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Oral antiplatelet therapies have no effect on circulating levels of RANTES in patients with coronary artery disease

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RANTES (Regulated upon Activation Normal T-cell Expressed and Secreted) belongs to the family of C−C chemokines and attracts macrophages and lymphocytes to chronic inflammatory sites [1, 2]. Elevated circulating levels have been found in inflammatory, immune-mediated disease and atherosclerosis [3–7]. This chemokine bridges macrophage adhesion to endothelial cell in atherosclerotic vessels. Thus, a link between atherosclerosis and inflammatory mediators has been detected. Thrombin-activated platelets produce and release RANTES [8–10]. Therefore this study aimed to determine whether platelet inhibition by different antiplatelet therapies in patients with coronary artery disease has a significant role on the serum concentration of RANTES.

The study population consisted of 46 patients (mean age 65 ± 11 years, 16 women, 30 men) with coronary artery disease, diagnosed by cardiac catheterization. 100 mg of aspirin was given to 17 patients, 75 mg of clopidogrel to 10 patients, the combination of both to 11 patients and non-medication to 8 patients (controls). The non-medication group included patients who were admitted to hospital with a history of unstable angina pectoris. None of them had taken antiplatelet drugs prior to the collection of blood samples. Cardiac catheterization established the diagnosis of coronary artery disease in the course of their hospital stay. Clinical characteristics of the four study groups are shown in Table 1.

Blood samples for the measurement of RANTES were collected after the patients had taken their medication for a period of 13 ± 2 days. The blood samples of the non-medication group were drawn after admission to hospital and prior to administering antiplatelet therapy. Following venepuncture of the antecubital vein, serum was collected on ice into serum separator tubes and was centrifuged at $1000 \times g$ for 10 min within 30 min. Then the samples were aliquoted and stored at $-80\,^{\circ}C$. For the quantitative determination of human RANTES concentrations in serum we used the Quantikine immunoassay (R & D Systems, Minneapolis, USA).

Changes in the quantitative parameters of the groups were assessed using the one-way ANOVA. If the Levene's test assumed that the variances of the groups were all equal the one-way ANOVA post hoc test LSD was used to determine which groups differed. Small significance values $P < 0.05$ indicated group differences. All data were expressed as mean $\pm$ standard deviation. All calculations were done with SPSS for Windows (Version 11.0.1, SPSS, Chicago, USA).

The clinical characteristics of the groups did not reveal any significant differences (Table 1). We found a mean RANTES value of 20.1 ± 19.9 ng/ml for the non-medication group. It did not differ significantly from the other three groups. The lowest concentration of RANTES was shown in the clopidogrel group and the highest in the aspirin group (Table 2).

Increased circulating levels of C−C chemokines have been demonstrated in inflammatory and immune-mediated disease [3–7]. Atherosclerosis is seen as a chronic inflammatory disease in which an over-recrutiment of macrophages and T-lymphocytes may be central to the pathogenesis [11]. RANTES mRNA and protein is present in atherosclerotic lesions but not in normal coronary arteries [12]. In inflamed tissues chemokines including RANTES have been identified in many different cell types [13]. A recent study showed that chemokines produced by endothelial cell cross talk with inflammatory cells and may aggrevate the inflammatory process by inducing the secretion of more chemokines [14]. Thrombin-activated platelets can also induce the expression and secretion of C−C chemokines [15]. Lately von Hundelshausen indicated that RANTES can participate in macrophage adhesion to endothelial cell by functioning as a bridge [9].

The non-medication group in our study exclusively consisted of patients with unstable angina. Nomura et al. found a higher RANTES level of 41 ± 5 ng/ml in their control group including patients with cardiovascular, neurological and autoimmune disease [6]. Otherwise Aukrust et al. revealed in their control group including healthy individuals a lower RANTES level of 11.2 ng/ml [7]. The RANTES level of our non-medication group with coronary artery disease lied in between (20.1 ng/ml) and was comparable to the level that Aukrust published for patients with congestive heart failure (22.2 ng/ml).

In our study antiplatelet drugs like aspirin, an irreversibly inhibitor of the cyclooxygenase-1 whithin platelets, and/or

Table 1: Clinical characteristics of patients and controls

	ASS	Clopidogrel	ASS + Clopidogrel	Non-medication	P Value
Sex (female : male)	4/13	4/6	3/8	5/3	NS
BMI (kg/m^2)	28.2 ± 5	27.7 ± 3	27.1 ± 3	30.0 ± 7	NS
HR (beats/min)	72 ± 13	74 ± 7	72 ± 7	76 ± 11	NS
SBP (mmHg)	128 ± 14	139 ± 18	135 ± 21	140 ± 24	NS
DBP (mmHg)	76 ± 13	78 ± 17	76 ± 11	80 ± 7	NS
EF (%)	56 ± 15	66 ± 11	52 ± 19	61 ± 22	NS
CRP (µg/dl)	11 ± 14	9 ± 10	14 ± 13	11 ± 10	NS
Leucocyte ($10^3/mm^3$)	8 ± 3	8 ± 4	8 ± 2	9 ± 2	NS
PLT ($10^3/mm^3$)	254 ± 44	243 ± 68	267 ± 79	262 ± 68	NS
T-CHOL (mg/dl)	4.9 ± 1.6	5.0 ± 1.1	5.1 ± 1.4	5.7 ± 1.2	NS
TG (mg/dl)	2.3 ± 1.9	1.4 ± 0.7	1.3 ± 0.4	1.8 ± 0.8	NS
HBA_{1c} (%)	6.4 ± 1.2	6.1 ± 0.9	5.9 ± 0.9	6.1 ± 1.0	NS

Abbreviations: ASS = aspirin; BMI = body mass index; HR = heart rate; SBP = systolic blood pressure; DBP = diastolic blood pressure; CRP = C-reactive protein; EF = ejection fraction; PLT = platelet count; T-CHOL = total cholesterol; TG = triglycerides; HBA_{1c} = haemoglobin A_{1c}; Values are the means $\pm$ standard deviation; NS = not significant

Table 2: Results

Groups	RANTES (ng/ml)	P Value
ASS (n = 17)	28.5 ± 16.8	0.191
Clopidogrel (n = 10)	16.2 ± 10.02	0.579
ASS + clopidogrel (n = 11)	17.7 ± 11.9	0.731
Non-medication (n = 8)	20.1 ± 17.9	–

Values are the means ± standard deviation; P value lists the probability that the differences between each treatment group mean and non-medication mean is zero

clopidogrel, an adenosine diphosphate (ADP) antagonist, did not play a significant role in the manipulation of RANTES in comparison to the control group.

In contrast to our results Nomura et al. have shown that elevated RANTES level significantly decreased in diabetic patients under therapy with ticlopidine, an adenosine diphosphate (ADP) antagonist. The level of 124 ± 11 ng/ml before fell to 86 ± ng/ml after treatment [6]. However as compared to the present investigation the RANTES levels in that subset of patients were 5 times higher.

Aukrust et al. showed that serum levels of RANTES were significantly elevated only in patients with most severe heart failure (NYHA class IV). In patients with heart failure (NYHA class II and III) due to coronary artery disease, idiopathic dilated cardiomyopathy, valvular or congenital heart disease they found elevated RANTES levels compared to healthy control subjects. But the differences were not significant [7]. In our study none of the patients belonged to NYHA class IV. Therefore our results are in agreement with this trial. In an editorial Libby and Simon have pointed out that we can regard anti-inflammatory therapies as potential antithrombotic and/or vice versa [10].

Our study shows that (1) in patients with coronary artery disease without heart failure RANTES levels are in a near normal range and (2) cannot be affected by antiplatelet therapy, indicating that platelet activation is not a result of RANTES secretion.

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Retraction

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Two new chlorinated amides from *Nicotiana glauca* R. Graham

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This paper, published in PHARMAZIE 57, 206–208 (2002) is officially retracted by the editors and has been removed from indexes and databases. The results presented there are invalid as they were based on a serious scientific misunderstanding.