Microbial Pathogenesis 104 (2017) 17–27

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

Pathological vicissitudes and oxidative stress enzyme responses in mice experimentally infected with reptarenavirus (isolate UPM/MY01)

Yusuf Abba [a, c, 1], Hasliza Hassim [b], Hazilawati Hamzah [a], Omar Emad Ibrahim [d], Mohd Azmi Mohd Lila [a], Mohamed Mustapha Noordin [a, *, 1]

[a] Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[b] Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[c] Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Maiduguri, PMB 1069, Maiduguri 600233, Borno State, Nigeria
[d] Department of Pathology, Faculty of Dentistry, Universiti Teknologi Mara, 47000 Sungai Buloh, Selangor Darul Ehsan, Malaysia

ARTICLE INFO

Article history:
Received 24 August 2016
Received in revised form
25 December 2016
Accepted 2 January 2017
Available online 3 January 2017

Keywords:
Boid inclusion body disease
Reptarenavirus
Oxidative stress
Hematology
Serum biochemistry
Histopathology

ABSTRACT

Boid inclusion body disease (BIBD) is a viral disease of boid snakes believed to be caused by reptarenavirus belonging to the family *Arenaviridae*. Unlike most mammalian arenaviruses, the reservoir host for reptarenavirus is still unknown. In this study, the pathological responses were evaluated in a mouse model for a period of 28 days. Blood and tissue samples (lung, liver, spleen, heart, kidney and brain) were collected for evaluation of hematology, biochemistry, histopathology and oxidative enzyme levels at six time points (1, 3, 7, 14, 21 and 28 days), after viral infection (2.0×10^6 pfu/mL) in the infected and normal saline in the control groups. An initial increase ($p < 0.05$) in white blood cell (WBC), neutrophil and lymphocyte counts were observed in the infected group at day 3 post infection, and a decline ($p < 0.05$) on day 7 and 4 post infection. Significant ($p < 0.05$) increases in alanine transaminase (ALT), aspartate transaminase (AST), creatinine, total protein and globulin levels were also observed in the infected group. An increased ($p < 0.05$) level of hydrogen peroxide, total antioxidant capacity (TAC), superoxide dismutase (SOD) activity and catalase activity (CAT) were frequently observed on different days in the infected group. The MDA activity was increased ($p < 0.05$) in the infected group on day 7 and 14. Histopathological changes observed in the liver, kidney, spleen, brain and lungs were mainly associated with degeneration, necrosis and infiltration of lymphocytes. Viral counts were low on days 7 and 14 but surged in both the liver and spleen on day 21 and 28. This study has shown that reptarenavirus replicates in mammalian host and induces oxidative stress. Furthermore, the resultant hematobiochemical and histopathological changes observed in infected mice were similar to what has been reported in mammarenavirus infections. This suggests that rodents may serve as potential reservoir hosts for reptarenavirus.

1. Introduction

Boid inclusion body disease is an often fatal viral disease of snakes' ranging from the B*oidae* and *Pythonidae* family. Its clinical course may run an acute or chronic course, with some snakes becoming asymptomatic carriers. Even though most recent reports of viral detection and identification were from snakes that are either dead of the disease or showing some clinical signs, the virus was recently detected in live snakes that were not showing clinical signs of infection [1]. Clinical signs of the disease include; regurgitation, anorexia, dysecdysis, torticollis, star gazing and stomatitis. Non-specific lesions observed in some snakes with BIBD include tumors such as lymphoblastic lymphoma [2,3]. The demonstration of eosinophilic intracytoplasmic inclusion bodies at histology is believed to be the gold standard for diagnosis [4,5]. Recently, the use of next generation sequencing technologies has assisted in discovering a new virus genus belonging to the *Arenaviridae* family in snakes with inclusion body disease, these groups of viruses, are now known as reptarenaviruses [6–9].

Members of the family *Arenaviridae* have been known to cause

* Corresponding author. Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
E-mail address: noordinmm@upm.edu.my (M.M. Noordin).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.micpath.2017.01.003
0882-4010/

highly fatal hemorrhagic fevers in both humans and laboratory model animals. Based on the new classification the genus, Arenavirus is now divided into Mammarenavirus and Reptarenavirus: common among them is the lymphocytic choriomeningitis virus (LCMV), which causes an aseptic meningitis in humans and choriomeningitis in mice [10]. Other members of the family, such as Lassa, Parital, Machupo, Junin and Pichinde viruses have all been shown to cause serious diseases in humans and laboratory animals characterized by fever, thrombocytopenia, increased levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), leukopenia or leukocytosis, histopathological changes in the spleen, liver, brain and kidneys and finally death [11–13]. In infected snakes with inclusion body disease, clinical signs were not always specific and liver enzyme changes have not been previously reported, while histological findings are characterized by presence of inclusion bodies in the cytoplasm of peripheral blood cells, visceral organs and the brain [4,5,14].

Oxidative stress is a condition that arises due to an imbalance of reactive oxygen species (ROS) and nitrogen oxide species (NOS) in the cell [15]. These accrued levels of ROS and NOS further results in an imbalance of oxidative enzyme such catalase, glutathione, and superoxide dismutase, ultimately deterring the cellular function. Several viruses have been shown to be associated with increased oxidative stress in the cell and in most cases; increased ROS levels in the cell are required by these viruses for replication and transcription within the host. Therefore, evaluation of the levels of these important oxidative enzyme will provide invaluable information about the association of a particular viral infection and oxidative stress in the host [16].

With the recent discovery of arenaviruses from reptiles, phylogenetic studies have shown a clear divergence from the mammalian associated Arenaviruses [7,8]. Our earlier study documented the first report of reptarenavirus in Malaysia, and we reported the presence of cytopathic effects in both rat embryonic fibroblast and vero cell lines infected with the virus [17]. Recently, the presence of different reptarenavirus genotypes was reported co-existing in snakes with BIBD. This was believed to be as a result of recombination and reassortment of the viral large and small genome segments in situ in the host [18,19]. This valuable information provides more insight into the etiopathogenesis of BIBD infection in snakes. Even though several *in vitro* studies have shown the possibility of propagating reptarenavirus in cell lines derived from snakes and mammals [7,20], there is a paucity of information on the pathology of reptarenavirus *in vivo,* and if rodent hosts can serve as potential reservoir hosts by propagating the virus without showing clinical signs. This work was hence designed in order to evaluate experimental reptarenavirus infection in mice (*Mus musculus*) by investigating changes in blood parameters, tissue pathology, viral load and oxidative enzyme levels.

2. Materials and methods

2.1. Cell, reagents and kits

Vero cell (P 12) was obtained from the Virology Laboratory, Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Roswell-Park medium (RPMI-1640; Necalai, Japan), Fetal bovine serum (Gibco, USA), PEROXsay (GBioscience, USA), total antioxidant capacity assay kit (Abnova, USA), catalase assay kit (Abnova, USA), malondialdehyde assay kit (Abnova, USA), superoxide dismutase assay kit (Sigma, Singapore), glutathione assay kit (Sigma, Singapore), GeneAll®Ribospin RNA extraction kit (GeneAll, USA), TetrocDNA kit (Bioline, UK), Sensifast™ SYBR HiRox kit (Bioline, UK).

2.2. Animal management and grouping

A total of forty eight male ICR (Institute of Cancer Research, USA) mice, aged 6–8 weeks were used for this study. The mice were acquired from the Universiti Putra Malaysia, animal farm and housed at the Faculty of Veterinary Medicine animal house for two weeks in order to acclimatize them. The animal house was an enclosed unit with controlled ventilation and air conditioning. The room temperature was controlled at 30 °C. Routine antibacterial medication was instituted using Trimethoprim and Sulphamethaxazole at 15 mg/kg, orally for 3 days. Pelleted commercial feed and water were provided *ad libitum*. Lighting period was 12 h daily (6am-6pm). After a period of two weeks, the mice were divided into two groups of 24 mice each with 8 mice per cage; group 1 (infected) and group 2 (control).

2.3. Ethical statement

The study was conducted in strict compliance with the guidelines of the Institutional Animal Care and Use Committee, Universiti Putra Malaysia (UPM/IACUC/AUP-R020/2014). All *in vivo* experimental procedures were performed under sterile conditions at the Animal Experimental Unit, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Animals were humanely handled and euthanized at stipulated dates (1, 3, 7, 14, 21 and 28 days) during the experimental period, using a CO_2 chamber after anesthesia with Ketamine + Xylazine (50 mg/kg+5 mg/kg). The euthanasia protocol used was approved by the institutional Animal Care and Use Committee, Universiti Putra Malaysia. As it was a time series experiment, a standard endpoint strategy was put in place to euthanize the animals in case of any undue suffering or development of severe clinical signs. However, the animals did not exhibit any adverse clinical signs or symptoms that warranted the initiation of the endpoints. Hence, the experiment was completed and the animals were euthanized only on the stipulated dates stated earlier.

2.4. Viral propagation and purification

Viral stock culture (Genbank Accession no KU198322.1; Reptarenavirus isolate UPM/MY01) derived from the kidneys of a Boa constrictor with BIBD was propagated in confluent Vero cell line until the development of cytopathic effect (CPE). Infected flasks were then withdrawn, freeze-thawed and passaged onto new monolayer cells for four subsequent passages (P1-P4). The infected cell culture homogenate was centrifuged at 6000 rpm for 30 min at 4 °C (Beckman J2-21M, USA). The supernatant was collected in fresh 50 mL tubes and centrifuged at 20,000 RPM, 4 °C for 2½ hours (Beckman Coulter, Avanti J-26S XPi, USA). The resulting pellet was collected, re-suspended in PBS and layered on a sucrose gradient made up of 10%–50% sucrose concentration and centrifuged using an ultra-centrifuge at 40,000 RPM, 4 °C for 2½ hours (Beckman Coulter, Optima XPN-100, USA). Resulting bands were carefully aspirated, transferred into a new tube and dissolved in PBS. The solution was further centrifuged at 40,000 RPM, 4 °C for 4 h (Beckman Coulter, Optima XPN-100, USA), and the resultant virus pellet was collected, re-suspended in PBS and kept at - 70° C until further use [17].

2.5. Evaluation of $TCID_{50}$/mL and pfu/mL

The purified reptarenavirus isolate was titrated using methods previously described through an end point dilution assay [21]. Vero cells were cultured using RPMI media in 24-well plates until 70–80% confluent. Virus stock (KU198322.1; Reptarenavirus isolate