SHORT COMMUNICATION

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PRELIMINARY STUDY OF SPONTANEOUS HEPATITIS IN LONG-EVANS CINNAMON RATS: A BLOOD EXCHANGE MAY IMPROVE FETAL HEPATITIS

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ABSTRACT

Long-Evans Cinnamon rats are a Wilson disease model highly susceptible to fulminant hepatitis around the age of 20 weeks, and hepatoma over the age of one year. Although prophylaxis has been established for the otherwise fatal hepatitis, effective treatment remains unknown. A blood exchange was tested to determine whether the prognosis of spontaneous hepatitis could be modified in icteric female rats. When bilirubinuria appeared, the rats immediately underwent surgery. Rats under anesthesia were first cannulated into the right atrium via the carotid vein, followed by 2.5 mL of blood exchange with heparinized fresh blood from Long-Evans agouti rats. Treated rats and controls were then observed for 2 months. Compared to the 50% mortality of untreated rats, all icteric rats that received a blood exchange survived the acute episode. We confirmed that Wilson disease animals are highly susceptible to acute hepatitis and show a poor prognosis. However, a single blood exchange improved spontaneous hepatitis in this animal model. This would serve as a first step for establishing a treatment for fatal hepatitis in animals. A blood exchange may improve fulminant hepatitis of Wilson disease model rats.

Key Words: Blood exchange, Hepatitis, Long-Evans Cinnamon rat

INTRODUCTION

Long-Evans Cinnamon rats are highly susceptible to fulminant hepatitis around the age of 20 weeks,[1)] and most survivors from acute hepatitis develop hepatoma over the age of one year.[2,3)] A genetic study showed that the rats have a deletion in the copper-transporting ATPase gene homologous to that in the Wilson disease gene.[4)] It is of interest to note that the livers of Wilson model rats are loaded not only with copper,[5)] but also iron.[6)] Copper is primarily deposited due to a dysfunction of the copper transporter in Wilson disease, and iron accumulation might be secondary to a ceruloplasmin deficiency shown in male patients with Wilson disease.[7)] Therefore, the rats are not only an animal model of Wilson disease, but may also be suitable in vivo subjects for the study of carcinogenesis by the potential elements of copper and iron. Prophylaxis for fulminant hepatitis in these rats has already been established. The standard treatments for

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Wilson disease are copper chelation[8,9] and zinc replacement,[10] which are effective for an acute episode of otherwise fulminant hepatitis. A low iron diet[11] and iron removal by phlebotomy[12] are also effective, probably due to their suppression of iron-induced oxidative stress. However, a treatment that will effectively modify the poor prognosis of rats that have already progressed to spontaneous hepatitis is not yet known.

MATERIALS AND METHODS

Because female LCE rats are more sensitive to fulminant hepatitis than male rats, females aged 10 weeks were purchased from Chubu Kagaku Shizai Inc. (Nagoya), and were fed commercial chow (CRF-1, Oriental Yeast Co., Ltd., Tokyo) containing 15.8 mg/100g Fe, and 0.9 mg/100 g Cu ad libitum. Body weight was determined twice a week. When the linear increase in body weight subsided, daily urinalysis was conducted along with body weight measurements. On the day bilirubinuria was detected, rats were divided into two groups of control and treatment animals. Controls underwent 0.5 mL blood sampling from a tail vein, followed by a 2-month observation period without treatment of any kind. Treated animals underwent surgery for blood exchange. The right atrium was cannulated through the carotid vein under anesthesia, followed by 2.5 mL of blood exchange with heparinized fresh blood from Long Evans agouti (LEA) rats. Some removed blood was kept for tests. Finally, the carotid vein was ligated after withdrawing the cannula. It usually took 20 min to complete the procedure. Although a transient manual assist was sometimes needed for respiratory distress, there were no fatal accidents during surgery, nor was there any supplement of fluid during the postoperative period of 2 months. Blood samples were tested for alanine aminotransferase activity (ALT). All animal experiments were carried out in accordance with the guidelines of the Nagoya University School of Medicine for the care and use of laboratory animals.

Mortality rates of the control and treatment groups were compared using Kaplan-Meier estimates and the log rank test. Any difference in serum ALT levels between control and treatment groups was evaluated by Student *t* test.

RESULTS

All the rats between the age of 19 and 25 weeks developed bilirubinuria associated with weight loss. Survival curves for control and treatment groups are shown in Fig. 1. Mortality and the pre-treatment levels of serum ALT are summarized in Table 1. There were no deaths among the treatment group despite transient respiratory distress requiring manual support in some rats. Five of 10 rats without treatment survived icteric hepatitis, while the other 5 died within 3 days after bilirubinuria development. All 10 rats that underwent a blood exchange survived icteric hepatitis. There was a statistical difference in mortality between the control and treatment groups ($p = 0.012$, log rank test) (Fig. 1). Blood tests confirmed acute hepatitis in all rats regardless of their prognosis. Pre-treatment ALT levels did not differ between the control and treatment groups, nor was there any difference in serum ALT levels between the survivors and victims of the control group. No recurrent icteric hepatitis appeared in both survivors of either the control or treatment groups during the subsequent 2 months.

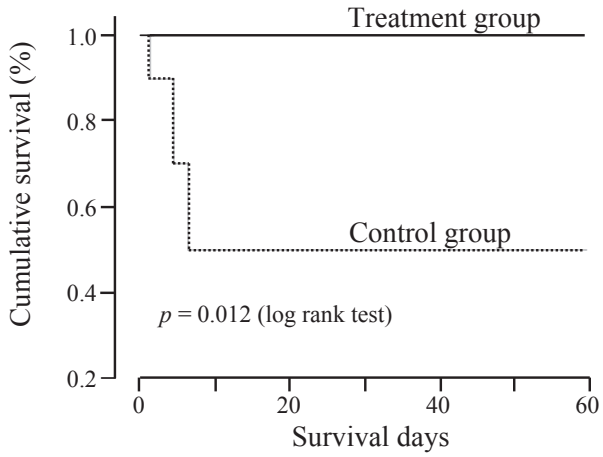

Fig. 1 Kaplan-Meier survival analysis for control and treatment groups

Table 1 Mortality and serum ALT levels of icteric LEC rats

		Survivors		Victims	
	Mortality	$N^{a)}$	ALT[b)] (U/L) [mean ± SD (range)]	$N^{a)}$	ALT[b)] (U/L) [mean ± SD (range)]
Control	50	5	558 ± 116 (442–703)	5	689 ± 229 (423–1028)
Treatment	0	10	638 ± 110 (508–873)	0	none

Serum ALT levels of icteric rats are no different between the control and treatment groups. There is no difference in serum ALT levels between the survivors and victims of control group.
[a]*N*: number of observations; [b]ALT: alanine transaminase; [c]SD: standard deviations

DISCUSSION

As far as we know, this is the first successful trial for treating spontaneous hepatitis in LEC rats. All the trials reported to date were a prophylaxis of hepatitis,[8-12] whereas our experiment was performed in animals with icteric hepatitis. The treatment of non-viral hepatitis could become important in time since patients with metabolic disease may increase in number in the near future. Therefore, this treatment, now widely applied in human disease, could be a first step in an animal study establishing procedures safer and more economical than whole blood exchange.

Although LEC rats are an animal model of Wilson disease, our results obtained from female rats sensitive to fulminant hepatitis were more favorable than those of human studies. Although the prognosis of fulminant hepatitis was somewhat improved after the introduction of blood purification therapy (BPT), the prognosis of its subacute form remains poor.[13] The standard volume of plasma exchange, one of the most common BPTs procedures, is 3,600 mL per session (45 units × 80 mL), indicating that any plasma exchange is intended to completely exchange patient plasma. Even after extensive BPT, Wilsonian fulminant hepatitis (WFH) is so virulent that the first choice of treatment is a liver transplant.[14] Despite clinical experiences in WFH, all LEC rats treated with a single blood exchange (about a 26% exchange of circulating blood) survived spontaneous hepatitis. The infused fresh plasma obtained from healthy LEA rats may contain sufficient amounts of the coagulants and ceruloplasmin ferroxidases that are deficient in

LEC rats.[15] The effect of a ceruloplasmin supplement may have played a role in the treatment model, though different responses to blood exchange under the 2 pathological conditions suggest that fulminant hepatitis in Wilson model rats may have a different etiology than that in the subacute form of WFH.

One cycle of blood exchange might temporarily affect the potential of copper and iron, or have no effect at all. A 50% loss of experimental animals at the age of 20 weeks was avoided by a simple treatment that might provide a major benefit for long-term experiments on liver fibrosis and carcinogenesis due to those hepatotoxins. Studies using a large number of LEC rats may be needed to confirm the effects of a single blood exchange.

In summary, a single blood exchange improved spontaneous hepatitis in Wilson disease model rats.

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