Hyperlipidemia in Rabbit Hemorrhagic Disease

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Abstract: Clinically healthy rabbits were inoculated with rabbit hemorrhagic disease virus (RHDV) and the kinetics of their serum lipid parameters and liver enzymes were monitored. After RHDV inoculation, hyperlipidemia was observed (P_triglyceride<0.0001, P_cholesterol=0.0003) along with significant increases in serum aspartate aminotransferase, alanine aminotransferase and γ-glutamyltransferase (P<0.0001). An exponential increase in serum triglyceride was also seen. Thus, the presence of hyperlipidemia (from 30 h post-inoculation) in the infected rabbits points to impairment in lipid metabolism. This is the first demonstration that RHDV infection leads to hyperlipidemia, probably due to the disorder of liver enzymes associated with lipid metabolism.

Key words: cholesterol, rabbit hemorrhagic disease, triglyceride

Rabbit hemorrhagic disease (RHD), first reported in 1984 in China [9], has been characterized as an acute fatal necrotic viral hepatitis in both wild and domestic adult European rabbits (Oryctolagus cuniculus). The highly contagious causative agent is a single strand, non-enveloped RNA virus which belongs to the family Caliciviridae [10]. The viral particles mainly accumulate in the membrane-bound cisternae and scatter around vacuoles in the cytoplasm of many necrotic hepatocytes [12]. Marked elevations of liver enzymes in serum of RHDV infected rabbits has been observed [7, 15].

The liver plays an important role in energy metabolism. Most plasma proteins and endogenous lipoproteins are synthesized in the liver. Besides storing glucose as glycogen, converting amino acids to glucose, and degrading lipids, also produces carrier proteins for cholesterol and the triglycererols. Primary liver disease does not usually cause an increase in triglyceride (TG), but may result in an increase in the level of cholesterol (CHOL) [4]. Increases in TG and CHOL reflect impairments in liver lipid metabolism which might severely disable cell functions that rely on lipid and may also cause systemic damage to organs. Although extensive hepatocyte vacuolization, severe changes in mitochondria, and depletion of glycogen granules were demonstrated ultra-structurally in RHDV-infected hepatocytes [6], changes in lipid metabolism have not been reported. In this study, we experimentally infected 15-week-old New Zealand White rabbits with RHDV, and monitored the serum parameters of lipids and liver enzymes at different stages of the damage due to RHDV to elucidate the impairment of lipid metabolism in the liver.

Twelve, 15-week-old, clinically healthy RHD-free New Zealand White rabbits, as confirmed by the absence of antibody against RHDV in the hemagglutination inhibition (HI) test, were obtained from the National Animal Health Research Institute in Taiwan. The microorganism monitoring of the rabbits in the institute...
includes the following diseases: rabbit hemorrhagic disease, myxomatosis, tularemia, pasteurellosis, bordetellosis, salmonellosis, pseudomoniasis, coccidiosis, aural acariasis and oxyurisosis (pinworm). None of the rabbits, 7 females and 5 males, had previously been vaccinated with inactivated RHD vaccine. The rabbits were kept in sanitized wire cages and separated by sex. All were fed commercial dry rabbit feed that contained 19% crude protein, 10% crude fiber and 2,500 kcal/kg metabolic energy (“Laboratory Rabbit” feed, Fwu-Sow Industry Co., Taiwan). The rabbits were kept and handled according to the regulations laid down by the Institutional Animal Care and Use Committee (IACUC) of National Chung Hsing University (NCHU).

RHDV was obtained from a liver specimen of a field case. A 10% suspension of homogenized liver in 0.01 M phosphate-buffered saline was made, and centrifuged at 7000 \(\times g\) for 30 min at 4°C. The supernatant was then filtered through a 0.22 \(\mu m\) filter and stored at –70°C until use. A 50% rabbit lethal dose (RLD\(_{50}\)) viral titration of the supernatant was carried out by intra-muscularly inoculating groups of four 2-month-old rabbits (weighing over 2 kg) with serial dilutions of \(10^{-4}\) to \(10^{-9}\) in Eagle’s minimal essential medium. A total of 24 rabbits, 4 rabbits for each titer, were used. The rabbits were monitored continuously for 7 days. Rabbits that died with typical RHD signs of hemorrhages in the respiratory system, liver, spleen and cardiac muscle were recorded. The Reed and Muench method was used to calculate the RLD\(_{50}\) [5].

In the present experiment, 12 rabbits were each inoculated intramuscularly with 0.5 ml RLD\(_{50}\) RHDV. Blood samples were collected from the auricular artery of the rabbits after 12 h of fasting and used as a base line (0 hour post-inoculation; HPI). After virus inoculation, 1.5 ml blood samples were collected at 18 HPI, and then every 6 h until a rabbit died or until 60 HPI. Blood collected in syringes without anticoagulant was left at room temperature for 30 min and then at 4°C for 30 min to elute the serum. Whole blood was then centrifuged at 1000 \(\times g\) for 10 min and the sera collected. Triglyceride (TG), cholesterol (CHOL), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and \(\gamma\)-glutamyltransferase (\(\gamma\)-GT) were measured spectrophotometrically with a Hitachi 7150 autoanalyzer (Hitachi Co., Tokyo).

Data collected at different HPI were analyzed using Statistic Analysis System (SAS 9.1). The means and standard deviations were calculated by the Means Procedure for time and sex. Least Squares Means of the General Linear Model (GLM) Procedure were applied to the analysis of the differences on the effect of time and sex. P values less than 0.05 were considered significant. The strength of the relationship that existed between the parameters was defined by the statistics of the Pearson correlation coefficient and its significance by the correlation procedure. Regression analysis was used to determine the increasing pattern.

Following the progression of the disease, fever, loss of appetite, and marked depression were observed. Seventy-five percent of the infected rabbits died between 27 HPI and 45 HPI with typical RHD signs of hemorrhages in the respiratory system, liver, spleen and cardiac muscle. The remaining 3 rabbits that survived after 54 h were sacrificed at 60 HPI. The survival rate at different periods of time is shown in Fig. 1. Changes in lipid parameters and liver enzymes are shown in Table 1. Hyperlipidemia developed significantly \(P_{TG}<0.0001, P_{CHOL}=0.0003\) and was coupled with significant increases in serum AST, ALT, and \(\gamma\)-GT \(P<0.0001\). Compared with the baseline values (0 HPI), significant elevations of AST and ALT \(P<0.05\) were seen at 24 HPI, and for \(\gamma\)-GT, TG, and CHOL at 30 HPI. By regression analysis, TG values were fitted to a logarithmic curve:

![Fig. 1: The survival rate of 12 rabbits at different hours post-inoculation (HPI) after RHD virus inoculation.](image-url)
Hyperlipidemia developed with significant increases in TG and CHOL. TG and fatty acids are synthesized within the cytosol [3]. Impaired metabolism of fatty acids leads to accumulation of TG (fat) which forms the non-membrane-bound vacuoles in cells [2]. With the progression of RHD, massively increasing hepatic necrosis led to the release of TG into the blood and caused serum TG to increase exponentially. Hypertriglyceridemia is also observed in the early phase of acute hepatitis in humans [16]. We propose that there is a relationship between TG and ALT. The raised serum ALT activity in viral hepatitis may reflect a compensative increase in muscle ALT increasing transamination of alanine for post-absorptive glucose homeostasis [16]. This would explain why TG correlated significantly with ALT ($r=0.56$, $P<0.0001$, $R^2=0.33$). Since hypertriglyceridemia serves to provide substrate to meet the energy requirement of the muscle, this condition spares glucose for alanine synthesis to maintain the alanine content of the muscle in viral hepatitis [1]. This might be the reason why the surviving rabbits had high TG in RHD. In humans, the increased composition of triglycerides in LDL (low density lipoprotein) seen in many liver diseases can be attributed to deficiency in lipoprotein lipase, hepatic triglyceride lipase, and cholesterol acyltransferase activity [13]. Hence, the development of hyperlipidemia in RHDV infected rabbits might be due to the deficiency of several enzymes responsible for lipid metabolism.

Cholesterol, which is synthesized in smooth endoplasmic reticulum, is synthesized in smooth endoplasmic reticulum, and is synthesized in smooth endoplasmic reticulum, and is synthesized in smooth endoplasmic reticulum, and is synthesized in smooth endoplasmic reticulum, and is synthesized in smooth endoplasmic reticulum.

![Fig. 2. Exponential increment of serum TG (mg/dl) in RHD virus infected rabbits.](image-url)
mic reticulum [3], can be synthesized by all body cells except the brain cell. It is removed from the circulation only by the liver [14]. Bile acids are derived from the metabolism of cholesterol and then directly secreted into the bile. Hence, the massive secondary biliary obstruction seen in RHD can lead to an increase in serum CHOL. The $\gamma$-GT is generally a less sensitive but a more specific indicator of cholestasis than ALP [8]. This explains why $\gamma$-GT was significantly increased from 30 HPI together with increasing CHOL. CHOL increase can also indicate the severity of cholestasis in RHD. The surviving rabbits had significantly higher cholesterol than those that died earlier ($P<0.0001$). Although this reflects the progressing cholestasis and the impairment of CHOL metabolism in the surviving rabbits, we observed that rabbits with higher lipidemia had higher survivability. Further investigation will be needed to elucidate this phenomenon.

It has been suggested that the clinical, biochemical and histological features of RHD resemble those of fulminant liver failure syndrome in humans [15]. However, our study also showed evidence of hyperlipidemia (from 30 HPI), following the massive leakage of liver enzymes (from 24 HPI), indicating that RHDV infection also impaired the lipid metabolism function of the liver. Since the surviving rabbits had higher lipidemia, the significance of hyperlipidemia in RHDV infection after the acute fulminant phase in RHD requires further study.

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**References**

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