ETHANOL, ACETALDEHYDE AND LACTIC ACID STIMULATE TYPE III COLLAGEN SYNTHESIS OF CULTURED CHANG LIVER CELLS—IMMUNOELECTRON MICROSCOPE STUDY

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Perihepatocellular fibrosis is often encountered in cases of alcoholic liver injury without a remarkable increase in the number of mesenchymal cells. Tissue culture experiments have shown that under certain conditions hepatocyte, an epithelial cells, may produce collagen fibers(1). To study the effects of ethanol and its oxidation product acetaldehyde and associated metabolite lactate on liver cell collagen synthesis, Chang liver cells, an established adult human liver cell line with positive ADH and albumin in the cytoplasm and ALDH in the mitochondria, as revealed by immunohistochemical as well as immunoelectron microscope study, were used as target cells. The results showed that ethanol, acetaldehyde and lactic acid all stimulated Chang liver cells to produce collagen fibers when the cells were cultured for 28 days of longer(1). It is the purpose of the present study to characterize the kinds of collagen produced by cultured Chang liver cells.

MATERIAL AND METHODS

Chang liver cells were cultured in Falcon tissue culture flasks. The basic culture fluid consisted of Eagle's MEM with 12% fetal calf serum and ascorbate(50 ug/ml). The cells were cultured in the following fluids: group 1: basic fluid + ethanol(100 mM); group 2: basic fluid + ethanol and 4-methyl-pyrazole(2 mM, an inhibitor of ADH); group 3: basic fluid + acetaldehyde(200 mM) and group 4: basic fluid + lactic acid(5 mM). Each group consisted of 50 samples, which were cultured in a 5% CO₂ incubator at 37 degrees centigrade for 28 days. The culture fluid was renewed every three days.

COLLAGEN IMMUNOTYPING: Chang liver cells attached to the bottom of the tissue culture flasks were fixed in 4% paraformaldehyde for 10 min. 1st antibody( anti type I or type III collagen rabbit IgG, ADVANCE CO., Tokyo) or control rabbit serum was applied for 60 min. at room temperature. Then, 2nd antibody( peroxidase-labelled anti-rabbit IgG goat antibody, TAGO., U.S.A.) was applied for 60 min. The specimens were colorized with 3,3'-DAB·H₂O₂ for 5 min. for light micro-
Fig. 1. Immunoperoxidase staining of type III collagen (arrows) present outside Chang liver cells cultured in the fluid with ethanol. x400.

Fig. 2. Electron micrograph of Fig. 1. Type III collagen fibrils reveal cross striation with periodicity (arrow heads). x40,000.

scopy. The specimens were refixed in 2% glutaraldehyde for electron microscopy, followed by coloration with 3',3''-DAB·H₂O₂. Finally, the specimens were fixed in 2% OsO₄ and embedded in epon for ultrathin sectioning.

RESULTS AND DISCUSSION

Large portions of the specimen from each group revealed the presence of type III collagen fibers as shown in figure 1. They were present outside the cells. Cross striation with periodicity characteristic of collagen fibrils was discernible on electron microscopy (Fig. 2). Immunoperoxidase staining of type I collagen was negative in all the specimens examined. Peroxidase negative collagen fibrils were found on electron microscopy, and argentaffine fibers were observed on the same specimens. Therefore, these fibers were assumed to be negative to anti type I collagen IgG.

REFERENCE