Interleukin-8 is not involved in the increased chemotactic activity of peripheral blood plasma during acute myocardial infarction

T. Siminiak¹, J.-M. Schröder², M. Sticherling², and H. Wysocki²

¹ Department Intensive Therapy, Academy of Medicine, Poznan, Poland
² Department of Dermatology, University of Kiel, FRG

Summary: Polymorphonuclear neutrophils (PMN) are known to participate in the development of tissue injury during myocardial infarction due to both free oxygen radicals release, as well as to their involvement in the "no-reflow" phenomenon. We have previously shown that peripheral blood plasma (obtained from patients with acute myocardial infarction) has chemotactic activity for PMN and is able to induce PMN adherence as well as superoxide anion production. To investigate whether interleukin-8 (IL-8/NAP-1), a potent chemotactic factor for PMN, is involved in plasma-mediated PMN stimulation, we measured plasma levels of IL-8 in five patients with transmural myocardial infarction with highly sensitive enzyme-linked immunosorbent assay (ELISA) using specific antibodies. Blood samples were taken immediately after patients' admission, within 15 and 30 min of treatment with intravenous nitrates, as well as after 1, 2, 3, and 7 days. All samples expressed IL-8 activity within the detection limit (0.4 ng/ml) as observed at the basal state. Thus, IL-8 may not be considered as responsible for the chemotactic activity in peripheral blood in patients with myocardial infarction.

Key words: Polymorphonuclear neutrophils – acute myocardial infarction – interleukin-8 – chemotactic activity

Introduction

Participation of polymorphonuclear neutrophils (PMN) in the development of tissue injury during acute myocardial infarction is well documented. Activated PMN aggregate and adhere to endothelium (6) that leads to subsequent leukoembolization of coronary capillaries and impairment of blood flow. Furthermore, activated PMN release large amounts of free oxygen radicals and proteolytic enzymes (8, 15) that exert a direct cytotoxic effect on myocytes. Increased PMN migration to ischemic myocardium in response to locally released chemotactic stimuli was shown in several experimental studies to enhance the extent of irreversibly injured myocardium.

We have previously reported that peripheral blood plasma from patients with myocardial infarction has chemotactic activity for PMN obtained from healthy donors and is able to augment PMN adherence (9, 10), as well as inducing PMN superoxide anion production (26). Systemic activation of PMN as a result of myocardial infarction was shown by evaluation of PMN aggregation in peripheral blood (1, 25).

The purpose of the present study was to verify if interleukin-8, a potent chemotactic factor for PMN, is involved in increased chemotactic activity of peripheral blood plasma during AMI.
Material and methods

Blood samples were collected by venipuncture from five consecutive patients (A–E), aged 36–65 years, with transmural acute myocardial infarction. Patients were admitted to our department 6–12 h after acute ischemia onset. Since we have observed modification of the chemotactic plasma activity by intravenous nitrates (unpublished observations), the blood samples were collected as follows: immediately after patients' admission, within 15 and 30 min of treatment with intravenous isosorbide dinitrate as well as 1, 2, 3, and 7 days after onset of symptoms, i.e., within the period of previously reported (9, 10) increased chemotactic activity in peripheral blood. Diagnosis was based on clinical investigation, electrocardiograms and echocardiography as well as on enzymes evaluation. Patients with concomitant infection, diabetes, neoplasma, rheumatoid arthritis, and skin disease were excluded from the study group; also data from patients with shock were not taken into consideration. Patient C, a 59-year-old man, died on the 6th day due to recurrent ventricular fibrillation.

Interleukin 8 was measured with an enzyme-linked immunosorbent assay as described previously (12). Monoclonal anti-IL-8 antibodies were purified from culture supernatants by ammonium sulfate precipitation and subsequent chromatography on protein A fast protein liquid chromatography columns (Pharmacia-LKB, Uppsala, Sweden). For sandwich-ELISA testing, polystyrene microtiter plates were coated with purified antibodies at 10 µg/ml overnight at 4°C and blocked thereafter with 1% bovine serum albumin in phosphate-buffered saline. Samples to be tested were incubated in duplicate for 1 h with coated wells. After washing, biotinylated antibody was added at optimal concentration determined in advance, as well as avidin-biotin-peroxidase complex (Vector Labs, Burlingame, California) was added. Using o-phenylenediamine as substrate, the enzymatic color reaction was measured at 492 nm in a Behring ELISA processor II.

The study protocol was accepted by the Regional Committee for Human Research.

Results

The extinction values of serial IL-8 dilutions for the standard curve were as follows: 
E = 0.5 for 3.1 ng/ml; E = 0.36 for 1.6 ng/ml; E = 0.24 for 0.8 ng/ml; E = 0.15 for 0.4 ng/ml.

Mean values of duplicates of the extinction observed with plasma samples obtained from patients with acute myocardial infarction are shown in Table 1. Since IL-8 concentrations capable to induce PMN chemotaxis in Boyden chambers were shown (23) to range between $1 \times 10^{-10}$ M and $5 \times 10^{-10}$ M, i.e., far above the detection limit of our ELISA immunoassay and IL-8 was not detected in the peripheral blood of patients with myocardial infarction, it could not be considered as involved in the generation of chemotactic activity.

Table 1. The extinction values of ELISA immunoassay using anti-IL-8 antibodies of plasma samples obtained from patients (A–E) with acute myocardial infarction: immediately after admission, in 15 and 30 min of treatment with intravenous nitrates as well as 1, 2, 3 and 7 days after acute ischemia onset. Mean values of duplicates.

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