakkan peranan yang lebih jelas dari terapi eradikasi dengan antibiotik dalam upaya pencegahan sekunder pada penderita sindroma koroner akut.

Abstract

Although many seroepidemiological and case-controlled studies have shown an association of Chlamydia pneumoniae infection and coronary heart disease (CHD), the role of this organism in the pathogenesis of atheroslerosis remains controversial. Most of these studies found odd-ratios of 2 or more, and some reported increasing ratios with increasing antibody titers. C. pneumoniae has also been detected in atherosclerotic plaque either by electron microscopic study, PCR or immunocytochemistry methods. This organism was detected in atheromatous lesions about ten times more frequently than in control samples of arterial tissue. Until now, the causal relationship between C. pneumoniae and atherosclerosis remained unresolved. However, various potential causative mechanisms that may act either acutely (e.g precipitating plaque rupture) or chronically (e.g promoting plaque growth) have been proposed for the reported association between infection and CHD. Preliminary reports showed that short courses of macrolide antibiotic therapy can reduce recurrent coronary events in patients with recent myocardial infarction or unstable angina pectoris and elevated anti C. pneumoniae anti body titers. Although the findings were encouraging, they came from small pilot studies. Large randomized, double-blind, placebo-controlled studies are needed to establish the value of antibiotic eradication therapy in patients with acute coronary syndromes.

Keywords : C. pneumoniae, atherosclerosis, antibiotic, lipid.

Chlamydia pneumoniae (C. pneumoniae) is a human respiratory pathogen that causes acute respiratory tract infections and 10 to 21% of the community acquired pneumoniae. The infections are geographically widespread. Antibody prevalence studies have shown that everyone is infected with the *C. pneumoniae* organism at one time in their life and that reinfection is common. In addition to respiratory disease, seroepidemiologic studies have shown an association of this organism with cardiovascular disease.[1]

In the 1940s. it was found that *Chlamydia trachomatis*, one of the species of Chlamydia that caused lymphogranuloma venereum (LGV), could occlude arteries in addition to occluding lymphatic. However, after the introduction of sulfonamides to treat the LGV cases, these findings were not explored any further.[2] In 1988, Leinonen conducted a study of acute myocardial infarction (AMI) cases. It was found that 70% of the AMI patients had a positive sera conversion to a bacterial mutant lipopolysaccharide (LPS) of *C. pneumoniae*. The LPS was known to cross-react with

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several chlamydial lipopolysaccharides. The sera were also tested for *C. pneumoniae* with several immunofluorescence method. It was also demonstrated that AMI patients had an elevated stable titers to *C. pneumoniae*. In coronary heart disease (CHD) cases, Leinonen et al, also found that patients have elevated IgG and IgA titers to *C. Pneumoniae*.[3]

The study concluded that AMI or unstable angina possibly could be associated with exacerbation of chlamydial infection at the origin of plaque rupture. As for CHD, the cases might be associated with chronic chlamydial infection.

EPIDEMIOLOGY

The genus chlamydia contains three species : *C. psittaci, C. trachomatis* and *C. pneumoniae*. The third chlamydial species, *C. pneumoniae,* can be grown in a variety of cell cultures, eventhough it is considerably more difficult than the other chlamydial species. Knowledge of the epidemiology of *C. pneumoniae* infections has been derived primarily from serologic studies. *C. pneumoniae* is considered to be one of the most prevalent infectious agent worldwide.

Infections begin to occur in late childhood and adolescence, and continue throughout adult life. Prevalence that has been tested in many populations throughout the world, increases with age. The prevalence was 10% in children and more than 50% in adults. Prevalence in persons older than 40 years is 60-70% in tropical areas. A percentage of up to 85% have been mentioned in Israel. Secondary episodes (reinfections) appear to occur in older adults throughout life. Chlamydial infection is also found to be responsible for up to 21% of the community acquired pneumoniae.[4] In Scandinavia, *C. pneumoniae* produces epidemics of pneumonia and respiratory illness followed by periods of infrequent infection. Transmission appears to be from person to person probably primarily in schools and family units.

The *C. pneumoniae* infection is 25% more numerous in men than in women probably due to smoking habits. It is also more frequent in elderly, in AID patients and in patients with combined infections.

Furthermore, several epidemiologic studies have demonstrated an association between serologic evidence of *C. pnemoniae* infection and coronary atherosclerotic disease.

Diagnosis of *C. pneumoniae* infection

Previous studies have shown that *C. pneumoniae* was very difficult to isolate by tissue culture technique. Antigen detection, using enzyme immunoassay (EIA) and direct immunofluorescence (DIF) are rarely used in scientific studies. The EIA and DIF techniques generally have low detection rates and they are not very specific either. To date the investigations have mostly relied on serologic diagnosis. Serologic diagnosis of *C. pneumoniae* largely depends on microimmunofluorescence (MIF) and complement fixation (CF) tests.[5] For the MIF test, Grayston and associates have proposed criteria for serologic diagnosis of *C. pneumoniae* infection.[6] For acute infection, the patient should have a four-fold increase in the IgG titer or a single IgM titer ≥ 1/16 or a single IgG titer ≥ 1/512. Past or pre-existing infection is defined as an IgG titer ≥ 1/16 and ≤ 1/512. Grayson further proposed that the pattern of antibody response in primary infection may differ from that seen in reinfection. In initial infection, the IgM response at 6 to 8 weeks. In reinfection, the IgM response may be absent and the IgG response occurs earlier, usually within 1 to 2 weeks.

For the CF test, Grayson et. al, have also suggested that a four-fold titer rise or a titer ≥ 1/64 could be considered as diagnostic for *C. pneumoniae* infection. Early studies suggested that CF test was more likely to be positive in the initial infection rather than during reinfection. To improve the efficacy of laboratory diagnosis of *C. pneumoniae* it has been developed other non-culture method, such as polymerase chain reaction (PCR). In 1992, Campbell et al, described a PCR assay using a genomic *C. pneumoniae* specific primers.[7]

They found good correlation with the culture method. Gaydos and her colleaques developed an assay using primers from 16 S rRNA gene with the amplication products being detected by EIA.[8] They have compared PCR-EIA to culture. The PCR specificity and sensitivity are 98% and 75%, respectively.[8] However PCR method is much more expensive than serology and antigen detection tests, and needs special laboratory and expertise.

Other marker for detecting *C. pneumoniae* infection is the presence of immune complex (IC) which contain chlamydial LPS and the respective antibodies. The discovery of IC containing antibodies to chlamydial proteins suggested that an ongoing chlamydial infection could result in the direct transport of chlamydial proteins into the blood circulation. So, IC has been

considered as a better marker for an intravascular chronic infection than IgG antibodies.[2]

Seroepidemiological studies

A study by Saikker (1988) showed that men under 50 years of age with elevated IgG or IgA had elevated odds ratios for AMI and chronic CHD.[9] Another extensive, prospective investigation was conducted by the Helsinki Heart Study, in 1992. The study demonstrated that, there were increased risks of the first cardiac incidents associated with elevated *C. pneumoniae* antibodies, in middle-aged men.[10]

Other research groups in various countries have recently reported similar findings. The association of elevated *C. pneumoniae* antibodies with CHD has been verified in Seattle and Alaska (USA) Sweden, UK, Germany and the Netherlands (table 1). However there were few negative reports, where control groups had high antibody prevalence due to old age or an ongoing epidemic, which could not confirm this serological association.[2]

Table 1. *C. pneumoniae* in atherosclerosis : summary of epidemiological studies

Country	Study (marker)	Odds ratio*
Finland	Saikku et al, 1988 (IgG, IgA)	2.8, 6.5
	Leinonen et al, 1990 (IC)	8.8
	Saikku et al, 1992 (IgA, IC)	2.7, 2.1
	Linnanmaki et al, 1993 (IC)	3.9
USA	Thom et al, 1991 (IgG)	1.9
	Thom et al, 1992 (IgG)	2.2
	Melnick et al, 1993 (IgG)	1.8
	Davidson et al, 1995 (IgG)	5.3
Sweden	Haidl et al, 1992 (IgG)	Not given
	Dahlen et al, 1995 (IgG)	3.8
Germany	Leinonen et al, 1994 (IC)	3.9
	Gieffers & Maass (IgG, IgA)	1.8, 1.8
UK	Mendall et al, 1995 (IgG, IgA)	2.1, 11.1
	Petel et al, 1995 (IgG)	3.1
	Cook et al, 1995 (IgG)	2.2
The Netherlands	Ossewaarde et al, 1995 (IgG)	4.0

(Adapted from Saikku P. *Chlamydia pneumoniae* and atherosclerosis-an update. Scand J Infect Dis 1997 ; (S) 104 : 53-6).

Presence of *C. pneumoniae* in lesions

The presence of chlamydial protein containing immune complexes in the circulation, indicated the presence of chlamydial infection in the immediate vicinity of the vascular interior.

Shor et al, in Johannesburg, found the pear-shaped, double walled vesicles in coronary arterial fatty streaks and atheromatous plaques, seen by electron micrographs to be *C. pneumoniae* elementary bodies.[11] The finding were later confirmed by immunocytochemistry and PCR.

The presence of *C. pneumoniae* particles in various athrosclerotic lesions has been verified in Caucasians in South Africa, USA, Italy and Finland.*C. pneumoniae* has also been found in athrosclerotic lesions of Negroes, Japanese, Indians, Alaskan Indians and Eskimos. The findings of *C. pneumoniae* seems to be a constant factor associated with atherosclerotic lesions worldwide.[2]   In 13 published studies of *C. pneumoniae* in human pathology samples, evidence of presence in arterial tissue was defined as presence of chlamydial DNA, antigens, or elementary bodies. Overall local infection was judged to be present in 52% of atheromatous lesions but in only 5% of control samples of arterial tissue, yielding a weighted odds ratio of about 10.[12]

Proposed Pathogenesis

Chlamydia is known to cause localized infections of long duration leading to grave complications years later. *In vitro* studies have shown that *C. pneumoniae* is able to infect macrophages, as well as endothelial and smooth-muscle cells. These are the cells in which the organisms are found in diseased areas of vessel walls in atherosclerosis. Another hypothesis of the organism causing atherogenesis and plaque instability is, when macrophages become infected with *C. pneumoniae*, the immune cells carry the pathogens from the respiratory system into the systemic circulation. Macrophages transfer the bacteria into the arterial cell walls. The infected endothelial cells would attract more macrophages and set a vicious cycle of inflammation.

The result is large fibrous lesions or plaques, that narrow the blood vessel and rupture of the plaques could then cause blood clots and AMI.[13]

C. pneumoniae posseses LPS with strong acidic ketodeoxy-octonate immunoreactive residue. which may react with low-density lipoprotein (LDL)-cholesterol particles.[10] The LDL particles could then be oxidized. It was also found that *C. pneumoniae* could multiply in cells of the arterial walls. The multiplication is also associated with proteolytic activity which could cause endothelial alteration or destruc-

tion. *C. pneumoniae* infection in the vessel wall may also actively induce the release of immune activation cytokine as well as thrombogenesis leading to atherogenesis.[14] More recent investigation revealed that chronic *C. pneumoniae* infection seems to be associated with a serum lipid profile considered to increase the risk of atherosclerosis. The serum triglyceride and total cholesterol were higher and the HDL cholesterol were lower significantly in the subjects with a chronic *C pneumoniae* infection than in the subjects with no antibodies.[15] This finding supports the hypothesis that infections play a role in the pathogenesis of atherosclerosis.

C. Pneumoniae and cardiovascular disease

There were several studies which investigate the association between *C. pneumoniae* and cardiovascular diseases, such as hypertension, unstable angina, AMI and stroke or transient ischemic attack (TIA). A study done by Cook et al, in Birmingham, UK found that *C. pneumoniae* infection was associated with hypertension. It was demonstrated that 32.8% of patients with hypertension had chronic chlamydial infection, compared to only 16.8% of the controls (P = 0.001). The presence of infection and hypertension may contribute to atherosclerotic vascular complications in hypertensive patients.[16]

Gurfinkel et al, in 1996 showed that patients with unstable angina had significantly higher *C. pneumoniae* antibody and C- reactive protein (CPR) titers compared to healthy volunteers.[17] Other study, conducted by Cook et al, also showed a positive association between acute or chronic *C. pneumoniae* infection, and unstable angina and AMI. The genetic or environmental determinants of the immune response to *C. pneumoniae* infection, may in part contribute to the ethnic differences in cardiovascular disease patterns.[16]

Gupta et al, in 1977, has done a study in St George's Hospital Medical School, London. It was demonstrated that a significant number of post MI patients are *C. pneumoniae* seropositive, and the antibody titers remain persistently elevated over time.[18] For cerebrovascular disease, Wimmer et al. and Cook et al. presented data that gave support to the association of CVD with previous *C. pneumoniae* infection and the association of acute stroke and TIAs with acute recrudescence of infection.[19,20]

Intervention Studies

Because of the accumulated evidences that support the involvement of *C. pneumoniae* in atherogenesis and plaque instability, several studies have tested the hypothesis that treatment of patient with acute coronary syndrome with an antibiotic would reduce plaque instability and associated clinical events. Saikku et al, studied the effect of doxycycline 100 mg once daily for 4 months versus placebo in CHD patients but found no effect of the treatment on markers of *C pneumoniae* and hemostatic parameters.[21]

Another study was conducted by Gurfinkel et al, from Favoloro Foundation and Durand Hospital in Buenos Aires, Argentina.[22] It was a double-blind, randomized. prospective, multicenter, parallel-group, placebo-controlled study. A total of 202 patients, from eight coronary-care units in Argentina, were recruited into the study.

Patients with unstable angina or acute non Q-wave myocardial infarction were randomized to receive either roxithromycin 150 mg twice daily or placebo twice daily for 30 days, in addition to conventional therapy (aspirin, heparin, nitrate, betablocker or calcium channel blockers whenever appropriate).

The study demonstrated a statistically significant reduction in cardiac events in the roxithromycin group compared to the placebo group (p=0.032).

The rates of severe recurrent ischaemic, myocardial infarction and ischaemic death were reduced from 5.4%, 2.2% and 2.2% in the placebo group to 1.1%, 0% and 0% in the roxithromycin group respectivelly. No significant drug-releted adverse effects were observed. These preliminary result suggested that treatment with roxithromycin can reduce adverse cardiac events. They also supported the hypothesis of a new mechanism for plaque instability. There are at least two possible explanations for the findings of the study. First, roxithromycin through its anti-chlamydial activity, supress the reactivation of chronic infection within the atherosclerotic plaque. Secondly, roxithromycin also has an anti-inflammatory activity, which may attenuate the persistent inflammation in the plaque leading to a more stable state.[22]

However, there is dilema of giving antibiotic since antibody positive of *C. pneumoniae* is not always connected with the existence of bacteria and antibiotic long-term can only be given if the presence bacteria is proven (isolated). The most difficult question is the

length of antibiotic treatment of *C. pneumoniae* for secondary prevention study.[23] Knowledge of the biology of chronic chlamydia infection suggests that short periods treatment will be inadequate for lasting benefits, while long-term antibiotic therapy could contribute to the development of resistance in other organisms.

CONCLUSIONS

Studies on the role of *C. pneumoniae* as an active part in athrosclerosis are of the most importance. Various potential mechanisms that may act either acutely (precipitating plaque rupture) or chronically (promoting plaque growth), have been proposed for the reported associations between the chlamydial infection and cardiovascular disease.

The antibiotics, especially macrolides with antichlamydial activity such as roxithromycin, could play a role in the eradication of this organism.

The study conducted by Dr. Gurfinkel, was the first prospective study, that focused on the anti-chlamydial and anti-inflammatory effect of roxithromycin. Perhaps, it was not only the eradication effect of the organism that was responsible for the positive thrombotic effects. Other properties of the antibiotic, such as anti-inflammatory, anti-oxidant, anti thrombotic effect and broad actions against other infectious organisms, were also the cause of those effects.[13] However, further large-scale trials are required to confirm these findings, and more researchs are needed to investigate the detail of the mechanisms involved.

Acknowledgments

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