

论著

## 冷冻复苏对大鼠肝细胞代谢活性的影响

潘琼<sup>1,2</sup>, 陈彦<sup>1</sup>, 梅黎<sup>1</sup>, 戴仁科<sup>1\*</sup>

(1. 中国科学院广州生物医药与健康研究院, 广东 广州 510663; 2. 中国科技大学生命科学院, 安徽 合肥 230026)

收稿日期 2008-4-2 修回日期 网络版发布日期 2008-11-28 接受日期 2008-8-15

**摘要** 目的 研究冷冻复苏对肝细胞代谢活性及细胞色素P450 (CYP) mRNA表达调控的影响, 为冷冻复苏肝细胞用于实验研究提供依据。方法 采用程序冷冻降温仪冷冻新鲜分离的大鼠肝细胞, 1个月后复苏。实时荧光定量PCR法检测细胞CYP1A2, CYP2B1和CYP3A1 mRNA的表达; 高压液相色谱-串联质谱法检测细胞内咪达唑仑 $\alpha$ -羟基化、双氯芬酸4-羟基化和右美沙芬去甲基化的代谢产物生成量。结果 肝细胞冷冻复苏后存活率与新鲜细胞无明显差异, 显微镜下可见细胞贴壁并连成片状, 细胞核圆而亮。冷冻复苏肝细胞中CYP1A2和CYP2B1 mRNA的诱导表达与新鲜细胞无明显差异, CYP3A1 mRNA无明显表达。与新鲜细胞相比, 咪达唑仑 $\alpha$ -羟基化代谢产物水平无明显差异; 双氯芬酸4-羟基化产物约为1/2; 右美沙芬去甲基化产物则增加了1倍。结论 冷冻复苏对肝细胞的代谢活性有一定的影响。在药物代谢研究中须考虑冷冻复苏因素对肝细胞代谢活性的影响, 有助于对实验结果做出客观和恰当的判断。

**关键词** [肝细胞](#) [低温保存](#) [代谢解毒, 药物](#) [细胞色素P450](#)

分类号 [R965](#)

## Effect of cryopreservation on metabolic activities of hepatocytes

PAN Qiong<sup>1,2</sup>, CHEN Yan<sup>1</sup>, MEI Li<sup>1</sup>, DAI Ren-Ke<sup>1\*</sup>

(1. Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510663, China; 2. School of Life Sciences, University of Science and Technology of China, Hefei 230026, China)

### Abstract

**AIM** To study the effect of cryopreservation on metabolic activities and cytochrome P450 (CYP) mRNA expression in hepatocytes and provide support for application of cryopreserved hepatocytes in experimental research. **METHODS** Freshly isolated rat hepatocytes were cryopreserved with rate-controlled freezer, and thawed after 1 month. Real-time quantitative PCR was used to detect expressions of CYP1A2, CYP2B1 and CYP3A1 mRNA, and LC-MS/MS was used to measure contents of metabolites of midazolam-1'-hydroxylation (OH-Mid), diclofenac-4'-hydroxylation (OH-Dic) and dextromethorphan-O-demethylation (Dex) in hepatocytes, respectively. **RESULTS** There was no significant difference in cell viability between fresh and cryopreserved hepatocytes. The cryopreserved hepatocytes attached and established extensive cell-cell contact, with round and bright nucleus. CYP1A2 and CYP2B1 mRNA expressions induced by  $\beta$ -naphthoflavone and phenobarbital in cryopreserved hepatocytes were similar to that in the fresh primary cells. However, CYP3A1 mRNA expression did not induced by pregnenolone-16 $\alpha$ -carbonitrile in cryopreserved hepatocytes. In cryopreserved hepatocytes, the content of OH-Mid was remained as almost the same as the fresh primary hepatocytes, while contents of OH-Dic decreased approximately as a half, and Dex was double as fresh hepatocytes. **CONCLUSION** Cryopreservation exerts different effects on metabolic activities of hepatocytes. To acquire objective and appropriate results, it is necessary to consider the different influence of cryopreservation on cell metabolic activity in drug metabolic research.

**Key words** [hepatocytes](#) [cryopreservation](#) [metabolic detoxication](#) [drug](#) [cytochrome P-450](#)

DOI: 10.3867/j.issn.1000-3002.2008.06.011

通讯作者 戴仁科 [dai\\_renke@gibh.ac.cn](mailto:dai_renke@gibh.ac.cn)

### 扩展功能

#### 本文信息

- ▶ [Supporting info](#)
- ▶ [PDF\(1311KB\)](#)
- ▶ [\[HTML全文\]\(0KB\)](#)
- ▶ [参考文献](#)

#### 服务与反馈

- ▶ [把本文推荐给朋友](#)
- ▶ [加入我的书架](#)
- ▶ [加入引用管理器](#)
- ▶ [复制索引](#)
- ▶ [Email Alert](#)
- ▶ [文章反馈](#)
- ▶ [浏览反馈信息](#)

#### 相关信息

- ▶ [本刊中 包含“肝细胞”的 相关文章](#)
- ▶ [本文作者相关文章](#)

- [潘琼](#)
- [陈彦](#)
- [梅黎](#)
- [戴仁科](#)