Differences Expression of Caspase-3 In Hepar and Spleen of Rattus norvegicus Infected with Methicillin Resistant Staphylococcus aureus and Enterococcus faecalis

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Abstract: Bacteria are organisms that can cause infection. Methicillin Resistant Staphylococcus aureus (MRSA) and Enterococcus faecalis are gram-positive bacteria that can cause nosocomial infections. Virulence factors of MRSA thus play a role in the infection process, such as are polysaccharide, surface proteins such as adhesins, glycoprotein, hemagglutinin, and fibronectin, while enterococcus such as gelatinase, cytolysin, enterococcal surface protein (Esp) and aggregation substance (AS) are the virulence factors of Enterococcus that can cause infection. This research is True Experimental research with post-test only for the Control Group Design. The MRSA and E. faecalis bacteria were injected intraperitoneally to R. norvegicus and observed after 24 hours. Animals were placed into three groups: the control group, treatment with MRSA, and Enterococcus faecalis. The hepar and spleen were isolated from the dead R. norvegicus and a Immunohistochemistry (IHC) test was conducted to observe the expression of caspase-3 by light microscope. The result showed that the caspase-3 expression increased in the infected group of MRSA and Enterococcus faecalis compared with the control group. The expression of caspase-3 in the hepar was higher than in the spleen. The hepar serves as the receiver of the portal and systemic circulation. The hepar also plays an important role in the host defense that is exposed to and will catch pathogens, followed by cleaning. The increased expression of caspase-3 suggests the cell death also increased.

1 INTRODUCTION

Infectious diseases still occupy the top causes of morbidity and mortality in developing countries (Triana, 2014). Bacteria is one of the organisms that can cause infectious diseases (Kaufmann et al., 2011). The spread of diseases is caused by various intermediaries, including air, animals, objects, humans themselves, and even unconsciously, the hospital becomes a high-risk source of transmission (Triana, 2014). These bacteria include MRSA (Sandi et al., 2015) and Enterococcus faecalis (Chen and Zervos, 2009).

Increased incidence of the S. aureus infection, especially MRSA with the phenomenon of antibiotic resistance, is considered as one of the biggest barriers to infection control. MRSA is a bacteria that causes nosocomial infection (Sandi et al., 2015) and Enterococcus faecalis (Tortora, 2016). Enterococcus faecalis is a commonly found species of Enterococcus (Chen and Zervos, 2009). MRSA and Enterococcus faecalis are gram-positive bacteria that can cause infections involving the death of cells (Chen and Zervos, 2009; Sandi et al., 2015).

Apoptosis is the programming of cell death used to prevent inflammation and limit cell damage (Martinez, 2017). Increased toxins, produced by bacteria, will increase apoptosis (Baudouin, 2008). Apoptosis is also used for homeostasis and protection against bacterial infections (Upton and Chan, 2014).

The hepar is an organ that plays an important role in the defense of the host against the invasion of microorganisms (Talwani et al., 2013), while the spleen is the main filter for pathogens and antigens carried by blood. The spleen is an area of regulation host immune response to initiate innate and adaptive immune responses to pathogens and the establishment of specific antigens in immune
responses that protect bacterial, viral, and blood-borne fungal infections (Bronte and Pijtet, 2013).

Caspase is the leading cause of cell death by dividing cell proteins to disassemble cells that will die. In the arrangement, it is important that caspase be maintained to avoid unexpected cell death (Parrish et al., 2013). Caspase-3 is a member of the caspase family that plays a role in the execution phase in the apoptosis (Prakosa et al., 2013). Caspase-3 is also the best effector caspase and an apoptotic signal, if there is activation of caspase-3, there is no cell rescue (Parrish et al., 2013).

2 MATERIALS AND METHOD

2.1 Animal model

The animal model used in the research was the Rattus norvegicus with the criteria of being male, healthy, age three months, and a body weight of 150–200 grams.

2.2 Bacteria

The bacteria of MRSA and Enterococcus faecalis were obtained from the Installation of Clinical Microbiology RSUD Dr. Soetomo Surabaya. The concentration was $10^5$ CFU bacteria with Phosphate-buffer saline (PBS).

2.3 Sample

The animal models were placed in three groups, each consisting of four rats. The groups were infected with MRSA and Enterococcus faecalis, as a control were given PZ (1 ml in the peritoneum of each rat; the concentration was $10^5$ CFU bacteria with PBS). Observation was performed for 24 hours post infection. Rats were sacrificed for removal of the hepars and spleens. Organs were fixed in formalin buffer and preparation (formalin fixed and paraffin embedded section were performed) and Immunohistochemistry for Caspase-3 using a rabbit and mouse antibody. The expression cells were observed under a light microscope with an objective 100x in a five field of view. The analysis of data using was carried out using GraPhad prism.

3 RESULTS

3.1 Expression of Caspase-3 in Hepar

Of the mean number of the cells expressed in the caspase-3 in hepar, the group infected with MRSA were higher than the group infected with Enterococcus faecalis (Figure 1).

The highest expression of caspase-3 that infected was the MRSA group (96.75%), than the Enterococcus faecalis (84.25%), and finally the control group (9.5%) (Figure 1).

Figure 1: Bar-graph Expression of Caspase-3 in hepar.

Figure 2: Expression of caspase-3 in hepar, (a) control group of R. norvegicus, (b) Enterococcus faecalis group, (c) MRSA group. Magnification x1000.
3.2 Expression of caspase-3 in the Spleen

The mean number of cells was expressed by caspase-3 in the spleen; the group infected with MRSA were higher than the group infected with Enterococcus faecalis (Figure 3).

![Expression of Caspase-3 (%)](image)

Figure 3: Bar-graph Expression of Caspase-3 in the Spleen.

The highest expression of caspase-3 was MRSA (19.25%) then Enterococcus faecalis (11.5%), and then the control group (4.25%) (Figure 4).

![Expression of caspase-3 in the spleen](image)

Figure 4: Expression of caspase-3 in the spleen, (a) control group of R. norvegicus, (b) Enterococcus faecalis group, (c) MRSA group. Magnification x1000.

4 DISCUSSION

Apoptosis plays a major role in removing infected cells, mutating (or causing damage during development), tissue homeostasis, and the effects of aging. Apoptosis is associated with morphological and biochemical changes, including the release of cytochrome c, the activation of protease caspase, chromatin condensation, and DNA fragmentation (Jafari et al., 2015).

The virulence of MRSA plays a role in an infection, such as polysaccharides and surface proteins (Gordon and Lowy, 2008). The surface protein is responsible for colonization. Polysaccharides and protein-A are inhibit phagocytosis by polymorphonuclear leukocytes. The enzyme catalase is also a factor that supports the bacteria for survival inside phagocytic cells (Sandi et al., 2015). Bacteria produces various virulence factors that allows them to escape from the body’s immunity, infecting and spreading to remote organs (Ramachandran, 2014).

The virulence of Enterococcus faecalis causes infections such as gelatinase, enterococcal surface protein (Esp), aggregation substance (AS) and cytolysine (Uysal, 2013). Cytolysine can lyse various target cells; it can lyse erythrocyte cells, polymorphonuclear neutrophils, and macrophages. Cytolysine may also increase toxicity and bacteriocin activity for colonization. Other factors, such as gelatinase may help bacteria to avoid the immune system (Tyne et al., 2013). Aggregation substance promotes direct binding, independent opsonin to polymorphonuclear leukocyte can survive in different phagocytes. Enterococcal surface proteins with multiple repetitive motives in the encoding gene may be important in immune avoidance. Products that cause direct tissue damage are cytolysine and gelatinase (Waar, 2004).

Apoptosis can within or outside the host cell because of stimulation of microbial infections, oxidative stress, DNA damage or DNA errors or when the cells have reached the final cycle. Started enzymes form a cascade signal, send a danger signal through the caspase initiator and continue to capase-3 as the executor. The enzyme initiates cell disassembly by degrading the DNA. The toxins of bacteria cause inflammation and increased caspase-3 and apoptosis (Wall and Beth, 2014). The molecular strategies by bacteria to interact with the host can be unique to a particular pathogen (Wilson et al., 2002).

Hepar is the portal defense for bloodstream infections. Hepar contains immune cells to bind and clean pathogens (Talwani et al., 2013). The spleen is...
the filter for pathogens and the antigen carried in blood. Establishment of the immune system to specific antigens from bacterial, viral, and fungal infections. The spleen uses the regulation of immune response (Bronte and Pijttet, 2013) and filters to remove blood cells due to aging or pathological changes (Pivkin et al., 2016).

MRSA and Enterococcus are bacteria that can cause tissue damage especially for hepar. During infection in the bloodstream, the majority of bacteria are sequestered immediately by Kupffer cells in the hepar and increased enzymes in the hepar (Kolaczkowska et al., 2015). Abnormal enzyme levels may indicate hepar damage (Giannini et al., 2005). Response hepar damage to infection is occurs increased caspase-3 and apoptosis (Kolaczkowska et al., 2015).

5 CONCLUSIONS

In conclusion, there was an increase of expression caspase-3 in the hepar and spleens of R. norvegicus when the group infected with MRSA was higher than that infected with Enterococcus faecalis. Increased expression of caspase-3 suggests the cell death also increased.

ETHICS APPROVAL

All documents for ethics approval as well as proposal of the research have been reviewed by the ethics committee of Universitas Airlangga Faculty of Dental Medicine, as described on the ethical approval No. 265/HRECC.FODM/X/2017.

REFERENCES


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