Physiological Research Pre-Press Article

Hepatocyte transplantation attenuates the course of acute liver failure induced by thioacetamide in Lewis rats.

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Running head: hepatocyte transplantation in acute liver failure

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Summary

Acute liver failure (ALF) is a clinical syndrome resulting from widespread damage of hepatocytes, with extremely high mortality rate. Urgent orthotopic liver transplantation was shown to be the most effective therapy for ALF but this treatment option is limited by scarcity of donor organs. Therefore, hepatocyte transplantation (Tx) has emerged as a new therapeutical measure for ALF, however, the first clinical applications proved unsatisfactory. Apparently, extensive preclinical studies are needed. Our aim was to examine if hepatocytes isolated from transgenic "firefly luciferase" Lewis rats into the recipient liver would attenuate the course of thioacetamide (TAA)-induced ALF in Lewis rats. Untreated Lewis rats after TAA administration showed a profound decrease in survival rate; no animal survived 54 hours. The rats showed marked increases in plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, in plasma level of bilirubin and ammonia (NH_3), and in a significant decrease in plasma albumin. Hepatocyte Tx attenuated the course of TAA-induced ALF Lewis rats which was reflected by improved survival rate and reduced degree of liver injury showing as lowering of elevated plasma ALT, AST, NH_3 and bilirubin levels and increasing plasma albumin. In addition, bioluminescence imaging analyses have shown that in the TAA-damaged livers the transplanted hepatocyte were fully viable throughout the experiment. In conclusion, the results show that hepatocyte Tx into the liver can attenuate the course of TAA-induced ALF in Lewis rats. This information should be considered in attempts to develop new therapeutic approaches to the treatment of ALF.

Key Words: Acute liver failure, Thioacetamide, Hepatocyte transplantation.

Introduction

The liver is one of the most versatile and biologically active organs whose key functions include: a) filtration and storage of blood; b) crucial participation in body metabolism of carbohydrates, lipids, proteins and hormones, as well as the synthesis of albumin and coagulation factors); c) detoxification and biotransformation of foreign chemicals, and d) excretion of protein-bound/lipid-soluble waste products (Suchy 2009).

Therefore liver failure or insufficiency is a life-threatening condition. Acute liver failure (ALF) is a clinical syndrome resulting from widespread damage of hepatocytes with resultant loss of liver function. ALF is a rare orphan disorder which according to the statistics of the Acute Liver Failure Study Group (ALFSG, a consortium of investigators and academic clinical centers and the most reliable source of ALF data - www.acuteliver.org) affects yearly 2000 people in the U.S. (Bernal and Wendon 2013, Lee 2012, Lee *et al.* 2012, Saliba and Saumel 2013, Zhao *et al.* 2013). The cardinal signs displayed by ALF patients are coagulopathy and hepatic encephalopathy, which are the consequence of the loss of hepatocyte capability of synthesizing clotting factors and of removing ammonia (NH_3), respectively. Treatment of ALF remains supportive and continues to be one of the most challenging problems in clinical medicine (Bernal and Wendon 2013, Lee 2012, Lee *et al.* 2012, Saliba and Saumel 2013), and despite the progress in this field and unique liver regeneration ability only 45% of patients with ALF will spontaneously recover (Bernal and Wendon 2013, Lee 2012, Lee *et al.* 2012, Saliba and Saumel 2013). For the remaining 55% emergency orthotopic liver transplantation (OLT) is currently the only effective therapeutical approach. However, this treatment option has limited application, primarily due to the scarcity of donor organs in general and especially those available on short notice. The other problems are the invasiveness of OLT procedure in seriously ill patients and also the high cost of this procedure (Berg et al. 2011, Bernal and Wendon 2013, Germani *et al.* 2012, Yamashiki *et al.* 2012). Moreover, currently existing prognostic criteria are inadequate to predict who will recover from ALF without the need for OLT and who will die (Berg *et al.* 2011, Bernal and Wendon 2013, Mao *et al.* 2014, Wlodzimirow *et al.* 2012, Zhao *et al.* 2013). Therefore, in order to develop bridging techniques enabling survival until an organ becomes available for OLT or until liver function recovers, other treatment approaches, such as artificial and bioarteficial liver support have been investigated for more than three decades

(Banares *et al.* 2013, Booth *et al.* 2012, Fukumitsu *et al.* 2011, Pless 2010, Sgroi *et al.* 2009, Struecker *et al.* 2014). However, the effectiveness of these new methods is inadequate: it is now clear that artificial devices that would satisfactorily replace diverse liver functions and support a sophisticated process of the regeneration of a deteriorating organ are still not available (Banares *et al.* 2013, Booth *et al.* 2012, Fukumitsu *et al.* 2011, Pless 2010, Sgroi *et al.* 2009, Struecker *et al.* 2014). Moreover, it is important to emphasize that for ALF, a rare orphan disease affecting only a small number of patients, large controlled clinical trials are not available and therefore evidence-based medicine in this field is almost absent (Banares *et al.* 2013, Struecker *et al.* 2014).

All these facts considered, hepatocyte transplantation (Tx) has emerged as a new therapeutical approach to ALF (Dhawn *et al.* 2010, Hughes *et al.* 2012, Pareja *et al.* 2014, Sgroi *et al.* 2009), however, first therapeutic attempts proved unsatisfactory (Dhawn *et al.* 2010, Hughes *et al.* 2012, Pareja *et al.* 2014, Tritto *et al.* 2012, Yu et al. 2012). Therefore the need for extensive preclinical testing became obvious (Amano *et al.* 2011, Eguchi *et al.* 1997, Wertheim *et al.* 2012). One crucial challenge is to unequivocally identify and track the viability of the transplanted hepatocytes in the host throughout the study. A recent development of transgenic rats expressing ubiquitously the gene for luciferase derived from the firefly (*Photinus pyralis*) is a major technological achievement in this field because it allows bioluminescence imaging (BLI) of the transplanted cells *in vivo* (Hakamata *et al.* 2006). Using this technique, the same recipient can be repeatedly examined throughout the study without the need for animals to be killed at each time point (Hakamata *et al.* 2006, Kasahara et al. 2013, Maeda *et al.* 2013). Moreover, it was shown that an immunological reaction or immunosuppressive therapy substantially altered the function of the transplanted cells (Kawahara et al. 2010, Loukopoulos *et al.* 2014, Wu *et al.* 2010). Since the transgenic “firefly luciferase rat” was developed on the background of an inbred rat strain (Lewis rats), the studies of transplanted hepatocytes can be performed without a need for post-Tx immunosuppression (Hakamata *et al.* 2006, Kasahara *et al.* 2013, Maeda *et al.* 2013). It is also noteworthy that in our recent study (Koblihová et al. 2014) we developed and characterized a highly reproducible model of thioacetamide (TAA)-induced ALF in Lewis rats which, in our opinion, is optimal for evaluation of new approaches to the therapy of ALF.

Based on the above considerations, we decided to examine if hepatocyte Tx into the liver via portocaval application would attenuate the course of TAA-induced ALF in Lewis rats. Furthermore, to gain more insight in the viability of transplanted hepatocytes, longitudinal *in vivo* BLI analysis of the transplanted cells in healthy and TAA-induced ALF Lewis rats was performed.

Methods

Ethical approval, animals, chemicals, and isolation and transplantation (Tx) of hepatocytes

The studies were performed in accordance with guidelines and practices established by the *Animal Care and Use Committee of the Institute for Clinical and Experimental Medicine*, Prague, which accord with the *European Convention on Animal Protection and Guidelines on Research Animal Use.* All the animals used in the study were housed in facilities accredited by the Czech Association of Laboratory Animal Care. The experiments were performed in male Lewis rats that were purchased from Charles River Laboratories (Velaz, Prague, Czech Republic) at the age of 9 weeks. Before starting experiments the rats were acclimatized in our vivarium during two weeks. The animals were kept on a 12-hour/12-hour light/dark cycle. The luciferase Lewis transgenic rats (luc-Lew-Tg) were supplied as stock animals from the Division of Organ Replacement Research, Center for Molecular Medicine, Jichi Medical School, Tochigi, Japan. The generation and characterization of luc-Lew Tg inbred strain model is described in the original study by Hakamata and co-workers (Hakamata *et al.* 2006). Throughout the experiments rats were fed a normal salt, normal protein diet (0.45% NaCl, 19-21% protein, SEMED, Prague, Czech Republic) and had free access to tap water. TAA (Sigma, Prague, Czech Republic) was dissolved in physiological saline and the appropriate dose was injected i.p. In the present study freshly prepared TAA was administrated i.p. in two injections, on day 0 at 8:00 AM and 20:00 PM in the total amount 525 mg.kg^{-1} ^{i}of BW. This dose was chosen based on our recent study evaluating the optimal doses of TAA for induction of ALF; we showed that after this dose all Lewis rats developed ALF and without treatment succumbed within first 48 hours (Koblihová *et al.* 2014). Control rats received i.p. injections of physiological saline. Under general anesthesia (pentobarbital sodium 30 mg. kg^{-1} of BW) male luc-Lwe-Tg weighing 250 – 280 g underwent abdominal “U” incision for hepatocytes isolation which was performed by the two-step collagenase perfusion method (Berry *et al.* 1991, Garnol T *et al.* 2014, Seglen 1976). The viability of isolated hepatocytes was assessed by the trypan blue exclusion test (Berry *et al.* 1991, Garnol T *et al.* 2014, Seglen 1976) and in our experiments ranged between 93 to 95 %. Isolated hepatocytes from luc-Lwe-Tg in the amount 2 x 10^6 cells dissolved in the 600 µl physiological saline solution were implanted into the liver through the portal vein. In this regard, it is important to highlight that the amount of transplanted hepatocytes

employed in in the present study was derived from previous studies that successfully used hepatocytes transplantation as a tool for the treatment of liver-based metabolic defects (for review see Filippi and Dhawan 2014). Thus, the optimal cell number to be transplanted was calculated at 5 x 10^6.kg^{-1} BW, which represent about 5 % of parenchymal cell mass from the liver and it has been reported that this quantity of transplanted hepatocytes is sufficient to restore metabolic liver function in liver-based metabolic diseases (for review see Filippi and Dhawan 2014). Based on this notion and in accordance with the BW of animals, the aforementioned amount of hepatocytes (i.e. 2 x 10^6) was transplanted. In accordance with these findings in liver-based metabolic diseases, also other investigators have recently started to use similar amount of cells for the treatment of ALF (Amano *et al.* 2011, Fukumitsu *et al.* 2011, Jitraruch *et al.* 2014, Loukopoulos *et al.* 2014, Puppi *et al.* 2014). Untreated rats (without hepatocyte Tx) received 600 µl of physiological solution by the same route.

Experimental design

Series 1: Effects of hepatocytes transplantation on the course of TAA-induced ALF in Lewis rats.

Twenty-four hours before i.p. administration ("-24 h") blood sample (about 600 µl) was taken from the tail vein, for biochemical analyses (Fuji Drive-Chem 4000 Analyser).[ii] Plasma levels of albumin, bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, and ammonia level (NH_3) were determined. Blood samples for the same analyses were also taken 24, 48, 72, 96 and 168 hours after first administration of TAA. The follow-up period in this series was 168 hours and at the end of experiments surviving animals were killed by an overdose of pentobarbital. Since during ALF development the animals' food and water intake are dramatically reduced, 5% glucose solution, 2 ml/100 g BW, was administered subcutaneously every morning to prevent dehydration; our recent study demonstrated that this procedure is an effective remedy (Koblihová *et al.* 2014). Hepatocyte Tx was performed 24 hours after first TAA administration. The survival rate was monitored every 8 hours, BW was monitored every 24 hours and blood samples were taken as described above.

The following experimental groups were investigated (initial n = 20 in each group):

1. Lewis rats + physiological saline + hepatocyte Tx (Lewis rats + hepatocytes Tx)
2. Lewis rats + TAA + physiological saline (Untreated Lewis rats with ALF)
3. Lewis rats + TAA + hepatocyte Tx (Lewis rats with ALF + hepatocytes Tx)

Series 2: Hepatocyte viability assessed by BLI analysis after Tx in Lewis rats.

The reliability of BLI analyses of transplanted cells isolated from luc-Lew-Tg was described and validated in previous studies (Hakamata *et al.* 2006, Kasahara *et al.* 2013, Maeda *et al.* 2013). Therefore in our experiments the same technique was used. In this series, in vivo luciferase imaging of hepatocytes after Tx was obtained by using noninvasive IVIS XR system (Caliper, Hopkinton, MA, USA). Rats were anesthetized with 2 % isoflurane and D-luciferin (30 mg/ml solution in physiological saline, Caliper, Hopkinton, MA, USA) was administrated at a dose of 50 $mg.kg^{-1}$ of BW via the femoral vein. The signal intensity was quantified as photon flux in units of $photons.sec^{-1}.cm^{-2}$ in the region of interest by using Living Image software. BLI analyses were performed 24, 48, 72, 96 and 168 hours after hepatocyte Tx. The following experimental groups were examined (n = 8 in each group):

1. Lewis rats + hepatocyte Tx

2. Lewis rats with ALF + hepatocyte Tx

It is important to underscore that in this series a separate groups of animals were used as compared with series #1 (in another words BLI analyses was performed on different animals than in series #1).

Statistical analysis

Statistical analysis of the data was performed using Graph-Pad Prism software (Graph Pad Software, San Diego, CA, USA). ANOVA for repeated measurements, followed by Student-Newman-Keuls test, was performed for analysis of changes within the groups. Statistical comparison of other results was made by Student´s t-test or one-way ANOVA. Unless indicated otherwise, values are expressed as mean ± S.E.M. A p-value less than 0.05 was considered statistically significant.

Results

Series 1: Effects of hepatocyte transplantation on the course of TAA-induced ALF in Lewis rats.

As shown in Figure 1A, TAA administration in untreated Lewis rats dramatically decreased survival rate (48 hours after TAA administration only 25% of animals were alive) and no animal survived 54 hours. Hepatocyte Tx markedly improved the survival rate after TAA (all animals remained alive at 54 hours after TAA administration) with the final survival rate of 15%. One of healthy Lewis rats died within 36 hours after Tx

As shown in Figure 1B, already in the first 24 hours after TAA administration a marked increase in plasma NH_3 levels was seen; this was significantly attenuated by hepatocyte Tx. In the animals which survived till the end of experiment (168 h after first TAA injection) NH_3 levels were only modestly higher as compared with healthy Lewis rats (34.2 $\pm$ 2.6 vs. 24.9 $\pm$ 2.3 mmol/l, $p<0.05$).

As shown in Figures 2A and 2B, TAA administration caused significant increases in plasma ALT and AST activities and hepatocyte Tx substantially attenuated these increases: as soon as 96 hours after first TAA administration there were no significant differences in plasma ALT and AST activities as compared with healthy Lewis rats.

As shown in Figure 3A, TAA administration elicited significant elevations in plasma bilirubin level and hepatocyte Tx attenuated this increase, similarly as in the case of ALT and AST activities. Already 72 hours after first TAA administration there were no significant differences in plasma bilirubin levels as compared with healthy Lewis rats.

As shown in Figure 3B, TAA administration resulted in a progressive decrease in plasma albumin levels, with the maximal decrease seen 48 hours after first TAA administration (13.4 $\pm$ 1.4 vs. 34.9 $\pm$ 2.9 g/l in healthy Lewis rats, $p<0.05$). Hepatocyte Tx significantly attenuated the decrease in plasma albumin levels and beginning from 96 h after first TAA administration the plasma albumin levels were not significantly lower as compared with healthy Lewis rats.

Series 2: Hepatocyte viability after Tx as assessed in Lewis rats by BLI analysis.

As shown in Figure 4, BLI analyses revealed that hepatocytes transplanted into the liver of healthy Lewis rats exhibited only minimal viability from the beginning of the study, and 72 hours after Tx no sign of viability was seen. In contrast, 24 h after Tx into the liver of Lewis rats with ALF the viability of transplanted hepatocytes was well visible (Figure 5A), and was further augmented 48 and 72 hours after Tx (Figures 5B and 5C). It remained appreciable until the end of experiment i.e. 168 hours after hepatocyte Tx (Figure 5D).

Discussion

The major finding of the present study is that transplantation of hepatocytes isolated from luc-Lew-Tg markedly attenuated the course of TAA-induced ALF in Lewis rats. This was reflected by improved survival rate and reduced degree of liver injury as indicated by lowering of plasma ALT, AST, NH_3 and bilirubin levels. In this context, it is worthy to underscore the course of plasma bilirubin levels, because even if a major portion of this endogenous organic anion is derived from aging red blood cells, its plasma levels are still accepted as a golden biomarker for the assessment of liver function especially in toxically-induced liver injury (Poynard and Imbert-Bismut 2012). In addition, hepatocyte Tx not only restored the function of detoxification, but also that of biosynthesis : plasma albumin levels in surviving TAA-induced ALF Lewis rats ultimately returned to levels that were not significantly different from those in healthy Lewis rats. Furthermore, BLI analysis of liver tissue clearly demonstrated that in TAA-damaged livers transplanted hepatocytes were fully viable throughout the experiment. Taken together, our present findings strongly indicate that hepatocyte Tx into the liver via portocaval application can attenuate the course of TAA-induced ALF in Lewis rats.

The value of these findings is enhanced by the experimental design which mimics the clinical situation: hepatocyte Tx was initiated after the signs of ALF became apparent. This is of special importance because in some previous studies the transplantation was performed before induction of ALF, then the translation of the results into clinical practice is doubtful (Amano *et al.* 2011, Hughes *et al.* 2012, Sgroi *et al.* 2009, Struecker *et al.* 2014). Second, the animal experimental model applied should resemble the clinical condition as closely as possible (Butterworth *et al.* 2009, Ternblache and Hickman 1991, Tunon *et al.* 2009). We have recently provided evidence (Koblihová *et al.* 2014) that TAA-induced ALF is the optimal model which fulfills most of the required criteria (Butterworth *et al.* 2009, Rahman and Hodgson 2000).

In this regard, it should be emphasized that attempts to create artificial and bioarteficial systems supporting regeneration of the patient´s liver (”bridging” to regeneration or to a time point when a suitable liver for OLT becomes available) have not brought satisfactory results (Struecker *et al.* 2014, Stutchfield *et al.* 2011). Some meta-

analyses (Kjaergard *et al.* 2003, Struecker *et al.* 2014) have reported no beneficial effects of application of artificial and bioarteficial liver support systems on the mortality of patients with ALF. It was shown that while such support devices are safe for acute application and can improve biochemical markers (Prazak *et al.* 2013, Ryska *et al.* 2009, Ryska *et al.* 2012, Struecker *et al.* 2014, Stutchfield *et al.* 2011), they do not generally improve survival rate of patients with ALF (Pless 2010, Struecker *et al.* 2014, Stutchfield *et al.* 2011) and, remarkably, no bioarteficial liver support system has obtained approval from U.S. Food and Drug Administration for standard clinical uses (Arenas-Herrera *et al.* 2013). It will be noted that the original notion that the critical "failed" function in ALF is detoxification, and thus approaches ensuring this are adequate for treatment of ALF, proved inaccurate (Banares *et al.* 2013, Selden and Hodgson 2004). The commonly used systems cannot fully replace the diversity and complexity of liver function: detoxification is more complex than simple removal of albumin-bound toxins, and regulation of homeostasis means more than normalization of plasma electrolyte, glucose and hydrogen ions levels (Suchy 2009). Therefore, alternative strategies for treatment of ALF that would address the complexity of disturbed physiological functions of the liver are heavily investigated. In the light of recent research hepatocyte Tx which restores missing liver functions in a natural way seems to be a very promising therapeutical approach (Pless 2010).

So far, hepatocyte Tx has been performed for treatment of inborn metabolic liver diseases which are caused by a deficiency of a single hepatic enzyme or protein (e.g. Crigler-Najjar syndrome type I, urea cycle defects and Wilson´s disease) and can be corrected with engraftment of hepatocytes expressing the gene involved. Such applications were relatively successful, at least as a method of "bridging to transplantation" (Dhawan *et al.* 2010, Filippi and Dhawan 2014, Yu *et al.* 2012, Waelzlein *et al.* 2009). In contrast, clinical studies of hepatocyte Tx in ALF yielded poor results, and it was proposed that the number of transplanted hepatocytes might be sufficient to correct single inborn metabolic errors but not to provide full compensation of liver function (Struecker *et al.* 2014). However, our present data show that hepatocyte Tx into the liver in the amount of 5% of parenchymal cell mass is sufficient to attenuate the course of TAA-induced ALF in Lewis rats. In this regard, it is important to emphasize that the amount of transplanted hepatocytes in the present study was increased in accordance with recent findings of studies employing the hepatocyte Tx for

the treatment of liver-based metabolic diseases to the quantity 2 x 10^6 (for review see Filippi and Dhawan 2014). The exact justification for the amount of used hepatocytes in our present study is given in the Methods section. Our current findings indicate that hepatocyte Tx could serve as a therapeutical method either as a "bridge to transplantation" or even as a "bridge to regeneration".

The second major finding of the present study is that BLI analyses revealed that hepatocytes transplanted into the liver of Lewis rats with TAA-induced ALF (but not into the liver of healthy Lewis rats) exhibited distinct viability until the end of the experiment. The finding demonstrates also that our current approach and technique to transplant hepatocytes directly into the TAA-damaged liver is feasible and safe. This is very important because direct delivery into the liver has been earlier questioned for safety reasons, and alternative sites, such as the spleen and peritoneal cavity have been suggested (Struecker *et al.* 2014, Waelzlein *et al.* 2009). However, even though hepatocyte Tx into these other sites have been shown to be safe and to some extent effective, a major limitation of this approach is that it does not support regeneration of damaged liver tissue (Struecker *et al.* 2014, Wertheim et al. 2012, Waelzlein *et al.* 2009). Of particular interest is also our finding that hepatocytes transplanted into the liver of healthy Lewis rats exhibited minimal viability immediately after Tx, and 72 hours later no sign of viability was seen. These findings resemble the situation in another newly examined liver support strategy, the technique of recellularization of decellularized liver. The studies showed that in order to keep the masses of hepatocytes metabolically active outside their original environment, it is necessary to remove first all cells and other antigenic material from the liver (e.g. porcine) and obtain a non-immunogenic extracellular matrix (decellularization step). Only then can the matrix be repopulated with cells and after their maturation the neo-liver can be used as a bioreactor (recellularization concept) (Arenas-Herrera *et al.* 2013, Struecker *et al.* 2014, Yagi et al. 2013). Taken together, our present and the above-quoted findings suggest that for successful preservation of viability of transplanted hepatocytes, the natural cells and other antigenic material from the recipient liver should first be destroyed. We are aware that our present study was not designed to evaluate such a proposal and interpretation of our BLI data remains hypothetical. Future studies are required to address the mechanism(s)

responsible for the different course of viability of transplanted hepatocytes in healthy Lewis rats compared to Lewis rats with ALF. However, it is important to emphasize that BLI analyses was performed in a separate groups of from those where the effects of hepatocyte on the course of TAA-induced ALF was examined. The reason for the separate series of experiments is that BLI analyses requires repetitive anesthesia with 2 % isoflurane and this procedure could negatively alter the major followed end-point of the present study (i.e. effects of hepatocyte Tx on the survival rate in TAA-induced ALF). Moreover, it is important to recognize that despite marked attenuation the course of TAA-induced ALF by hepatocyte Tx the final survival rate was still only 15 %. The limitation of our present study is that we cannot provide a sufficient explanation for this fact, because e.g. we do not have data about the viability of transplanted hepatocyte in animals that died during the first 72 hours of our experiment. It is obvious that future studies are needed to address this issue.

In this context, it is worthwhile to briefly discuss, why in our studies TAA-induced ALF model is used. The major reason is that we have in our recent study unequivocally proven (Koblihova *et al.* 2014) that this model meets criteria that were originally suggested for an ideal animal model of ALF (if TAA is administrated in accordance with our design) (Terblanche and Hickman 1991) and are as follow: a) reversibility, i.e. that induced liver failure should be potentially reversible; b) reproducibility, i.e. that degree of liver damage should be standardized, especially within the major endpoint which is the death of the animal; c) death cause, i.e. that death should be a direct result of the insult to the liver; d) therapeutic window, i.e. that sufficient time should available between the insult and the death; e) minimum hazard to personnel, i.e. that the toxin should present minimal risk to investigators. We are aware that our current findings are limited only to the course of TAA-induced ALF, however recently hepatocyte Tx was successfully used in the treatment of another chemical models of ALF (e.g. D-galactosamine-induced ALF) (Puppi *et al.* 2014) and therefore we believe that our present results should be reproducible in other models of ALF and such studies are necessary to be performed in future.

In conclusion, the results of the present study show that hepatocyte Tx into the liver can attenuate the course of TAA-induced ALF in Lewis rats. This information should be considered in attempts to develop new therapeutic approaches or tools for treatment of ALF.

Conflict of interest

None.

Acknowledgments

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Figure Legends

Figure 1. The survival rate (**A**) and plasma ammonia (NH_3) levels (**B**) in healthy Lewis rats exposed to hepatocyte transplantation (Lewis rats + hepatocyte Tx), in Lewis rats with acute liver failure without treatment (untreated Lewis rats with ALF) and in Lewis rats with acute liver failure treated with hepatocyte transplantation (Lewis rats with ALF + hepatocyte Tx). *P<0.05 versus the value for Lewis rats + hepatocyte Tx at the same time point. $^{\#}$ P<0.05 versus all the other values at the same time point.

Figure 2. Changes in plasma alanine aminotransferase (ALT) (**A**) and aspartate aminotransferase (AST) (**B**) activities in healthy Lewis rats exposed to hepatocytes transplantation (Lewis rats + hepatocyte Tx), in Lewis rats with acute liver failure without treatment (untreated Lewis rats with ALF) and in Lewis rats with acute liver failure treated with hepatocyte transplantation (Lewis rats with ALF + hepatocyte Tx). *P<0.05 versus the value for Lewis rats + hepatocytes Tx at the same time point. $^{\#}$ P<0.05 versus all the other values at the same time point.

Figure 3. Changes in plasma bilirubin (**A**) and albumin (**B**) levels in healthy Lewis rats exposed to hepatocyte transplantation (Lewis rats + hepatocyte Tx), in Lewis rats with acute liver failure without treatment (untreated Lewis rats with ALF) and in Lewis rats with acute liver failure treated with hepatocyte transplantation (Lewis rats with ALF + hepatocyte Tx). *P<0.05 versus the value for Lewis rats + hepatocyte Tx at the same time point. $^{\#}$ P<0.05 versus all the other values at the same time point.

Figure 4. Representative images from in vivo luciferase bioluminescence analyses in healthy Lewis rats exposed to hepatocyte transplantation at 24 (**A**), 48 (**B**), 72 (**C**) and 168 (**D**) hours post-Tx.

Figure 5. Representative imagines from in vivo luciferase bioluminescence analyses in Lewis rats with acute liver failure treated with hepatocyte transplantation 24 (**A**), 48 (**B**), 72 (**C**) and 168 (**D**) hours after hepatocyte transplantation.

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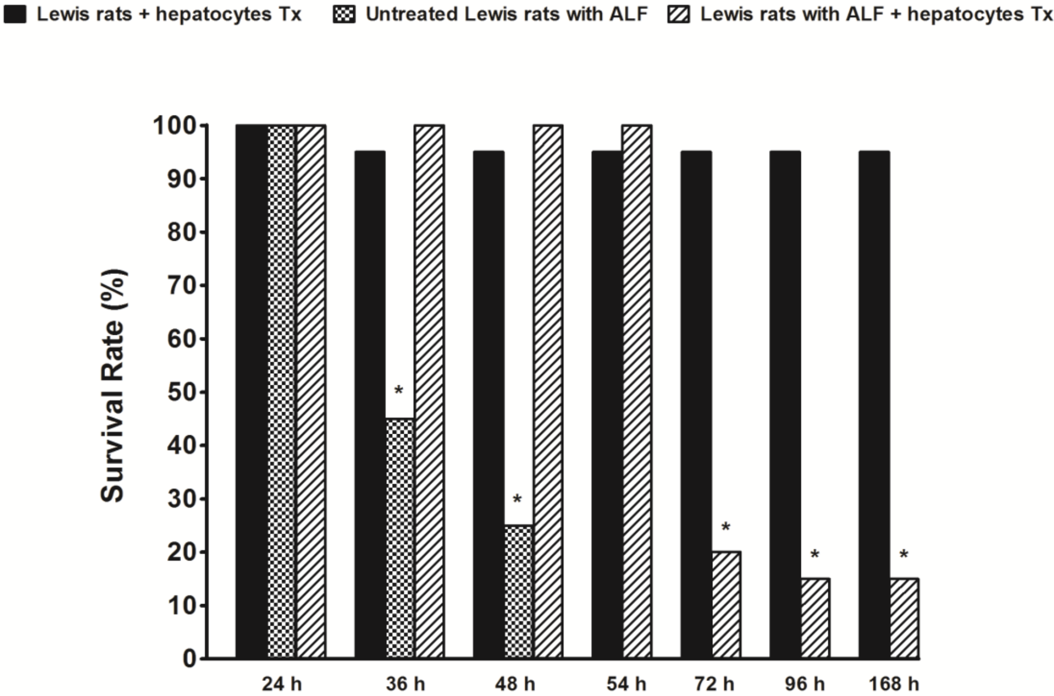
Figure 1

A



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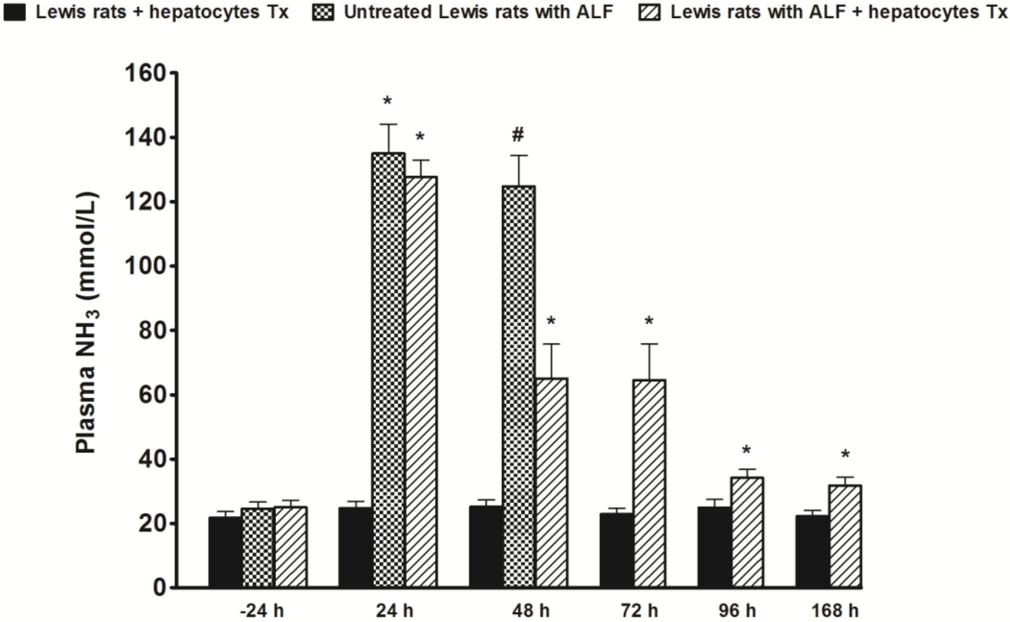

Figure 2

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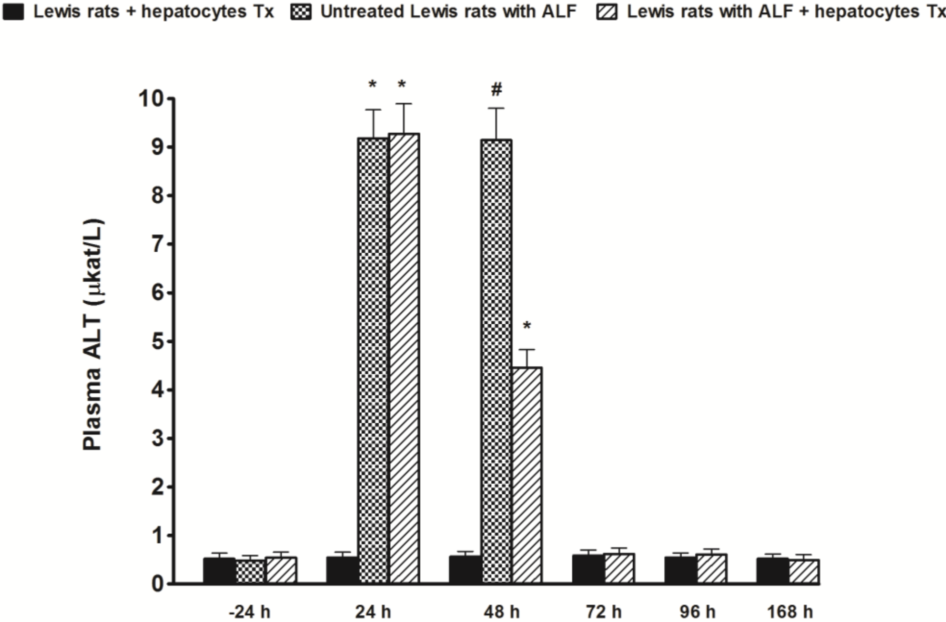

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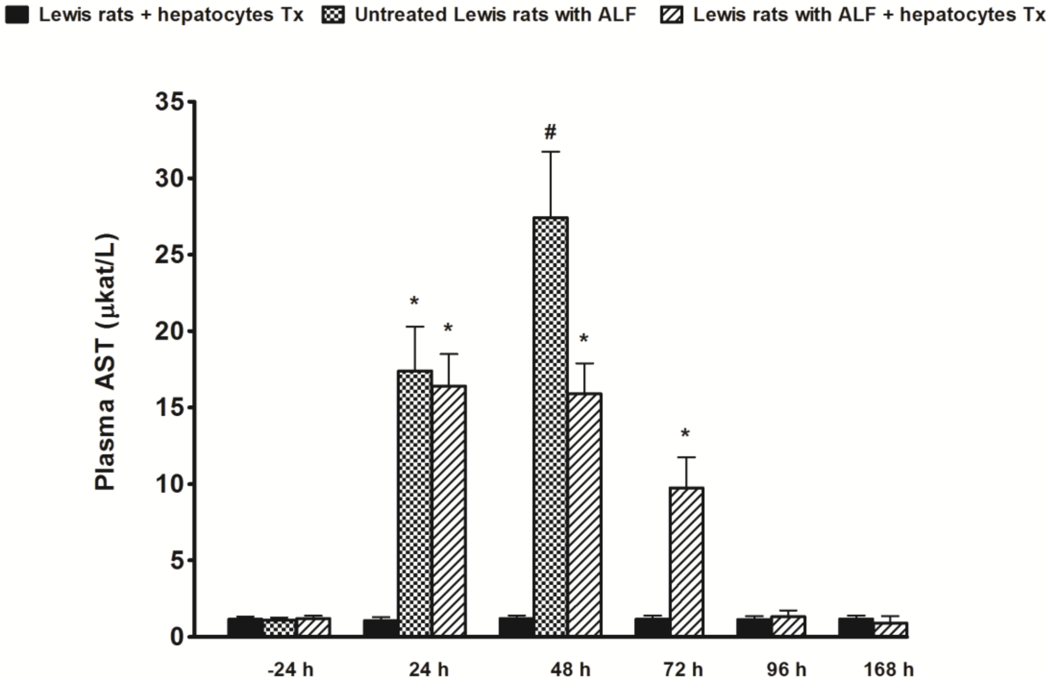

Figure 3

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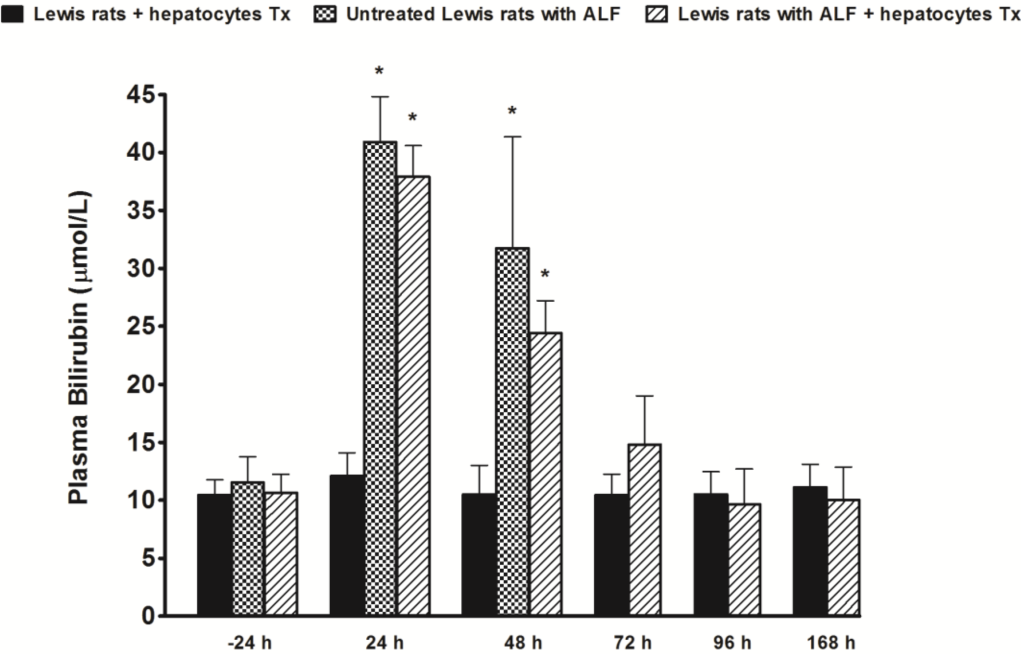

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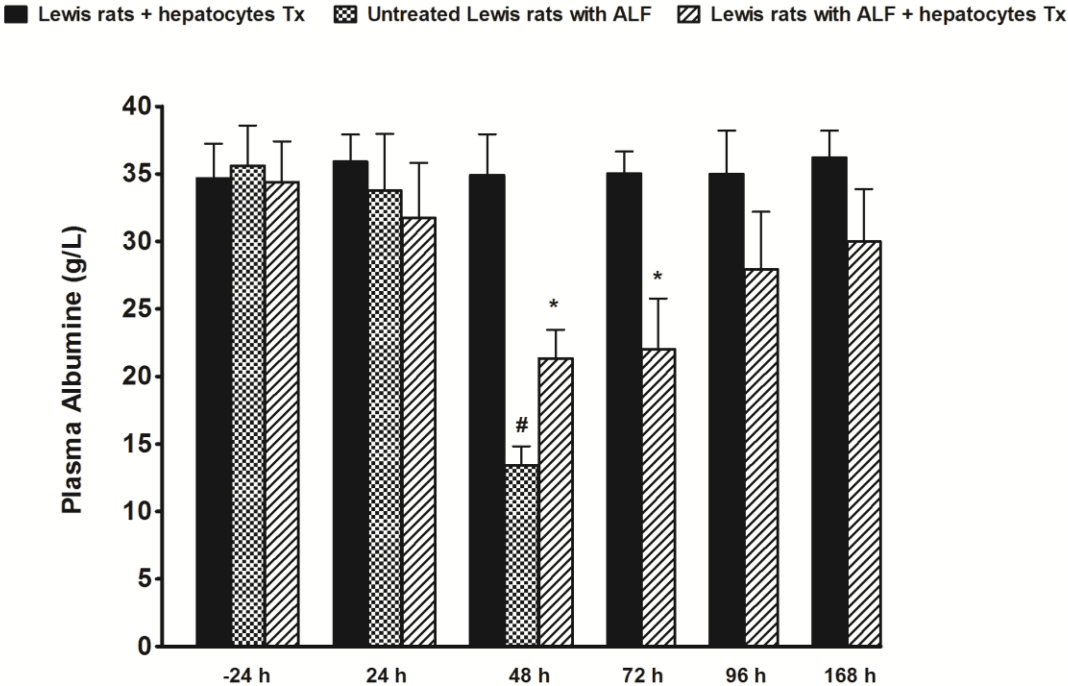

Figure 4

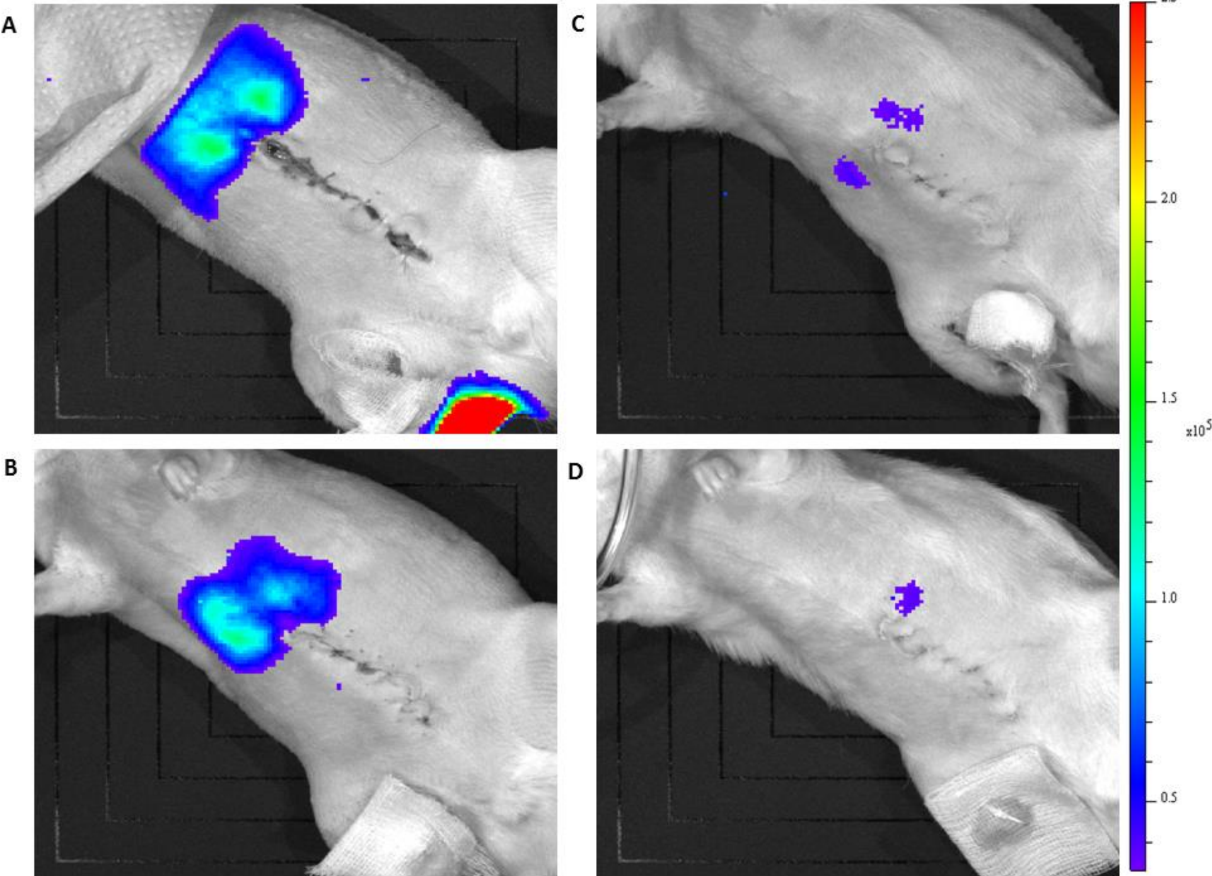

Figure 5