

Clarithromycin Treatment of Bacteremia Produced by Mycoplasmas in Patients with Antiphospholipid Syndrome or Systemic Lupus Erythematosus

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Abstract

Microorganisms have been associated with the induction of autoimmune diseases. Mycoplasmas are able to produce chronic diseases and autoimmune response, there are previous reports of the presence of mycoplasmas in blood of patients with Antiphospholipid syndrome (APS) so the main purpose of this study was to treat bacteremia produced by mycoplasmas in patients with antiphospholipid Syndrome or Systemic Lupus Erythematosus (SLE). Fifteen female patients with APS and twenty two patients with SLE who had bacteremia caused by mycoplasmas were treated with clarithromycin, mycoplasmas were eradicated from blood and patients showed a clinical improvement.

Keywords: Mycoplasma, Antiphospholipid Syndrome, Systemic Lupus Erythematosus

1. Introduction

Mycoplasmas are the smallest self-replicating bacteria. Mycoplasmataceae family encompasses more than 200 well described species, many of which are human or animal pathogens. (Citti et al. 2013)

Mycoplasmas are small size bacteria, with a minute genome and total lack of a cell wall. Owing to their limited biosynthetic capabilities, most mycoplasmas are parasites exhibiting strict host and tissue specificities. (Rottem 2003)

Within the host, biofilms can also offer a niche, protecting mycoplasmas from host defense as well as from antibiotics, which explains in part the persistence of mycoplasmas in susceptible hosts for months or even years. (Citti et al. 2013)

Antiphospholipid syndrome (APS) is an autoimmune condition characterized by vascular thrombosis and or pregnancy morbidity in the presence of antiphospholipid antibodies (Espinosa et al. 2007)

Systemic Lupus Erythematosus (SLE) is a multisystemic autoimmune disease characterized by variable inflammatory destruction of skin, joints, blood elements, kidneys, serosa, nervous system and other tissues, various autoantibodies are produced during the disease (Agmon-Levin et al.2009,James et al.1997). Systemic Lupus Erythematosus is the prototype of a systemic autoimmune disorder in which immune complexes or cytotoxic antibodies give rise to tissue damage. (Manger et al. 2002)

The general consensus is that autoimmune diseases have a multifactorial etiology, depending on both genetic and environmental factors. Microorganisms may induce the disease by a variety of mechanisms like molecular mimicry (Shoenfeld et al. 2006)

In a previous study *Mycoplasma penetrans* was isolated from the blood and throat of a patient with APS who developed a severe hemolytic anemia, she received treatment with clindamycin and Vancomycin and 3 days later she improved clinically and was released from the intensive care unit (Yáñez et al. 1999). In a later study we searched for antibodies against *M. penetrans* in patients with APS or SLE, patients showed a high proportion of IgG and IgM antibodies. (Herrera-Saldívar et al.2012)

The aim of this study was to treat bacteremia produced by mycoplasmas in patients with antiphospholipid Syndrome or Systemic Lupus erythematosus.

2. Methods

2.1. Patients

Fifteen female patients with antiphospholipid Syndrome (APS) and twenty two female patients with systemic lupus erythematosus (SLE) were enrolled in the study. Patients had medical assistance by a rheumatologist at the Hospital de Especialidades del Centro Médico Nacional Gral. Manuel Ávila Camacho del IMSS. Patients were diagnosed according to international criteria for APS and SLE. Patients signed an informed consent form and the protocol was approved by the Ethics Committee of the Hospital. All patients had a bacteremia produced by mycoplasmas. Mycoplasmas were detected in blood cultures and identified by polymerase chain reaction (PCR).

2.2. Mycoplasma Detection

Mycoplasmas were cultured in SP4 media and PCR was performed from positive cultures. Five milliliters of blood were collected from each patient; 100 microliters of plasma were added to 900 microliters of SP4 media. Three tenfold dilutions were seeded with plasma.

Cultures were incubated under aerobic conditions at 37°C for 30 days or until the phenol red indicator of the media turned yellow. Ten microliters of positive broths were seeded on SP4 agar plates and incubated under aerobic conditions at 37°C for 30 days. DNA was obtained from positive cultures.

A Polymerase Chain Reaction (PCR) test was used to confirm the presence of mycoplasmas in blood. The oligonucleotide primers used for PCR detection were a) AR1: 5' ATG RGG RTG CGG CGT ATT AG 3' y b) AR2: 5' CKG CTG GCA CAT AGT TAG CCRT 3', (where K represents a mixture of nucleotides G_T and R contains A-G), which amplified a 301-nucleotide specific gene sequence that exist in the genome of 30 mycoplasma species (Sidhu et al.1995). Before we used this PCR based test to detect *Mycoplasma*, we confirmed the specificity of these primers.

The reaction mixture contained 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris-HCL (pH 8.3), 0.2 mM of each deoxynucleotide triphosphate, 6 μM of each primer and 1 unit of AmpliTaq® (Perkin Elmer Cetus, Emerville, CA.) in a total volume of 50 μl. The sample to be analyzed (5 μl) was always added last. A diluted lysate of *M. fermentans* PG-18 corresponding to 100 CCU and sterile water were used as positive and negative controls respectively. The amplification involved 40 cycles, each consisted of denaturation at 95°C for 25 s, primers annealing at 60°C for 60 s and extension at 72°C for 60 s. The amplified products were analyzed by electrophoresis in 2% agarose gels and visualized by UV light after ethidium bromide staining.

All patients had bacteremia produced by mycoplasma confirmed by PCR.

3. Results

Clinical history of patients with APS showed that 5/15 had a history of abortions, 3/15 had cerebral vascular events, 2/15 had pulmonary thromboembolism, 1/15 had acute myocardial infarction, 1/15 had peripheral venous thrombosis of lower extremities.

Clinical history of patients with SLE showed that 2/22 had a history of abortions, 1/22 had pulmonary thromboembolism.

3.1. Therapeutic Treatment

Patients with APS: Eighty percent were under prednisone treatment, 33% were receiving non-steroid anti-inflammatory drugs, 40% received anticoagulant drugs and 13% received immunosuppressive treatment.

Patients with SLE: Sixty eight percent of patients were under prednisone treatment, 41% were receiving non-steroid anti-inflammatory drugs, 5% received anticoagulant drugs and 27% received immunosuppressive treatment.

3.2. Antibiotic Treatment

All patients received Clarithromycin 500 mg twice a day for 10 days. They did not receive antibiotics during the following 20 days. This treatment was repeated two more times.

After treatment blood cultures did not show mycoplasma growth. PCR from blood was negative for all samples, suggesting that the antibiotic was effective against mycoplasma bacteremia.

The clinical condition of the patients was evaluated by the Rheumatologist. All patients showed a clinical improvement after the antibiotic treatment. Patients did not suffer vascular events during the treatment and six months later. Patients who suffered of arthritis also showed a clinical improvement, once they finished their antibiotic treatment; arthritis was less severe during the next six months.

4. Discussion

Several studies have concluded that autoimmune diseases have a multifactorial etiology depending on genetic and environmental factors. Bacteria and viruses can induce autoimmune diseases by different mechanisms. (Shoenfeld et al. 2006, Blank et al. 2002, Ramos-Casals et al. 2004) Proteins of certain bacteria can act as polyclonal activators of lymphocytes; viruses can infect and destroy a specific subset of T cell and may alter the balance of the T subsets and then alter the immune response.

Some microorganisms can act as superantigens and induce an exaggerated antibody response. Microbes can also direct the release of cytokines and chemokines which can act as growth, differentiation or chemotactic factors for different cell populations.

Immune system is tolerant to the molecules of our cells but some microorganisms share with us antigens from conserved protein families and may induce the production of autoantibodies, this phenomenon is called molecular mimicry. (Shoenfeld et al. 2006)

Infectious agents play an important role in APS and SLE (Ramos-Casals et al. 2008, Labarca et al. 1997, Nived et al. 1985) Infectious agents may play an etiopathogenic role in the clinical expression of some patients with APS, with bacterial infections probably acting as acute triggering agents of catastrophic APS, whereas viruses, such as HCV and HIV, might act in some patients as chronic triggering agents that induce a heterogeneous atypical presentation of APS (Ramos-Casals 2004)

Mycoplasmas cause acute and chronic diseases in several animal species and men. Also they have been associated with autoimmune diseases like arthritis. They evade the immune response and persist in a same host for weeks, months or even years. Mycoplasmas enter an appropriate host in which they multiply and survive for long periods of time. These microorganisms have evolved molecular mechanisms needed to deal with host immune response; these mechanisms include mimicry of host antigens, survival within phagocytic and non-phagocytic cells and phenotypic plasticity. Molecular mimicry refers to antigenic epitopes that have been shown to be shared by different mycoplasmas and host cells and were proposed as putative factors involved in evasion of host defense mechanisms or induction of autoantibodies produced during some mycoplasmal infections. Mycoplasmas have been shown to activate monocytes, macrophages, and brain astrocytes and induce secretion of proinflammatory cytokines. (Rottem 2003) Mycoplasmas apparently lost genes involved in the biosynthesis of amino acids, fatty acids, cofactors and vitamins and therefore depend on the host microenvironment to supply the full spectrum of biochemical precursors required for the biosynthesis of macromolecules. Competition for these biosynthetic precursors by mycoplasmas may disrupt host cell integrity and alter host cell function. The intimate contact of the mycoplasma with the host cell membrane may also result in the hydrolysis of host cell phospholipids catalyzed by the potent membrane bound phospholipases present in many mycoplasma species. This could trigger specific signal cascades or release cytolytic phospholipids capable of disrupting the integrity of the host cell membrane. *M. penetrans* stimulates host phospholipases to cleave membrane phospholipids and some antigens like cardiolipin may be exposed to the immune cells. (Rottem 2003) It has been reported that a substantial number of patients with *M. pneumoniae* respiratory disease have anti-cardiolipin antibodies. Furthermore, many clinical criteria for APS have also been well documented in patients with *M. pneumoniae* infection including Guillain Barré like illness and other central nervous system manifestations, hemolytic anemia, positive Coombs test, thrombocytopenia and arthritis. (Asherson et al.1994, Snowden et al. 1990)

Antiphospholipid antibodies were also found in more than 50 % of patients with *M. pneumoniae* pneumonia (Snowden et al. 1990). Catteau et al., described two cases of Steven Johnson syndrome associated with *M. pneumoniae* infection and the presence of antiphospholipid antibodies (Catteau et al. 1995) Yáñez et al. reported a case of a 17 year old woman sexually inactive who had an acute onset of arthritis, fever, hemolytic anemia who was diagnosed with APS. *Mycoplasma penetrans* was isolated from blood, throat and tracheal aspirate, she received Clindamycin and Vancomycin, and she improved clinically after antibiotic treatment and was released from the intensive care unit 9 days after admission. (Yáñez et al. 1999)

Herrera et al. determined the presence of antibodies to *M. penetrans* in patients with primary antiphospholipid syndrome, secondary antiphospholipid syndrome and systemic lupus erythematosus and controls. They found antibodies in up to 40% of patients in contrast to 4% of controls. (Herrera-Saldívar et al. 2012). We took blood samples from patients who had antibodies to mycoplasmas in order to culture mycoplasmas; those patients with mycoplasmas were enrolled in this project. Patients with mycoplasmas received clarithromycin and during the treatment showed clinical improvement. After treatment no mycoplasmas were detected in blood. Since most patients were receiving corticosteroids it is probable that steroids could favored mycoplasma proliferation. On the other hand clarithromycin itself may contribute to the improvement of arthritis in patients because of its anti-inflammatory action. (Labro 1998, Scaglione et al. 1998, Ianaro et al. 2000)

Two reports determined the effectiveness of antibiotics in the case of APS. In the first report one patient with APS associated with *Helicobacter pylori*, all disease manifestation disappeared upon eradication of the bacteria. In the other report an experimental model of APS was developed where the manifestations were abrogated by parallel treatment with ciprofloxacin.

(Shoenfeld et al. 2006) The main contribution of this study is the clinical improvement of patients who were treated with clarithromycin probably due to the eradication of mycoplasmas from blood. It could be interesting to do a similar study in a larger group of APS and SLE patients and to determine in a long follow study their evolution.

5. References

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