

The significance of platelet activation in rheumatoid arthritis

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Abstract We evaluated the significance of platelet activation in patients with rheumatoid arthritis (RA). The expression of CD62P and CD63 by platelets was determined using flow cytometry in 18 active RA patients, 10 remission RA and 15 normal controls. Meanwhile, the erythrocyte sedimentation rate (ESR) and C-reactive protein was also determined in all groups. The expression of CD62P in active RA patients ($11.88 \pm 2.47\%$) was significantly higher than that in remission RA group ($2.85 \pm 1.60\%$; $P < 0.01$) and control group ($2.78 \pm 1.04\%$; $P < 0.01$). The expression of CD63 in active RA patients ($9.90 \pm 3.02\%$) was significantly higher than that in remission RA group ($4.11 \pm 2.00\%$; $P < 0.01$) and control group ($4.13 \pm 1.85\%$; $P < 0.01$). The level of CRP (54.33 ± 23.35 mg/l) and ESR (86.06 ± 33.67 mm/h) in active RA patients was higher than that in remission RA group (2.55 ± 1.01 mg/l, 14.70 ± 4.57 mm/h; $P < 0.01$ for both) and normal control group (3.21 ± 2.18 mg/l, 12.25 ± 5.05 mm/h; $P < 0.01$ for both). There was a positive correlation between CD62P and ESR ($r = 0.5224$, $P < 0.01$) and also a positive correlation between CD62P and CRP ($r = 0.7048$, $P < 0.01$) as well as between CD63 and ESR ($r = 0.4476$, $P < 0.05$) but no correlation

between CD63 and CRP. Platelet activation may be a sign of RA exacerbation.

Keywords C-reactive protein · Platelet activation · Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of unknown cause, which primarily affects the peripheral joints in a symmetric pattern. Patients with RA may present constitutional symptoms such as fatigue, malaise, and morning stiffness [1–3]. Extra-articular involvements of the skin, heart, lungs, and eyes can be significant. RA can result in joint destruction and thus often leads to considerable morbidity and mortality. It is believed that an RA patient may present an increasing account of platelets during active stages and that will decline in number with the remission of the inflammation [4–6]. But the activation status of the platelets is unknown. In our study, the expression of CD62P and CD63 by platelets was determined using flow cytometry to evaluate the relation between activation of platelets and the progression of RA.

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Materials and methods

Diagnosis criteria

The American College of Rheumatology developed the following criteria for the classification of RA: morning stiffness, arthritis of three or more joint areas, arthritis of hand joints of at least one area swollen (in a wrist, metacarpophalangeal, or proximal interphalangeal joint),

symmetric arthritis with simultaneous involvement of the same joint areas on both sides of the body, rheumatoid nodules, serum RF, and radiographic changes. A patient can be diagnosed to have RA if four of the seven criteria are met.

Clinical information

Eighteen active RA patients and 15 remission RA patients were enrolled in this study during 2004–2005. There were 6 men and 12 women aged from 27 to 78 years old (averagely 42 years old) in acute RA patients group. There were four men and six women aged from 23 to 68 years old (averagely 41 years old) in remission RA patients group. All the patients according to the criteria were excluded the possibility of a heart disease, hepatic disease, thrombosis, and kidney disease. There were six men and nine women volunteers aged from 22 to 56 years old (averagely 39 years old) in control group. The therapy regime was maxtrex 7.5 mg qw po and salazosulfapyridine 1.0 bid po in all patients, Mobic 7.5 mg bid po in ten patients, Celecoxib 100 mg bid po in two patients, and Arava 20 mg qd po in two patients additionally.

Measurements

The fasting blood samples were collected in test tubes with 2% ethylebediaminetetraacetic acid. After the disposal with flourescein isothiocyanate (FITC)-labeled monoclonal antibody bought from HUAMEI, the expression of CD62P and CD63 by platelets was determined using flow cytometry in 30 min. At the same time C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) was determined in all groups.

Statistical analysis

All results were expressed as means±standard deviation. Comparisons between two groups were performed using SAS software (ver 6.04) and Student’s *t* test. *P* values of less than 0.05 were considered to be significant.

Table 1 The Expression of CD62P ($\bar{X} \pm s$)

Group	Number	CD62P (%)
Control	15	2.78±1.04
Remission RA	10	2.85±1.60 ^a
Active RA	18	11.88±.47 ^b

^a Compared with the control, *P*>0.05.

^b Compared with the remission RA group, *P*<0.01; compared with the control, *P*<0.01.

Table 2 The Expression of CD63 ($\bar{X} \pm s$)

Group	Number	CD63 (%)
Control	15	4.13±1.85
Remission RA	10	4.11±2.00 ^a
Active RA	18	9.90±3.02 ^b

^a Compared with the control, *P*>0.05.

^b Compared with the remission RA group, *P*<0.01; compared with the control, *P*<0.01.

Results

The expression of CD62P by platelets

Compared with the control group patients, the expression of CD62P by platelets in the active RA patients group was rather higher, whereas the expression of CD62P in remission RA patients was much lower than that in active patients (*P*<0.01) but had no statistical difference from that in the control (*P*>0.05; Table 1).

The expression of CD63 by platelets

The expression of CD63 in the control group was very low but higher in active RA patients. But the expression of CD62P in remission RA patients was much lower than that in active patients (*P*<0.01) and had no difference from that in the control (*P*>0.05; Table 2).

CRP and ESR

CRP and ESR in the control group was very low but higher in active RA patients. In addition, CRP and ESR in remission RA patients was much lower than that in active patients (*P*<0.01) and had no difference from that in the control (*P*>0.05; Table 3).

Table 3 ESR and CRP ($\bar{X} \pm s$)

Group	Number	CRP(mg/l)	ESR(mm/h)
Control	15	3.21±2.18	12.25±5.05
Remission RA	10	2.55±1.01 ^a	14.70±4.57 ^c
Active RA	18	54.33±23.35 ^b	86.06±33.67 ^d

^a Compared with the control, *P*>0.05.

^b Compared with remission RA group, *P*<0.01. Compared with the control, *P*<0.01.

^c Compared with the control, *P*>0.05.

^d Compared with remission RA group, *P*<0.01. Compared with the control, *P*<0.01.

The correlation between CD62P, CD63 and CRP, ESR in active RA patients

There were positive correlations between the expression of CD62P and CRP, ESR. The correlation coefficients were 0.5224 ($P < 0.01$) and 0.7048 ($P < 0.01$), respectively. There were positive correlations between the expression of CD63 and ESR, and the correlation coefficient was 0.4476 ($P < 0.05$). The correlation coefficient between CD63 and CRP was -0.0620 ($P > 0.05$), but it had no statistical significance.

Discussion

In clinical practice, it is very important to evaluate the curative effect and treatment guidance by the indicator of ESR and CRP. And they are undoubtedly considered to be two classical signs of RA [7–10]. As is known, the account of platelets correlates with the rheumatoid activity, but there are still no studies on the correlations between platelet activation and rheumatoid activity. When platelets were activated, CD62P could be found in the secretory granules of platelets and CD63, a lysosome membrane glycoprotein, could also be expressed on the platelets' surface. CD63 and CD62P were considered as the symbols of platelet activation, and the latter was the gold standard [11–13].

CD62P, a 140-kDa protein, is the largest member of the known selectin families. It contains nine consensus repeats and extends approximately 40 nm from the endothelial surface. Other names of CD62P include P-selectin, granule membrane protein 140, and platelet activation-dependent granule to external membrane protein. CD62P is expressed in α -granules of activated platelets and granules of endothelial cells. Within a few minutes of stimulation by inflammatory mediators such as histamine, thrombin, or phorbol esters (it doesn't belong to the inflammatory mediators), the endothelial cells would express CD62P on the surface. Expression of CD62P also occurs in the surgical trauma endured during preparation of the tissues for intravital microscopy. The expression pattern of CD62P is short-lived, reaching its peak only in 10 min. Additional synthesis of CD62P is brought about within 2 h stimulation with cytokines such as interleukin-1 or tumor necrosis factor α . The primary ligand for CD62P is PSGL-1, which is constitutively found on all leukocytes. Other ligands for CD62P include CD24 and uncharacterized ligands. The transient interactions between CD62P and PSGL-1 cause leukocytes to roll along the venular endothelium. Accordingly, CD62P is largely responsible for the rolling phase of the leukocyte adhesion cascade. At the same time, CD62P is one of the causes that make inflammation arising and lasting. Therefore, it is also a symbol of chronic inflammations or inflammation activation [14–16].

In this study, we found that CD62P and CD63 were over expressed on the surface of platelets, which implied platelet activation. Markers of inflammation, such as ESR and CRP are associated with disease activity. Additionally, the CRP value over time correlates with radiographic progression. From the results, it could be seen that the expression of CD62P was higher in acute RA group than that in remission group, which assimilates to ESR or CRP. CD62P value positively correlated with ESR ($r = 0.5224$, $P < 0.01$) and CRP ($r = 0.7048$, $P < 0.01$). Why was the correlation coefficient between CD62P and CRP larger than that between CD62P and ESR? It may be caused of which ESR was affected by a lot of factors, such as infection, tumor, dislipidemia, etc. The expression of CD63 in acute RA group was higher than that in remission group, but there was no statistical difference between remission group and normal control group. CD63 value positively correlated with ESR ($r = 0.4776$, $P < 0.05$), but it didn't correlate with CRP. In this study, compared with CD63, it is more sensitive to predict inflammation activity by the CD62P. We deduced that the over-expression of CD62P in active RA patients may be the result of platelet activation, and it also could be a novel marker of rheumatoid activity. Additionally, the over-expression of CD62P could be an aftereffect of the disease activity. The real mechanism needs to be further studied.

At present, the estimation of RA severity depends on morning stiffness, articulation's swollenness and pain, synovitis, ESR and CRP, among which ESR and CRP are two important impersonal laboratory markers but are affected by various factors. As a marker of inflammation activity, could CD62P become a foundation that the RA activity was judged on? It needs more clinical validations performed.

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