Research Journal of Allergy and Immunology

Protozoan Resistance Ability against Detoxification and Oxidative Stress.

Aydin H[1], Celik VK[1], Tekin YK[2*], Kapancik S[1], Eren SH[3], Korkmaz I[2]

[1]Department of Biochemistry, Faculty of Medicine, Cumhuriyet University, Sivas, Turkey

[2]Department of Emergency Medicine, Faculty of Medicine, Cumhuriyet University, Sivas, Turkey

[3]Department of Emergency Medicine, Faculty of Gaziantep University, Medicine, Gaziantep, Turkey

Abstract

In this study, we aimed to show the resistance reflex of protozoa by examining enzyme levels and capacities of oxidants/antioxidants engaged in detoxification of drugs. All strains were sonicated in ice bath twice with 15 second intervals, 1/1 ratio of 50 mm Tris/H_2O_2 buffer was added after fractionation and then the mixture was centrifuged at 15000 g for 10 min, and supernatant portion collected. The levels of malondialdehyde (MDA), catalase (CAT), glutathione S-transferase (GST) and paraoxonase 1 (PON1) and total antioxidant capacity (TAS) were measured from the supernatants obtained. The levels of MDA, CAT, GST, PON1 and TAS were found as 23.3 mm, 0.47 U/ml, 0.182 mol/L, 5.7 mmol/L and 0.562 mm in 11PS strain; 26.0 mm, 0.69 U/ml, 0.702 mol/L, 2.46 mmol/L and TAS: 0.521 mm in PAT strain; 39.5 mm, 0.72 U/ml, GST: 0.382 mol/L, PON1: 6.78 mmol/L and 0.4661 mm in 2 HH strain and 1.166 mm, 0.15 U/ml, 0.185 mol/L, 1.523 mol/L and 0.236 mm in *A. castellanii* strain, respectively. It is obvious that protozoan has defence systems against the drugs used in medical treatment and against oxidative stress. It is thought considering their defence systems against pathogenic protozoan that, they will be more successful in struggle against pathogens.

Keywords: *Acanthamoeba castellanii*, *Acanthamoeba keratitis*, Malondialdehyde (MDA), Total antioxidant capacity (TAS), Catalase (CAT), Paraoxonase-1 (PON1), Glutathione S transferase (GST).

Accepted on December 13, 2016

Introduction

Acanthamoeba castellanii and *Acanthamoeba keratitis* are amoeba species living freely in the nature and cause especially keratitis. They have been found to cause infections for the first time in 1970s [1]. As protozoans live freely in environments such as the nature, soil, spring water, air and swimming pools; the risk of their transmission to humans is very high. A. keratitis is usually caused by the use of contact lenses due to the reaction with contaminated environmental factors. It is reported that Acenthamoeba infections gain importance as the number of immunocompromised patients using soft contact lens increases [1-3].

Some of these free-living protozoans can settle in central nervous system in humans, causing severe diseases such as primary amoebic meningo-encephalitis or granulomatous amoebic encephalitis and besides brain and eye, they can localized in the skin, bones, lungs, urogenital system and adrenal glands [1,4-9]. Also it has been reported in the studies that *Acanthamoeba* species play a role as a host for pathogenic viruses, bacteria and fungi [10]. There are still unclear parts in many studies conducted for understanding the pathogenesis and pathophysiology of *Acanthamoeba* infections. For this reason; further studies are required to understand and reveal pathogenic features of protozoa parasites.

In healthy individuals, oxidant levels are stabilized by anti - oxidants. These anti-oxidant molecules prevent the damage caused by free radicals to the target biomolecules such as lipids, proteins and nucleic acids [11]. CAT catalyses the transformation of H_2O_2 to water and oxygen and protects the cell against the respiration explosions [12,13]. The oxygen analogs are hydrolyzed by the serum Arilesterase, paraoxonase 1 (PON1), which appears to play a central role in their detoxification and toxicity [14,15].

Reactive Oxygen Species (ROS) are highly toxic for organisms. ROS and free radicals play an important role in many physiologic and pathologic processes. Organisms are protected against hazardous effects of endogenous ROSs by enzymatic and non-enzymatic antioxidant enzyme systems. ROSs are mainly naturalized by anti-oxidant enzymes such as superoxide dismutase (SOD), and catalase (CAT) [16]. The most affected molecules from free radicals are biomolecule lipids. Some ROSs cause lipid peroxidation in the cytoplasm, mitochondria, cell nucleus and endoplasmic reticulum membranes; which in turn results in increased membrane permeability and cellular damage. Lipid peroxidation causes

irreversible membrane damage [17,18]. 3D structure of albumin and IgG can be destroyed. Cells and mitochondria DNA can be oxidized, causing damage in cellular life [19]. As the half-time of ROS is short, it is difficult to determine the concentrations in the body. Therefore, enzymes related to free radicals such as SOD, CAT, GST and paraoxonase can be obtained by determination of MDA which is a biomarker of lipid peroxidation.

The Glutathione S-transferase (GST) (GST; E.C. 2.5.18) isoenzymes involved in the cellular detoxification of both xenobiotic and endobiotic compounds are ubiquitously distributed in nature, ranging from microbes to insects, plants, fish, birds and mammals [20]. Glutathione S-Transferases that have multifunctional and wide spectrum substrate specificity plays a defense role in the organisms, which are exposed to potentially toxic chemicals. GST detoxification fulfills its role by neutralizing electrophilic zones of the compounds related with -SH group of the reduced glutathione (GSH) [21,22].

It is known that protozoans such as *Acanthamoeba keratitis* and *A. castellanii* mediate the formation of diseases and variety of bacterial infections in human -beings. Many drugs are used in the fight against these kinds of protozoans.

The objective of this study was to investigate the level of enzymes, which are involved in detoxification and capacity of oxidant/antioxidants in protozoa, in order to increase the effectiveness of these type of drugs.

Material and Methods

Cell Fractionation: All strains were sonicated in ice bath twice with 15 second intervals, 1/1 ratio of 50 mM Tris/H_2O_2 buffer was added after fractionation and then the mixture was centrifuged at 15000 g for 10 min, and supernatant portion was collected.

Enzyme activity measurement: GST activities were spectrophotometrically measured at 340 nm according to the method defined by Habig et al. [23]. The principle of the experiment is based on monitoring the formation of thioeter link between GSH and 1-chloro-2,4-dinitrobenzene (CDNB) in a spectrophotometer at 340 nm. One unit of enzyme activity has been calculated as the enzyme activity which catalyzes the formation of 1 mol product in one minute under 30°C and it has been defined as mol/L.

Catalase activity was determined by measuring the amount of H_2O_2 reduced at 240 nm wavelength according to the method defined by Beers and Sizer [24].

PON1 activities were measured using a the commercial kit [25] Syncron LX autoanalyzer and antioxidant activity (TAS) was spectrophotometrically identified at 532 nm according to the method for the measurement of antioxidant activity in human fluids which has been described by Koraceviv et al. [26]. Total antioxidant activity is expressed as mmol/L. MDA was spectrophotometrically measured according to the thiobarbituric acid (TBA) method [27].

Results

MDA level, CAT, GST, PON1 enzyme activities and TAS levels were measured as the end product of lipid peroxidation of three strains in *Acanthamoeba keratitis* (11PS, PAT and 2HH) and *A. castallanii* homogenates. These values were measured as follows:

11PS strain; MDA: 23.3 µmol/L, CAT: 0.47 U/ml, GST: 0.182 µmol/L, PON1: 5.7 µmol/L ve TAS: 0.562mmol/L.

PAT strain; MDA: 26.0 µmol/L, CAT: 0.69 U/ml, GST: 0.702 µmol/L, PON1: 2.46 µmol/L ve TAS: 0.521 mmol/L.

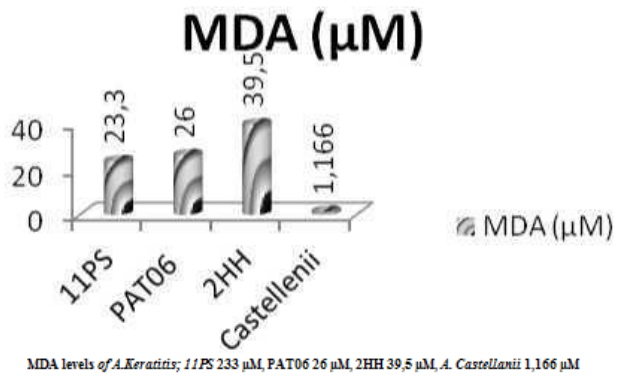
2HH strain; MDA: 39.5 µmol/L, CAT: 0.72 U/ml, GST: 0.382 µmol/L, PON1: 6.78 µmol/L ve TAS: 0.4661 mmol/L.

A. castellanii strain; MDA:1.166 µmol/L, CAT:0.15 U/ml, GST:0.185 µmol/L, PON1: 1.523 µmol/L ve TAS: 0.236 mmol/L.

Discussion

Organisms have a variety of defence systems in order to survive against adverse conditions such as oxidative damage and external chemicals (e.g. fungicide, insecticide and drugs). Besides the genetic defects in many diseases that threaten human health; there are many other microorganisms involved such as infection causing bacteria, viruses and fungi. Effects of the drugs are reduced or eliminated as the microorganisms develop resistance to many drugs used to fight against these kinds of organisms. Understanding the defense systems of the microorganisms used against oxidative damage and the drugs sustaining their lives has gained importance for development of new generation medications against these organisms. It is known that, *Acanthamoeba* species are the most important cause of microbial keratitis that causes numerous ocular inflammations and loss of vision [28]. In addition, as they serve as a host for microorganisms such as pathogenic viruses, bacteria and fungi; these species form a basis for many different diseases together with keratitis [29-31]. Even though, information related to the pathogenesis and pathophysiology of the infections that are caused directly or indirectly by these protozoa and strains, is not clear. In this study; we examined the biochemical parameters involved in A keratitis (11PS, PAT06 and 2HH) and *A. castellanii* oxidant/antioxidant (MDA, TAS, CAT), and PON1 and detoxification (such as GST and PON1).

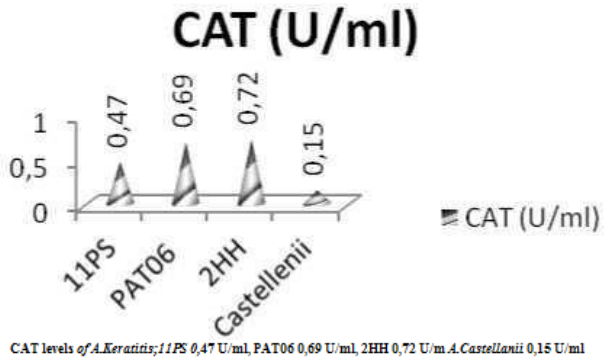
When the biochemical parameters of A. keratitis (11PS, PAT06 and 2HH) and *A. castellanii* were compared; it was found that the lowest values belong to *A. castellanii* (Figure 1).



MDA levels *of A.Keratitis; 11PS* 233 µM, PAT06 26 µM, 2HH 39,5 µM, *A. Castellanii* 1,166 µM

Figure 1. *MDA Levels in Akantomoeba sp.*

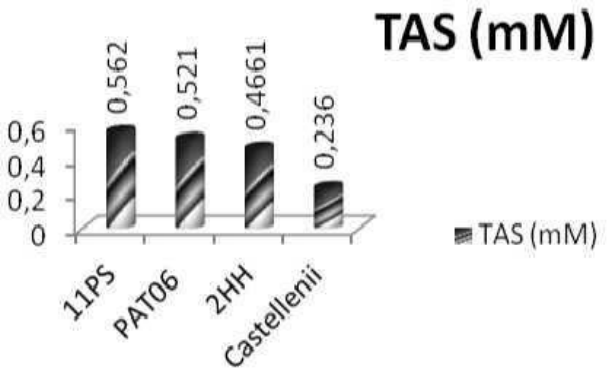
When A. keratitis strains were compared, the highest values of MDA and PON1 level was seen in 2HH strain; the lowest value of MDA was seen in 11PS strain and the lowest value of PON1 was seen in PAT06 (Figures 2-5).



CAT levels *of A.Keratitis;11PS 0,47* U/ml, PAT06 0,69 U/ml, 2HH 0,72 U/m *A.Castellanii* 0,15 U/ml

Figure 2. *CAT Levels in Akantomoeba sp. Homogenates*

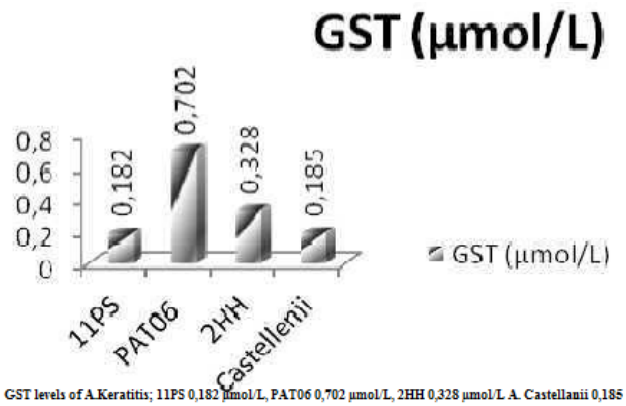
The highest value of TAS level was found in 11PS strain and the lowest was found in 2 HH strain (Figure 2).



TAS levels of A.Keratitis; 11PS 0,562 mM, PAT06 0,521 mM, 2HH 0,466 mM, A. Castellanii 0,236 mM

Figure 3. *TAS Levels in Akantomoeba sp. Homogenates*

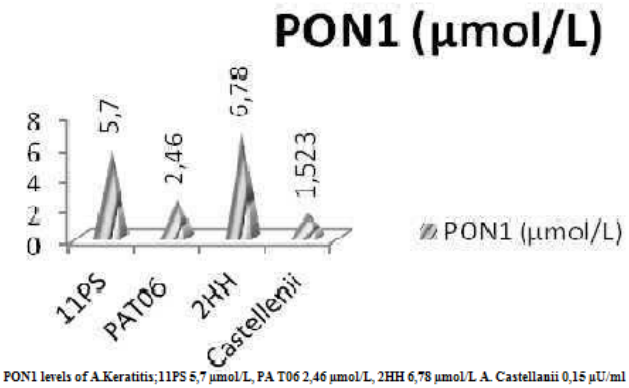
The highest value of CAT level was seen in 2HH strain and the lowest was seen in 11PS strain (Figure 4).



GST levels of A.Keratitis; 11PS 0,182 µmol/L, PAT06 0,702 µmol/L, 2HH 0,328 µmol/L A. Castellanii 0,185 µmol/L

Figure 4. *GST. Levels in Akantomoeba sp. Homogenates*

The highest value of GST activity was found in PAT06 and lowest in 11PS strain. According to our findings, the highest values of MDA, CAT and PON1 levels were seen in 2HH strain (Figure 5).



PON1 levels of A.Keratitis;11PS 5,7 µmol/L, PA T06 2,46 µmol/L, 2HH 6,78 µmol/L A. Castellanii 0,15 µU/ml

Figure 5. *PON1 levels in Akantomoeba sp. homogenates*

In living organisms; there is a balance between oxidants and antioxidants. Many macromolecules expose to oxidative damage (such as lipid peroxidation in the event of disruption of this balance). High MDA, but low TAS level in 2 HH strain indicates a disruption of this balance and thus, lipid peroxidation is seen. The reason for the increase in the level of PON1 is the increased lipid peroxidation. The increase in CAT activity may develop due to oxidative stress. These results show that 2HH strain is exposed to oxidative stress and that the current antioxidant systems are inadequate. We could not compared our findings with other studies as there is no a study in the literature about these parameters of the strains of interest. According to the levels of biochemical parameters (MDA, TAS, CAT, GST and PON1), the groups involved in A. keratitis oxidant/antioxidant strains were respectively found as follows: 2HH>PAT06>11PS according to MDA level, PAT06>2HH>11PTS according to PON1, GST activity level, 2HH>PAT06>11PTS according to CAT activity, 11PTS> PAT06> 2HH according to TAS level.

Recent studies have found a significant correlation between the use of contact lens and pathogenesis of keratitis [3,32]. Some of the solutions used as contact lens disinfectants includes

hydrogen peroxide (H_2O_2) [33,34]. Efficacy of contact lens care solutions in killing *Acanthamoeba castellanii* were demonstrated by in vitro testing and live-imaging [35]. There are two opposite opinions proposed for the effect of H_2O_2 on *A. keratitis*. According to a study by Kilvington and Anger [35] H_2O_2 is effective on *A. keratitis* as a disinfectant, while Hiti et al. [34] reported that it is not effective. However, our findings indicate that, the protozoa species causing keratitis has antioxidant systems (CAT, PON1, GST and TAS). Therefore, we suggest that antioxidants of these protozoa species reduce oxidative effect of H_2O_2 and thus, H_2O_2 included in the content of contact lens solution is not a good disinfectant. Our findings support the study of Hiti et al. [34] which suggests that H_2O_2 has not sufficient effect on *Acanthamoeba* cysts.

There are several studies about the oxidants/antioxidants and detoxification enzymes in highly structured organisms. It has proven in the studies that these enzymes play an important role in pathophysiology/pathogenesis of many diseases. In our study, we observed that protozoans have oxidant/antioxidant and detoxification enzymes.

Conclusion

Results of our study obviously indicated that, the protozoans have defense systems against the drugs used in medical treatment and also against oxidative stress. It is thought considering their defence systems against pathogenic protozoan that, they will be more successful in struggle against pathogens. More comprehensive studies for understanding the biochemical features of protozoan will meet the deficits in this area.

References

1. Graffi S, Peretz A, Jabaly H, et al. Acanthamoeba keratitis. Isr Med Assoc J 2013;15:182-5.
2. Marciano-Cabral F, Cabral G. Acanthamoeba spp. as agents of disease in humans. Clin Microbiol Rev 2003;16:273-307.
3. Lorenzo-Morales J, Martin-Navarro CM, Lopez-Arencibia A, et al. Acanthamoeba keratitis: an emerging disease gathering importance worldwide?. Trends Parasitol 2013;29:181-7.
4. Saygı G, Polat Z. Free Living Amoebae and Parasitosis caused by them (Primary Amoebic Meningoencephalitis - Granulomatous Amoebic Encephalitis - Keratitis) C U. Facult Med J 2003;140-9.
5. Galarza M, Cuccia V, Sosa FP, et al. Pediatric granulomatous cerebral amebiasis: A delayed diagnosis. Pediatr Neurol 2002;26: 153-56.
6. Healy JF. Balamuthia amebic encephalitis: Radiographic and pathologic findings. Am J Neuroradiology 2002;486-9.
7. Singh B, Fleury C, Jalalvand F, et al. Human pathogens utilize host extracellular matrix proteins laminin and collagen for adhesion and invasion of the host. FEMS Microbiol Rev 2012;36:1122-80.
8. Patel A, Hammersmith K. Contact lens-related microbial keratitis: recent outbreaks. Curr Opin Ophthalmol 2008; 19:302-06.
9. Massoni F, Giorgi D A, Palmieri S, et al. Prevalence of legionella in hospital water systems: medicolegal and occupational considerations. Acta Medica Mediterranea 2013;527.
10. Siddiqui R, Khan NA. War of the microbial worlds: who is the beneficiary in Acanthamoeba-bacterial interactions? Exp Parasitol 2012;130:311-13.
11. Yazici C, Köse K. Melatonin: Antioxidant power of dakness [in Turkish]. Erciyes Üniversitesi Sağlık Bilimleri Dergisi (E.ÜJournal of Health Sciences) 2004;56-65.
12. Chelikani P, Ramana T, Radhakrishnan TM. Catalase: A repertoire of unusual features. Indian J Clin Biochem 2005;131-35.
13. Çimen C, Oter C, Demir H, et al. Investigation of characterization and kinetic of catalese enzyme to obtain from erythrocytes rat [in Turkish]. YYU Vet. Fak. Derg 2005;15-20.
14. Costa LG, Li WF, Richter RJ, et al. The role of PON1 in the detoxication of organophosphates and its human polymorphism. Chem Biol Interact 1999;429- 38.
15. Akgur SA, Ozturk P, Solak I, et al. Human serum paraoxonase (PON1) activity in acute organophosphorous insecticide poisoning. Forensic Sci Int 2003;136-40.
16. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, orconsequence? Lancet 1994;344: 721-4.
17. Kiselyov K, Muallem S. ROS and intracellular ion channels. Cell Calcium. 2016;60:108-14.
18. Santandreu FM, Valle A, Oliver J, et al. Resveratrol potentiates the cytotoxic oxidative stress induced by chemotherapy in human colon cancer cells. Cell Physiol Biochem 2011;28: 219-28.
19. Smith C, Marks AD, Lieberman M. Marks' Basic Medical Biochemistry: A Clinical Approach. Lippincott Williams and Wilkins 2004;439-57.
20. Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol. 1995;30:445-600.
21. William BJ. Glutathione-s-transferases; An overiew. enzymatic basis of detoxication, New York. 1 and 2 Acad. Press 1980.
22. Thomas DB. The Glutathione-s-transferases; An update. Hepatology 1989;9:486-96.
23. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130-9.
24. Beers RF, Sızer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 1952;195:133-40.
25. Furlong CE, Suzuki SM, Stevens RC et al. Human PON1, a biomarker of risk of disease and exposure. Chem Biol Interact 2010;187:355-61.

26. Koracevic D, Koracevic G, Djordjevic V, et al. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol 2001;54:356-61.
27. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 1979;95:351-8.
28. Alkharashi M, Lindsley K, Law HA, et al. Medical interventions for acanthamoeba keratitis. Cochrane Database Syst Rev 2015.
29. Alsam S, Jeong SR, Sissons J, et al. Escherichia coli interactions with Acanthamoeba: a symbiosis with environmental and clinical implications. J Med Microbio 2006;55: 689-94.
30. Willcox MD. Microbial adhesion to silicone hydrogel lenses. Eye Contact Lens, 2013;39:61-6.
31. Scheid P, Schwarzenberger R. Acanthamoeba spp. as vehicle and reservoir of adenoviruses. Parasitol Res 2012;111:479-85.
32. Kuzman T, Kutija MB, Juri J, et al. Lens wearers non-compliance-Is there an association with lens case contamination? Cont Lens Anterior Eye, 2014;37:99-105.
33. Kilvington S, Anger C. A comparison of cyst age and assay method of the efficacy of contact lens disinfectants against Acanthamoeba. Br J Ophthalmol 2001; 85:336-40.
34. Hiti K, Walochnik J, Faschinger C, et al. One-and two-step hydrogen peroxide contact lens disinfection solutions against Acanthamoeba: How effective are they? Npg, 2005;19:1301-05.
35. Kolar SS, Manarang JC, Burns AR, et al. Cont Lens Anterior Eye. 2015;38:442-50.

*Correspondence to

Yusuf Kenan Tekin

Departments of Emergency Medicine

Cumhuriyet University

Sivas, Turkey