Evaluating the best time to intervene acute liver failure in rat models induced by d-galactosamine[1]

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ABSTRACT

PURPOSE: To describe an animal model for acute liver failure by intraperitoneal d-galactosamine injections in rats and to define when is the best time to intervene through King's College and Clichy´s criteria evaluation.

METHODS: Sixty-one Wistar female rats were distributed into three groups: group 1 (11 rats received 1.4 g/kg of d-galactosamine intraperitoneally and were observed until they died); group 2 (44 rats received a dose of 1.4 g/kg of d-galactosamine and blood and histological samples were collected for analysis at 12 , 24, 48 , 72 and 120 hours after the injection); and the control group as well (6 rats).

RESULTS: Twelve hours after applying d-galactosamine, AST/ALT, bilirubin, factor V, PT and INR were already altered. The peak was reached at 48 hours. INR > 6.5 was found 12 hours after the injection and factor V < 30% after 24 hours. All the laboratory variables presented statistical differences, except urea ($p = 0.758$). There were statistical differences among all the histological variables analyzed.

CONCLUSION: King's College and Clichy´s criteria were fulfilled 12 hours after the d-galactosamine injection and this time may represent the best time to intervene in this acute liver failure animal model.

Key words: Liver Failure, Acute. Galactosamine. Models, Animal. Rats.

Introduction

Acute liver failure (ALF) is a rare and severe manifestation resulting from sudden and intense hepatocellular necrosis, associated with jaundice, coagulopathy and a certain degree of liver encephalopathy within the first 8 weeks of the appearance of the symptom and the absence of any previous liver condition[1,2].

This is associated with a high mortality rate of 40-50%, depending on the cause and the therapeutic management[3]. The etiologies are much varied and the most common are drug intoxication and viral hepatitis[4].

Liver presenting acute failure may recover and regenerate, however in most cases the deterioration might be quick for a possible recovery. Currently, in the USA, 45% of the adults have spontaneous recovery and 25% require liver transplant as the only life saving therapy[5]. Early identification in patients who will not survive with support therapy alone is fundamental for indicating liver transplantation. The prognostic evaluation criterion mostly used for this is King's College[6]. Meta-analysis studies have confirmed its specificity (80%), but reported limited sensitivity (70%)[7]. To overcome this limitation, other prognostic criteria might be used, such as Clichy´s criteria, which takes into consideration the alterations of factor V coagulation, the patient's age and the presence of encephalopathy[8].

Hepatocytes transplant has been proposed for ALF treatment. In this technique, the human hepatocytes are infused in the splenic vascular, the portal or the peritoneal cavity to provide liver function to the sick liver. Although there are reports of successful errors of congenital metabolism the challenges used in acute liver failure are considerable[9].

Therefore it becomes highly desirable to have an adequate experimental model for available acute liver failure in order to provide a better understanding of this condition and enables to develop new therapeutic approaches with the aim of extending the survival until the liver transplant takes place or the sick liver regenerates.

In the worldwide literature, the model for inducing acute liver failure via toxins in rats has been mostly used to test support therapies consist of d-galactosamine intraperitoneal administration, because it is potentially reversible, presenting non-significant extra-hepatic toxicity and has the best-defined hepatotoxic dosage[10].

However, the descriptions of this model are very superficial, thus the reproducibility in other research centers are becoming more difficult. Moreover, studies using d-galactosamine induced ALF model to test therapies have been empirical and interventions were separated without following clinical parameters.

The experiment report was conducted with the aim provide a more detailed description of d-galactosamine induced ALF that would enable to apply King's College and Clichy´s criteria, in order to establish clinical time to intervene.

Methods

This project was approved by the Research Ethics Committee at UNIFESP (0197/12) and all the experiments using laboratory animals followed the norms.

Experiments were performed at Laboratory of Surgical Technique and Experimental Surgery, Department of Surgery, Universidade Federal de São Paulo (UNIFESP). The rats were acquired at the Development Center for Medical and Biological Experimental Models (CEDEME), UNIFESP.

Sixty-one Wistar female rats weighing between 195 g and 251 g were used. The rats were maintained in an animal facility inside plastic cages measuring 40 x 34 x 17 cm with a maximum of five rats in each cage under a controlled temperature of about 22ºC with artificial illumination, a sleep-wake cycle control, water and available food *ad libitum*.

The animals were distributed into the following groups:

Group 1 – Composed of eleven rats were being observed regarding mortality for a period of time, after applied an intraperitoneal d-galactosamine injection of 1.4 g/kg. Craniotomy, thoracotomy and laparotomy were performed after their death, so that the brain, heart, lung, kidneys, spleen, pancreas and liver could be removed for histological analysis.

Group 2 - Composed of 44 rats intraperitoneal d-galactosamine injection of 1.4 g/kg was applied. After each 12, 24, 48, 72 and 120 hours, six rats were chosen according to the severity criteria of the clinical manifestation presented by each rat. The rats were subjected to intraperitoneal anesthesia using 50 mg/kg of ketamine (Dopalen, Vetbrands Brazil) and 20 mg/kg of xylazine (Virbaxyl 2%, Virbac, Brazil), followed by laparotomy and thoracotomy in order to collect blood by intracardiac puncture until exsanguination and death. After the animals´ death, heart, lung, kidney, spleen, pancreas, and liver were removed for histological analysis. Soon after, craniotomy was performed to remove the brain.

Group 3 – The control group was composed by six rats and were observed for a period of seven days. Then they were anesthetized intraperitoneally by applying 50 mg/kg of ketamine (Dopalen, Vetbrands Brazil) and 20 mg/kg of xylazine

(Virbaxyl 2%, Virbac, Brazil) and subjected to laparotomy and thoracotomy in order to collect blood by intracardiac puncture until exsanguination and death. After the animals´ death, heart, lung, kidney, spleen, pancreas, and liver were removed for histological analysis. Soon after, craniotomy was performed to remove the brain.

D-galactosamine preparation

Hydrochloride d-galactosamine ($C_6H_{13}NO_5$-HCl) (Genese diagnostic product Inc.) is found in the form of white colored powder in bottles of 5g. To dilute, the drug was aliquoted under laminar flow, using a sterile spatula being removed from the bottle of origin and placed in a 50ml falcon tube. A semi-analytical scale (Crystal 200 smi) with a precision of 0.001g, previously calibrated and tared with the weight of an empty falcon tube used for weighing the drug. The falcon tube was added 3.5g of D-galactosamine, 40ml of 0.9% saline solution and used a vortex shaker mixing the solution until it obtained a clear liquid, without residues. This concentrated solution, 8.75% (3.5g of D-galactosamine: 40ml of saline solution) were injected 16 ml/Kg of weight in each rat intraperitoneally, equivalent to a dose of 1.4g/kg of D-galactosamine residues. Taking the risk of skin irritation and contact with dermatitis, each person used individual protection material (gloves, apron and glasses) to manipulate the drugs.

Intraperitoneal injection

To proceed to the intraperitoneal injection of the drugs, the rats were held with their belly up and with the head slightly inclined downwards in order to move the viscera and intestinal loops toward the diaphragm and to reduce the risk of puncturing. The rats were weighed and the dose to be administered was calculated with one decimal place and pipette was transferred from the falcon tube to a sterile test tube. The solution was removed by a 5ml syringe coupled to a 22G needle (0.7 x 25mm) and applied in the lower left quadrant of the abdomen. The needle was introduced making an angle of approximately 45° and before injecting the drug, a mild aspiration was performed to be certain that it did not reach the bladder, the intestine or a blood vessel.

Common surgical procedure for all rats

To remove the organs, the rats were anesthetized with 50mg/kg of Ketamine (Dopalen, Vetbrands, Brazil) and 20mg/kg of xylazine (2% Virbaxyl, Virbac, Brazil), applied intraperitoneally, except group 1 which had their organs removed after their death.

The rats were placed in the dorsal decubitus position on the surgical board and had their limbs laid down. Antisepsis containing 70% alcohol was performed previously on the abdominal and thoracic walls and the rats were submitted to laparotomy and thoracotomy of the abdominal wall and thoracic anatomic planes section.

With a 5 ml syringe coupled to a 22G needle (0.7x25mm), a heart puncture was performed to remove blood until the rat died. The blood was distributed in microsampling tubes (0.5ml for the purple tube EDTA; 0.6ml for the yellow dry tube and 1.8ml for the sodium citrate blue tube) for later analysis, and the rest was discharged.

Liver, spleen, pancreas, right kidney, heart and right lung were removed from the abdominal and thoracic cavity. After the removal of the organs, the abdominal and thoracic walls were closed by stitching.

Subsequently the rat was placed in ventral decubitus position on the surgical board with the limbs laid down and submitted an opening of the skin to expose the cranial followed by a craniotomy and a withdrawal of the brain. The organs were prepared on the surgical board and submitted to thin cuts with a cold bladed scalpel. The cut of each component was placed in a 10% formaldehyde solution in sterilized jars identified with a number for each rat.

Histological analysis

All the rats in this study were subjected to histological analysis of the liver, spleen, pancreas, kidney, heart, lung and brain.

The histological slides were analyzed by the same professional with an optical microscope, blindly, without knowing which slide belonged to which rat.

The semi-quantitatively hepatic histological variables were analyzed, as followed:

- Hepatocellular necrosis: 0- absent; 1- slight (foci of necrosis, sometimes confluent); 2- moderate (extensive areas of confluent necrosis); 3- intense (extensive confluent necrosis as bridge or in panacinar form);
- Apoptosis: 0- absent; 1- slight; 2- moderate; 3- severe;
- Bleeding: 0- No; 1- Yes;

- Steatosis was evaluated regarding the following types: predominantly macrovesicular; or predominantly microvesicular and mixed.

Laboratory analysis

The rats in group 2 were sacrificed at 12, 24, 48, 72 and 120 hours after the intraperitoneal d-galactosamine injection. Blood samples were collected by intracardiac puncture at a time after applying the anesthesia using ketamine and xylazine. The withdrawal of blood samples from the rats for analysis was also submitted from the control group 3. The following variables were analyzed:

- Aspartate aminotransferase (AST) and alanine aminotransferase (ALT);
- Bilirubin (total and fractions);
- Urea and creatinine;
- Factor V, prothrombin time (PT) and INR;
- Albumin.

Statistical analysis

To compare the numerical laboratory variables of the rats, the Kruskal-Wallis non-parametric test was used because of the small sample size (maximum of 6). Regarding to detect the differences between evaluation times, Dunn-Bonferroni´s multiple comparisons were used to identify such differences.

The existence of the associations between the histological categorical variables was ascertained by using Fisher's exact test because of the small sample size. P values <0.05 were considered statistically significant.

Results

Macroscopic findings

Macroscopically the dead rats´ livers presented a pale coloring (Figure 1) when compared to a normal liver (Figure 2) and with some hemorrhagic focuses. Hemorrhagic focuses in the lung could also be observed, as well as food stasis in the stomach and intestinal loops.

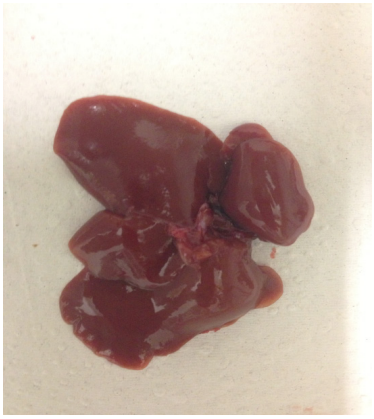

FIGURE 1 – A rat´s liver that received D-gal.

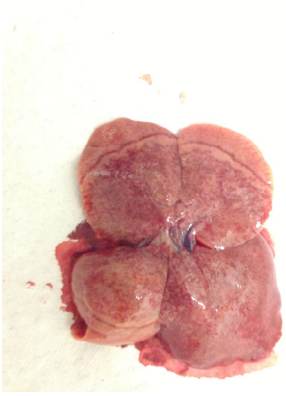

FIGURE 2 – A controlled rat´s liver.

Clinical findings and mortality

From observing the rats in group 1, that received doses of 1.4 g/kg of d-galactosamine, was noted that 27 hours after the injection, some rats began to present hemorrhagic suffusions on the tail and nose and, after 49 hours, they presented limb spasms and seizures. Other symptoms presented were restlessness, itching or hyporesponsiveness.

The first death occurred 44 hours after the d-galactosamine injection. The mortality peak occurred between 49 and 60 hours (n = 5). The last death was registered at 76 hours after taking d-galactosamine.

120 hours after applying the injection, three of the eleven rats were still alive (mortality rate of 73%).

In group 2, after 48 hour there were only ten animals alive. So, it was possible to sacrifice only 4 animals at 72h and 120h period.

Laboratory findings

Twelve hours after applying d-galactosamine injection, it was already possible to observe the alterations in the liver

enzymes (Table 1) and the decrease of the coagulation factors (Table 2). The peak for this alteration occurred 24 hours after taking d-galactosamine.

All the laboratory variables studied, presented a statistically significant difference according to Kruskal-Wallis test, except urea (Tables 3 and 4). Using the Dunn-Bonferroni test to identify the differences, it could be seen that the values reached the variables and were similar at zero hour and at 120 hours after applying the injection, and these were different from the values presented at 24 and 48 hours after applying the injection.

King's College criterion (INR > 6.5) were fulfilled at 12 hours after taking the drug and Clichy´s criterion (Factor V < 30%) was after 24 hours (Table 2).

TABLE 1 – Median value of ALT and AST evaluated in control group and group 2 for a period of time.

	Time (hour)						
	Control Group (n=6)	12h (n=6)	24h (n=6)	48h (n=6)	72h (n=4)	120h (n=4)	P value[1]
AST	91.5[A]	4.810	16.015[B]	7.778[B]	1.120	77[A]	<0.001
ALT	60[A]	3.581	16.002[B]	9.498[B]	1.083	43.8[A]	<0.001

[1]Descriptive level of Kruskal-Wallis test
(A) and (B) are distinct according to Dunn-Bonferroni multiple comparisons

TABLE 2 – Median value of factor V, INR and PT evaluated in control group and group 2 for a period of time.

	Time (hour)						
	Control Group (n=6)	12h (n=6)	24h (n=6)	48h (n=6)	72h (n=4)	120h (n=4)	P value[1]
Factor V	260.5[A]	125	23,5[B]	40	288.5[A]	268[A]	0.001
INR	1.54[A]	7.52	10[B]	10[B]	1.84	1.46[A]	<0.001
PT	18.4[A]	58.5	71[B]	71[B]	20.6[A]	17.3[A]	<0.001

[1]Descriptive level of Kruskal-Wallis test
(A) and (B) are distinct according to Dunn-Bonferroni multiple comparisons

TABLE 3 – Median value of albumin, urea and creatinine evaluated in control group and group 2 for a period of time.

	Time (hour)						
	Control Group (N=6)	12h (n=6)	24h (n=6)	48h (n=6)	72h (n=4)	120h (n=4)	P value[1]
Albumin	4.4[A]	4.2 [A,a]	3.7[B]	3.3[B,b]	3.4	4.2	0.001
Urea	46.5	47.5	42.5	42	39	43	0.758
Creatinine	0.4	0.4	0.6[A]	0.2[B]	0.4	0.3	0.005

[1]Descriptive level of Kruskal-Wallis test
(A) and (B) are distinct according to Dunn-Bonferroni multiple comparisons
(a) and (b) are distinct according to Dunn-Bonferroni multiple comparisons

TABLE 4 – Median value of BT and BD evaluated in control group and group 2 for a period of time.

	Time (hour)						
	Control Group (n=6)	12h (n=6)	24h (n=6)	48h (n=6)	72h (n=4)	120h (n=4)	P value[1]
BT	0.05[A]	0.22	1.6[B]	3.99[B]	4.91[B]	0.09	<0.001
BD	0.04[A]	0.06	1.28	3.34[B]	4.17[B]	0.06	0.001

[1]Descriptive level of Kruskal-Wallis test
(A) and (B) are distinct according to Dunn-Bonferroni multiple comparisons

Microscopic findings

Histological sections through the rats´ livers in group 1 showed extensive areas of confluent necrosis, sometimes compromising zones 1 and 2, accompanied by lymphomononuclear or lymphohistiocytic inflammatory infiltrate, sometimes with the presence of neutrophils. The remaining parenchyma showed ballooned hepatocytes, retraction figures and acidophilus corpuscles, along with slight macro and microvesicular steatosis. In some rats, slight ductular reaction in zone 1 was observed.

Histological analysis on liver sections over the course of time showed that 12 hours after taking the drug, foci of necrosis and apoptosis could be seen (Figure 3). These became more intense after 24 and 48 hours (Figures 4 and 5), but then practically normalized after 72 hours (Figure 6 and Table 5). Ballooning and ductular reaction appeared later on, thus suggesting that parenchymal regeneration was occurring (Figure 7).

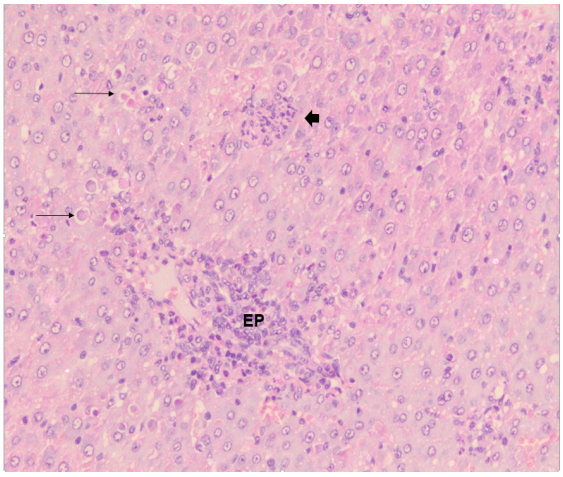

FIGURE 3 - Histological section of a sacrificed rat´s liver at 12 hours, featuring multiple apoptotic bodies (*thin arrows*) and foci of necrosis (wide arrows) accompanied by mixed inflammatory infiltrate. (HE x200)

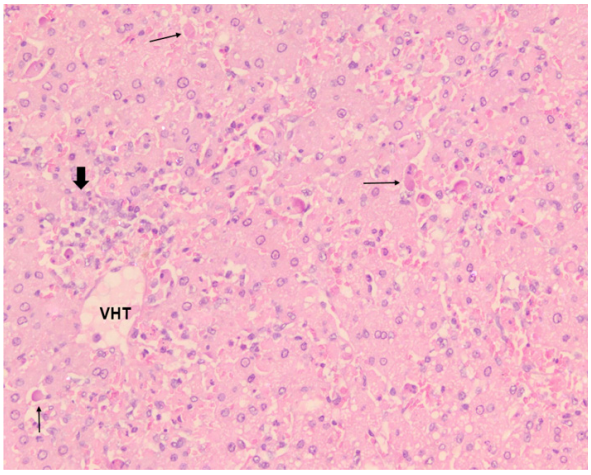

FIGURE 4 - Histological section of a sacrificed mouse´s liver at 24 hours with a large quantity of apoptotic bodies (*thin arrows*) and foci of necrosis (*wide arrows*) located predominantly near hepatic terminal vein (VHT). (HE x150)

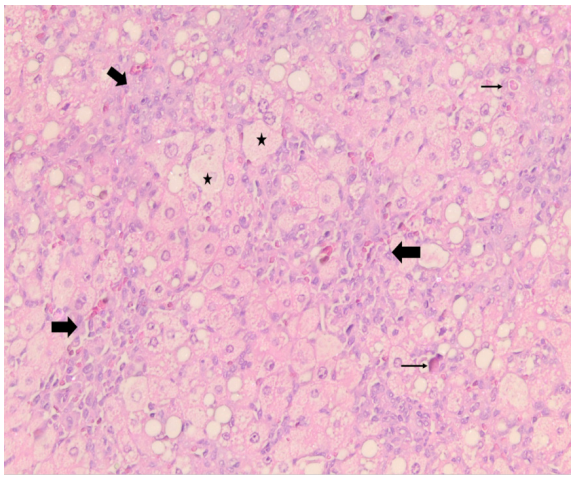

FIGURE 5 - Histological section of a sacrificed rats´ livers at 48 hours presenting confluent areas of necrosis accompanied by inflammatory infiltrate lymphomononuclear cells (*wide arrows*), numerous apoptotic bodies (*thin arrows*), ballooned hepatocytes (□) and steatosis macrogoticular located predominantly in zone 3. (HE x150)

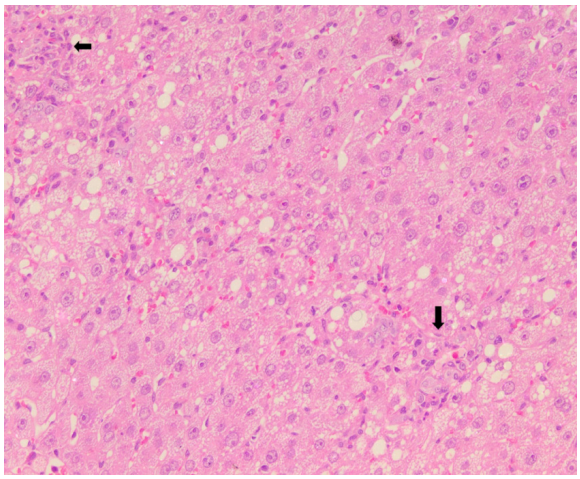

FIGURE 6 - Histological section of a sacrificed rats´ livers at 72 hours showing rare foci of necrosis accompanied by inflammatory infiltrate lymphomononuclear cells (*arrows*) and macro and microgoticular steatosis. (HE x100)

TABLE 5 – Semi-quantitative analysis of the histopathological changes in the liver presented by the animals in the control group and group 2 for a period of time.

	Time (hour)												
	Control Group*		12h		24h		48h		72h		120h		P value[1]
	N	%	N	%	N	%	N	%	N	%	N	%	
HEMORRHAGE	5	100%	6	100%	6	100%	6	100%	4	100%	4	100%	<0.001
No	5	100%	5	83.3%	0	0%	2	33.3%	4	100%	4	100%	
Yes	0	0%	1	16.7%	6	100%	4	66.7%	0	0%	0	0%	
STEATOSIS	5	100%	6	100%	6	100%	6	100%	4	100%	4	100%	<0.001
Absent	5	100%	0	0%	0	0%	0	0%	1	25%	2	50%	
Macrogoticular	0	0%	1	16.7%	0	0%	0	0%	1	25%	0	0%	
Microgoticular	0	0%	5	83.3%	5	83.3%	1	16.7%	0	0%	0	0%	
Mixed	0	0%	0	0%	1	16.7%	5	83.8%	2	50%	2	50%	

*Material loss of one animal
[1]Descriptive level of Kruskal-Wallis test

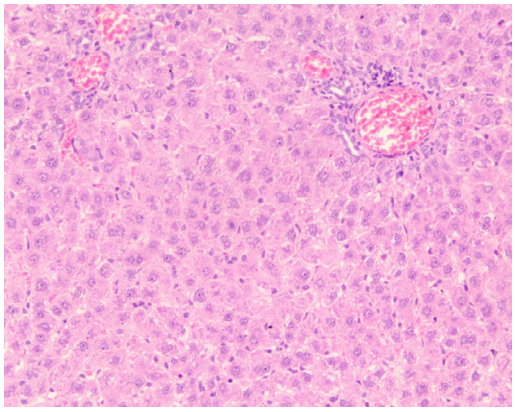

FIGURE 7 - Histological section of a sacrificed rats´ livers at 120 hours presenting discrete not specific reactive changes represented by hyperplasia and hypertrophy of Kupffer cells. (HE x200)

All histological variables analyzed by Fisher's exact test presented statistically significant differences (Tables 5 and 6).

Portal and lobular infiltrates, along with steatosis, could be observed 12 hours after drug administration.

The remaining analyzed organs (lung, heart, kidney, brain, spleen and pancreas) did not present any significant alterations, except for the kidneys of some rats that were sacrificed 48 hours after the d-galactosamine injection. These presented isomeric intracytoplasmic microvacuolation in contorted tubules. One rat was also sacrificed 48 hours after the injection presented pancreatic steatosis.

TABLE 6 – Semi-quantitative analysis of the histopathological changes in the liver presented by the animals in the control group and group 2 for a period of time.

	Time (hour)												
	Control Group*		12h		24h		48h		72h		120h		P value[1]
	N	%	N	%	N	%	N	%	N	%	N	%	
NECROSIS	5	100%	6	100%	6	100%	6	100%	4	100%	4	100%	<0.001
Absent	5	100%	0	0%	0	0%	0	0%	1	25%	4	100%	
Mild	0	0%	4	66,7%	1	16.7%	1	16.7%	1	25%	0	0%	
Moderate	0	0%	2	33.3%	2	33.3%	2	33.3%	1	25%	0	0%	
Intense	0	0%	0	0%	3	50%	3	50%	1	25%	0	0%	
APOPTOSiS	5	100%	6	100%	6	100%	6	100%	4	100%	4	100%	<0.001
Absent	4	80%	0	0%	0	0%	0	0%	1	25%	3	75%	
Mild	1	20%	0	0%	0	0%	2	33.3%	3	75%	1	25%	
Moderate	0	0%	2	33.3%	2	33.3%	2	33.3%	0	0%	0	0%	
Intense	0	0%	4	66,7%	4	66.7%	2	33.3%	0	0%	0	0%	

*Material loss of one animal
[1]Descriptive level of Kruskal-Wallis test

Discussion

Acute liver failure is a severe condition and in liver transplants, most of the time constitutes to as the only life saving therapy. Several animal models for acute liver failure, using rats have been described and the use of d-galactosamine to induce liver failure is the most widely used.

D-galactosamine is an amino sugar metabolized in the liver via galactose, which leads to depletion of uridine nucleotides and blocks the liver transition, thus resulting in massive diffuse necrosis of the liver[11]. Studies using radio labeled d-galactosamine have established the specific hepatotoxicity of this drug: virtually 100% of the drug is metabolized by the liver[12].

Keppler *et al*.[13] were among the first researchers to use d-galactosamine to induce acute liver failure in Wistar rats. After injecting intraperitoneally 1.5 g/kg of d-galactosamine, divided into 6 doses of 0.25 g/kg, AST, ALT, prothrombin activity, bilirubin, prothrombin time, total proteins and Hb in sacrificed rats after 25.5 and 48 hours were evaluated. Similar to what was found in this present study, these authors reported that AST, ALT and bilirubin levels and prothrombin time were greater at 25.5 hours after the d-galactosamine injection. Histologically, they found intense parenchymatous necrosis and intense lobular and periportal inflammatory infiltrate with progression of injury at 48 hours after the injection, thus showing complex dissociation of the parenchyma. These data were similar to what was found in this present study.

Watanabi *et al*.[14] injected a dose of 1.3 g/kg of d-galactosamine intraperitoneally into three Sprague-Dawley rats. In addition to ALT, bilirubin and prothrombin time, these authors ascertained the ammonia, cholesterol and glucagon levels and evaluated liver and brain histology at 24 and 48 hours after the injection. They also produced electroencephalograms on the rat to identify encephalopathy findings. The EEGs showed slow high-amplitude waves compatible with hepatic encephalopathy, while histological analysis showed brain edema. Unlike this, the histological analysis on the brain among the rats in this present study was normal, although the clinical findings of numbness, spasms, seizures and coma were presented, also in Watanabi *et al*.[14] study, compatible hepatic encephalopathy were reported.

Dixit *et al*.[15] correlated the clinical neurological findings from rats subjected to intraperitoneal injection of 0.85 mg/kg of d-galactosamine, with enzymatic alterations and hepatic histology. The peak hepatic enzyme alterations coincided with the highest degree of histological liver failure, which occurred 48 hours after the injection, the rats presented second degree of encephalopathy (confusion and numbness). The authors also affirmed that d-galactosamine did not have any direct effect on the blood-brain barrier, thus suggesting that the brain edema found in the histological analysis was due to a cytotoxic effect that led to the formation of vasogenic edema.

From the analysis on liver failure evolution over the course of time and laboratory alterations in this present study was that the peak for liver enzymes also coincided with the time of greater intensity of necrosis, apoptosis and hepatic bleeding was noted, as Dixit *et al*.[15] had also observed. However, this peak occurred 24 hours after the d-galactosamine injection, i.e. earlier than what Dixit *et al*.[15] had observed. One explanation for this is the difference between the doses used in each study, thus, it could be asked whether the evolution of the liver failure is dependent on the dose administered.

Zhang *et al.*[16] analyzed rats for a period longer than 48 hours. To evaluate the use of granulocytic colony stimulating factor in ALF, 160 Wistar rats were analyzed and subjected to intraperitoneal d-galactosamine injection of 1.4 g/kg. Eighty rats received d-galactosamine subcutaneous, followed by saline solution 2 hours after the d-galactosamine injection, once a day for five consecutive days. For the other eighty rats, 2 hours after the d-galactosamine injection, granulocytic colony stimulating factor was administered once a day for five days. In the d-galactosamine + saline group, thirty rats were observed over the period of time to evaluate the mortality and 50 rats were sacrificed at different hours after the injection (six at a time): 6, 12, 24, 72, 120 hours and 7 days.

Those authors only analyzed hepatic histology and ALT enzyme alterations. In the group that only received d-galactosamine + saline, the mortality rate was found to be 66.7% (20/30), i.e. similar to the results in this present study, and the progression of histological lesions through different hours also corresponded to the findings in this present study. However, the peak of ALT and the histological injury found by those authors was at a later stage, occurring 72 hours after the d-galactosamine injection. Reversibility of liver failure was also proven by Zhang *et al*.[16] showing liver histological sections with regeneration of hepatocytes and vacuolar degeneration five days after the injection, and normal ALT values seven days after the injection. The group that received granulocytic colony stimulating factor presented a lower mortality of 46.7% (14/30), lower ALT levels and lower liver failure[16]. The intervention was done empirically, without considering any clinical aspects.

D-galactosamine is an expensive drug, and it cannot be applied on large animals and for this reason rats are commonly

used, however unfortunately we are not able to get blood samples from the same rat during different periods of time, in which it would be ideal, because of its low blood volume. D-galactosamine is likely to cause irritability to the skin and eyes, it must be handled using personal protection equipment.

The prognosis for acute liver failure improved significantly after the emergency liver transplant has been performing. The one-year survival rate reached 77%. When evaluating the prognosis for fulminant hepatitis, the decision regarding the need for and the time to proceed a liver transplant should be made as early as possible[17]. A series of prognostic criteria have been developed with the aim to select candidates for a liver transplant the best way possible.

The criterion developed by King's College is the most commonly used. It forms the basis to register for emergency liver transplant in the United Kingdom and has been widely disseminated internationally[8].

Meta-analysis evaluated the survival among patients with ALF that was not induced by paracetamol showed moderate sensitivity (70%, 95% CI 61-79%) and higher specificity (80%, 95% CI 73-87%), in which the capacity to discriminate between the need for transplant and the lack to need were evaluated by ROC curve, with Q of 0.783[7]. Another meta-analysis performed to evaluate King's College criterion among patients with ALF, secondary to paracetamol, revealed sensitivity of 69% and specificity of 92%, but Q was 0.61[18]. To overcome these limitations, other alternative prognostic systems might be used[6].

Clichy-Villejuif criteria are mostly used in France, and include hepatic encephalopathy and factor V level < 20% among patients under 30 years of age and < 30% among patients over 30 years of age. Using Clichy´s criteria, a recent study conducted in France evaluated 808 patients who underwent an emergency liver transplant due to ALF between 1997 and 2010. The sensitivity, specificity and positive and negative predictive values for Clichy´s criteria were 75%, 56%, 50% and 79%, respectively, for ALF due to paracetamol, were 69%, 50%, 64% and 55%, respectively, for ALF unrelated to paracetamol[17].

Comparing this present study with other similar ones, it was found that this was the first study to evaluate King's College and Clichy´s criteria in an animal model for ALF. It was observed that 12 hours after d-galactosamine injection, the mice presented a median INR > 6.5, thus fulfilling King's College criterion and that, 24 hours later, factor V reached levels lower than 30%, fulfilling Clichy´s criterion. This finding may indicate the best time to intervene in this model by testing new therapeutic approaches.

Transplantation of hepatocytes, encapsulated hepatocytes and stem cells may contribute as a support therapy until the liver has recovered from the acute event, possibly avoiding liver transplantation. There are advantages of having less surgical risk and limited duration of taking immunosuppressive medication.

Conclusion

King's College and Clichy´s criteria were fulfilled 12 hours after the d-galactosamine injection and this time may represent the best time to intervene in this acute liver failure animal model.

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