Coculture of Hepatocytes with Islets

C.-H. Kuo, J.-H. Juang, and H.-L. Peng

ABSTRACT

Bioartificial liver support (BAL) systems are potential new therapeutic approaches for use as liver support to prevent nutrient deficiencies, hypoxia, or ischemia before the acquisition of donated organs. To investigate whether islets are beneficial for hepatocyte function and survival, we cocultured BALB/c mouse islets with C57BL/6J hepatocytes to assess hepatocyte viability, function, and apoptosis. We observe cell viability to decrease progressively by 50% from day 0 to day 3 among isolated hepatocytes (group A) and hepatocytes cocultured with islets (group B). However, group A was prone to necrosis and reduced albumin secretion during culture. In contrast, at day 7 group B maintained albumin secretion (0.3351 ± 0.0581 vs 0.1451 ± 0.0329 μg/h/mL; $P < .05$). Early apoptosis was observed at day 3 among group A but at day 7 in group B. In addition, quantitative analysis of the apoptotic cells revealed group B to show a delayed phenotype of both early and late apoptosis compared with group A. Our results indicated that islets could retain hepatocyte function and delay apoptosis, suggesting that the coculture system is potentially applicable to develop a high-performance BAL.

Liver failure shows an annual mortality exceeding 40,000 deaths in the United States.[1] Liver transplantation, though the only established successful treatment of end-stage liver disease, is limited by the availability of donor livers; as a result, many patients die each year while on the waiting list.[2,3] In 1987, a bioartificial liver support (BAL) bioreactor was first introduced to treat a single patient with acute liver failure.[4] Ideal patients for the treatment are those suffering from acute liver failure who need to be "bridged" before they can receive a liver transplant or until they recover.[5–8]

Ideally, a stem cell inoculum could be added to the BAL bioreactor to overcome the cell supply problem.[9] Pig hepatocytes grown in extracorporeal bioreactors are being tested clinically to support liver failure patients until transplantation.[10] However, maintenance of hepatocyte function remains an important issue. Pancreatic islets produce many factors, such as insulin, glucagon,[11] somatostatin,[12] and hepatocyte growth factor.[13] In the absence of pancreatic islets, Ricordi et al[14] observed hepatocyte degeneration, suggesting a possible role of "trophic factors" produced by the islets to maintain hepatocyte function, suggesting the benefit of intrahepatic islet transplantation in portosystemic shunt procedures or heteropic liver transplantation to help prevent atrophy and degeneration. Kaufmann et al[15] have also demonstrated that the effect of pancreatic islets to affect hepatocyte number or albumin secretion per cell was mediated exclusively by soluble factors such as insulin and glucagon rather than cell-to-cell contact.[15] The transplantation of rat hepatocytes alone or with pancreatic islets has been performed to determine whether hepatocellular allografts can survive in an ectopic site.[9]

We sought to investigate whether islets and liver cells from different sources could be useful for clinical hepatocyte transplantation. The cocultured hepatocytes and islets were isolated from C57BL/6J and BALB/c mice, respectively to assess viability, function, and apoptosis.

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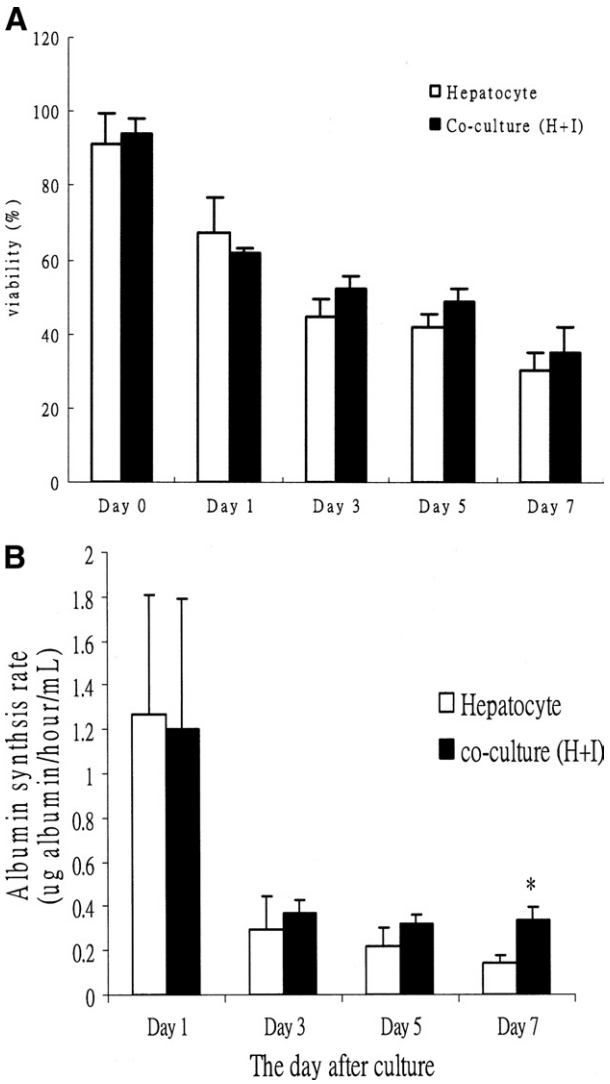

Fig 1. **(A)** Cell viability assay. **(B)** Albumin secretion rate per unit volume. Open bars correspond to hepatocytes and solid bars to the coculture of hepatocytes with islets. *$P < .05$.

MATERIALS AND METHODS

Islets Isolation

Essentially as previously described,[16–22] we anesthetized BALB/c mice with sodium amobarbital to distend the pancreas using 2.5 mL RPMI-1640 medium containing 1.5 mg/mL collagenase (*Clostridium histolyticum* type XI; Sigma-Aldrich St Louis, MO) before excision. After incubation in a water bath at 37°C, islets were separated by density gradient (Histopaque-1077; Sigma-Aldrich).

Isolation and Culture of Hepatocytes

A 2-step perfusion used some modifications of the method of Seglen et al.[23] Hepatocytes were isolated from 6-week-old male C57BL/6J mice perfused with 10 mL collagenase IV (1 mg/mL; Sigma-Aldrich) using Ca^{2+}- and Mg^{2+}-free Hank balanced salt solution.[24] Approximately 1×10^5 hepatocytes were cultured with or without the isolated islets (~150 islets) in hypatocyte growth medium or type I collagen (BD biosciences)–coated plates in 5% CO_2 at 37°C.[25]

Cell Viability Assay

Hepatocytes were counted with the use of light microscopy on culture days 0, 1, 3, 5, and 7. The viability was evaluated by trypan blue staining at each time.

Albumin Assay

Aliquots of hepatocyte or hepatocyte-islet bathing media were collected on days 1, 3, 5, and 7 for analysis of secreted albumin using an enzyme-linked immunoadsorbent assay with the antibodies specific for mouse albumin (Immunology Consultants Laboratory).

Apoptosis Assay

Cellular apoptosis reporters annexin V–fluorescein isothiocyanate and propidium iodide were analyzed by a 2-laser flow cytometry system (BD FACScalibur flow cytometer) using 488-nm argon and 633-nm HeNe air-cooled lasers.

Statistical Analysis

Data expressed as mean ± SEM were compared using unpaired Student t test with $P < .05$ scored as significant.

RESULTS

Figure 1A shows that cellular viability decreased progressively to about 50% from day 0 to day 3 among both isolated hepatocytes (group A) and hepatocytes cocultured with

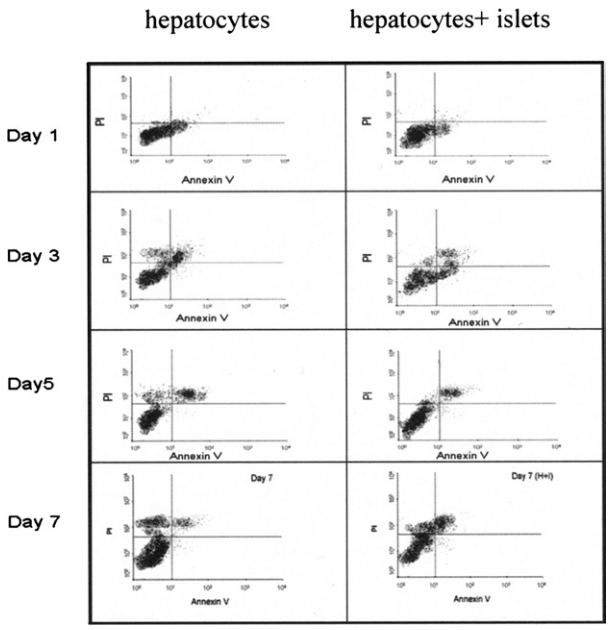

Fig 2. Flow cytometry analysis: The representative profile is shown and apoptotic cells evaluated by annexin V/propidium iodide (PI) staining. Values shown are representative experiments in triplicate with similar results.

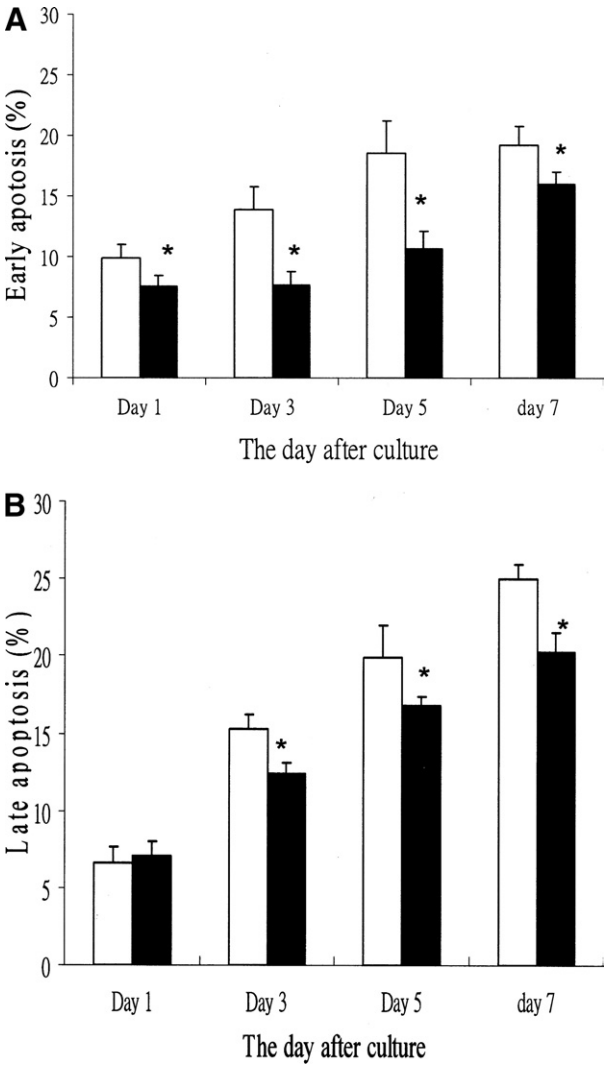

Fig 3. Apoptosis analysis. **(A)** Early apoptosis: values shown are representative experiment in upper left quadrant. **(B)** Late apoptosis: values shown are representative experiment in upper right quadrant. Open bars correspond to hepatocytes and solid bars to the coculture of hepatocytes with islets. $^{*}P < .05$.

islets (group B). On day 3, the albumin synthesis rate in both groups had decreased to one-third compared with those on day 1 (Fig 1B). As shown in Fig 1B, group B maintained the albumin synthesis rate since day 3; the level of 0.3351 ± 0.0581 μg/h/mL at day 7 was significantly higher than 0.1451 ± 0.0329 μg/h/mL in group A. The apoptotic analysis determined by the dot shift to the upper left quadrant was reported as early apoptosis, and that to the upper right quadrant as late apoptosis. Figure 2, shows early apoptosis at day 3 in group A but at day 7 in group B. Nonetheless, the late apoptosis patterns, beginning to show at day 3, appeared to be similar for both groups (Fig 2). Quantitative analysis of the apoptotic cells revealed that group B, compared with group A, had delayed appearance of not only early apoptosis (Fig 3A) but also late apoptosis (Fig 3B).

DISCUSSION

Islets seem to exert stimulatory effects on hepatocyte number and functions in vitro. The beneficial effects have been shown to be mediated by signal transfer via insulin and glucagon.[19] We demonstrated positive effects of islets on hepatocyte survival and function, which were most probably conferred by soluble factors of islets, because both cells were separated from the membranes. Moreover, the beneficial effects appeared to be independent of the sources of the islets and the hepatocytes. Death rate of the hepatocytes could be much faster than the proliferation rate because of the poor oxygen and nutrient supply. It is speculated that the islets probably provided hepatocytes insulin and glucagon to balance the rate of proliferation and death.

In summary, coculture of hepatocytes with islets may be helpful for high-performance BAL.

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