The Interference Effect on Determination of Myeloperoxidase Levels by Developed Assay Kit
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ABSTRACT

Myeloperoxidase (MPO) is used as an inflammatory marker for several diseases. The MPO reaction can be interfered by some reducing agents. The aim of this study is to check whether some substances found in some biological fluids can interfere the reaction of this developed assay kit. The color of the MPO kit were evaluated by the naked eye and spectrophotometer in present of albumin, ascorbic acid and glucose at the specific concentration of white blood cells. The absorbance of the reaction mixture was measured at the wavelength 586 nm. This study demonstrated that the detection of MPO level could be interfered by albumin and ascorbic acid, but not glucose.

บทคัดย่อ

ไมอีโลเพอร์ออกซิเดสถูกใช้เป็นตัวบ่งชี้อย่างหนึ่งเกี่ยวกับภาวะอักเสบในโรคหลายชนิด ซึ่งปฏิกิริยาของไมอีโลเพอร์ออกซิเดสสามารถถูกรบกวนได้ด้วยสารบางอย่าง ดังนั้นคณะผู้วิจัยจึงมีวัตถุประสงค์เพื่อตรวจสอบว่าสารบางอย่างที่พบในของเหลวชีวภาพสามารถรบกวนปฏิกิริยาการตรวจหาไมอีโลเพอร์ออกซิเดสของชุดน้ำยาที่พัฒนานี้หรือไม่ โดยทำการศึกษาการเกิดสีของชุดน้ำยาที่พัฒนาขึ้นด้วยตาเปล่าและเครื่องสเปกโทรโฟโตมิเตอร์ในภาวะที่มีอัลบูมิน กรดแอสคอร์บิก และกลูโคส เมื่อมีความเข้มข้นของเม็ดเลือดขาวที่จำเพาะ โดยวัดค่าการดูดกลืนแสงของการเกิดปฏิกิริยาที่ความยาวคลื่น 586 นาโนเมตร จากการศึกษาครั้งนี้แสดงให้เห็นว่าอัลบูมินและกรดแอสคอร์บิกสามารถรบกวนการเกิดปฏิกิริยาของการตรวจหาไมอีโลเพอร์ออกซิเดส ในขณะที่กลูโคสไม่รบกวนการตรวจวัดในปฏิกิริยานี้

Keywords: Myeloperoxidase, Interference, Developed assay kit
คำสำคัญ: ไมอีโลเพอร์ออกซิเดส สารรบกวน ชุดน้ำยาที่พัฒนา

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Introduction

Microbial invasion can cause various diseases in human. The first defense system against the microbial invasion is neutrophils. Granules in neutrophils contain myeloperoxidase (MPO), a heme-containing enzyme in oxido-reductase class (E.C.1.11.1.7). In various inflammatory diseases, MPO is a local mediator of tissue damage by releasing from lysis neutrophil in the setting of inflammatory process and it acts as an important therapeutic target in blood, or urine for treatment of inflammatory conditions (Brovkovych et al., 2008). At present, MPO is a well-studied enzyme in health and disease.

MPO has multiple substrates including hydrogen peroxide (H_2O_2). These substrates donate 2 electrons to the intermediate of myeloperoxidase compound I, such as include chloride, bromide and thiocyanate (Furtmuller et al., 1998, 2000). Because chloride is the mostly found in phagosome (van Dalen, et al., 1997, MPO oxidation of chloride can form hypochlorous acid (HOCl) for killing microorganisms (Winterbourn and Kettle, 2013).

PST, is a code of this novel substrate, developed by our research group with a petty patent, for MPO bromination reaction. A test kit containing PST, potassium bromide (KBr), H_2O_2 change the color from yellow to purple when MPO is added. This test kit has a potential to use in medical laboratory for assaying MPO in biological fluids (such as blood and urine) as a marker of inflammatory. The color of the reaction can be measured by using a spectrophotometer. However, several reducing agents, such as uric acid, can interfere the reaction of this developed test kit. Uric acid competing with chloride by MPO catalysis causes decreasing HOCl production (Larissa et al., 2018).

Objectives of the study

The aim of this study is to check whether some substances found in some biological fluids, including albumin, ascorbic acid and glucose can interfere the reaction.

Methodology

Collection of blood

This study was reviewed and approved by the Institutional Ethical Committee at Khon Kaen University (Ethical number; HE 611605). Blood were collected from Blood bank, Srinagarind hospital, Khon Kaen University during 2019. It was centrifuged at 3,000 rpm for 10 min at 4^oC and buffy coat was collected.

White blood cells (WBCs) counting

The buffy coat (white blood cells) was diluted with normal saline and counted by automated XS-800i hematology analyzer. In this study stock WBC concentration was $298.17 x 10^3$ cells/μL (neutrophil 51.2%)

MPO preparation from buffy coat

Buffy coat was washed with 0.9% sodium chloride solution follow by centrifugation at 3,000 rpm for 10 min at 4°C. This step was repeated several times until no contaminated red blood cells were in the centrifuge tubes. The white blood cells obtained was diluted with 0.9% sodium chloride. After that, the diluted white blood cells were sonicated for 10 min to break cell membrane. The solution was centrifuged for 10 min and the supernatant which contained MPO was kept at -20°C for further analysis.

Assay of MPO activity

The assay mixture composed of 1mM PST 75 μL, 0.75 M KBr 300 μL in a 0.1 M phosphate buffer pH 6.4 2,475 μL, 25 μL stock interference reagents (albumin, creatinine and glucose) per each concentration and 25 μL of MPO (contain WBC 1,295.28 cells/μL) was mixed together at room temperature. 6 mM H_2O_2 100 μL was finally added to the reaction mixture. The absorbance of the reaction mixture was measured at the wavelength 586 nm at 10 min. The experiments were performed in triplicate.

Results

Effect of albumin on the developed assay kit

Colorimetric method for MPO detection may be interfered by high concentrations of albumin, ascorbic acid and glucose in the artificial samples. To test this possibility, the artificial samples were supplemented with albumin, ascorbic acid and glucose to give final concentrations between 0 and 2.0 g/L (Figure 1), 0 and 500 g/dL (Figure 2) and 0 and 5,000 mg/dL (Figure 3), respectively. Albumin was found to interfere at 0.5 and 1 g/L albumin by the naked eye and spectrophotometer for this developed assay kit, while ascorbic acid at all concentration used interfered the reaction. However, glucose showed no effect on this MPO detection. The results showed good recovery for glucose with approximately 100%.

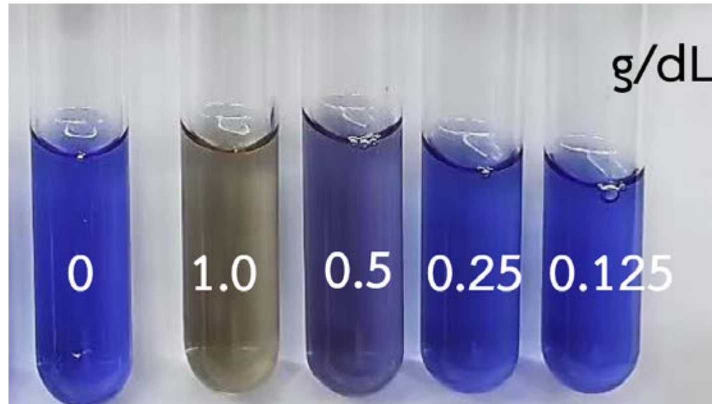

Figure 1 Effect of albumin on the MPO assay. Artificial samples were supplemented with albumin (final albumin concentrations between 0 and 1.00 g/dL).

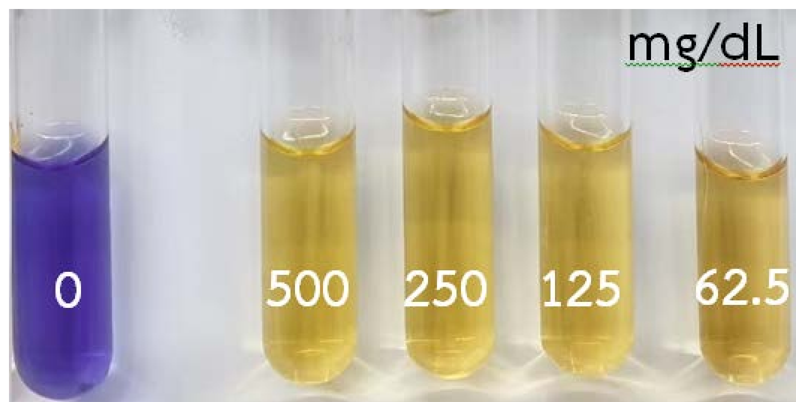

Figure 2 Effect of ascorbic acid on the MPO assay. Artificial samples were supplemented with ascorbic acid (final creatinine concentrations between 0 and 500 g/dL).

Figure 3 Effect of glucose on the MPO assay. Artificial samples were supplemented with glucose (final glucose concentrations between 0 and 5,000 mg/dL).

Discussion and Conclusions

MPO is used as an inflammatory marker for detection of urinary tract infection (Ciragil et al., 2014), septicemia (Schrijver et al., 2017) and periodontal disease (Klangprapan et al., 2016). It acts as an important therapeutic target for treatment of inflammatory conditions (Brovkovych et al., 2008). At present, MPO is a well-studied enzyme in health and disease. PST is a novel substrate, developed by our research group with a petty patent, for MPO bromination reaction. This test kit has a potential to use in medical laboratory for assaying MPO in biological fluids as a marker of inflammatory. The color of the reaction can be measured by using a spectrophotometer. However, several reducing agents, such as uric acid, can interfere the reaction of this developed test kit. Uric acid competing with chloride by MPO catalysis causes decreasing HOCl production (Larissa et al., 2018). The main finding of our study is that albumin and ascorbic acid could interfere the MPO detection. Albumin was found to interfere at 0.5 and 1 g/L by the naked eye and spectrophotometer for this MPO assay kit. However, in healthy person can be detect albumin in urine less than 30 mg/dl and glucose had the minimal effect on this MPO kit Ascorbic acid interfered at all concentrations used in this study. Albumin and ascorbic acid may produce their hydrolysis products which change the pH in buffer system of artificial samples.

In summary, the present study demonstrated that the detection of MPO could be interfered by albumin and ascorbic acid, but not glucose in artificial samples. For further work, this developed kit should be studied by using the other interferences before use with biological specimen such as urine.

Acknowledgements

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