Roxithromycin in Prevention of Acute Coronary Syndrome Associated with *Chlamydia pneumoniae* Infection: A Randomized Placebo Controlled Trial

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Abstract

The role of *Chlamydia pneumoniae* infection in precipitating acute coronary syndrome (ACS) is unclear. Some studies have indicated that intervention with macrolide antibiotics might reduce coronary events in patients with ACS. A double blind, randomized, placebo-control trial was conducted on 84 ACS patients. Patients were randomized to 30 days of treatment with roxithromycin (150 mg, twice daily) or matching placebo. The follow-up period was 90 days, and the primary clinical end point included cardiovascular death, unplanned revascularization and recurrent angina/MI. Anti-*C. pneumoniae* IgG positive in 24 of 43 (55.8%) patients in the roxithromycin group and 23 of 41 (56.1%) patients in the placebo group. Anti-*C. pneumoniae* IgA positive in 20 of 43 (46.5%) patients in the roxithromycin group and 13 of 41 (31.7%) patients in the placebo group. Thirty-three cardiac events occurred (2 cardiovascular deaths, 9 CABG, 12 PTCA and 10 recurrent angina/MI) with 17 events in the roxithromycin group and 16 events in the placebo group. There was no significant difference of cardiac events between the roxithromycin and placebo groups. The present study suggests that antibiotic therapy with roxithromycin is not associated with reduction of cardiac events as reported by other investigators. However, therapeutic interventions may need to be specifically targeted to a group of patients who are confirmed with chronic *C. pneumoniae* infection.

Key word : *Chlamydia pneumoniae*, Randomized Placebo Control, Roxithromycin, Acute Coronary Syndrome

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The spectrum of clinical syndromes caused by coronary atherosclerosis ranges from asymptomatic disease and stable angina (SA) to acute coronary syndrome (ACS), which include unstable angina (UA), acute myocardial infarction (AMI), and sudden cardiac death. The pathophysiology of atherosclerosis involves injury, inflammation, infiltration, degeneration, and thrombosis. The stimulus of inflammatory response in plaque and systemic inflammation in patients at increased risk for coronary events is unknown. The possibility that infectious agents may directly or indirectly trigger the cascade has been raised recently[1-3].

Established cardiovascular risk factors such as cigarette smoking, diabetes mellitus, hypertension, and hypercholesterolemia do not fully explain the temporal and geographical causation in the prevalence of coronary heart disease over the past century[4]. Endemic of a potential role of infectious agents in the pathogenesis of atherosclerosis has been reported in several studies[5-8]. In particular, an infection with *Chlamydia pneumoniae* has been claimed to be associated with atherosclerotic cardiovascular disease in seroepidemiological and angiographically studies[9-13]. Furthermore, *Chlamydia pneumoniae* has been identified and cultured from atheromatous lesions of coronary arteries and can infect vascular cells *in vitro*[14-16]. The organism can also induce atherosclerosis in animal models and the macrolide antibiotic, azithromycin, was shown to prevent accelerated intimal thickening in cholesterol-fed rabbits that had repeated nasal inoculations of *Chlamydia pneumoniae*[17-19]. Three small secondary prevention trials using macrolide treatment in humans have been presented[20-22]. Two of them have been promising for the preventive effect of the antibiotic but the other one has a negative result. A randomized placebo controlled trial was conducted to explore the effect of roxithromycin in secondary prevention of acute coronary syndrome associated with *Chlamydia pneumoniae* infection. The aim of the study was to determine whether roxithromycin in addition to conventional therapy would reduce cardiovascular events in patients with documented acute coronary syndrome.

MATERIAL AND METHOD

Patients

Patient recruitment began in October 1998 and was completed by September 2000. A total of 84 consecutive patients with acute coronary syndrome who satisfied clinical inclusion criteria and were admitted to the ward or attended the outpatient clinic were evaluated. For all patients, clinical history, physical examination, and blood samples for the following determinations were obtained: *Chlamydia pneumoniae* IgG and IgA, cholesterol, triglycerides, HDL-C, LDL-C.

The inclusion criteria were as follows: age between 40 and 75 years; evidence of acute coronary syndrome (Q-wave myocardial infarction (MI), non-Q-wave myocardial infarction and unstable angina). The exclusion criteria were as follows: left-bundle-branch block, hepatic failure, renal failure, congestive heart failure and contraindication to macrolide therapy. The primary endpoints were as follows: severe recurrent ischemia, acute MI, percutaneous transluminal coronary angioplasty (PTCA), urgent coronary artery bypass graft (CABG) surgery and cardiac death.

Laboratory methods

Serum *Chlamydia pneumoniae* -specific IgG, IgA was determined by ELISA method (SeroCP IgG IgA tests: Savyon Diagnostics, Ashdod, Israel). This ELISA method used elementary bodies as test antigen. Fifty μL of positive control, 3 of 50 μL of negative control and 50 μL of 1:105 diluted specimens were added to the microtiter strips. The ELISA plate was covered and incubated at 37°C for one hour in 100 per cent humidity. After washing 3 times with buffer, 50 μL of 1:300 diluted horseradish peroxidase (HRP) was added and conjugated with anti-human immunoglobulin. The plate was covered and incubated again for one hour at 37°C in 100 per cent humidity. After washing 3 times with the buffer, 100 μL of tetramethylbenzidine (TMB) substrate was added to stop the reaction. The strips were measured at 450 nm for which the positive control absorbance value was > 1.00 and the average absorbance value of negative control was > 0.01 and ≤ 0.40. Total cholesterol, triglycerides, HDL-C were measured by using standard enzymatic methods; the LDL-C levels were calculated from the Friedewald formula.

Study Design

This was a double blind, randomized placebo controlled trial. After conventional therapy had been started and informed consent had been obtained, the patients were randomly allocated either roxithromycin 150 mg orally twice a day or placebo

for 30 days. Follow up visits were scheduled at day 14, day 30 and day 90 after the start of the study treatment. Random allocation was done according to a computer–generated list. A set of sealed envelopes containing patient codes and study treatment was prepared and stored in a secure place.

Statistical analysis

The roxithromycin group and placebo group were compared according to baseline characteristics in a descriptive way. The proportion of test was employed to compare lipid profiles. The proportion of patients experiencing an adverse event was compared between the roxithromycin and placebo group by use of the x^2 test. The odd ratio (OR) for adverse cardiovascular events in each group was calculated by EpiInfo 6.04 (Center for Disease Control, Atlanta, Ga.). A p valve of less than 0.05 was regarded as a significant level.

RESULTS

There were no significant differences between the two groups with regard to age, sex, risk factors and lipid profiles (Table 1). Anti-*C. pneumoniae* IgG were positive in 24 (55.8%) of 43 patients who were treated with roxithromycin and 23 (56.1%) of 41 patients in the placebo group. With regard to anti-*C. pneumoniae* IgA, 20 (46.5%) of 43 patients who received roxithromycin and 13 (31.7%) of 41 patients in the placebo group were positive. After 3 months, of the patients participating in the roxithromycin group, 17 adverse cardiovascular events occurred (10 between day 0 and day 30 and 7 between day 31 and day 90). In the placebo group 16 events occurred (13 between day 0 and day 30 and 3 between day 31 and day 90). Table 2 shows the type of cardiovascular events that occurred in both groups. There was no significant difference between the patients receiving roxithromycin and the patients receiving placebo. The prevalence of anti-*C. pneumoniae* IgG and IgA were almost identical in the roxithromycin and placebo groups. The risk factors between the two groups were also not significantly different. The composite of clinical cardiovascular events that occurred within 3 months in the roxithromycin group was 17 *versus* 16 events in the placebo group. The distribution of events was also generally similar in the two groups.

DISCUSSION

Recent data suggested that chronic bacterial infections involved in the genesis of ischemic heart disease (IHD), in particular, *C. pneumoniae* infection. It may be involved both by a direct mechanism of colonization and atherosclerotic plaque instability and by an indirect mechanism of activation of inflammation[23]. Two recent randomized studies about treatment of *C. pneumoniae* infection in IHD reported that treatment with macrolide antibiotics (azithromycin, roxithromycin) could reduce adverse cardiovascular events. The study of Gupta et al showed that azithromycin treatment of *C. pneumoniae* positive patients reduced the risk of adverse cardiovascular events during an 18-month

Table 1. Baseline characteristics, lipid profiles, anti-*C. pneumoniae* IgG and IgA.

	Roxithromycin (n=43)	%	Placebo (n=41)	%
Age, years (mean ± SD)	62.9 ± 9.6		60.4 ± 12.6	
Male	29	67.4	24	58.5
Family history	10	23.3	12	29.3
Hypertension	24	55.8	20	48.8
Smoking	19	44.2	25	61.0
Diabetes mellitus	22	51.2	15	36.6
Hyperlipidemia	25	58.1	28	68.3
Total cholesterol (mg/dL)	222.3 ± 61.5		225.7 ± 57.9	
Triglycerides (mg/dL)	185.1 ± 82.6		167.4 ± 72.7	
HDL-C (mg/dL)	37.4 ± 10.4		37.9 ± 13.6	
LDL-C (mg/dL)	147.1 ± 52.9		153.0 ± 54.0	
Anti-*C. pneumoniae* IgG	24	55.8	23	56.1
Anti-*C. pneumoniae* IgA	20	46.5	13	31.7

Table 2. Cardiac events that occurred from day 0 to day 90.

	Roxithromycin (n=43)	Placebo (n=41)	p value
Recurrent angina/MI	4	6	NS
PTCA	7	5	NS
CABG	5	4	NS
Death	1	1	NS
Total events (%)	17 (39.5)	16 (39.0)	NS

follow-up period[21]. In addition, Gurfinkel et al also reported on a pilot antibiotic trial from Argentina: 202 patients presenting with unstable angina or non-Q-wave MI were randomized on hospital admission to roxithromycin 150 mg twice daily, or placebo for 30 days[20]. They found that roxithromycin could reduce morbidity and mortality during a one-month period. These studies suffered from the small number of patients and small number of cardiac events, poor characterization of patients, and short follow-up.

One randomized, placebo controlled trial study (ACADEMIC) reported by Anderson et al showed study designs contrast with those earlier trials in several ways such as a much larger sample (300 patients), gave more intensive antibiotic treatment (2.5 g of azithromycin over 3 months)[22]. However, they did not confirm a dramatic early reduction in clinical events (9 in the azithromycin group *versus* 7 in the placebo group).

The present study is the first randomized, placebo controlled for secondary prevention antibiotic trial in Thailand. Study design was similar with Gurfinkel et al but the result was different especially the number of cardiac events. In the Roxis pilot study[20], cardiac events occurred in only 14.6 per cent (14/96) in the roxithromycin group and 8.7 per cent (8/92) in the placebo group. On the other hand, in the present study the cardiac events occurred more often than in the Roxis pilot study, cardiac events occurred in 39.5 per cent (17/43) in the roxithromycin group and in the placebo group it occurred 39.0 per cent (16/41). This means that the patients in the present study seem to be more severe than the patients in the study of Gurfinkel et al. However, cardiac events that occurred in the study of Gupta et al were nearly the same as in our study (30% in the non randomized group, 25% in the placebo group and 15% in the *C. pneumoniae* intermediate titer group). However, the difference in the study population in these 4 antibiotic trials could not be compared in many aspects.

The data in the present study do not support the important role of roxithromycin in secondary prevention of acute coronary syndrome as reported by Gurfinkel et al[20]. The patients treated with roxithromycin for a period of 30 days did not show a significant reduction in the occurrence of major cardiovascular event compared with the placebo group. There are at least two possible explanations for the findings of the present study. First, roxithromycin, through its antichlamydial activity, may reduce but not completely eliminate the organism within atherosclerotic plaque. Gieffers et al reported that *C. pneumoniae* infection in circulating human monocytes is refractory to azithromycin or rifampin treatment[24]. In this regard, prevention of vascular infection by antichlamydial treatment may be problematic. Because circulating monocytes carrying a pathogen with reduced antimicrobial susceptibility might initiate reinfection or promote atherosclerosis[25]. Secondly, although roxithromycin also has anti-inflammatory activity, the data in the present study do not demonstrate this effect[26].

SUMMARY

In the present study of 84 ACS patients followed-up for 90 days, a 1-month course of roxithromycin was not associated with an overall reduction in cardiovascular events compared with placebo. Further large-scale trials are required to assess the potential role of antibiotic therapy in CAD patients before this regimen will be the standard treatment in cardiac patients in the future.

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การใช้ยาร็อกซิโทรมัยซินเพื่อป้องกันการเกิดกลุ่มอาการหลอดเลือดหัวใจเฉียบพลันที่มีความสัมพันธ์กับการติดเชื้อ *คลามิเดีย นิวโมนิอี* โดยศึกษาแบบสุ่มให้ยาจริงเปรียบเทียบกับยาหลอก

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ผลของการติดเชื้อ *คลามิเดีย นิวโมนิอี* ที่มีต่อการเกิดกลุ่มอาการหลอดเลือดหัวใจเฉียบพลันยังไม่เป็นที่แน่ชัด มีนักวิจัยบางกลุ่มสรุปว่าการใช้ปฏิชีวนะยากลุ่ม แมคโครไลด์สามารถลดความรุนแรงของการเกิดกลุ่มอาการดังกล่าวได้ คณะผู้วิจัยจึงได้ทำการศึกษาซ้ำอีกครั้งเพื่อพิสูจน์ว่าผลของการให้ยาปฏิชีวนะกลุ่มดังกล่าวจะสามารถลดความรุนแรงได้จริงหรือไม่ โดยทำการศึกษาแบบสุ่มตัวอย่างให้ยาร็อกซิโทรมัยซิน หรือยาหลอก ในผู้ป่วยกลุ่มอาการหลอดเลือดหัวใจเฉียบพลันจำนวน 84 ราย ทั้งแพทย์ผู้รักษาและผู้ป่วยจะไม่ทราบว่ายาที่ได้เป็นยาจริงหรือยาหลอก ขนาดของยาที่ใช้คือ 150 มก. วันละ 2 ครั้ง นาน 30 วัน และติดตามดูต่อเนื่องจนครบ 90 วันโดยดูความรุนแรงของโรคที่เกิดขึ้นเพื่อประเมินความแตกต่างระหว่างผู้ป่วยทั้งสองกลุ่มจากการเกิดการเสียชีวิตด้วยโรคหัวใจ การต้องทำการขยายหลอดเลือดหัวใจหรือผ่าตัดเปลี่ยนหลอดเลือดหัวใจ และการเจ็บหน้าอกซ้ำ/การเกิดกล้ามเนื้อหัวใจตาย ผลการตรวจเลือดเพื่อดูภูมิต้านทานต่อเชื้อ *คลามิเดีย นิวโมนิอี* ชนิด ไอจีจี พบว่า กลุ่มที่ได้รับยาร็อกซิโทรมัยซิน ให้ผลบวก 55.8% (24/43) ส่วนกลุ่มที่ได้ยาหลอก ให้ผลบวก 56.1% (23/41) ในกรณีภูมิต้านทานชนิด ไอจีเอ พบว่าให้ผลบวก 46.5% (20/43) และ 31.7% (13/41) ในกลุ่มที่ได้รับยาร็อกซิโทรมัยซินและกลุ่มที่ได้ยาหลอกตามลำดับ หลังจากติดตามผู้ป่วยอยู่นาน 90 วัน พบว่าเกิดเหตุการณ์ที่เกี่ยวข้องกับโรคหัวใจทั้งสิ้น 33 ครั้งประกอบด้วย การเสียชีวิตจากโรคหัวใจ 2 ราย ต้องทำการผ่าตัดเปลี่ยนหลอดเลือดหัวใจ 9 ราย ต้องขยายหลอดเลือดหัวใจ 12 ราย และเกิดอาการเจ็บหน้าอกซ้ำ/กล้ามเนื้อหัวใจตาย 10 ราย โดยกลุ่มผู้ป่วยที่ได้รับยาร็อกซิโทรมัยซิน เกิด 17 ครั้ง ส่วนกลุ่มที่ได้รับยาหลอกเกิด 16 ครั้ง ซึ่งไม่มีความแตกต่างกันทางสถิติระหว่าง 2 กลุ่ม โดยสรุปพบว่าการใช้ยาปฏิชีวนะชนิด ร็อกซิโทรมัยซิน ในผู้ป่วยกลุ่มอาการหลอดเลือดหัวใจเฉียบพลันไม่สามารถลดความรุนแรงของการเกิดเหตุการณ์เกี่ยวกับหัวใจลงได้เหมือนกับการศึกษาที่รายงานก่อนหน้านี้ อย่างไรก็ตามการให้ยาดังกล่าวกับผู้ป่วยโรคหลอดเลือดหัวใจที่สามารถตรวจยืนยันการติดเชื้อ *คลามิเดีย นิวโมนิอี* แบบเรื้อรังน่าจะมีประโยชน์บ้างไม่มากก็น้อย

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