Effect of Clarithromycin on Inflammatory Markers in Patients with Atherosclerosis

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Atherosclerosis can to a certain extent be regarded as an inflammatory disease. Also, inflammatory markers may provide information about cardiovascular risk. Whether macrolide antibiotics, especially clarithromycin, have an anti-inflammatory effect in patients with atherosclerosis is not exactly known. To study this phenomenon, a placebo-controlled, randomized, double-blind study was performed. A total of 231 patients with documented coronary artery disease received a daily dose of either 500 mg of slow-release clarithromycin or placebo until the day of surgery. Levels of inflammatory markers (C-reactive protein, interleukin-2 receptor [IL-2R], IL-6, IL-8, and tumor necrosis factor alpha) were assessed during the preoperative outpatient visit, on the day of surgery, and 8 weeks after surgery. Also, changes in the levels of inflammatory markers between visits were determined by delta calculations. Baseline patient characteristics were balanced between the two treatment groups: the average age was 66 years (standard deviation [SD] = 9.0), 79% of the patients were male, and the average number of tablets used was 16 (SD = 9.3). The inflammatory markers of the groups as well as the delta calculations were not significantly changed. Treatment with clarithromycin did not influence the inflammatory markers in patients with atherosclerosis.

Despite the use of pharmaceutical therapy against known risk factors and changes in lifestyle and behavior, cardiovascular disease remains a leading cause of death worldwide (4). Apart from well-known risk factors such as elevated and modified low-density protein cholesterol, free radicals (caused by smoking), hypertension, diabetes mellitus, genetic alterations, and hyperhomocysteinemia, infections caused by various microorganisms are also considered potential causes of atherosclerosis. For example, cytomegalovirus, Helicobacter pylori, and Chlamydia pneumoniae have been linked to the pathogenesis of atherosclerosis (5). In addition, atherosclerosis can be regarded as an inflammatory disease (10).

All these risk factors can cause endothelial injury and dysfunction, which in many studies is considered the first step in the pathogenesis of atherosclerosis. It forms the basis of the so-called response-to-injury hypothesis (19). This response to endothelial injury is mediated at every stage of atherosclerosis by monocyte-derived macrophages and specific subtypes of T lymphocytes (10, 19).

Elevated levels of C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), or interleukin-6 (IL-6) are associated with an increased risk of future myocardial infarction (12, 16, 17, 18). Patients with elevated CRP levels (>2.0 mg/liter) are at risk of recurrent angina pectoris and acute myocardial infarction. Conversely, in patients with unstable angina pectoris, CRP levels are elevated (13). Researchers have now suggested that inflammatory markers such as CRP may provide information about cardiovascular risk (16).

Inflammatory markers such as IL-2R, IL-6, IL-8, TNF-α, and CRP can provide information about the mechanism of the inflammatory reaction associated with atherosclerosis. IL-2R, IL-6, IL-8, and TNF-α derive from activated monocytes, macrophages, T cells, or endothelial cells. These inflammatory markers stimulate fibroblasts and smooth muscle cells to proliferate. They may also induce free radical generation by neutrophils, and this may facilitate oxidation of low-density protein cholesterol and attract monocytes and other inflammatory cells to the area of endothelial damage (6, 10). Cytokines promote the production of endogenous tissue plasminogen activator and plasminogen activator inhibitor 1, which stimulate thrombus formation (14).

There is much speculation about the anti-inflammatory potential of macrolides independent of their well-established role in the chemotherapy of infectious diseases. Macrolides have potentially relevant in vitro, ex vivo, and in vivo immunomodulatory effects (11). To investigate the effect of clarithromycin on inflammatory markers in patients prior to coronary artery surgery, we performed a prospective, double-blind, randomized, placebo-controlled study.

MATERIALS AND METHODS

Between July 1999 and July 2001, patients with documented coronary artery disease were enrolled in the study. All these patients were scheduled for coronary artery bypass graft surgery. Patients were evaluated for inclusion in the study during a visit to the preoperative outpatient clinic at the department of thoracic surgery of the Amphia Hospital. Exclusion criteria included the following: concomitant administration of terfenadine (Triludan), rifabutin (Mycobutin), cisapride (Propulsid), or antipyrine; antibiotic therapy with a macrolide, tetracycline, or quinolone within 3 months prior to inclusion in the study or during the study period; the presence of renal failure (serum creatinine concentrations above 150...
μmol/liter), elevated liver function (alanine aminotransferase levels of >55 U/liter, aspartate aminotransferase levels of >45 U/liter, total bilirubin levels of >27 μmol/liter, alkaline phosphatase levels of >180 U/liter), and for female patients capable of childbearing, not taking adequate birth control precautions. After the patients gave their informed consent, they were randomized in a double-blind, placebo-controlled study. From that point on, they began receiving the study medication until the day of surgery. The study medication consisted of either a daily dose of 500 mg of slow-release clarithromycin (clarithromycin SR) or a matching placebo tablet (both from Abbott laboratories, Ltd., Queensborough, Kent, England). Clarithromycin SR is well absorbed following oral administration and well distributed in body fluids and tissues, where it achieves high and persistent concentrations (half-life in tissue, 5.3 h).

From each patient, 10 ml of blood (extracted with EDTA) was obtained at the following stages: (i) during the initial preoperative outpatient visit, (ii) on the day of surgery just before the operation, and (iii) 8 weeks after surgery. Blood samples were stored at 4°C immediately after collection and centrifuged within 2 h. Plasma was then stored at −70°C pending further testing. This plasma was analyzed with an IMMULITE analyzer according to the manufacturer’s instructions (EURO/DPC Ltd., Glyn Rhonwy, United Kingdom) for quantitative estimation of soluble IL-2R (units per liter), IL-6 (picograms per liter), IL-8 (picograms per liter), TNF-α (picograms per liter), and CRP (milligrams per liter).

The study was approved by the local medical ethics committee.

Statistical analysis. A sample size calculation was made to measure the effect on the CRP value based on the results from a previous study (2). In this study the placebo group had a mean CRP value of 8.7 mg/liter and a standard deviation (SD) of 6.0. For a hypothesized reduction of 30% (2.6 mg/liter) of the CRP with on the CRP value based on the results from a previous study (2). In this study the placebo group had a mean CRP value of 8.7 mg/liter and a standard deviation (SD) of 6.0. For a hypothesized reduction of 30% (2.6 mg/liter) of the CRP with

RESULTS

A total of 231 patients were enrolled in the study. After randomization, 117 patients received 500 mg of clarithromycin SR and 114 received a placebo. Table 1 shows that the baseline patient characteristics were well balanced between the two treatment groups. The mean numbers of tablets used before surgery were 16 (SD = 9.5) for the clarithromycin group and 17 (SD = 9.6) for the placebo group. The mean age was 66 years (SD = 8.9) in the clarithromycin group versus 64 years (SD = 9.3) in the placebo group. Eighty percent of the clarithromycin group was male versus 78.1% in the placebo group. No significant differences were found.

Eight patients who during the study period simultaneously used antibiotics other than a macrolide, tetracycline, or quinolone were excluded from this treatment analysis due to possible interference from additive anti-inflammatory effects.

The baseline levels of inflammatory markers for both treatment groups on the day of the preoperative visit (visit 1), as well as the values after treatment on the day of surgery (visit 2) and 8 weeks after surgery (visit 3), are shown in Table 2. As the data in the table indicate, there were no significant differences in the levels of inflammatory markers between the two groups at visit 1, 2, or 3 (Table 2). Changes in the levels of inflammatory markers between visits were also calculated. There were no significant differences in these changes between the treatment groups.

DISCUSSION

Treatment with a daily dose of 500 mg of clarithromycin SR in patients with coronary artery disease prior to cardiac surgery did not significantly alter the inflammatory markers directly after treatment on the day of surgery or 8 weeks after surgery. Clarithromycin can therefore be considered to have no or little anti-inflammatory potential.

The hypothesis that atherosclerosis has an infectious etiology has promoted studies of the effect of antibiotics, especially macrolides. The activity against C. pneumoniae and the general anti-inflammatory effect on the outcome in patients with cardiovascular disease were studied retrospectively as well as prospectively.

For example, Østergaard et al. in a retrospective cohort study (15) assessed the time-dependent effect of macrolide therapy versus that of penicillin therapy on the risk of hospitalization due to cardiovascular disease. The authors concluded that the decreased relative risk of hospitalization (0.48) due to Cardiovascular disease in users of macrolides within 3 months indicated a possible direct anti-inflammatory effect on diseased vessels, but this was not further studied.

In several prospective intervention studies using either azithromycin (AZ) or roxithromycin, the anti-inflammatory effect was examined. Gupta et al. (7, 8) performed the first

TABLE 1. Patient characteristics

| Characteristic | Clarithromycin (n = 113) | Placebo (n = 110) | P value |
|---------------|-------------------------|------------------|--------|
| Age, yr       | 66 (9.0)                | 64 (9.4)         | NS     |
| Wt, kg        | 82 (12.4)               | 80 (13.4)        | NS     |
| Ht, cm        | 172 (8.7)               | 172 (9.1)        | NS     |
| NYHA          | 2.8 (0.8)               | 2.8 (0.8)        | NS     |
| Preoperative period (days) | 17.7 (10.4) | 20.4 (15.8) | NS     |
| No. of tablets used | 16 (9.3)     | 17 (9.6)         | NS     |
| Medical history |                       |                  |        |
| Male          | 92 (81.4)               | 85 (77.3)        | NS     |
| Smoker        | 24 (21.2)               | 24 (21.8)        | NS     |
| COPD          | 11 (9.7)                | 12 (10.9)        | NS     |
| Diabetes mellitus |                 |                  |        |
| IDDM          | 8 (7.1)                 | 8 (7.3)          | NS     |
| NIDDM         | 13 (11.5)               | 8 (7.3)          | NS     |
| Dyslipidemia  | 66 (58.4)               | 70 (63.6)        | NS     |
| Malignancy    | 8 (7.1)                 | 3 (2.7)          | NS     |
| Severe underlying disease | 6 (5.3)    | 5 (4.5)          | NS     |
| CVA           | 10 (8.8)                | 7 (6.4)          | NS     |
| Angina pectoris | 108 (95.6)             | 110 (100)        | NS     |
| Myocardial infarction | 54 (47.8)   | 49 (44.5)        | NS     |
| Heart valve insufficiency | 4 (3.5)    | 3 (2.7)          | NS     |
| Hypertension  | 54 (47.8)               | 56 (50.9)        | NS     |
| Earlier vascular surgery | 21 (18.6)  | 18 (16.4)        | NS     |
| Family history |                       |                  |        |
| Heart disease | 86 (76.1)               | 83 (75.5)        | NS     |
| Diabetes mellitus | 20 (17.7)            | 17 (15.5)        | NS     |
| Medication    |                        |                  |        |
| Anticholesterol | 66 (58.4)             | 69 (62.7)        | NS     |
| Antihypertensives | 113 (100)            | 109 (99.1)       | NS     |
| Immunosuppressives | 0 (0)            | 3 (2.7)          | NS     |

a NYHA, stage of heart failure according to the New York Heart Association classification; COPD, chronic obstructive pulmonary disease; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin dependent diabetes mellitus; CVA, cerebrovascular accident. Values for medical and family histories and medication are given as number (percent) of patients. Other values (for the first six characteristics) are given as means (SDs).

b NS, not significant.

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intervention trial in which 60 survivors of acute myocardial infarction with persistently elevated anti-Chlamydia antibody titers were treated with a 3-day course of AZ, followed by a single dose per week for 3 months, or with a placebo. In a subgroup of patients treated with a double dose of AZ, significant decreases in total monocyte tissue factor and CD11b were found after 6 months (but not 3 months). This indicates a stronger anti-inflammatory effect after 6 months when a double dose of AZ was used. However, no significant differences were noted in the levels of any of the inflammatory markers in either of the normal groups receiving AZ or placebo. In a similar study by Gurfinkel et al. (9), 202 patients with coronary heart disease were treated with roxithromycin for 30 days. CRP levels decreased in both the placebo and the roxithromycin treatment groups at 30 days, but the declines did not reach statistical significance. Moreover, CRP was not associated with the significant reduction in ischemic events. This result indicates that the reduction in cardiovascular events in these studies is based on a stabilizing effect on atherosclerotic plaques, caused by an anti-anchyamidal effect, rather than on an anti-inflammatory effect.

More recently, Anderson et al. (2) treated 302 patients who had coronary artery disease and a seropositive reaction to C. pneumoniae with AZ or placebo (in a similar treatment protocol as that described by Gupta et al. [7, 8]). The levels of four inflammatory markers were unchanged at 3 months but showed minor changes after 6 months. The changes in the levels of inflammatory markers before and after treatment were significantly lower for CRP ($P = 0.011$) and IL-6 ($P = 0.043$) after 6 months (not after 3 months). It must be stressed that no difference was found in clinical events and that these results are based on minor changes; a major anti-inflammatory effect was not shown. The overall minor changes in inflammatory markers are consistent with our study results.

The duration of treatment could be of critical importance in explaining the different results in various studies. Our study patients were treated, on average, with 16 tablets (a number equivalent to the number of treatment days). We therefore repeated our analysis on a subgroup of 114 patients who were treated with 14 or more tablets, but we found no differences in levels of inflammatory markers between the two groups at visit 1, 2 or 3. A repeated analysis of a small subgroup of 56 patients treated with 21 or more tablets also did not show any differences. For that reason we can conclude that the possible anti-inflammatory effect of clarithromycin, if any at all, does not increase with the duration of treatment. In addition, in the intervention studies mentioned above, even though patients received long-term treatment for 1 to 3 months (2, 7, 8), such treatment had little or no effect on inflammatory markers. Other data about a difference between the effects of short-term and long-term administration of macrolides on the immune response have been reported in a review by Labro (11). These studies are, however, mainly ex vivo studies, so comparison to the results with our study population is not appropriate.

In conclusion, several studies have demonstrated an anti-inflammatory effect of macrolides in vitro and in treatment of diseases involved with the bronchial epithelium (1, 3, 20). The present study is, however, in our opinion the first reported in vivo study about the effect of clarithromycin in patients with atherosclerosis and indicates that clarithromycin has no measurable anti-inflammatory effect in such patients.

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