

# Adenosine and neutrophil CD11b/CD62L and oxidative burst in stable ischemic heart disease

Jacek Rysz<sup>1</sup>, Robert Błaszczak<sup>1</sup>, Kornelia Kędziora-Kornatowska<sup>2</sup>,  
Krzysztof Kujawski<sup>1</sup>, Sławomir Jabłoński<sup>3</sup>, Leszek Markuszewski<sup>4</sup>

<sup>1</sup>Department of Family Medicine, Medical University of Lodz

<sup>2</sup>Department and Clinic of Geriatrics Collegium Medicum Medical UMK of Bydgoszcz

<sup>3</sup>Department of Surgery and Thoracosurgery of Hospital No. 2, Medical University of Lodz

<sup>4</sup>Clinic of Cardiology and Cardiometabolism Hospital No. 2, Medical University of Lodz

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**Corresponding author:**

Jacek Rysz, MD PhD  
Department of Family Medicine  
Medical University of Lodz  
Zeromskiego 113  
90-549 Lodz, Poland  
Phone/fax: +48 42 639 34 65  
E-mail: jacek.rysz@skwam.lodz.pl

## Abstract

**Objectives:** The immune response to endogenous as well exogenous stimuli is modified by various factors including drugs. Adenosine is an endogenous nucleotide involved in cellular energy metabolism. Adenosine receptors may influence the biological activity of numerous cells, regardless of sporadic or continuous manner of cellular adhesion under physiological conditions. These receptors are capable of specific recognition of cellular surface antigens, cellular segregation and the control of cellular morphology.

**Material and methods:** We evaluated the effect of a single, intravenous dose of adenosine on the respiratory burst of polymorphonuclear leucocytes and the expression of their cell surface adhesion antigens CD11b and CD62L in 20 male patients with stable ischaemic heart disease. Expression of CD11b and CD62L adhesion molecules was determined with flow cytometry using respective monoclonal antibodies, while the neutrophil oxidative burst was evaluated with the Burst-test assay.

**Results:** The current study results demonstrate that Adenocor does not affect the respiratory burst of polymorphonuclear leucocytes after *Escherichia coli* phagocytosis or PMA stimulation. The expression of adhesion molecules after intravenous adenosine at the dose of 140 µg/kg/min was not altered either.

**Conclusions:** The analysis of the presented results provides unequivocal evidence to characterize the effects of adenosine on the selected parameters of immune reactions and the statistically insignificant increase of CD11b and CD62L was only moderate after a single intravenous dose of adenosine.

**Key words:** ischaemic heart disease, CD11b and CD62L, adhesion molecules, adenosine.

## Introduction

Adenosine is a common, endogenous nucleotide involved in cellular energy metabolism. After intravenous administration, it is rapidly degraded and its half-life in serum is approximately 10 seconds. Adenosine uptake is primarily regulated by the red blood and endothelial cells, where it is degraded by adenosine deaminase to inosine and adenosine monophosphate (AMP). Adenosine receptors are cell surface glycoproteins intermediating protein interactions as well as carbohydrate and protein interactions, as it is in the

case of selectins. They play an important role in adhesion to the extracellular matrix (ECM) and cellular migration within ECM [1]. Adenosine receptors may influence the biological activity of numerous cells, regardless of the sporadic or continuous manner of cellular adhesion under physiological conditions. These receptors are capable of specific recognition of cellular surface antigens, cellular segregation and the control of cellular morphology. Cell surface antigen panel controls the biological activity and determines the different behaviour of various cell types [1, 2]. The regulation of expression of various types of adhesion molecules plays a role in many physiological processes, including hemostasis and leucocyte recirculation. The alternation of expression of a given set of adhesion antigens, in both quantitative and qualitative terms, may often underlie pathological processes as in metastasis, tissue remodelling or inflammatory processes. Immune cells react to various stimuli and change their function, particularly phagocytosis, transformation or cytokine release. These reactions require energy resources and the increased turnover of compounds capable of storing the energy for cellular requirements [3, 4]. The application of modern research assays allows for monitoring of biochemistry of biologically active compounds and their effects on physiological processes. Some pharmaceuticals (Adenocor, Preductal) may directly affect cellular energy resources, which consequently alter the cellular phenotype and cell activity. Therefore, the current study was designed to evaluate the effect of a single, intravenous dose of adenosine on the respiratory burst of polymorphonuclear leucocytes and the expression of their cell surface adhesion antigens CD11b and CD62L.

## Materials and methods

### Patients and treatment

The study included 20 male patients with stable ischaemic heart disease, aged 38-66 years (mean  $51.8 \pm 8.2$  years) with the diagnosis confirmed with clinical evaluation, non-invasive tests (including electrocardiographical and echocardiographical exercise tests) and coronarography. The exclusion criteria included bronchial asthma, chronic obstructive pulmonary disease, and symptoms of infection, concomitant therapy with theophylline or any other medications known to affect the metabolism of adenosine. The study protocol was approved by the Medical University of Lodz Bioethics Committee.

Adenosine (Adenocor; Sanofi-Winthrop, Gentilly, France) was administered with the infusion pump at the rate  $140 \mu\text{g}/\text{kg}/\text{min}$  no longer than for 6 minutes. The echocardiographical adenosine exercise test was performed simultaneously to determine resting conditions and then it was followed up during adenosine infusion and up to four minutes after the infusion was discontinued.

### Expression of CD11b and CD62L adhesion molecules

Peripheral blood samples (5 ml) were drawn from the antecubital vein to tubes containing heparin. These samples were obtained before heparin administration (reference group) and 5 minutes after the intravenous injection of adenosine. Expression of CD11b and CD62L adhesion molecules on the cell surface was determined with monoclonal antibodies provided by DAKO in a flow cytometry assay performed with automated flow cytometer FACStar (Becton-Dickinson). Incubation with monoclonal antibodies was performed in whole blood samples following the monoclonal antibodies manufacturer recommendations.

### Assay of respiratory burst

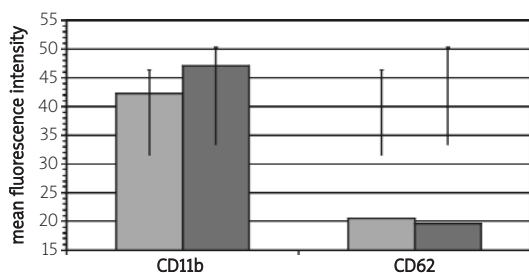
The respiratory burst was assayed with the Burst-test (ORPEGEN Pharma, Heidelberg, Germany). The samples of whole, heparinised blood ( $100 \mu\text{l}$ ) after cooling on ice for 10 minutes were supplemented with  $20 \mu\text{l}$  suspension of non-pretreated opsonised *E. coli*, phorbol 12-myristate 13-acetate (PMA) or N-formyl-methionyl-leucyl-phenylalanine (fMLP) and incubated in a water bath at  $37^\circ\text{C}$  for 10 minutes, then  $10 \mu\text{l}$  solution of 1-, 2-, 3-dihydrorodamine was added before consecutive incubation at  $37^\circ\text{C}$  for another 10 min. After the incubation, the samples were lysed for 20 minutes after the addition of 1 ml of lysing solution (Orpegen Pharma, Heidelberg, Germany) diluted 1:10 with distilled water and centrifuged for 5 minutes at  $4^\circ\text{C}$ . Phosphate buffered solution was used for washing and then, the samples were centrifuged. Then,  $20 \mu\text{l}$  DNA Staining Solution (ORPEGEN Pharma, Heidelberg, Germany) was added and samples were incubated on ice for 15 minutes. The samples were evaluated with flow cytometry and the analysis included 10 000 cells with size and intracellular granules respective for polymorphonuclear leucocytes to determine the percentage of cells with the respiratory burst and the mean fluorescence intensity was measured.

### Statistical analysis

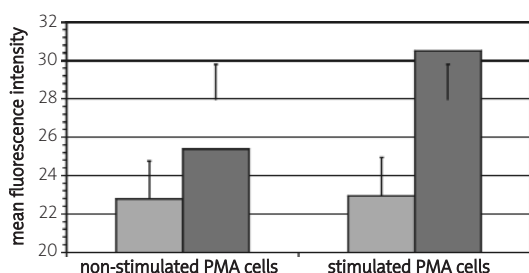
Results from fluorescence assays are expressed as arithmetic mean  $\pm$  standard deviation. The differences between groups were analysed with Mann-Whitney test. The outcomes with the likelihood of null hypothesis rejection at  $P$  of 0.05 or less were considered statistically significant.

## Results

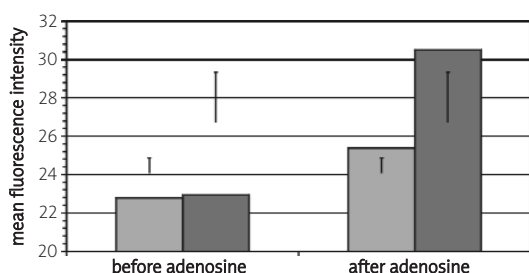
The expression of CD11b and CD62L on polymorphonuclear leucocyte surface presented as the arithmetic mean of fluorescence before and after adenosine administration is depicted in Figure 1. The



**Figure 1.** The expression of CD11b and CD62L on polymorphonuclear leucocyte before and after adenosine



**Figure 2.** Polymorphonuclear leucocyte respiratory burst before and after adenosine (non-stimulated PMA cells and stimulated cells)



**Figure 3.** Polymorphonuclear leucocyte respiratory burst before and after adenosine (non-stimulated PMA cells and stimulated cells)

differences between their expression before and after adenosine administration were not statistically significant.

The assay of the polymorphonuclear leucocyte respiratory burst before and after adenosine administration demonstrated the increase of mean fluorescence intensity both in PMA stimulated cells and in the case of non-stimulated cells. The increase in relation to reference group did not however, reach a significance level in statistical terms (Figures 2 and 3).

### Discussion

The immune cells are the first line defence against invading pathogens and the most immediate response to external stimuli including predominantly

recruited polymorphonuclear leucocytes [3, 5, 6]. Adhesion antigens present on their surface are integral glycoproteins mediating protein interactions or protein-carbohydrate interactions as in the case of selectins. Selectins play an important role in cellular adhesion to the extracellular matrix (ECM) and the processes of cellular migration within ECM. The adhesion molecules may influence the biological activity of immune cells regardless whether the cellular adhesion is incidental or takes place for extended time periods. These cells are capable of specific recognition, segregation and morphological control of other cells. They express numerous surface adhesion molecules with regard to the cell type, stage of maturity or the functional state. The alternation of the surface antigen panel may be associated with cellular stimulation and de novo synthesis, the release from cell storage pools or reversible transformation from inactive into active receptor forms. The entire composition of the surface antigen panel may determine the biological activity and the differences in the responsiveness of various cell types. The control of expression of adhesion antigens is responsible for physiological reactions including hemostasis and leucocyte recirculation [7]. However, the unexpected change of expression panel of adhesion antigens both in quantitative and qualitative terms may underlie different pathology as metastasis, tissue remodelling or inflammatory processes. Cell surface adhesion receptors are classified based on their structural and functional properties into kadherins, adhesins, selectins and integrins. The group of selectins, including CD62L antigen, include protein family mediating inflammatory reaction and share common structural features. They are expressed on the surface of leucocytes and activated endothelial cells. CD62L antigen is constitutively present on the surface of monocytes, polymorphonuclear leucocytes and lymphocytes. The antigen is regarded as a precursor of phagocyte inactivation and their migration into the inflammatory site. It is apparently related to the increased expression of CD62L and consequently enhanced expression of surface  $\beta_2$  integrins, especially CD11b. These mechanisms are guiding phagocyte migration towards their target sites [1, 7]. Integrins, including CD11b antigens, are major adhesion receptors mediating cellular interactions with ECM and intercellular signal processing. After binding a specific ligand, they mediate downstream signal transduction leading to alternation of phosphorylation of membrane internal proteins, intracellular  $Ca^{2+}$  concentration, cytoplasm pH, inositol turnover and eventually the entire cell functional state with regard to adhesion, aggregation, secretion and proliferation. The final biological effects of downstream and upstream intracellular signal transduction are variable depending on the cell and ligand type [8-10].

The oxidative stress is defined by a fine balance between the activity of oxidants and antioxidants. The uncontrolled increase of reactive species including the single atoms of molecules with an unpaired electron is associated with the pathogenesis of atherosclerosis, diabetes mellitus and cancer [3, 4]. Under physiological conditions, the defence mechanisms with various antioxidants are able to dispose of the excess of oxygen reactive species. The increased generation of reactive species and the disturbed redox equilibrium may lead to the cellular and tissue damage. The elucidation of the precise mechanisms of reactive species generation may lead to novel areas of therapeutical intervention. Polymorphonuclear leucocytes after stimulation generate large amounts of reactive oxygen species in a process of the respiratory burst and the release of superoxide anion and hydrogen peroxide. The respiratory burst occurs as a result of increased glucose turnover through the pentose shunt that provides reduced form of nicotinadeninenucleotide phosphate (NADPH) catalysed by NADP/NADPH<sup>+</sup> oxidase within the cellular membrane of polymorphonuclear leucocytes.

The immune response is commonly modulated with various medications. The current study results demonstrate that Adenocor does not affect the respiratory burst of polymorphonuclear leucocytes after *Escherichia coli* phagocytosis or PMA stimulation. The probable explanation of these effects might be related to the short half-life of the drug in systemic circulation after intravenous administration. The expression of adhesion molecules after intravenous adenosine at the dose of 140 µg/kg/min was not altered either. The increased expression of a single receptor is thought to be related to an increased energetical demand requiring adenine phosphate substrates (ATP, ADP, AMP) [2, 11]. The intravenous dose of Adenocor might have not reached the sufficient serum concentration to enhance the turnover of energetical substrates and subsequently could not alter the expression of the surface antigen assayed in this study. It does not, however, exclude the possible role of adenosine in the regulation of the immune response at higher doses. This apparently merits further research. The analysis of the presented results provides unequivocal evidence to characterize the effects of adenosine on the selected parameters of immune reactions and the statistically insignificant increase of CD11b and CD62L was only moderate after a single intravenous dose of adenosine.

## Conclusions

These results demonstrate that a single intravenous dose of adenosine does not affect the respiratory burst of peripheral blood polymorphonuclear leucocyte and the expression of their surface adhesion molecules CD11b and CD62L.

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