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• BASIC RESEARCH •

Effects of electroporation on primary rat hepatocytes *in vitro*

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Abstract

AIM: To investigate the effects of electroporation on primary rat hepatocyte and to optimize the electroporation conditions introducing foreign genes into primary hepatocytes.

METHODS: A single-pulse procedure was performed at low voltage (220-400 V) but with high capacitance (500-950 μF). Hepatocytes were divided into 4 groups according to the electroporation conditions: group Ⅰ, 220 V and 500 μF; group Ⅱ, 220 V and 950 μF; group Ⅲ, 400 V and 950 μF, and group IV. The control group was freshly isolated hepatocytes and directly cultured under the same conditions as those of electroporation groups. The effects of electroporation on primary rat hepatocytes were detected by trypan blue exclusion (TBE) and MTT analysis. Besides, albumin (Alb), alanine transaminase (ALT) and lactate dehydrogenase (LDH) in the supernatants of cultured hepatocytes were measured by biochemical assay.

RESULTS: Between day 1 and day 15 after incubation, primary rat hepatocytes of each electroporation group appeared normal, being the same with those of control group. TBE staining showed that slight hepatocyte damage and high survival rate were found in the electroporation groups and the control group. Cultured for 3, 7, 11 and 15 days, hepatocyte viability was approximatly 92.6±2.5 %, 89.5±3.3 %, 82.0±3.5 % and 74.3±1.2 %, respectively. MTT analysis indicated that the viabilities of hepatocytes had no significant difference between each electroporation group, and those were similar to that of control group. At the 36th hour after electroporation, Alb, ALT and LDH in the supernatants of control group were 5.3±0.1 g·L^{-1}, 183.7±8.4 nkat·L^{-1} and 896.8±58.5 nkat·L^{-1}; those of group Ⅱ were 5.7±0.1 g·L^{-1}, 215.4±16.7 nkat·L^{-1} and 1063.8±51.8 nkat·L^{-1}; and those of group Ⅲ were 5.8±0.2 g·L^{-1}, 217.1±8.4 nkat·L^{-1} and 1063.8±10.0 nkat·L^{-1}. Statistically, the proteins of group Ⅱ and group Ⅲ were significantly higher than those of control group (P<0.05), whereas the protein production of group Ⅰ, Alb, ALT and LDH were 5.3±0.2 g·L^{-1}, 205.4±3.3 nkat·L^{-1} and 1035.4±116.9 nkat·L^{-1}, were similar to those of control group. At the same time, TBE and MTT analysis indicated that there was no significant cell viability difference between electroporation groups and control group.

CONCLUSION: This single-pulse electroporation procedure performed at low voltage (220-400 V) but with high capacitance (950 μF) is one of the optimal choices to introduce foreign genes into primary rat hepatocyte.

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INTRODUCTION

Electroporation technique has been widely used in the experiments of foreign gene transfection into cultural cells. Little is known about the effect of electroporation on the primary culture cells, especially on the primary hepatocytes, for most of the target cells are immortal carcinoma cells. At present, in the *in vitro* study of liver diseases, primary cultured hepatocytes have been used as one of the most important target cells, which are increasingly used in the research of basic and clinical medicine[1-17], and electroporation has also been used as preferred means introducing foreign genes into primary hepatocytes. It is known that primary hepatocyte does not proliferate in common culture, so the biological activities of electroporated hepatocytes must be the key points affecting the efficiency of transfection and expression of foreign genes in the target cells. In order to optimize the electroporation conditions introducing foreign genes into hepatocytes, we studied the effect of electroporation on the biological activities of primary cultured rat hepatocyte.

MATERIALS AND METHODS

Isolation and culture of hepatocytes

Wistar rats, male, about 200 g of body mass, were provided by the Center for Laboratory Animal at Chongqing University of Medical Sciences. Hepatocytes were harvested from adult Wistar rats using the *in situ* collagenase perfusion technique[18]. Injected into the peritoneal cavity with pentobarbital sodium (100 mg·kg^{-1} body weight) and heparin (200 IU·kg^{-1} body weight), the abdomen of the animals were opened and the portal vein was exposed and cannulated. Then the liver was perfused at 37 ℃ *in situ* first with a calcium-free Hanks' Balanced Salt Solution (HBSS) for 10 min, next with 0.2 g·L^{-1} collagenase (Type Ⅳ, 390 kU·g^{-1}) in calcium-presented HBSS for 15 min. The liver was removed and the cells were combed gently in tissue culture medium. Hepatocytes were pelleted, washed, and separated from nonparenchymal cells by differential centrifugations at 50×g. Viability of hepatocytes exceeded 98 % as determined by trypan blue exclusion (TBE).

Electroporation

Freshly isolated hepatocytes were diluted into 1×10^{10} cells·L^{-1} suspension with serum-free RPMI 1640 medium for

electroporation (electroporation apparatus is Bio Rad Gene Pulser Ⅱ), and were divided into three groups. In group Ⅰ, voltage was 220 V, and capacitance was 500 μF, in group Ⅱ, 220 V and 950 μF, and in group Ⅲ, 400 V and 950 μF. All of three groups were electroporated by single-pulse procedure, and electroporation time was about 19 ms. Before and after electroporation hepatocytes were put onto ice for 10 min. Electroporated hepatocytes diluted into 3×10^8.cells.L^{-1} suspension with hepatocyte culture medium was inoculated into 96-well plastic cell-culture plates (3×10^4 cells in each well). Medium contained RPMI 1640 with insulin (100 $U\cdot L^{-1}$), penicillin, streptomycin and 100 $ml\cdot L^{-1}$ fetal bovine serum. Control group, group Ⅳ, was freshly isolated hepatocytes and was directly cultured under the same conditions as those of electroporation groups. The medium was changed after 36 h incubation and later changed every two days.

Hepatocyte viability and function

To detect the viabilities of hepatocytes of electroporatin groups and control group, TBE staining was used from day 1 to day 15 after incubation. Besides, MTT analysis was also used to measure the cell viability. At 36 h after incubation, the medium was changed with phenol red-free RPMI 1640 medium (0.1 mL each well), 10 μL MTT (5 $g\cdot L^{-1}$) was added into each well, and incubated for another 4 h. Then discard the supernatants, add 0.1 ml dimethyl sulfoxide into each well and oscillate the culture plate for 5 min. Next, the 96-well culture plate was put into Elx800 type automatic reader and absorbance values in each well was detected at 405 nm wave length. Each group had more than 6 wells each time. Three more of these tests were repeated. To measure albumin (Alb), alanine aminotransferase (ALT) and lactic dehydrogenase (LDH) in the supernatants of cultured hepatocytes of electroporation groups and control group, biochemical assays were used.

RESULTS

Effect of electroporation on the viability of hepatocytes

From day 1 to day 15 after incubation, primary rat hepatocytes of each electroporation group appeared normal, the same as those of control group. TBE staining showed that the viabilities of hepatocytes had no significant difference between each electroporation group, which were similar to that of control group (Table 1). Culture for 7 d showed that hepatocyte viability was still around 90 %, and about 74 % hepatocytes survived 15 d after electroporation. MTT analysis also showed the same results, after 36 h of culture, primary rat hepatocytes of electroporation groups had the same viabilities as that of control group ($P>0.05$, Student's *t* test), the absorbance values (405 nm wave length) of group Ⅰ to Ⅳ were 0.317±0.069, 0.369±0.059, 0.279±0.085 and 0.332±0.072, respectively.

Table 1 Viabilities of electroporated hepatocytes measured by TBE

Culture time (day)	Survival rate (%)
1	95.4±1.8
3	92.6±2.5
5	91.3±2.4
7	89.5±3.3
9	87.2±4.6
11	82.0±3.5
13	77.0±0.9
15	74.3±1.2

Effect of electroporation on hepatocyte function

To detect the effect of electroporation on Alb, ALT and LDH production of primary rat hepatocytes, biochemical assays were used to measure these proteins in the supernatants of cultured hepatocytes. Amounts of Alb, ALT and LDH in the supernatants were assayed 36 h after incubation. The results (Table 2) showed that Alb, ALT and LDH production of electroporation groups Ⅱ and Ⅲ were significantly higher than those of control group, whereas the protein production of group Ⅰ was similar to those of control group.

Table 2 Alb, ALT and LDH production in the supernatants of primary cultured rat hepatocytes

Groups	Alb/($g\cdot L^{-1}$)	ALT/($nkat\cdot L^{-1}$)	LDH/($nkat\cdot L^{-1}$)
Ⅰ	5.3±0.2	205.4±3.3	1035.4±116.9
Ⅱ	5.7±0.1[a]	215.4±16.7	1063.8±51.8[a]
Ⅲ	5.8±0.2[a]	217.1±8.4[a]	1063.8±10.0[a]
Ⅳ	5.3±0.1	183.7±8.4	896.8±58.5

[a]$P<0.05$, *vs* control.

DISCUSSION

Primary hepatocytes have been increasingly used in basic and clinical medicine researches, such as the study of hepatocyte function, the mechanism of hepatocyte injuries and the protective approaches of hepatocyte biological activities[19-25], and the use of hepatocytes in bioartificial liver support system (BALSS)[26,27] and gene therapy[28]. Besides, more and more researchers utilize primarily cultured hepatocyte model to study foreign genes' activities and effects on host cells, for example, hepatitis B and C viruses replication and expression in hepatocytes[29-31]. In order to keep the normal biological activities and functions *in vitro* of hepatocytes, more methods have been used to optimize primary hepatocyte model, especially the introduction techniques of foreign genes into hepatocytes.

The introduction and stable expression of foreign genes in mammalian hepatocytes have been demonstrated by several techniques[32-37], including the use of physical approaches such as direct injection of a DNA calcium phosphate precipitate, electroporation of plasmid DNA and the exposure to liposome-erythrocyte ghost complexes as well as the biological approach of infection of primary hepatocyte cultured with retrovirus vectors. Among these techniques, electroporation has been considered as one of the most useful methods introducing foreign into target cells[38-41]. However, it is not defined that which electroporation condition is the best one under which foreign genes can be efficiently transduced into primarily cultured hepatocytes, and this condition could cause little damage to target cells.

It is known that primarily cultured hepatocytes are well-differentiated cells, most of which are resting cells, arrested in G0, and do not proliferate in common culture conditions. To be an ideal model, in which foreign gene can get highly efficient transfection and expression, primarily cultured hepatocytes after electroporation could not only keep a high survival rate, but also maintain excellent cellular functions. Therefore, optimizing electroporation conditions has become the key point affecting the biological activities of primary hepatocytes.

Our results showed that a single-pulse procedure performed at low voltage (220-400 V) but with a high capacitance (950

μF) caused minimal cell damage and kept hepatocyte with a high survival rate, cultured for 7 d and 15 d, greater than 90 % and 74 % respectively, and hepatocyte viabilities of electroporation groups were similar to that of control group and much higher than those reported by other savants. Our current data further indicated that this electroporation procedure could not only maintain hepatocyte function, but also facilitate Alb, ALT and LDH expression in hepatocytes and production in the culture medium. Its mechanism is not clear, possibly related to two reasons: first, Alb, ALT and LDH might leak into culture medium through electroporated hepatocyte membrane, while minimal cell damage had been found by TBE and MTT assay. Second, this electroporation condition may have a potentiation to stimulate wave of DNA synthesis in electroporated rat hepatocytes. In conclusion, this single-pulse procedure performed at low voltage (220-400 V) but with a high capacitance (950 μF) could not only cause minimal cell damage, but also potentiate DNA synthesis in rat hepatocytes. For instance, up to date, we have used this electroporation procedure in transduceing hepatitis B virus gene into primary rat hepatocytes and primary duck hepatocytes and successfully observed that hepatocytes are competent for transfection with HBV gene, which can stably replicate and express in primary hepatocytes[42,43]. Therefore, this single-pulse procedure can be used as one of the optimized electroporation procedures introducing foreign genes into primarily cultured hepatocytes.

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