THE FUNCTIONAL AND BIOCHEMICAL CHANGES OF PLATELETS IN EXPERIMENTAL DECOMPRESSION SICKNESS OF RABBITS

BY

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ABSTRACT

The functional and biochemical changes of rabbit platelets were studied after an exposure to 6 ATA (atmosphere absolute) for 40 min (bottom time). Platelet counts significantly decreased after the decompression. Platelet aggregation induced by collagen was not changed. Although there was no change in the mode volume of platelets after the decompression, the transient appearance of circulating smaller or fragmented platelets suggested a random over-destruction of platelets. Whole and releasable adenine nucleotide contents of platelets were decreased significantly after the decompression. There were no significant changes in cytoplasmic adenine nucleotide contents. Therefore, in decompression sickness, the circulating platelets behaved similarly to those in acquired storage pool disease. Platelet thrombi were found in the pulmonary arteries. These findings suggest that circulating air-bubbles interact with platelets, causing the platelet release reaction, and these activated platelets participate in the formation of thrombi in experimental decompression sickness.

Key words: acquired storage pool disease, decompression sickness, platelets, adenine nucleotides

INTRODUCTION

The pathological cause and abnormality in decompression sickness is principally bubble formation by supersaturated inert gas in the circulation and tissues by pressure exposure and inadequate decompression (Bert [1]). It is known that decompression sickness is associated with coagulation abnormalities (Swindle [2]; Carson [3]). Philp et al. [4] suggested that there were similarities between decompression sickness and disseminated intravascular coagulation (DIC). They [5, 6] also discovered the effectiveness of antiplatelet drugs such as aspirin in decompression sickness. Since these results suggest that platelets may have an important role in the genesis of this disorder, the functional and biochemical changes of platelets in experimental decompression sickness of rabbits were studied.

MATERIALS AND METHODS

I. Experimental procedures

Twenty four male albino rabbits weighing from 2.8 to 3.4 kg were used. After intraperitoneal injection of 40 mg/kg body weight of pentobarbital (Nembutal, Abbott, USA), the femoral arteries were exposed bilaterally for later blood samplings. As controls, blood samples were taken from a central ear artery before the compression. The animals

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were kept in a hyperbaric chamber (Ishiyama [7]). The pressure was increased at a rate of 1 ATA (atmosphere absolute) per min for 5 min (compression). Then the rabbit was exposed to 6 ATA for 40 min (bottom time), and the pressure was decreased at the same rate (decompression) (Fig. 1). Immediately after the decompression, an elastic catheter filled with heparinized saline to prevent coagulation was inserted into the femoral artery for blood sampling. Respiratory rates and heart rates were continuously monitored during the experiment.

II. Platelet study
A. Counts and volumes

Before the compression, blood (0.9 ml) was drawn from the central ear artery into a disposable plastic syringe containing 0.1 ml of sodium citrate solution. After the decompression, the same volume of blood was drawn from the femoral artery through the catheter at intervals of 5 min. The citrated blood was centrifuged at 800×g for 15 min at room temperature to obtain platelet rich plasma (PRP). Platelet counts and volumes were determined by a Coulter counter (Model ZB 1) and Coulter channelizer (C-1000 with a 30 μ aperture tube, Hialeah, Florida, USA) (Yamazaki et al. [8]).

B. Platelet aggregation

Before the compression, blood (9 ml) was drawn from a central ear artery into a disposable plastic syringe containing 1 ml of sodium citrate solution. At 30 min after the decompression, the same volume of blood was drawn from the femoral artery. Platelet counts in PRP were adjusted to 500,000/μl by autologous plasma. Collagen (Collagen-reagent Horm, Hormon-Chemie, Munich, Germany) at final concentrations of 5, 10, and 30 μg/ml was used as the aggregation inducer. Platelet aggregation was measured with an aggregometer (Model DP-247E, Sienco Co., Morrison, Colo., USA).

The lag time, initial velocity and maximum aggregation were measured according to the method of Yamazaki et al. [9].

C. Adenine nucleotides in platelets

An equal volume of cold EDTA (ethylenediaminetetraacetic acid)-ethanol solution (1 vol. 100 mM EDTA and 9 vol. 99.5% ethanol) was added to each of the above samples of PRP. After keeping this mixture at 4°C for 30 min, the supernatant was obtained by centrifugation at 10,000×g for 2 min using a Fisher centrifuge and stored at −80°C until the determination of whole platelet ATP (adenosine triphosphate) and ADP adenosine diphosphate) contents.

The amounts of ATP and ADP released from platelets were measured as follows: at 5 min after the aggregation induced by 50 μg/ml collagen, the PRP-collagen mixture was centrifuged at 10,000×g for 1 min, and the supernatant containing released adenine nucleotides was removed. An equal volume of cold EDTA-ethanol solution was added to the supernatant, and the mixture was kept at 4°C for 30 min. After centrifuging the
mixture at 10,000×g for 2 min, the supernatant was stored at −80°C.

Amounts of whole and released adenine nucleotides in the samples obtained as described above were measured by the firefly luciferine-luciferase method (Holmsen et al. [10]) using an ATP photometer (JRB, La Jolla, Cal, USA).

Amounts of cytoplasmic adenine nucleotides were defined as the whole platelet adenine nucleotides minus the released adenine nucleotides.

III. Histological examination

For the histological examinations, immediately after the cessation of breathing, the rabbits were perfused with saline containing 10% formalin through the femoral artery. As the control, another three rabbits were sacrificed by KCL injection, perfused and histologically examined.

IV. Statistical examination

The matched pairs method was used.

RESULTS

I. Respiratory and heart rates

Figure 2 shows the observed changes in respiratory rates of six rabbits. All rabbits died within 44 min after the decompression due to apnea. The intervals from the decompression to the cessation of breathing varied between 5 and 44 min. At about 10 min after the decompression, the respiratory rates increased rapidly to about twice the pre-compression values and then decreased. It was noted that cessation of breathing always preceded heart arrest. In contrast to respiratory rates, heart rates were not changed markedly until the cessation of breathing (Fig. 3).

II. Platelet counts

Figure 4 shows the changes in platelet counts of six rabbits observed in the experimental decompression sickness. The abscissa indicates time in minutes after the decompression, and the ordinate is the percentage changes in platelet count, taking the pre-compression value as 100%. Platelet counts decreased during the time course of the experiment. A regression line can be drawn

![Fig. 2. Respiratory Rates in Experimental Decompression Sickness](image-url)

The abscissa indicates time after the decompression, and the ordinate indicates the respiratory rate as a percent of the pre-compression value. At about 10 min after the decompression, respiratory rates increased rapidly to about twice the pre-compression values and then decreased.
between the changes in platelet count and the period after the decompression ($Y=100.2-0.8X$, $r=-0.87578$, $n=43$, $p<0.001$). The platelet counts measured just prior to the apnea were 56 to 72% (65.7±6.13% in M±SD) of the pre-compression value and showed a statistically significant difference compared to the pre-compression value ($p<0.05$).

III. Platelet volume
Changes in platelet volume induced by the decompression were observed in six rabbits. A typical volume distribution before and after decompression is shown in Fig. 5. The mode volume of platelets tended to shift slightly to the right, although the change was not significant. In the smaller volume portion of the curve, from channel zero to channel 15, there occurred a small, about 3%, increase after the decompression. This suggested that there may be an increase of smaller platelets or platelet fragments.

However, two rabbits showed completely different distribution patterns of platelet volume 10 min after the decom-
Fig. 7. Scanning Electron Micrographs Before and 10 min After Decompression
a. Before the compression
b. Ten minutes after the decompression

There was an abnormal increase of small platelets (arrows) and formation of numerous pseudopods 10 min after the decompression.
pression (Fig. 6). They showed a relative decrease in platelets with normal volumes and an extreme increase in platelets with small volumes at 10 min after the decompression. Twenty minutes after the decompression, a distribution pattern similar to the control pattern was observed in these rabbits. Therefore, it was thought that these drastic changes in the volume distribution pattern occurred transiently.

Scanning electron micrographs confirmed the abnormal increase of small platelets with formation of numerous pseudopods 10 min after the decompression (Fig. 7).

IV. Platelet aggregation

Maximum aggregation, lag time and initial velocity in collagen-induced aggregation were measured in five rabbits. None of them showed any significant change (Fig. 8).

V. Adenine nucleotides in platelets

Changes in ATP and ADP contents in platelets after the decompression were observed in six rabbits. Before the compression, the whole ATP contents of platelets were 5.61±0.38 μmoles/10^{11} platelets (M±SD), and it significantly decreased to 5.10±0.57, 30 min after the decompression (p<0.05). The whole ADP contents of platelets were 1.40±0.41 before the compression and decreased to 1.05±0.85 after the decompression. However, these changes were not significant.

The amounts of ATP released from 10^{11} platelets after induction by collagen was 1.57±0.27 μmoles before the compression and 0.89±0.52 after the decompression; these values were significantly different (p<0.05). The amounts of released ADP tended to decrease from 0.87±0.28 before the compression to 0.67±0.30 after the decompression, although it was not significant at the level of p<0.05.

Amounts of cytoplasmic ATP were 4.04±0.25 μmoles/10^{11} platelets before the compression and 4.22±0.30 after the decompression. Amounts of cytoplasmic ADP were 0.61±0.14 before the compression and 0.62±0.41 after the decompression. There were no significant differences (Table 1).

VI. Histological changes
Fig. 9. Histological Changes in Lung in Experimental Decompression Sickness
a. Thrombus formation ×400, HE (hematoxylin eosin) stain
b. Fibrin fibers are found in the thrombus. ×400, PTAH (phosphotungstic acid-hematoxylin) stain
c. Dilatation of perivascular lymph vessels. ×400, HE stain
d. Thrombi which mainly consist of platelet aggregates and are surrounded by granulocytes and monocytes. ×400, MSB (martius-scarlet-blue) stain
Table 1. Adenine Nucleotides in Platelets
(μmoles/10^11 Platelets) in Experimental
Decompression Sickness
(n=6, M±SD)

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<tr>
<td>Whole</td>
<td>5.61±0.38</td>
<td>5.10±0.57*</td>
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<tr>
<td>Releasable</td>
<td>1.57±0.27</td>
<td>0.89±0.52*</td>
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<tr>
<td>Cytoplasmic</td>
<td>4.04±0.25</td>
<td>4.22±0.30</td>
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*p<0.05, as compared to before

Histological changes induced by the decompression were examined in six rabbits. The findings were compared to the control with the sham operation (three rabbits). In all cases, numerous microthrombi mainly consisting of platelet aggregates mixed with fibrin, some of which were surrounded by granulocytes and monocytes, were found in the medium-sized and large arteries of the lung. Dilatation of perivascular lymph vessels was also observed in the lungs (Fig. 9). Constriction of medium-sized arteries was suggested by the presence of vascular wall thickening and the dilatation of perivascular lymph vessels. Vacuolar degeneration was present in the liver cells.

Discussion

In 1878, Bert [1] established that gas embolism is the principal pathology of decompression sickness. Since the 1930's, several workers (Swindle [2]; Carson [3]) have reported the presence of hemostatic abnormalities in decompression sickness. Philp et al. [11–13] have observed thrombocytopenia and platelet microthrombi in lung vessels in the experimental decompression sickness of rats. They suggested that platelets recognized the bubble as a foreign surface and adhered to it. At the same time, antiplatelet drugs such as aspirin have been reported to be effective in the treatment of decompression sickness (Philp et al. [5, 6]). Therefore, it has been hypothesized that the platelet plays an important role in decompression sickness. Furthermore, Philp et al. [12] reported that platelet counts decreased 24–32% in experimental decompression sickness of rats. Our study with rabbits also showed that platelet counts continuously decreased with time after the decompression, suggesting the abnormal destruction of platelets.

Although we did not observe the decrease in platelet volume distribution as a whole, there occurred a small increase in the smallest volume range, suggesting that there may be an increase in smaller platelets or platelet fragments. Moreover, the transient appearance of circulating smaller platelets or fragmented platelets was evidenced in at least two experimental rabbits by platelet volume examination (Fig. 6) and by scanning electron micrographs (Fig. 7). A similar transient fragmentation of platelets has been observed clinically in patients with severe burns (Tanoue et al. [14]). In such cases, the reticulo-endothelial system is considered to be responsible for the removal of these fragments from the circulating blood. Although it is not determined whether these platelet fragments are caused by a shortened survival of platelets or they are produced in massive phagocytosis of damaged platelets, the presence of these fragments suggests the over-destruction of platelets. It also coincided with the thrombocytopenia observed after the decompression.

It has been reported that aggregation
induced by ADP was lowered in rats after moderate or severe decompression (Philp [11]). We used collagen as the aggregation inducer and measured maximum aggregation, lag time and initial velocity. However, none of these parameters were changed. Platelets have two pools of adenine nucleotides; one is a metabolic pool and the other is a storage pool. Adenine nucleotides in the metabolic pool (cytoplasmic adenine nucleotides) provide energy for cellular metabolism, and they are retained in the platelet during the release reaction. Adenine nucleotides in the storage pool (releasable adenine nucleotides) do not participate in cellular metabolism, but are released from platelets in the release reaction. This released ADP plays an important role in further aggregation (Holmsen et al. [15, 16]; White [17]). Our study showed that both whole ATP and releasable ATP contents of platelets decreased significantly after the decompression. Amounts of ADP in platelets also decreased, but not significantly. Amounts of cytoplasmic adenine nucleotides did not change.

Platelets have been reported to be heterogeneous in their volume, metabolic activity, function and other properties (Karpatkin [18–20]). The decrease in adenine nucleotide contents after the decompression suggests two possibilities: one is that platelets which have more adenine nucleotide contents are more easily consumed than those which have less adenine nucleotide contents, as suggested by Yamazaki et al. [8], and the other is that the whole platelet population is randomly activated and consumed, and the remaining platelets are those which were less activated and had a partial release reaction. Since we did not observe the decrease of platelet volume as a whole, the first possibility is less likely. Circulating platelets in decompression sickness are, therefore, similar to those in acquired storage pool disease (Fukami et al. [21]; Zahavi [22]) which has been recently described in clinical diseases, such as DIC (Pareti et al. [23]), idiopathic thrombocytopenic purpura (ITP) (Malpass et al. [24]), collagen disease (Zahavi et al. [25]) and vascular prostheses (Savage et al. [26]).

As suggested by Warren et al. [27], circulating bubbles that occur in decompression sickness will interact with both platelets and endothelium resulting in platelet activation and the exposure of the endothelium, respectively.

Activated platelets will be easily incorporated into the formation of thrombi, which were found mainly in the pulmonary arteries as shown in Fig. 9. They were often mixed with fibrin and surrounded by granulocytes and monocytes. Since aggregated platelets are known to release vasoactive substances such as thromboxane A₂, those vessels where thrombi were formed will be constricted by those substances and will contribute to the respiratory changes (Motomiya et al. [28]) that were seen in the experimental animals (Fig. 2).

Thus, platelets play an important role in decompression sickness. Therefore, inhibition of platelet activation by antiplatelet drugs could, at least partially, prevent the progress of decompression sickness.

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