Determination of lipid peroxidation biomarkers in Vero cell line inoculated with Bovine Ephemeral Fever Virus

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Abstract

Avci O, Yavru S, Dik I. Determination of lipid peroxidation biomarkers in Vero cell line inoculated with Bovine Ephemeral Fever Virus.

Aim: The aim of the present study was to determine of lipid peroxidation biomarkers in Vero cell line inoculated with Bovine Ephemeral Fever Virus (BEFV, Genbank No: GQ229452.1).

Materials and Methods: Cell supernatants were collected 4 h/day for 5 days after BEFV inoculation. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) enzymes, glutathione (GSH) and malondialdehyde (MDA) values were analyzed from the test media by commercially available ELISA kits. In addition to this, cytopathogenic effects (CPE) of BEFV in cell culture were evaluated periodically by invert microscope.

Results: In this research, CPE of BEFV was observed at 72 h post-inoculation. Maximum level of SOD was determined at 56 h, while minimum levels of CAT and GPX were determined at 8 and 104 hours, respectively. Maximum GSH levels were determined at 30, 60, 84 and 120 hours while minimum GSH concentrations were measured at 44, 92 and 112 hours. A sudden decrease of MDA level was observed in the first 8 hours occurred. In addition, CAT, MDA and SOD düzeylerinin BEFV’ın neden olduğu CPE oluşumundan önce azaldığı tespit edildi.

Conclusion: It is concluded that lipid peroxidation biomarkers can be useful in the pathogenesis of BEFV. It may prove helpful in the design of future protect from decreasing of oxidative damage associated with BEFV infection.

Keywords: BEFV, SOD, CAT, GPX, MDA
**Introduction**

Bovine Ephemeral Fever (BEF), caused by Bovine Ephemeral Fever Virus (BEFV, order Mononegavirales, family Rhabdoviridae, genus Ephemerovirus), is a viral vector borne disease (Murray 1997), and virus causes acute infection, suddenly fever, anorexia, depression (Wang et al 2001), noncontagious, that affects both cattle and buffalo (Uren et al 1992). BEFV has a negative sense RNA five structural proteins (G, N, P, M, L), and propagated in one-day old baby mice after intracerebral inoculation. Although bovine kidney, hamster lung, Aedes albopictus (St. George 1985), Baby Hamster Kidney-21 (Nandi and Negi 1999) and Vero (Yeruham et al 2010) cell cultures can be preferred for virus propagation in vitro.

Oxidative stress can be determinate in all cells during physiologic events for vitality or harbinger of physiopathology damage. Unpaired electron in atomic or molecular structure is called free radical. Free oxygen radicals on biological systems firstly reported by Gershman et al. in 1954 (Turrens 1991). Oxidant structures are produced during various metabolic events in organism. Autoxidation of polyunsaturated fatty acids (PUFAs) has been realizable with oxygen. Malondialdehyde (MDA) is formed as a result of lipid peroxidation. MDA, is a dialdehyde, contains 3-carbon and carbonyl group at C1 and C3. It can be learned the severity of lipid peroxidation by measurement of MDA levels (Fernandez et al 1997, Aydin et al 2009, Aydin et al 2010). Infectious diseases are reported to be sources of free oxygen radicals (Maksimenko and Vavaev 2012). Oxidative stress in viral infections was reported in the studies (Raju et al 2000, Crist et al 2013, Yahya et al 2013, Fraisier et al 2014).

It was hypothesized that oxidative stress and free radicals can be formed in permanent Vero cell which inoculated with BEFV. The purpose of the present study was to determine and compare the lipid peroxidation markers in Vero cell inoculated with BEFV.

**Material and Methods**

**Cell culture and virus**

Vero cell line and BEFV (Genbank No: GQ229452.1) were supplied from Virology Department, Faculty of Veterinary, University of Selcuk. Cells were cultivated in 25 cm² flasks (Corning, USA) with Dulbecco’s Modified Eagle Medium (DMEM, Biological Industries, Israel) supplemented with 5% heat-inactivated fetal calf serum (FCS, Biological Industries, Israel), and 1% antibiotics (10000 IU/mL penicillin G, 10 mg/
mL streptomycin, and 25µ/mL amphotericin B; Biological Industries, Israel) at 37°C and 5% CO2 incubator. A total of 30 flasks were used during experiment. After 100 TCID50 (0.5 mL) of the BEFV was inoculated by non-adsorption method medium were collected at 4 h intervals for 5 days. The supernatants were centrifuged for 10 min at 5000 g (+4°C) and stored at -80°C until use.

**ELISA Analyses**

Levels of SOD (Cayman, USA), CAT (Cayman, USA), GPX (Cayman, USA), GSH (OxisResearch, USA) and MDA (OxisResearch, USA) in the supernatants of the Vero cell cultures were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits. The tests were performed as per the manufacturer’s instructions. The plates were then read on an automatic micro plate reader (Rayto RT 2100C, China). This study protocol has been approved by ethics committee of Veterinary Medicine of Selcuk University (Report No: 2014/02).

**Results**

Cytopathogenic effects of BEFV on Vero cell line after 72 and 120 h post-inoculation were shown in Figure 1 and 2, respectively. SOD, CAT, GPX, GSH and MDA concentrations of BEFV were shown in Graphics 1, 2, 3, 4 and 5, respectively.

**Discussion**

It has been recognized that oxidative stress plays an important role in diverse viral infections (Valyi-Nagy and Dermody 2005, Hahn et al 2008, Williams et al 2010). In the current research, BEFV changed oxidative status of Vero cell line. BEFV caused fluctuation in the enzymatic antioxidants including SOD (Graphic 1), CAT (Graphic 2) and GPX (Graphic 3) activities and non-enzymatic antioxidant GSH (Graphic 4) level. In the experimentally induced infectious studies, fluctuations of antioxidant enzymes were reported when these enzymes were measured from tissues (Yazar and Tras 2001). In this study, maximum level of SOD was measured at 56 h, whereas minimum levels of CAT and GPX were determined at 8 and 104 h, respectively. Maximum GSH concentrations were measured at 30, 60, 84 and 120 h, while minimum concentrations were determined at 44, 92 and 112 h, respectively. In addition, CAT and SOD activities decreased before developing CPE. These results may be reflect that BEFV change oxidative status in Vero cell line in vitro, and BEFV firstly effect CAT and SOD activities. It has been shown that oxidative damage is related with acute encephalitis in mice experimental infected with herpes simplex virus type 1 (Schachtele et al 2010). Also it has also been reported that oxidative injury can be occur in rabies by Jackson et al. (2011). It is known that different viral proteins may target cell mitochondria and impair mitochondrial morphology (Lichty et al 2006). Oxidative stress is strongly induced by stimulation of the mitochondrial Ca2+ in hepatitis C infection (Li et al 2007). The significantly reduced (P<0.01) level of Ca, inorganic phosphor, and total protein in blood sera of cattle infected with BEFV reported by Sahin et al (2004).
Interestingly, MDA levels, which is accepted oxidative stress biomarker, dramatically decreased at 8 hour after BEFV inoculation (Graphic 5). It has been reported that decreased MDA level may be measured when samples contain insufficient lipid substrate (Shukla et al 1997). It has been speculated that Vero cell line may has lower lipid substrate in the current research.

Conclusion

It has been concluded that SOD, CAT, GPX and MDA can be used as a novel early lipid peroxidation biomarkers of oxidative stress in Vero cell inoculated with BEFV. Antioxidant supplementations may be useful for decreasing of oxidative damage caused by BEFV in vitro studies.

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References


