Platelet Tocopherol Levels in Mucocutaneous Lymph Node Syndrome (MCLS: Kawasaki Disease)

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Summary Studies of changes in platelet tocopherol in 16 children with mucocutaneous lymph node syndrome (MCLS) were undertaken during the disease course.

1. Plasma tocopherol was lowest at the beginning of the disease (during the first 1 to 2 weeks), while it increased with improvement in symptoms at 4 to 5 weeks.

2. Platelet tocopherol however was lowest in the first 2 to 3 weeks, and at about the 4th week or later increased. When compared with normal levels, determined in 24 children without MCLS (5 to 14 years old), the lowest level seen throughout the disease course coincided with it. Thus, platelet tocopherol may be generally higher in MCLS.

3. There was little correlation between plasma and platelet tocopherol levels which were simultaneously assayed, \( r = 0.26, p < 0.1, n = 44 \), while, the correlation between platelet tocopherol and plasma tocopherol/total lipid ratio was increased significantly to 0.55 \( (p < 0.001, n = 38) \).

4. Individual patients in whom platelet tocopherol and plasma tocopherol/total lipid ratios were repeatedly assayed, were divided into two groups based on the Asai scoring, as a high risk group and a low risk group. No correlation was observed between the risk grades and either platelet tocopherol or tocopherol/total lipid ratios in plasma.

Key Words platelet tocopherol, plasma tocopherol, mucocutaneous lymph node syndrome (MCLS) (Kawasaki disease)

Mucocutaneous lymph node syndrome (MCLS) is an acute febrile illness of unknown etiology usually seen in infants and children, first described by Kawasaki in 1967 \( (1, 2) \). It is characterized by fever lasting one to two weeks not responding to antibiotic therapy; conjunctival congestion; red, swollen, fissured diffuse erythema of the lips and oral cavity; swollen hands and feet; a polymorphic rash; acute nonsuppurative swelling of cervical lymph nodes; leukocytosis and thrombocytosis. The most serious complication of MCLS is aneurysm formation in coronary
vessels (3, 4) that seems to be related to pathological changes occurring in the vascular wall and in platelets, resulting in vasculitis and thrombocytosis with possible hyperaggregability.

For treatment, aspirin is usually used for its anti-inflammatory action, including its inhibitory effect on prostaglandin synthesis (5, 6).

Vitamin E deficiency is known to reduce platelet life span and enhance the aggregation induced by ADP, epinephrine and collagen (7–9). Complete reversal of the platelet abnormalities occurs following the attainment of normal plasma vitamin E levels. These findings have also been confirmed by in vitro studies (10–12). From this standpoint, vitamin E has recently been used in MCLS as a therapeutic agent (13). However, there have been few reports examining vitamin E behavior in MCLS, while, to our knowledge, there have been no reports on platelet tocopherol changes.

In this study we examined changes in platelet tocopherol concentrations in MCLS patients and compared them with changes in plasma tocopherol.

**MATERIALS AND METHODS**

The subjects were 16 patients with MCLS admitted to our hospital during the experimental period from June 1, 1980 to May 31, 1981, who were all routinely treated with one or more anti-inflammatory drugs including aspirin, steroid hormones and flubioprofen as listed in Table 1, but were never given any vitamin E preparations. Asai scoring which is cited in this table, is very useful in assessing the

| No. | Sex  | Age   | Asai scoring | Therapy |
|-----|------|-------|--------------|---------|
| 1   | TM   | 1y6m  | 9            | ASA, SH |
| 2   | SD   | 4m    | 7            | ASA, SH |
| 3   | KM   | 2y0m  | 6            | ASA     |
| 4   | MY   | 1y6m  | 4            | ASA     |
| 5   | SS   | 1y11m | 1            | ASA     |
| 6   | NK   | 4y0m  | 1            | ASA     |
| 7   | YY   | 1y6m  | 1            | ASA     |
| 8   | KT   | 5y4m  | 1            | ASA     |
| 9   | YA   | 1y8m  | 1            | ASA     |
| 10  | AM   | 7y0m  | 7            | ASA, FP |
| 11  | MN   | 1y7m  | 5            | ASA     |
| 12  | EH   | 4y0m  | 4            | ASA     |
| 13  | TN   | 5y0m  | 2            | ASA     |
| 14  | MH   | 10m   | 1            | ASA     |
| 15  | HT   | 4m    | 1            | ASA     |
| 16  | MK   | 3y11m | 0            | ASA     |

ASA, aspirin; SH, steroid hormones; FP, flubioprofen.

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value of coronary angiography for detection of aneurysm formation in MCLS patients; the higher the score the greater the risk (14).

On the other hand, 24 children without MCLS, aged 5 to 14 years, were selected for estimation of normal levels of platelet tocopherol. They had recovered from acute illnesses, such as nephritis, respiratory infections, urinary tract infections, or anaphylactoid purpura, all of which did not affect platelet function and lipid metabolism. They were not completely age-matched to the children with MCLS examined in this study. Since alpha-tocopherol in plasma and erythrocytes did not significantly differ among children more than 2 months of age in our previous experience (15), fasting blood specimens were randomly collected to estimate normal levels from children who had recovered from their illnesses before discharge.

Blood was drawn after overnight fasting. Procedures for blood collection and cell separation were carried out using plastic ware. Platelets were isolated from 5 ml or more of blood collected in 3.8% sodium citrate (0.5 ml/5 ml blood), which was first spun at 200 × g for 10 min, and the platelet-rich plasma was then separated from RBC layer. The platelets were then pelleted by centrifugation at 800 × g for 10 min, and washed twice with 10 ml of normal saline. The washed platelet lower layer was sonicated at 20 Kc in 1.0 ml of saline solution added to 1.0 ml of tocol solution (2 μg/ml ethanol). This was used for determination of alpha-tocopherol and protein, while plasma was used for the determination of alpha-tocopherol and three major plasma lipids (cholesterol, triglycerides, and phospholipids).

Alpha-tocopherol, separated from the other analogues in platelets and plasma was analyzed by performance liquid chromatography using a slight modification of Ishibashi’s method (16) devised for determination of RBC tocopherols, as described in our previous paper (17). The recovery of authentic alpha-tocopherol added to the platelet solution was 90% or more. The values listed for tocopherol show only the alpha-form.

Total cholesterol, triglycerides and phospholipids were measured by the methods of Heiskell et al. (18), Allain et al. (19), and Takayama et al. (20), respectively. Total lipids were estimated by summing the three major lipids and protein content in platelets was assayed by the method of Lowry et al. (21).

RESULTS

1. Plasma tocopherol (Fig. 1)

Plasma tocopherol in patients with MCLS was lower at the early stage of disease (1 to 2 weeks), when compared with the normal level of 735 ± 48 μg/dl (M ± SEM) estimated from the controls in this study. The difference was however statistically not significant. During the course of the disease, plasma levels increased with an improvement in symptoms. The level at the 5th week of disease was significantly higher than that at the beginning (p < 0.05).
2. Plasma tocopherol/total lipid ratio (Fig. 2)

The plasma tocopherol/total lipid ratio has been proposed as an expression which represents the most reliable index of vitamin E status routinely available in humans\(^\text{(22–24)}\). The ratio was lowest at the 2nd week of the disease, 1.3, which increased thereafter and reached 2.2 at the 4th week. The difference in the ratio between the 2nd and 4th week was significant \((p<0.02)\). This showed that the available tocopherol was lower in the early stage of disease when compared with the recovery period.

3. Platelet tocopherol

Changes in platelet number are shown in Fig. 3 and the number was generally higher throughout the disease course, as compared with that of the control group \((237,000 \pm 47,000)\). Figure 4 shows changes in platelet tocopherol based on platelet
Fig. 3. Changes in platelet number during the course of MCLS (M ± SEM).

Fig. 4. Changes in platelet tocopherol levels during the course of MCLS (M ± SEM).

protein. Tocopherol in platelets decreased to its lowest level at the 2nd and 3rd week and then increased again thereafter. The normal level of platelet tocopherol was 0.24 ± 0.02 µg/mg protein in 24 children without MCLS. The lowest level during the disease course nearly coincided with the mean normal level and the tocopherol content in platelets in patients with MCLS was commonly higher throughout the disease course than that of the controls.

4. Relationship of tocopherol level in plasma and platelets

Changes in platelet tocopherol during the disease course were somewhat different from those in plasma. The correlation in tocopherol between plasma and platelets, which were simultaneously assayed, was poor \( r=0.26, p<0.1, n=44 \) as shown in Fig. 5. However, the correlation between platelet tocopherol and plasma tocopherol/total lipid ratio, increased significantly to 0.55 \( p<0.001, n=38 \), as shown in Fig. 6.

5. Changes in platelet tocopherol and tocopherol/total lipid ratio in plasma in individual cases with MCLS

Individual patients in whom platelet tocopherol and plasma tocopherol/total lipid ratio were assayed repeatedly during the disease course are shown in Figs. 6...
Fig. 5. Correlation between plasma tocopherol and platelet tocopherol.

Fig. 6. Correlation between platelet tocopherol and tocopherol/total lipid ratio in plasma in MCLS.

and 7, respectively. They were divided into two groups based on Asai scoring, a high risk group (more than 7 points of score) and a low risk group (less than 6 points). No difference was observed between the risk grades in either platelet tocopherol (Fig. 7) or tocopherol/total lipid ratios (Fig. 8).

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DISCUSSION

Increase in thrombocyte numbers during the course of MCLS is assumed to be related to an exaggeration of the vascular changes in coronary vessels followed by aneurysm. There have been several reports supporting this assumption, in which hyperaggregability of platelets occurred in MCLS (3, 4). Thrombus formation was also observed in coronary vessels accompanying aneurysms in all autopsied patients, in whom generalized vasculitis was also seen in the small and middle-sized arteries (26).

Since vitamin E is known to have an inhibitory effect on platelet aggregation (7-12), the use of vitamin E, in association with other anti-inflammatory drugs in MCLS may prevent thrombus formation. Our study shows that plasma tocopherol levels at the early active stage of disease were the lowest in
the normal range and increased to the mean normal level during recovery. This finding did not agree with Senaga's report (13), in which plasma tocopherol remained unchanged during the first 4 to 5 weeks of disease while tocopherol in HDL fractions decreased in the acute stage. Although the assessment of human vitamin E status from the clinical standpoint has generally relied on serum or plasma tocopherol concentration, there is some question about the validity of this relationship, since circulating tocopherol is closely related to plasma total lipids (22, 26–28). Tocopherol is widely assumed to have its biological function in biomembranes. From this point of view, the tocopherol/total lipid ratio in plasma has been proposed as the best index of vitamin E status routinely available (22–24).

We thus examined the tocopherol/total lipid ratio in plasma and directly measured platelet tocopherol levels. The tocopherol/total lipid ratio decreased in the early stage of disease, while platelet tocopherol levels were lowest in the first 2 to 3 weeks throughout the disease course. This indicates that tocopherol may be mainly consumed as the scavenger of free radicals which are generated in large amounts by the metabolic processes of inflammation (25, 26), since the first 3 weeks is the most active stage in MCLS. In addition, limitation of tocopherol intake due to impaired appetite may explain the relatively lower levels. However, the lowest concentration found in the course of disease coincides with the mean normal level observed in children without MCLS. This suggests that platelet tocopherol in MCLS is generally maintained at higher levels and helps to inhibit aggregation. The precise mechanism is however unknown.

Simultaneous determination of platelet and plasma tocopherol showed a better correlation between platelet tocopherol and tocopherol/total lipid ratio, than that between platelet tocopherol and plasma tocopherol alone, which indicates the reliability of the ratio as an index of vitamin E status.

The risk grade indicated by Asai scoring was not related to tocopherol status. In our 16 patients with MCLS, neither thrombus nor aneurysm was seen after precise examination by echocardiography and coronary angiography. Further studies of more severe cases are needed.

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