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Minireview

The importance of liver lesions and changes to biochemical and coagulation factors in the pathogenesis of RHD

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RHDV (rabbit haemorrhagic disease virus) is an etiologic factor of RHD (rabbit haemorrhagic disease), which is highly morbid and mortal viral infection of an adult European rabbit. Although three decades have passed since the first outbreak of rabbit haemorrhagic disease, the pathogenesis of RHD has still not been fully elucidated. It is known that RHDV replicates in the liver within the first hours following infection, causing necrotic and apoptotic cell death of hepatocytes. Anatomopathological changes are also observed in other organs of infected rabbits, i.e. lungs, spleen, kidneys, heart, as well as central nerve system. These changes leading to animals death are predominantly caused by systemic hemorrhagic diathesis with disseminated intravascular coagulation (DIC), appearing most likely as a consequence of liver cell loss through RHDV-induced apoptosis. In this paper, we presented previously described changes in biochemical and coagulation factors in RHDV infection.

Key words: rabbit, RHDV, biochemical factors, coagulation factors

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The rabbit haemorrhagic disease virus (RHDV), belonging to the *Caliciviridae* family – Lagovirus genus (International Committee on Taxonomy of Viruses), is an etiologic factor of rabbit haemorrhagic disease (RHD) (Abrantes *et al*., 2012). It is a highly contagious and lethal disease in rabbits of *Oryctolagus cuniculus* species, first described in 1984 in China, which then spread across the world causing high losses in rabbit farms and in populations of wild rabbits (Abrantes *et al.,* 2012). RHD usually occurs in three clinical forms: hyperacute, acute and subacute, rarely chronic, but it is also very often asymptomatic. Usually, death of infected rabbits occurs suddenly after approx. 1–3 days from infection with RHDV (Buczek *et al.,* 1991; Tokarz-Deptuła, 2009; Abrantes *et al.,* 2012; Hukowska-Szematowicz, 2013; Niedźwiedzka-Rystwej, 2013). Among the symptoms of the disease, body temperature increases by 1–1.5°C, which before the death of animals falls below physiological temperature, while other symptoms include loss of appetite, apathy, depression, as well as symptoms on the part of respiratory system (problems with breathing) and nervous system (convulsions, cramps, ataxia, and paralysis of legs), usually recorded shortly before animal death (Buczek *et al.,* 1991; Marcato *et al.,* 1991; Tokarz-Deptuła, 2009; Abrantes *et al*., 2012; Hukowska-Szematowicz, 2013; Niedźwiedzka-Rystwej, 2013). In the course of RHD, also anatomopathological lesions in the liver, lungs, kidneys, heart, pancreas, in the brain, spinal cord, as well as in thymus, spleen, lymph nodes and tonsils have been noticed (Buczek *et al.,* 1991; Marcato *et al.,* 1991; Plassiart *et al.,* 1992; Park *et al.,* 1995; Abrantes *et al*., 2012). Such lesions are manifested with enlargement of the organs, particularly the liver and spleen, as well as petechial haemorrhages mainly in the lungs, causing acute failure with symptoms of dyspnoea, particularly exacerbated just before the death of infected animals (Buczek *et al.,* 1991; Marcato *et al.,* 1991; Plassiart *et al.,* 1992). However, the most intensive lesions in RHD are recorded in the liver, where replication of RHDV occurs (Kimura *et al.,* 2001; Sanchez-Campos *et al.,* 2004; Ferreira *et al.,* 2006a, 2006b; San-Miguel *et al.,* 2006; Chen *et al.,* 2008; Garcia-Lastra *et al.,* 2010). In this organ, inflammatory infiltrates are observed, principally rich with PMN cells (Plassiart *et al.,* 1992; Park *et al.,* 1995; Ferreira *et al.,* 2005), as well as reduced number of T and B-cells is noticed (Marques *et al.,* 2010). Also, multi-foci lesions of hepatocytes are visible (Marcato *et al.,* 1991; Plassiart *et al.,* 1992; Park *et al.,* 1995; Alonso *et al.,* 1998; Jung *et al.,* 2000; Sanchez-Campos *et al.,* 2004; San-Miguel *et al.,* 2006; Garcia-Lastra *et al.,* 2010; Marques *et al.,* 2010), which were initially assumed as a result of necrosis (Marcato *et al.,* 1991; Plassiart *et al.,* 1992; Park *et al.,* 1995), yet later studies proved that these are basically as a result of apoptosis (Alonso *et al.,* 1998; Jung *et al.,* 2000; Sanchez-Campos *et al.,* 2004; San-Miguel *et al.,* 2006; Garcia-Lastra *et al.,* 2010; Marques *et al.,* 2010). At present, it was also evidenced that hepatocyte death is also preceded with the phenomenon of autophagy (Vallejo *et al.,* 2014), as in early hours of rabbit infection with RHDV, in liver cells, due to stress of endoplasmatic reticulum of such cells, increased expression of ATG proteins and beclin-1 was recorded, as well as other autophagy-related proteins. Such studies (Vallejo *et al.,* 2014) suggest that the process can probably contribute to more effective replication of RHDV, and thus to its spreading, as only after its deactivation the process of apoptosis is activated, which leads to damage of the infected hepatocytes and escaping RHDV from them

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Abbreviations: ALT, alanine transaminase; AP, alkaline phosphatase; APPT, activated partial thromboplastin time; AST, aspartate transaminase; DIC, disseminated intravascular coagulation; HGF, hepatocyte growth factor; LDH, lactate dehydrogenase; NAC, N-acetyl cysteine; OSPT, one-stage prothrombin time; PMN, polymorphonuclear leukocytes; RHD, rabbit haemorrhagic disease; RHDV, rabbit haemorrhagic disease virus; ROS, reactive oxygen species; SOD, superoxide dismutase; STAT, signal transducer and activator of transcription; TG, triglycerides; TNF, tumor necrosis factor; γ-GT, gamma-glutamyl transferase

(Vallejo *et al.,* 2014). It was also evidenced that, during rabbit infection with RHDV, apoptosis in hepatocytes occurs both as a result of activation of external (receptor) path, and internal (mitochondrial) path (Sanchez-Campos *et al.,* 2004; San-Miguel *et al.,* 2006; Garcia-Lastra *et al.,* 2010; Tunon *et al.,* 2011a, 2011b). It was found that, during the rabbit infection with RHDV, between 12 and 36h, there is an increase in the expression of TNF-α and ligand for Fas receptor (FasL), which points to apoptosis activation *via* the receptor (external) path (San-Miguel *et al.,* 2006; Garcia-Lastra *et al.,* 2010; Tunon *et al.,* 2011a). Observations of the infected hepatocytes also revealed an increase in reactive oxygen species (ROS), increased oxidised to reduced glutathione ratio (GSSG/GSH), as well as reduced volume of superoxide dismutase (SOD) (Sanchez-Campos *et al.,* 2004; San-Miguel *et al.,* 2006; Tunon *et al.,* 2011a), which would prove distorted oxidation balance and the increasing oxidation stress that directly contributes to damage of mitochondrial membrane and activation of internal path of hepatocyte apoptosis. Moreover, during the infection, from 12h, increased volume of cytochrome c is recorded, which is released from the mitochondria as a result of damage to the mitochondrial membrane and its increased permeability. This fact also proves activation of apoptosis via the internal path (San-Miguel *et al.,* 2006; Tunon *et al.,* 2011b). It was also evidenced that from 36–48 h, in the infected hepatocytes of rabbits infected with RHDV, increased activity of caspase-3 is recorded, and a much higher expression of pro-apoptotic Bax protein as compared to anti-apoptotic Bcl-2 protein (San-Miguel *et al.,* 2006, Tunon *et al.,* 2011b). Previous studies indicate that administration of N-acetyl cysteine (NAC) (San-Miguel *et al.,* 2006), cardiotrophin (CT-1) (Tunon *et al.,* 2011a) and melatonin (Tunon *et al.,* 2011b), which have antiapoptotic effects related to: reduced oxidative stress, expression of Bax, cytosolic cytochrome c release and inhibition caspase 3, could attenuate liver damage and prolong survival RHDV-infected rabbits. It was, however, found that during the infection, already at 24, 36 and 48 h from infection, the expression of the hepatocyte growth factor (HGF) and its receptor c-met decreases (Sanchez-Campos *et al.,* 2004, Tunon *et al.,* 2011a), and there is no activation of STAT1 and STAT3 (*signal transducer and activator of transcription*) in hepatocytes of rabbits infected with RHDV (Garcia-Lastra *et al.,* 2010), which probably contributes to reduced capacity for liver regeneration. Hence, liver condition, caused by apoptosis of hepatocytes and weakened or complete lack of regeneration, leads to its dysfunction resulting in the recorded increased level of liver enzymes in serum of the animals infected with RHDV, in particular including aspartate transaminase (AST) and alanine transaminase (ALT), bilirubin, gamma-gutamyl transferase (γ-GT), alkaline phosphatase (AP), and lactic dehydrogenase (LDH) (Kęsy *et al.,* 2000; Sanchez-Campos *et al.,* 2004; Ferreira *et al.,* 2006a; 2006b; San-Miguel *et al.,* 2006; Chen *et al.,* 2008; Garcia-Lastra *et al.,* 2010; Marques *et al.,* 2010). Increased levels of AST and ALT were recorded as from 24-36 h from rabbit infection with RHDV, and they were always higher for AST than for ALT (Kęsy *et al.,* 2000; Sanchez-Campos *et al.,* 2004; Ferreira *et al.,* 2006a; 2006b; San-Miguel *et al.,* 2006; Chen *et al.,* 2008; Garcia-Lastra *et al.,* 2010; Marques *et al.,* 2010). Furthermore, it was evidenced that increase in AST positively correlates with the degeneration of hepatocytes where mitochondrial dysfunction occurs, which refers to over half volume of the enzyme (Ferreira *et al.,* 2006a). It was also found that exceeding AST concentration above 6000 IU/l is related to death of the infected rabbits within 6h (Ferreira *et al.,* 2006b). During the infection, increased level of bilirubin is also observed (Kęsy *et al.,* 2000; Sanchez-Campos *et al.,* 2004; Ferreira *et al.,* 2006a; 2006b; San-Miguel *et al.,* 2006), although not as high as for AST and ALT, yet its increase is recorded slightly earlier, as from 18 h from animal infection with RHDV, namely before increase in AST and ALT concentration (Ferreira *et al.,* 2006a, 2006b). From 30 h from rabbit infection with RHDV, increase in γ-GT (Chen *et al.,* 2008) is also found, and from 36 to 48 h in LDH (Sanchez-Campos *et al.,* 2004), as well as increase in AP recorded several hours before the death of the animals (Ferreira *et al.,* 2006b). During the RHD infection in rabbits, fat metabolism disorders are also noted, namely from 30 h from infection, hypolipidaemia is recorded, manifested with increased cholesterol level and triglycerides (TG) (Chen *et al.,* 2008). It is assumed that cholesterol increase is caused by cholestasis, namely obstruction in biliary tract resulting from dysfunction of liver enzymes (Chen *et al.,* 2008), and manifested with increased bilirubin level (Kęsy *et al.,* 2000; Sanchez-Campos *et al.,* 2004; Ferreira *et al.,* 2006a; 2006b; San-Miguel *et al.,* 2006). In the course of the infection, hypoglycaemia is also observed (Ferreira *et al.,* 2006b), manifested with decreased level of blood glucose, which reveals an image reverse to the image recorded for AST. It is assumed that decrease in blood glucose accompanies the shortage of glycogen in the liver, and is probably caused by energy deficit as a result of mitochondrial damage in the process of hepatocyte apoptosis, which causes activation of anaerobic glycogenolysis to achieve reserve ATP in such cells (Ferreira *et al.,* 2006a; 2006b). Moreover, it is assumed that hypoglycaemia accompanying rabbit infection with RHDV can cause symptoms such as convulsion seizures observed shortly before the death of the infected animals (Ferreira *et al.,* 2006a; 2006b).

In the course of rabbit infection with RHDV, haemorrhagic diathesis is also observed with disseminated intravascular coagulation (DIC) manifested with changes in blood coagulation, which leads to multi-organ failure and quick death of the animals. The image of changes related to coagulation disorders is characterised with many clots in minor blood vessels of many organs (Buczek *et al.,* 1991; Marcato *et al.,* 1991; Plassiart *et al.,* 1992; Ueda *et al.,* 1992; Park *et al.,* 1997). It is assumed that this condition is due to distortion of both external and internal blood coagulation activation path, manifested with extended One-Stage Prothrombin Time (OSPT) and Activated Partial Thromboplastin Time (APTT) (Plassiart *et al.,* 1992; Ueda *et al.,* 1992; Park *et al.,* 1997; Paździor *et al.,* 2011). Also during rabbit infection with RHDV, significant decrease in the number of platelets is recorded, reduced degree of platelet aggregation, and changed activity of coagulation factors V, VII and X, as well as increased volume of soluble fibrin and its degradation products (D-dimers) (Plassiart *et al.,* 1992; Ueda *et al.,* 1992; Park *et al.,* 1997; Kęsy *et al.,* 2000; Paździor *et al.,* 2011). Observations also include initially increased, and later, in terminal phases of the infection, reduced activity of antithrombin III, with simultaneous lack of significant changes to the volume of fibrinogen (Plassiart *et al.,* 1992; Ueda *et al.,* 1992; Park *et al.,* 1997; Kęsy *et al.,* 2000; Paździor *et al.,* 2011). The results testify to the developing haemorrhagic diathesis with DIC syndrome, beginning from 24–30 h from rabbit infection with RHDV (Plassiart *et al.,* 1992; Ueda *et al.,* 1992; Park *et al.,* 1997; Kęsy *et al.,* 2000; Paździor *et al.,* 2011). The pathogenesis of DIC in RHD is unknown but few hypothesis exist.

At first, it was believed that DIC syndrome, observed in this infection, is caused by the formed antigen-antibody complexes, but this was excluded, as RHDV was not detected around the fibrin clots (Park *et al.,* 1997). Another hypothesis points out that DIC develops as a result destruction of the endothelium of blood vessels by RHDV, but it is increasingly discarded because no pathological lesions are found in tissues near the clots (Ueda *et al.,* 1992; Park *et al.,* 1997). In turn, Ramiro Ibanez et. al (Ramiro Ibanez *et al.,*1999) hypothesis that mechanism to develop of DIC may be due to the adjacency to the vessels walls of RHDV-infected intravascular monocytes. Yet another authors (Ueda *et al.,* 1992; Park *et al.,* 1997) suggest that DIC syndrome with haemorrhagic diathesis, observed in RHD, are a result of damage to the liver, as it is in this organ that the factors are synthesised, including coagulation inhibitors, and it is this organ that is responsible for clearing the activated coagulation factors. It is assumed that the aforementioned liver dysfunction contributes to activation of serum coagulation factors and causes faulty clearing of the activated factors by the liver, which, accompanied with decrease in the coagulation inhibitors in serum, leads to DIC (Ueda *et al.,* 1992; Park *et al.,* 1997).

Taking all the above facts into the consideration, one may conclude that the liver lesions and changes in biochemical and coagulation indicate that main reason of rabbits death are liver damage and DIC syndrome. Furthermore, the lesions recorded in the course of rabbit infection with RHDV and the pace of the infection point to the fact that RHD can serve not only as an animal model for acute viral diseases in mammals, including humans, such as haemorrhagic fevers in humans (infection with Ebola, Marburg viruses), but also for acute viral hepatitis.

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