Age-Dependent Decrease in Platelet Aggregation in Rats

Kouichi OSHIMA, Tadanori MORIKAWA, and Masaki HAGIWARA*

Fuji Chemical Industries Ltd., 530 Chokeiji, Takaoka, Toyama 933, Japan.
Received January 24, 1994; accepted April 1, 1994

The degree of platelet aggregation induced by ADP and collagen was examined in platelet-rich plasma from 7- to 90-week-old rats. The aggregation induced by ADP was unchanged at 90 weeks. Collagen-induced aggregation decreased remarkably at 60 and 90 weeks. Therefore, collagen-induced platelet activation is highly age-dependent, with rats of advanced age showing a lower response than younger rats.

Keywords platelet aggregation; collagen; ADP; age

The rat is one of the small mammals used very often in a variety of experimental studies. In addition, relatively young rats are used because of their low cost and easy handling. However, experimental studies of human thrombotic diseases such as myocardial infarction, cerebral thrombosis and disseminated intravascular coagulation should sometimes be performed in aged animals, since these diseases are highly age-dependent with higher incidence in the elderly. In addition, it is well known that the aging process involves an imbalance or a reduction in body functions. We have previously obtained evidence that the effect of the thyrotropin-releasing hormone on the central nervous system decreases in middle-aged rats (50–60 weeks). An age-dependent increase in thrombosis might be due to changes in platelet functions, blood coagulation factors, or fibrinolysis systems. In order to study thrombosis using aged rats, it is necessary in the first instance to have basic information regarding the influences of aging on blood coagulation in rats. Many studies on age-related changes in platelet function have been described, but there are few reports about the age-dependent changes in platelet aggregation in rats. A number of reports of platelet aggregation induced by ADP, collagen, and other aggregants have described enhanced functions and increased sensitivity of platelets taken from elderly people (Johnson et al., Scarabin and Samama, Chao et al., Yokoyama et al., Yamanishi et al., Kasjanovova and Balaz). However, the evidence provided in this paper indicates that while the degree of platelet aggregation is age-dependent, it is lower in old rats.

MATERIALS AND METHODS

Male Wistar-ST rats (obtained from Sankyo Lab. Co., Ltd.) were housed for 1 to 90 weeks in our animal house prior to the experiments. They were kept at a constant temperature (23 ± 2°C) under a controlled 12-h light: dark cycle (lights turned on at 6 a.m.). Blood (9 parts blood: 1 part 3.3% trisodium citrate) was collected from the abdominal aorta of rats anesthetized with ether. The blood was centrifuged at room temperature for 10 min at 150 g and 1670 g to obtain platelet-rich plasma (PRP) and platelet-poor plasma (PPP), respectively. Platelet numbers were adjusted to 5 x 10^5/mm^3. Platelet aggregation was induced in PRP, at 37°C, in an aggregometer (Hema Tracer (NBS, Tokyo)). Aggregation was carried out in the 4-channel aggregometer with simultaneous incubation of 4 concentrations of stimulants (ADP and collagen; Horm). Cuvettes containing 200 µl of PRP were preincubated for 3 min prior to the addition of the stimulants. The degree of aggregation was expressed as a maximum percentage during 7 and 13 min periods after adding ADP and collagen, respectively. The difference in optical density between PPP and PRP was defined as 100%. Two concentrations of stimulants were selected for each sample; ADP (2.5 and 5 µM) and collagen (5 and 10 µg/ml). The results were expressed as means ± S.E. The statistical significance of results was determined using the multiple-comparison test (Bonferroni) after a one-way variance analysis.

RESULTS AND DISCUSSION

ADP and collagen induced platelet aggregation in a concentration-related manner in the PRP of 7-week-old rats. The maximum percentage aggregation by ADP was 28.9 ± 3.0, 43.4 ± 2.2, 52.6 ± 2.2 and 58.4 ± 1.7 at concentrations of 1.25, 2.5, 5 and 10 µM (n = 7), respectively. In collagen the corresponding figures were 17.9 ± 10.0, 55.6 ± 4.2, 65.9 ± 1.3 and 69.4 ± 1.4 at 2.5, 5, 10 and 20 µg/ml (n = 8), respectively. Collagen and ADP are platelet agonists used very often in the primary screening of potential antiplatelet compounds. Therefore, in this study we chose concentrations of collagen (5, 10 µg/ml) and ADP (2.5, 5 µM) based on their concentration response described above and examined the degree of platelet aggregation induced by them in aged rats.

Table I summarizes the degree of platelet aggregation induced by ADP and collagen in the PRP of 7- to 90-week-old rats. The aggregation induced by 5 µM ADP was unchanged at 90 weeks, but that induced by 2.5 µM decreased slightly, although not significantly, at 90 weeks. Collagen-induced aggregation induced by 10 µg/ml decreased markedly at 90 weeks and that induced by 5 µg/ml already showed a decrease, although not significantly, at 15 weeks. At 60 and 90 weeks, the aggregation produced by 5 µg/ml collagen decreased significantly.

Thrombotic diseases depend on platelet aggregation, blood coagulation factors, fibrinolytic components and changes in the blood vessels such as atherosclerosis. Although the study of only one factor, platelet aggre-
Table I. Age-Dependent Changes in Platelet Aggregation in Rats

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>7</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP 2.5 μm</td>
<td>+2</td>
<td>+1</td>
<td>±3</td>
<td>+2</td>
<td>±4</td>
</tr>
<tr>
<td>5 μm</td>
<td>+1</td>
<td>+1</td>
<td>±3</td>
<td>±2</td>
<td>±2</td>
</tr>
<tr>
<td>Collagen</td>
<td>+3</td>
<td>±10</td>
<td>±8</td>
<td>±10</td>
<td>±11</td>
</tr>
</tbody>
</table>

Data are means ± S.E. of the percentage aggregation (%). a) p < 0.05, b) p < 0.001 vs. 7 weeks, c) p < 0.05 vs. 30 weeks (Bonferroni).

The response involving ADP. On the other hand, collagen produces a 2nd release from the alpha-granules in addition to release I. Furthermore, the release reaction of ADP is inhibited by indomethacin, a cyclooxygenase inhibitor, but that of collagen is not. The fact that platelet aggregation mechanisms differ between ADP and collagen may lead to the different responses to ADP and collagen seen in aged rats. However, the responses to platelet agonists vary depending on the concentration used. For example, Vericel et al. demonstrated an increase in the aggregation reaction of platelets from elderly people at extremely low concentrations of thrombin, but no age difference in platelet aggregation at normal concentrations of thrombin. Therefore, a concentration of ADP lower than 2.5 μm might produce low aggregation in aged rats.

The blood level of fibrinogen, one of the platelet aggregation factors, increases in elderly people, but it is not clear whether blood components influenced the collagen-induced aggregation in aged rats seen in this study. The observation that 5-HT release induced by collagen was lower in 52–60-week-old rats than in young rats has led to the suggestion that some functions of platelets change in aged rats.

The present study demonstrates that platelet aggregation activity decreases with advancing age, although further studies are required to examine the behavior of platelets from even older rats (over 90 weeks).

Acknowledgements The authors would like to thank Mr. David Horsley for comments about the English presentation of the manuscript.

REFERENCES