DEMONSTRATION AND MAINTENANCE OF MUCUS SECRETION IN CULTURED HUMAN GALLBLADDER EPITHELIAL CELLS

SOICHI YOSHITOMI, KOHJI MIYAZAKI, AND FUMIO NAKAYAMA

Department of Surgery I, Kyushu University Faculty of Medicine, Fukuoka, 812 Japan

(Received 27 May 1986; accepted 19 January 1987)

SUMMARY

The method of human gallbladder epithelial cell culture has been developed successfully with active mucus secretory function. Human gallbladder epithelial cells were dissociated by Disparse digestion from the specimens obtained by cholecystectomy for uncomplicated gallbladder stone cases. The dissociated cells formed a monolayer in Eagle’s minimum essential medium supplemented with 10% fetal bovine serum within 24 h after the inoculation. These cells were maintained for at least 2 wk without fibroblastic overgrowth. Cultured cells contained periodic acid Schiff-positive material in cellular cytoplasm for 3 d. On transmission electron microscopy these materials were identified as mucous secretory granules. Mucous secretory function was determined by [3H]glucosamine incorporation. Sixty percent of the secreted glycoproteins labeled with [3H]glucosamine was eluted in excluded fractions of Sepharose 4B gel filtration, which were considered to be mucous glycoprotein, because they were found to be resistant to proteoglycan-specific enzymes such as hyaluronidase, chondroitinase ABC, heparitinase, and heparinase. The mucous glycoprotein secretion was maintained for 3 d and found to be inhibited in a dose-dependent manner by monensin (10⁻⁷ to 10⁻⁵ M) which is a known blocker of secretory function.

Key words: human gallbladder epithelium; primary culture; mucus secretion; ultrastructure; monensin.

INTRODUCTION

Much attention has been focused on the role of mucin as a nucleating agent in gallstone formation (1,5,13), but very little is known about the physiologic or pharmacologic control of gallbladder mucus secretion (11) because of the methodological limitations.

For investigation of the mechanism of mucus secretion in gallbladder, the in vitro approach is advantageous because of its simplicity, i.e. it is free from the influence of hormone, nerve system, or blood supply. Recently, explant culture or tissue incubation of animal gallbladder has been introduced to study mucus secretion (10,16). However, these systems could maintain mucus secreting function only for 24 h. Furthermore, species difference is known to exist such as in hormonal response and mucus secretion in gallbladder (2,12). Therefore, if we are to clarify the pathologic process in human gallbladder such as gallstone formation, human gallbladder epithelial function should be studied because wide species difference is also known to exist in gallstone formation.

In the present study the method of dissociation and culture of human gallbladder epithelial cells is presented. The morphologic and functional integrities of these cells dissociated were established by demonstrating inhibitory effect of monensin on mucus secretion.

MATERIALS AND METHODS

[3H]Thymidine (specific activity 19.3 Ci/mmol) and D-[6-3H]glucosamine hydrochloride (specific activity 28.2 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Nuclear trace emulsion Sakura NR-M2 was obtained from Konishiroku Co., Ltd. (Tokyo, Japan). Monensin was purchased from Calbiochem-Behring Co. (La Jolla, CA). Trichloroacetic acid (TCA) and phosphotungstic acid (PTA) were obtained from Ishizu Pharmaceutical Co. (Osaka, Japan). Trypsin (1:250) was obtained from Sanko Pure Chemical Industries Ltd. (Tokyo, Japan).

Preparation of gallbladder epithelial cells. Gallbladder was obtained from patients with a well-functioning gallbladder undergoing cholecystectomy for gallstones. The gallbladder was placed in a chilled saline solution immediately after removal. Neck of the gallbladder was incised and everted. Mucosa was checked for signs of inflammation. Only noninfamed gallbladders were used. Bile was carefully removed with sterile gauze and rinsed thoroughly with ice cold Hanks’ balanced salt solution. The gallbladder was inverted and transected in the
middle and the distal half was used with the opening closed with forceps, which was subsequently filled with CMF-Hanks' balanced salt solution containing 1000 protease U/ml of Dispase I (Gohdo Shusei Co., Ltd., Chiba, Japan). Thereafter, the gallbladder sac was immersed in CMF-Hanks' solution and shaken in a water bath at 100 strokes/min at 37° C for 45 min. The initial content was discarded and the transected gallbladder was refilled with CMF-Hanks' solution. Isolated cells and cell clumps were harvested by repeated pipetting of the content. The cell suspension thus obtained was filtered through one layer of nylon stocking mesh. The cell clumps can be separated selectively by low speed centrifugation (50 g for 5 min, twice). The pellet was resuspended in a culture medium for subsequent incubation.

Culture. Cells were inoculated in 35-mm plastic dishes (Falcon 3001), with Eagle's minimum essential medium (MEM) (Nissui Co., Ltd., Tokyo, Japan) supplemented with heat-inactivated (at 56° C for 30 min) 10% fetal bovine serum (M.A.B. Bioproducts, Walkersville, MD), and incubated in a humidified atmosphere gassed with 5% CO₂ in air. Medium was changed daily.

Light microscopy. Cultures were observed daily with phase contrast microscope. For determination of mucus-producing cells, periodic acid-Schiff (PAS) reaction with or without 1.0% diastase was employed. For determination of mitotic index (M.I.), the cells were stained with Giesma

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**Fig. 1.** Scanning electron micrograph of epithelial cell clumps from human gallbladder immediately after dissociation. a, b. The clump is composed of cylindrical epithelial cells and luminal surface is covered with microvilli. a, ×239, b, ×726 c. Microvilli are well preserved after dissociation procedure. ×12 832 d. Basal aspect of the clump is demonstrated. Basal pole of each cell is free from submucosal tissues. ×1327.

**Fig. 2.** Phase contrast micrograph of human gallbladder epithelial cells in culture, 2-d-old cells. ×180. Polygonal shaped cells with oval nucleus form a monolayer. Cellular cytoplasm is dark and some granules are observed in perinuclear region.