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In vitro and in vivo inhibition of myeloperoxidase with 5-fluorouracil

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Abstract Objective: Myeloperoxidase (MPO) exists in neutrophils and has an important bactericidal role. During phagocytosis, MPO catalyzes a peroxidative re- action using chloride ion and hydrogen peroxide (H₂O₂) as substrate. The aim of the present study was to in- vestigate whether 5-fluorouracil (5-FU), a chemothera- peutic agent, has a direct inhibitory effect on MPO and to evaluate some properties of this inhibition.

Methods: The inhibitory effect of 5-FU on MPO was studied in rat tissue, human leukocytes, and leukocytes from cancer patients under 5-FU therapy. MPO was solubilized in a detergent-containing buffer. MPO activity was measured spectrophotometrically through the oxidation of a synthetic substrate tetramethyl benzidine in the presence of H₂O₂.

Results: 5-FU inhibited tissue-associated MPO activity in a dose-dependent but not time-dependent manner with an IC₅₀ value of 0.6 mg/ml. 5-FU also inhibited MPO activity in isolated human leukocytes in a dose-dependent manner, and the IC₅₀ value was 0.75 mg/ml. Using this 5-FU concentration, the inhibitory effect was monitored at different substrate concentrations. Leu- kocyte MPO activities of patients receiving 5-FU ther- apy were compared before treatment and after the first, second, and third administration cycles. 5-FU treatment resulted in a significant decrease in leukocyte MPO ac- tivity, and repeated 5-FU treatment caused additional decrease.

Conclusion: Our data showed that 5-FU directly inhib- ited the MPO activity of human leukocytes in vitro and in vivo. We concluded that, the patients treated with 5-FU should be intensively followed for the risk of infections.

Keywords Myeloperoxidase · 5-Fluorouracil · Myelosuppression

Introduction

Myeloperoxidase (MPO, EC 1.11.1.7) is localized in the primary granules of polymorphonuclear (PMN) leuko- cytes and lysosomes of monocytes [1]. It has an impor- tant role in killing bacteria during phagocytosis [2, 3]. MPO is a heme-containing enzyme which catalyzes the formation of hypochlorous acid (HOCl) from hydrogen peroxide (H₂O₂) and chloride ion (Cl⁻) during phago- cytosis, and this antimicrobial system is called the MPO/ H₂O₂/Cl⁻ system [4, 5].

5-Fluorouracil (5-FU), a fluorinated analog of uracil, is the drug of choice for the treatment of metastatic colorectal carcinoma [6, 7]. The substitution of fluorine for hydrogen in the 5 position of the uracil molecule results in the formation of a compound that is recog- nized as deoxyuridine monophosphate (dUMP) by metabolizing enzymes after being anabolized to 5-fluo- rodeoxyuridine monophosphate (FdUMP). 5-FU has been proven to be a potent inhibitor of thymidylate synthase and is incorporated into DNA and RNA in the form of fluorinated nucleotides [8, 9]. Leukocytes are the most affected group of cells at bone marrow during chemotheray, and leukopenia is the cause of many systemic fatal infections. For that reason, an ideal chemotherapeutic agent should have a minimal immuno- suppressive effect [10, 11]. If antimicrobial function of leukocytes is impaired in addition to myelosuppression, the risk of infections increases.

In a previous study, we have shown that tissue MPO activity was decreased in 5-FU-treated rats when compared with the untreated group. Pathological examination has shown that the inhibition of MPO activity was
much more important than the inhibition of leukocyte infiltration [12]. The aim of the present study was to evaluate a possible direct inhibitory effect of 5-FU on MPO in both in vitro and in vivo conditions.

**Materials and methods**

**Chemicals**

3,3′,5,5′-Tetramethyl benzidine (TMB), \( \text{H}_2\text{O}_2 \) (3% solution), bovine serum albumin (BSA), Histopaque 1077, and hexadecyltrimethylammonium bromide (HETAB) were obtained from Sigma Chemical Company (St.; dimethyl formamide (DMF) was from BDH Chemicals Ltd. (UK). All other reagents were of analytical grade.

**Biological material**

In this study, rat colon tissue and human leukocytes were used as a sample. All experiments were conducted in accordance with the appropriate ethics guidelines. The tissue sample was obtained from a male Wistar rat colon as described previously [13] and homogenized in 1:10 (w/v) 50 mM potassium phosphate buffer (pH 7.4) using a Dounce homogenizer. The homogenate was centrifuged at 15000×g, and the pellet was resuspended in HETAB-containing buffer.

Leukocytes isolated from the blood of healthy volunteers were used for in vitro experiments. For in vivo studies, leukocytes were isolated from the blood of ten patients (five male and five female, with an age range of 24–68 years) with gastrointestinal tumors and treated only with 5-FU. These patients were treated for five consecutive days per month (one administration cycle) by i.v. bolus injections of 5-FU at 400–600 mg/m². A 10-ml venous blood sample was taken from each patient before chemotherapy and after the first, second, and third administrations. Leukocytes were isolated using Histopaque 1077, and leukocyte pellets were homogenized with phosphate-buffered saline (PBS). Of this leukocyte homogenate, 20 μl was used for protein determination. The remaining leukocyte homogenate was centrifuged at 15000×g, and the pellet was resuspended in HETAB-containing buffer and assayed for MPO activity.

**MPO assay**

The method of Suzuki et al. [14] was used with slight modification. Standard reaction mixture consisted of 500 μl detergent-containing buffer (160 mM potassium phosphate buffer, pH 5.4, 1% HETAB), 100 μl TMB (16 mM, dissolved in DMF), 50 μl sample homogenate, and 300 μl water. The reaction was started by the addition of 50 μl \( \text{H}_2\text{O}_2 \) (diluted to 0.06% for tissue samples and 0.003% for leukocytes) at 37°C. The rate of MPO-catalyzed oxidation of TMB was followed by recording the increase of absorbance at 655 nm. Considering the initial and linear phase of the reaction, we measured the absorbance change per minute, and one enzyme unit was defined as the amount of enzyme producing one absorbance change per minute under assay conditions. Enzyme activity was calculated as units per gram of wet tissue for tissue samples and units per milligram of protein for leukocytes.

To follow the in vitro effect of 5-FU, the drug was added to the reaction mixture just before measuring MPO activity. Enzyme activity was first measured at different 5-FU concentrations and the IC\(_{50}\) value was determined for tissue and leukocyte samples. Using this 5-FU concentration, the inhibition of leukocyte MPO activity was evaluated at varying TMB and \( \text{H}_2\text{O}_2 \) concentrations. To investigate the in vivo effect of 5-FU, MPO activity was measured in 5-FU-treated patients’ leukocytes, before and after treatment. The Lowry method was used for quantitative protein analysis, where BSA was used as the protein standard [15].