L-ASCORBIC ACID 2-PHOSPHATE AND CULTURED KERATOcyTES

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We examined the effect of L-ascorbic acid 2-phosphate (P-Asc) on the growth of cultured rabbit keratocytes and their collagen production.

MATERIALS AND METHODS

(1) Rabbit keratocytes were cultured in the presence or absence of 0.1 mM P-Asc. After 10, 20 and 30 days, the number of cells was determined. The cells in 30-day culture were observed under a TEM.

(2) Confluent keratocytes were incubated for 10 hr with or without 0.1 mM P-Asc and observed immunohistochemically with anti-type I and III collagen antibodies.

(3) Confluent cells were cultured for 6 days with or without 0.1 mM P-Asc. Types I and III collagens in the media were measured by ELISA.

RESULTS AND DISCUSSION

Growth of the keratocytes was enhanced by the addition of P-Asc (Fig. 1). In TEM observations, cells were more multilayered in the presence of P-Asc (Fig. 2). P-Asc decreased collagen-immunoreactive cytoplasmic granules (Fig. 3). Cisternae of the endoplasmic reticulum (ER) were dilated without P-Asc, probably showing an accumulation of contents. In immunoassay, P-Asc promoted the production of collagens. (The amount of collagens produced by a single keratocyte with P-Asc was as follows: type I: 2.0 X 10⁻³ ng, type III: 8.5 X 10⁻⁴ ng. No collagens were detected in the control culture.) P-Asc may have a therapeutic effect on
L-ascorbic acid 2-phosphate
corneal wound repair.

Fig. 1

Cell No./Well

0 1.0 x 10^3
10 2.0 x 10^3
20 3.0 x 10^3
30 4.0 x 10^3
5.0 x 10^3

- 0.1 mM P-Asc
- Bar : 50 µm

Fig. 2
Fine structure of the cells cultured in the presence (B) or absence (A) of 0.1 mM P-Asc for 30 days. The cells with P-Asc are more multilayered than those without P-Asc. Cisternae of ER of the cells in the control culture show an extensive dilation. N; nucleus, M; mitochondrion

Fig. 3
Immunostaining of types I (A, C) and III (B, D) collagens in cultured keratocytes. Immuno-reactive cytoplasmic granules are observed in the cells cultured without P-Asc. They are not found in the cells incubated with P-Asc

REFERENCES

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