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Original Research Article

# Sustained decrease in plasma sphingosine-1-phosphate concentration and its accumulation in blood cells in acute myocardial infarction

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ABSTRACT

Sphingosine-1-phosphate (S1P) is a cardioprotective sphingolipid present at high concentration in plasma and blood cells. However, effect of the myocardial infarction on S1P metabolism in blood is poorly recognized. Therefore, we aimed to examine the dynamics of changes in concentration of sphingolipids in blood of patients with acute ST-segment elevation myocardial infarction (STEMI). The study was performed on two groups of subjects: healthy controls ($n = 32$) and patients with STEMI ($n = 32$). In the latter group blood was taken upon admission to intensive heart care unit, and then on the second, fifth and thirtieth day, and approximately two years after admission. STEMI patients showed decreased plasma S1P concentration and accumulation of free sphingoid bases and their 1-phosphates in erythrocytes. This effect was already present upon admission, and was maintained for at least thirty days after the infarction. Interestingly, two years post-infarction plasma S1P level recovered only partially, whereas the content of erythrocyte sphingolipids decreased to the values observed in the control subjects. The most likely reason for the observed reduction in plasma S1P level was its decreased release or increased degradation by vascular endothelial cells, as we did not find any evidence for downregulation of S1P synthesis or release by blood cells. We conclude that patients with STEMI are characterized by marked alterations in sphingolipid metabolism in blood which could be a consequence of the infarction itself, the antiplatelet treatment given or both. Our data suggest that cardioprotective action of S1P may be diminished in patients with acute myocardial infarction.



## 1. Introduction

Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid metabolite that regulates a number of cellular functions including cell proliferation, differentiation, survival and migration [1]. S1P is produced by the enzyme sphingosine kinase (SPHK) which phosphorylates free sphingosine. SPHK can also use free sphinganine as substrate to form sphinganine-1-phosphate (SA1P) [2]. S1P can be either dephosphorylated by specific and nonspecific lipid phosphatases or irreversibly cleaved by the enzyme sphingosine-1-phosphate lyase in the final degradative step of sphingolipid metabolism (Fig. 1) [1].

S1P was found to play a dual role in cellular signaling, acting either as an intracellular messenger or by binding to a family of five plasma membrane G protein-coupled receptors ($S1PR_{1-5}$) [3]. It should be noted that SA1P was shown to activate these receptors with a potency comparable to S1P [4]. S1PRs are ubiquitously expressed in tissues, including the myocardium. In cardiomyocytes $S1PR_1$ is the dominating isoform, however, $S1PR_2$ and $S1PR_3$ are also present in these cells [3]. In human and rodent plasma S1P is found at high nanomolar concentrations sufficient to activate S1PRs [5].

S1P has recently attracted much attention as an important factor protecting the heart against ischemia-reperfusion injury [6–8]. It was shown that preincubation of rat neonatal cardiomyocytes with S1P prevents hypoxia-induced cell death [9]. Cardioprotective action of extracellular S1P was subsequently confirmed in rodent models of ischemia/reperfusion injury [10,11] as well as in human myocardial tissue [12]. Consistently, deletion of the SPHK1 gene increased susceptibility of the heart to ischemia/reperfusion injury [13], whereas adenovirus-mediated SPHK1 gene transfer was found to attenuate post-ischemic heart failure [14]. It was also suggested that cardioprotective properties of high-density lipoprotein (HDL) involve S1P, as most of plasma S1P is contained within HDL [15].

S1P was also found to mediate ischemic preconditioning (IPC) in the heart. SPHK1 is activated in response to IPC [16] which

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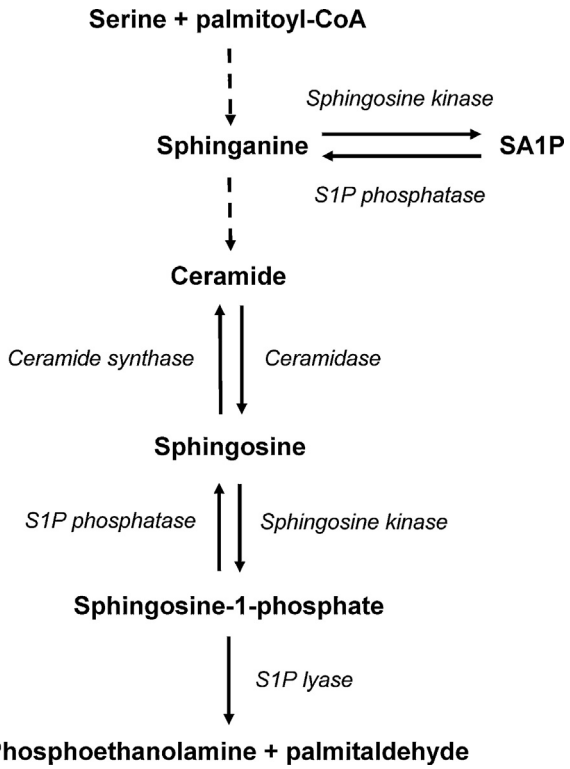


**Fig. 1.** Schematic representation of metabolism of free sphingoid bases and their 1-phosphates. S1P – sphingosine-1-phosphate, SA1P – sphinganine-1-phosphate.

to a large extent prevents decrease in the enzyme activity and myocardial S1P level upon ischemia/reperfusion [17]. Consistently, pharmacological or genetic inhibition of SPHK1 abolished IPC-induced cardioprotection in the murine heart [13,16]. Vessey et al. [18] suggested that the mechanism of IPC involves export of produced S1P from cardiomyocytes to the extracellular space and stimulation of S1PRs. Interestingly, it was found that ischemic postconditioning, similarly to IPC, protected isolated mouse hearts against ischemia/reperfusion injury via SPHK1 activation [19]. S1P was shown to exert its cardioprotective action via activation of Akt kinase and STAT-3 transcription factor [20]. Mitochondrial S1P produced by SPHK2 may be involved in this effect as well [21]. In addition, recent data indicate that S1P may exert cardioprotection by stimulating phosphorylation of connexin43 [22].

We previously found that plasma concentration of S1P and SA1P in patients with acute myocardial infarction was markedly lower as compared to healthy controls [23]. In the present study we aimed to gain more insight into the mechanism underlying this phenomenon by investigating the effect of myocardial infarction on sphingoid base-1-phosphate metabolism in blood cells.

## 2. Materials and methods

### 2.1. Subjects

The investigation conforms with the principles outlined in the Declaration of Helsinki and was approved by the Ethical Committee for Human Studies of the Medical University of Bialystok. All patients gave their informed consent prior to their inclusion in the study.

Two groups of subjects were recruited for the study: (1) patients with acute ST-segment elevation myocardial infarction (STEMI) ($n = 32$) and (2) controls matched for age, sex and body mass index with no history of cardiovascular disease or other chronic diseases ($n = 32$). Subjects from this group did not take any medications at the time of the study. The characteristics of these groups are given in Table 1. In the control group, blood was taken from the antecubital vein after an overnight fast. In the patients with acute STEMI, blood was taken upon admission to intensive heart care unit and then on the second, fifth and thirtieth day, and approximately two years after admission in the fasting state. The heart infarct was diagnosed according to the European Society of Cardiology guidelines basing on the presence of chest pain lasting over 20 min, changes in ECG and elevation of markers of myocardial necrosis [24]. Before admission to the hospital (in the ambulance) each patient obtained the following treatment: aspirin (300 mg), clopidogrel (600 mg) and unfractionated heparin (5000 units). Treatment with aspirin (75 mg/day) and clopidogrel (75 mg/day) was continued in the following days. Each patient underwent successful primary coronary intervention with bare stent implantation.

**Table 1**
Clinical characteristics of the subjects.

| | Control | Infarction |
|---|---|---|
| Age (years) | 55.2 ± 1.8 | 65.4 ± 2.2 |
| Sex (males/females) | 22/10 | 22/10 |
| BMI | 28.0 ± 1.2 | 28.6 ± 1.1 |
| Blood glucose (mg%) | 99.1 ± 2.0 | 107.1 ± 4.3 |
| Total cholesterol (mg%) | 194 ± 7 | 191 ± 9 |
| HDL cholesterol (mg%) | 61.2 ± 3.2 | 42.1 ± 2.4*** |
| LDL cholesterol (mg%) | 118 ± 7 | 124 ± 8 |
| Triglyceride (mg%) | 122 ± 13 | 134 ± 22 |
| Red blood cell count ( $\times 10^6/\mu l$) | 4.74 ± 0.07 | 4.61 ± 0.1 |
| Hemoglobin (g/dl) | 14.3 ± 0.3 | 14.0 ± 0.3 |
| Platelet count ( $\times 10^3/\mu l$) | 259 ± 10 | 267 ± 16 |
| Diabetes (%) | 0 | 25 |
| Hypertension (%) | 0 | 59 |
| Hyperlipidemia (%) | 0 | 48 |
| ACE inhibitors/ARBs (%) | 0 | 87 |
| Beta blockers (%) | 0 | 75 |
| Statins (%) | 0 | 100 |
| Proton pump inhibitors (%) | 0 | 79 |
| Insulin (%) | 0 | 19 |
| Metformin (%) | 0 | 12 |

The results are means ± SEM.
ACE – angiotensin converting enzyme; ARB – angiotensin II receptor blocker; BMI – body mass index; HDL – high-density lipoprotein; LDL – low-density lipoprotein.
*** $p < 0.001$ vs. the control group (by Student's $t$-test).

### 2.2. Blood fractionation

Immediately after sampling into 4 ml sodium citrate tubes blood was centrifuged at 300 × $g$ for 10 min at room temperature, and the platelet-rich plasma was transferred to a fresh plastic tube. The leukocyte-rich buffy coat was thoroughly removed. Separated erythrocytes were suspended in phosphate-buffered saline (PBS), centrifuged at 800 × $g$ for 10 min and the upper layer as well as the remaining buffy coat were discarded. Red blood cells were then resuspended in PBS and flash frozen in liquid nitrogen. Platelet-rich plasma was centrifuged at 1000 × $g$ for 10 min to separate platelets. Supernatant was then transferred to a fresh plastic tube and recentrifuged at 5000 × $g$ for 10 min to obtain platelet-free plasma. Isolated thrombocytes were washed with platelet wash buffer (5 mM $KH_2PO_4$, 5 mM $Na_2HPO_4$, 0.1 M NaCl, 1% glucose, 0.63% sodium citrate, pH 6.6), suspended in PBS, and flash frozen in liquid nitrogen. All samples were stored at −80 °C until analysis.

Hemoglobin concentration in erythrocyte suspensions was determined colorimetrically using Drabkin's reagent kit (Sigma). Protein concentration in platelet samples was measured with the BCA protein assay kit (Sigma). Bovine serum albumin (fatty acid free, Sigma) was used as a standard.

In order to test for possible S1P release from platelets activated during isolation procedure blood samples were taken from six healthy volunteers. Plasma was then isolated from each sample by