NEW BIOMEDICAL TECHNOLOGIES

Functional Activity of Hepatocytes in Tissue Fragments in a New Bioreactor Biological Artificial Liver

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Functional activity of hepatocytes in a new bioreactor designed for culturing of liver tissue fragments under perfusion conditions was tested. Specific hepatic functions such as ammonium detoxification, urea and protein synthesis, and P-450-dependent metabolism of p-nitroanisole were maintained for 1-1.5 days. The bioreactor can be used as a bioartificial liver support apparatus.

Key Words: *acute liver insufficiency; bioartificial liver support; bioreactor; hepatocytes; liver fragments*

The systems aimed at compensation of hepatic functions during liver failure are now developed and applied in clinical practice [1,7,10,11]. These systems contain biologically active donor hepatocytes placed into a bioreactor, through which patient's blood or plasma is perfused. It is assumed that under these conditions hepatocytes execute tissue-specific functions and probably stimulate regenerative process in patient's liver [1,11]. The efficiency of these systems in acute liver failure caused by inherited metabolic diseases was previously demonstrated [1,7,11]. Nevertheless, the system, which could be widely used in clinical practice has not yet been created.

Isolated hepatocytes are used in most proposed artificial liver support systems. Long-term maintenance of their functional activity requires immobilization, creation of an artificial extracellular matrix, or formation of spheroid hepatocyte aggregates [10,11]. An alternative approach is the use of tissue fragments or sections [9,12]. Preparation of liver fragments is a technologically simple process. It requires no expensive enzymes and media [8] and preserve natural extracellular matrix maintaining high functional activity of hepatocytes [6]. Previously we studies biochemical activity of hepatocytes in cultured liver fragments, determined their optimal size, and demonstrated high functional activity of hepatocytes in this system [3]. However, liver tissue fragments cannot be used in most available bioreactors. Here we developed a new bioreactor for culturing of liver tissue fragments under perfusion conditions and at cell concentrations close to that in the liver tissue.

The aim of this work was to investigate functional activity of hepatocytes in the new bioreactor and to evaluate the possibility of using this bioreactor as a bioartificial liver support apparatus.

MATERIALS AND METHODS

Williams-E medium containing 10^{-8} M insulin, 10^{-6} M dexamethasone, 50 mg/liter gentamicin, agarose, β-glucuronidase (Sigma), and p-nitroanisole (PNA, Aldrich) were used.

Wistar rats weighing 100-150 g were narcotized with ether, the liver was removed, placed in cold (4°C) Hanks' solution, and 10-15 min later minced to 0.5×

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0.5×1 mm fragments [8]. Carrier particles (2% agarose gel) were cut in the same way. Carrier particles (8 g) and tissue fragments (1.0-1.5 g) were placed in a 20-ml bioreactor perfused with 100-150 ml culture medium (40 ml/min perfusion rate, recirculation regimen). pH was maintained at 7.2-7.4 by saturating the medium with air-CO_2 (95%+5%) mixture.

The contents of urea (with Lachema kits), ammonium [5], p-nitrophenol (PNP) [13] in the medium, and glycogen in cells [5] were measured.

Secretory activity of hepatocytes was assayed by adding ^{14}C-amino acids (10 μCi/ml, UVVVR) to the medium for 15 min. Radioactivity of secreted proteins was measured as described previously [2].

The content of viable and dead cells in tissue fragments was evaluated by double luminescent staining with ethidium bromide and Bisbenzimide Hoechst 33342 stain (Sigma) [4].

The data are presented as $X \pm m$.

RESULTS

Column bioreactor (Fig. 1) consists of a body and carrier particles evenly distributed among liver fragments. Culture medium flows from the top to bottom with a peristaltic pump. Carrier particles and liver fragments are restrained in the bioreactor with a filter. Metabolic processes crucial for efficient functioning of the bioreactor as bioartificial liver support were investigated, in particular, clearance of ammonium ions playing an important role in the development of encephalopathy during acute liver failure. The mean rate of urea synthesis after 26-h culturing was 11.4 μmol/g tissue/h (Fig. 2.), which corresponds to values obtained for human liver (7-14 μmol/g tissue/h) [13]. The duration of hepatocyte active functioning was limited by medium exhaustion because of high tissue: medium ratio (1 g:100 ml) characteristic of high-density cultures. Under these conditions, the synthesis of urea stopped after on average 34±3 h (Fig. 2.). Nevertheless, this considerably surpassed the duration of detoxification session with bioartificial liver support systems (4-8 h) in clinical practice [1,11].

The state of the cytochrome P-450-dependent metabolic function of hepatocyte microsomes was assessed by the rate of PNA demethylatation measured by the yield of PNP. The specific rate of PNP accumulation was 2.3±0.4 μmol/g tissue/h, which corresponds to known data for isolated hepatocytes and perfused rat liver (2.0 and 2.4 μmol/g tissue/h, respectively) [13].

The liver is the major source of plasma proteins, therefore protein synthesis and secretion by hepatocytes in the bioreactor were also investigated. To this end, pulse labeling with ^{14}C-amino acids was carried out after 24 h of the bioreactor functioning. The release of labeled proteins into the medium sharply increased 1.5 h after removal of unbound label, but after 30 min this parameter returned to the initial level. Thus, the cells retain the ability to synthesize and secrete proteins during this period.

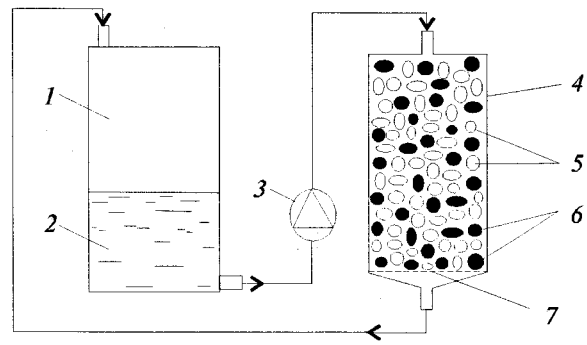

Fig. 1. Setup for studying hepatocyte functional activity in the bioreactor biological artificial liver. *1*) reservoir, *2*) nutrient medium, *3*) peristaltic pump, *4*) bioreactor body, *5*) carrier particles, *6*) liver fragments, *7*) filter.

After 24 h of culturing, liver fragments were incubated with 1 μM epinephrine for 1 hour. Epinephrine reduced glycogen content in hepatocytes from 0.58±0.03 to 0.23±0.05 μmol glucose residues/g tissue. In the control (without hormone) the glycogen content remained unchanged. This effect of epinephrine similar to *in vivo* effect of the hormone suggests that hepatocytes retain the ability to respond to hormonal signals.

The content of viable cells in fragments gradually decreased from 85±5 to 55±5% after 30 h in culture.

The bioreactor is the principal element of the bioartificial liver support systems. It must contain enough hepatocytes (no less than 3-5% of patient's liver weigh

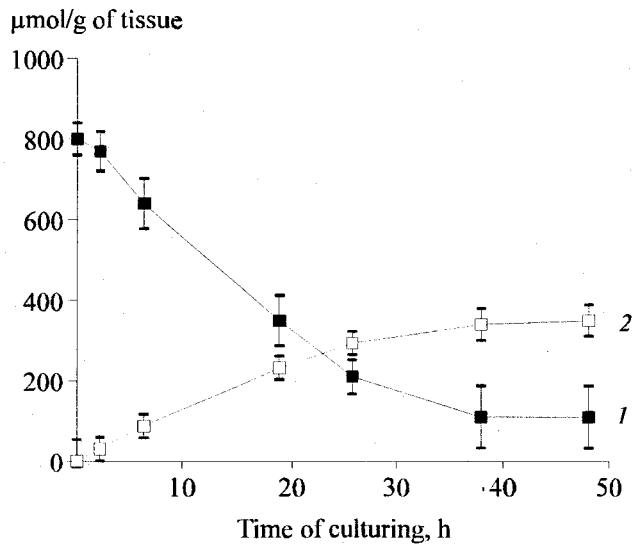

Fig. 2. Time course of ammonium (*1*) and urea (*2*) concentration in medium during culturing of liver fragments in the bioreactor. Ordinate: content of ammonium and urea.

[11]) and low volume of fluid, at the same time providing the conditions for good exchange of substances between blood and hepatocytes. Moreover, the apparatus intended for clinical use has to be simple and practically feasible. The bioreactor examined in this work meets all these requirements. Our experiments demonstrated high functional activity of liver cells in the bioreactor. The duration of hepatocyte active functioning exceeded 24 h, which is quite enough for practical use.

Thus, our study of hepatocyte functional activity in tissue fragments in a new bioreactor testifies that it can be used as a bioartificial liver support apparatus.

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