Comprehensive Studies on Rat Primary Cultured and Isolated Hepatocytes in the Evaluation of a Therapeutic Agents for Liver Disease

Koji SAKAMOTO, Mayumi TONOOKA, Koichiro ABE*, Kenichi USAMI and Takako KASAHARA

Department of Pharmacology, *Department of Plastic and Reconstructive Surgery, Showa University, School of Medicine, 1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142, Japan

Accepted May 6, 1986

Abstract—We have investigated the effect of a therapeutic agent for liver disease, Laennec, on the GOT leakage from freshly isolated and primary cultured rat hepatocytes which were treated with CCl₄. By treatment with Laennec together with CCl₄, the GOT leakage from isolated hepatocytes increased and that from cultured hepatocytes decreased, compared to those incubated only with CCl₄. The results suggest that it is better to use primary cultured hepatocytes than to use freshly isolated hepatocytes to evaluate therapeutic agents for liver disease.

The present study was designed to make a comparison between freshly isolated and cultured hepatocytes that were with Laennec. We have investigated the effect of various drugs on isolated hepatocytes (1) and discovered that the isolated hepatocytes do not possess cell functions. Laennec, which contains many components, is the hydrolyte of human placenta (2). Laennec is reported to absorb the proliferated connective tissue (3) and to decrease the lipidosis (4) and serum transaminase activity (5) in liver cirrhosis induced by treatment with CCl₄.

Male S.D. rats weighing about 200 g were fed a standard laboratory diet and water ad libitum. The rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.), and the hepatocytes were isolated by a modification of Seglen’s two-step procedure (6). The liver was perfused, via the portal vein, for 10 min with Ca²⁺-Mg²⁺-free Hanks’ balanced salt solution (CMF-HBSS) containing 0.5 mM EGTA (ethyleneglycol-bis (β-aminoethylether) N,N’-tetraacetic acid) and 10 mM HEPES (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid), pH 7.5, at a constant flow of 15 ml/min at 37°C with continuous bubbling of oxygen. Then the perfusate was changed to 0.05% collagenase (Sigma, type IV) CMF-HBSS, pH 7.5, and was also recirculated for 15 ml/min with continuous bubbling of oxygen. The liver was removed and shaken in the same collagenase CMF-HBSS. The cell suspension was passed through mesh (φ=150 µm) and centrifuged at 50×g for 2 min. The supernatant fluid was aspirated, and the cell pellet was resuspended with Williams E medium (WE). This was repeated twice. The exclusion of trypan blue test showed that the hepatocytes had a viability index of approx. 90–98%. Cell suspension with a concentration of 1×10⁵ cells/ml was used in the experiment with freshly isolated cells. The freshly isolated hepatocytes were treated with Laennec and CCl₄ at various dosages and incubated for 1 hr at 37°C. Williams E medium was supplemented with bovine serum (10%), insulin (10 µg/ml), dexamethasone (1×10⁻⁵ M), penicillin (100 U/ml), streptomycin (100 µg/ml) and fungizone (0.25 µg/ml), at pH 7.4. Suspensions of 1×10⁶ cells/ml were plated in plastic dishes (d=60 mm) and cultured as monolayers in a humidified incubator at 37°C under 5% CO₂ and 95% air. After the first 24 hr culture, the medium was replaced and hepatocytes were treated with various dosages of Laennec and CCl₄.
and then they were cultured for another 24 hr. CCl₄ was dissolved in DMSO (dimethyl sulfoxide) and then diluted in WF. Suspensions containing the same final concentrations of DMSO (1%) in WE were used as a control. The medium was collected from incubated and cultured hepatocytes where the GOT (glutamate oxaloacetate transaminase) activity was measured. The GOT activities were determined by the POP-TOOS method (7).

Figure 1 shows the effect of Laennec on the leakage of GOT from isolated hepatocytes which were treated with CCl₄. The leakage of GOT from the isolated hepatocytes exposed to the control medium was 29.8±6.6 (mean±S.D.) K.U./ml of medium. When the isolated hepatocytes were incubated in 5 mM and 10 mM CCl₄, the leakage of GOT from hepatocytes increased significantly compared to that of the 1% DMSO control. On the other hand, the leakage of GOT from hepatocytes significantly decreased by incubation in Laennec. When the isolated hepatocytes were incubated in Laennec and 5 mM CCl₄, the leakage of GOT from hepatocytes increased significantly compared to those incubated only in 5 mM CCl₄. Figure 2 shows the effect of Laennec on the leakage of GOT from cultured hepatocytes treated with CCl₄. The leakage of GOT from cultured hepatocytes exposed to the control medium was 178.6±14.1 (mean±S.D.) K.U./ml of medium. When the hepatocytes were incubated in 5 mM CCl₄, the leakage of GOT from hepatocytes increased significantly compared to that of the 1% DMSO control. The leakage of GOT from cultured hepatocytes significantly decreased by incubation in Laennec. When the cultured hepatocytes were incubated in Laennec and 5 mM CCl₄, the leakage of GOT from hepatocytes decreased significantly compared to those incubated only in 5 mM CCl₄.

In the present study, the leakage of GOT from hepatocytes decreased significantly by

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**Fig. 1.** Effect of Laennec on leakage of GOT from rat isolated hepatocytes by treatment of CCl₄. Hepatocytes were suspended in Williams E medium and incubated for 1 hr at 37°C. Each of the values was expressed as Karmen units/ml of medium and represents the mean±S.D. of four to six experiments. ■■■P<0.01 and ■■■■P<0.001, different from 1% DMSO control values; ▲P<0.05, different from control values; ***P<0.001, different from CCl₄ values.

**Fig. 2.** Effect of Laennec on leakage of GOT from rat cultured hepatocytes by treatment of CCl₄. The hepatocytes were cultured for 24 hr in Williams E medium and then incubated with Laennec in the presence or absence of CCl₄ for another 24 hr. Other conditions were the same as Fig. 1. ■■■P<0.01 different from 1% DMSO control values; ▲P<0.05 and ▲▲P<0.01, different from control values; ***P<0.001 different from CCl₄ values.
the treatment with Laennec. Recently, we investigated that the effect of Laennec on the 50% hypotonic hemolysis of rat erythrocytes. Laennec protected against hypotonic hemolysis at concentrations from 10 µl/ml to 200 µl/ml which could suggest that Laennec has a membrane stabilizing action. Also, the present study shows a difference between the isolated and cultured hepatocytes. By treatment with Laennec together with CCl₄, the leakage of GOT from the isolated hepatocytes increased significantly compared to cells treated with 5 mM CCl₄ alone. This result may be due to the additional effects of a damage caused by CCl₄ and a membrane action of Laennec. There are reports that the cell function of isolated hepatocytes becomes impaired by perfusion with collagenase solution. That is, the freshly isolated hepatocytes, which have impaired cell membranes, were given more severe damage by Laennec with CCl₄. On the contrary, the leakage of GOT from cultured hepatocytes by treatment with Laennec together with 5 mM CCl₄ decreased significantly compared to those treated only with 5 mM CCl₄. There are reports that the cell function of isolated hepatocytes is impaired by perfusion with collagenase solution. It is also reported that there are decreases in cell membrane function (8, 9), ATP content (10), and protein metabolism (11) in isolated hepatocytes. However, there are reports that the function of hepatocytes recover in a 24 hr culture (12, 13). Therefore, it is doubtful whether isolated hepatocytes are suitable for use in studies on liver functions. The results of our study suggests that in the evaluation of therapeutic agents for liver disease, the maintenance of liver function in the primary cultured hepatocytes is superior to that of the isolated hepatocytes.

References
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