Use of Plateable Cryopreserved Rat Hepatocytes as a Model to Assess Hepatotoxicity and Drug-Drug Interactions

Timothy A. Moeller, Scott Lloyd, Paul M. Silber, and Neil S. Jensen

Abstract
Freshly isolated rat hepatocytes are widely used to study drug metabolism, toxicity, and drug-drug interactions. However, the availability of freshly isolated hepatocytes is limited and can be subject to inter-day variability with respect to both the quantity and quality of isolated cells. We describe the use of cryopreserved rat hepatocytes (PCR) that form monolayers on collagen-coated plates, and which can be used to assay long-term (>24 h) toxicity and drug-drug interactions. To evaluate the PCR model, we compared metabolism of ethoxyresorufin in freshly isolated rat hepatocytes from different breeds of rats to that observed in PCR. The in vitro metabolism of ethoxyresorufin was measured on day 5. An 8-fold increase in ethoxyresorufin was measured on day 5. An 8-fold increase in ethoxyresorufin O-deethylase (EROD) activity was observed following exposure to 3-methylcholanthrene. An 8-fold increase in testosterone 6ß-hydroxylase activity of 1100% to 4000% (Fig. 3). Induction of CYP1A with either 3-methylcholanthrene (1 µM) or dexamethasone (50 µM) resulted in an increase in testosterone 6ß-hydroxylase activity of 200% to 1500% (Fig. 2). Induction of CYP3A with 3-methylcholanthrene (1 µM) induction of CYP1A and CYP3A activities can be demonstrated in PCR. PCR have the ability to maintain a viable monolayer for at least five days. Viability and CYP1A and CYP3A induction and metabolism. MTT was added to PCRH incubated with inducers or vehicle control to determine viability (Fig. 1). Induction of CYP1A with either 3-methylcholanthrene (1 µM) or 3-methylcholanthrene (1 µM) induction of CYP3A as measured by the metabolism of ethoxyresorufin to resorufin in four lots of plateable cryopreserved rat hepatocytes.

Introduction
Hepatocytes from different breeds of rats have been successfully isolated and cultured for over 20 years. Rat hepatocytes have been in a broad range of studies including drug metabolism, drug-drug interaction, compound toxicity, drug-protein, and experiments for liver assist devices. Freshly isolated rat hepatocytes have been the gold standard for many of these studies. This is due to its ability to activate metabolic compounds when induced in cell suspension, or to attach to collagen-coated tissue culture plates where they can be cultured for up to 5 days. Traditionally, cryopreserved rat hepatocytes have only been used for short-term studies. They have not been shown to attach to collagen-coated plates in numbers sufficient for use in multi-day studies. The use of cryopreserved rat hepatocytes is limited by the availability of freshly isolated hepatocytes. In the work presented here, we demonstrate the use of plateable cryopreserved rat hepatocytes. These hepatocytes can be thawed and cultured in collagen-coated plates where they can attach, grow for at least five days (>75% confluence) and be used for long-term toxicity studies and induction studies. The availability of cryopreserved rat hepatocytes with these characteristics can provide researchers with a consistent adherent rat hepatocyte model that avoids the indiscriminate requirement of fresh hepatocytes.

Materials and Methods
Plateable cryopreserved rat hepatocytes. Plateable rat cryopreserved hepatocytes (PCR) were obtained from BioreclamationIVT, 1450 South Rolling Road, Baltimore, MD. PCR samples were thawed at room temperature in a humidified tissue culture incubator for 1 hour before being transferred to a collagen coated 48-well plate (140,000 cells per well). Plates were then incubated overnight in a 37°C, 5% CO2 humidified incubator to allow attachment of the hepatocytes. Medium was replaced after 24 hr.

Results

Figure 1. Viability of PCR at 96 hours after plating as determined by MTT reduction.

Figure 2. Dexamethasone (50 µM) induction of CYP3A as measured by the metabolism of ethoxyresorufin to resorufin in four lots of plateable cryopreserved rat hepatocytes.

Figure 3. 3-methylcholanthrene (1 µM) or 3-methylcholanthrene (1 µM) induction of CYP3A as measured by the metabolism of ethoxyresorufin to resorufin in four lots of plateable cryopreserved rat hepatocytes.

Figure 4. Toxicity of tamoxifen at 24 hours as measured by MTT reduction and ATP levels using plateable cryopreserved rat hepatocytes.

Figure 5. Toxicity of tamoxifen at 48 hours as measured by MTT reduction and ATP levels using plateable cryopreserved rat hepatocytes.

Conclusion
- PCR have the ability to maintain a viable monolayer for at least five days.
- Induction of CYP1A and CYP3A activities can be demonstrated in PCR.
- Toxicity of compounds can be assessed by multiple day inductions using PCR.
- PCR offer a reliable and convenient alternative to freshly plated rat hepatocytes.

Abstract
Freshly isolated rat hepatocytes are widely used to study drug metabolism, toxicity, and drug-drug interactions. However, the availability of freshly isolated hepatocytes is limited and can be subject to inter-day variability with respect to both the quantity and quality of isolated cells. We describe the use of cryopreserved rat hepatocytes (PCR) that form monolayers on collagen-coated plates, and which can be used to assay long-term (>24 h) toxicity and drug-drug interactions. To evaluate the PCR model, we compared metabolism of ethoxyresorufin in freshly isolated rat hepatocytes from different breeds of rats to that observed in PCR. The in vitro metabolism of ethoxyresorufin was measured on day 5. An 8-fold increase in ethoxyresorufin O-deethylase (EROD) activity was observed following exposure to 3-methylcholanthrene. An 8-fold increase in testosterone 6ß-hydroxylase activity of 1100% to 4000% (Fig. 3). Induction of CYP1A with either 3-methylcholanthrene (1 µM) or dexamethasone (50 µM) resulted in an increase in testosterone 6ß-hydroxylase activity of 200% to 1500% (Fig. 2). Induction of CYP3A with 3-methylcholanthrene (1 µM) induction of CYP1A and CYP3A activities can be demonstrated in PCR. PCR have the ability to maintain a viable monolayer for at least five days. Viability and CYP1A and CYP3A induction and metabolism. MTT was added to PCRH incubated with inducers or vehicle control to determine viability (Fig. 1). Induction of CYP1A with either 3-methylcholanthrene (1 µM) or 3-methylcholanthrene (1 µM) induction of CYP3A as measured by the metabolism of ethoxyresorufin to resorufin in four lots of plateable cryopreserved rat hepatocytes.

Introduction
Hepatocytes from different breeds of rats have been successfully isolated and cultured for over 20 years. Rat hepatocytes have been in a broad range of studies including drug metabolism, drug-drug interaction, compound toxicity, drug-protein, and experiments for liver assist devices. Freshly isolated rat hepatocytes have been the gold standard for many of these studies. This is due to its ability to activate metabolic compounds when induced in cell suspension, or to attach to collagen-coated tissue culture plates where they can be cultured for up to 5 days. Traditionally, cryopreserved rat hepatocytes have only been used for short-term studies. They have not been shown to attach to collagen-coated plates in numbers sufficient for use in multi-day studies. The use of cryopreserved rat hepatocytes is limited by the availability of freshly isolated hepatocytes. In the work presented here, we demonstrate the use of plateable cryopreserved rat hepatocytes. These hepatocytes can be thawed and cultured in collagen-coated plates where they can attach, grow for at least five days (>75% confluence) and be used for long-term toxicity studies and induction studies. The availability of cryopreserved rat hepatocytes with these characteristics can provide researchers with a consistent adherent rat hepatocyte model that avoids the indiscriminate requirement of fresh hepatocytes.

Materials and Methods
Plateable cryopreserved rat hepatocytes. Plateable rat cryopreserved hepatocytes (PCR) were obtained from BioreclamationIVT, 1450 South Rolling Road, Baltimore, MD. PCR samples were thawed at room temperature in a humidified tissue culture incubator for 1 hour before being transferred to a collagen coated 48-well plate (140,000 cells per well). Plates were then incubated overnight in a 37°C, 5% CO2 humidified incubator to allow attachment of the hepatocytes. Medium was replaced after 24 hr.

Results

Figure 1. Viability of PCR at 96 hours after plating as determined by MTT reduction.

Figure 2. Dexamethasone (50 µM) induction of CYP3A as measured by the metabolism of ethoxyresorufin to resorufin in four lots of plateable cryopreserved rat hepatocytes.

Figure 3. 3-methylcholanthrene (1 µM) or 3-methylcholanthrene (1 µM) induction of CYP3A as measured by the metabolism of ethoxyresorufin to resorufin in four lots of plateable cryopreserved rat hepatocytes.

Figure 4. Toxicity of tamoxifen at 24 hours as measured by MTT reduction and ATP levels using plateable cryopreserved rat hepatocytes.

Figure 5. Toxicity of tamoxifen at 48 hours as measured by MTT reduction and ATP levels using plateable cryopreserved rat hepatocytes.

Conclusion
- PCR have the ability to maintain a viable monolayer for at least five days.
- Induction of CYP1A and CYP3A activities can be demonstrated in PCR.
- Toxicity of compounds can be assessed by multiple day inductions using PCR.
- PCR offer a reliable and convenient alternative to freshly plated rat hepatocytes.