Mini Review
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Pharmacokinetics and isolated hepatocyte

Abstract

Hepatic metabolism is an important contributor affecting the bioavailability of chemicals. Today isolated hepatocytes are widely used to study the biological assessment of xenobiotics and drug metabolisms. In this regard, methods have been developed to implement systems to evaluate in vitro biological properties of natural and synthetic compounds. This mini review will provide a brief introduction of two isolated hepatocyte preparation methods.

Keywords: hepatocytes, drug metabolism, bioavailability, xenobiotics

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Introduction

Two main groups of collagen in the body which is present in an extracellular matrix have been identified: fibrillar (Type I, II, III, V, XI) and non-fibrillar collagen.[1] A mixture of collagens is embedded in the collagenous fibrillar network of the liver. Approximately 80% of the total hepatic collagen is made up of type I and type III collagens.[2] Moreover, these types of collagen are present in the renal interstitium and blood vessels.[3] By means of this structural protein the hepatocytes are attached to other neighboring hepatocytes. Knowledge about this key component has shaped the methods of isolation of not only hepatocytes but also other mammalian cells such as kidney glomeruli.

Clostridium species excrete collagenases which break the peptide bonds in collagen and contribute to its pathogenesis. This enzyme is widely used to isolate both parenchymal and non-parenchymal cells. Non-parenchymal cells and Kupffer cells could be isolated from liver by pronase and collagenase perfusion.

Hepatocyte isolation methods

Method 1: In this method liver is perfused continuously with SC-1 solution at 37°C for 5 min followed by 15 min perfusion with 0.03% collagenase which is prepared by dissolving in SC-2 solution (Table 1). After these perfusion procedures, the liver is cut, and the cells are suspended in Geys balance salt solution (GBSS). The cell suspension is filtered using a steel mesh and centrifuged at 50 g for 1 min. The cell pellet was resuspended in GBSS solution, and the washing procedure was repeated three times.[4]

Method 2: Calcium-free Hanks balanced salt solution with HEPES (HBSSH) is prepared by combining 100 ml of each stock solution (A,B,C,D) with 600 ml deionized, distilled water and pH is adjusted to 7.5 by adding NaOH. Deionized, distilled water is added to each stock up to 1L (Table 2).

Table 1 List of chemicals used in Method 1

Ph 7.25 (All in Mg/L)									
SC-1 Solution	NaCl	KCl	$NaH_2PO_4 \cdot H_2O$	Na_2HPO_4	HEPES	$NaHCO_3$	EGTA	Glucose	
	8,000	400	88.7	120.45	2,380	350	190	900	
SC-2 Solution	NaCl	KCl	$NaH_2PO_4 \cdot H_2O$	Na_2HPO_4	HEPES	$CaCl_2 \cdot 2H_2O$			
	8,000	KCl	88.7	120.45	2,380	560			
Geys Balance Salt Solution	NaCl	KCl	$MgCl_2 \cdot 6H_2O$	$MgSO_4 \cdot 7H_2O$	NaH_2PO_4	KH_2PO_4	Glucose	$NaHCO_3$	$CaCl_2 \cdot 2H_2O$
	8,000	370	210	70	120	30	991	227	225

Table 2 List of chemicals used in Method 2

All in G/L								
Stock A	**Stock B**			**Stock C**				**Stock D**
Glucose	$MgCl_2 \cdot 6H_2O$	$MgSO_4 \cdot 7H_2O$	NaCl	KCl	KH_2PO_4	Na_2HPO_4	Phenol Red	HEPES
10 g	1 g	1 g	80 g	4 g	0.6 g	0.49 g	0.1 g	48 g
Collagenase stock buffer (pH 7.4)								
NaCl	KCl	$CaCl_2.2H_2O$		HEPES			NaOH (1N)	
3.9 g	0.5 g	0.7 g		24 g			66 ml	

To prepare 1% collagenase buffer solution dissolve 50 mg collagenase type I in 50 ml collagenase stock buffer. To obtain non parenchymal cells pronase which effectively digest parenchymal cells could be used. After these procedures the liver is embedded in a dish containing HBSSH. The cell suspension is centrifuged at 50 g for 4 min at 4°C. Then supernatant is discarded and cells are resuspended in fresh medium.[5]

Conclusion

Isolated hepatocytes provide a suitable tool which facilitates evaluating not only chemical metabolisms but also the effects of drugs on organelles in both human and animal cells. In various studies cells viability was between 85% and 97%.[6] Since in method 1 and 2 the weight/volume percentage concentration of the collagenase solution is respectively 0.03 and 1 and the washing time period differ in both methods, it is suggested that the efficacy of different methods should be simultaneously assessed in a similar experiment.

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Conflict of interest

The author declares no conflict of interest.

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