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Acute liver failure induced by D-galactosamin: Selection of optimal dosage in rabbit

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ABSTRACT

We sought to investigate the course of acute liver failure (ALF) when induced by different doses of D-galactosamin to select optimal dosage for different research objectives. Thirty healthy New Zealand White rabbits were randomly divided into three groups, which were administered with D-galactosamine via ear vein at doses of 1.2 (high-dose group), 0.6 (mediumdose group) and 0.3 g·kg⁻¹ (low-dose group), respectively, to induce ALF. At 0, 24, 48, 72, and 96 h after administration, blood samples were collected for measurement of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKP), and total bilirubin (TB). The histology of liver tissue was accessed after 24 hours D-galactosamin adminstration. All rabbit were dead within 24 hours in the high dose groups and 80% of rabbit were dead within 96 h in the medium dose group, and the mean survival time was 65.7 h ± 10.2 h. Only one animal died at 96 hours after treatment in the low-dose group. Serum levels of ALT, AST, AKT and TB were significantly increased, while serum levels of glucose was significantly decreased in the medium dose groups. All these parameters were normal at 96 hours in the low-dose group. The rabbit in model groups showed severe damage to liver tissue 24 hours after D-galactosamin administration in dose-dependent manner. Conclusion: A stable and reproducible rabbit model of ALF has been successfully established by 0.6 g·kg⁻¹ D-galactosamin administration, and can be used for future ALF research.

KEYWORDS

Acute liver failure; Rabbit; D-galactosamine.



INTRODUCTION

Acute liver failure (ALF) is a clinical condition with very high mortality rate. Its pathophysiological background is still poorly understood, which necessitates a search for optimal experimental ALF models with features resembling those of the human disorder. Numerous studies have been performed in an attempt to develop optimal models to study pathophysiology of ALF, both in large (dogs, goats and pigs) and small (rats and mice) animals. After considering factors like: knowledge of the genomes, accessibility for physiological and biochemical measurements, life span of animals, and economical acceptance, a consensus has been reached (similar as in cardiovascular research)^[1] that laboratory rabbit is the animal species most suitable for studies of ALF. Rabbit were used widely in preclinical models for evaluation of regenerative medicine in human^[2,3]. In in vivo models, the rabbit has the advantage due to its relatively large size compared with the rat and mouse. In addition, it is inexpensive and relatively easy to handle compared with other large animal models^[4,5].

D-galactosamine is a molecule which, when metabolized via the galactose pathway in the liver, causes serious metabolic alterations and hepatic necrosis through depletion of different uridine intracellular mediators^[6], and has therefore been used to develop ALF models^[7]. However, most studies have focused on the effect of high-dose D-galactosamine on animal, in which the animal occurred a serious ALF and were died within 24 hours. In some experiment, such as cell therapy of ALF, it is important for animal to have a relative long survival time with liver injury after D-galactosamine for long time evaluation of efficiency of cell transplantation. In this study, we sought to investigate the course of ALF when induced by different doses of D-galactosamin to select optimal dosage for different research objectives.

EXPERIMENTAL SECTION

Animals

New Zealand white rabbits (12 male and 12 female, 2 ± 0.3 Kg in weight) were supplied by Experimental Animal Center of Shaoxing University and placed in the animal house of the Center. They were housed individually in cages under standard laboratory conditions with a period of 12 hours light/dark at 25 to 30°C and 70 to 80% relative humidity in the animal house. The animals were allowed to acclimatize for at least 10 days before the start of the experiments. The rabbits were fed with a standard rabbit chow pellet and allowed to drink water ad libitum.

Experimental design

Animal were assigned into three groups which are considered as control, low, and moderate dose of D-galactosamine via ear vein injection (10 animals in each group). The low dose group was injected with $0.3 \text{ g} \cdot \text{kg}^{-1}$ D-galactosamine, the moderate dose group was injected with $0.6 \text{ g} \cdot \text{kg}^{-1}$ D-galactosamine, and the high dose group was injected with $1.2 \text{ g} \cdot \text{kg}^{-1}$ D-galactosamine. According to the Shaoxing University research regulation, all research projects dealing with human or animal subjects must be approved in advance by the university research ethics committee. The current project was approved by the committee as complying with the country and university research ethics (approval No. S20130029).

Blood samples assay

Serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AKP), total bilirubin (TB), and glucose was determined by routine biochemical methods using a Hitachi Automatic Analyzer (Hitachi, Inc. Japan).

Histological examination

All rabbits were sacrificed after 24 hours D-galactosamine injection and resected liver specimens of each rabbit in all groups were fixed in 10% buffered formaldehyde for 24 hours and embedded into

paraffin after 16 hours of alcohol process. 4-6 μm thick sections were obtained from the paraffin blocks and stained with hematoxylin and eosin (H&E). Each slide was examined under a light microscope.

Statistical analysis

All data were expressed as mean \pm standard error of the mean (SEM).

RESULTS

Dose-dependent effect of D-galactosamine on rabbit survival

All rabbit were dead in the high dose groups, and the mean survival time was 18.5 h \pm 5.1 hours. 60% of rabbit were dead at the 24 hours, and 80% of rabbit were dead at the 48, 72, and 96 hours in the medium dose group, and the mean survival time was 65.7 h \pm 10.2 h. Only one animal died at 96 hours after treatment in the low-dose group.

Assessment of histological liver changes in response to D-galactosamin

HE staining showed that the liver of control rabbits showed the common characteristic lobular organization of the mammalian liver. The hepatic cords were well organized and radiated from a central vein. Neither obvious congestion nor hepatic necrosis/degeneration were observed (Figures 1A, B). However, in the D-galactosamin administration groups, arrange of hepatic cord were disorganized in a dose dependent manner. Blood vessel congestion were obviously visible and the blood sinusoids were dilated between the cords of hepatocytes (Figures 1C-H). The necrotic and degenerative hepatocellular conditions were characterized by decrease of cell diameter as well as few vacuolated hepatocytes without usual polyhedral shape (Figures 1C-H). These changes were observed at the low dose of administration and became more significant at the moderate dose of administration. After high dose of D-galactosamin administration for 24 days, there was serious hepatocellular necrosis, congestion of blood vessel, and increase of blood sinusoids dilation between the cords of hepatocytes. Thus, our results demonstrated that D-galactosamin administration resulted in hepatocytes necrosis in a dose-dependent manner.

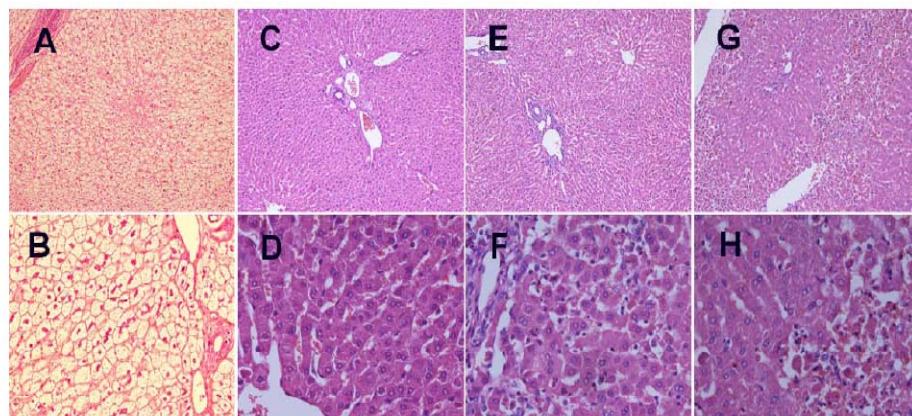


Figure 1: Liver damage of rabbits after exposure to D-galactosamin administration (A, B) normal liver tissue; (C, D) rabbits treated with D-galactosamin at 0.3 g·kg⁻¹; (E, F) rabbits treated with D-galactosamin at 0.6 g·kg⁻¹; (G, H) rabbits treated with D-galactosamin at 1.2 g·kg⁻¹. A, C, E, and G100; B, D, F, and H400.

Assessment of blood samples changes in response to D-galactosamin

As shown in TABLE 1, in the medium dose groups, serum levels of ALT, AST, AKT and TB were significantly higher at all time points than at baseline (0 h), while serum levels of glucose was significantly lower at all time points than at baseline (0 h)(TABLE 1). Because all animal were died within 24 hours in the high dose groups, blood samples assay were not performed.

TABLE 1: Changes in plasma ALT, AST, AKP, TB and Glucose activity in response to different D-galactosamin induction

Group	ALT(U/L)	AST(U/L)	AKP(U/L)	TB ($\mu\text{mol/L}$)	Glucose (mmol/L)
1.2 g. kg^{-1}					
24 h	--	--	--	--	--
48 h	--	--	--	--	--
72 h	--	--	--	--	--
96 h	--	--	--	--	--
0.6 g. kg^{-1}					
24	2451 \pm 213.5	3697 \pm 513.2	489 \pm 23.2	34.1 \pm 5.9	2.1 \pm 0.4
48	3648 \pm 223.1	5893 \pm 687.2	505 \pm 40.6	45.2 \pm 7.3	2.5 \pm 0.4
72	1157 \pm 89.2	987 \pm 42.6	395 \pm 20.8	27.3 \pm 5.4	3.9 \pm 0.5
96	768 \pm 56.1	458 \pm 23.2	183 \pm 18.6	15.6 \pm 3.3	5.5 \pm 0.6
0.3 g. kg^{-1}					
24	78.3 \pm 6.5	44.6 \pm 3.2	102.9 \pm 9.3	1.2 \pm 0.05	6.5 \pm 0.5
48	85.6 \pm 10.2	56.3 \pm 9.4	139.5 \pm 8.6	0.8 \pm 0.02	7.1 \pm 0.5
72	44.5 \pm 6.3	39.7 \pm 5.5	121.6 \pm 8.8	0.7 \pm 0.02	7.9 \pm 0.3
96	50.2 \pm 4.8	42.3 \pm 6.8	109.8 \pm 7.5	0.4 \pm 0.03	7.5 \pm 0.6
0 g. kg^{-1}					
24	55.4 \pm 3.3	30.1 \pm 1.9	101.6 \pm 6.2	0.4 \pm 0.02	8.9 \pm 0.6

DISCUSSION

In this context, it is important to acknowledge that a search for optimal animal model for ALF is still ongoing and factors such as reproducibility, adequate animal size, cost of animals, an appropriate “therapeutic window” (time required for treatment, between the insult and death of animals), minimal risk to investigators etc. must be considered. Currently, surgical models of ALF in large animals are preferred (e.g. devascularization of the liver in pigs) because of high reproducibility; moreover, large size of animals enables evaluation of different bioartefical liver support systems. However, the large size of animals is also a disadvantage because experiments are extremely costly (housing and handling of animals, induction of ALF requires a team of investigators). Therefore, chemical agents, such acetaminophen, galactosamine, carbon tetrachloride and many others are used in relative small laboratory animals for induction of ALF. Rabbit were used widely in preclinical models for evaluation of regenerative medicine in human^[3,8]. In in vivo models, the rabbit has the advantage due to its relatively large size compared with the rat and mouse. In addition, it is inexpensive and relatively easy to handle compared with other large animal models^[4,5]. After consideration of the aforementioned aspects, especially of the reproducibility of ALF induction and safety for investigators, the use of D-galactosamin is at present most recommended.

Consistent with previous studies, the present data showed that rabbits suffered the sever ALF after 0.6 g·kg⁻¹ of D-galactosamin administration, in which all rabbit were dead within 24 hours. On the basis of this results, we further found that 40% of rabbit were dead within 24 hours and 80% of rabbit were dead within 96 hours after injection with 0.6 g·kg⁻¹ of D-galactosamin. In addition, serum levels of ALT, AST, AKT and TB were significantly increased and hepatocytes necrosis in in this groups. Thus, 0.6 g·kg⁻¹ administered i.p. as a single injection is an optimal dose for long time evaluation of ALF and 1.2 g·kg⁻¹ administered i.p. as a single injection is an optimal dose for induction of severe ALF. There is no effect on rabbit afer 0.3 g·kg⁻¹ administration. Collectively, our present findings provide a sound methodological background for experimental studies aimed at evaluation of pathophysiology and development of new approaches in the therapy of ALF.

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