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Isolation and protective effect in UW solution of human hepatocytes during cold storage

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Abstract

Background: To get isolated human hepatocytes and investigate the protective effect of UW solution on human hepatocytes during cold storage at 0–4 °C. *Methods*: A well-perfused liver lobe weighed 60–80 g were used for hepatocytes isolation. Human hepatocytes were isolated by collagenase recirculating perfusion method (Table 1) and then stored in UW solution (pH = 7.4, 37 °C). After storage for 1, 12, 24 and 48 h, hepatocytes viability was determined by trypan blue exclusion. LDH release rate was assessed by biochemical method. ATP levels during cold storage and after incubation at 37 °C were assessed by HPLC. *Results*: Hepatocytes viability just after isolation was 88 ± 1.4%. LDH release was 10.1 ± 0.6%. There were no great changes of viability and LDH release rate after preserved for 12, 24 and 48 h. ATP level during cold storage decreased significantly, but after incubated at 37 °C, there were no great difference of ATP level (Table 2). *Conclusions*: UW solution could protect hepatocytes effectively during cold storage at 0–4 °C.

Keywords: UW solution; Cold storage; Hepatocyte

1. Background

The aim of the study is to get isolated human hepatocytes and to investigate the protective effect of UW solution on human hepatocytes during cold storage at 0–4 °C. In this study, human livers were harvested from non-heart beating donors.

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Table 1
Perfusion solution for human hepatocytes isolation (pH = 7.4, 37 °C)

	Initial perfusion solution	Perfusion solution
Hanks solution	+	+
Ca++	–	+
Mg++	–	+
Gentalmycin	–	–
EGTA	0.5 mmol/l	–
HEPES	–	15 mmol/l
Collagenase II	–	0.5 mmol/l
Hyaluronidase	–	0.5 mmol/l

2. Methods

Well-perfused liver lobes weighing 60–80 g were used for hepatocytes isolation. Human hepatocytes were isolated by collagenase recirculating perfusion method (Table 1) and then stored in UW solution (PH = 7.4, 37 °C). After storage for 1, 12, 24 and 48 h, hepatocytes viability was determined by trypan blue exclusion. LDH release rate was assessed by biochemical method. ATP levels during cold storage and after incubation at 37 °C were assessed by HPLC.

3. Results

Hepatocytes viability just after isolation was 88 ± 1.4%. LDH release was 10.1 ± 0.6%. There were no great changes of viability and LDH release rate after preserved for 12, 24 and 48 h. ATP level during cold storage decreased significantly, but after incubation at 37 °C, there were no great difference of ATP level (Table 2).

4. Conclusions

Human hepatocytes could be isolated by collagenase recirculating method successfully. UW solution could protect hepatocytes effectively during cold storage at 0–4 °C.

Table 2
Hepatocytes viability and ATP volume (μmol/g wet weight)

	0	12 h	24 h	48 h
Viability (%)	88.0 ± 1.4	86.2 ± 1.2	83.6 ± 1.8	82.5 ± 1.5
LDH Release rate (%)	10.1 ± 0.6	10.6 ± 0.3	11.2 ± 0.9	12.1 ± 0.7
ATP during cold storage	12.6 ± 1.1[a]	8.1 ± 0.8[a]	7.2 ± 0.9[a]	5.8 ± 0.6
ATP after incubation	14.5 ± 0.9	14.3 ± 1.0	14.0 ± 1.2	13.2 ± 1.1

[a] Compared with initial isolated hepatocytes (0 h), $p < 0.01$.