Published in final edited form as:

J Dig Dis. 2012 November ; 13(11): 579–587. doi:10.1111/j.1751-2980.2012.00632.x.

Pharmacogenetic selection of transplanted human hepatocytes in immunocompetent rats

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Abstract

Objective—To introduce a genetic survival advantage for transplanted human hepatocytes over host cells in rats.

Methods—Green fluorescent protein (GFP) was introduced into Huh7 human hepatoma cells to create fluorescent GFP-Huh7 cells. mRNA of *CYP2E1*, the enzyme that converts acetaminophen (APA) into hepatotoxic intermediates, was quantified by real-time PCR. Effects of APA on GFP-Huh7 and control Huh7 cells were determined in cell culture. Immunological tolerance was induced by injection of GFP-Huh7 cells into fetal rats *in utero*. After birth, GFP-Huh7 cells were transplanted into tolerant rats followed by APA treatment. Serum alanine aminotransferase (ALT) levels and liver histological data were obtained. GFPHuh7 cells were detected by real-time PCR and microscopy.

Results—*CYP2E1* mRNA levels in GFP-Huh7 cells were 2.7% of parental Huh 7 cells. In 1 mmol/L APA, parental Huh7 cells decreased by 60% while GFP-Huh7 cells increased to within 95% of untreated controls after 5 days. In rats transplanted with GFP-Huh7 cells and treated with APA, serum ALT increased to a peak of 210 U/mL on day 1 and returned to normal levels by day 3. Fluorescence microscopy of liver specimens from rats transplanted with GFP-Huh7 cells showed substantial increases in GFP-Huh7, but not Huh 7 cells by day 7 after APA treatment. Real-time PCR confirmed a 10-fold increase of GFP mRNA in APA-treated rats, but not in those without APA treatment.

Conclusions—The difference in *CYP2E1* gene expression between GFP-Huh7 and rat hepatocytes provides a convenient means for enrichment of transplanted human cells in rat liver.

Keywords

hepatocytes transplantation; pharmacological selection; animal models

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Conflict of Interest: None

INTRODUCTION

The development of preventive and therapeutic agents for liver disease has been hampered by the lack of suitable animal models.¹ Liver of rats and mice have substantial differences compared with human liver, especially in terms of susceptibility to hepatitis viruses.¹ Chimpanzees and other primates are much better experimental models, but their use has been limited by ethical concerns, restricted availability and prohibitively high costs.² Recently, experiments on chimpanzees has been forbidden in the USA.³ Therefore, it has become increasingly important to develop suitable small-animal models of liver disease for preclinical testing, as well as for facilitating studies on the pathogenesis of diseases.

Previously, we reported on an approach to generate an animal model of hepatitis B (HBV)⁴ and hepatitis C (HCV)⁵ infections in which rodent livers were humanized by xenotransplantation of human hepatocytes, showing that human hepatocytes could be transplanted and survive in rats when the hosts are rendered tolerant to human hepatocytes. In this model, immunotolerance was generated by intrafetal inoculation of human hepatocytes during the embryological development of the fetal immune system.⁶ However, although the rats were able to host human hepatocytes and subsequent HCV infection, the fraction of human cells in the HCV rat model was relatively low, and the amount of virus generated in the model was correspondingly small.

The metabolism and toxicity of acetaminophen (APA), a commonly used analgesic-antipyretic drug, have been widely studied. APA is a safe drug at recommended levels, but in cases of overdose can cause acute liver⁷ and renal failure⁸. At pharmacological doses, APA is mainly metabolized by glucuronidation,⁹ and to a lesser extent, by cytochrome P450 2E1 (CYP2E1) which produces a reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI)¹⁰. Toxicity of APA occurs when large amounts of APA are metabolized by the CYP450 pathway, leading to the depletion of glutathione (GSH)¹¹ and covalent binding of NAPQI to vital cellular components¹⁰. This ultimately disrupts cellular function which leads to apoptosis and necrosis.¹²

Many P450 enzymes are involved in the metabolism of APA, including CYP1A2, CYP2A6, CYP3A and CYP2E1, the latter of which is considered to be the main enzyme responsible for the bioactivation of APA as evident by enzyme kinetics, and timing of APA intoxication^{13,14}. Furthermore, it has been found that *CYP2E1*-knockout mice were highly resistant to toxic doses of APA.¹⁵ When the human *CYP2E1* gene was reintroduced into *CYP2E1*-null mice, APA-mediated hepatotoxicity was restored,¹⁶ suggesting that manipulation of the expression of *CYP2E1* might be useful in creating a selective survival advantage for transplanted human cells after APA treatment.

The overall objective of the current research was to increase the relative numbers of transplanted human hepatocytes in normal rats to create a rat model for viral and other infectious processes which is more similar to the human condition.

MATERIALS AND METHODS

Cell lines and cell culture

E47 cells (a generous gift from Dr. Arthur I. CEDERBAUM, Mount Sinai School of Medicine, New York, NY, USA), which were derived from HepG2 (a hepatoblastoma cell line constitutively expressing human CYP2E1), was used as a positive control. The cells were grown in Dulbecco Modified Eagle Medium (DMEM; 12800-017, Life Technologies, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS; 10438-026, Life Technologies, Grand Island, NY, USA) and 0.5 mg/mL Geneticin® Selective Antibiotic (G418 Sulfate; 11811-031, Life Technologies, Grand Island, NY, USA) supplemented with 1× penicillin-streptomycin solution (15140-122, Life Technologies, Grand Island, NY, USA) in 5% CO₂ at 37°C using a standard humidified CO₂ incubator. Cells were subcultured at a ratio of 1:5 once a week.

Huh7, a well-differentiated human hepatoma cell line,¹⁷ was maintained in DMEM supplemented with 10% FBS and 1× penicillin-streptomycin solution (all from Life Technologies, Grand Island, NY, USA as shown above). Cells were passaged once a week to maintain 75% confluent conditions.

Green fluorescent protein (GFP)-Huh7 cells were obtained by stable transfection of Huh7 cells with a murine retroviral vector, *MG-1* (a generous gift from Dr. David I. DORSKY, University of Connecticut Health Center, Farmington, CT, USA), containing *GFP* and *neomycin* resistance genes.¹⁸ Briefly, 3×10^4 viral particles/mL medium were mixed with polybrene (8 µg/mL as final concentration) and placed in media of Huh7 cells at 25% confluency at 37°C. After 2 h, the inoculation medium was removed, and then the culture was incubated with fresh medium for 24 h. Cells were trypsinized and replated onto new dishes containing medium with 200 µg/mL G418. Fluorescent cells resistant to G418 due to the presence of the *neomycin* resistance gene were harvested after 3 weeks.

For the use in animals, trypsinized cells were washed extensively in sterile phosphate-buffered saline (PBS; pH 7.0). A total of 1×10^7 cells per mL PBS were used for tolerization (see below), and 5×10^7 cells/mL PBS were used for transplantation in animals (see below).

As a negative control, NIH 3T3, a mouse fibroblast cell line known to be lack of *CYP2E1* gene expression, was obtained from American Type Culture Collection (ATCC) and cultured in DMEM supplemented with 10% FBS and 1× penicillin-streptomycin solution. Cells were grown at 37°C in a humidified atmosphere of 5% CO₂. Cells were passaged every 3–4 days to maintain 75% confluent conditions.

APA treatment of cultured cells and assessment of cell viability

To assess the toxicity of APA (A3035; Sigma-Aldrich Co., St. Louis, MO, USA), equal numbers of cells of the three cell lines (Huh7, GFP-Huh7 and E47) were seeded in microtiter plates. On day 1, fresh culture medium with 1 mmol/L APA was added to each cell line. At daily intervals, viable numbers of cells were assessed using MTT Cell Proliferation Assay Kit (M6494, Life Technologies, Grand Island, NY, USA) according to

the manufacturer's instructions. In brief, 10 mL of 12 mmol/L MTT stock in sterile saline was added to 100-μL fresh DMEM in each well, and incubated for 4 h at 37°C. Cells were solubilized with 100 μL SDS-HCl solution (10% SDS in 0.01 mol/L HCl) followed by 4 h of incubation at 37°C. Absorbance was read at 570 nm using a Safire 2 Microplate Reader (Tecan USA, Research Triangle Park, NC, USA). Assays were performed in quadruplicate, and readings of treated cells normalized to the absorbance of control untreated cells at the same time point and were expressed as mean ± standard deviation (SD).

Measurement of *CYP2E1* gene expression in various cell lines by reverse transcription-polymerase chain reaction (RT-PCR) and quantification by real-time polymerase chain reaction (PCR)

RNA was isolated from 5×10^5 cells with Trizol (15596-026, Life Technologies, Grand Island, NY, USA) and treated with RQ1 RNase-Free DNase (M6101, Promega, Madison, WI, USA). One mg of DNase-treated total RNA was reversely transcribed using iScript™ cDNA Synthesis Kit (170-8890, Bio-Rad Laboratories, Hercules, CA, USA). After incubation at 25°C for 5 min, at 42°C for 30 min and at 85°C for 5 min, the resulting cDNA was amplified by PCR carried out with 1 U *Taq* DNA Polymerase, Recombinant (10342-046, Life Technologies, Grand Island, NY, USA) with 20 pmol/L of *CYP2E1* specific primers: forward: 5'-CCTACATGGATGCTGTGGTG-3' and reverse: 5'-TCCAGCACACACTCGTTTTTC-3' and with 20 pmol/L of lactate dehydrogenase A (*LDHA*) specific primers: forward: 5'-TAATGAAGGACTTGGCAGATGAACT-3' and reverse: 5'-ACGGCTTTCTCCCTCTTGCT-3'. Thirty cycles of PCR reactions were performed at 95°C for 2 min, 95°C for 30 s, 58.3°C for 30 s and 72°C for 30 s. The final PCR products were analyzed on 2% agarose gels. cDNA levels were further measured by quantitative PCR (qPCR) with FastStart SYBR Green Master (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's protocol using *CYP2E1* specific primers: forward: 5'-AGCAGATTTCTGGGAGCCTCAGTT-3' and reverse: 5'-AGTGGAAGTGTGACTTGGAGCTGT-3'.

Human *LDHA* mRNA levels in each sample were simultaneously quantified to normalize the values of *CYP2E1* RNA. The primer sequence for *LDHA*: forward: 5'-TAATGAAGGACTTGGCAGATGAACT-3' and reverse: 5'-ACGGCTTTCTCCCTCTTGCT-3'. Assays were performed in triplicate and results were expressed as mean ±SD of *CYP2E1* gene expression as a percentage of the control cell line.

Animals

Sprague-Dawley female rats, 15 days timed-pregnant, were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN, USA), maintained on 12 h light-dark cycles and fed *ad lib* with standard rat chow. Animal handling and surgery were performed in accordance with protocols approved by the University of Connecticut Animal Care and Use Committee, and conformed to USDA and National Institute of Health (NIH) animal usage guidelines.

Tolerization of normal rats to GFP-Huh7 cells

Tolerization of rats was performed as described previously.¹⁸ In brief, at 17 days of gestation, pregnant rats were anesthetized with ketamine/xylazine (90 mg/kg and 10 mg/kg,

respectively), and the abdominal cavities and gravid uteri were exposed by laparotomy under aseptic conditions. GFP-Huh 7 cells of 1×10^5 in 10 μ L PBS were injected through the uterine wall into intraperitoneal cavity of fetal rats.

Transplantation of GFP-Huh 7 cells and selection by APA administration

Five days after birth, groups of tolerized rats were transplanted with GFP-Huh 7 cells by slowly injecting 5×10^6 cells in 100 μ L PBS through the translucent abdominal skin wall directly into the spleen. Two weeks after transplantation, tolerized rats were treated with toxic doses of APA at 1250 mg/kg ($n = 20$) or normal saline ($n = 20$) by intraperitoneal injection. At varying times after APA treatment, groups of rats were sacrificed and their blood and livers were collected. Sera from collected blood were frozen and used for liver enzyme assessment. Portions of liver sections were flash frozen in liquid nitrogen for cryosectioning, whereas other portions of livers were fixed for immunohistochemical (IHC) analysis or fixed in formalin and processed for histological analysis.

Visualization of transplanted GFP-Huh7 cells in rat livers

The presence of human GFP-Huh7 cells in rat livers was confirmed by quantitating the numbers of GFP (+) cells, and measuring levels of GFP mRNA in rat livers using qPCR.

Detection of GFP (+) cells in rat liver by fluorescence microscopy—Cryostat sections (5 μ m) from unfixed frozen tissues were processed conventionally, and GFP (+) cells in rat liver sections were detected using a fluorescence microscopy (Observer. 21, with Axio Cam; Carl Zeiss, Inc. Thornwood, NY, USA) at $\times 200$ magnification.

Quantitation of GFP mRNA in rat liver by quantitative real-time PCR—To quantitate the changes of the numbers of transplanted GFP-Huh7 cells following APA treatment, RNA from liver specimens with and without APA treatment was extracted and subjected to quantitative real-time PCR. RNA was isolated from 50 mg liver tissue with Trizol, and treated with RNase-free DNase (Promega, Madison, WI, USA). One mg of DNase-treated total RNA was reversely transcribed using iScript cDNA Synthesis Kit (BioRad Laboratories, Hercules, CA, USA). After incubation at 25°C for 5 min, at 42°C for 30 min and at 85°C for 5 min, the resulting cDNA was quantified by qPCR with FastStart SYBR Green Master (Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's protocol using *GFP* specific primers: forward: 5'-CAGCAGAACACCCCCATC-3' and reverse: 5'-CTCGTCCATGCCGAGAGT-3'. Human *LDHA* mRNA levels in each sample were simultaneously quantified to normalize values of *GFP* RNA. The primer sequence for *LDHA*: forward: 5'-TAATGAAGGACTTGGCAGATGAACT-3' and reverse: 5'-ACGGCTTTCTCCCTCTTGCT-3'. Assays were performed in triplicate and results expressed as mean $\pm$ SD of liver *GFP* gene expression as a percentage of control treated animals.

Serum aminotransferase assays

To detect possible liver damage following APA administration, 10- μ L aliquots of rat serum, were assessed spectrophotometrically for alanine aminotransferase (ALT) one day prior to

as well as 1–7 days after APA injection using an Infinity ALT solution (Thermo Electron, Melbourne, Australia) according to the manufacturer's instructions. All assays were performed in triplicate and results expressed as mean $\pm$ SD (U/L).

Liver Histology

To observe the histological effects of APA treatment, liver specimens at various time after APA administration were fixed in 10% formalin and stained with hematoxylin and eosin (HE), and were evaluated for liver architecture and cellular reaction by a pathologists who was blinded to the protocol.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2008 (Microsoft Corp., Farmington, CT, USA). Student's *t*-test was performed for the analysis of MTT, liver enzyme assessment and qPCR. $P < 0.05$ was regarded as statistically significant.

RESULTS

Expression of the *CYP2E1* gene in cultured cells

Figure 1 shows the results of RT-PCR detection of *CYP2E1* RNA and *LDHA* RNA. The final products of the PCR reactions amplified with specific primers were analyzed on 2% agarose gels. To ensure equal loading of final products of PCR amplification of *CYP2E1* mRNA, the same amount of cDNA amplified from isolated mRNA using specific *LDHA* primers were loaded simultaneously. Final products of PCR with specific primers to *CYP2E1* mRNA in cell lysates of Huh7 cells, positive control E47 cells and GFP-Huh7 cells all showed bands corresponding in migration to an expected 236 bp fragment of the amplified *CYP2E1* sequence, compared with the size markers. In contrast, final PCR products for *CYP2E1* mRNA extracted from cell lysates of negative control NIH 3T3 cells showed as expected no signal under the same conditions. It was further noted that the signal in GFP-Huh7 cells was visibly less than that in parent human hepatoma cell line Huh7 cells, as well as that in positive control E47 cells, a human hepatoma HepG2 cell subclone which constitutively expresses human *CYP2E1*.

To quantify the observed difference in expression of human *CYP2E1* gene in the various human hepatoma cell lines, qRT-PCR was performed using specific primers to human *CYP2E1* mRNA and the results are shown in Table 1. Human *CYP2E1* mRNA levels in GFP-Huh7 cells were only 2.69% of that in parental Huh7 cells. As expected, expression of human *CYP2E1* in the positive control E47 cells was 16 times more abundant than that in Huh7 cells whereas expression of human *CYP2E1* was undetectable in negative control NIH 3T3 cells under the same conditions.

Effect of APA treatment on cultured human liver cell lines, Huh7, GFP-Huh7, and E47

To determine whether the differential expression of *CYP2E1* gene in the liver cell lines could result in differences in susceptibility to APA in cell culture, equal numbers of Huh 7, GFP-Huh7 and E47 cells were exposed to 1 mmol/L APA at day 1 in culture. The numbers of viable cells were assessed daily by MTT assays. Fig. 2 shows that cells with the highest

levels of *CYP2E1* mRNA, E47 cells, were the most susceptible to APA toxicity. Of E47 cells treated with 1 mmol/L APA, viable cell numbers were decreased to 76% and 42% at 2 and 5 days after APA treatment, respectively ($P < 0.0003$). Similarly, the number of viable parental Huh7 cells treated with 1 mmol/L APA was decreased to 40% after 5 days ($P < 0.0003$). In contrast, Huh7-GFP cells were the least susceptible to APA. The number of viable GFP-Huh7 cells steadily increased at rates similar to those of the untreated cells (> 95%) after 5 days of APA exposure. At a higher APA concentration of 2.5 mmol/L, the numbers of viable GFP-Huh 7 cells also declined, but after 5 days the numbers of viable GFP-Huh 7 cells were still almost double (1.7 times) those of parental Huh 7 cells (data not shown).

Effect of APA treatment on ALT levels in tolerized rats transplanted with GFP-Huh7 cells

On day 1 after APA treatment, serum ALT increased to a maximum of 210 U/mL, and then decreased to 160 U/mL on day 2 (Fig. 3). The level of ALT with APA treatment was significantly higher at day 1 and 2 after APA treatment compared with that of controls ($P = 0.03$ and $P = 0.01$ for day 1 and 2, respectively). ALT levels in APA-treated rats decreased to almost baseline levels by day 3 after treatment.

Effect of APA treatment on liver histology in tolerized rats transplanted with GFP-Huh7 cells

Fig. 4 shows that on day 1 and day 2 after APA administration, there was significant patchy central necrosis corresponding in time to the ALT peak level in Fig. 3. The changes in histological patterns were not seen in control animals which were tolerized and transplanted with GFP-Huh 7 cells but without APA treatment.

Detection of GFP (+) cells in rat liver by fluorescence microscopy

Fig. 5 shows the presence of GFP (+) cells in rat livers using fluorescence microscopy. On day 2 after APA treatment, livers from tolerized rats with transplanted GFP-Huh7 cells had definite but few scattered fluorescent cells. The numbers of GFP (+) cells counted by microscopy increased from 2 ± 1 on day 1 to approximately 45 ± 10 cells per high power field on day 7 after APA treatment. In contrast, liver specimens from rats which were transplanted with GFP-Huh7 cells but those untreated with APA did not show significant increase in numbers of fluorescent cells, showing that APA was required for the increase of the numbers of human hepatocytes.

Quantitation of GFP mRNA in rat liver by real-time qRT-PCR

RNA from liver specimens with and without APA treatment was extracted and subjected to quantitative PCR. Fig. 6 shows that the average Ct of GFP mRNA when normalized to that of LDHA were 0.54, 0.42, 0.18 on day 1, 2 and 3, respectively, post-APA treatment. Normalized Ct were corresponded to 0.9, 0.9 and 0.7 fold increases. On day 5, the Ct of normalized GFP mRNA was 1.46, which was an increase of 2.5-fold above baseline, and reached a peak of 3.83, a 6-fold increase on day 7 following APA treatment compared with the level on day 1 ($P < 0.001$). GFP mRNA levels in liver specimens of rats treated with only saline showed no significant changes in GFP mRNA levels at any time under identical

conditions. These data quantitatively confirm the qualitative increases in numbers of GFP (+) Huh7 cells observed by fluorescent microscopy in Fig. 5.

Discussion

It has long been known that in spite of its high degree of differentiation, the mammalian liver has the ability to regenerate under the conditions of injury¹⁹. This natural phenomenon has been used previously to create chimeric human-rodent livers.²⁰ For example, Rhim *et al.*²¹ created an elegant urokinase-type plasminogen activator (uPA) transgenic mouse in which the host hepatocytes were destroyed by transgenic expression of *uPA* gene. Transplantation of human hepatocytes into these animal models rescued them from liver failure, and resulted in replacement of large portions of the mouse liver by human hepatocytes through regeneration. Thus, in terms of the ratio of transplanted to host cells, the strategy of inducing injury to host hepatocytes not only decreases the numbers of host cells, but simultaneously provides the stimulus for proliferation of transplanted cells. However, the ratio of transplanted to host cells cannot be controlled in this model, and if insufficient numbers of normal hepatocytes are not provided, liver failure ensues. An advantage to a pharmacogenetic selection mechanism is that the destruction of host cells can be controlled by the parameters of administration of the hepatotoxin. It is, therefore, theoretically possible to tailor the extent to which the host liver is replaced by human cells by the dose and/or frequency of doses of the hepatotoxin. This might be useful in comparing the effects of drugs on human *vs.* rodent hepatocytes, especially since preclinical animal studies on drug toxicity are currently performed on rats and mice. Our results indicated that human hepatocytes transplanted into a rat liver environment are capable of responding to the regenerative stimuli as was observed in transgenic *uPA* mice, and implies that at least a portion of the receptors and downstream pathways involved in hepatocyte regeneration in human cells responds to rat regenerative stimuli.

A stable GFP-Huh7 cell line was generated by transfection of Huh7 cells with *GFP* gene and neomycin resistance (*NR*) gene. The finding that this GFP-Huh7 cell line had extremely low expression of *CYP2E1* gene was surprising as we had anticipated the necessity of employing knockdown methods to achieve the desired selective advantage. It is not clear how the transfection resulted in this phenotype. It is tempting to speculate that insertional mutagenesis was involved which affected *CYP2E1* gene expression. However, we have no evidence to support this. We did not measure *CYP2E1* enzyme activity or the activity of any other cytochromes. Therefore, it is possible that *CYP2E1* enzyme levels may not have been completely downregulated, or that effects on other cytochromes contributed to the observed effects. However, the current data indicated that the downregulation of *CYP2E1* is associated with pharmacologic selection in cell culture, and in animals exposed to APA. To demonstrate the principle of pharmacogenetic selection, this stably transfected well-differentiated hepatoma cell line was convenient. However, to prove that the full complement of hepatocyte function could be provided by this technique would best be established by transplantation of primary hepatocytes. Pharmacogenetic selection of primary cultured hepatocytes are planned.

The levels of *CYP2E1* mRNA levels measured by real-time PCR in GFP-Huh7 cells were only 2.7% of the parental Huh cell line. However, it is possible that enzyme levels could have been induced to some extent by APA exposure in the transplanted rats. If this had occurred, and some transplanted cells had been destroyed by APA, then it is possible that the numbers of human liver cells could potentially be increased by additional genetic methods to further knockdown the *CYP2E1* gene. This and longer duration studies to determine the fate of transplanted human hepatocytes using pharmacogenetic selection are in progress.

In the current studies, ALT was used as a convenient surrogate marker of liver injury. The assay measured enzyme activity, and as has been published previously, APA in sufficient doses causes a rapid increase in activity levels⁶. However, this activity does not provide information about the source of the enzyme, *i.e.* whether the source was rat or human. Because the *CYP2E1* mRNA levels were extremely low in the human GFP-Huh7 cells, and the fact that the number of GFP (+) cells persistently increased after APA administration, it is highly likely that the origin of the vast majority of the ALT elevations was the rat hepatocytes. Nevertheless, we are developing an immunological assay to distinguish between rat from human ALT.

In summary, to the best of our knowledge, this is the first report of the use of a pharmacogenetic technique to increase survival of human hepatocytes in a chimeric rat liver by administration of a hepatotoxic selection agent *in situ*.

Acknowledgments

This work was supported in part by funding from the Herman Lopata Chair in Hepatitis Research and NIDDK RO1-DK042182.

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Fig. 1.

Expression of cytochrome P450 2E1 (CYP2E1) in various cell lines estimated by reverse transcription-polymerase chain reaction (RT-PCR). To ensure equal loading of final products of PCR amplification of *CYP2E1* mRNA, the same amount of cDNA amplified from isolated mRNAs as determined by using specific *LDHA* primers were loaded simultaneously. Lanes 1 and 2, cDNA from Huh7 cells; lanes 3 and 4, NIH 3T3 cells; lanes 5 and 6, E47 cells; lanes 7 and 8, GFPHuh7 cells. cDNAs were amplified with CYP2E1 primers (lanes 2, 4, 6 and 8) and LDHA primers (lanes 1, 3, 5 and 7). Lane 9, molecular marker. Arrow denotes expected migration of CYP2E1 PCR product.

Fig. 2.

Cell viability after acetaminophen (APA) treatment measured by MTT assays. Cells with the highest levels of *CYP2E1* mRNA, E47 cells, were the most susceptible to APA toxicity. The percentage of the number of E47 and Huh7 cells decreased whereas the Huh7-GFP cells steadily increases at rates similar to those of the untreated cells after APA treatment. * $P = 0.01$, ** $P < 0.0003$.

Fig. 3. Serum alanine aminotransferase (ALT) levels in rat treated with acetaminophen (APA) and saline. (■) Rats tolerized, transplanted with GFP-Huh 7 cells and treated with APA; (□) untreated controls. * $P = 0.03$, ** $P = 0.01$.

Fig. 4. Liver sections from rats tolerized, and transplanted with GFP-Huh7 cells at (a) day 1 and (b) day 2 after treatment with acetaminophen (APA), and (c) from control rat tolerized, transplanted without APA treatment on day 2 (HE stain, $\times 200$).

Fig. 5. Fluorescence microscopy of liver cryosections of representative liver sections from tolerized, transplanted and acetaminophen (APA)-treated rats ($\times 200$). (a-e) Day 2, 3, 4, 5 and 7 post-APA treatment, respectively, and (f) rat liver sections from tolerized, transplanted but without APA treatment on day 7.

Fig. 6.

Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) of green fluorescent protein (GFP) mRNA levels in the livers from tolerized rats transplanted with GFP-Huh7 cells at various times after acetaminophen (APA) treatment. C7, a representative sample from a control tolerized, transplanted rat treated with saline instead of APA and assayed on day 7. * $P < 0.001$, ** $P < 0.01$.

Table 1

Relative levels of cytochrome P450 2E1 (CYP2E1) mRNA in various cell lines analyzed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and specific primers.

Human hepatoma cell lines	Ct	CYP2E1 mRNA Levels (normalized relative to Huh7)
Huh7	0	100%
E47 (HepG2 w/ CYP2E1)	− 4.01	1611%
GFP-Huh7	5.217	2.69%