KnE Life Sciences

DonAgro
International research conference on Challenges and Advances in Farming, Food Manufacturing, Agricultural Research and Education
Volume 2021

Knowledge E
enriching | engaging | empowering

Conference Paper

Modification of the Method for Determining Myeloperoxidase in Blood Neutrophils

Tatyana R. Korablyeva[1], Ivan V. Senchuk[1], and Elizaveta E. Ageeva[2]

[1]Academy of Life and Environmental Sciences of V.I. Vernadsky Crimean Federation University
[2]Medical Academy named after S.I. Georgievsky of V.I. Vernadsky Crimean Federation University

ORCID:
Tatyana R. Korablyeva: http://orcid.org/0000-0002-5650-617X

Abstract

Myeloperoxidase is a heme-containing peroxidase expressed primarily in neutrophils and to a lesser extent in monocytes. Determining the activity of myeloperoxidase in blood cells is one of the tests of the immune status of animals. Conventional methods are based on the oxidation of benzidine by the peroxide system to the unstable benzidine blue, which spontaneously turns into stable brown benzidine. The aim of this study was to develop a modification of the cytological determination of the myeloperoxidase enzyme using metol. The relative percentage of peroxidase-positive neutrophils in the peripheral blood of animals was determined after 100 neutrophils had been counted. The task was achieved by using the reaction with metol in the method of cytological determination of the activity of neutrophil myeloperoxidase in animal blood smears, which was based on the oxidation of metol by a peroxide system. Images of micropreparations were digitized using a Sony device for processing the received images of the cells. The Image Tool computer program was used for this purpose. The biological substrate was processed from a buffer-incubation mixture with subsequent drying and microscopy. The main new modification of the method was using metol. Metol does not have the ability to inhibit the activity of myeloperoxidase. The research showed easy and fast results. This method is economical and perspective for using in practice.

Keywords: myeloperoxidase, blood, neutrophils, metol

Corresponding Author:
Tatyana R. Korablyeva
astemenkolp@gmail.com

Published: 5 April 2021

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the DonAgro Conference Committee.

1. Introduction

Enzymes regulate biochemical processes of the organism. Many drugs show their effect through the changing of the activity of enzymes inside the cells. Among the intracellular enzymes, myeloperoxidase is of interest. This enzyme is involved in oxidative metabolism and, accordingly, in antimicrobial protection. In pathological conditions, myeloperoxidase activity can significantly change [1-4]. Myeloperoxidase is the proinflammatory enzyme stored in the azurophilic granules of neutrophilic granulocytes [5].

The myeloperoxidase system is formed together with hydrogen peroxide and a halide cofactor. It forms the most effective microbicidal and cytotoxic mechanism of leukocytes [5].

The enzyme plays a fundamental role in the production of oxidizing agents by neutrophils. This heme enzyme uses hydrogen peroxide and chloride for catalysis and production of hypochlorous acid, which is the main strong oxidizing agent generated by neutrophils [6].

In the presence of hydrogen peroxide, myeloperoxidase oxidizes the chlorine anion to hypochlorite, which has a strong antibacterial effect due to induced oxidative stress [7, 8].

Myeloperoxidase plays a critical role in the destruction of Klebsiella pneumoniae, Cryptococcus neoformans [9, 10].

The evaluation of mieloperoxidase activity is the crucial for determining the level of inflammation.

In blood cells of clinically healthy animals, there was a high determined myeloperoxidase activity, and in thick animals blood it was reduced. Myeloperoxidase activity is reduced in neutrophils having infectious leukocytosis and some metastatic tumors. Determining the activity of myeloperoxidase in blood cells is one of the tests of the immune status of animals [11, 12].

For studying the influence of various substances and pathological processes on the activity of intracellular enzymes, the methods of destructing cells followed by purification and concentration of the studied substances are used [13, 14].

The disadvantage of this model is the studying of the properties of the enzymes isolated from their intracellular environment. Protein conformation in the extracellular environment can change significantly, which will lead to a change of the kinetic parameters of the enzyme.

This does not allow correctly assess the changes of the enzyme activity. The cytological studies that doesn't provide for cell destruction, determining of myeloperoxidase is most often carried out using a benzidine test. Conventional methods are based on the oxidation of benzidine by the peroxide system to an unstable benzidine blue, which spontaneously turns into a stable benzidine brown [15]. One of the most common cytological methods for the determination of myeloperoxidase is the Graham method (1966). According to this method the enzyme activity is detected using alcohol solution saturated with benzidine.

The aim of the study was to develop a modification of the cytological determination of the myeloperoxidase enzyme using metol.

2. Methods and Equipment

Neutrophils of stabilized blood of 10-day old calves were investigated. Identification of peroxidase was carried out according to the Graham-Knoll method [15] and our modified technique. In modern methods of cytological detection of myeloperoxidase in blood cells, an alcohol solution saturated with benzidine is used. The intensity of staining in myeloperoxidase stained smears of peripheral blood was classified exploring 100 consistent neutrophils. Using the Graham-Knoll method (1966), the enzyme activity was detected in an alcohol solution saturated with benzidine with various concentrations of hydrogen peroxide for 5 minutes at a temperature of 37 degrees Celsius. Smears were washed in running water and air dried in the dark. Dry smears were studied under the microscope during immersion. Images of micropreparations were digitized using a Sony analog camcorder and image capture systems. For processing the received images of the cells, the Image tool computer program was used.

3. Results

One of the disadvantages of the above methods for detecting myeloperoxidase activity is the using of benzidine, which has a carcinogenic effect.

The assigned task is achieved by using the reaction with metol in the method of cytological determination of the activity of neutrophil myeloperoxidase in animal blood smears, which is based on the oxidation of metol by a peroxide system.

To solve this problem, we prepared the biological substrate, processing it with a buffer-incubation mixture, incubated the biological substrate with subsequent drying and microscopy.

For preparing the buffer-incubation mixture, 4-methyl para-aminophenol sulfate (the commercial name is methol) was used – a non-deficient substance that is used in combination with hydroquinone for the photo and film development. An alcohol solution saturated with benzidine is cheaper than benzidine, and most importantly, it is harmless to the researcher's body.

To obtain a buffer-incubation mixture, 0.1 M Tris-HCl buffer (pH 7.2-7.5) was prepared. On the day of the study, 10 mg of methol was dissolved in 10 ml of distilled water, 13.5-14 ml of 0.1 M was added to the resulting solution of Tris-HCl buffer and 2-3 drops of a

1% hydrogen peroxide solution, and incubated blood smears in the dark for 5 minutes. After the incubation of blood smears, they were washed with distilled water, dried and additionally colored with a 0.5% aqueous solution of safranin or neutral red, which makes it possible to determine blood neutrophils by the shape of the nuclei. The stained blood smears were washed with distilled water, dried and microscopic using an immersion lens of a light microscope. The relative percentage of peroxidase-positive neutrophils in the peripheral blood of animals was determined after counting 100 neutrophils. The activity of the studied enzyme was judged by the number of granules specifically colored in brown in the cytoplasm of peroxidase-positive neutrophils of animal blood.

To compare the results and comparison with the known method of the detection of myeloperoxidase using a benzidine reagent, blood smear microscopy was performed using an immersion system at a magnification of (90×10). The activity of the determined enzyme was analysed by staining intensity and the number of brown granules in the neutrophil cytoplasm. For visual evaluation of cytochemical reaction, different degrees of enzyme activity were taken into account.

0 degree – only the nucleus of neutrophils is stained, the cytoplasm is not stained, the contours of granules are not visible;

1st degree – the entire cytoplasm is diffusely colored into light brown or no more than 1/4 of the cytoplasm is colored;

2nd degree – well-browned granules are visible in the cytoplasm;

3rd degree – granules occupy the entire cytoplasm, but the nucleus is free of granules (Figure 1);

4th degree – granules occupy the entire cytoplasm and are layered on the nucleus.

The average cytochemical index (SCI) was derived according to the following formula, preliminarily counting 100 neutrophils, where a, b, c, d, e are the number of cells, respectively 0, 1, 2, 3, and 4 degrees.

The novelty is due to the fact that the proposed method uses metol, which does not have the ability to inhibit the activity of myeloperoxidase of animal blood granulocytes and does not have a carcinogenic effect.

4. Discussion

Activated neutrophils use myeloperoxidase (MPO) to generate many potent toxic oxidizing agents.

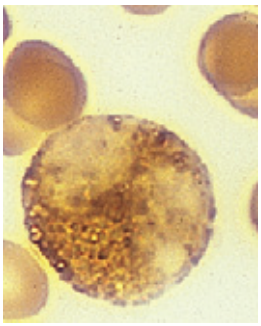

Figure 1: Microphotographs of activity of myeloperoxidase neutrophils of animal blood

Benzidine ($C_{12}H_{12}N_2$) is used in medical diagnostics (benzidine test, synonym for Gregersen reaction) to detect blood. Owing to the oxidation of benzidine by hydrogen peroxide due to the catalytic peroxidase activity of the blood, we observe the appearance of green or blue color in the tested samples.

During the oxidation of benzidine used in reactions, intensely colored compounds are formed, if myeloperoxidase is present in the cell, it forms a hypochlorite anion, which, being a strong oxidizing agent, oxidizes benzidine converting it to a colored compound. In cytochemical reactions, the enzyme activity is determined by the oxidation of chromogens (benzidine, o-dianisidine, and others) according to the Graham-Knoll method or its modifications.

In our research we applied an organic substance parametaminaminophenol sulfate with the formula (C7H10NO)2SO4 which is easily oxidized and passes into a colored compound just like benzidine.

The sample size of this pilot study was small. The technique is simple and economical. Further studies are needed to test the sensitivity and specificity of the method for determining the myeloperoxidase enzyme in blood neutrophils under various pathological conditions in animals.

5. Conclusion

The proposed method is an express method for cytochemical analysis of the activity of myeloperoxidase in neutrophilic granulocytes in blood smears, it allows one to determine the relative percentage of peroxidase-positive blood neutrophils and the intracellular activity of the studied enzyme, which makes it possible to assess the state of cellular microbicidal blood systems receiving participation in the formation of nonspecific resistance of the body.

Funding

The scientific work was carried out as part of the implementation of the initiative theme at the faculty of veterinary medicine of the Federal state Autonomous Educational Institution of Higher Education of V.I. Vernadsky Crimean Federation University.

Acknowledgement

The authors would like to thank their colleague for their contribution and support of the research. They are also thankful to the Academy of bioresources and environmental sciences for provision of logistics, and the university staffs for material and logistic supports, and their cooperation in completing this research.

Conflict of Interests

The authors have no conflict of interest to declare.

References

[1] Aratani, Y. (2018). Myeloperoxidase: Its Role for Host Defense, Inflammation, and Neutrophil Function. Journal Archives of Biochemistry and Biophysics vol. 64, pp. 47–52.

[2] Mandelkorn, J., *et al*. (1980). Immunofluorescent Demonstration of Myeloperoxidase of Phi Bodies and Rods in Leukaemic Leucocytes. *Journal of Histochemistry and Cytochemistry .,* vol. 12, pp. 449–456.

[3] Kettle, A. and Winterbourn, C. (1997). Myeloperoxidase: A Key Regulator of Neutrophil Oxidant Production *Journal.Redox Report*. vol. 3, issue 1, pp. 3–15.

[4] Ndrepepa, G. (2019). Myeloperoxidase - A Bridge Linking Inflammation and Oxidative Stress with Cardiovascular Disease. *Journal Clinica Chimica Acta*. vol. 493, pp. 36–51.

[5] Schultz, J. and Kaminker, K. (1962). Myeloperoxidase of the Leucocyte of Normal Human Blood. I. Content and Localization.*Journal Archives of Biochemistry and Biophysics*, vol. 96, pp. 465–467.

[6] Hampton, M., Kettle, A. and Winterbourn, C. (1998). Inside the Neutrophil Phagosome: Oxidants, Myeloperoxidase, and Bacterial Killing. *Blood*. vol. 92, issue 9, pp. 3007–3017.

[7] Blair-Johnson. M., Fiedler. T. and Fenna, R. (2009). Human Myeloperoxidase: Structure of a Cyanide Complex and its Interaction with Bromide and Thiocyanate Substrates. *Biochemistry Journal,* vol. 40, pp. 13990–13997.

[8] Klebanoff, S. and Hamon, C. (1972). Role of Myeloperoxidase-Mediated Antimicrobial Systems in Intact Leukocytes. Journal of the Reticuloendothelial Society, vol. 12, issue 2, pp. 170–196.

[9] Hirche, T., *et al*. (2005). Myeloperoxidase Plays Critical Roles in Killing Klebsiella Pneumoniae and Inactivating Neutrophil Elastase: Effects on Host Defense *Journal of Immunology*. vol. 174, issue 3, pp. 1557–1565.

[10] Aratani, Y., *et al.* (2006). Contribution of the Myeloperoxidase-Dependent Oxidative System to Host Defense against Cryptococcus Neoformans. *Journal of Medical Microbiology*, vol. 55, issue 9, pp. 1291–1299.

[11] Klebanoff, S., *et al*. (2013). Myeloperoxidase: A Front-Line Defender against Phagocytosed Microorganisms.*Journal of Leukocyte Biology,* vol. 93, pp. 185–198.

[12] Theilgaard–Mönch, K., Porse, B. and Borregaard, N. (2006). Systems Biology of Neutrophil Differentiation and Immune Response.*Journal* Current Opinion in Immunology, vol. 18, pp. 54–60.

[13] Dri, P., *et al*. (1982). New Approaches to the Detection of Myeloperoxidase Deficiency. *Blood*, vol. 60, pp. 323–327.

[14] Kitahara, M., Simonian, Y. and Eyre, H. (1979). Neutrophil Myeloperoxidase: A Simple, Reproducible Technique to Determine Activity.Journal of Laboratory and Clinical Medicine., vol. 93, issue 2, pp. 232–237.

[15] Graham, R. and Karnovsky, M. (1966). The Early Stages of Absorption of Injected Horseradish Peroxidase in the Proximal Tubules of Mouse Kidney: Ultrastructural Cytochemical Studies using Peroxidases as Protein Tracers.*Journal of Experimental Medicine.*, vol. 124, pp. 1123–1133.