The present technique is best suited for routine qualitative assessment of CK-MB activity. Simple detection is adequate for most clinical conditions. The technique can be used to quantitate CK-MB activity with total CK activities of less than 150 U/liter, but suffers from a lack of precision.

References

Carotenoids in the Leukocytes of Carotenemic and Non-Carotenemic Individuals

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Leukocytes and leucocyte membranes isolated from the blood of carotenemic (serum carotene, 4.0 mg/liter) and non-carotenemic individuals were found to contain small amounts of carotenoid pigments. The principal pigment isolated from the leucocyte membranes of carotenemic individuals was beta-carotene. Traces of beta-carotene and other carotenoids were found in the leucocytes and leucocyte membranes of the non-carotenemic individuals.

Carotenoid pigments have been found in erythrocytes (1, 2) and platelets (3), but I find no report of the presence of this substance in leucocytes. Leukocytes, isolated from the blood of carotenemic and non-carotenemic individuals, were analyzed for carotenoid pigments.

Materials and Methods

Leukocytes were isolated from the blood of patients with erythropoietic protoporphyria who were being treated with beta-carotene for the relief of their photosensitivity (4) and from blood of non-carotenemic individuals.

The leukocytes (70% polymorphonuclear cells) were isolated by mixing equal volumes of whole anticoagulated blood (heparin or EDTA) and a 30 g/liter solution of dextran (T500, Pharmacia) in isotonic saline and allowing the mixture to settle for 30 min. The leukocyte-rich supernate was removed and centrifuged at 250 × g for 10 min. The erythrocyte membranes remaining in the resulting pellet were lysed by resuspending it in saline (2 g/liter) for 20 s, then normalizing the toxicity with more concentrated saline (16 g/liter). The suspension was then centrifuged again at 250 × g for 10 min, and the lysing-washing procedure was repeated until no erythrocytes could be seen in the pellet.

Polymorphonuclear leukocytes were separated from lymphocytes and monocytes as follows. Whole anticoagulated blood was diluted with an equal volume of isotonic saline. Four parts of the diluted blood were layered on top of a mixture of 2.4 parts of aqueous Ficoll solution (Ficoll, 90 g/liter; Sigma) and one part of aqueous sodium diatrizoate solution (339 g/liter, Hypaque; Winthrop). The blood/Ficoll/Hypaque mixture was centrifuged at 400 × g for 35 min. The top layer, containing lymphocytes and monocytes, was removed, centrifuged, and washed with hypotonic saline as described above to remove any contaminating erythrocytes. From the bottom layer (i.e., the material below the lymphocyte layer), as much as possible of the Ficoll/Hypaque mixture was removed and discarded, and 1 ml of homologous plasma was added to the pellet of polymorphonuclear cell/erythrocyte mixture. An equal volume of dextran (30 g/liter solution) was added and the mixture was allowed to settle for 30 min. The polymorphonuclear cell-rich supernatant fluid was then removed, centrifuged, and washed with hypotonic saline as described above, to remove contaminating erythrocytes.

The various pellets were extracted in the presence of antioxidants as previously described (1). The extracts were chromatographed on silica gel thin-layer chromatographic sheets (Eastman Kodak Co.) and developed with petroleum ether. Crystalline beta-carotene (Nutritional Biochemicals Co.) was used as a standard. The absorption spectra of the extracts were recorded with a Cary Model 14 recording spectrophotometer.

Results and Discussion

The absorption spectra of the extracts of leukocytes from both carotenemic and non-carotenemic individuals indicate

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1. Ficoll is a nonionic synthetic polymer of sucrose, $M_r \sim 400,000$. 

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This is the presence of a mixture of carotenoid pigments (Figure 1). This mixture reflects the dietary intake of foods containing different kinds of carotenoids. However, beta-carotene appears (Figure 1) to be a predominant part of the mixture. Moreover, when the carotenoid extracts of leukocytes were separated by thin-layer chromatography, the $R_f$ value for the principal band of the extracts was identical to that for crystalline beta-carotene standard. As in my study of erythrocytes (1), relatively faint bands representing other carotenoids were also seen. I found an average of 0.35 mg of total carotenoids per gram of leukocytes (wet weight). There was $<$10% difference in the carotenoid content of polymorphonuclear cells and of lymphocytes and monocytes. The carotenoid content of the leukocytes from carotenemic individuals was not significantly different from that of the non-carotenemic individuals (less than 15%). However, the carotenes concentration in the blood of the patients was only moderately above normal (4.0 mg/liter); further studies on the leukocytes of carotenemic individuals are needed to determine if the carotenoid content of leukocytes increases any with higher blood carotene concentrations.

To determine whether the carotenoids were components of the leukocyte membrane or were from adsorbed serum lipids, leukocytes from non-carotenemic individuals were isolated and an aliquot washed 10 times with isotonic saline. Less than 15% of the carotenoid was removed by the washing procedure. This would suggest that the carotenoid is either an integral part of the membrane, or very firmly bound to it. This finding is similar to that in erythrocytes (1).

Carotenoids have now been isolated from human erythrocytes, platelets and leukocytes. The exact relationship of these pigments to the cell membrane and their function in the cell require further investigation.

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References