ORIGINAL RESEARCH

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The effect of the duration of clopidogrel use on hsCRP levels after stenting the target vessel in patients with acute coronary syndrome

Abstract

Purpose: The aim of this study is to investigate the relationship between the duration of clopidogrel use and the inflammation process after acute coronary syndrome in patients who received bare metal stent (BMS) or drug eluting stent (DES).

Method: Sixty patients with acute coronary syndrome who received a stent were divided into three groups: 20 patients with BMS receiving clopidogrel for one month (BMS1 group), 20 patients with BMS receiving clopidogrel for 6 months (BMS6 group), and 20 patients with DES receiving clopidogrel for 6 months (DES group). High sensitive C-reactive protein (hsCRP, mg/dL) was measured at baseline, and then at first, third and sixth post-operative month.

Results: The initial hsCRP levels were similar and decreased significantly in all groups by one month of clopidogrel treatment (from 7.1 ± 1.9 to 3.8 ± 2.3 in BMS1 group, $p=0.002$, from 6.5 ± 2.8 to 4.3 ± 2.5 in BMS6 group, $p=0.01$ and from 7.7 ± 2 to 3.6 ± 2.4 in DES group, $p<0.001$). In the BMS1 group, after termination of the clopidogrel therapy after the first month, hsCRP levels increased again at the third and sixth months. In the BMS6 and DES groups, hsCRP levels continued to show a decrease at the third month and sixth months.

Conclusions: Clopidogrel decreases hsCRP levels in patients with acute coronary syndrome. It might be desirable to lengthen the duration of the clopidogrel therapy to maintain its anti-inflammatory effects.

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In spite of important progress made in the field of percutaneous coronary interventions (PCI), having a significant place in the treatment of coronary artery disease, it remains a large problem that the frequency of restenosis and thrombosis remains high. The inflammation process has been shown to influence the prognosis after PCI [1]. Inflammation plays a role from the initiation and development of the atherosclerotic event to the manifestation of thrombotic complications [2,3]. In comparison with other inflammation markers, the strongest evidence of a correlation has been observed with CRP. The vascular trauma occurring during PCI causes local cytokine expression and a systematic inflammatory response [1]. After PCI, CRP increases with time and reaches a maximum level within 48-72 hours [4]. After the role of inflammation in the occurrence of atherosclerotic and atherothrombotic complications was evident, studies were done to determine the prognostic role of the inflammatory markers, especially that of high sensitive CRP (hsCRP), in patients who underwent PCI. hsCRP measurements are now used for determining the vascular risk and prognosis. A series of studies has shown an increase in CRP after stent implantation and a correlation with death, myocardial infarction, recurrent angina and restenosis [1,5-8]. In other studies, the relationship between inflammatory markers and post-PCI atherothrombotic events have been demonstrated [9,10]. In these studies, it was proposed that repressing the secondary inflammatory response after coronary stent application might reduce the ischemic complications. It has been suggested that the use of clopidogrel therapy before coronary stent application has a positive impact on the prognosis, due to its known anti-inflammatory effects [11,12].

This study was designed to demonstrate both the effects of clopidogrel on the inflammatory process in patients with bare metal stents or drug eluting stents after acute coronary syndrome (ACS), and the effect of the duration of clopidogrel use on this process.

Methods

Patient selection and treatment

Sixty-five patients with ACS who received a stent following diagnosis of angiographically proven coronary artery disease ($\geq 70\%$ coronary artery stenosis) were included in the study prospectively. Patients with history of systematic infections, coronary artery disease, inflammatory diseases, chronic systemic disease, and patients with the habit of smoking, alcohol or drug use were excluded from the study. In addition, two patients were excluded from the study because they would not give blood samples for hsCRP, two patients required another revasculari-

zation and one patient was excluded for not using medication regularly. The remaining 60 patients were divided into three groups according to the type of stent and the duration of clopidogrel use. The BMS1 group group consisted of 20 patients with bare metal stent (BMS) who received clopidogrel for only one month after stent implantation with optimal medical therapy (see below). The BMS6 group consisted of 20 patients with BMS who received clopidogrel for six months after stent implantation with optimal medical therapy. The DES group consisted of 20 patients with paclitaxel eluting stent who received clopidogrel for six months after stent implantation with optimal medical therapy.

Optimal medical therapy included aspirin (1x300 mg/day in the first three months, 100 mg/day in the last three months), beta-blocker (metoprolol 2x50 mg/day), angiotensin converting enzyme inhibitor (perindopril 1x5 mg/day) and statin (atorvastatin 1x20 mg/day). All patients received an initial 600 mg load of clopidogrel and then 75 mg daily after the stent implantation.

Blood samples for hsCRP were obtained from all patients, before stent application, at 1, 3 and 6 months follow-up. Blood samples were centrifuged, then stored at -20°C . hsCRP levels were measured using the immunonephelometric method. Results expressed in mg/dL.

This study was approved by the University Ethics Committee. All patients were fully informed about the study, and their written consents were obtained.

Statistical evaluation

Data are expressed either as mean $\pm$ SD or as percentage. Comparisons between the groups were carried out using Oneway Anova tests. To compare the change of measurements between baseline and first, third and sixth month in each group, the Student's paired t-test was used. Correlation analyses were performed using the Pearson coefficient of correlation. A value of $p < 0.05$ was considered to be significant. SPSS 15.0 software was used for statistical analysis (Version 15, SPSS Inc., Chicago, IL, USA).

Results

There were no significant differences between the groups with regards to age, gender, incidence of diabetes mellitus or hypertension, and the targeted arteries that necessitated revascularization ($p>0.05$) (Table 1).

No statistically significant differences were found in the hsCRP levels before stent implantation among the three groups (7.1 ± 1.9 mg/dL in the BMS1 group, 6.5 ± 2.8 mg/dL in

TABLE 1. Demographic features of the groups

	BMS1 Group (n=20)	BMS6 Group (n=20)	DES Group (n=20)	P
Age (year)	61.2±7.4	57.1±11.2	58.9±9.1	NS
Male (n, %)	11 (55%)	7 (35%)	8 (40%)	NS
Hypertension (n, %)	11 (55%)	9 (45%)	10 (50%)	NS
Diabetes mellitus (n, %)	3 (15%)	2 (10%)	3 (15%)	NS
Total cholesterol (mg/dL)	184.1±47.1	177.3±47	157.2±41	NS
HDL cholesterol (mg/dL)	38.8±8.4	36.8±9	38.8±9.9	NS
Triglyceride (mg/dL)	176.7±87.1	183.1±82.4	184.5±90.6	NS
Family history (n, %)	6 (30%)	5 (25%)	5 (25%)	NS
BMI (kg/m ²)	29±4	28±4	29±5	NS
<i>Acute coronary syndrome (n, %)</i>				
USAP	8 (40%)	7 (35%)	6 (30%)	NS
Non- STEMI	6 (30%)	7 (35%)	7 (35%)	NS
STEMI	6 (30%)	6 (30%)	7 (35%)	NS
<i>Targeted artery</i>				
LAD	9 (45%)	11 (55%)	8 (40%)	NS
CX	5 (25%)	4 (20%)	7 (35%)	NS
RCA	6 (30%)	5 (25%)	5 (25%)	NS

BMS1: The group with the bare metal stent, using clopidogrel for 1 month. BMS6: The group with the bare metal stent, using clopidogrel for 6 months, DES: The group with the drug inducing stent, using clopidogrel for 6 months. BMI: Body mass index, USAP: Unstable angina pectoris, Non-STEMI: Non-ST elevation myocardial infarction, STEMI: ST elevation myocardial infarction, LAD: Left anterior descending artery CX: Circumflex artery, RCA: Right coronary artery, NS: Non significant ($p < 0.05$).

FIGURE 1. hsCRP levels at 1, 3 and 6 month follow-up in BMS1, BMS6 and DES groups. BMS1: The group with the bare metal stent, using clopidogrel for 1 month. BMS6: The group with the bare metal stent, using clopidogrel for 6 months, DES: The group with the drug eluting stent, using clopidogrel for 6 months. Comparisons between three groups were carried out using Oneway Anova test at baseline, first, third and sixth months, non-significant, $p > 0.05$.

the BMS6 group and 7.7 ± 2 mg/L in the DES group, $p = 0.4$). By the first post-operative month, hsCRP values (3.8 ± 2.3 , 4.3 ± 2.5 , 3.6 ± 2.4 mg/L, in the BMS1, BMS6 and DES groups, respectively) decreased significantly compared with the baseline values in all groups. This decrease was significant with $p < 0.001$ for all three groups. In the BMS1 group, there was a significant increase in the hsCRP levels at third and sixth month compared with the first month (3.8 ± 2.3 mg/dL at first post-operative month, 4.7 ± 2.1 mg/dL at third month ($p = 0.03$) and 5.7 ± 2.2 mg/dL ($p = 0.01$) at sixth month after stent implantation). In the BMS6 group, these decreased to 3.0 ± 1.7 mg/dL, $p = 0.02$ at the third month and 2.4 ± 2.5 mg/dL, $p = 0.03$ at the 6th month. In the DES group, the decrease in the hsCRP levels were 2.4 ± 1.6 mg/dL, $p = 0.03$ at the third month and 2.3 ± 2.4 mg/dL, $p = 0.03$ at the 6th month.

When the BMS1 and BMS6 groups were compared, the levels of hsCRP were significantly higher in BMS1 group at the 3rd month and the 6th month (Figure 1).

The patients in the BMS1 group also had significantly higher hsCRP levels than the patients in the DES group at the 3rd month ($p = 0.01$) and the 6th month ($p < 0.001$, Figure 1). There were no statistically significant differences in hsCRP levels between the patients in the BMS6 and DES groups at the 3rd month ($p = 0.2$) and the 6th month ($p = 0.4$).

Discussion

Our study showed that clopidogrel decreased hsCRP levels, and that this decrease directly correlated with the duration of clopidogrel treatment after ACS. The evaluation at the end of the first month showed that there was a significant decrease in the hsCRP levels in all three groups receiving clopidogrel treatment. The extent of decrease was not significantly different among the groups. In the BMS1 group, where the clopidogrel therapy was finished after the first month, hsCRP levels were higher at the 3rd month in comparison with the levels at the first month and continued to increase at the 6th month. In contrast, in the BMS6 and DES groups, in which the treatment with clopidogrel was continued for 6 month, the hsCRP levels were significantly lower at both the 3rd and 6th months. In light of the data, it can be said that a continuous decrease in the hsCRP levels was achieved during the clopidogrel therapy of 6 months.

Since the relationship between atherosclerosis and inflammation has been clearly defined, the levels of inflammatory markers in circulation could help to determine cardiovascular event risk. In comparison with other inflammatory markers, the ability of CRP to point to cardiovascular disease risk stands out [3]. CRP activates a number of events through

inflammatory reaction, as has been demonstrated by a number of *in vitro* studies [13]. The CRP levels secreted by the atheroma plaques during the subclinical inflammation of the atherosclerosis are very low, and highly sensitive tests are needed for accurate measurement. After it was demonstrated that even acceptable CRP levels, within normal limits in healthy individuals, are independent determinants of atherosclerotic vascular disease, highly sensitive methods such as hsCRP were developed to measure CRP levels. Currently, hsCRP is the standard method for determining vascular risk and prognosis. The numerous reports, broad range of studies, randomized and prospective in design, suggest that basic hsCRP levels will be used to determine cardiovascular risk in the future [14-17]. hsCRP levels have a proven short and long term prognostic value for patients with acute coronary syndrome [18,19].

Studies evaluating restenosis after percutaneous coronary intervention stress that hsCRP can be a useful marker [9,20, 21]. Recent studies with patients with DES show that CRP levels show significant correlations with stent-related thrombosis, death and myocardial infarction. It was argued that CRP can be used for risk evaluation [22].

It has been proposed that the thrombocytes play a large part in the destabilization of plaques [23]. Thrombocyte-related inflammatory response is believed to trigger acute coronary syndromes [24]. Understanding the inflammatory features of the thrombocytes led researchers to investigate the effects of the antithrombosis treatment on the inflammatory markers. Clopidogrel, with a proven efficacy after PCI, partly blocks the ADP receptor, but this blockage varies from case to case [25]. In addition to inhibition of the activation of the ADP-dependent thrombocyte activation, it is thought that clopidogrel may exert effects via other mechanisms. Zhihui *et al.* have demonstrated that, in cases with ACS, the sCD40 ligand secretion of the thrombocyte decreases significantly with clopidogrel therapy [26]. The expected anti-inflammatory effect could not be achieved with other antithrombotic agents. It was proven that aspirin does not reduce sCD40 ligand secretion [27-28]. Chew *et al.* have demonstrated a decrease in hsCRP levels with clopidogrel treatment before PCI [29]. Another study investigating the CD40 and CRP levels demonstrated that the CD40 and CRP levels drop with clopidogrel therapy after PCI [30]. In contrast, a number of studies have revealed contradictory results. In a study by Azar *et al.*, it has been shown that clopidogrel has no effect on hsCRP levels with stable coronary artery disease [31]. Many studies concluded that clopidogrel reduced ischemic complications after PCI in ACS [11]. Woodward *et al.* treated patients

with acute myocardial infarction with a daily dose of 75 mg clopidogrel for 6 months and observed the change in their CRP levels. In this study, the decrease of CRP levels was observed at the first month and the decrease was even stronger at the sixth month [32]. It was suggested that inflammation plays an important role in cases where PCI was performed, both with regards to the success of the operation, as well as during the follow up period. A body of evidence showed that, in cases where PCI was performed, inflammation plays a key role on the development of ACS and restenosis [33,34]. Some studies determined the CRP levels before the application to be a strong marker for the early and late development of cardiac events and stenosis [6,8,34]. In contrast to bare metal stents, in DES, depending on the medication or polymer endued on the stent due to delayed healing on the artery wall, an accumulation of fibrin, eosinophil and lymphocytes was reported. It has been demonstrated that inflammation delays endothelialization and continues even as long as 220 days after the stent implantation [35]. In one study published recently, it was demonstrated that an increase in intimal hyperplasia was related to an increase in the inflammatory response, as measured by hsCRP [36]. Summary sentence here

The limited number of patients was the most important limitation of our study. A second limitation was that inflammatory markers other than CRP were not measured. A third limitation was the relatively short duration of the follow-up period (6 months). Therefore, large and long-term follow-up studies are needed.

Conclusions

In our study, it was established that clopidogrel decreases hsCRP levels following stent implantation relative to non-clopidogrel-treated patients, and that the extent of decrease correlated with the duration of the clopidogrel therapy. We believe that the anti-inflammatory effect of clopidogrel is proven, as demonstrated by the decrease in the hsCRP levels. As the anti-inflammatory effect of clopidogrel is dependent on the duration of the drug therapy, we believe that a longer duration of therapy with clopidogrel might be beneficial. Further research work is needed to demonstrate the anti-inflammatory effects of clopidogrel, and the correlation between those effects, clinical outcomes and the duration of the therapy.

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